

# Abstracts

EDITOR: F.A. Kummerow

ABSTRACTORS: J.C. Harris, M.A. Kokatnur, F.A. Kummerow, G. List, B. Matijasevic, R.A. Reiners, and P.Y. Vigneron

## Biochemistry and nutrition

**STUDIES ON THE ACTIVITY OF ACYL-CoA:CHOLESTEROL o-ACYLTRANSFERASE AND ACID CHOLESTEROL ESTER SYNTHETASE IN RAT AORTAS.** D.L. Severson and T. Fletcher (Dept. of Pharmacol. and Therapeutics, Fac. of Med., Univ. of Calgary, Calgary, Alberta, Canada) *Biochim. Biophys. Acta* 664: 475-486 (1981). Acyl-CoA: cholesterol o-acyltransferase (ACAT) activity was measured in microsomal preparations from rat aorta (intima-media) with (<sup>14</sup>C)oleoyl CoA and endogenous cholesterol as substrates. The specific activity of ACAT in liver and adrenal microsomal preparations was 10-20-times greater than ACAT activity in aortic microsomes; no ACAT activity could be detected in fat pad microsomes. ACAT activity in liver and adrenal microsomes was enhanced by the addition of exogenous cholesterol. In contrast, exogenous cholesterol did not increase ACAT activity in rat aortic microsomes. Levels of endogenous cholesterol and ACAT activity in microsomal preparations from rat aorta were not reduced when circulating plasma cholesterol levels were decreased by the administration of 4-aminopyrazolopyrimidine to rats. Acid cholesterol ester synthetase activity was not detectable in high-speed supernatant fractions from rat aorta; low levels of activity could be measured in rat aorta microsomal preparations but this was less than 10% of ACAT activity. Thus, ACAT would seem to be the principal enzymatic route for the synthesis of cholesterol esters in aorta.

**DEVELOPMENT OF THE CAPACITY OF MOUSE MAMMARY GLANDS FOR MEDIUM CHAIN FATTY ACID SYNTHESIS DURING PREGNANCY AND LACTATION.** S. Smith and A. Stern (Bruce Lyon Memorial Res. Lab., Children's Hosp. Med. Center, 51st and Grove St., Oakland, CA) *Biochim. Biophys. Acta* 664: 611-615 (1981). The time-course for appearance of fatty acid synthetase and thioesterase II, enzymes required for the synthesis of medium chain (C<sub>8</sub>-C<sub>12</sub>) fatty acids by the mammary gland, has been studied in the mouse and compared with that in the rat. The development of high levels of fatty acid synthetase in the mouse mammary gland during the early days of lactation coincided with an observed increase in the overall lipogenic capacity of the gland, assessed by measuring in incorporation of radioactive acetate into fat in tissue slices. Both the level of thioesterase II in the gland and the proportion of medium chain fatty acids synthesized from acetate by tissue slices began to increase in late gestation but did not reach a maximum until lactation was well established. In this regard the mouse is distinctly different from the rat, which establishes maximum levels of thioesterase II in the mammary gland prior to parturition. The significance of this difference as it relates to mammary gland development is discussed.

**EFFECT OF VITAMIN A DEFICIENCY ON LUTEINIZING HORMONE RECEPTORS AND ADENOSINE 3',5'-MONOPHOSPHATE-MEDIATED STEROIDOGENESIS IN RAT TESTICULAR TISSUE.** K.K. Steinberg and D.S. Sgoutas (Dept. of Path. and Lab. Med., Sch. of Med., Emory Univ., Atlanta, GA) *Pro. Soc. Exp. Bio. Med.* 167:110-116 (1981). Weanling male Sprague-Dawley rats were maintained on a vitamin A (retinol)-deficient retinoic acid supplemented diet for a period of 8 to 14 weeks. After 10 weeks, Leydig cell-enriched preparations from vitamin A-deficient animals had 45% fewer gonadotropic receptors for human luteinizing hormone (hLH). Testicular slices from vitamin A-deficient animals demonstrated decreased formation of adenosine 3',5'-monophosphate (cyclic AMP) and decreased testosterone content in response to hLH stimulation compared to vitamin A-supplemented rats (P<0.01). Our findings suggest that changes which cause hyporesponsiveness in testosterone production from vitamin A-deficient rats can be attributed to a reduction in gonadotropin testicular receptors for hLH and a decreased cyclic AMP production. Testicular morphology was not altered during the first 10 weeks of the experiment, although, after 14 weeks on the experimental diet there was marked degeneration of tubules, cessation of spermatogenesis, and testicular atrophy.

**ANIMAL FATTY ACID SYNTHETASE, A NOVEL ARRANGEMENT OF THE β-KETOACYL SYNTHETASE SITES COMPRISING DOMAINS OF THE TWO SUBUNITS.** J.K. Stoops and S.J. Wakil (Marrs-McLean Dept. of Biochem., Baylor Col. of Med., Houston, TX) *J. Bio. Chem.* 256(10):5128-5133 (1981). The fatty

acid synthetase of animal tissues is a homodimer with a  $M_r = 480,000$ . Active enzyme centrifugation studies showed that the dimer form of the enzyme is active in palmitate synthesis while the monomer is not since it lacks β-ketoacyl synthetase activity. Evidence presented in this paper shows that β-ketoacyl synthetase activity requires the juxtapositioning of two thiol groups at the active center, with each subunit contributing one of these thiols. The latter have tentatively been identified as the active cysteine-thiol of the β-ketoacyl synthetase of one subunit and the cysteine-thiol of the acyl carrier protein site of the other subunit.

**SERUM LIPOPROTEINS MODULATE OXYGENATED STEROL INSERTION INTO HUMAN RED CELL MEMBRANES.** R.A. Streuli, J. Chung, A.M. Scanu, S. Yachnin (Sect. of Hematol./Oncology, Dept. of Med., Univ. of Chicago, Chicago, IL) *Science* 212:1294-1296 (1981). The insertion of oxygenated sterol compounds into human red blood cell membranes as well as the consequent transformation of the red cells to an echinocyte shape and the expansion of the membrane are impeded by the presence of serum lipoproteins in the incubation medium. All density classes of human serum lipoproteins bind oxygenated sterol compounds, and lipoproteins can act as acceptors of oxygenated sterols previously inserted into red cells. Since oxygenated sterols have been reported to be atherogenic, the modulating and possibly protective effects of serum lipoproteins on oxygenated sterol-induced derangement of cell membrane structure and function may provide a useful model for further study.

**ISOLATION AND IDENTIFICATION OF 23,25-DIHYDROXY-VITAMIN D<sub>3</sub>, AN IN VIVO METABOLITE OF VITAMIN D<sub>3</sub>.** Y. Tanaka, J.K. Wichmann, H.K. Schnoes and H.F. DeLuca (Dept. of Biochemistry, College of Agricultural and Life Sciences, Univ. of Wisconsin-Madison, Madison, WI 53706) *Biochemistry* 20(13): 3875-3879 (1981). Vitamin D supplemented rats produce a metabolite of 25-hydroxy-[3α-<sup>3</sup>H]vitamin D<sub>3</sub> that is easily separated from known metabolites by using high-performance liquid chromatography. The production of this metabolite in vivo as well as 1,25-dihydroxyvitamin D<sub>3</sub>, 24(R),25-dihydroxyvitamin D<sub>3</sub>, and 25-hydroxyvitamin D<sub>3</sub> 26,23-lactone is largely if not totally eliminated by nephrectomy. Kidney homogenates from vitamin D supplemented chickens incubated with 25-hydroxyvitamin D<sub>3</sub> produce significant quantities of the new, unknown metabolite. This metabolite was isolated in pure form from such incubation mixtures by using both straight phase and reversed-phase high-performance liquid chromatography. This metabolite has been positively identified as 23,25-dihydroxyvitamin D<sub>3</sub> by ultraviolet absorption spectrophotometry, mass spectrometry, and derivatization. This structure was confirmed by chemical synthesis of both C-23 stereoisomers. Although the natural product exactly comigrates with one of the synthetic isomers, the exact stereochemistry of the natural product remains unknown. It is possible that this new metabolite is an intermediate in the biosynthesis of 25-hydroxyvitamin D<sub>3</sub> 26,23-lactone.

**FATTY ACID SYNTHESIS IN MOUSE BROWN ADIPOSE TISSUE, THE INFLUENCE OF ENVIRONMENTAL TEMPERATURE ON THE PROPORTION OF WHOLE-BODY FATTY ACID SYNTHESIS IN BROWN ADIPOSE TISSUE AND THE LIVER.** P. Trayhurn (Dunn Nutrition Laboratory, University of Cambridge and Medical Research Council, Milton Road, Cambridge CB4 1XJ, U.K.) *Biochim. Biophys. Acta* 664(3):549-560 (1981). Fatty acid synthesis has been measured in vivo with <sup>3</sup>H<sub>2</sub>O in mice acclimated at different environmental temperatures and the importance of brown adipose tissue and the liver to whole-body fatty acid synthesis at each temperature assessed. At 33 C, when non-shivering thermogenesis is minimal, the rate of fatty acid synthesis in interscapular brown adipose tissue was lower than in the liver, but higher than in white adipose tissue and the carcass. At 4 C, when non-shivering thermogenesis is maximal, the fatty acid synthesis rate in interscapular brown adipose tissue was many times greater than in any other tissue. In mice maintained at 22 C the rate of fatty acid synthesis was also higher in brown adipose tissue than in other tissues. Overall, the relative importance of brown adipose tissue as a site of fatty acid synthesis increased with lower environmental

temperatures, while that of the liver decreased. An estimate of the contribution that de novo synthesis makes to total fatty acid utilization by interscapular brown adipose tissue suggests that fatty acid synthesis and breakdown constitutes a significant heat-dissipating 'cycle' in brown adipose tissue of cold-acclimated mice. Such a cycle is not evident in suckling animals since fatty acid synthesis in brown adipose tissue is very low during early development.

