Abstracts

• Fats and Oils

COLORED PIGMENTS IN COTTONSEED. II. ROLE OF HEXANE ISOMERS ON OIL QUALITY. F. Osman et al. *Nahrung* **20**, 475–82 (1976). Isohexane is the most advantageous isomer. The refining and decolorization processes become better as the percentage of isohexane in the normal hexane increases. Benzene is the least desirable constituent in commercial hexane. Its presence is responsible for the darkest color of the oil which indicates the selectivity of benzene for colored pigments. The most important losses during refining were registered during the extraction of the oil with benzene or benzene-hexane. Cyclohexane in n-hexane (35–60%) and methylcyclopentane (6–12%) do not increase the degree of extraction of the colored pigments but increase the loss of the extracted oil during refining. (Rev. Fr. Corps Gras)

MODIFICATION OF THE TOCOPHEROLS AND OF THE PLASTOCHROMANOL DURING THE AUTOXIDATION OF THE CRUDE AND REFINED OILS. M. Gogolewski et al. *Tluszcze jadalne* **1976**(3), 1–7. The greatest resistance to the autoxidation process belongs to the δ-tocopherol present in the soybean oil. The level of this compound is still about 50% of the initial value, at the moment when the other tocopherols and the plastochromanol submit to an almost total decomposition. The differences in the speed of decomposition of individual tocopherols can be explained by a different aptitude of these compounds to form dimers. The highest aptitude to form these compounds belongs to the δ-tocopherol and to the plastochromanol. (Rev. Fr. Corps Gras)

STUDY OF THE INFLUENCE OF THE NATURE OF THE STATIONARY PHASE ON THE PRECISION OF DETERMINATIONS OF THE COMPOSITION OF METHYL ESTERS OF FATTY ACIDS BY GAS-LIQUID CHROMATOGRAPHY. EXAMPLE: THE RAPESEED OIL. W. Kubacka et al. *Prace Inst.* **26**(1976), 89–100. A study of the influence of the stationary phase on the precision and the repeatability of fatty acid methyl ester determination is presented in this paper. The study has been done on the most frequently used stationary phases: BDS, PEGA, and EGSS-X. It was established that an influence of the nature of the stationary phase on the results of determinations of fatty acid methyl esters in rapeseed oil exists, the differences being, however, minimal and, in the most cases, not exceeding 1%. (Rev. Fr. Corps Gras)

RAPID METHOD FOR DETERMINATION OF THE COMPOSITION OF METHYL ESTERS OF FATTY ACIDS IN RAPESEEDS WITH THE STATISTIC EVALUATION OF ITS REPEATABILITY. S J. Kubacki et al. *Prace Inst.* 26, 81–8 (1976). The gas-liquid chromatographic method for determination of the composition of methyl esters of fatty acids in the rapeseeds, used for a great series of analyses, is presented. These have been done on a column 1.5 m length filled with 10% BDS on 100/120 Diatomite CAW, the duration being about 15 min. The repeatability of the method, expressed by the standard deviation, varies from 0.21% for stearic acid to 1.89% for erucic acid. (Rev. Fr. Corps Gras)

DETERMINATION OF CHLOROGENIC ACID IN SUNFLOWER SEEDS. A.N. Oumanskaya et al. *Pishch. Promst.* **1976**(4), 173–4. Complete extraction of free chlorogenic acid from sunflower seeds is realized if the seeds are submitted to a triple treatment for 30-40 min with 70% ethanol at 50–55C. The use of thin layer chromatography for the separation of phenolic compounds allows a reduction of 5–6 times of the time of development of the chromatogram with a good reproducibility of the results. (Rev. Fr. Corps Gras)

RHEOLOGICAL CHARACTERISTICS OF MARGARINES. L.K. Nikolaiev. *Pishch. Promst.* **1976**(4), 134–6. The viscosity properties of different margarines are characterized with a biparametric dependence and vary considerably in function, not only of the temperature, but also of the speed gradient. The effective viscosity of margarines decreases with increase of these two parameters. For all the margarines studied, there exists a characteristic independent of temperature and another dependent of temperature, and with these, it is possible to determine the viscosity value of the studied margarines. (Rev. Fr. Corps Gras)

MODIFICATIONS OF CULINARY FATS DURING THE HEATING. V.P. Maksimetz et al. *Pishch. Promst.* **1976**(4), 67–8. The values of peroxide, of thiobarbituric acid, and of benzidine, and the optical density at 227 nm (unsaturated aldehydes) and 283–285 nm (saturated aldehydes) cannot be used for evaluation of the degree of thermooxidation. However, these values are interesting for the study of the dynamics of oxidation processes in the heating conditions. The color, the refractive index at 40C, and the viscosity at 40C are in correlation with the duration of heating but these values increase highly only after 8 hours. Although the acidity value increases in proportion to the increase of duration almost linearly, a direct dependence between the acid value and the oxidation degree doesn't exist. Absorption in the cyclohexane at 232 nm can be used for the evaluation of dienic polymerization products in heated fats. (Rev. Fr. Corps Gras)

THE LIPIDIC OXIDATION PRODUCTS AND THE ANTIRADICAL ACTIVITY OF LIPIDS OF SUNFLOWER SEEDS DURING THE MATURATION. V.G. Shtcherbakov et al. *Pisch. Promst.* **1976**(4), 37–9. The oxidation processes of lipids in oil rich sunflower seeds occur even in the normal physiological state of the maturing seed. A high content of tocopherols and a high antiradical activity of lipids in the hulls was found. In the lipids from seeds harvested by the two phase process, the oxidation processes occur more intensively than in the seeds which became mature in the plant. (Rev. Fr. Corps Gras)

PARTICULARITIES OF THE PREVENTED OXIDATION OF THE LIPIDS. V.N. Oushkalova et al. *Pishch. Promst.* **1976**(4), 27–30. The use of a model permits one to establish the particularities of the inhibition of oxidation of lipids. The classic phenols are 10–15 times less effective in preventing the oxidation of the lipids than the model esters. Despite the different activity, the consumption of phenols in the oxidation of lipids and models occurs at equal speeds. The cause of the weak effectiveness of the additions of phenols into unsaturated lipids is determined by a rupture of the chains of the natural inhibitors. (Rev. Fr. Corps Gras)

MODIFICATION OF THE LIPIDS IN THE PROCESS OF PREPARATION AND PRESERVATION OF BREAD. L.I. Poutchkova et al. *Pishch. Promst.* **1976**(4), 23–6. In the preparation and subsequent preservation of bread, the total content of lipids and lipidic products formed remains unchanged, but not the ratio between the free and bound forms. The new distribution of the free and bound forms of the lipids is a function of the composition and of the properties of lipidic products formed. During the period of conservation of the bread, the samples with an addition of sunflower oil and hydrogenated cottonseed oil show a decrease of the bound lipids, to the contrary of those with an addition of a mixture of 80% of sunflower oil and 20% of hydrogenated cottonseed oil, as well as a mixture obtained by transesterification of sunflower oil and 15–20% of tallow. The increase of the bound lipids improves the quality of the bread. (Rev. Fr. Corps Gras)

SYNTHETIC PHOSPHOGLYCERIDES. PART 2. PREPARATIVE AND QUANTITATIVE THIN-LAYER CHROMATOGRAPHY. M. Ranny, J. Silhanek, A. Bradikova, R. Seifert and M. Zbirovsky. *Tenside Deterg.* **14**(5), 246–50 (1977). Components were isolated from synthetic phosphoglycerides, by means of preparative thin-layer chromatography, which were recognized to be salts of phosphatide, semilysophosphatide and bisphosphatide acid and as a mixture of lysophosphatide and phosphatide acid. Methods for the quantitative determination of glycerides and phos-

phoglycerides by quantitative thin-layer chromatography were investigated and a commercial product analyzed.

PALM OIL MILL PROCESS DESCRIPTION. T.D. Tjeng and J.J. Olie (Stork Apparatus Construction B. V., Amsterdam). *Seifen, Ole, Fette, Wachse* 103(16), 453–7 (1977). Covers harvesting and mill processing operations. Discussed under processing are reception; storage; sterilization; stripping; digesting; pressing; clarification and purification of oils.

PHYSICO-CHEMICAL CHARACTERISTICS OF LIPID-ORGANIC SOLVENT MIXTURES INTERESTING THE VEGETABLE OIL EXTRACTION AND THEIR TRANSFORMATIONS ON MISCELLA. STUDIES ON THE SURFACE TENSION, DENSITY AND VISCOSITY FOR THE MIXTURES OF LAURIC ACID, OLEIC ACID OR VIRGIN OLIVE-OIL WITH HEXANE OR CYCLOHEXANE. V. Flores Luque and C. Gomez Herrera. *Rev. Fr. Corps Gras* 24, 433–8 (1977). A miscella presents an extraction capacity for the oil placed inside capillary pores of oilseed flakes. This capacity increases with the value of «combined function», defined by the quotient «surface tension/kinematic viscosity». The authors study the «combined function» values for miscellas obtained mixing hexane or cyclohexane with olive oil, oleic acid or lauric acid. These values are compared to those obtained from miscellas of soybean oil with several organic solvents. The «combined function» variations with miscella concentration and temperature depend principally on changes in its dynamic viscosity.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. A. Karleskind. *Rev. Fr. Corps Gras* 24, 419–25 (1977). Recent advances in liquid chromatography result from important technological progress: improvement in the instrumentation (pumps and, specially detectors); development of supports consisting of very small diameter particles, between 5 and 10 μ, allowing more efficient separations. Thus, the column-chromatography which is become the high performance liquid chromatography (HPLC) has been shown as particularly unique for studying big molecules and thermolabile compounds. It is particularly efficient for determining the aflatoxins and analysing the fats and oils.

DETERMINATION OF TRACE METALS BY ATOMIC ABSORPTION SPECTROPHOTOMETRY. A. Prevot. *Rev. Fr. Corps Gras* 24, 409–18 (1977). The instrumentation is more efficient thanks to recent improvements: electrodeless discharge lamps, new atomization sources (plasma), tantalum furnaces necessary for some elements (Si, Sn), control of the temperature. The automatic sample changers for graphite furnace improve the repeatibility and the time for determining Fe and Cu in the oils. The microcomputers memorizing the results allow automated calibrations. The evolution of Fe, Cu and Ni contents in oils during refining is reviewed and the determination of different elements: As, Cd, Pb, Hg, Mn in fats is described. Comparatively with other foods thier concentrations in fats are extremely low.

EVALUATION OF THE LIMPIDITY IN OILS BY LASER. J.E. Caupeil. *Rev. Fr. Corps Gras* 24, 427–31, (1977). Traditional control of wintering is carried out by a «Cold test». This test is simple, but the results require several hours. The laser allows to detect and evaluate the crystallisation germs in waxes so that a «cold test» requires only a few minutes. A method for determining in 15 mn the waxes at a level of mg/kg in wintering oils has been developed. This rapid method is able to control the crystallisation and filtration of waxes during the wintering.

COMPREHENSIVE EVALUATION OF FATTY ACIDS IN FOODS. J. Exler and J.L. Weihrauch. (Consumer and Food Economics Institute, Agricultural Research Service, U.S. Department of Agriculture, Hyattsville, Maryland) *J. Am. Diet. Assoc.* 71, 518–21 (1977). Based on production volume and value and on per capita consumption, shellfish are an important food group in the United States. The nutrient composition of shellfish indicates that this food group is low in fat and relatively high in protein. Tabulated data on fatty acids in the different edible portions of seven species of crustaceans and over twenty species of mollusks are presented. The proportionately high level of polyunsaturated fatty acids is shown by the polyunsaturation index calculated for some important species of shellfish.

^{13}C NMR MEASUREMENTS OF UNSONICATED PHOSPHATIDYLCHOLINE BILAYERS OF DIFFERENT FATTY ACID AND STEROL COMPOSITION. A.M.W. Lancee-Hermkens and B. De Kruijff (Dept. of Biochem., State Univ. of Utrecht, University Centre "De Uithof", Padualaan 8, Transitorium 3, Utrecht, The Netherlands) *Biochim. Biophys. Acta* 470, 141–51 (1977). ^{13}C NMR linewidths were measured for various ^{13}C resonances in unsonicated dispersions of synthetic and natural phosphatidylcholines both in the absence and presence of cholesterol at temperatures where the acyl chains are in the liquid-crystalline state. In the absence of cholesterol the linewidths of the various resolved chain resonances were decreased with increasing unsaturation and decreasing chain length. The motion of the 9-*cis* olefinic carbon atoms in dioleoylphosphatidylcholine was more restricted than the motion of the 9-*cis*olefinic carbon atoms in 1-stearoyl-2-oleoyl-phosphatidylcholine despite the higher overall fluidity of dioleoylphosphatidylcholine. The polar head-group motion was not dependent upon the fatty acid composition. Incorporation of cholesterol broadens all observed chain resonances of all phosphatidylcholines, thus demonstrating a reduction in chain motion by cholesterol. For both the saturated and unsaturated phosphatidylcholines the reduction of the chain motion is decreased with increasing chain length.

LATERAL PHASE SEPARATIONS IN BINARY MIXTURES OF PHOSPHOLIPIDS HAVING DIFFERENT CHARGES AND DIFFERENT CRYSTALLINE STRUCTURES. E.J. Luna and H.M. McConnell (Stauffer Lab. for Physical Chem., Stanford Univ., Stanford, Ca.) *Biochim. Biophys. Acta* 470, 303–16 (1977). Synthetic dipalmitoyl phosphatidylserine exhibits a sharp chain-melting transition temperature at 51°C as judged by partitioning of the spin label 2,2,6,6-tetramethylpiperidine-1-oxyl. Phase diagrams representing lateral phase separations in binary mixtures of dipalmitoyl phosphatidylserine with dipalmitoyl phosphatidylcholine as well as with dimyristoyl phosphatidylcholine are derived from paramagnetic resonance determinations of 2,2,6,6-tetramethylpiperidine-1-oxyl partitioning, freeze-fracture electron microscopic studies and theoretical arguments that limit the general form of acceptable phase diagrams. The reported phase diagrams are the first to describe binary mixtures in which one lipid is charged and the second lipid uncharged. These phase diagrams also are the first to include the problem of solid phases with different crystalline conformations as it related to the occurrence of a pretransition in phosphatidylcholines and its absence in phosphatidylserines. In addition to the phase diagrams reported here for these two binary mixtures, a brief theoretical discussion is given of other possible phase diagrams that may be appropriate to other lipid mixtures with particular consideration given to the problem of crystalline phases of different structures and the possible occurrence of second-order phase transitions in these mixtures.

OCCURRENCE OF FATTY ACID CHLOROHYDRINS IN JELLYFISH LIPIDS. R.H. White and L.P. Hager (Rodger Adams Lab., Dept. of Biochem., Univ. of Illinois, Urbana, Il.) *Biochemistry* 16, 4944–8 (1977). Fatty acid chlorohydrins are characterized as lipid components of an edible jellyfish. The four isomers 9-chloro-10-hydroxypalmitic acid, 10-chloro-9-hydroxypalmitic acid, 9-chloro-10-hydroxystearic acid, and 10-chloro-9-hydroxystearic acid were identified by gas chromatography-mass spectrometry comparison of the methyl esters and their trimethylsilyl derivatives with known synthetic samples. Two additional isomers, 11-chloro-12-hydroxystearic acid and 12-chloro-11-hydroxystearic acid, were also found in the lipid by the identification of the expected mass spectral fragments of the trimethylsilyl (Me_3Si) derivative of their methyl esters. These six isomeric compounds represented approximately 1.4% of the total extractable jellyfish lipid and were released from the lipid as methyl esters by boron trifluoride-methanol treatment. These isomers account for only about 30% of the organic chlorine in the lipid. Evidence is given that the remaining organic chlorine is also present as fatty acid chlorohydrins containing more than one hydroxyl group.

THE CRYSTAL STRUCTURE OF CHOLESTERYL 17-BROMOHEPTADECANOATE. S. Abrahamsson and B. Dahlen (Dept. of Structural Chem., Faculty of Med., Univ. of Goteborg, P.O.B., S-400 33 Goteborg 33, Sweden) *Chem. Phys. Lipids* 20, 43–56 (1977). Crystals of cholesteryl-17-bromoheptadecanoate ($C_{44}H_{77}BrO_2$) are monoclinic (P_{21}) with a = 7.663(2), b = 10.311(5), c = 55.96(2) Å and β = 103.10(3°). These are two molecules in the asymmetric unit which have different conformations of the cholesterol side chain and about the ester bond. The molecules pack with regions of only steroid skeleta alternating with regions of hydrocarbon chains. Due to the packing requirements of the skeleta the carbon chains are forced into a hybrid type packing which contains features

of the earlier known O⊥ and T|| subcells. The subcell (HS1) is orthorhombic with a_s = 10.3, b_s = 7.5 and c_s = 2.54 Å. The molecular packing is such that the ω-bromine atoms do not continue the *trans*-carbon chains but adopt a *gauche* conformation.

PERFLUOROALKANES. A MODEL FOR THE HEXAGONAL METHYLENE SUBCELL? D.L. Dorset (Molecular Biophys. Dept., Med. Foundation of Buffalo, Inc., Buffalo, N.Y.) *Chem. Phys. Lipids* 20, 13-9 (1977). High resolution hkO electron diffraction intensity data from hexagonally packed helices in the crystalline perfluoroalkane, $nC_{36}F_{74}$, are well fit by a CF_2 rotor model similar to the CH_2 rotor model used earlier for the α-form of long chain lipids. Other crystallographic evidence for similar helical twisting of polymethylene chains is adduced in a consideration of the structure as a model for the hexagonal methylene subcell.

MASSENSPEKTROMETRISCHE UNTERSUCHUNGEN AN ATHER-ESTERN DER 2,3-DIHYDROXYPROPIONSAURE. H.-J. Drexler, H. Schiller and A. Seher (Bundesanstalt fur Fettforschung, Puisallee 68–76, 4400 Munster, Germany) *Chem. Phys. Lipids* 20, 71–6 (1977). The mass spectra of two homologous series of 2,3-dialkoxypropionic acid alkylesters with ester moieties ranging from 8 to 22 C-atoms and either chains having 12 or 16 C-atoms have been studied and were discussed. Fragmentation starts by splitting off one of the two alkoxy groups. Consequently, two courses of fragmentation could be observed. This was proved by the mass spectrum of the newly synthesized DL-2-tetradecyl-3-hexadecyloxypropionic acid dodecylester.

MOLECULAR INTERACTIONS IN THE MODEL LIPOPROTEIN COMPLEX FORMED BETWEEN GLUCAGON AND DIMYRISTOYLGLYCEROPHOSPHOCHOLINE. R.M. Epand, A.J.S. Jones, and B. Sayer (Dept. of Biochem., McMaster Univ., Hamilton, Ontario L8S 4J9, Canada) *Biochemistry* 16, 4360–7 (1977). Glucagon forms water-soluble complexes with dimyristoylglycerophosphocholine below the phase transition temperature of the lipid. The peptide has no effect on the proton spin-lattice relaxation times of the lipid but causes a marked broadening of the line width of the terminal methyl and methylene resonances of the lipid. These results are interpreted as arising from a decrease in the rate of lateral diffusion of the lipid caused by the presence of glucagon. The thermal-transition temperature of the lipid is found to be unaltered by glucagon as monitored with the fluorescent probe, pyrene, or with differential scanning calorimetry. The latter technique also indicates that there is no change in the enthalpy of this transition, although some broadening is detected. Thus, there is no major alteration in the bilayer structure of the lipid caused by the presence of glucagon. Ultraviolet difference spectra indicate that the tyrosine and tryptophan residues of glucagon enter a more hydrophobic environment in the lipoprotein complex.

FATTY ACIDS, PART 14. SYNTHESIS OF FURANOID ESTERS FROM NATURALLY OCCURRING UNSATURATED FATTY ESTERS. M.S.F. Lie Ken Jie and C.H. Lam (Dept. of Chem., Univ. of Hong Kong, Hong Kong) *Chem. Phys. Lipids* 20, 1–12 (1977). Methyl 9,10,12,13-diepoxystearate yields a mixture of isomeric C_{18}-furanoid esters on treatment with propyl iodide-sodium iodide-dimethyl sulphoxide (PrI-NaI-DMSO). Pure methyl 9,12-epoxyoctadeca-9,11-dienoate can be obtained from methyl 12-oxo-octadec-*cis*-9-enoate by mercuration-demercuration reaction; methyl 9,10-epoxy-12-oxostearate, in the presence of PrI-NaI-DMSO, BF_3 or *p*-TsOH; and methyl 9-hydroxy-12-oxo-octadec-*trans*-10-enoate when treated with BF_3 or *p*-TsOH. The synthesis of a methyl substituted C_{18}-furanoid ester (methyl 9,12-epoxy-10-methyl-octadeca-9,11-dienoate) is also described.