**FETAL RAT LUNG PHOSPHATIDYLCHOLINE SYNTHESIS IN DIABETIC AND NORMAL PREGNANCIES: A COMPARISON OF PRENATAL DEXAMETHASONE TREATMENTS.** M.Y. Tsai, M.W. Josephson and D.M. Brown (Departments of Laboratory Medicine and Pathology and Pediatrics, University of Minnesota Medical School, Minneapolis, MN 55455) *Biochim. Biophys. Acta* 664(1): 174-181 (1981). The effects of maternal diabetes upon fetal lung surfactant phospholipid metabolism were studied using 19-day gestation age fetal rats from mothers with streptozotocin-induced diabetes mellitus. In this experimental animal model, maternal glucose intolerance significantly impaired fetal body and lung development. However, incorporation of [<sup>14</sup>C]palmitate and [<sup>3</sup>H]choline into lung total and disaturated phosphatidylcholine was unimpaired in offspring of diabetic mothers. Dexamethasone, which is known to promote fetal lung maturation in normal pregnancies, was administered to diabetic and control mothers during late gestation. Prenatal dexamethasone inhibited lung growth in both diabetic and control pregnancies. While this agent slightly stimulated [<sup>14</sup>C]palmitate incorporation into total phosphatidylcholine and markedly enhanced [<sup>3</sup>H]choline incorporation into both disaturated and total phosphatidylcholine in control pregnancies, it failed to stimulate incorporation of either precursor into fetal lung from diabetic pregnancies.

**LYMPHATIC ABSORPTION OF SHELLFISH STEROLS AND THEIR EFFECTS ON CHOLESTEROL ABSORPTION.** G.V. Vahouny, W.E. Connor, T. Roy, D.S. Lin and L.L. Gallo (Department of Biochemistry, George Washington Univ., School of Med. and Health Sciences, 2300 Eye Street, N.W., Washington, DC 20037) *Am. J. Clin. Nutr.* 34(4):507-513 (1981). Studies have been conducted on the absorbability of individual sterols from a mixture of oyster sterols when administered intragastrically to rats with indwelling catheters in the left thoracic duct. In addition, the effect of oyster sterols on cholesterol absorption has been assessed using [<sup>14</sup>C] cholesterol in the mixture, and comparison against absorption of cholesterol alone. The order of absorbability (percentage absorption) of individual sterols from the mixture of oyster sterols was: cholesterol > 26-carbon sterols > dehydrocholesterol > 24-methylene cholesterol > brassicasterol > plant sterols. The absorption of noncholesterol sterols was 8.2 ± 0.8% of the fed dose, or less than half of that for an equivalent level of cholesterol alone. The presence of these sterols in mixtures containing cholesterol reduced lymphatic absorption of cholesterol by 25 to 40% compared to absorption of the same amount of cholesterol administered alone, or to an amount of cholesterol equal to the total oyster sterols, respectively. These studies suggest that shellfish sterols are poorly absorbed, and, like plant sterols, effectively reduce dietary and/or endogenous cholesterol absorption from the intestine.

**INFLUENCE OF SEPTIC SHOCK UPON PHOSPHATIDYLCHOLINE REMODELING MECHANISM IN RAT LUNG.** P. Von Wichert, M. Temmesfeld and W. Meyer (I. Medizinische Univ., Martinstrasse, Hamburg, Germany) *Biochim. Biophys. Acta* 664:487-497 (1981). Septic shock in rats leads to pulmonary disorders associated with alterations of phospholipid metabolism. The ratio between phosphatidylcholine and lysophosphatidylcholine is lowered both in lung tissue and in pulmonary surfactant because enzymes of phosphatidylcholine remodeling mechanism are distinctly affected by septic shock. Specific activity of phospholipase A<sub>2</sub> in enhanced 5-fold while specific activities of lysolecithin acyltransferase and lysolecithin:lysolecithin acyltransferase are only slightly increased or remain unchanged. Beyond that, palmitic acid content of lung tissue phosphatidylcholine is significantly reduced and replaced mainly by arachidonic acid. The release of this fatty acid by action of phospholipase A<sub>2</sub> may lead via intermediates to the generation of potent mediators such as prostaglandins, thromboxane or slow-reacting substance.

**DISTRIBUTION OF LIPOPROTEIN TRIGLYCERIDE AND LIPOPROTEIN CHOLESTEROL IN AN ADULT POPULATION BY AGE, SEX, AND HORMONE USE.** R.W. Wahl, G.R. Warnick, J.J. Albers, J.J. Hoover, C.E. Walden, R.O. Bergelin, J.T. Ogilvie, W.R. Hazzard, and R.H. Knopp (Northwest Lipid 39:111-124 Res. Clinic Dept. of Med., School of Med. Univ. Washington, Seattle Wash. *Atherosclerosis* (1981). This report describes the distribution of lipoprotein triglyceride and lipoprotein cholesterol in employees of the Pacific Northwest Bell Telephone Company. Means, medians and selected percentiles are presented for very low, low and high density lipoproteins (VLDL, LDL and HDL, respectively) in 606

randomly selected white subjects aged 20-59. Women who use sex hormones have significantly higher concentrations of triglycerides in all of the fractions across all age decades from 20 to 59 than do women not taking hormones. Men have the highest average VLDL triglyceride value but their average triglyceride concentrations in the LDL and HDL fractions approximate those of women not taking hormones. This study in a well-defined population provides reference standards for lipoprotein triglyceride concentrations. These results can be used to evaluate the effect of sex hormone treatment on the lipoprotein triglyceride content as a potential risk factor in men and older women.

**LIPIDS AND LIPOPROTEINS IN HYPERLIPIDEMIA TYPE IIa DURING TREATMENT WITH DIFFERENT LIPID LOWERING DRUGS.** J.G. Wechsler, V. Hutt, H.-U. Klör and H. Ditschuneit (Dept. of Med., Div. of Metab., Nutr. and Gastro., Univ. of Ulm, West-Germany) *Artery* 8(6):519-529 (1980). In the present study we examined the effect of different lipid lowering drugs on lipids and lipoproteins in hyperlipoproteinemia type IIa. We have treated over 24 weeks 10 patients with 900 mg β-pyridylcarbinol daily, 11 patients with 3 g of xantinolnicotinate, 4 patients with 600 mg bezafibrate daily and 10 patients with a combination of 2.4 g inositolnicotinate and 1.5 g colfibrate daily. One patient with familial hypercholesterolemia (untreated total cholesterol 800 mg%) received a combined drug treatment during 5 years. Total cholesterol decreased to 200 mg%, mainly due to decrease in LDL-cholesterol. However in HDL significant decreases of about 50% could be observed. Treatment with the four different drugs showed significant decrements in low density lipoproteins (LDL) whereas an increase of protective high density lipoproteins could not be observed.

**LACK OF EFFECT OF SOMATOSTATIN ON THE GLUCAGON-INDUCED ALTERATIONS OF HEPATIC METABOLISM OF (1-<sup>14</sup>C) OLEATE.** I. Weinstein, I. Wasfi and M. Heimberg (Dept. of Pharm., Univ. of Missouri, Sch. of Med., Columbia, MO) *Biochim. Biophys. Acta* 664(1):124-132 (1981). Livers from normal fed male rats were perfused in a recycling system in vitro. Glucagon was infused in varying quantities to give final concentration in the cell-free perfusate of 4.9 · 10<sup>-10</sup> - 4.9 · 10<sup>-7</sup> M after 3 h of perfusion, assuming no degradation of the hormone. Where indicated, cyclic somatostatin was infused simultaneously to give a final concentration of 3.0 · 10<sup>-6</sup> M. In the absence of somatostatin, glucagon at a concentration as low as 4.9 · 10<sup>-10</sup> M increased the release of glucose and increased ketogenesis, but impaired the synthesis and release of perfusate triacylglycerol and very low density lipoprotein lipids. Somatostatin did not affect these actions of glucagon. Somatostatin alone, however, did reduce the output of very low density lipoprotein. It is suggested that the alteration of fatty acid metabolism by somatostatin in vivo results from modulation of pancreatic glucagon secretion, not from interference by somatostatin of the action of glucagon on the liver.

**PLASMA APOLIPOPROTEIN B AND VLDL-, LDL, AND HDL-CHOLESTEROL AS RISK FACTORS IN THE DEVELOPMENT OF CORONARY ARTERY DISEASE IN MALE PATIENTS EXAMINED BY ANGIOGRAPHY.** T.F. Whyne, P. Alaupovic, M.D. Curry, E.T. Lee, P.S. Anderson and E. Schechter (Lab. of Lipid and Lipoprotein Studies, Oklahoma Med. Res. Found., Depts. of Med., Biochem. and Mol. Bio., and Biostat. and Epidem., Univ. of Oklahoma Health Sic. Center, Oklahoma City, OK) *Atherosclerosis* 39:411-424 (1981). To assess the potential use of plasma apolipoprotein B (ApoB) as a risk factor for coronary artery disease, this apolipoprotein was quantified by electroimmunoassay in 161 male patients with angiographically documented coronary atherosclerosis and 72 male patients with normal coronary arteries. In addition to ApoB, the analyzed lipoprotein profile included plasma total cholesterol, plasma triglyceride and VLDL-, LDL-, and HDL-cholesterol levels. Results of this study indicate that measurement of ApoB may offer important predictive value for coronary artery disease, especially at lower levels of plasma cholesterol. Whether this and other conclusions also apply to general population, remains to be established in further studies.