CONJUGATED OCTADECADIENOIC ACIDS OF MILK FAT. P.W. Parodi (The Butter Marketing Board, Hamilton Central, 4007 Queensland, Australia). *J. Dairy Sci.* 60, 1550–3 (1977). Conjugated *cis, trans- (trans, cis)* octadecadienoic acids of milk fat were isolated by preparative gas liquid chromatography and preparative thin layer chromatography of their methyl esters. Reductive ozonolysis and partial hydrazine reduction followed by reductive ozonolysis of resulting *cis*- and *trans*-monoene fractions showed that the conjugated *cis, trans- (trans, cis)* octadecadienoic acids of milk fat consisted essentially of *cis*-9, *trans*-11-octadecadienoic acid. Gas liquid chromatography fatty acid profiles with 50-m wall-coated open tubular columns showed components having the same equivalent chain length as conjugated *trans, trans*-octadecadienoic acid and *cis,cis*-octadecadienoic acid.

AB INITIO MOLECULAR ORBITAL CALCULATIONS ON THE CONFORMATIONS OF SOME UNSATURATED FRAGMENTS OF LIPID MOLECULES: 1-BUTENE, CIS-2-PENTENE AND 1,4-PENTADIENE. W.T.M. Schurink and S. De Jong (Inst. of Theoretical Chem., Univ. of Nijmegen, The Netherlands) *Chem. Phys. Lipids* 19, 313–22 (1977). Conformational energies have been calculated for the title compounds which are basic fragments of physiologically active polyunsaturated fatty acids. For 1-butene extremely good accordance has been obtained with experimental data. In *cis*-2-pentene a strong influence of the *cis* methyl group on the potential energies for internal rotation has been found. For 1,4-pentadiene no great influence has been found from a possible coupling of the two rotors on the relative stabilities of the two stable conformations. Reasonable agreement has been observed with similar calculations published recently.

AUTOXIDATION OF ALKOXYLIPIDS. III. KINETICS OF PEROXIDE FORMATION. N. Yanishlieva and H.K. Mangold (Bundesanstalt fur Fettforschung, 44 Munster, Westf., Germany) *Chem. Phys. Lipids* 20, 21–9 (1977). The influence of the type and position of various functional groups in saturated glycerol-derived alkoxylipids on the kinetics of peroxide formation is studied. The autoxidation of the glycerol-derived compounds is compared with that of some structural analogs. As a rule, ethers are oxidized much faster than ether-esters and esters. Free hydroxy groups exert an accelerating effect on the rate of autoxidation.

• Biochemistry and Nutrition

SELF-ASSOCIATION OF APO-C-I FROM THE HUMAN HIGH DENSITY LIPOPROTEIN COMPLEX. J.C. Osborne, Jr., T.J. Bronzert, and H.B. Brewer, Jr. (Molecular Disease Branch, Natl. Heart, Lung, and Blood Inst., Natl. Insts. of Health, Bethesda, Md.). *J. Biol. Chem.* 252, 5756–60 (1977). The molecular properties of apo-C-I, isolated from the human high density lipoprotein complex, have been evaluated as a function of pH, solvent composition, and protein concentration by sedimentation equilibrium and circular dichroic measurements. This protein self-associates in aqueous solution at neutral pH with concomitant changes in secondary structure. In contrast, in the acid pH range, apo-C-I is monomeric and its ellipticity is independent of protein concentration. The results are discussed in terms of the interpretation of experiments where changes in the physical properties of apolipoproteins have been used to monitor ligand binding and lipid-apolipoprotein recombination.

COMBINED GAS CHROMATOGRAPHY—CHEMICAL IONIZATION MASS SPECTROMETRY OF SPHINGOLIPIDS. I. Glucosyl sphingosine, ceramides and cerebrosides of the spleen in Gaucher's disease. M. Oshima, T. Ariga and T. Murata (Dept. of Biochem. Schl. of Med., Kitasato Univ., Asamizodai Sagamihara-shi, Kanagawa, 228, Japan) *Chem. Phys. Lipids* 19, 289–99 (1977). Trimethylsilylated glucosyl sphingosine, ceramides and glucocerebrosides were analysed by combined gas chromatography (GC)—chemical ionization (CI) mass spectrometry. Isobutane, methane and ammonia were used as reactant gases for chemical ionization. Essentially the same fragment ions were detected in the spectra with the different reactant gases. Purified glucocerebrosides isolated from the spleen of a patient with Gaucher's disease were clearly separated into their 8 molecular species by gas chromatography. Three other minor components were detected by spectrometry, and these 11 molecular species of glucocerebrosides from the spleen of the patient with Gaucher's disease have been analysed. The ceramides obtained by periodate oxidation and then alkaline reduction of the glucocerebrosides were also separated into 11 molecular species by GC-CI mass spectrometry. Molecular weight could be determined using the major fragment ion of m/e (M^+-90) in the spectra of ceramides and cerebrosides. The chemical ionization method is useful for structural analyses and determination of the molecular species of sphingoglycolipids.

THE ISOLATION AND CHARACTERIZATION OF SPHINGOMYELINASE FROM HUMAN PLACENTAL TISSUE. P.G. Pentchev, R.O. Brady, A.E. Gal and Sue R. Hibbert (Dept. of Health, Education and Welfare, Natl. Insts. of Health, Bethesda, MD) *Biochim.*

Biophys. Acta **488**, 312–21 (1977). Human placental sphingomyelinase activity was eluted as a single symmetrical peak from Sephadex G-200 with a molecular weight of 290,000; however, the enzyme behaved heterogeneously on ion exchange chromatography. A specific species of sphingomyelinase was purified approx. 10,000-fold to a constant specific activity of 274,000 nanomol of sphingomyelin hydrolyzed per mg protein per h. When the purified enzyme was examined on sodium dodecyl sulfate disc gel electrophoresis, two distinct protein bands in approximately equal proportions with molecular weights of 36,800 and 28,300 were found. The specificity of the enzyme is directed towards both the hydrophilic phosphocholine and the hydrophobic ceramide moieties of sphingomyelin. Possible interrelationships between the heterogenous forms of placental sphingomyelinases are discussed.

EFFECT OF FAT AND CASEIN ON INTRACELLULAR KILLING OF STAPHYLOCOCCUS AUREUS BY MILK LEUKOCYTES. M.J. Paape and A.J. Guidry (Animal Physiol. and Genetics Inst., Agr. Res. Ctr., ARS, USDA, Beltsville, MD) *Proc. Soc. Exp. Biol. Med.* **155**, 588–93 (1977). Suspensions of PMN isolated from milk and *S. aureus* were incubated together at different ratios and with different types and concentrations of mammary fluids. Results showed that greater numbers of *S. aureus* were left unphagocytosed and alive within PMN at the higher *S. aureus*:PMN ratios (2:1) than at the lower ratios. This effect was more noticeable in whole milk than in skimmed milk. Phagocytosis and destruction of *S. aureus* by PMN (ratio 1:10) were reduced ($p < 0.01$) to a greater extent in whole milk or in skimmed milk containing 10% cream than in either skimmed milk or whey. There was no difference between skimmed milk or whey. Increasing the concentration of either whole milk or skimmed milk in the incubation mixture from 10 to 40% resulted in fewer ($p < 0.01$) *S. aureus* phagocytosed but had no affect on intracellular kill. The data show that the milk fat globule is a major deterrent to the phagocytosis and destruction of *S. aureus* by PMN. Casein inhibited phagocytosis, but only when the concentration of skimmed milk in the incubation medium was increased to 40%. Casein did not affect intracellular kill.

THE RELATIONSHIP BETWEEN CHOLINEPHOSPHATE PHOSPHATASE (ALKALINE PHOSPHATASE) AND PHOSPHATIDYLCHOLINE BIOSYNTHESIS IN HELA CELLS. H.B. Paddon and D.E. Vance (Dept. of Biochem., Univ. of British Columbia, Vancouver, British Columbia V6T 1W5, Canada) *Biochim. Biophys. Acta* **488**, 181–9 (1977). Enzymatic activity that hydrolyzes cholinephosphate and *p*-nitrophenyl phosphate (alkaline phosphatase, EC 3.1.3.1) has been found in two strains of HeLa cells. Both phosphatase activities were primarily associated with the microsomal fraction and had pH optima of 9–9.5. Double reciprocal plots indicated that cholinephosphate might be hydrolyzed by two distinct enzymes. *p*-Nitrophenyl phosphate competitively inhibited both cholinephosphate phosphatase activities. The role of cholinephosphate phosphatase in phosphatidylcholine biosynthesis was investigated by comparative studies between the two strains of HeLa cells. The data suggest that under normal growth conditions, cholinephosphate phosphatase does not influence the rate of phosphatidylcholine biosynthesis in HeLa cells.

MYCOBACTERIUM SMEGMATIS FATTY ACID SYNTHETASE. LONG CHAIN TRANSACYLASE CHAIN LENGTH SPECIFICITY. D.O. Peterson and K. Bloch (James Bryant Conant Labs., Harvard Univ., Cambridge, Mass.) *J. Biol. Chem.* **252**, 5735–9 (1977). Long chain transacylase activity, acyl-CoA + enzyme $\rightleftarrows$ acyl-enzyme + CoA, catalyzed by the multienzyme complex fatty acid synthetase from *Mycobacterium smegmatis* was measured by exchange of radioactive coenzyme A into even numbered fatty acyl-CoA substrates 14 to 24 carbon atoms long. This transacylase activity decreases sharply with increasing chain length. It is suggested that C_{24} transacylation may be rate-limiting in *denovo* fatty acid synthesis catalyzed by the mycobacterial system. Mycobacterial polysaccharides stimulate the rate of transacylation, and this enhancement becomes more marked as the chain length of the substrate increases. The magnitude of the effect is similar to polysaccharide stimulation of overall synthetase activity. It is therefore proposed that terminal transacylation is the specific and perhaps only partial reaction catalyzed by the *M. smegmatis* fatty acid synthetase which is facilitated by polysaccharide. The product distribution of the synthetase is distinctly bimodal, with peaks for acyl chains 16 and 24 carbon atoms long. A scheme based on nonoverlapping unimodal chain length specificities for the rates of two activities, elongation and terminal transacylation, is offered to explain this bimodal distribution.

INFLUENCE OF DIETARY COPPER AND ZINC ON RAT LIPID METABOLISM. H.G. Petering, L. Murthy, and Ellen O'Flaherty (Kettering Lab., Dept. of Environmental Health, Col. of Med., Univ. of Cincinnati, Cincinnati, Ohio) *J. Agric. Food Chem.* **25**, 1105–9 (1977). The effects of varying both dietary zinc and copper over wide ranges of concentrations on lipid and nonlipid metabolic parameters in male rats fed a nonatherogenic semipurified diet were investigated. Body weight was directly related to both zinc and copper, and as expected serum zinc and copper levels were directly dependent on dietary zinc and copper, respectively. Liver and kidney copper concentration were directly related to serum copper and ceruloplasmin. The most important findings were those which showed an inverse relationship between serum copper or ceruloplasmin concentrations and levels of serum cholesterol, triglyceride, and phospholipid. There was the expected direct relationship among serum levels of cholesterol, triglycerides, and phospholipids. Other correlations among levels of lipids and metals in serum and tissue were also found, but interestingly no correlation was found between serum or dietary zinc and serum cholesterol.

THERMODYNAMICS OF LIPID PROTEIN ASSOCIATIONS. THERMODYNAMICS OF HELIX FORMATION IN THE ASSOCIATION OF HIGH DENSITY APOLIPOPROTEIN A-I (APOA-I) TO DIMYRISTOYL PHOSPHATIDYLCHOLINE. H.J. Pownall, F.J. Hsu, M. Rosseneu, H. Peeters, A.M. Gotto and R.L. Jackson (Div. of Athero. and Lipoprotein Res., Dept. of Med., Baylor College of Med. and The Methodist Hosp., Houston, TX) *Biochim. Biophys. Acta* **488**, 190–7 (1977). The structure and phospholipid-binding properties of human plasma high density apolipoprotein A-I (apoA-I) has been studied at pH 7.4 and 3.1 by microcalorimetry, circular dichroism and density gradient ultracentrifugation. At pH values of 7.4 and 3.1, apoA-I binds to dimyristoyl phosphatidylcholine (DMPC) to form complexes of similar composition (molar ratio of DMPC/apoA-I of 100) and helical content (67%). At pH 7.4, the lipid-protein association is accompanied by an increase in helical content from 58 to 67% and an exothermic enthalpy of binding (ΔH_B) of −90 kcal/mol apoA-I. At pH 3.1, the helical content of apoA-I is increased from 48 to 67% on binding to DMPC and the enthalpy of binding was −170 kcal/mol. We suggest that the difference in the enthalpies of binding (−80 kcal/mol) at pH 3.1 compared to 7.4 is due to the greater coil → helix transition at the lower pH.

CATION DIFFUSION SELECTIVITY IN A PORE MODEL. THE PHOSPHATIDYLCHOLINE/WATER LAMELLAR PHASE. J.L. Rigaud and C.M. Gary-Bobo (Lab. de Physiol. Cellulaire, Col. de France, Paris 5e, France) *Biochim. Biophys. Acta* **469**, 246–56 (1977). The diffusion coefficients D (cm^2/s), of four monovalent cations K^+, Na^+, Rb^+ and Cs^+ and of Ca^{2+} have been measured in phosphatidylcholine/water lamellar phase as a function of phase hydration and temperature and in the presence of divalent cations. Diffusion rates vary strongly with phase hydration, between 10^{-7} and 10^{-6} cm^2/s for monovalent and 10^{-8} and 10^{-7} for Ca^{2+}. The activation energies obtained are relatively small (5–10 kcal/mol). As the phase water content increases, a series of diffusion sequences is obtained, corresponding to the sequences predicted by Eisenman's theory of alkali ion equilibrium selectivity. This diffusionnal selectivity, which depends exclusively upon non-equilibrium parameters (mobility) within the hydrophilic path is discussed in respect to current theories of pore selectivity.

DYSLIPOPROTEINAEMIA IN PATIENTS WITH ACTIVE, CHRONIC POLYARTHRITIS. A STUDY ON SERUM LIPOPROTEINS AND TRIGLYCERIDE CLEARANCE (INTRAVENOUS FAT TOLERANCE TEST). S. Rössner and Camilla Löfmark (Dept. of Rheumatol., Karolinska Hosp. and King Gustaf V Res. Inst., S-104 01 Stockholm 60, Sweden) *Atherosclerosis* **28**, 41–52 (1977). Serum lipoproteins and the intravenous fat tolerance test were determined in 28 patients with active chronic polyarthritis, in most cases classical rheumatoid arthritis (RA). Two groups of RA patients were studied. One group was treated with only salicylates or other antiphologistic drugs, the other also with steroids, cytostatics etc. The low concentration of total serum cholesterol, well known in RA, was found to result from low concentrations in all three lipoprotein fractions VLDL, LDL and HDL compared to randomly selected healthly male and female controls. A slight reduction of the total serum triglyceride (TG) concentration was observed, which corresponded to

minor TG reductions in each lipoprotein class. The cholesterol/TG ratio in VLDL in RA patients was higher than in controls. One possible explanation of these findings could be that in RA an accumulation of the intermediate density lipoprotein (IDL) occurred. The HDL cholesterol concentration in RA patients was very low compared to controls. The clinical implication of this finding may be of interest since it has been demonstrated that a low HDL-cholesterol concentration is associated with an increase risk for atherosclerotic manifestations.

STIMULATION OF ACYL-CoA: CHOLESTEROL ACYLTRANSFERASE ACTIVITY BY HYPERLIPEMIC SERUM LIPOPROTEINS. G.H. Rothblat, M. Naftulin and L.Y. Arbogast (Med. Col. of Pennsylvania, Dept. of Physiol. and Biochem., 3300 Henry Ave., Philadelphia, PA) *Proc. Soc. Exp. Biol. Med.* **155**, 501–6 (1977). Cholesteryl ester synthesis by the microsomal fraction from rat hepatoma cells was stimulated by preincubation of the microsomal fraction with lipoproteins obtained from hyperlipemic rabbit sera. Lipoproteins from normolipemic sera had no stimulatory effect. Growth of cells in hyperlipemic rabbit sera also increased microsomal cholesteryl ester synthesis. Increased cholesteryl ester synthesis is accompanied by an increased microsomal-free cholesterol content.

EFFECTS OF CHRONIC ETHANOL CONSUMPTION ON THE CATABOLISM OF CHYLOMICRON TRIACYLGLYCEROL AND CHOLESTERYL ESTER IN THE RAT. T.G. Redgrave and G. Martin (Dept. of Physio., Univ. of Melbourne, Parkville, Victoria 3052, Australia) *Atherosclerosis* **28**, 69–80 (1977). Rats were fed for 24 days a liquid diet with ethanol as 36% of calories to produce hyperlipemia and hepatic steatosis. The catabolism of chylomicrons doubly-labeled in the triacylglycerol and cholesteryl ester moieties was studied in conscious rats after ingestion of their usual liquid diets with or without ethanol. A constant intravenous infusion of chylomicrons revealed a defect in chylomicron catabolism after chronic treatment with ethanol. The plasma clearance of chylomicron cholesteryl ester was impaired to a greater extent than clearance of chylomicron triacylglycerol. These findings are consistent with defective catabolism of chylomicron remnants, and suggest that the accumulation of chylomicron remnants in the plasma contributes to the development of increased post-prandial hyperlipemia and chronic hyperlipemia in association with excessive ethanol consumption.

PHOSPHATIDYLCHOLINE ^{13}C-LABELED CARBONYLS AS A PROBE OF BILAYER STRUCTURE. C.F. Schmidt, Y. Barenholz, C. Huang and T.E. Thompson (Dept. of Biochem., Univ. of Virginia Schl. of Med., Charlottesville, Va.) *Biochemistry* **16**, 3948–54 (1977). Dipalmitoyl- and dihexanoylphosphatidylcholine have been synthesized using fatty acids which have the acyl carbonyl carbons enriched with carbon-13. The chemical shifts of these carbonyl carbons, which are known to be sensitive to intermolecular interactions, have been measured in a variety of solvents, including aqueous dispersions. The use of dihexanoylphosphatidylcholine permits the observation of molecules in both monomer and micelle forms in aqueous solutions. Carbon-13-proton two- and three-bond coupling constants have also been measured. From these data, it can be concluded that, when the molecules are in bilayers, the observed shifts are determined by hydrogen bonding of the carbonyl oxygens with the water, even though there is partial exclusion of water molecules from this region of the bilayer. The extent of water exclusion can be quantified and taken as a measure of molecular packing. The hydration difference between carbonyls of molecules on the inside and outside of small single-walled vesicles is found to be 0.05. Furthermore, the relative shifts of the two carbonyl carbon-13's indicate that the fatty acid esterified to the 1-carbon of the glycerol is less accessible to water than that esterified to the 2-carbon of glycerol.

PROTON NUCLEAR MAGNETIC RESONANCE STUDY OF THE DECAY OF TRANSBILAYER COMPOSITIONAL ASYMMETRY GENERATED BY A PHOSPHATIDYLCHOLINE EXCHANGE PROTEIN. J.M. Shaw, W.C. Hutton, B.R. Lentz, and T.E. Thompson (Dept. of Biochem., Univ. of Virginia Schl. of Med., Charlottesville, Va.) *Biochemistry* **16**, 4156–63 (1977). Transbilayer compositional asymmetry was generated in single-lamellar vesicles formed from $-N(CD_3)_3$ egg phosphatidylcholine by incubation with erythrocyte ghost membranes in the presence of a purified phosphatidylcholine exchange protein prepared from beef liver. In a series of experiments, between 50 and 85% of the $-N(CD_3)_3$ phosphatidylcholine in the external face of the bilayer vesicles was replaced by $-N(CH_3)_3$ phosphatidylcholine from ghost membranes after a 24-h incubation at 37°C. Proton NMR studies utilizing Pr^{+3} as a shift reagent showed that 82 to 89% of the exchanged $-N(CH_3)_3$ phosphatidylcholine was found on the external face of the vesicle wall. During preparation of the asymmetric vesicles, spontaneous cholesterol movement from the cholesterol-depleted ghosts resulted in final cholesterol concentrations in the asymmetric vesicles of between 5 and 16 mol %.