**SERUM LIPIDS AND LIPOPROTEINS IN HEALTHY CHILDREN 11 TO 14 YEARS OF AGE: AGE DEPENDENCY AND TRACKING.** K. Widhalm, W. Strobl and G. Westphal (Dept. of Ped. and Insti. for Med., Stat., Univ. of Vienna, Med. Sch., Vienna, Austria) *Artery* 8(6):530-536 (1980). In order to determine if tracking of serum lipids and lipoproteins occurs in childhood, a 4 year longitudinal study in 109 children (54 boys 55 girls) aged 11 to 14 years was carried out. All children were apparently healthy as indicated by physical examination and a routine clinical chemistry profile. Venous blood was drawn in May 1976, 77, 78 and 79 after an overnight fast. Cholesterol (C) and triglycerides (TG) were determined using full enzymatic methods. HDL-C and LDL-C were estimated by means of the LRC-Method, NIH. Our data strongly

suggest that C and LDL-C track well during the period between 11 and 14 years of age in boys and in girls. The decrement of lipids and lipoproteins during this age is interpreted as a physiological, dynamic pattern.

**CALCIUM/MAGNESIUM SPECIFICITY IN MEMBRANE FUSION: KINETICS OF AGGREGATION AND FUSION OF PHOSPHATIDYL SERINE VESICLES AND THE ROLE OF BILAYER CURVATURE.** J. Wilschut, N. Duzgunes, and D. Papahadjopoulos (Cancer Res. Inst. and Dept. of Pharm., Sch. of Med., Univ. of Calif., San Francisco, CA) *Biochemistry* 20:312603133 (1981). We have investigated the relative abilities of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  to induce the fusion of phospholipid vesicles composed of pure bovine brain phosphatidylserine (PS). Vesicle aggregation was monitored by light-scattering measurements, fusion by the terbium/dipicolinic acid assay for mixing of internal vesicle volumes, release of vesicles contents by carboxyfluorescein fluorescence, and changes in vesicle size by freeze-fracture electron microscopy. Either small unilamellar vesicles prepared by sonication, or large unilamellar vesicles, prepared by reverse-phase evaporation and extrusion through a polycarbonate membrane, were used. The results are discussed in terms of the degree of dehydration and the change in lipid fluidity induced by interaction of the divalent cations with the vesicles. Crucial to the specificity of  $\text{Ca}^{2+}$  in inducing the fusion of PS vesicles seems to be its ability to form an anhydrous complex between apposed bilayers.

**ROLE OF MATRIX PROTEIN IN ASSEMBLING THE MEMBRANE OF VESICULAR STOMATITIS VIRUS: RECONSTITUTION OF MATRIX PROTEIN WITH NEGATIVELY CHARGED PHOSPHOLIPID VESICLES.** J.J. Zakowski, W.A. Petri, Jr., and R.R. Wagner (Dept. of Microbiology, the University of Virginia School of Medicine, Charlottesville, VA 22908) *Biochemistry* 20(10):3902-3907 (1981). The matrix (M) protein of vesicular stomatitis virus (VSV) was reconstituted into phospholipid vesicles by detergent dialysis. Reconstitution of the positively charged M protein occurred only in the presence of negatively charged phospholipids, such as phosphatidylserine, phosphatidic acid, or phosphatidylinositol. Preformed vesicles containing negatively charged phospholipids also bound free M protein. Derivatization of the positively charged lysines in M protein with acetic anhydride or succinic anhydride prevented M protein reconstitution but did not affect the biological property of M protein to inhibit in vitro VSV transcription. An additional indication of the electrostatic nature of the M protein binding to the vesicles was that M protein could not be reconstituted in the presence of 0.5 M NaCl. Nonelectrostatic forces also appear to be involved in the association of the M protein with vesicles, since previously reconstituted M protein remained associated with the vesicles upon subsequent exposure to 0.5 M NaCl.

**MEMBRANE ACTIVE AGENTS. EFFECT OF VARIOUS ANESTHETICS AND CHLORPROMAZINE ON ARTERIAL LIPID METABOLISM.** F.P. Bell and E.V. Hubert (Diabetes and Atherosclerosis Res., The Upjohn Company, Kalamazoo, MI 49001) *Atherosclerosis* 39(4):517-525 (1981). The local anesthetic lidocaine was studied for its effects on lipid metabolism in aortas from normal rats, rabbits, and cholesterol-fed (atherosclerotic) rabbits in vitro. Incubation of aortas in the presence of 3-5 mM lidocaine resulted in a statistically significant reduction in the incorporation of [ $^{14}\text{C}$ ]oleate into cholesteryl esters and phosphatidylcholine. Additionally, significant increases in [ $^{14}\text{C}$ ]oleate incorporation into the diglyceride fraction of atheromatous rabbit aortas was observed with a trend to greater incorporation into the diglyceride fraction of normal rat and rabbit arteries as well. The most significant overall effect of lidocaine was its inhibition (50-90%) of the arterial sterol esterification. Assays of acylCoA:cholesterol acyltransferase (ACAT, EC 2.3.1.26) in isolated arterial microsomes revealed that, in addition to local anesthetics (e.g., lidocaine), other membrane-active agents such as chlorpromazine and methoxyflurane inhibit ACAT: this suggests ACAT may be regulated by alterations in the biphasical properties of its membrane milieu.

**PHOSPHOLIPASE A<sub>2</sub> ACTIVITY SPECIFIC FOR PHOSPHATIDIC ACID. A POSSIBLE MECHANISM FOR THE PRODUCTION OF ARACHIDONIC ACID IN PLATELETS.** M.M. Billah, E.G. Lapetina and P. Cuatrecasas (Dept. of Molecular Biol., Wellcome Res. Lab., Res. Triangle Park, NC) *J. Biol. Chem.* 256(11): 5399-5403 (1981). Activation of platelets induces the formation of phosphatidic acid, which results from the combined actions of a phosphatidylinositol-specific phospholipase C and a 1,2-diacylglycerol kinase. It has been proposed that this phosphatidic acid leads in some way to the subsequent production of arachidonic acid. We propose that this phosphatidic acid-specific phospholipase A<sub>2</sub> might play an important role in the formation of arachidonic acid in stimulated platelets.

**REGULATION OF 1,25-DIHYDROXYVITAMIN D<sub>3</sub> RECEPTORS IN CULTURED MOUSE BONE CELLS. CORRELATION OF RECEPTOR CONCENTRATION WITH THE RATE OF CELL DIVISION.** T.L. Chen and D. Feldman (Dept. of Med., Stanford Univ., Sch. of Med., Stanford, CA) *J. Biol. Chem.* 256(11):5561-5566 (1981). We have previously described a receptor for 1,25-(OH)<sub>2</sub> vitamin D<sub>3</sub> in primary cultures of mouse bone cells. We now report that the concentration of this receptor per cell is not fixed but appears to be a regulated function which varies during the culture period. These findings are important to consider in any attempt to demonstrate or quantitate 1,25-(OH)<sub>2</sub>-vitamin D<sub>3</sub> receptors in primary cultures, and may be a crucial factor in determining the level of responsiveness of cells to 1,25-(OH)<sub>2</sub> vitamin D<sub>3</sub> treatment.

**A SPECIFIC BINDING PROTEIN FOR 1 $\alpha$ ,25-DIHYDROXY-VITAMIN D IN THE CHICK EMBRYO CHORIOALLANTOIC MEMBRANE.** W.A. Coty, C.L. McConkey, and T.A. Brown (Dept. of Bio. Chem., UCLA Sch. of Med., Los Angeles, CA) *J. Biol. Chem.* 256(11):5545-5549 (1981). A 3.7 S binding protein for the steroid hormone and vitamin D metabolite 1 $\alpha$ ,25-dihydroxyvitamin D (1,25-(OH)<sub>2</sub>-D) was observed in high salt cytosol extracts of chick embryo chorioallantoic membrane. The binding protein was characterized after partial purification of cytosol extracts by ammonium sulfate fractionation. The binding of 1,25-(OH)<sub>2</sub>-D was saturable, had a high affinity ( $K_D = 0.16$  nM), and was specific for hormonally active vitamin D metabolites. Analysis of the displacement of [ $^3\text{H}$ ]1,25-(OH)<sub>2</sub>-D by unlabeled analogues showed the affinities of vitamin D metabolites to be in the order of 1,25-(OH)<sub>2</sub>-D = 1,24R, 25-(OH)<sub>3</sub>-D >> 25-OH-D = 1-OH-D > 24R, 25-(OH)<sub>2</sub>-D. Hormone binding was sensitive to pretreatment with sulfhydryl-blocking reagents.