SERUM BILE ACID ANALYSIS: A RAPID, DIRECT ENZYMATIC METHOD USING DUAL-BEAM SPECTROPHOTOFLUORIMETRY. P.A. Siskos, P.T. Cahill, and N.B. Javitt (Div. of Gastroenterology and Nuclear Med., New York Hospital—Cornell Med. Center and The Polytechnic Inst. of New York) *J. Lipid Res.* **18**, 666–71 (1977). The direct quantitative measurement of total bile acids in serum has been achieved using an enzymatic fluorescent method with a dual-beam spectrophotofluorimeter. By use of a 3-hydroxysteroid dehydrogenase, oxidation of bile acids with NAD is completed in 200 seconds with the observed NADH fluorescence being proportional to the concentration of serum bile acids. This method is rapid (8 minutes per individual sample), has an intrinsic sensitivity of ± 1 μM of total bile acids, requires no sample preparation and less than 0.8 ml of serum. Paired data analysis using enzymatic fluorescence and gas-liquid chromatographic methods gives a correlation coefficient (r) of 0.99 for 34 samples ranging from 2 to 530 μM.

INVESTIGATION OF REGULATION OF MICROSOMAL HYDROXYMETHYLGLUTARYL COENZYME A REDUCTASE AND METHYL STEROL OXIDASE OF CHOLESTEROL BIOSYNTHESIS. J.T. Spence and J.L. Gaylor (Div. of Nutr. Sci., and Sec. of Biochem., Mole. and Cell Biol., Cornell Univ., Savage Hall, Ithaca, NY) *J. Biol. Chem.* **252**, 5852–8 (1977). Parallel changes in the activities of three microsomal enzymes of rat liver cholesterol metabolism, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), 4α-methyl sterol oxidase, and cholesterol 7α-hydroxylase, have been observed. Methyl sterol oxidase activity oscillates in a diurnal rhythm. Administration *in vivo* of either an intestinal bile acid sequestrant or Triton WR-1339 increases both HMG-CoA reductase and methyl sterol oxidase activities. Cholesterol esters and other derivatives of cholesterol added *in vitro* inhibit both HMG-CoA reductase and 4-methyl sterol oxidase. The results suggest that, at least for these two microsomal enzymes of hepatic cholesterol biosynthesis, activities may be regulated similarly.

EFFECT OF PETROSELINIC AND STEARIC ACIDS ON THE ALKYL DIACYL GLYCERIDES OF NOVIKOFF HEPATOMA CELLS. W. Steele and H.M. Jenkin (The Hormel Inst., Univ. of Minnesota, 801 16th Ave. N.E., Austin, Minn.). *Proc. Soc. Exp. Biol. Med.* **155**, 410–5 (1977). Differences in the metabolism of the petroselinic and stearic acids were compared in Novikoff hepatoma cells cultivated *in vitro*. There was no change in the amount of total lipid with time of incubation when the cells were grown in unsupplemented medium, whereas when the cells were grown in the presence of n12–18:1 or 18:0, 30 and 25%, respectively, of the total fatty acids were utilized in 48 hours.

THE CONCOMITANTLY MEASURED TRANSFER OF FREE CHOLESTEROL, ESTERIFIED CHOLESTEROL, PHOSPHOLIPIDS AND PHOSPHOPROTEIN FROM PLASMA INTO THE AORTIC WALL OF STILBOESTROL-TREATED COCKERELS. S. Stender and S. Christensen (Dept. of Clin. Chem. CL, Rigs-hospitalet, Univ. Hosp. of Copenhagen, Blegdamsvej 9, DK-2100 Copenhagen, Denmark) *Atherosclerosis* **28**, 15–28 (1977). In vivo labelled plasma containing free and esterified [^{3}H]cholesterol, [^{32}P]phospholipids and [^{32}P]phosphoprotein was injected intravenously into eight cockerels and the radioactivity in the various fractions per square centimeter of thoracic aorta (uptake) was determined 0.2–12 h after the injection. Using the uptake and the integrated plasma decay curve, an uptake coefficient for the inner aorta in μl plasma per square centimeter per hour was calculated for each fraction. The results indicated that the labelled free and esterified plasma cholesterol taken up by inner aorta remained there as free and esterified cholesterol during the first 5–6 h after the injection. The uptake of labelled cholesterol can therefore within that period be used for calculation of the influx of plasma cholesterol into the aortic wall.

CHANGES IN METABOLIC PROPERTIES OF RABBIT VERY LOW DENSITY LIPOPROTEINS BY DIETARY CHOLESTEROL, AND SAT-

URATED FAT. E. Stange, M. Alavi and J. Papenberg (Medizinische Universitatsklinik, Heidelberg, W. Germany) *Atherosclerosis* **28**, 1–14 (1977). As was shown in a recent investigation, there is a marked change in the structural properties of rabbit lipoproteins by dietary cholesterol, saturated and polyunsaturated fats. Based on these electrophoretic, chemical and electron microscopic findings we examined the metabolic behaviour of ^{125}I-labeled VLDL ($d <$ 1.006 g/ml) from normal rabbits (group I) as well as animals fed 1% cholesterol (group II), 1% cholesterol + 5% coconut oil (group III) or 1% cholesterol + 5% corn oil (group IV). The curve peeling technique resulted in a fractional catabolic rate per hour (FCR/h) of 0.082 in the normal VLDL. All hypercholesterolemic fractions II, III and IV were metabolised significantly faster ($P < 0.05$) with FCR/h values of 0.119, 0.157 and 0.173 respectively. The difference in decay between VLDL II and both VLDL III or IV was statistically significant only during the late phase of metabolism, 2 h after injection. The apoprotein decay curves gave similar results. Ten min after the injection, most of the activity was recovered in the IDL class (d: 1.006–1.019 g/ml). The LDL fraction of the normal group had the lowest decay rate, whereas in all other groups HDL metabolism was delayed. It therefore may be concluded, that the metabolic behaviour of hypercholesterolemic VLDL differs from normal VLDL both quantitatively and qualitatively.

EFFECT OF CHOLESTEROL FEEDING AND ESTROGEN TREATMENT ON SYNTHESIS OF FATTY ACIDS IN LIVER. K. Srinivasan and T.I. Pynadath (Dept. of Chem., Kent State Univ., Kent, Ohio). *Atherosclerosis* **27**, 407–7 (1977). The effect of cholesterol feeding and estrogen administration on synthesis of fatty acids in liver mitochondria, microsomes and cytoplasm of male rabbits has been investigated. The synthesis was measured by the incorporation of (1-^{14}C)acetyl CoA or (2-^{14}C)malonyl CoA into long chain fatty acids under optimal conditions. It was found that atherogenesis markedly decreased the fatty acid synthesis in cytoplasm. The mitochondrial fatty acid synthesis was not affected by the disease. There was a small but measurable decrease in the synthesis of fatty acids in microsomes. Estrogen had no effect on the synthesis of fatty acids in mitochondria or microsomes. But if effectively counteracted, after a short lag period, the decreased synthesis of cytoplasmic fatty acids observed in atherosclerosis. It is possible that liver fatty acid synthetase is one of the enzyme systems through which estrogens exert their atherosclerosis-retarding effect. The decreased cytoplasmic fatty acid synthesis observed in atherosclerosis might account for the low levels of saturated fatty acids reported in liver and plasma lipids of atherosclerotic animals.

CHARACTERIZATION OF TWO MAJOR NEUTRAL GLYCEROGLUCOLIPIDS OF THE HUMAN GASTRIC CONTENT. B.L. Slomiany, A. Slomiany, and G.B.J. Glass (Gastroenterology Res. Lab., Depts. of Med. and Biochem., New York Med. Col., New York, N.Y.) *Biochemistry* **16**, 3954–8 (1977). Two major neutral glycerogluco lipids (A and B) have been isolated from lipid extract of human gastric content by the procedure involving column fractionation on DEAE-Sephadex, silicic acid, and thin-layer chromatography. Both glycolipids contained glucose, glyceryl ethers, and fatty acids. The structures of these glycolipids were identified by mild alkaline methanolysis, oxidation with periodate and chromium trioxide, and permethylation studies. Based on the obtained data, we propose that glycolipid A is a monoalkylmonoacylglyceryl hexaglucoside and glycolipid B is a monoalkylmonoacyl-glyceryl octaglucoside. The diglyceride portion of these glycolipids consists mostly of 1-*O*-alkyl-2-*O*-acylglycerol.

PARTIAL PURIFICATION OF TWO LITHOCHOLIC ACID-BINDING PROTEINS FROM RAT LIVER 100,000G SUPERNATANTS. R.C. Strange, R. Cramb, J.D. Hayes and I.W. Percy-Robb (Univ. Dept. of Clin. Chem., Royal Infirmary, Edinburgh EH3 9YW, Scotland, U.K.) *Biochem. J.* **165**, 425–9 (1977). The partial purification of two lithocholic acid-binding proteins from liver 100,000*g* supernatants is described. Gel-filtration, $(NH_4)_2SO_4$ fractionation, $Ca_3(PO_4)_2$ fractionation and ion-exchange chromatography were used. Both proteins exhibited glutathione *S*-transferase activity; one may be the non-specific anion-binding protein ligandin. Glutathione *S*-transferase activity of one of the binding proteins was inhibited by lithocholic acid.

EFFECT OF DIETARY CHOLESTEROL AND TALLOW ON CHOLESTEROL SYNTHESIS IN THE CASTRATED GOAT. J.R. Thompson, D.C. Beitz and N.L. Jacobson (Dept. of Animal Sci., Iowa State Univ., Ames, IA) *J. Nutr.* **107**, 1632–9 (1977). The effect of dietary cholesterol plus tallow on cholesterol content of several tissues and on in vivo cholesterol biosynthesis was determined in castrated male, mature Saanen goats. The control group consisted of the same type of goats and was fed a dry concentrate mixture. The lipid-supplemented group was fed the same diet plus supplemental cholesterol and tallow at 250 mg and 3 g, respectively, daily per kg body weight for 15 to 18 weeks. Cholesterol content of liver, spleen, and small intestine was increased significantly by cholesterol feeding. Liver, muscle, and skin control goats contained 47%, 12%, and 20% respectively, of total body cholesterol. In cholesterol plus tallow-fed goats, liver, muscle, and skin contributed 72%, 12%, and 11%, respectively. The cholesterol plus tallow feeding reduced cholesterolgenesis in the small intestine, spleen, and kidney to about 25% of that in corresponding tissues of controls; that in adrenal cortex was reduced to 45% of control. Cholesterol plus tallow feeding caused a three-fold increase in cholesterogenesis in adipose tissue. No significant changes occurred in other tissues.

SYNTHESIS OF SULFATE ESTERS OF LITHOCHOLIC ACID, GLYCOLITHOCHOLIC ACID, AND TAUROLITHOCHOLIC ACID WITH SULFUR TRIOXIDE-TRIETHYLAMINE. K.-Y. Tserng and P.D. Klein (Div. of Biol. and Med. Res., Argonne Natl. Lab., Argonne, Il.) *J. Lipid Res.* **18**, 491–5 (1977). The facile synthesis of lithiocholic acid sulfates by a procedure that produced the desired products in over 90% yield is described. Lithocholic acid sulfate and glycolithocholic acid sulfate were synthesized by reacting lithocholic acid or glycolithocholic acid with sulfur trioxidetriethylamine complex in dimethylformamide for 0.5–1 hr. Tauraolithocholic acid sulfate was obtained by conjugating lithocholic acid sulfate with taurine in dimethylformamide at 90°C for 0.5 hr. The one-pot synthesis of taurolithocholic acid sulfate starting from lithocholic acid is also described. This procedure, which generated lithocholic acid sulfate in situ, produced taurolithocholic acid sulfate in 98% yield, compared to an overall yield of less than 10% obtained by previously published procedures.

CHEMICAL SYNTHESIS AND SURFACE PROPERTIES OF AN ANALOG OF THE PULMONARY SURFACTANT DIPALMITOYL PHOSPHATIDYLCHOLINE. J.G. Turcotte, A.M. Sacco, J.M. Steim, S.A. Tabak and R.H. Notter (Dept. of Med. Chem., Col. of Phar., Univ. of Rhode Island, Kingston, R.I.) *Biochim. Biophys. Acta* **488**, 235–48 (1977). An analog, (*S*)- 3- 2,3-bis(hexadecyloxy) propoxyl hydroxyphosphinyl -propyl trimethylammonium hydroxide, inner salt, hydrate (DPPC-analog), of dipalmitoyl phosphatidylcholine (DPPC) was synthesized. The analog differs from pulmonary DPPC in that it: is a dipalmityl diether rather than a dipalmitoyl diester; has a trimethylammonium propylene phosphono polar head instead of a trimethylammonium ethylenoxy phosphate head; and has an absolute configuration opposite that of pulmonary DPPC. To complement the dynamic surface pressure-area determinations, differential scanning calorimetry and dilatometry measurements were carried out on DPPC and DPPC-analog water dispersions. The results showed that the liquid-crystalline transition temperature of the DPPC-analog is 45°C, slightly higher than the 41°C found for DPPC. Thus, the superior interfacial respreading found for the DPPC-analog at 23° and 37°C indicates that its bulk phase liquid-crystalline transition temperature is not as directly related to its surface properties as it is in the case of DPPC.

MISCIBILITY PROPERTIES OF BINARY PHOSPHATIDYLCHOLINE MIXTURES. A CALORIMETRIC STUDY. P.W.M. Van Dijck, A.J. Kaper, H.A.J. Oonk and J. De Gier (Biochem. Lab., State Univ. of Utrecht, Transitorium III, Padualaan 8, Univ. Centre "De Uithof," Utrecht, The Netherlands) *Biochim. Biophys. Acta* **470**, 58–69 (1977). From data obtained by differential scanning calorimetry phase diagrams were constructed, using a thermodynamically based fitting method. The following binary mixtures of phosphatidylcholines in water were studied: 14:0/14:0-glycerophosphocholine/16:0/16:0-glycerophosphocholine, 14:0/14:0-glycerophosphocholine/18:0/18:0-glycerophosphocholine, 12:0/12:0-glycerophosphocholine/16:0/16:0-glycerophosphocholine, 18:1$_t$/18:1$_t$-glycerophosphocholine/14:0/14:0-glycerophosphocholine and 18:1$_t$/18:1$_t$-glycerophosphocholine/16:0/16:0-glycerophosphocholine. A comparison is made of the present results with those obtained using probe techniques and the differences are discussed.

UTILIZATION OF L(+)-3-HYDROXYBUTYRATE, D(−)-3-HYDROXYBUTYRATE, ACETOACETATE, AND GLUCOSE FOR RESPIRATION AND LIPID SYNTHESIS IN THE 18-DAY-OLD RAT. R.J. Webber and J. Edmond (Dept. of Biol. Chem. and Mental Retardation Res. Ctr., School of Med., Univ. of California, Los Angeles, CA) *J. Biol. Chem.* **252**, 5222–6 (1977). A comparison has been made *in vivo* between L(+)-3-hydroxy[3-^{14}C]butyrate, D(−)-3-hydroxy[3-^{14}C]butyrate, [3-^{14}C]acetoacetate, and D-[2-^{14}C]glucose for sterol and fatty acid synthesis and respiration in the 18-day-old suckling rat. Sterols and fatty acids in spinal cord, brain, and skin were preferentially labeled by these metabolites over sterols and fatty acids in the liver and kidneys. The evidence that the L(+)-3-hydroxy[3-^{14}C] butyrate is a favored substrate for the synthesis of sterols and fatty acids but less favored for oxidation, while D(−)-3-hydroxy[3-^{14}C]butyrate is a favored substrate for oxidation but less favored for the synthesis of sterols and fatty acids, suggests that these isomers are preferentially metabolized in different compartments.

CONTRASTING EFFECTS OF ETHANE-1-HYDROXY-1,1-DIPHOSPHONATE (EHDP) ON THE REGRESSION OF TWO TYPES OF DIETARY-INDUCED ATHEROSCLEROSIS. W.D. Wagner, T.B. Clarkson and J. Foster (Arteriosclerosis Res. Center, Bowman Gray Schl. of Med., Wake Forest Univ., Winston-Salem, N.C.). *Atherosclerosis* **27**, 419–35 (1977). Atherosclerosis was induced in White Carneau pigeons by feeding cholesterol either continuously or intermittently during a 14-month period. Animals were then treated daily with either saline, or 0.5 or 2.5 mg EHDP/kg body wt during which time a cholesterol-free "regression" diet was fed. Subgroups of pigeons were studied after four and eight months of treatment. After the progression period, aortas and brachiocephalic arteries of those pigeons that were subjected to intermittent hypercholesterolemia accumulated more cholesterol, were more complicated, and showed a different pattern of lesion regression than arteries in animals continuously hypercholesterolemic. EHDP treatment had no effect on plasma cholesterol, triglyceride, calcium, or phosphorus concentrations. No effect was seen on collagen concentrations in either the intermittent or the continuous cholesterol fed animals. The beneficial effect of EHDP on the more advanced atherosclerosis in the pigeons fed cholesterol intermittently may be due to an action on lipid-calcium-connective tissue complexes within the atherosclerotic plaque. The opposite effect of EHDP in pigeons with relatively uncomplicated atherosclerosis induced by continuous cholesterol feeding is less easily understood, but a possible role implicating lysosomes is suggested.

MYCOBACTERIUM SMEGMATIS FATTY ACID SYNTHETASE. A MECHANISM BASED ON STEADY STATE RATES AND PRODUCT DISTRIBUTIONS. W.I. Wood, D.O. Peterson and K. Bloch (James Bryant Conant Labs., Harvard Univ., Cambridge, Mass.) *J. Biol. Chem.* **252**, 5745–9 (1977). The initial steady state rate and product distribution of fatty acid synthesis catalyzed by *Mycobacterium smegmatis* fatty acid synthetase has been investigated as a function of various concentrations of acetyl-CoA, malonyl-CoA, mycobacterial polysaccharide, and bovine serum albumin. Polysaccharide has a large effect on both rate and chain length. The steady state rate stimulation by polysaccharide is not duplicated by other acyl-CoA-binding molecules such as bovine serum albumin. It is concluded that relief of product inhibition does not adequately explain the specific effects of the mycobacterial polysaccharide. A general mechanism is presented which accounts for variation in reaction rate and product pattern over a wide range of experimental conditions. We propose that the diffusion of long chain acyl-CoA (C_{14} to C_{24}) from the enzyme is the rate-limiting step in fatty synthesis catalyzed by the *M. smegmatis* synthetase. Polysaccharide facilitates this rate-limiting step by forming a ternary complex with enzyme-bound acyl-CoA causing rapid release of product.

CEREBROSIDE ANALOGUES FROM 3-PHENYLSERINES. B. Weiss (Div. of Neuroscience, New York State Psychiatric Inst., Dept. of Biochem., Col. of Physicians and Surgeons, Columbia Univ., New York, N.Y.) *Chem. Phys. Lipids* **19**, 347–55 (1977). Cerebroside analogues were synthesized from DL-*threo*- and DL-*erythro*-3-phenylserines by the following sequence of reactions: esterification, N-acylation, reduction with sodium bis (2-methoxyethoxy)-aluminum hydride (SMEAH), and condensation with acetobromoglucose followed by deacetylation. Mass spectrometry disclosed that the glycosidic bond was formed at the primary hydroxy group.

PANCREATIC LIPASE EFFECTORS EXTRACTED FROM SOYBEAN MEAL. F. Widmer (Nestlé Products Technical Assistance Co. Ltd., Res. Dept., Biochem. Sec., CH-1814 La Tourde-Peilz, Switzerland) *J. Agric. Food Chem.* **25**, 1142–5 (1977). The activity of procine pancreatic lipase is shown to be affected by proteinaceous compounds extracted from raw soybean meal. Both activators and inhibitors were detected. They are heat stable and only moderately susceptible to proteolysis. Their apparent effects on lipolysis are assumed to be due to interaction with the substrate emulsion.

DIFFUSION AND PATCHING OF MACROMOLECULES ON PLANAR LIPID BILAYER MEMBRANES. D.E. Wolf, J. Schlessinger, E.L. Elson, W.W. Watt, R. Blumenthal and P. Henkart (Cornell Univ., Ithaca, N.Y.) *Biochemistry* **16**, 3476–83 (1977). We have developed a model system for biological membranes in which amphipathic macromolecular antigens are bound to planar lipid bilayers. The effect of antibodies on the diffusion and distribution of these antigens has been studied. The antigens used are derivatives of dextran (mol/wt: 82,000) to which controlled amounts of fatty acid, rhodamine, and the antigenic hapten, 2,4,6-trinitrophenyl (TNP), have been covalently bound. These fluorescent amphipathic antigens bind to artificial planar lipid bilayer membranes from the aqueous medium. The diffusion coefficients of these macromolecules were measured by fluorescence photobleaching recovery. Cross-linking one population of dextran derivative retarded the diffusion of another non-cross-reacting population. Sodium azide, colchicine, and cytochalasin B did not affect the motion of these molecules on oxidized cholesterol bilayers.