**VESICLE-MEDIATED TRANSFER OF PHOSPHOLIPIDS TO PLASMA MEMBRANE DURING CELL AGGREGATION OF Dictyostelium discoideum.** N.S. DeSilva and C.-H. Siu (Banting and Best Dept. of Med. Res., C.H. Best Inst., Univ. of Toronto, Toronto, Ontario, Canada) *J. Biol. Chem.* 256(11):5845-5850 (1981). We have previously reported that synthesis of phospholipids increased 4-fold at the onset of chemotactic migration during development of *Dictyostelium discoideum* and that the newly synthesized phospholipids are preferentially incorporated into the plasma membranes. To test the hypothesis that the rapid transfer of phospholipids to the plasma membrane is mediated by vesicles, we isolated phospholipid-rich vesicles from cells at 6 h of development. These results indicate that transfer of newly synthesized phospholipids from their site of synthesis to the plasma membrane probably occurs through a special class of phospholipid-rich vesicles.

**GEMFIBROZIL—THE EFFECT ON BILIARY CHOLESTEROL SATURATION OF A NEW LIPID-LOWERING AGENT AND ITS COMPARISON WITH CLOFIBRATE.** M.J. Hall, L.M. Nelson, R.I. Russell and A.N. Howard (Gastroenterology Unit, Royal Infirmary Glasgow G4 OSF and Dept. of Med., Univ. of Cambridge, Cambridge, Great Britain) *Atherosclerosis* 39(4):511-516 (1981). Clofibrate is known to increase cholesterol saturation of bile and the prevalence of gallstones. We studied 10 healthy volunteer subjects to determine the effect of gemfibrozil (a new lipid-lowering agent) on biliary cholesterol saturation and to compare it with that of clofibrate. Biliary cholesterol saturation indices were calculated on fasting duodenal bile samples collected before and after administration of each drug for 4 weeks using a 4-week "washout" period between each preparation. There was a statistically significant rise in the cholesterol saturation index from a control value, taken as the mean of 2 samples, of 1.226 (0.785-1.526), median (range), to 1.547 (0.807-1.781) after clofibrate, but the rise to 1.352 (0.840-2.686) after gemfibrozil was not significant. However, direct comparison of the cholesterol saturation indices on clofibrate and gemfibrozil revealed no statistically significant difference. Only prospective clinical trials will establish definitely the risk of cholelithiasis on gemfibrozil but these results suggest that this drug is unlikely to have an advantage over clofibrate in this respect.

**LIPOPROTEIN BINDING TO CANINE HEPATIC MEMBRANES. METABOLICALLY DISTINCT APO-E AND APO-B,E RECEPTORS.** D.Y. Hui, T.L. Innerarity, and R.W. Mahley (Gladstone Found. Lab. for Cardiovascular Disease, Cardiovascular Res. Inst. and Dept. of Path., Univ. of Calif., San Francisco, CA) *J. Biol. Chem.* 256(11):5646-5655 (1981). Hepatic membranes from adult dog livers have receptors which bind to lipoproteins containing the E apoprotein (the apo-E HDL<sub>c</sub>) revealed nonlinearity of the binding which could be resolved into two components, suggesting the presence of two separate binding sites. The binding site for apo-E HDL<sub>c</sub> that possessed the highest affinity was calcium-dependent and was sensitive to proteolytic digestion with pronase. The lower affinity binding site for apo-E HDL<sub>c</sub> did not require calcium and was resistant to pronase digestion. Chemical modification of the

arginyl or lysyl residues of the apo-E HDL<sub>c</sub> prevented the HDL<sub>c</sub> from binding to the higher affinity receptor but had no effect on their binding to the lower affinity site. Adult canine liver membranes also bound canine <sup>125</sup>I-HDL. However, the binding of HDL was of lower affinity did not require calcium, was not blocked by modification of the lysyl or arginyl residues, and may not be of physiologic significance.

**LIPOPROTEIN COMPOSITION AND TRANSPORT IN THE PIG AND DOG CARDIAC LYMPHATIC SYSTEM.** P. Julien, E. Downar, and A. Angel (Dept. of Med., Faculty of Med., Univ. of Toronto, Toronto, Canada) *Circ. Res.* 49:248-254 (1981). The cardiac lymphatic system of pigs was found to consist of valved efferent lymph vessels draining into a cardiac lymph node, which is similar to the lymphatic system found in dog and man. The interstitial lipoproteins of the heart were studied by sampling cardiac efferent lymph in anesthetized fasting pigs and dogs. The high concentration and turnover of cholesterol-rich lipoprotein in cardiac lymph suggest that the predilection of the coronary bed for atherosclerotic degeneration may be a function of the interstitial lipoprotein concentration and composition as well as the plasma concentration of lipoprotein species.

**MODULATION OF RAT LIVER 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE BY LIPID INHIBITORS, SUBSTRATES, AND CYTOSOLIC FACTORS.** G. Lehrer, S.R. Panini, D.H. Rogers, and H. Rudney (Dept. of Biol. Chem., Univ. of Cincinnati Med. Center, Cincinnati, OH) *J. Biol. Chem.* 256(11):5612-5619 (1981). Oleic acid and oleoyl coenzyme A were found to be inhibitors of microsomal and purified 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (EC 1.1.1.34) activities. Inhibition of the microsomal enzyme was time-dependent and complete in 10 min. The addition of oleic acid, but not of oleoyl-CoA, resulted in gross morphological changes in the microsomal vesicles. Purification of the enzyme increased its sensitivity to both inhibitors, indicating that the microsomal membrane was not a necessary component of the inhibition. In all instances, oleoyl-CoA was a more potent inhibitor than oleic acid. These data strongly indicate that substrates (or inhibitors) may induce conformational changes in the enzyme molecule and that a regulatory mechanism possibly exists in the cell involving substrate levels, cytosolic factors, and naturally occurring inhibitors such as fatty acids and their acyl-CoA derivatives.

**MECHANISM OF CHOLESTEROL AND PHOSPHATIDYLCHOLINE EXCHANGE OR TRANSFER BETWEEN UNILAMELLAR VESICLES.** L.R. McLean and M.C. Phillips (Dept. of Physiology and Biochemistry, The Medical College of Pennsylvania, Philadelphia, PA 19129) *Biochemistry* 20(10):2893-2900 (1981). The mechanism of cholesterol and phosphatidylcholine exchange has been investigated by following the transfer of radiolabeled cholesterol and phosphatidylcholine from negatively charged, unilamellar cholesterol-egg yolk phosphatidylcholine donor vesicles to neutral acceptor vesicles of similar composition. Transfer of cholesterol molecules from the inner to outer monolayer of the vesicle bilayer is not rate limiting in exchange. In contrast to cholesterol exchange, the half-time for 1-palmitoyl-2-oleoyl[1-<sup>14</sup>C] phosphatidylcholine exchange was 48 ± 5 h so that more than six molecules of cholesterol were transferred for each molecule of phosphatidylcholine. Transfer of cholesterol across a dialysis membrane is shown to be slow process whose rate may be predicted by application of Fick's first law of diffusion. These results are only consistent with a mechanism of lipid exchange in which cholesterol and phosphatidylcholine diffuse through the aqueous phase; the experimental activation energy is associated with desorption of lipid from the donor bilayer into the aqueous phase.

**SYNTHESIS AND BIOLOGICAL ACTIVITY OF VITAMIN D<sub>3</sub> 3β-SULFATE. ROLE OF VITAMIN D<sub>3</sub> SULFATES IN CALCIUM HOMEOSTASIS.** S. Nagubandi, J.M. Londowski, S. Bollman, P. Tietz, and R. Kumar (Dept. of Med., Mayo Clinic and Found., Rochester, MN) *J. Biol. Chem.* 256(11):5536-5539 (1981). To determine the biological activity of vitamin D sulfates, we synthesized vitamin D<sub>3</sub> 3β-sulfate and tested its biological activity in vitamin D-deficient hypocalcemic rats. When vitamin D<sub>3</sub> sulfate was administered as a single oral dose of 208,000 or 416,000 pmol (100 μg or 200 μg), it increased active calcium transport in the duodenum and was able to mobilize calcium from bone and soft tissue. We conclude that vitamin D<sub>3</sub> sulfate, a metabolite of vitamin D<sub>3</sub> of heretofore unknown biological activity, is considerably less active than vitamin D<sub>3</sub> itself.

**INSULIN-RICIN B CHAIN CONJUGATE. A HYBRID MOLECULE WITH RICH-BINDING ACTIVITY AND INSULIN BIOLOGICAL ACTIVITY.** R.A. Roth, B.A. Maddux, K.Y. Wong, Y. Iwamoto, and I.D. Goldfine (Cell Biol. Res. Lab. and Dept. of Med., Mount Zion Hosp. and Med. Center, San Francisco, CA) *J. Biol. Chem.*

256(11):5350-5354 (1981). The polypeptide hormone insulin and the binding portion of ricin toxin, the B chain, were linked via a disulfide bond. This insulin-ricin B chain conjugate bound to insulin receptors with a potency one-twentieth that of native insulin. Rat HTC hepatoma cells, a cultured cell line that has relatively few insulin receptors, bound the conjugate to a much greater degree than insulin. Binding occurred predominantly via the ricin B chain portion of the conjugate since binding was not inhibited by insulin but was inhibited by galactose, a known inhibitor of the interaction of B chain to its receptor.