AN IMPROVED ASSAY OF GANGLIOSIDES SEPARATED BY THIN-LAYER CHROMATOGRAPHY. A.J. Yates and D. Thompson (Div. of Neuropathology, Dept. of Pathology, The Ohio State Univ., Columbus, Oh.) *J. Lipid Res.* **18**, 660–5 (1977). There were no differences in the recoveries of ganglioside sialic acid from silica gel G thin-layer chromatograms when they were sprayed with resorcinol reagent varying in normality between 3 and 10. However, both the temperature at and the time for which the plates were heated after spraying did affect the recoveries, which can reach 100% when the plates are heated for 6 min at 135°C.

EFFECT OF HIGH LEVELS OF DIETARY VITAMIN E ON HEMATOLOGICAL INDICES AND BIOCHEMICAL PARAMETERS IN RATS. N.Y.J. Yang and I.D. Desai (Div. of Human Nutr., Schl. of Home Econ., Univ. of British Columbia, Vancouver, B.C., Canada). *J. Nutr.* **107**, 1410–7 (1977). The effects of low, normal and high levels of dietary vitamin E on rats were assessed by feeding diets containing 0, 25, 250, 2,500, 10,000, and 25,000 IU vitamin E/kg diet for prolonged periods of 8 and 16 months. All nutrients in the basal diet except vitamin E were adequate. Excess vitamin E and vitamin E deficiency depressed body weight over a period of time. High levels of dietary vitamin E increased the relative heart weight after 8 months and relative spleen weight after 16 months. Urinary excretion of creatine and creatinine were apparently normal in all rats with the exception of vitamin E deficient rats which had significantly higher creatine and lower creatinine levels in urine. Results of these studies clearly indicate that excess vitamin E has deleterious effects and that further investigations are urgently warranted to test the implications of these findings on the long range metabolic and physiological consequences in animal and man.

REGULATION OF HYDROXYMETHYLGLUTARYL-CoA REDUCTASE IN RAT LEUKOCYTES. N.L. Young and V.W. Rodwell (Dept. of Biochem., Purdue Univ., West Lafayette, In.) *J. Lipid Res.* **18**, 572–81 (1977). Methods were developed for the assay of hydroxymethylglutaryl-CoA reductase (NADPH) activity in microsomes from rat leukocytes. The activity in freshly isolated leukocytes is low compared to rat liver but can be assayed reliably. The patterns of response of leukocyte reductase in the assay to variation in substrate concentration, protein concentration, and time mimic those of rat liver reductase. Reductase activity in leukocyte microsomes, as in liver microsomes, is depressed by dietary cholesterol and by fasting and is elevated by dietary cholestyramine. Unlike liver reductase, leukocyte reductase activity does not exhibit a detectable diurnal rhythm. We conclude that the assay of reductase in freshly isolated leukocytes holds promise as a technique for detecting the effects of various factors on cholesterol synthesis in vivo.

THE MOLECULAR ORGANIZATION OF BIMOLECULAR LIPID MEMBRANES. THE EFFECT OF KCL ON THE LOCATION OF INDOLE-

ACETIC ACID IN THE MEMBRANE. U. Zimmerman, R.G. Ashcroft, H.G.L. Coster and J.R. Smith (Biophysics Lab., Schl. of Physics, Univ. of New South Wales, Sydney, N.S.W., Australia). *Biochim. Biophys. Acta* **469**, 23–32 (1977). The effect of indoleacetic acid on the individual dielectric and conductance parameters of the polar-head, hydrocarbon and unstirred surface-layer regions of bimolecular lipid membranes was studied using low frequency (0.1–100 Hz) impedance dispersion measurements. It was found that the effect of 10^{-6} M indoleacetic acid on these parameters was dependent on the concentration of KCl in the external solution. The hydrocarbon capacitance was insensitive to the presence of indoleacetic acid at all KCl concentrations. It is concluded that at low KCl concentrations indoleacetic acid is located only on the surface and in the polar-head regions of the membrane while at high concentrations of KCl indoleacetic acid is absorbed into the hydrocarbon region, leaving the surface largely in its unmodified state. These results correlate with the known salt dependence of the action of the hormone in plant cells.

IMMUNOLOGICAL AND CATALYTIC CROSS REACTIVITY STUDIES OF FATTY ACID SYNTHETASE COMPLEXES FROM AVIAN AND MAMMALIAN LIVERS. S. Kumar, K.R. Srinivasan and N. Asato (Dept. of Biochem., Col. of Med., and Dentistry of New Jersey, New Jersey Med. School, Newark, N.J.) *Biochim. Biophys. Acta* **489**, 32–47 (1977). Fatty acid-synthetase complexes from avian and mammalian livers catalyse similar series of enzymatic reactions. We have studied the nature of immunological similarities and differences among these antigens by double diffusion analysis. Anti-chicken and anti-pigeon liver fatty acid-synthetase-γ-globulins give precipitin reactions with the antigens from avian livers, but not from rat liver, and the antibody prepared against the mammalian enzyme does not cross react with the avian enzymes. Minor antigenic differences seem to exist between the enzymes from avian livers as shown by the formation of spurs in immunodiffusion analysis. To further characterize the nature of antigenic fragment, we have determined immunological and catalytic efficiency of the chicken enzyme modified with iodoacetic acid, iodoacetamide, palmitoyl CoA, trypsin and urea. The first four modifying agents lead to complete inhibition of activity for fatty acid-synthesis, but to only partial inhibition of activity for the reduction of acetoacetyl-*N*-acetyl cysteamine. However, the immunological reactivity is completely retained. The enzyme treated with 2 M urea gives results similar to other modifying agents but treatment with 4 and 6 M urea leads to complete loss of catalytic and immunological reactivity.

EFFECT OF PHOSPHOLIPASE C AND CHOLESTEROL OXIDASE ON MEMBRANE INTEGRITY, MICROVISCOSITY, AND INFECTIVITY OF VESICULAR STOMATITIS VIRUS. N.F. Moore, E.J. Patzer, Y. Barenholz and R.R. Wagner (Depts. of Microbiol. and Biochem., Univ. of Virginia School of Med., Charlottesville, Va.). *Biochemistry* **16**, 4708–15 (1977). Exposure of intact vesicular stomatitis (VS) virus or intact mixed-lipid vesicles to phospholipase-free cholesterol oxidase resulted in $< 5\%$ oxidation of membrane cholesterol compared with $> 95\%$ oxidation of cholesterol in detergent-disrupted virus or liposomes. Phospholipase C hydrolyzed $\sim 55\%$ of phospholipids in intact VS virion membranes and $\sim 90\%$ of phospholipids in small, single-walled lipid vesicles. Prior or simultaneous exposure of VS virions or liposomes to phospholipase C resulted in $> 90\%$ oxidation by cholesterol oxidase of membrane cholesterol to cholest-4-en-3-one. Phospholipase A_2 did not expose VS virion cholesterol to oxidation by cholesterol oxidase. Treatment with phospholipase C and cholesterol oxidase did not greatly alter the integrity of VS virion membrane, as determined by electron microscopy and protein electropherograms: only minimal amounts of oxidized cholesterol were released from virions exposed to both enzymes. Exposure to cholesterol oxidase alone resulted in only minor alterations in VS viral infectivity and membrane microviscosity monitored by fluorescence depolarization.

THE INTRINSIC STRUCTURAL ASYMMETRY OF HIGHLY CURVED PHOSPHOLIPID BILAYER MEMBRANES. A. Chrzeszczyk, A. Wishnia and C.S. Springer, Jr. (Dept. of Chem., State Univ. of New York, Stony Brook, N.Y.) *Biochim. Biophys. Acta* **470**, 161–9 (1977). Phosphorus-31 NMR studies of solutions of small L- -dipalmitoyl phosphatidylcholine bilayer vesicles containing sodium dimethyl phosphate uniformly distributed between the continuous external and the intravesicular aqueous spaces, with the paramagnetic shift reagent Pr^{3+} present only in the external space, are reported. These studies give the distribution both of dipalmitoyl phosphatidylcholine in the vesicle inner and outer monolayers and of dimethyl phosphate in the aqueous spaces. With the third necessary parameter obtained from the vesicle sedimentation coefficient, the very different packing parameters of dipalmitoyl phosphatidylcholine in inner and outer monolayers can be determined. The vesicle outer radius is 109 A. Although the total bilayer thickness is virtually identical to that of planar bilayers, the outer monolayer is thicker (20 A) and the inner monolayer thinner (15 A). The area per head group at the inner surface, 68 A^2, is like the planar value, but the tails are much more folded, so as to decrease the radial lengths and increase the tangential spread (to 94 A^2). The reverse is true in the outer layer: the surface per head group is 76 A^2, tapering to 51 A^2 in the tail region, so that outer layer tails are relatively extended. The difference is equivalent to a shift of about two 2 gl kinks from outer to inner layers; the uneven packing certainly affects fluidity, and may have important biological consequences.

COMMON CHARACTERISTICS OF THE CYTOCHROME P-450 SYSTEM INVOLVED IN 18- AND 11β-HYDROXYLATION OF DEOXYCORTICOSTERONE IN RAT ADRENALS. I. Bjorkhem and K.-E. Karlmar (Dept. of Clinical Chem., Huddinge Univ. Hosp., and Dept. of Chem., Karolinska Institutet, Stockholm, Sweden). *J. Lipid Res.* **18**, 592–603 (1977). 18- and 11β-Hydroxylation of deoxycorticosterone and side chain cleavage of cholesterol were studied in mitochondria and submitochondrial reconstituted systems prepared from rat and bovine adrenals. A mass fragmentographic technique was used that allows determination of hydroxylation of both exogenous and endogenous cholesterol. From the results obtained, it is concluded that 18- and 11β-hydroxylation have similar properties and that the binding site for deoxycorticosterone is similar or identical in the two hydroxylations. The possibility that the same specific type of cytochrome P-450 is responsible for both 18- and 11β-hydroxylation of deoxycorticosterone is discussed.

SOME CHARACTERISTICS OF SOLUBLE FATTY ACID SYNTHESIS IN GERMINATING PEA SEEDS. P. Bolton and J.L. Harwood (Dept. of Biochem., Univ. College, P.O. Box 78, Cardiff CF1 1XL, U.K.). *Biochim. Biophys. Acta* 489, 15–24 (1977). Soluble fractions from germinating pea synthesize palmitic acid de novo and stearic acid by elongation. Malonyl CoA, acyl carrier protein and NADPH are required for both reactions. In contrast to some other plant systems, no requirement was found for divalent cations. On the other hand, the formation of both stearate and palmitate was inhibited by sulphydryl reagents and palmitate elongation was sensitive to arsenite. The products of the reactions were examined and found to be principally acyl-acyl carrier proteins and unesterified fatty acids. Unlike the pea microsomal fractions, the soluble enzymes are stimulated only slightly by the addition of exogenous lipids. The substrate for palmitate elongation is palmitoyl-acyl carrier protein, which is quantitatively elongated to stearate. Comparisons are made with membrane-localised fatty acid synthesis from the same tissue.

ANALYSIS OF MOLECULAR SPECIES OF ETHER ANALOGUES OF PHOSPHATIDYLCHOLINES FROM BIOLOGICAL SAMPLES. T. Curstedt (Dept. of Chem., Karolinska Inst., Stockholm, Sweden). *Biochim. Biophys. Acta* **489**, 79–88 (1977). A method is described for quantitative analysis of molecular species of ether analogues of phosphatidylcholines. The choline-containing phospholipids are isolated by chromatography on a lipophilic weak anion exchanger and are hydrolyzed with phospholipase C. The neutral lipids formed are separated by straight-phase chromatography on Lipidex-5000 into three classes: 1-alkyl-2-acyl-, 1-alk-1′-enyl-2-acyl- and 1,2-diacylglycerols. The trimethylsilyl ethers of the ether lipids are then separated into molecular species by reversed-phase chromatography on Lipidex -5000 and the species are analyzed by gas chromatography-mass spectrometry. For a more detailed analysis, the ester bond is hydrolyzed and the fatty acids are separated from the 1-alkyl- and 1-alk-1′-enylglycerols on a lipophilic strong anion exchanger. The glyceryl ethers are then analyzed as trimethylsilyl ethers by gas chromatography-mass spectrometry. The method can be used for analysis of 50–1,000 μg of ether analogues present in samples containing a 100–1,000-fold excess of 1,2-diacylglycerophosphocholines.

PHOSPHOLIPID EXCHANGE BETWEEN BILAYER MEMBRANES. G. Duckwitz-Peterlein, G. Eilenberger and P. Overath (Institut

fur Theoretische Physik der Universitat zu Koln, D5 Koln 41 and the Max-Planck-Institut fur Biologie, D74 Tubingen, G.F.R.). *Biochim. Biophys. Acta* **469**, 311–25 (1977). The mode of interaction of aqueous dispersions of phospholipid vesicles is investigated. The vesicles (average diameter 950 Å) are prepared from total lipid extracts of *Escherichia coli* composed of phosphatidylethanolamine, phosphatidylglycerol and cardiolipin. One type of vesicle contains *trans*-Δ^9-octadecenoate, the other type *trans*-Δ^9-hexadecenoate as predominant acyl chain component. A mixture of these vesicles is incubated at 45°C and lipid transfer is studied as a function of time using the phase transition as an indicator. At a given molar ratio of the two types of vesicles the rate of lipid transfer is independent of the total vesicle concentration. It is concluded that lipid exchange through the water phase by way of single molecules or micelles is the mode of communication of these negatively charged lipid vesicles.

THE INFLUENCE OF MICELLE FORMATION ON LIPOXYGENASE KINETICS. J.R. Galpin and J.C. Allen (Biochem. Dept., The Med. Col. of St. Bartholomew's Hosp., Charterhouse Sq., London, ECIM 6BQ, U.K.). *Biochim. Biophys. Acta* **488**, 393–401 (1977). The effects of a series of *n*-alcohols and *n*-carboxylic acids on lipoxygenase activity was studied. It was shown that to a large extent the effects of these compounds could be ascribed to physiochemical interaction with the substrate solution rather than a direct action on the enzyme itself. The effect of better substrate analogues such as stearate and oleate could also be ascribed to this effect. A type-2 lipoxygenase was found to have a very unusual velocity-substrate relationship which could be normalized by addition of calcium chloride in amounts stoichiometric with the substrate. An excess of calcium inhibited the enzyme. By comparison of results with linoleoyl sulphate/linoleoyl alcohol mixed micelles, an explanation for this unusual velocity-substrate activity is presented.

LIPIDS OF DAUCUS CAROTA CELL SUSPENSION CULTURES. H.-D. Gregor (Res. Inst. for Food Sci., Kyoto Univ., Uji, Kyoto 611, Japan). *Chem. Phys. Lipids* **20**, 77–85 (1977). In carrot cell suspension cultures greater amounts of phospholipid were detected than in carrot root material, but the major phospholipid classes were the same in both materials, and their fatty acid composition was very similar. In contrast to the cultured cells, no significant amounts of free fatty acids and monoglycerides, could be detected in the carrot root. The fatty acid composition of the major lipid classes from cell cultures is reported for the first time in this report. The degree of unsaturation was higher in triglyceries and phospholipids than in free fatty acids. In a study of phospholipid biosynthesis, [^{3}H] glycerol was shown to be incorporated into 4-phospholipids (PE, PC, PG, PS) to different extents. The highest specific activity was observed in PC and PG. Five molecular species were isolated from each of the 4 phospholipids and analyzed by GC-MS and LSC.

THE INTERACTION OF SPECTRIN · ACTIN AND SYNTHETIC PHOSPHOLIPIDS. C. Mombers, P.W.M. Van Dijck, L.L.M. Van Deenen, J. De Gier and A.J. Verkleij (Lab. of Biochem. and Inst. of Molecular Biol., State Univ. of Utrecht, Padualaan 8, Utrecht, The Netherlands) *Biochim. Biophys. Acta* **470**, 152–60 (1977). Using differential scanning calorimetry and freeze fracture electron microscopy interactions were studied between lipids and a spectrin · actin complex isolated from human erythrocyte membranes. With dispersions of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine, 1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol and mixtures of these two compounds, which for experimental reasons were chosen as the lipid counterpart, such an interaction could clearly be deduced from changes in the temperature and the enthalpy of the phase transition. Furthermore it was demonstrated that the interaction with this membrane protein protects the bilayer against the action of Ca^{2+} and Mg^{2+} and prevents fusion of lipid vesicles which easily occurs in some of the systems when divalent ions were added to the pure lipid vesicles.

A NOVEL PACKING OF THE HYDROCARBON CHAINS IN LIPIDS. THE LOW TEMPERATURE PHASES OF DIPALMITOYL PHOSPHAITDYLGLYCEROL. J.L. Ranck, T. Keira and V. Luzzati (Ctr. de Genetique Moleculaire, C.N.R.S., 91190 Gif-sur-Yuette, France). *Biochim. Biophys. Acta* **488**, 432–41 (1977). The system dipalmitoyl phosphatidylglycerol-water displays several phases in the temperature-concentration range explored in this work. All the phases are lamellar; they differ by the organization of the hydrocarbon chains. In the high temperature phase the conformation of the chains is liquid-like. In the low temperature phases the chains are stiff and parallel and they interdigitate (in others words the CH_3 ends of the chains of one layer are near to the polar groups of the opposite layer). Moreover, several types of packings of the stiff chains are observed which differ by the symmetry of the two-dimensional lattices. The observed lattices are p6, cmm and pgg.

SPECIFIC INTERACTION OF CONCANAVALIN A WITH GLYCOLIPID MONOLAYERS. B.D. Read, R.A. Demel, H. Wiegandt and L.L.M. van Deenen (Biochem. Lab., State Univ. of Utrecht, Padualaan 8, Utrecht 2506, The Netherlands). *Biochim. Biophys. Acta* **470**, 325–30 (1977). The effect of ^{131}I-labelled concanavalin A on the surface pressure and surface radioactivity of monolayers formed from phospholipids and from natural and synthetic glycolipids has been studied. The lectin binds to and penetrates dipalmitoyl phosphatidylcholine monolayers at a surface pressure of 15 dynes/cm and this interaction is inhibited by the presence of α-methyl mannose in the subphase. At surface pressures of 25 dynes/cm or higher, concanavalin A will interact with monoglucosyl diglyceride or diglucosyl diglyceride from *Acholeplasma laidlawii* and with synthetic glycolipids containing 2 or 3 α 1 → 4-linked D-glucose residues in the headgroup, but not with phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, or with the ganglioside II^{3}NeuAc-GgOse$_4$-Cer. The binding to the glycolipid sugar group and penetration of the hydrocarbon region seem to occur simultaneously, as the time courses for the development of surface pressure and surface radioactivity coincide.

STRUCTURAL STUDIES ON BRANCHED FUCOSPHINGOLIPIDS OF HOG GASTRIC MUCOSA. B.L. Slomiany and A. Slomiany (Gastroenterology Res. Lab., Dept. of Med. and Biochem., New York Med. College, New York, N.Y.) *Chem. Phys. Lipids* **20**, 57–69 (1977). A structural studies have been performed on new complex glycolipids extracted from hog gastric mucosa by 0.4 M sodium acetate in methanol-chloroform-water, and which were purified to homogeneity by DEAE-Sephadex and Florisil column chromatography and by preparative thin-layer chromatography in three solvent systems. Five branched fucolipids have been purified from this extract, three of which have been characterized previously and remaining two were subject of this investigations.

KETOGENIC RESPONSE TO MEDIUM-CHAIN TRIGLYCERIDE LOAD IN THE RAT. A. Bach, H. Schirardin, A. Weryha and M. Bauer (Lab. de la Clin. Med. A, Hosp. Civil 67005 Strasbourg-Cedex, France). *J. Nutr.* **107**, 1863–70 (1977). We studied ketonemia induced in rats by a single oral load of medium-chain triglycerides (MCT) ($C_{8:0}$ 50.5%, $C_{10:0}$ 48.0%, $C_{12:0}$ 1.0%). Medium-chain fatty acids, rather than being incorporated into the lipids synthesized by the liver, are oxidized there, with high production of ketone bodies. Severe and long-lasting hyperketonemia developed rapidly. With increased MCT loads, ketonemia also increased, although not linearly. The level of the hyperketonemia seemed equal in the two sexes. Ingestion of MCT by fasting rats caused an additional rise in ketonemia. Long-chain triglycerides were not ketogenic, since their constituent fatty acids are incorporated into lipids and are thus less subject to oxidation. Lipids induce less severe ketonemia in genetically obses rats than in normal-weight rats.

AN ANALYSIS OF PARTIAL REACTIONS IN THE OVERALL CHAIN ELONGATION OF SATURATED AND UNSATURATED FATTY ACIDS BY RAT LIVER MICROSOMES. J.T. Bernert Jr. and H. Sprecher (Dept. of Physiol. Chem., Col. of Med., The Ohio State Univ., Columbus, OH). *J. Biol. Chem.* **252**, 6736–44 (1977). A direct correlation between rates of condensation and overall chain elongation has been observed with palmitoyl-CoA, 6,9-octadecadienoyl-CoA, and 6,9,12-octadecatrienoyl-CoA. Rates of condensation and overall chain elongation were depressed when microsomes were used from rats raised on a balanced *versus* a fat-free diet. This effect was more pronounced for palmitoyl-CoA than for the unsaturated substrates. The rates of the β-hydroxyacyl-CoA dehydrase and 2-*trans*-enoyl-CoA reductase reactions were always much higher than for condensation and were not altered by dietary change. These findings show that condensation is rate-limiting and implicate it in overall control of chain elongation. In addition, a difference in the rate of *N*-ethylmaleimide inhibition for condensation activity with

palmitoyl-CoA *versus* unsaturated substrates provides direct evidence for the involvement of at least one different enzyme in the condensation systems using saturated *versus* polyunsaturated primers.

THE ACTION OF BILE SALTS AND OTHER DETERGENTS ON PANCREATIC LIPASE AND THE INTERACTION WITH COLIPASE. B. Borgstrom (Dept. of Physiol. Chem., Univ. of Lund, Lund, Sweden). *Biochim. Biophys. Acta* **488**, 381–91 (1977). The effect of inhibiting pancreatic lipase seems to be a property common to most detergents irrespective of charge and structure. Inhibition has no absolute relation to the critical micellar concentration (CMC) of the detergent; for most anionic detergents, inhibition is complete in the CMC range; for cationic and nonionic detergents, inhibition occurs generally at concentrations well below the CMC. Lipase inhibition occurs parallel to a displacement of lipase from the substrate interface to the aqueous phase and most probably is a general detergent effect caused by a competition at the substrate interface for hydrophobic interactions. Bile salts inhibit also the activity of lipase against "water soluble" substrates. These interactions may still be interfacial. Nonionic detergents in contrast to anionic and cationic detergents do not bind to lipase or colipase.

EFFECT OF DIETARY FAT SATURATION ON ACYLCOENZYME A: CHOLESTEROL ACYLTRANSFERASE ACTIVITY OF EHRLICH CELL MICROSOMES. D.E. Brenneman, T. Kaduce and A.A. Spector (Depts. of Biochem. and Med., Univ. of Iowa, Iowa City, IA). *J. Lipid Res.* **18**, 582–91 (1977). Ehrlich cells grown in mice fed coconut oil diets (highly saturated) contained about twice as much cholesteryl ester as those grown in mice fed sunflower oil diets (highly polyunsaturated). Our results indicate that dietary modification of the microsomal fatty acid composition is associated with alterations in the activity of ACAT, an enzyme that is tightly bound to the microsomes. These changes in ACAT activity may be partly responsible for the differences in cholesteryl ester contents of Ehrlich cells grown in mice fed the coconut and sunflower oil diets.

CAROTENOID BIOSYNTHESIS IN RHODOMICROBIUM VANNIELII. EXPERIMENTS WITH NICOTINE AND 2-(4-CHLOROPHENYLTHIO)-TRIETHYLAMMONIUM CHLORIDE (CPTA). G. Britton, R.K. Singh and T.W. Goodwin (Dept. of Biochem., Univ. of Liverpool, P.O. Box 147, Liverpool, L69 3 BX, U.K.). *Biochim. Biophys. Acta* **488**, 475–83 (1977). Nicotine and 2-(4-chlorophenylthio)triethylammonium chloride (CPTA) each inhibit production of the normal carotenoids of *Rhodomicrobium vannielii* (Rhodospirillaceae), especially rhodopin, β-carotene and spirilloxanthin, and cause the accumulation of lycopene. The inhibition of hydration of the C-1,2 double bond as well as cyclization is in agreement with proposals that these two reactions involve similar mechanisms. After removal of nicotine, cells reincubated in buffer solution or in the presence of diphenylamine convert accumulated lycopene into rhodopin. Under other conditions rhodopin is synthesized, on removal of nicotine, not from accumulated lycopene but from early precursors. The pathway of rhodopin and spirilloxanthin biosynthesis in *Rm. vannielii* is discussed briefly, and the possible involvement of enzyme aggregates in carotenoid biosynthesis is considered.

ROLE OF VITAMIN E IN GLUTATHIONE-INDUCED OXIDANT STRESS: METHEMOGLOBIN, LIPID PEROXIDATION, AND HEMOLYSIS. N.R. Brownlee, J.J. Huttner, R.V. Panganamala, and D.G. Cornwell (Dept. of Physiological Chem., Ohio State Univ., Columbus, OH). *J. Lipid Res.* **18**, 635–44 (1977). Red blood cells (RBC) from normal and vitamin E-deficient rats were incubated in a hypertonic solution of reduced glutathione adjusted to pH 8. Methemoglobin formation occurred in intact RBC from both normal and vitamin E-deficient rats. Hemolysis was significantly greater in RBC from vitamin E-deficient rats. Experiments with catalase, superoxide dismutase, and methional showed that H_2O_2 was the primary extracellular source of oxidant stress. Extracellular superoxide and hydroxyl radical were not involved in oxidant stress. Experiments with dimethyl sulfoxide showed that intracellular hydroxyl radical, generated from H_2O_2, was the hemolytic agent. Neither methemoglobin formation nor lipid peroxidation involved hydroxyl radical.

MODIFICATION OF THE FATTY ACID COMPOSITION OF L1210 MURINE LEUKEMIA CELLS. C.P. Burns, D.G. Luttenegger, S-P.L. Wei and A.A. Spector (Depts. of Med. and Biochem., Univ. of Iowa Col. of Med., Iowa City, IA). *Lipids* **12**, 747–52 (1977). We have compared the effect of diets containing 16% sunflower seed oil (polyunsaturated fat-rich) or 16% coconut oil (saturated fat-rich) fed for 3–7 weeks on the composition of L1210 murine leukemia cells which were transplanted into the peritoneal cavity during the final week of feeding. The L1210 phospholipids of mice fed the sunflower oil diet contained 43% polyenoic fatty acids and an average of 1.5 double bonds per fatty acid molecule as compared to only 25% polyenoic fatty acids and 1.2 double bonds in the coconut oil group. In contrast, the cells from the sunflower oil group contained only 13% monoenoic fatty acids as compared to 33% in those from the coconut oil group. These results indicated that the fatty acid saturation of tumor cell phospholipids can be altered appreciably. The changes in fatty acid composition were not associated with any change in the sterol/phospholipid ratio of the cells. Therefore, our results suggest that it may be possible to alter the physical properties and function of a tumor cell membrane by dietary modification of its phospholipid composition.

CHARACTERIZATION OF THE LACTATION-DEPENDENT FATTY LIVER IN MYO-INOSITOL DEFICIENT RATS. L.E. Burton and W.Y. Wells (Dept. of Biochem., Michigan State Univ., East Lansing, Michigan). *J. Nutr.* **107**, 1871–83 (1977). The characteristics of the lipid deposited during lactation-dependent fatty liver development in *myo*-inositol-deficient rats were studied. After only 4 days of lactation, cholesterol esters and triglyceride levels were significantly elevated, whereas liver phospholipid levels were significantly depressed in the livers of *myo*-inositol deficient dams compared with rats fed *myo*-inositol supplemented diets. Electron microscopy revealed the presence of numerous large intracellular lipid droplets in the livers of dams deprived of *myo*-inositol after 14 days of lactation. Plasma lipoprotein lipid levels were depressed for *myo*-inositol deprived dams during lactation, suggesting a block in hepatic lipoprotein secretion. No differences in lipid composition were observed in kidney and intestinal tissues from 21-day lactating dams fed the *myo*-inositol deficient or control diets.

HEPATIC METABOLISM OF THE GENETICALLY DIABETIC (DB/DB) MICE. II. LIPID METABOLISM. T.M. Chan and J.H. Exton (Dept. of Physiol., Vanderbilt Univ., School of Med., Nashville, TN). *Biochim. Biophys. Acta* **489**, 1–14 (1977). Hepatic lipogenesis measured in vivo with ^3H_2O was elevated 2- to 4-fold in diabetic (*db/db*) mice aged 4–8 weeks. Lipogenesis in adipose tissue was 2-fold normal at 4 weeks, but declined to normal by 8 weeks. Hepatocytes from *db/db* mice also showed increased lipogenesis. Incorporation of ^{14}C from labeled glucose, lactate, acetate and glycerol into total lipid and fatty acid, sterol and glycerol fractions were markedly elevated in hepatocytes from 5–6 week old *db/db* mice, but declined with age. Activities of hepatic malic enzyme, ATP citrate lyase, citrate synthase, acetyl-CoA carboxylase and pyruvate dehydrogenase were increased in *db/db* mice. Oxidation of [1-^{14}C]octanoate or [1-^{14}C]oleate to $^{14}CO_2$ was similar in hepatocytes from normal and *db/db* mice, but endogenous and fatty acid-stimulated ketogenesis were markedly reduced. The incorporation of radioactivity from 0.5 mM [1-^{14}C]oleate into glyceride and sterol fractions was increased 2- to 3-fold in hepatocytes from *db/db* mice. These data indicate that hepatic fatty acid synthesis and esterification are elevated in *db/db* mice as early as 4–5 weeks. It is suggested that these changes are due to hyperinsulinemia and contribute to the development of obesity in these animals.

THE METABOLISM OF CHYLOMICRON REMNANTS BY ISOLATED PERFUSED RAT LIVER. A.D. Cooper (Dept. of Med., Div. of Gastroenterol., Stanford Univ. School of Med., Stanford, CA). *Biochim. Biophys. Acta* **488**, 464–74 (1977). When whole chylomicrons containing radiolabeled lipids were added to the perfusate of an isolated liver, virtually no cholesterol ester or triacylglycerol was removed during the first hour, while a small amount of free cholesterol disappeared. After 3 hr, 40% of the cholesterol ester was removed by the liver. In contrast, when chylomicron remnants, prepared by incubation of chylomicrons with post heparin plasma and containing the same amount of cholesterol, were added to the perfusate, 76 ± 7% of the cholesterol ester was removed in the first hour. It is concluded that the chylomicron remnant is the lipoprotein of alimentary origin which regulates hepatic cholesterol synthesis, and that its metabolism by isolated liver appears to reflect the hepatic component of chylomicron metabolism in vivo.

DIFFERENCE DECOUPLING NUCLEAR MAGNETIC RESONANCE: A METHOD TO STUDY THE EXCHANGE OF FATTY ACIDS BETWEEN PHOSPHOLIPID MOLECULES. J.E. Cronan, Jr., and J.H. Prestegard (Dept. of Molecular Biophysics and Biochem. and of Chem., Yale Univ., New Haven, Conn.). *Biochemistry* **16**, 4738–42 (1977). A nuclear magnetic resonance technique has been developed to study the exchange of oleic acid moieties between phospholipid molecules of *Escherichia coli*. The method relies on the splitting of the NMR spectrum of the proton on position 2 of a diglyceride glycerol backbone caused by a ^{13}C atom in the carboxyl of the fatty acid esterified to position 2. By subtracting decoupled from coupled spectra, the amount of diglycerides having ^{13}C in the carboxyl of the position 2 fatty acid moiety can be measured. We have used these techniques to measure the exchange of oleic acid moieties between phospholipid molecules in both normally growing cultures of *E. coli* and cultures starved for oleic acid. In neither case did we observe fatty acid exchange. The implications of this result and the general usefulness of the technique are discussed.

LIPID METABOLISM IN AN INOSITOL-DEFICIENT YEAST, SACCHAROMYCES CARLSBERGENSIS. THE INFLUENCE OF TEMPERATURE AND ANAEROBIOSIS ON THE CELLULAR LIPID COMPOSITION. G. Daum, H. Glatz and F. Paltauf (Inst. of Biochem., Tech. Univ. of Graz, Graz, Austria). *Biochim. Biophys. Acta* **488**, 484–92 (1977). *Saccharomyces carlsbergensis* ATCC 9080 was grown at temperatures of 9, 25, 30 and 35°C, respectively. At all temperatures inositol-deficient cells contain less phosphatidylinositol than supplemented cells. Total acylglycerols increase in supplemented cells as the temperature decreases whereas in deficient cells total acyl glycerols are higher at 35°C than at 9°C. Temperature has little influence on the fatty acid composition of the supplemented as well as on that of the deficient cells. Under anaerobic growth conditions two major effects of inositol deficiency observed under aerobic conditions, i.e. accumulation of cellular triacylglycerols and increased production of acetoin, do not occur. Other effects of inositol deficiency, such as increased glucose utilization, increase of ethanol production, decrease of phosphatidylinositol and aggregation of cells, are observed under anaerobic conditions as well as under aerobic conditions.

EFFECT OF HIGH-OLEIC AND HIGH-LINOLEIC SAFFLOWER OILS ON MAMMARY TUMORS INDUCED IN RATS BY 7,12-DIMETHYLBENZ-(α)ANTHRACENE. S. Dayton, S. Hashimoto and J. Wollman (Res. Service, Med. Service, and Lab. Service, VA Wadsworth Hosp. Ctr., Los Angeles, CA) *J. Nutr.* **107**, 1353–60 (1970). A mutant safflower oil, rich in oleic acid, was used for a critical test of the hypothesis that polyunsaturated fats act as co-carcinogens. Weanling female rats were each given 5 mg of 7,12-dimethylbenz(α)anthracene. They were then pair-fed diets containing 20%, by weight, of conventional high-linoleic safflower oil; a mutant high-oleic safflower oil; or coconut oil. Half of each group received supplementary DL-α-tocopherol. Tumors were identified by two observers, by palpation. Data on incidence of tumors and on numbers of tumors per affected rat led to similar conclusions. When the data for tocopherol-supplemented and unsupplemented subgroups were combined, the high-oleic safflower oil group had significantly more tumors than did the coconut oil group. The high-linoleic safflower oil group was not significantly different from either of the other groups. In all groups, histologic examination of the largest tumor in each rat revealed more benign tumors, mostly duct papillomas, than carcinomas.

GANGLIOSIDES AND PHOSPHOLIPIDS OF THE MEMBRANES FROM BOVINE ADRENAL MEDULLARY CHROMAFFIN GRANULES. H. Dreyfus, D. Aunis, S. Harth and P. Mandel (Centre de Neurochimie du CNRS, 11 Rue Humann, 67085 Strasbourg Cedex, France). *Biochim. Biophys. Acta* **489**, 89–97 (1977). The lipid and ganglioside compositions of membranes of chromaffin granules isolated from bovine adrenal medulla have been investigated. The detailed lipid analysis revealed the presence of high levels of lysophosphatidylcholine, in agreement with previous studies, but also of sphingomyelin and plasmalogens. From these membranes, gangliosides have been extracted and separated by thin-layer chromatography and analysed. 95% of the total recovered gangliosides were hematosides (G_{M3}), which migrated as three major species. Sugar analyses have been performed, as well as the fatty acid compositions. The three hematoside gangliosides appeared to differ on the basis of their fatty acid composition. Compared with the brain, chromaffin granule membranes showed a simple ganglioside composition, thus offering a good model for the study of the metabolism and the role of gangliosides. The simple ganglioside composition of chromaffin granule membranes has allowed us to state that there are 60 mol phospholipid and 30 mol cholesterol per mol ganglioside.

PREFERENTIAL UPTAKE AND UTILIZATION OF MEVALONOLACTONE OVER MEVALONATE FOR STEROL BIOSYNTHESIS IN ISOLATED RAT HEPATOCYTES. P.A. Edwards, J. Edmond, A.M. Fogelman and G. Popjak (Dept. of Biol. Chem. and the Div. of Cardiol., Dept. of Med. Univ. of California, School of Med., Los Angeles, CA) *Biochim. Biophys. Acta* **488**, 493–501 (1977). Mevalonolactone at micromolar concentrations is taken up by rat hepatocytes and is converted into non-saponifiable lipids much faster than mevalonate. Although the first evidence of decarboxylation of both mevalonolactone and mevalonate (as determined by the release of $^{14}CO_2$ from the 1-^{14}C-labelled substrates) was observed at the same time (2 min), the subsequent rate of decarboxylation of mevalonolactone was approx. 43-fold of that found with mevalonate. The more rapid utilization of micromolar concentrations of mevalonolactone, compared to mevalonate, can be partly explained by the approx. 2-fold faster entry of the unchanged mevalonolactone into intact cells compared to the anionic mevalonate. This difference in uptake into the cell was observed with both the pure (*R*)- and the biologically inactive (*S*)-enantiomers and was independent of temperature.

THE ENERGY BALANCE OF TRIACYLGLYCEROL METABOLISM IN ISOLATED RAT ADIPOCYTES AND THE EFFECT OF INSULIN. R.M. Evans and C.J. Garratt (Dept. of Chem., Univ. of York, Heslington, York Y01 5DD, U.K.). *Biochim. Biophys. Acta* **489**, 48–57 (1977). Isolated rat fat cells have been incubated with various concentrations of glucose with and without insulin in the presence of either [U-^{14}C]glucose or [^{3}H]H_2O. Incorporation of ^{14}C into CO_2, fatty acids and triacylglycerol, and of ^3H into fatty acids has been measured. By using both labels it has been possible to compare the total rate of fatty acid synthesis with the rate of fatty acid synthesis from exogenous glucose. The data have been used to calculate the overall energy balance associated with triacylglycerol metabolism.

COMPARATIVE EFFECTS OF METHYLMALONYL COENZYME A ON FATTY ACID SYNTHETASE DERIVED FROM RAT AND MAN. E.P. Frenkel and R.L. Kitchens (Evelyn L. Overton Hematology-Oncology Res. Lab., Dept. of Internal Med., Univ. of Texas Southwestern Med. Schl., Dallas, Texas). *Proc. Soc. Exp. Biol. Med.* **156**, 151–4 (1977). The effect of methylmalonyl CoA on fatty acid synthetase activity was compared on enzyme derived from rats and man. Partially purified fatty acid synthetase from both sources was shown to be strikingly similar. Enzyme from both sources catalyzed propionyl CoA equally well (approximately 90% the rate of acetyl CoA at equimolar concentrations), giving rise to odd-chain fatty acids. Neither enzyme catalyzed methylmalonyl CoA in measurable activities over a wide range of concentrations. Michaelis constants for malonyl CoA were essentially the same (K_m = 4×10^{-5} and 3×10^{-5} M for rat and human liver, respectively). Methylmalonyl CoA was demonstrated to be a competitive inhibitor of malonyl CoA, with K_i values of 2.4×10^{-5} M for the enzyme from both sources. Human liver fatty acid synthetase was immunoreactive with antiserum prepared against purified rat liver fatty acid synthetase.

STUDIES ON TETRAHYMENA MEMBRANES. PALMITOYL-COENZYME A DESATURASE, A POSSIBLE KEY ENZYME FOR TEMPERATURE ADAPTATION IN TETRAHYMENA MICROSOMES. H. Fukushima, S. Nagao, Y. Okano and Y. Nozawa (Dept. of Biochem., Gifu Univ. School of Med., Tsukasamachi 40, Gifu, Japan). *Biochim. Biophys. Acta* **488**, 442–53 (1977). Microsomes from a thermotolerant Tetrahymena NT-1 catalyze the conversion of palmitoyl-CoA to palmitoleate. Palmitoyl-CoA desaturase enzyme requires molecular oxygen and NADH or NADPH as cofactor and its activity is inhibited by cyanide. A pH optimum range 7.0–7.3 is observed. There is a clear break at 30°C and a slight bend around 15°C in the Arrhenius plots of palmitoyl-CoA desaturase activity. After quenching from 39.5°C, at 26°C microsomal membranes show small particle-free areas, when examined by freeze-fracture electron microscopy, indicating the onset of phase separation. Larger smooth areas devoid of membrane-intercalated particles are observed in microsomes at 23 and 15°C. The results support evidence that the thermally induced transition of desaturase enzyme activity is related to the altered membrane properties due to temperature change.