**EPOXIDATION OF UNSATURATED FATTY ACIDS BY A SOLUBLE CYTOCHROME P-450-DEPENDENT SYSTEM FROM *Bacillus megaterium*.** R.T. Ruettinger and A.J. Fulco (Dept. of Bio. Chem., UCLA Sch. of Med. and the Lab. of Biomed. and Environ. Sci., Univ. of Calif., Los Angeles, CA) *J. Biol. Chem.* 256(11):5728-5734 (1981). In previous publications from this laboratory we have described a soluble, partially purified cytochrome P-450-dependent monooxygenase complex that, in the presence of NADPH and O<sub>2</sub>, catalyzes the monohydroxylation of long chain fatty acids, alcohols, and amides at the ω - 1, ω - 2 and ω - 3 positions. We have now found that this preparation catalyzes the epoxidation as well as the hydroxylation of palmitoleic acid and a variety of other monounsaturated fatty acids. The experimental results reported here strongly support the concept that both hydroxylation and epoxidation are catalyzed by an identical cytochrome P-450 complex utilizing the same active and binding sites.

**BOAR ACROSIN. ASSOCIATION OF AN ENDOGENOUS MEMBRANE PROTEINASE WITH PHOSPHOLIPID MEMBRANES.** J.W. Straus, R.F. Parrish and K.L. Polakoski (Dept. of Obstetrics/Gynecol. and Urology, Washington Univ. Sch. of Med., St. Louis, MO) *J. Biol. Chem.* 256(11):5662-5668 (1981). Acrosin, an enzyme required for fertilization, is an endogenous proteinase associated with membranes of the sperm acrosome. Liposomes were utilized as a model system to evaluate the mode of association between highly purified boar acrosin and phospholipid bilayer membrane. Acrosin was observed to bind to liposomes containing acidic phospholipids such as phosphatidylglycerol, cardiolipin, and phosphatidylserine. There was no apparent binding of acrosin to liposomes which consisted of nonacidic phospholipids, thus indicating that an ionic phospholipid constituent was required for binding. Equilibrium binding experiments, with anionic liposomes, suggest the presence of either multiple classes of independent binding sites, or apparent negative cooperativity, with a range in the apparent affinity constant (K<sub>a</sub>) from 2 × 10<sup>11</sup> M<sup>-1</sup> at low acrosin concentrations to 3 × 10<sup>8</sup> M<sup>-1</sup> at high acrosin concentrations. The results demonstrate that acrosin-liposome binding is due in part to electrostatic charge interactions and indicate that the enzyme has properties of an extrinsic membrane protein.

**AORTIC CHOLESTEROL ESTERASE AND OTHER LYSOSOMAL ENZYME ACTIVITIES IN DOCA-SALT, RENAL AND SPONTANEOUS HYPERTENSION IN THE RAT.** T. Tomita, Y. Shirasaki, Y. Takiguchi, T. Okada and E. Hayashi (Dept. of Pharmacology, Shizuoka College of Pharmaceutical Sciences, 2-2-1 Oshika, Shizuoka, Japan 422) *Atherosclerosis* 39(4):453-461 (1981). In spontaneously hypertensive rats, prolonged hypertension caused a decrease in aortic cholesterol esterase activity with N-acetyl-β-D-glucosaminidase activity increased and acid phosphatase activity unchanged [3]. The present study was undertaken to compare these changes with those caused by other experimentally induced types of hypertension. Treatment with DOCA-salt for one month significantly elevated both aortic cholesterol esterase and acid phosphatase activities. In contrast to spontaneous hypertension, venous changes were also observed. An intake of 1% NaCl ad libitum produced results similar to those with the DOCA-salt treatment, despite the fact that blood pressure did not increase. This suggested that humoral factors were the main cause of the elevated enzyme activities in DOCA-salt treatment, despite the fact that blood pressure did not increase. This suggested that humoral factors were the main cause of the elevated enzyme activities in DOCA-salt hypertension. In rats made hypertensive by unilateral renal arterial constriction with contralateral nephrectomy (one clip-one kidney hypertension) or without contralateral nephrectomy (one clip-two kidney hypertension), aortic cholesterol esterase activities were unchanged, while aortic N-acetyl-β-D-glucosaminidase, and aortic and venous acid phosphatase activities were increased. These results show distinct differences in the response of lysosomal enzymes during the three hypertensive states.

**REGULATION OF 25-HYDROXY-VITAMIN D-1α-HYDROXYLASE IN CHICK ISOLATED RENAL TUBULES: EFFECT OF PROSTAGLANDIN E<sub>2</sub>, FRUSEMIDE AND ACETYSALICYLIC ACID.** J.D. Wark, R.G. Larkins, J.A. Eisman and K.R. Wilson (Univ. of Melbourne, Dept. of Med., Repatriation General Hosp., Heidelberg, Victoria, Australia) *Clin. Sci.* 61:53-59 (1981). Isolated renal

tubules were prepared from vitamin D-deficient chicks. The effects of added prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and agents which modify prostaglandin metabolism on the metabolism of 25-hydroxy-vitamin D<sub>3</sub> were studied. It is concluded that, in view of the effects of modulation of endogenous prostaglandin levels by frusemide in aspirin, and the stimulatory effect of exogenous PGE<sub>2</sub>, prostaglandins should be considered potential regulators of the renal 25-hydroxy-vitamin D-1 $\alpha$ -hydroxylase [25-(OH)D-1 $\alpha$ -hydroxylase] enzyme.

EFFECT OF POLYENEPHOSPHATIDYLCHOLINE ON CHOLESTEROL UPTAKE BY HUMAN HIGH DENSITY LIPOPROTEIN. O. Zierenger, G. Assmann, G. Schmitz and M. Rosseneu (A. Nattermann & Cie., Chemische Forschung, Abteilung Radiochemie, D-5000 Cologne, Germany) *Atherosclerosis* 39:527-542 (1981). The lipid and protein composition of human HDL was changed by incorporation of polyeneposphatidylcholine (PPC) into HDL in vitro. HDL with incorporated PPC (HDL-PPC) had a higher molar PC/apoprotein ratio than native HDL. PPC accounted for up to 50% of the PC fraction of HDL. The fluidity of HDL-PPC was higher than that of native HDL but lower than that of PPC liposomes. The transfer of cholesterol from LDL to HDL in human serum was studied by an in vitro [<sup>14</sup>C] cholesterol distribution test. In this test the lipoproteins of serum were labelled with [<sup>14</sup>C] cholesterol. An analytical procedure was developed to quantify the transfer of cholesterol from LDL to HDL after addition of PC. The transfer depended on the fluidity and the dose of the PC fraction used as well as on the initial LDL + VLDL/HDL ratio and was independent of LCAT activity.

## Fats and oils

AN ASPECT OF ANIONIC NUTRITION IN THE OIL PALM AND COCONUT. PROBLEM OF CHLORINE. G. de Taffin and P. Quencez, *Oléagineux*, 1980, 35, No. 12, p. 539-546. The importance of chlorine in the mineral nutrition of oil palm and coconut, brought to the fore in 1971 by Ollagnier and Ochs, is now accepted by most research workers. If, on the ferralitic soils of West Africa, potassium is one of the pivots of oil palm and coconut manuring, most often applied in the form of potassium chloride, Ollagnier et al. were able to show that potassium is not always necessary and that responses to potassium chloride often result from the effect of chlorine. On the outcome of the interpretation of results from very numerous experiments studying, case by case, the effect of potassium chloride applications in various situations, the authors have shown that leaf Cl levels are nearly always closely linked to those of the Ca, K, Mg or even N cations. Thus, in soils with a high exchangeable Ca content, potassium chloride is frequently inadequate to correct a potassic deficiency, whereas when there is a low exchangeable Ca level, on the contrary, KCl appears to be one of the best forms for the correction of a Cl deficiency and the K deficiency which may be associated with it. Finally, the authors demonstrate that where there is no chlorine deficiency, chlorated fertilizers, particularly KCl, can be used without problem.

BEHAVIOUR OF PALM OIL ON SOLIDIFICATION. E. Sambuc and M. Naudet, *Oléagineux*, 1980, 35, No. 12, p. 559-563. Palm oil must be considered as a plastic fat slow to solidify. Its behaviour during solidification is easy to follow using nuclear magnetic resonance spectrometry at low resolution. For refined palm oil this behaviour is characterized principally by an initial surfusion of low intensity, and by a secondary surfusion of lesser intensity which appears once the oil temperature reaches 10 C. The initial temperature at which solidification starts has no influence so long as it is not lower than the clear fusion point, and so long as the sample has been maintained at that temperature for a sufficient length of time. Water dispersed in the fat has no visible influence on the start of solidification, but leads to the disappearance of late secondary surfusion. Additives, such as partial glycerides or lecithins, have varying influences depending on whether they are used in the presence of water or not. In the absence of water the saturated monoglycerides have a clear accelerating effect. In the presence of water, lecithins have a marked delaying effect.

ION PAIR CHROMATOGRAPHY. RETENTION MECHANISMS AND APPLICATIONS. R. Rosset, *Rev. Franc. Corps Gras*, vol. 28, no. 3, 1981, p. 103-109, french., RFCG 81-08. The ionised or ionisable compounds may be retained on the stationary phases used in chromatography, thanks to the formation of ion pairs with a suitable counter-ion. Two techniques are used: by partition of ion pairs between the mobile phase and an aqueous immiscible phase immobilised by a silica gel; by partition of ion pairs between the mobile phase and a graft alkyl silica. Then, the counter-ion must have one or several hydrophobic chains able to react with the alkyl chains of silica: it's the most interesting method. A mechanism explaining the retention of hydrophobic silica is propounded; the influence of dif-

ferent parameters, pH, the mobile phase ionic strength is discussed, two examples of application are given.

THIN-LAYER CHROMATOGRAPHY ON ROD, WITH FLAME IONISATION DETECTOR, FOR THE CONTROL OF SOME LIPIDCHEMISTRY REACTIONS. I. ESTERIFICATION, ALCOHOLYSIS. J. Pore, J.P. Houis and I. Rasori, *Rev. Franc. Corps Gras*, vol. 28, no. 3, 1981, p. 111-115, french., RFCG 81-09. The TLC-FID is very useful in industry, particularly in lipidchemistry. This method is able to control the esterification and alcoholysis reactions giving industrial products mainly used as lubricants and emulsifiers. Examples of these reactions are given.

RAPID DETERMINATION OF DISATURATED MONOUNSATURATED GLYCERIDES IN THE OILS. APPLICATIONS TO PALM OIL AND ITS FRACTIONS. C. Ferrenbach-Bouvron, *Rev. Franc. Corps Gras*, vol. 28, no. 3, 1981, p. 117-121, french., RFCG 81-10. A moderate oxidation of the fat by the permanganate/periodate couple, in medium t-butanol, transforms the monounsaturated glycerides into azelaic glycerides. These may be directly determined by gas liquid chromatography, after methylation of the acid function. Quantitativity is made possible by using an internal standard (trimyristine) and by establishing the azelaic glyceride response coefficient. The response time is about 3 hours. This method is used for the different fractions from the palm-oil fractionation.

BUSH BUTTER TREE (SAFOU) FAT COMPOSITION. C. Tchendji, M. Severin, C. Deroanne and J.P. Wathelet, *Rev. Franc. Corps Gras*, vol. 28, no. 3, 1981, p. 123-125, french., RFCG 81-11. The Safou fruit is very abundant in Cameroun; in consequence, a carefully study of its oil seems interesting. The physical properties are given; the fatty acid composition and glyceridic structure are established.

LIPOLYTIC ACTIVITY OF *Bacillus pumilus*. A. Mourey, *Rev. Franc. Corps Gras*, vol. 28, no. 2, 1981, p. 55-58, french. RFCG 81-05. Production of lipolytic enzymes by *Bacillus pumilus* was investigated. Activity remained located in bacterial cells at all culture phases. Lysozyme, ultrasonic disruption or mechanical disruption with glass beads liberated various amounts of enzymes. The higher enzyme concentration was obtained by cell lysis with lysozyme. During the exponential growth phase, production of protoplasts was sufficient to liberate most of the lipolytic activity in the suspension medium.

STUDY OF THE CRYSTALLIZATION OF PLASTIC FATS. VI. INFLUENCE OF PARTIAL GLYCERIDES AND PHOSPHATIDES IN ABSENCE AND IN PRESENCE OF WATER. C. CASE OF EQUIPONDERAL MIXTURES OF STEAROPALMITIC MONOGLYCERIDES AND SOYBEAN LECITHINS. E. Sambuc, Z. Dirik and M. Naudet, *Rev. Franc. Corps Gras*, vol. 28, no. 2, 1981, p. 59-65, french. RFCG 81-06. The influence of stearopalmitic monoglycerides and soybean lecithins in equiponderal mixtures, on the spontaneous solidification of different model fats (hydrogenated sunflower oil, palm oil, coconut butter), with shaking, has been studied in absence and in presence of water. Monoglycerides and lecithins added simultaneously to fats, just as they are or in presence of water, produce a combination of effects peculiar to every additive, but these combinations don't add together. Specially, in presence of water, the delaying effect of lecithins is by far the most important though the monoglycerides favour the crystallization starting-up.

CHEMICAL COMPOSITION OF *Simarouba glauca* D.D. SEED. DETERMINATION OF THE GLYCERIDE STRUCTURE. G. Lognay, A. Ergo, J.-P. Wathelet and M. Severin, *Rev. Franc. Corps Gras*, vol. 28, no. 2, 1981, p. 67-70, french. RFCG 81-07. The kernel of an oleaginous seed: *Simarouba glauca* D.C. has been studied in detail. This tropical bush from Central America has been planted in Burundi for some years. The seed has been analysed and two methods for extracting the lipids have been tested. The fat characteristics have been established. The triglycerides have been analysed and their structure has been determined by the Van der Wahl and Wathelet methods. There are high contents in OOST - OOO - StOSt - POSt - OOP; the main triglycerides are symmetrical monounsaturated SIS, asymmetrical diunsaturated (SII) and triunsaturated (III).

AGRONOMIC PERFORMANCE OF < MALAYAN DWARF > COCONUT IN JAMAICA. D.H. Romney, *Oléagineux*, 1980, 35, no. 12, p. 551-554. Farm surveys of < Malayan Dwarf > showed 78 p. 100 of plants issued under a replanting scheme reaching bearing. Lethal yellowing disease killed only 0.2 p. 100. Mean annual yields for trees aged 7 years or more are 65 nuts per tree. Yields are significantly reduced by heavy weeds, banana competition or heavy clay soil : 22 p. 100 of fields have rat damage : 15 p. 100 of fields have nuts scarred by *Aceria* mites. An average of 5,727 nuts convert to 2,000 lb. copra.

A GENERAL SYNTHESIS OF LONG CHAIN  $\omega$ - AND ( $\omega$ -1)-HYDROXY FATTY ACIDS. S.R. Abrams (Prairie Regional Lab., Nat. Res. Council of Canada, Saskatoon, Saskatchewan, Canada) *Chem. Phys. Lipids* 28:379-384 (1981). A method for the synthesis of long chain fatty acid substituted at the  $\omega$  and  $\omega$ -1 positions has been developed. The key step is the isomerization of the triple bond of an alkyn-1-ol from an internal position in the chain to the free terminus with a new, convenient reagent, sodium aminopropylamine (NaPH). Standard functional group manipulations i.e., Jones oxidation, esterification and hydroboration of the triple bond are used to prepare  $\omega$ -hydroxy fatty esters. The generality of the method is illustrated with synthesis of  $\omega$ -hydroxy fatty esters with 24, 26, 28 and 30 carbon chains. In the 24 carbon series, hydration of the terminal triple bond of alkynoic ester **4a** followed by reduction gave the ( $\omega$ -1)-hydroxy ester.

A COMPARISON OF THE EFFICIENCY OF THE LIKENS AND NICKERSON EXTRACTOR FOR AQUEOUS, LIPID/AQUEOUS, AND LIPID SAMPLES. C.Y. Au-Yeung and A.J. MacLeod (Dept. of Chem., Queen Elizabeth Col., Univ. of London, Campden Hill Rd., London, England) *J. Agric. Food Chem.* 29:502-505 (1981). By use of a modified Likens and Nickerson apparatus, recoveries of four compounds were determined from aqueous, lipid/aqueous, and lipid media by using three different extracting solvents. Dichloromethane, with recoveries usually in excess of 80%, was the best general extractant, although efficiency did vary between solvents depending upon the solute. Similarly, solutes were recovered to different extents from different media, all other factors being constant. Recoveries generally increased with the time of extraction but were constant over a small concentration range. A further modification to the apparatus is described. When it was adapted for steam distillation of lipid samples, much improved recoveries were obtained, which were similar to those from aqueous samples.

MECHANISM OF CHOLESTEROL EXCHANGE BETWEEN PHOSPHOLIPID VESICLES. J.M. Backer and E.A. Dawidowicz (Biophysical Lab., Harvard Med. School, Boston, MA 02115) *Biochemistry* 20(13):3085-3810 (1981). The kinetics of cholesterol exchange between two populations of small unilamellar vesicles has been investigated. There is no change in the initial rate of this exchange process over a 100-fold change in the acceptor vesicle concentration at a constant donor concentration. These results are not consistent with a collision-dependent exchange mechanism. In support of transfer via the aqueous phase, the inclusion of a negatively charged lipid into the vesicles did not affect the exchange rate. These data together support our contention that the exchange of cholesterol between these vesicles involves a water-soluble intermediate.

OSMOTIC BEHAVIOR OF LIPOSOMES OF PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLSULFOCHOLINE AS A FUNCTION OF LIPID CONCENTRATION. R. Bittman, A.M. Leventhal, S. Karp, L. Blau, P.-A. Tremblay and M. Kates (Dept. of Chem., Queens College of the City Univ. of NY, Flushing, NY) *Chem. Phys. Lipids* 28:323-335 (1981). A relationship between the initial rate of liposome swelling,  $d(1/A)/dt$  and the reciprocal of the lipid concentration of the liposomes has been derived and the utilized to describe the osmotic swelling behavior of serially diluted liposomes and chloroplasts exposed to hypertonic urea solutions. The slopes of plots of  $d(1/A)/dt$  vs. the reciprocal of the lipid concentration of liposomes were not affected by differences in the initial absorbance of phosphatidylcholine bilayers, and were used to assess the ability of sterols to reduce the initial rates of urea permeation through dimyristoylphosphatidylcholine (DMPC) bilayers in the liquid-crystalline state.