MODIFICATION OF THE LIPID COMPOSITION OF NORMAL AND ROUS SARCOMA VIRUS-INFECTED CELLS. EFFECTS ON TRANSFORMATION-ASSOCIATED MEMBRANE PROPERTIES. A.H. Hale, J.E. Pessin, F. Palmer, M.J. Weber and M. Glaser (Depts. of Biochem. and Microbiol., Univ. of Illinois, Urbana, IL). *J. Biol. Chem.* 252, 6190–200 (1977). Procedures are described for modification of the phospholipid polar head group and fatty acid composition of normal and Rous sarcoma virus-infected chicken embryo fibroblasts. Lipid modification was carried out by growth of cells in delipidated medium containing either polar head group analogues or specific fatty acids. Normal and infected cells displayed similar kinetics of lipid alteration, and the modification was 50% complete in approximately 10 h. Since this is faster than can be accounted for by growth and dilution, extensive turnover of polar head groups and fatty acids must occur in this system.

HUMAN ENDOTHELIAL CELLS IN PRIMARY CULTURE. EFFECTS OF NORMAL LIPOPROTEINS ON THE INCORPORATION OF ACETATE INTO LIPIDS. T. Henriksen, S.A. Evensen, H. Torsvik and B. Carlander (Inst. for Surgical Res. and Med. Dept. A, Rikshospitalet, Univ. Hosp., Oslo, Norway). *Biochim. Biophys. Acta* 489, 64–71 (1977). Incorporation of [^{14}C]acetate into lipids was measured in primary cultures of human endothelial cells isolated from the intima of umbilical veins. Replacement of 20% normal human serum in the culture medium by lipoprotein-poor human serum resulted in a 10-fold increase in acetate incorporation into sterols, accompanied by a fall in the conversion of acetate into the fatty acid fraction. Addition of lipoproteins suppressed sterol synthesis. Low density lipoproteins inhibited acetate conversion to sterols to the same degree as normal human serum even at concentrations far below what is normally found in vivo, and also increased acetate incorporation into the fatty acid fraction. Very low density and high density lipoproteins were less potent and had no significant effects on fatty acid synthesis. Our findings suggest that vascular endothelial cells are subject to the same negative feedback control by lipoproteins as has previously been demonstrated for human skin fibroblasts and smooth muscle cells.

RESPONSE OF CHICK PARATHYROID GLANDS TO THE VITAMIN D METABOLITES. 1,25-DIHYDROXYCHOLECALCIFEROL AND 24,25-DIHYDROXYCHOLECALCIFEROL. H.L. Henry, A.N. Taylor and A W. Norman (Dept. of Biochem., Univ. of California, Riverside, CA). *J. Nutr.* 107, 1918–26 (1977). Under conditions of chronic hypocalcemia, e.g. in vitamin D depletion, the parathyroid glands undergo marked hypertrophy and hyperplasia. Seven days of treatment (100 IU/day; 6.5 nmoles) with cholecalciferol (CC) decreased parathyroid gland weight significantly from vitamin D depleted controls while increasing serum Ca from 6.3 to 8.6 mg/100 ml. 1,25-Dihydroxycholecalciferol (1.3 nmoles/day) also increased serum Ca to 8 6, but had no effect on gland weight. Both CC and 1,25-dihydroxycholecalciferol stimulated the production of intestinal calcium binding protein. This same dose of 1,25-dihydroxycholecalciferol in combination with small amounts of 24R,25-dihydroxycholecalciferol was as effective as CC in reducing parathyroid gland weight, but 24R-25-dihydroxycholecalciferol alone had no effect on gland weight or on calcium binding protein synthesis These results suggest that the dihydroxylated vitamin D metabolites may play a role in modulating parathyroid gland as well as intestinal function.

ESSENTIAL FATTY ACID DEFICIENCY INDUCED BY TOTAL PARENTERAL NUTRITION AND BY MEDIUM-CHAIN TRIGLYCERIDE FEEDING. H. Hirono, H. Suzuki, Y. Igarashi and T. Konno (Col. of Med. Sci. and the Dept. of Pediatrics, Tohoku Univ. School of Med., Sendai, Japan). *Am. J. Clin. Nutr.* 30, 1670–6 (1977). In hospitalized infants receiving either prolonged total parenteral nutrition without fat or a formula of medium-chain triglyceride, the fatty acid composition of platelet, red blood cell, and plasma lipids was determined. The results showed that the changes in the fatty acid composition occurred not only in plasma but also in platelets and red blood cells, and the decrease in linoleic and arachidonic acid and the concurrent increase in 5,8,11-eicosatrienoic acid were confirmed to be dramatic evidence of essential fatty acid deficiency. There was no effect of essential fatty acid deficiency upon the phospholipid distribution in red blood cells or plasma.

VITAMIN D BINDING FACTORS IN BOVINE BLOOD. B.W. Hollis, J.W. Hibbs and H.R. Conrad (Dept. of Dairy Sci., Ohio Agr. Res. and Development Ctr., Wooster, OH). *J. Dairy Sci.* 60, 1605–11 (1977). Both 25[25,26-hydrogen-3] hydroxycholecalciferol and [1α,2α-hydrogen-3] cholecalciferol were added to bovine plasma in vitro. Analysis by gel-filtration and ion exchange chromatography, electrophoresis, ultracentrifugation, competitive binding specificity studies, and plasma stripping showed that vitamin D circulated with a protein of α-globulin mobility. This globulin had a much higher affinity for 25-hydroxycholecalciferol while vitamin D_3 appeared to be associated first with an α-lipoprotein and with time became associated with the α-globulin. This α-globulin had a molecular weight of approximately 70,000 as determined by gel-filtration. Cholecalciferol appeared to bind tightly to the α-lipoprotein and resisted being stripped from the plasma. Thus, α-globulin appears to be the major carrier of vitamin D in the blood while the α-lipoprotein may aid in the transfer of cholecalciferol from the gut to the liver via the lymph system.

INTERACTION OF SALMONELLA TYPHIMURIUM WITH PHOSPHOLIPID VESICLES. INCORPORATION OF EXOGENOUS LIPIDS INTO INTACT CELLS. N.C. Jones and M.J. Osborn (Dept. of Microbiol., The Univ. of Connecticut Health Ctr., School of Med., Farmington, CN). *J. Biol. Chem.* 252, 7398–404 (1977). Incubation of intact cells of *Salmonella typhimurium* with bilayer phospholipid vesicles results in significant transfer of vesicle lipids to the cells. The transfer requires Ca^{2+} or spermine, and is dependent on time, temperature, the concentration and composition of the vesicles, and the nature of the cellular lipopolysaccharide. The process results in bulk transfer of vesicle lipids to the cells rather than reciprocal molecular exchange between vesicles and the outer membrane. All components of mixed lipid vesicles, including cholesteryl oleate and lipopolysaccharide, are transferred to the cells in a ratio similar to that of the donor vesicles. The properties of the transfer process are consistent with direct fusion of vesicles with the outer membrane of the cell.

THE EFFECT OF PHOSPHATIDYLCHOLINE DEPLETION ON BIOCHEMICAL AND PHYSICAL PROPERTIES OF A NEUROSPORA CRASSA MEMBRANE MUTANT. D. Juretic (Dept. of Biochem. and Biophys., The Pennsylvania State Univ., University Park, PA). *Biochim. Biophys. Acta* 469, 137–50 (1977). By using the choline starvation process it is possible to deplete the membranes of *Neurospora crassa* choline auxotroph *chol-1* of phosphatidylcholine, without affecting the viability of germinated spores or whole mycelium. Spin label probes were used to examine the possible dependence of the physical state of cellular lipids on the presence of phosphatidylcholine in the membranes.

EFFECT OF FATTY ACID SATURATION ON α-AMINOISOBUTYRIC ACID TRANSPORT IN EHRLICH ASCITES CELLS. T.L. Kaduce, A.B. Awad, L.J. Fontenelle and A.A. Spector (Depts. of Biochem. and Med., Univ. of Iowa, Iowa City, IA). *J. Biol. Chem.* 252, 6624–30 (1977). The Ehrlich ascites tumor was grown in mice fed different types of fat in order to modify the fatty acid composition of the cells. The phospholipids of a plasma membrane fraction isolated from cells grown on coconut oil contained considerably more monoenoic and less polyenoic fatty acids than those from cells grown on sunflower oil. Membrane phospholipids from cells grown in mice fed regular rodent chow had fatty acid composition intermediate between those of the cells grown on sunflower and coconut oil. Although the fatty acid compositions were altered, there was no appreciable ratio of phospholipid to cholesterol in any of the membrane preparations. The activation energies for the sodium-independent component also were highest in the cells grown on coconut oil and lowest in those grown on sunflower oil. These results suggest that amino acid transport systems in mammalian cells can be regulated to some extent by changes in the fatty acid composition of the cell membrane.

FAT CELL PLASMA MEMBRANES. I. PREPARATION, CHARACTERIZATION, AND CHEMICAL COMPOSITION. Y. Kawai and R.G. Spiro (Depts. of Biol. Chem. and Med., Harvard Med. School, The Elliott P. Joslin Res. Lab., and the Peter Bent Brigham Hosp., Boston, MA). *J. Biol. Chem.* 252, 6229–35 (1977). Perirenal adipose tissue from rabbit, rat, and calf was disrupted without the use of proteases by a sieving procedure to yield fat cells from which plasma membranes were prepared. These membranes were isolated from the homogenate of the cells by differential and sucrose density gradient centrifugation. The major plasma membrane fraction, which represented the lightest band resolved from the microsomal pellet, was obtained from rabbit in a yield of about 4 mg/100 g of

tissue and was purified about 17-fold in respect to 5'-nucleotidase activity. The membranes were rich in cholesterol and phospholipids (total lipid, 57% of membrane weight) and had high alkaline phosphatase activity. Only low levels of succinic dehydrogenase, NADPH-cytochrome *c* reductase, and nucleic acids were observed, indicating absence of significant contamination with intracellular components. The most striking observation was the presence of the same major glycoprotein band(s) in all six membrane fractions obtained by density gradient centrifugation of the microsomal pellet. This finding may have a bearing on plasma membrane biogenesis in the fat cell.

EFFECT OF ETHYNYLESTRADIOL ON BILIARY EXCRETION ON BILE ACIDS, PHOSPHATIDYLCHOLINES, AND CHOLESTEROL IN THE BILE FISTULA RAT. F. Kern, Jr., H. Eriksson, T. Curstedt and J. Sjovall (Dept. of Chem., Karolinska Institutet, Stockholm, Sweden). *J. Lipid Res.* 18, 623–34 (1977). The effects of ethynylestradiol on endogenous bile acids, their capacity to conjugate and excrete intravenously infused cholic acid, the concentrations of biliary cholesterol and lecithin, and the individual molecular species of phosphatidylcholine have been determined in male and female Sprague-Dawley rats. Endogenous biliary bile acids were analyzed by gas-liquid chromatography-mass spectrometry. Eleven bile acids were identified and several minor bile acids, primarily muricholates, could not be completely characterized. After 5 days of treatment with ethynylestradiol (1 mg/kg per day), the percentage of cholic acid decreased and the percentage of 6-hydroxylated bile acids, including several monounsaturated species, increased. Ethynylestradiol caused a decrease in bile acid-independent bile flow. Intravenous infusion of cholic acid at a high concentration caused cholestasis in control animals, but, after ethynylestradiol treatment, cholestasis developed during the infusion of a much lower concentration of cholate, indicating a lowered threshold for bile acid-induced cholestasis.

THE SEPARATION OF HUMAN SERUM HIGH DENSITY LIPOPROTEINS BY HYDROXYAPATITE COLUMN CHROMATOGRAPHY. EVIDENCE FOR PRESENCE OF DISCRETE SUBFRACTIONS. G.M. Kostner and A. Holasek (Inst. of Med. Biochem., Univ. of Graz, A-8010 Graz, Austria). *Biochim. Biophys. Acta* 488, 417–31 (1977). Human serum high density lipoprotein subfractions 2 and 3, isolated by preparative ultracentrifugation after blocking the enzyme phosphatidylcholine:cholesterol acyl transferase, have been subfractionated further by hydroxyapatite column chromatography. From subfraction 2 we reproducibly obtained 5 and from subfraction 3, 6 fractions differing in chemical composition and apolipoprotein content. The fractions eluting at low salt concentrations were composed primarily of apolipoprotein-A polypeptides while those eluting at high salt concentrations consisted primarily of apolipoprotein-C. Although small differences of their partial specific volumes existed, the obtained values indicate that all subfractions belonged to the parent density class.

MECHANISM FOR ELEVATION OF HEPATIC CHOLESTEROL SYNTHESIS AND SERUM CHOLESTEROL LEVELS IN TRITON WR-1339-INDUCED HYPERLIPIDEMIA. M. Kuroda, K. Tanzawa, Y. Tsujita and A. Endo (Fermentation Res. Lab., Sankyo Co., Ltd., 1-2-58 Hiromachi, Shinagawaku, Tokyo, 140, Japan). *Biochim. Biophys. Acta* 489, 119–25 (1977). During the first 3 hr after the intravenous injection of Triton (WR-1339) to rats at a dose of 400 mg/kg (first phase), a 2-fold increase in serum cholesterol levels was accompanied by a 17–35% decrease in hepatic cholesterol levels, but the liver plus serum cholesterol levels (mg/100 g body weight) were essentially unchanged. No increases were seen in the activity of either hepatic 3-hydroxy-3-methylglutaryl-CoA reductase or sterol synthesis during this period. These results strongly suggest that the increased serum cholesterol levels in the first phase was mainly caused by removing cholesterol from liver into the serum compartment, and that in the second phase the increased sterol synthesis in liver resulted from the elevation of 3-hydroxy-3-methylglutaryl-CoA reductase may explain the rise of serum cholesterol levels.

LIPID PHASE TRANSITIONS AND PHASE DIAGRAMS. I. LIPID PHASE TRANSITIONS. A.G. Lee (Dept. of Physiol. and Biochem., Univ. of Southampton, Southampton S09 3TU, U.K.). *Biochim. Biophys. Acta* 472, 237–81 (1977). The lipid composition of biological membranes is complex, and it is an article of faith amongst membranologists that this complexity serves a purpose. Quite what the purpose is is not yet clear, although it is generally thought to be connected with the necessity to provide the correct lipid micro-environment for the membrane-bound proteins. For the erythrocyte membrane, with its wide range of functions and consequent complexity of membrane proteins, a large number of lipid species might be expected. A large number of studies have shown that membrane proteins have fairly precise environmental requirements for optimum activity, some requiring a fluid environment, whereas others require much more rigid surroundings. Such studies are particularly well developed in bacterial systems, where it has been shown that both saturated and unsaturated fatty acids are required for incorporation into membrane lipids, and that at the growth temperature, lipids are present in both the solid, gel phase and fluid, liquid-crystalline phase.

EVIDENCE FOR PARTICIPATION OF CYTOCHROME B_5 IN MICROSOMAL Δ-6 DESATURATION OF FATTY ACIDS. T-C. Lee, R.C. Baker, N. Stephens and F. Snyder (Med. and Health Sci. Div., Oak Ridge Associated Univ., Oak Ridge, TN). *Biochim. Biophys. Acta* 489, 25–31 (1977). The Δ-6 desaturation of linoleic acid to γ-linolenic acid and oleic acid to 6,9-octadecadienoic acid by rat liver microsomes was investigated. Using a specific antibody prepared against purified rat liver cytochrome b_5, we demonstrated that cytochrome b_5 participated in Δ-6 desaturation of both fatty acids. The reaction products were identified as their methyl ester derivatives by argentation thin-layer chromatography, gas-liquid chromatography, and reductive ozonolysis followed by gas-liquid chromatography.

MORPHOLOGY OF ERYTHROCYTES FROM VITAMIN E-DEFICIENT LEAD-POISONED RATS. O.A. Levander, M. Fisher, V.C. Morris and R.J. Ferretti (Nutrition Inst. and Animal Physiology and Genetics Institute, Agricultural Res. Service, U.S. Dept. of Agr., Agr. Res. Center, Beltsville, MD). *J. Nutr.* 107, 1828–36 (1977). Weanling male rats were fed either a vitamin E-deficient Torula yeast diet fortified with selenium or the same diet supplemented with 100 ppm vitamin E. Of rats fed each diet, one group received 250 ppm lead in the drinking water, whereas another group received no lead. After 3 months, red cell filterability was measured and the red cell suspensions were examined by scanning electron microscopy (SEM). Red blood cells from vitamin E-supplemented, non-poisoned or lead-poisoned rats were filterable even after 6 hours of incubation in buffered saline. SEM revealed that these cells were largely echinocytes. Red cells from vitamin E-deficient rats gradually lost their filterability after incubation in vitro and lead poisoning accelerated this decline. These observations are consistent with the hypothesis that spherocytes develop more rapidly in E-deficient lead-poisoned than in E-supplemented non-poisoned rats and help explain the splenomegaly, increased erythrocyte mechanical fragility, and decreased red cell filterability observed in such rats.

FETAL AND MATERNAL VITAMIN A LEVELS IN TISSUES OF HYPERVITAMINOTIC A RATS AND RABBITS. C.A. Lorente and S.A. Miller (Dept. of Nutr. and Food Sci., Massachusetts Inst. of Tech., Cambridge, MA). *J. Nutr.* 107, 1816–21 (1977). Pregnant rats and rabbits were given excess vitamin A, in the form of retinyl acetate or retinoic acid, for the 3-day period just prior to palatal closure in the fetuses. Twenty-four hours later, the various forms of vitamin A, and their levels, were determined in fetal liver and carcass and in maternal liver and serum by thin-layer chromatography. The predominant forms of vitamin A found in both fetal and maternal tissues were retinyl palmitate, retinol and retinoic acid. In both species, the tissues from the groups treated with retinoic acid contained levels of vitamin A similar to those found in control tissues. After retinyl acetate treatment in the rat, both of the maternal tissues studied had elevated vitamin A levels, whereas in the rabbit only maternal liver levels increased. In the groups treated with retinyl acetate, the ratio of the vitamin A levels in fetal liver: maternal serum reflected a species difference: the ratio was lower than the control value in the rabbit and higher than controls in the rat. Radiotracer studies in the rat, using either ^{3}H-retinol or ^{14}C-retinoic acid, demonstrated vitamin A transport across the placenta, with vitamin A concentrating in the fetal liver.

INHIBITION OF LIPOPROTEIN BINDING TO CELL SURFACE RECEPTORS OF FIBROBLASTS FOLLOWING SELECTIVE MODIFICATION OF ARGINYL RESIDUES IN ARGININE-RICH AND B APOPROTEINS. R.W. Mahley, T.L. Innerarity, R.E. Pitas, K.H. Weisgraber, J.H. Brown, and E. Gross (Lab. of Experimental Athero., Natl. Heart, Lung and Blood Inst. and Sec. on Molecular Structure, Reproduction Res. Branch, Natl. Inst. of Child Health and

Human Dev., Natl. Insts. of Health, Bethesda, Md.). *J. Biol. Chem.* 252, 7279–87 (1977). Treatment of human low density lipoproteins (LDL) with 0.1 M 1,2-cyclohexanedione in borate buffer selectively modified half of the arginyl residues of the apolipoproteins and almost totally abolished the binding of the LDL to the high affinity cell surface receptors of human fibroblasts. Except for the modification of the arginyl residues, there were no apparent alterations in the other amino acid residues, lipid composition, size and morphologic appearance, or apoprotein pattern. Removal of cyclohexanedione by incubation of the modified LDL with 0.5 M hydroxylamine for 7 h restored more than 80% of the original activity as determined by competitive binding, internalization, and degradation studies with ^{125}I-LDL. Likewise, selective modification with cyclohexanedione of the arginyl residues of certain canine lipoproteins (LDL, HDL_1, and HDL_c) prevented their interaction with the cell surface receptors. In particular, the binding activity of the cholesterol-induced HDL_c which contain the arginine-rich apoprotein as the only detectable protein was abolished by cyclohexanedione.

EFFECT OF LARGE AMOUNTS OF VITAMIN E DURING PREGNANCY AND LACTATION. M.M. Martin and L.S. Hurley (Dept. of Nutr., Univ. of California, Davis, CA). *Am. J. Clin. Nutr.* 30, 1629–37 (1977). The effects of excessive intake of vitamin E during gestation and lactation on female rats and their progeny were studied. Pregnant rats receiving large doses of vitamin E (22.5 to 2252 mg/kg per day) had larger livers, higher levels of lipids and vitamin E in plasma, and higher concentrations of vitamin E in the livers than did controls. These deviations from normal were not, however, observed for all levels of supplementation. No obvious teratogenic effects were observed in the newborn young of the vitamin E-supplemented rats. Some eye abnormalities were seen in the older pups of rats given extremely high amounts of the vitamin. The survival rate, weight of the pups, and litter size were unaffected. However, the pups of the mothers who had received 500 mg of vitamin E per day (2252 mg/kg per day) during gestation and lactation had a much higher concentration of vitamin E in their livers and plasma than did controls. This study also confirmed the observation that vitamin E transfer across the placenta is negligible and that mammary transfer of this vitamin is quite efficient.