THERMODYNAMIC AND MOLECULAR BASIS FOR DISSIMILAR CHOLESTEROL-SOLUBILIZING CAPACITIES BY MICELLAR SOLUTIONS OF BILE SALTS: CASES OF SODIUM CHENODEOXYCHOLATE AND SODIUM URODEOXYCHOLATE AND THEIR GLYCINE AND TAURINE CONJUGATES. M.C. Carey, J.-C. Monter, M.C. Phillips, M.J. Armstrong, and N.A. Mazer (Dept. of Med., Harvard Med. Sch., Div. of Gastroenterology, Peter Bent Hosp., Boston, Mass.) *Biochem.* 20:3637-3648 (1981). The bile salts chenodeoxycholate (CDC) and its  $\beta$ -hydroxypiper ursodeoxycholate (UDC) are administered therapeutically (as acids) to dissolve cholesterol gallstones in man. Since their micellar solutions and those of their physiological conjugates differ strikingly in their capacities to solubilize cholesterol, we studied the interfacial and micellar properties of the epimers by a number of complimentary physical-chemical methods and correlated these with their solubilizing capacities. The critical micellar concentrations (cmc) estimated by surface tension, dye titration, and turbidimetry were similar (1-5 mM), varying slightly with the bile salt species, the method employed, NaCl concentration (0.1 M), and temperature (10-50° C). The data further suggests that the more hydrophilic bile salts

(taurine>glycine conjugates>free species and UDC>CDC, as inferred by HPLC mobility) solubilize cholesterol less well, presumably because the solubilized micelles interfere with hydrophilic interactions at the micellar surface. The possible molecular origins of this interaction are discussed.

GM1-GANGLIOSIDE-TRITON X-100 MIXED MICELLES: CHANGES OF MICELLAR PROPERTIES STUDIED BY LASER-LIGHT SCATTERING AND ENZYMATIC METHODS. M. Corti, B. Degiorgio, S. Sonnino, R. Ghidoni, M. Masserini and G. Tettamanti (CISE S.p.A., P.O. Box 12081-20100 Milano and Dept. of Bio. Chem., Med. Sch., Univ. of Milano, 20133 Milano, Italy) *Chem. Phys. Lipids* 28(3):197-214 (1981). The micellar properties of mixtures of GM1 ganglioside and the non-ionic amphiphile Triton X-100 in 25 mM Na phosphate-5 mM di Na EDTA buffer (pH = 7.0) were investigated by quasielastic light scattering in a wide range of Triton/GM1 molar ratios and in the temperature ranges 15-37 C. These measurements: (a) provided evidence for the formation of mixed micelles; (b) allowed the determination of such parameters as the molecular weight and the hydrodynamic radius of the mixed micelles; (c) showed the occurrence of statistic aggregates of micelles with increasing temperature and micelle concentration. Galactose oxidase was chosen for studying the relation between enzyme activity and micellar properties. The action of the enzyme on GM1 was found to be strongly dependent on the micellar structure. In particular: (a) galactose oxidase acted very poorly on homogeneous GM1 micelles, while affecting mixed GM1/Triton X-100 micelles; (b) fixed GM1 concentration the oxidation rate increased by enhancing Triton X-100 concentration and followed a biphasic kinetics with a break at a certain Triton X-100 concentration; (c) the formation of statistical micelle aggregates was followed by inhibition of the enzyme activity.

PREPARATION OF THIOESTER SUBSTRATES AND DEVELOPMENT OF CONTINUOUS SPECTROPHOTOMETRIC ASSAYS FOR PHOSPHOLIPASE A<sub>1</sub> AND MONOACYLGLYCEROL LIPASE. J.W. Cox and L.A. Horrocks (Dept. of Phys. Chem., The Ohio State Univ., 1645 Neil Ave., Columbus, OH 43210) *J. Lipid Res.* 22(3):496-505 (1981). Current assays of phospholipase A<sub>1</sub> and monoacylglycerol lipase activities in tissues are discontinuous, laborious, and expensive. Some spectrophotometric substrates were synthesized to alleviate this problem. Thioester analogs of phosphatidylcholine and phosphatidylethanolamine, rac-1,2-S,O-didecanoyl-3-phosphocholine-1-mercapto-2,3-propanediol and rac-1,2-S,O-didecanoyl-3-phosphoethanolamine-1-mercapto-2,3-propanediol, were synthesized from the diacylglycerol analog, rac-1,2-S,O-didecanoyl-1-mercapto-2,3-propanediol. The latter was prepared from triacylmercaptoglycerol by lipolysis and separation by chromatography on silica gel. Monoacylglycerol thioester analogs, 1-S-hexadecanoyl-1-mercapto-2,3-propanediol and 1-S-decanoyl-1-mercapto-2,3-propanedio, were synthesized by selective acylation of mercaptoglycerol. All of the substrates were hydrolyzed by *Rhizopus delemar* lipase to release sulfhydryl groups reactive towards 4,4'-dithiobispyridine. The hydrolysis could be followed continuously in a spectrophotometer with 0.2 absorbance unit corresponding to 5 nmol product. The structure and isomeric purity of the phospholipid analogs were verified by their behavior on thin-layer chromatography, elemental analyses, infrared spectra, and by the specificity of the colorimetric reaction with lipolytic enzymes. The results indicate that the phospholipase A<sub>1</sub> and monoacylglycerol lipase activities in *R. delemar* lipase are due to separate enzymes and that these enzyme specific assays will be of general utility for enzyme characterization and purification studies.

FORMATION, ISOLATION AND STRUCTURE DETERMINATION OF METHYL LINOLENATE DIPEROXIDES. D.T. Coxon, K.R. Price and H.W.-S. Chan (Ag. Res. Council, Food Res. Inst., Colney Lane, Norwich, England) *Chem. Phys. Lipids* 28:365-387 (1981). Autoxidation of methyl linolenate gives rise to isomeric mono-hydroperoxides by reaction with one mole of oxygen but further reaction with a second mole of oxygen readily occurs to produce an isomeric mixture of diperoxides. Autoxidation of individual pure methyl hydroperoxylinolenate isomers has been used as a method of obtaining less complex diperoxide mixtures which can be separated into their pure components by preparative high-pressure liquid chromatography (HPLC). The major diperoxide isomers arising from the autoxidation of pure 9R- and 13S-hydroperoxides of methyl linolenate have been isolated and characterized as isomeric epidioxyhydroperoxides of methyl linolenate. These same compounds have been identified as components of the more complex mixture of diperoxides produced during methyl linolenate autoxidation. The structures of the isolated diperoxides have been determined by physicochemical methods and a mechanism for their formation is proposed.

ORIENTATION OF THE CARBOXYL AND NH<sub>2</sub> TERMINI OF THE MEMBRANE-BINDING SEGMENT OF CYTOCHROME

**$b_5$  ON THE SAME SIDE OF PHOSPHOLIPID BILAYERS.** H.A. Dailey and P. Strittmatter (Dept. of Biochem., Univ. of Connecticut Health Center, Farmington, Connecticut) *J. Bio. Chem.* 256(8): 3951-3955 (1981). The present data show that the carboxyl terminal end of the membrane binding segment (nonpolar peptide) of cytochrome  $b_5$  is present on the same side of phospholipid bilayers as the hydrophilic, mene-containing,  $\text{NH}_2$ -terminal segment. This orientation was determined by observing rapid ionization of both tyrosyl residues at positions 5 and 8 from the carboxyl terminus upon addition of sodium hydroxide to the outer aqueous phase of vesicle preparations, and the reaction of one of these residues with a polar, impermeant reagent, diazotized sulfanilic acid. The rate of ionization of both aromatic residues occurred at least 1 order of magnitude faster than ionization of indigo trisulfonate trapped in the inner aqueous compartment of the vesicles. These data and consideration of our earlier characterization of cytochrome  $b_5$  structure and binding to membranes support a model for the membrane binding segment that is highly structured, penetrates to the middle of the bilayer, and loops back to the outer surface to place both the  $\text{NH}_2$  and the carboxyl termini on the same surface of the bilayer.

**PHOSPHATIDIC ACID, PHOSPHATIDYLINOSITOL, PHOSPHATIDYL-SERINE AND CARDIOLIPIN IN THE COURSE OF EARLY EMBRYONIC DEVELOPMENT, FATTY ACID COMPOSITION AND CONTENT IN WHOLE TOAD EMBRYOS AND IN MITOCHONDRIAL FRACTIONS.** I.C.B. De Romanelli, T.S. Alonso and N.G. Bazan (Inst. de Investigaciones Bioquímicas, Universidad Nacional del Sur, Consejo Nacional de Investigaciones Científicas y Técnicas, Avenida Alem, Bahía Blanca, Argentina) *Biochim. Biophys. Acta* 664:561-571 (1981). The fatty acid composition and content of phosphatidylinositol, phosphatidylserine and phosphatidic acid have been studied during the early development of toad embryos. Acidic phospholipids have been analyzed in whole oocytes and embryos and in the following subcellular fractions: yolk platelets, mitochondria and microsomes. Also cardiolipin, a mitochondrial phospholipid, has been analyzed. Arachidonate and stearate are the principal components of phosphatidylinositol. Cardiolipin shows the same composition up to gastrulation and linoleate comprises about 50% of the total acyl groups.