THE INFLUENCE OF CHOLESTEROL ON THE ACTIVITY, ON THE ISOTHERMIC KINETICS AND ON THE TEMPERATURE-INDUCED KINETICS OF 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE. K.A. Mitropoulos and S. Venkatesan (Med. Res. Council Lipid Metab. Unit., Hammersmith Hosp., Ducane Rd., London, W.12., U.K.). *Biochim. Biophys. Acta* 489, 126–42 (1977). The activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase was assayed in liver microsomal preparations from rats fed cholesterol, cholestyramine or the standard diet, without and after treatment with digitonin. The activity of the enzyme in the microsomal fraction was high in the preparations from rats fed cholestyramine and low in the preparations from rats fed cholesterol as compared with the activity of the enzyme in preparations from rats fed the standard diet. The concentration of free cholesterol and of cholesterol esters was different in the microsomal fraction from the rats in the three experimental conditions. Treatment of the microsomal fraction with digitonin resulted in all three cases in solubilization of free cholesterol and of cholesterol esters, but the specific activity of hydroxymethylglutaryl-CoA reductase was higher in the digitonin-treated preparation than in the corresponding microsomal fraction. There was no detectable activity of hydroxymethylglutaryl-CoA reductase in the supernatant obtained after treatment of the microsomal fraction with digitonin.

BINDING AND HYDROXYLATION OF SULFOCONJUGATED STEROIDS IN ADRENAL CORTEX MITOCHONDRIA. J. Montelius, J.-A. Gustafsson, M. Ingelman-Sundberg and J. Rydstrom (Dept. of Biochem., Arrhenius Lab., Univ. of Stockholm, Sweden). *Biochim. Biophys. Acta* 488, 502–11 (1977). Binding and hydroxylation of deoxycorticosterone 21-sulfate, testosterone 17-sulfate, cholesterol 3-sulfate and the corresponding free steroids by the hydroxylase systems in adrenal cortex mitochondria was studied. Deoxycorticosterone 21-sulfate binds to cytochrome *P*-450 and gives rise to a type I difference spectrum with a peak at 388 nm and a trough at 422 nm similar to that produced by deoxycorticosterone. The maximal extents of absorption change are similar for the free and the sulfoconjugated steroid. However, the concentration required for half-maximal binding to cytochrome *P*-450 is about two orders of magnitude lower for the free steroid as compared to the sulfoconjugated steroid. Similar results were obtained with testosterone and testosterone 17-sulfate.

LIPID-PROTEIN INTERACTIONS IN THE PLASMA LIPOPROTEINS. J.D. Morrisett, R.L. Jackson and A.M. Gotto, Jr. (Dept. of Med., Baylor Col. of Med., and the Methodist Hosp., Houston, TX). *Biochim. Biophys. Acta* 472, 93–133 (1977). The plasma lipoproteins have become the focus of extensive study in the last ten years. The clinical interest in the plasma lipoproteins is, in large part, due to accumulated evidence which has related elevated levels of plasma lipoproteins to premature coronary artery disease. From the biochemical point of view, the plasma lipoproteins represent an excellent system, in which lipid-protein interactions can be studied. The main purpose of this article is to review current evidence concerning the interaction between the protein (apoprotein) and lipid components within the plasma lipoproteins. To permit a timely and detailed discussion of these studies, we have limited our review to relatively recent investigations, most of which deal with the human lipoproteins. Earlier reviews have covered both structural and metabolic aspects of the plasma lipoproteins of man and other species.

DIET AND LIPOPROTEIN INFLUENCE ON PRIMATE ATHEROSCLEROSIS. R.J. Nicolosi, J.L. Hojnacki, N. Llansa and K.C. Hayes (Dept. of Nutr., Harvard Schl. of Public Health, Boston, MA). *Proc. Soc. Exp. Biol. Med.* 156, 1–7 (1977). Squirrel and cebus monkeys fed a coconut oil diet develop comparable hypercholesterolemias, but the squirrel monkey primarily expands its low-density lipoprotein cholesterol pool, whereas the cebus primarily increases its HDL pool of cholesterol. These results, coupled with the greater accumulation of aortic lipid, particularly cholesteryl ester, in the atherosclerotic-susceptible squirrel monkey, support the concept of the protective nature of high-density lipoproteins and the atherogenic potential of LDL. They also suggest that a species' genetic control of the lipoprotein response to diet is variable and has important biological implications.

IN VIVO METABOLISM OF CERAMIDES IN RAT BRAIN. FATTY ACID REPLACEMENT AND ESTERIFICATION OF CERAMIDE. H. Okabe and Y. Kishimoto (John F. Kennedy Inst., Dept. of Neurology, The Johns Hopkins Univ. Schl. of Med., Baltimore Md.). *J. Biol. Chem.* 252, 7068–73 (1977). Three double-labeled ceramides, [1-^{14}C]lignoceroyl D-*erythro*-[1-^{3}H]spingosine, [1-^{14}C]palmitoyl D-*erythro*-[1-^{3}H]sphingosine and D-[1-^{14}C]cerebronoyl D-*erythro*-[3-^{3}H]sphingosine, were prepared and injected separately into the brains of 18-day-old rats. The animals were killed after 2 h and various sphingolipids were isolated and purified. These lipids were further fractionated into subgroups depending on their fatty acid content (nonhydroxy or α-hydroxy, shorter chain or longer chain). $^{3}H/^{14}C$ ratios obtained in ceramides, cerebrosides, and sphingomyelins containing different fatty acids from the injected material were much higher than the initial ratio. This observation indicates that a replacement of the fatty acid occurred in the injected ceramide.

EFFECT OF MEAL-FEEDING ON INSULIN SENSITIVITY AND INCORPORATION OF [U-^{14}C]GLUCOSE INTO LIPIDS IN RAT AORTA. K. O'Dea, H. Kaplovitz and A. Marino (The Res. Div., Cleveland Clinic, 9500 Euclid Ave., Cleveland, OH). *J. Nutr.* 107, 1896–901 (1977). In vitro aortic lipogenesis from D-[U-^{14}C]glucose was significantly greater in meal-fed rats than in ad libitum fed controls. In addition, aortic tissue from meal-fed rats showed a sensitivity to insulin which was absent in the same preparations from nibbling rats. Two preparations of aortic tissue were used in this study. "Intima-media" contained intima and approximately the inner two-third of the media. "Adventitia" contained the remainder of the media and the inner, fat-free adventitia. This latter preparation was more sensitive to the change from a nibbling to a meal-eating regime, although the effects were clearly evident in the intima-media, suggesting that both adventital and medial cells were susceptible to the metabolic changes associated with the adaptation to meal-eating. The development of adaptive hyperlipogenesis and insulin sensitivity in these preparations is discussed in terms of possible variations in insulin receptor number in target tissues of meal-fed rats.

CHARACTERIZATION OF HUMAN VERY LOW DENSITY LIPOPROTEINS CONTAINING TWO ELECTROPHORETIC POPULATIONS: DOUBLE PREBETA LIPOPROTEINEMIA AND PRIMARY DYSBETALIPOPROTEINEMIA. A. Pagnan, R.J. Havel, J.P. Kane and Leila Kotite (Cardiovascular Res. Institute and Dept. of Med., Univ. of California,

San Francisco, CA). *J. Lipid Res.* **18**, 613–22 (1977). Two discrete populations of very low density lipoproteins, with fast and slow pre-beta electrophoretic mobility, were found in 50% of normolipemic and 30% of hyperlipemic individuals selected at random. The two populations were isolated by preparative electrophoresis from five hyperlipemic subjects. The particles comprising the slow component were smaller than those of the fast component and the slow component contained a larger proportion of cholesteryl esters, free cholesterol, B-apo-protein, and arginine-rich apoprotein and a smaller proportion of triglycerides and the two most anionic apoproteins (R-glutamic acid and R-alanine). The properties of the slow component thus closely resemble those of "remnant" very low density lipoproteins that accumulate in blood plasma of functionally hepatectomized rats. These results suggest that many individuals have "remnant" very low density lipoproteins in their plasma. However, the beta-migrating "remnant" that accumulated in large amounts in individuals with primary dysbetalipoproteinemia contains much more arginine-rich protein and this protein is structurally abnormal.

GLUTATHIONE PEROXIDASE SYSTEM AND MICROSOMAL LIPOPEROXIDATION IN PRENECROTIC STAGES OF DIETARY HEPATIC NECROSIS IN RATS. E.A. Porta, B.K.F. Ching and N.S. Joun (Dept. of Pathol., John A. Burns School of Med., Univ. of Hawaii, Honolulu, HA). *J. Nutr.* **107**, 1852–8 (1977). Simultaneous dietary deficiency of vitamin E and Se in weanling rats results in fatal hepatic necrosis. To investigate the possible relation between the state of hepatic cytosol glutathione peroxidase (GSH-Px) system (including glutathione reductase, GSH-Rd, and glucose-6-phosphate dehydrogenase, G-6-PD) and the hepatic microsomal lipoperoxidation (MLP), as well as the possible implications of these factors in the development of hepatic necrosis, weanling male rats were fed a basal diet low in Se and vitamin E, or were pair-fed with the same diet supplemented with either 3 mg/100 g diet of *dl*-α-tocopherol, with 0.36 mg/100 g diet of sodium selenite, or with both. Rats from the four groups were killed at 9, 20, and 26 days. Dietary vitamin E and/or Se had little effect on the activity of GSH-Rd, but both agents, singly or in combination, prevented the reduction of G-6-PD that occurred in rats deficient in vitamin E and Se. The increased in vitro MLP was only partially reduced by either Se or vitamin E, since in both cases there still were three-fold increases. These results suggest that: no clear-cut relation exists between the massive decline of cytosol GSH-Px and the development of hepatic necrosis; the state of GSH-Px is probably unrelated to MLP; and microsomes may not be the initial site of damage.

COMPARISON OF THE LIPID COMPOSITION OF RABIES VIRUS PROPAGATED IN NIL 2 CELLS MAINTAINED IN MONOLAYER VERSUS SPINNER CULTURE. J. Portoukalian, M. Bugand, G. Zwingelstein and P. Precausta (CNRS, Lab. de Physiol. Generale et Comparee, Univ. Claude Bernard-Lyon I, 69621 Villeurbanne, France). *Biochim. Biophys. Acta* **489**, 106–18 (1977). The lipid composition of purified rabies virus was determined after replication in Nil 2 amster fibroblasts grown either in monolayer or in spinner culture. The effect of viral infection on the lipids of both cell populations was also investigated. No qualitative difference appear in the lipids of the virions obtained from either cell culture, but the viral contents reflected some variation in the lipid patterns of the two host cells. The virus grown in cells maintained in spinner culture exhibited a lipid content and a cholesterol:phospholipid molar ratio significantly lower than those of the virus replicated in cells cultivated in monolayer. Furthermore, both virus populations contained a high amount of ceramide trihexoside, despite the lack of this component in the cells maintained in spinner culture. Since these lipids are structurally involved in the viral envelope, it was suggested that the differences in viral lipid composition might be related to some modifications noticed in the morphological and biological characteristics of the rabies virions grown in each cell population.

CATABOLISM OF CHYLOMICRON TRIACYLGLYCEROL AND CHOLESTERYL ESTER IN GENETICALLY OBESE RATS. T.G. Redgrave (Dept. of Physiology, University of Melbourne, Parkville, Victoria, 3052, Australia). *J. Lipid Res.* **18**, 604–12 (1977). The catabolism of chylomicrons was investigated in genetically obese rats and their nonobese littermates, and was compared with catabolism in older Sprague-Dawley rats with body weights similar to the obese rats and their younger controls. Labeled thoracic-duct lymph was collected from donor rats and the catabolism of the labeled chylomicrons was studied after a single intravenous injection or during steady intravenous infusion in unanesthetized, nonfasting, recipient rats. In the genetically obese rats clearances from the plasma of chylomicron triacylglycerol and cholesteryl ester were less than in their non-obese littermates. These results suggest that hyperlipidemia in genetically obese rats may be due in part to an accumulation of chylomicron remnants in the plasma. Flotation characteristics of plasma lipoproteins in the obese rats were consistent with this interpretation. However, separate experiments showed that genetically obese, fasting rats also accumulated more triacylglycerol in the plasma after injection of Triton WR 1339. The enlarged plasma triacylglycerol pool appears to derive from a mixture of hepatic and intestinal triacylglycerol-rich lipoproteins which, together, overload their common removal mechanism.

CONCENTRATION OF LIPOPROTEINS CONTAINING APOLIPOPROTEIN B IN HUMAN PERIPHERAL LYMPH. D. Reichl, N.B. Myant and J.J. Pflug (Med. Res. Council Lipid Metabolism Unit, Hammersmith Hosp., London W12 OHS, U.K.). *Biochim. Biophys. Acta* **489**, 98–105 (1977). The concentration of apolipoprotein B (apoB) in human serum and peripheral lymph was measured by quantitative immunoelectrophoresis with antiserum to human low-density lipoprotein. In four normal and six hyperlipidaemic subjects, total lymph apoB/ml was 5–10% of total serum apoB/ml in the same subject. These ratios were equivalent to lymph apoB concentrations of 60–120 μg/ml. When the assays were carried out under conditions in which unmasking of immunoreactive sites on lymph and serum apoB was assumed to be maximal (delipidation with Nonidet P40), the lymph/serum apoB concentration ratios in three normal subjects were similar to those obtained with untreated lymph and serum.

TWO CHOLESTEROL ESTER HYDROLASES. DISTRIBUTION IN RAT TISSUES AND IN CULTURED HUMAN FIBROBLASTS AND MONKEY ARTERIAL SMOOTH MUSCLE CELLS. M.C. Riddle, W. Fujimoto and R. Ross (Dept. of Med., Univ. of Oregon Health Sci. Ctr., Portland, OR). *Biochim. Biophys. Acta* **488**, 359–69 (1977). Hydrolytic activity against acetone-dispersed [4-^{14}C] cholesterol oleate has been assayed as a function of pH in seven parenchymal tissues, blood cells, and plasma of the rat, as well as in cultured human fibroblasts and monkey *(Macaca nemestrina)* arterial smooth muscle cells. Both acid and neutral hydrolytic activities were present in all of these except rat plasma. The pH optima were in all cases close to pH 4.5 and pH 6.8. Acid activity was quite constant from tissue to tissue, while neutral activity varied greatly, being greatest in adrenal, testies, and adipose tissue. Subcellular fractionation of human fibroblasts allowed demonstration that activities at pH 4.5 and pH 6.8 were concentrated in different fractions, apparently lysosomal and polysomal, respectively. It appears most cell types, including fibroblasts and smooth muscle cells, contain two separate enzymes capable of hydrolyzing cholesterol esters. The neutral pH polysomal enzyme, which is especially prominent in certain tissues, may have a function related to the specialized roles of these tissues.

MOBILIZATION OF ARACHIDONIC ACID IN HUMAN PLATELETS. KINETICS AND Ca^{2+} DEPENDENCY. S. Rittenhouse-Simmons, F.A. Russell and D. Deykin (Boston VA Hosp. and Depts. of Med. and Biochem., Boston Univ. School of Med., Boston, MA). *Biochim. Biophys. Acta* **488**, 370–80 (1977). The release and transfer of [^{3}H]arachidonic acid in human platelets was examined with regard to varied doses of thrombin, varied periods of incubation with thrombin, the presence of 4 mM Ca^{2+}, the degree of exposure of platelet phosphatidylethanolamine to the surface and the release of serotonin from platelet granules. The effect of thrombin on the incorporation of [^{3}H]arachidonic acid and [^{14}C]glycerol into platelet phosphatides was also studied. [^{3}H]Arachidonic acid was released in pre-labeled platelets exposed to thrombin from phosphatidylcholine and phosphatidylinositol and appeared increasingly esterified in alk-1-enyl-acyl-glycerophosphoethanolamine. Maximum transfer occurred with 5 U/ml of thrombin and 15 min of incubation and was dependent upon the presence of Ca^{2+}, whereas serotonin was maximally released with 0.04 U/ml of thrombin.

USE OF ANTIBODY SPECIFICITY TO STUDY THE SURFACE DISPOSITION OF APOPROTEIN A-I ON HUMAN HIGH DENSITY LIPOPROTEINS. G. Schonfeld, J-S. Chen and R.G. Roy (Lipid Res. Ctr., Depts. of Preventive Med. and Med., Washington Univ. School of Med., St. Louis, MO). *J. Biol. Chem.* **252**, 6655–9

(1977). Only ~10% of the apoprotein A-I (apo-A-I) in intact high density lipoprotein 2 (HDL_2, HDL_2-apo-A-I) is detected by radioimmunoassay, suggesting that not all of HDL_2-apo-A-I is immunologically reactive. Previous work had indicated that the COOH-terminal region of HDL_2-apo-A-I was immunologically more reactive than the NH_2 region, whereas the two terminal regions of the isolated apo-A-I molecule was equally reactive. To confirm these immunologic differences, apo-A-I and HDL_2 were used as immunogens and the specificities of antisera were compared. In addition, an anti-apo-A-I antiserum was absorbed with HDL_2 and the binding capacity of the absorbed antiserum for the COOH- and NH_2-terminal fragments of apo-A-I was ascertained.

HIGH AFFINITY LIPID BINDING SITES ON THE PERIPHERAL MEMBRANE ENZYME PYRUVATE OXIDASE. SPECIFIC LIGAND EFFECTS ON DETERGENT BINDING. H.L. Schrock and R.B. Gennis (Depts. of Chem. and Biochem., Univ. of Illinois, Urbana, IL). *J. Biol. Chem.* **252**, 5990–5 (1977). Pyruvate oxidase is a peripheral membrane enzyme which has been purified to homogeneity from *Escherichia coli*. When assayed in the presence of some amphiphiles the enzymatic activity is stimulated nearly 25-fold; these amphiphiles include phospholipids, neutral detergents, and charged detergents. Both anionic and cationic detergents, at concentrations well below their critical micelle concentrations, are capable of activating the enzyme. In this paper the interaction between sodium dodecyl sulfate (SDS) and pyruvate oxidase has been studied by equilibrium dialysis. It is demonstrated that pyruvate oxidase possesses a small number of high affinity detergent binding sites similar to those reported for serum apolipoproteins and that these sites are functionally significant. The primary mode of interaction between the detergent and the enzyme is hydrophobic and not electrostatic. It is also shown that the SDS-binding isotherm is strongly affected by specific ligands bound to the enzyme.

INFLUENCE OF THE ESTER CARBONYL OXYGENS OF LECITHIN ON THE PERMEABILITY PROPERTIES OF MIXED LECITHIN-CHOLESTEROL BILAYERS. F.T. Schwarz and F. Paltauf (Institut fur Biochemie der Technischen Universitat Graz, Scholgelgasse 9, A-8010 Graz, Austria). *Biochemistry* **16**, 4335–9 (1977). The passive diffusion of Na^+, Cl^-, and glucose across the bilayer membranes of single-shelled vesicles of diester-lecithin (1,2-dioctadecenoyl-*sn*-glycerophosphocholine), diether-lecithin (*rac*-1,2-dioctadecenylglycero-3-phosphocholine), and 1-ether-2-ester-lecithin (*rac*-1-octadecenyl-2-octadecenoylglycero-3-phosphocholine) with and without cholesterol has been measured at 4°C. The passive diffusion of Na^+ across pure membranes of diether-lecithin was found to be much slower as compared to membranes of diesterlecithin, but the opposite effect was observed with Cl^- and glucose. Mixed membranes of diesterlecithin and 30 mol % cholesterol showed strongly reduced diffusion rates for Na^+, Cl^-, and glucose; with mixed diether-lecithin-cholesterol vesicles, however, no significant reduction was observed. On the other hand, 1-ether-2-ester-lecithin showed a similar reduction of the glucose and Na^+ permeability upon addition of 30 mol % cholesterol as did the diesterlecithin. From these data it is concluded that the ester carbonyl oxygens of lecithin are strongly involved in the interaction between lecithin and cholesterol and that the diffusion of cations, on the one hand, and anions and uncharged solutes, on the other hand, is dictated by different mechanisms.

FETAL UTILIZATION OF MATERNALLY DERIVED KETONE BODIES FOR LIPOGENESIS IN THE RAT. D.W. Seccombe, P.G.R. Harding and F. Possmayer (Depts. of Physiol., Biochem., and Obstetrics and Gynaecol., Univ. Hosp., Univ. of Western Ontario, London, Ontario, N6A 5A5, Canada). *Biochim. Biophys. Acta* **488**, 402–16 (1977). When D-β-[3-^{14}C]hydroxybutyrate was injected via the femoral vein into pregnant Sprague-Dawley rats at 21 days of gestation, D-β-[3-^{14}C]hydroxybutyrate was enzymatically detected in fetal plasma within 5 min. The time course of the incorporation of DL-β-[3-^{14}C]hydroxybutyrate into fetal lipids was studied. Lipid extracts of brown adipose tissue exhibited the greatest relative incorporation followed by pancreas, liver and lung. Less radioactivity was incorporated into brain and placenta. The incorporation into fetal lipids was several-fold greater than into maternal lipids. The labelling of the individual phospholipids was similar in the different tissues with phosphatidylcholine accounting for more than 50%. 75% of the radioactivity in brown adipose tissue was in the triacylglycerol fraction.

LIPOLYSIS AND ADENOSINE 3′:5′-MONOPHOSPHATE METABOLISM IN ISOLATED WHITE FAT CELLS FROM GENETICALLY OBESE-HYPERGLYCEMIC MICE (OB/OB). R.E. Shepherd, C.C. Malbon, C.J. Smith, and J.N. Fain (Sec. of Physiol. Chem., Div. of Biol. and Med., Brown Univ., Providence, R.I.). *J. Biol. Chem.* **252**, 7243–8 (1977). Lipolysis and cyclic adenosine 3′:5′-monophosphate (cyclic AMP) accumulation were measured in isolated fat cells prepared from obese hyperglycemic mice (ob/ob) and lean littermates at 5 months of age. Fat cells from the ob/ob mice displayed near normal lipolysis even though the maximal cyclic AMP levels of these fat cells were significantly lower than the fat cells from lean littermates. Basal triglyceride lipase activity and activation by cyclic AMP were similar in the ob/ob and the lean mice. Adenylate cyclase in ghosts prepared from ob/ob mice fat cells was minimally responsive to norepinephrine. However, ghosts prepared from fat cells of lean or ob/ob mice were equally responsive to fluoride or 10 μM guanyl-5′-yl imidodiphosphate. Norepinephrine stimulation of adenylate cyclase was not potentiated by guanyl-5′-yl imidodiphosphate in fat cell ghosts from ob/ob mice. Total cyclic AMP phosphodiesterase activity measured at 0.125 and 1.025 μM cyclic AMP was more than 50% higher per cell in the fat cells obtained from ob/ob mice as compared to their lean littermates. These data indicate that although cyclic AMP accumulation by isolated fat cells in response to norepinephrine is markedly lower in ob/ob animals, lipolysis and triglyceride lipase activation were essentially unaltered.

LIPID PEROXIDATION AND ITS INHIBITION BY TINORIDINE. I. LIPID PEROXIDATION-INDUCED DISINTEGRATION OF MICROSOMAL MEMBRANE AND CYTOCHROME P-450 IN RAT LIVER. O. Shimada and H. Yasuda (Res. Lab., Yoshitomi Pharmaceutical Industries, Ltd., Yoshitomi-cho, Chikujo-gun, Fukuoka-ken 871, Japan). *Biochim. Biophys. Acta* **489**, 163–72 (1977). Incubation of rat liver microsomes with ascorbic acid resulted in the formation of malondialdehyde, the destruction of cytochrome *P*-450 and the decrease in the turbidity of the microsomal suspension. Tinoridine (2-amino-3-ethoxy-carbonyl-6-benzyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridine), at the concentration of 5 μM, completely inhibited the peroxidative damages of microsomes. A similar inhibition by tinoridine was observed in NADPH-induced lipid peroxidation of microsomes. The anti-peroxidative activity of tinoridine was about 50 times greater than that of α-tocopherol. From the relationship between the concentration of tinoridine and the amount of malondialdehyde formed, it was demonstrated that 1 mol of tinoridine blocks the formation of about 10 mol of malondialdehyde. This suggests that lipid peroxidation proceeds as a chain reaction and that tinoridine inhibits the reaction at the initial step.

AGE-DEPENDENT MODULATION OF 3′-PHOSPHOADENOSINE-5′-PHOSPHOSULFATE-GALACTOSYLCERAMIDE SULFOTRANSFERASE BY LIPIDS EXTRACTED FROM THE MICROSOMAL MEMBRANES AND ARTIFICIAL LIPID MIXTURES. H.P. Siegrist, H. Jutzi, A.J. Steck, T. Burkart, U. Wiesmann and N. Herschkowitz (Dept. of Pediatrics, Univ. of Berne, CH-3010 Bern, Switzerland). *Biochim. Biophys. Acta* **489**, 58–63 (1977). The 3′-phosphoadenosine-5′-phosphosulfate-galactosylceramide-sulfotransferase (cerebroside sulfotransferase) is a microsomal enzyme, which shows a definite developmental activity pattern. This report gives evidence that the enzyme activity of partially delipidated microsomes is modulated by the cholesterol:phospholipid ratio of the extracted microsomal lipids in an age-dependent manner. These findings suggest that in vivo the enzyme activity is modulated by the lipid surrounding.

NON-COVALENT CROSS-LINKING OF LIPID BILAYERS BY MYELIN BASIC PROTEIN. A POSSIBLE ROLE IN MYELIN FORMATION. R. Smith (Schl. of Chem., Univ. of Sydney, Sydney, N.S.W. 2006, Australia). *Biochim. Biophys. Acta* **470**, 170–84 (1977). Myelin basic protein associates with bilayer vesicles of pure egg phosphatidylcholine, L-α-dimyristoyl phosphatidylcholine and DL-α-dipalmitoyl phosphatidylcholine. Under optimum conditions the vesicles contain 15–18% of protein by weight. The binding to dipalmitoyl phosphatidylcholine is facilitated above its gel-to-liquid crystalline transition temperature. At low ionic strength the protein provokes a large increase in vesicle size and aggregation of these enlarged vesicles. Above a sodium chloride concentration of 0.07 M vesicle fusion is far less marked but aggregation persists. The pH- and ionic strength-dependence of this aggregation follows that of the protein alone; in both cases it occurs despite appreciable electrostatic repulsion between the associating species. A similar

interaction was observed with diacyl phosphatidylserine vesicles. These observations, which contrast with earlier reports in the literature of a lack of binding of basic protein to phosphatidylcholine-containing lipids, demonstrate the ability of this protein to interact non-ionically with lipid bilayers. The strong cross-linking of lipid bilayers suggests a role for basic protein in myelin, raising the possibility that the protein is instrumental in collapsing the oligodendrocyte cell membrane and thus initiating myelin formation.

PURIFICATION, CRYSTALLIZATION AND PROPERTIES OF TRIACYLGLYCEROL LIPASE FROM PSEUDOMONAS FLUORESCENS. M. Sugiura, T. Oikawa, K. Hirano and T. Inukai (The 2nd Dept. of Pharm., Tokyo Col. of Pharm., 1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan). *Biochim. Biophys. Acta* **488**, 353-8 (1977). Triacylglycerol lipase of *Pseudomonas fluorescens* was purified from the crude enzyme by ammonium sulfate precipitation and chromatographies on Sephadex G-75 and DEAE-cellulose. The crystallization of the lipase was successfully carried out. The purified lipase was demonstrated to be homogenous on disc electrophoresis and its molecular weight was calculated to be 32,000 by gel filtration. The optimum pH for hydrolysis of sesame oil was 7.0. The enzyme was stable up to 40° C under the condition of pH 7.0 for 30 min and had more than 80% of the remaining activity between pH 5.0–11.0 at 37° C for 60 min. The lipase was strongly inhibited by iodine and partially inhibited by $FeCl_3$ and *N*-bromosuccinimide, and showed the most activity on tricaproylglycerol, among the triacylglycerols used.

REDUCTION OF BLOOD AND LIVER CHOLESTEROL IN THE RAT BY STRAIGHT AND BRANCHED CHAIN ALKYL AMINES. J.A. Svoboda, T.R. Wrenn, M.J. Thompson, J.R. Weyant, D.L. Wood, and J. Bitman (Insect Physiology Lab. and Nutrient Utilization Lab., ARS, USDA, Beltsville, MD). *Lipids* **12**, 691–7 (1977). The activities of a branched chain and several straight chain amines (C_{12} to C_{18} chain length), and the azasteroid 25-aza-5α-cholestane were compared with those of 20,25-diazacholesterol dihydrochloride, which is a potent hypocholesterolemic agent in the rat. These amines and azasteroids inhibit the Δ^{24}-sterol reductase system in the tobacco hornworm, *Manduca sexta* (L.), and also block the conversion of C_{28} and C_{29} plant sterols to cholesterol, with a resulting accumulation of desmosterol. The effects of these compounds in the rat were determined on body weight gain, cholesterol, desmosterol, and lipid composition of blood, feces, liver, and epididymal fat pad weight. Our results demonstrate that the simple azasteroid, 25-aza-5α-cholestane, is a more potent inhibitor of cholesterol biosynthesis than the diazasterol and that the Δ^{24}-sterol reductase system in a mammal can be inhibited by simple, non-steroidal, acyclic amines.

CHARACTERIZATION OF THE LIPOPROTEINS OF ATHEROSCLEROTIC SWINE. A.R. Tall, D. Atkinson, D.M. Small, and R.W. Mahley (Biophys. Sec., Boston Univ. Schl. of Med., Boston, Mass.). *J. Biol. Chem.* **252**, 7288–93 (1977). Miniature swine fed high cholesterol, saturated fat diets develop hypercholesterolemia and accelerated atherosclerosis. Their lipoprotein pattern includes increased levels of β-migrating low density lipoprotein (LDL) and the appearance of HDL_c, a cholesterol ester-rich lipoprotein of α_2 mobility. To characterize the physical state of the cholesterol esters transported by the plasma lipoproteins of the hypercholesterolemic swine, we have examined their LDL, HDL_c, and high density lipoprotein (HDL_2) by differential scanning calorimetry, X-ray diffraction, polarizing microscopy, and negative-stain electron microscopy. Differential scanning calorimetry of HDL_c showed a reversible transition between 25 and 45°, corresponding to the smectic-cholesteric transition of isolated HDL_c cholesterol esters. X-ray scattering of HDL_c indicated a spherical particle in which the core cholesterol ester is smectic-like (layered) below the transition and more disordered above. Between 30 and 50° hypercholesterolemic swine LDL also showed a reversible transition of this cholesterol esters from a smectic to a more disordered state. Hypercholesterolemic swine HDL_2 showed no transition of its cholesterol esters. Hypercholesterolemic swine LDL ($\sim$ 240 Å diameter) was larger the HDL_c (150 to 190 Å), which was larger than HDL_2 (75 to 110 Å). Thus, HDL_c represents the smallest population of lipoprotein particles capable of having a smectic-like arrangement of core cholesterol esters.

YEAST MUTANTS BLOCKED IN REMOVING THE METHYL GROUP OF LANOSTEROL AT C-14. SEPARATION OF STEROLS BY HIGH-PRESSURE LIQUID CHROMATOGRAPHY. P.J. Trocha, S.J. Jasne and D.B. Sprinson (Dept. of Biochem., Columbia Univ., Col. of Physicians and Surgeons, New York, NY). *Biochemistry* **16**, 4721–6 (1977). Sterols of a nystatin resistant mutant and of the wild type parent of *Saccharomyces cerevisiae* were separated by a newly developed procedure involving high-pressure liquid chromatography and were identified. The mutant contained larger amounts of squalene and lanosterol (I) than the wild type, as well as 4,14-dimethylcholesta-8,24-dien-3β-ol (II), 4,14-dimethylergosta-8,24(28)-dien-3β-ol (III), and 14-methylergosta-8,24-(28)-dien-3β-ol (IV), which were not hitherto found in yeast. These results indicated a block in removal of the methyl group at C-14 of lanosterol. An ergosterol requiring derivative of the mutant which carried in addition a mutation in heme biosynthesis had the same sterols as the parent, but at one-third the concentration. The low level of sterols may be due to a requirement for a heme or cytochrome in oxygenation reactions between lanosterol and ergosterol.

PHOSPHOLIPID COMPOSITION OF LIVER HOMOGENATES AND MICROSOMES OF THE LEAN AND OBESE HYPERGLYCEMIC (OB/OB) MOUSE. P.T. Varandani, R.M. Darrow, and M.A. Nafz (Fels Res. Inst. and Biol. Chem. Program, Wright State Univ. Schl. of Med., Yellow Springs, Ohio). *Proc. Soc. Exp. Biol. Med.* **156**, 123–6 (1977). The phospholipid composition of livers obtained from obese-hyperglycemic (OB/OB) male mice and from their lean mates was compared. In unfractionated liver homogenates, the content (percentage of the total lipid phosphorus) of phosphatidylcholine, phosphatidylinositol, lysophosphatidylcholine, phosphatidic acid, phosphatidylethanolamine, and cardiolipin was similar in obese and lean mice; the content of spingomyelin was 38% lower ($P \leqslant 0.01$) in the obese liver than in the lean liver. In the microsomal fractions, the obese mouse liver contained a 20% lower content of phosphatidylethanolamine ($P \leqslant 0.001$) and a 40% higher content of phosphatidic acid ($P \leqslant 0.005$) than the lean mouse liver; the content of the remaining four phospholipids was similar in the two groups. The total phospholipid (micrograms per milligram of protein) concentrations in lean and obese livers were 127 and 124 for unfractionated homogenates (without statistical significance) and 422 and 373 for the microsomal fraction ($P < 0.005$), respectively. The possibility is discussed that the alterations in the phospholipid composition of obese liver microsomes might be partly responsible for the greater amount of a latent form of the insulin-degrading enzyme, glutathione-insulin transhydrogenase, in the obese liver microsomes.

RESPONSES OF ESSENTIAL FATTY ACID-DEFICIENT RATS TO FASTING-REFEEDING AND PARTIAL HEPATECTOMY. M.A. Williams, N. Waldeck, M.A. Ojakian, I. Hincenbergs and K.T. Tamai (Dept. of Nutr. Sci., Univ. of California, Berkeley, CA). *Proc. Soc. Exp. Biol. Med.* **155**, 482–6 (1977). Essential fatty acid-deficient rats were subjected to fasting-refeeding or partial hepatectomy to stimulate liver phospholipid and membrane formation under conditions of limited essential fatty acid (EFA) supply. These results indicate that: little of the arachidonate in EFA-depleted liver is used for lipoprotein formation and transport after fasting-refeeding, whereas eicosatrienoate is available; fasting-refeeding of EFA-deficient rats does not increase activities of liver glucose-6-phosphate dehydrogenase and fatty acid synthetase above the already elevated levels in EFA-deficient rats fed *ad libitum;* considerable new liver tissue can be formed after partial hepatectomy even when EFA levels in phospholipids are very low.

ENZYME AFFINITY IN THE ACYLATION OF LYSOPHOSPHATIDYLCHOLINE. B. Wittels and S. Hurlbert (Dept. of Pathol., Duke Univ. Med. Ctr., Durham, N.C.). *Biochim. Biophys. Acta* **489**, 72–8 (1977). Analogues of 1-palmitoyl-*sn*-glycero-3-phosphorylcholine were used to ascertain the respective roles of the 1-palmitoyl-*sn*-glycero and choline groups in binding this substrate to the transferase catalyzing the acylation reaction. 1-Palmitoyl-*sn*-glycero-3-phosphorylhomocholine proved to be an effective competitive inhibitor whereas 1-palmitoyl-2-deoxyglycero-3-phosphorylcholine was totally ineffective. The data support the view that the 1-palmitoyl-*sn*-glycero group plays the major role in determining enzyme affinity whereas the choline group functions primarily in the subsequent steps of the acylation reaction.

NEUTRAL LIPID BIOSYNTHESIS IN MYCOBACTERIUM SMEGMATIS. C-K. Wun, H.A. Barakat and R.W. Walker (Dept. of Environ. Sci., Univ. of Massachusetts, Amherst, MA). *Biochim.*

Biophys. Acta 488, 454–63 (1977). The biosynthesis of neutral lipids in *Mycobacterium smegmatis* was studied using cell-free extracts. Maximum neutral lipid production was obtained when the reaction mixture (400 µl) consisted of 0.25 M potassium phosphate buffer (pH 7.5), 0.125 mM oleoyl-CoA, 3.75 mM *sn*-glycerol-3-P, 10 mM $MgCl_2$ and 1.85 mg bovine serum albumin. No magnesium dependency for the acylation of *sn*-glycerol-3-P was observed. A slight stabilizing effect seemed to occur due to this ion. The enzyme phosphatidate phosphohydrolase, on the other hand, was shown to be magnesium dependent. The activity of this enzyme also appeared to be stimulated by high concentration (0.75 to 1.25 mM) of ATP which enhanced lipid formation at all concentrations tested (0.25 to 3.75 mM). A heat-stable protective factor having a molecular weight less than 16,000 which caused a stimulatory effect on *sn*-glycerol 3-phosphate acyltransferase activity was found in the cell-free extracts. Preliminary experiments suggest that the factor might be polysaccharide in nature.

PHOSPHOLIPID HEAD-GROUP CONFORMATIONS; INTERMOLECULAR INTERACTIONS AND CHOLESTEROL EFFECTS. P.L. Yeagle, W.C. Hutton, C.-h. Huang, and R.B. Martin (Dept. of Chem. and Biochem., Univ. of Virginia, Charlottesville, Va.). *Biochemistry* 16, 4344–9 (1977). The predominant orientation of the phosphorylcholine polar head group in phosphatidylcholine and sphingomyelin bilayers and cholesterol perturbations of the orientation have been identified by exploiting the ^{31}P ^{1}H nuclear Overhauser effect (NOE) in the ^{31}P NMR spectra of phospholipid bilayers. In pure egg phosphatidylcholine bilayers, a NOE of 40% is observed. The magnitude of the NOE has been measured as a function of continuous-wave proton-decoupler frequency in order to identify the proton source of the NOE. In pure egg phosphatidylcholine bilayers, the maximum NOE occurs at the N-methyl proton resonance position of the choline moiety. An analogous result was obtained with pure sphingomyelin. These results are explained by orienting the phosphorylcholine portion of the molecule parallel to the surface of the bilayer so that the positively charged N-methyl moiety is located close to the negatively charged phosphate on a neighboring phospholipid in an intermolecular interaction. Addition of cholesterol is shown to disrupt the intermolecular interaction in phosphatidylcholine bilayers.

REGULATION OF FATTY ACID SYNTHETASE IN PERINATAL CHICKS. IDENTIFICATION OF POLYSOMES SYNTHESIZING FATTY ACID SYNTHETASE. Z.E. Zehner, V.C. Joshi, and S.J. Wakil (Marrs McLean Dept. of Biochem., Baylor College of Med., Houston, Tx.). *J. Biol. Chem.* 252, 7015–22 (1977). Hepatic fatty acid synthetase activity in the developing chick is low in the embryo and increases 100-fold upon hatching and feeding by Day 3. Quantitative precipitin reactions show that the increase in fatty acid synthetase activity is due to an increase in the amount of enzyme. By immunochemical techniques the relative rate of synthetase synthesis increases 35-fold paralleling the increase in enzyme activity. Measurement of the turnover of fatty acid synthetase in 3- and 10-day-old chicks gave identical half-life ($t_{1/2}$) values of 33 h. These results indicate that the increase in synthetase activity upon hatching and development is related to an increase in synthesis of the enzyme at the level of translation.

ABOUT SOME METHODOLOGICAL APPROACHES FOR THE DETERMINATION OF BIOLOGICAL VALUE OF PROTEINS. M.P. Tchernikov et al. *Vopr. Pitan.* 1976(6), 8–14. The aminogramme of the free amino acids in the blood of portal vein of rats has a marked tendency toward a comparison with the aminogramme of the fed protein. It can be assumed that this comparison could be even greater if these experiments had been done on specially prepared animals. For this purpose, the rats must be maintained on a diet with a reduced content of protein, in order to decrease the catabolism of the amino acids. The data about the amino acid content of portal vein blood allows the judging of the speed of two first stages of assimilation of protein and especially of the speed of proteolysis of edible proteins in the gastro-intestinal tract and of the speed of resorption of amino acids. (Rev. Fr. Corps Gras)

INFLUENCE OF A REDUCED DIET ON HYPERLIPOPROTEINEMIA FROM METABOLIC OBESITY. I.A. Frolova et al. *Vopr. Pitan.* 1976(5), 39–43. Among the patients of an obesity of metabolic origin, one finds a hyperlipoproteinemia essential of the type IIb and less frequently of the types IIa and IV, the type III rarely being encountered. After a diet reduced in calories by limitation of carbohydrates and lipids, one observes, beside a reduction of weight, a decrease of the content of the atherogenic fractions of lipoproteins in the serum and a drop of the frequency of types IIb, III, and IV of hyperlipoproteinemia. The most effective action of a reduced diet on the lipoprotein composition of the blood serum was observed among the young patients. (Rev. Fr. Corps Gras) ●