**GAS-LIQUID CHROMATOGRAPHIC ANALYSIS OF EDIBLE OILS AND FATS.** Eyres, Laurence. (Abels Ltd., Auckland, New Zealand) *Chem. N.Z.* 43(6):237-239 (1979). Methyl esters of the fatty acids were prepared by trans-esterification of the sample with Na methoxide or tetramethylammonium hydroxide or by heating the saponified fat under reflux with methanol- $\text{H}_2\text{SO}_4$ - $\text{NH}_4\text{Cl}$ . The methyl esters were separated on a column (2 m  $\times$  1.6 mm) of 10% of silar 5CP on Gas-Chrom Q (100 to 120 mesh), with N as carrier gas (10 ml  $\text{min}^{-1}$ ) and temp.-programming from 195° to 220°. Hexyl heptadecanoate was used as internal standard. Triglycerides were chromatographed on columns (0.5 m  $\times$  1.6 mm) of 3% of OV-1 on Gas-Chrom Q (100 to 120 mesh) with a N flow of 20 ml  $\text{min}^{-1}$  and temp.-programming from 150° to 360° at 10°  $\text{min}^{-1}$ ; triheptadecanoic was used as internal standard.

**ASYMMETRIC INCORPORATION OF TRISIALOGANGLIOSIDE INTO DIPLMITOYLPHOSPHATIDYLCHOLINE VESICLES.** P.L. Felgner, E. Freire, Y. Barenholz and T.E. Thompson (Dept. of Biochem., Univ. of Virginia Sch. of Med., Charlottesville, Virginia) *Biochemistry* 20:2168-2172 (1981). Results are presented with demonstrate that purified trisialoganglioside spontaneously incorporates into preformed phospholipid vesicles. Determinations of the extent of incorporation were made by separating large unilamellar dipalmitoylphosphatidylcholine vesicles containing incorporated ganglioside from micellar ganglioside on a Sepharose-2B column. Incorporation occurs without appreciable altering the vesicular character of the phospholipid bilayer as judged by the maintenance of an outside/inside ratio, determined by  $^{31}\text{P}$  NMR, comparable to that of the original vesicles. All of the incorporated ganglioside is accessible to neuraminidase, indicating that incorporation occurs only on the outer face of the bilayer.

**HYDROLYSIS OF RAT CHYLOMICRON ACYLGLYCEROLS: A KINETIC MODEL.** D.M. Foster and M. Berman (Laboratory of Theoretical Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205) *J. Lipid Res.* 22(3):506-513 (1981). A quantitative model describing the kinetics of hydrolysis of rat chylomicron acylglycerols by bovine milk lipoprotein lipase has been developed using data from studies on rat lymph chylomicrons containing doubly labeled acylglycerols. The detailed analysis indicates that, in addition to hydrolysis from tri- to mono-, and monoacylglycerol to glycerol, an apparently direct hydrolysis pathway of tri- to monoacylglycerol is also present. This accounts for the transient accumulation of monoacylglycerol seen in some of the experiments. For most hydrolysis steps, a Michaelis-Menten mechanism adequately describes the rate of hydrolysis as a function

of lipoprotein lipase concentration. A higher order, more complex mechanism, however, is necessary for the apparent tri- to monoacylglycerol hydrolysis pathway. A mathematical function that describes the way free fatty acid released can control the rate of hydrolysis, and how the presence of the binding sites for free fatty acid on albumin in the incubation medium can modulate this, is included. The model simultaneously satisfies the kinetics of hydrolysis for tri-, di-, and monoacylglycerol together with the kinetics of the glycerol and fatty acid moieties for a wide range of albumin and lipoprotein lipase concentrations.

**REGIOSPECIFIC ATTACK OF METHOXIDE ION ON 4-ALKOXY-O-QUINONE IMINES. A NOVEL ROUTE TO *p*-QUINONE MONOACETALS.** S. Fujita (Res. Labs., Ashigara, Fuji Photo Film Co. Ltd., Minamishigara, Kanagawa, Japan) *J. Chem. Soc. Chem. Commun.* 9:425-426 (1981). In the solvolysis of 4-alkoxy-5-methyl-o-benzoquinone *N*-arylsulphonylimines (1a) and (1b) ( $\text{NaOH-MeOH-H}_2\text{O}$ ), the regiospecific attack of  $\text{MeO}^-$  occurs initially on the carbon atom substituted by the alkoxy-group to produce the *p*-benzoquinone monoacetals (3a) and (3b), respectively.

**MEASUREMENT OF 5,8,11,14-EICOSATETRAYNOIC ACID IN PLASMA BY GAS-LIQUID CHROMATOGRAPHY.** R.P. Goodman and A.R. Brash (Dept. of Pharm., Vanderbilt Univ., Sch. of Med., Nashville TN) *J. Lipid Res.* 22:541-543 (1981). A gas-liquid chromatography-flame ionization detection method is described for measuring plasma levels of eicosatetraenoic acid using eicosatrienoic acid as an internal standard. The technique is simple, rapid, and reproducible. Eicosatetraenoic acid and eicosatrienoic acid behave similarly in the extraction system chosen, yet can be easily resolved by gas-liquid chromatography. Under the conditions of our assay, most plasma fatty acids have a short retention time and are found near the solvent front, whereas, eicosatetraenoic acid and eicosatrienoic acid are retained for 6.9 and 3.75 minutes, respectively.

**HEXAGONAL PHASES IN PHOSPHOLIPIDS WITH SATURATED CHAINS: PHOSPHATIDYLETHANOLAMINES AND PHOSPHATIDIC ACIDS.** K. Harlos and H. Eibl (Max-Planck-Institut für biophysikalische Chemie, 34 Göttingen-Nikolausberg, Federal Republic of Germany) *Biochemistry* 20(10):2888-2892 (1981). The structure of phospholipids with saturated chains was investigated by differential scanning calorimetry and by X-ray diffraction. It is shown that both phosphatidylethanolamines and phosphatidic acids can exhibit a hexagonal phase at high temperature. The temperature of the transition to the hexagonal phase is dependent on chain length and sodium salt concentration. Increasing the chain length or the sodium salt concentration results in a decrease in the transition temperature. In addition to this transition at high temperature, a calorimetric transition at low temperature is detected in some phospholipids.

**INHIBITORY EFFECT OF BILE ACIDS ON THE ACTIVITY OF HUMAN  $\beta$ -GLUCURONIDASE AT ITS OPTIMAL pH.** K.-J. Ho and L.-H. C. Ho (Dept. of Path., Univ. of Alabama in Birmingham Med. Center, and Veterans Admin. Hosp., Birmingham, AL) *Pro. Soc. Exp. Biol. Med.* 167:304-309 (1981). The effect of bile acids on  $\beta$ -glucuronidase activity was studied with centrifuged and dialyzed urines as the source of enzyme and phenolphthalein glucuronide as the substrate. The enzyme activity was measured at 56° in acetate buffer pH 5.2. Since both urine and bile acids are two major determinants of  $\beta$ -glucuronidase activity in bile and their alteration may predispose to bilirubin pigment gallstone formation.

**PHOSPHONO-SPHINGOMYELINS. I: SYNTHESIS OF CERAMIDE (TRIMETHYL) AMINOETHYL PHOSPHONATE.** V.M. Kapoulas and M.C. Moschidis (Dept. of Biochem., Sch. of Natural Sci., Univ. of Ioannina, Ioannina, Greece) *Chem. Phys. Lipids* 28: 357-363 (1981). The synthesis of the phosphono-analogue of sphingomyelin is described. The *N*-acyl-D-erythro-sphingosyl-1-(*N,N,N*-trimethyl-2-aminoethyl) phosphonate was obtained by phosphorylation of *N*-acyl-3-*O*-benzoyl-D-erythro-sphingosine with (2-bromoethyl)phosphonic acid chloride and triethylamine, subsequent quaternization with anhydrous trimethylamine and benzene at 55-60 C for four days, and finally, consecutive removal of the protective group by mild alkaline hydrolysis. Comparison of the CD spectra of both, natural sphingomyelin and its phosphono-analogue, confirmed that their structures and configurations were identical.

**STEROLS IN MARINE INVERTEBRATES. 22. ISOLATION OF STRUCTURE ELUCIDATION OF CONICASTEROL AND THEONELLASTEROL, TWO NEW 4-METHYLENE STEROLS FROM THE RED SEA SPONGES *THEONELLA CONICA* AND *THEONELLA SWINHOEI*.** E. Kho, D.K. Imagawa, M. Rohmer, Y. Kashman and C. Djerassi (Depts. of Chem., Stanford Univ., Stanford, CA) *J. Org. Chem.* 46:1836-1839 (1981). Two new and unusual sterols with unsaturation in the  $\Delta^{14}$  position and a heretofore unprecedented 4-methylene nucleus, conicasterol (12d, 4-methyl-

ene-24(R)-methylcholest-8(14)-en-3 $\beta$ -ol) and theonellasterol (12d, 4-methylene-24(S)-ethylcholest-8(14)-en-3 $\beta$ -ol), were isolated as the principal sterol constituents from the Red Sea sponges *Theonella conica* (Kieschnick) and *Theonella swinhoei* (Gray), respectively. The structures were determined by chemical and spectral analysis and comparison to the spectral data of the newly synthesized 4-methylenecholestan-3 $\beta$ -ol (11a).

VOLUME DETERMINATION OF DEUTERATED DIMYRISTOYL-LECITHIN BY MASS AND SCATTERING LENGTH DENSITOMETRY. W. Knoll (Abteilung für Biophysik, Univ. Ulm, Oberer Eselsberg, Ulm, F.R.G.) *Chem. Phys. Lipids* 28:337-345 (1981). By a combination of mass densitometry with scattering length densitometry it was possible to determine for dimyristoyllecithin with perdeuterated chains the degree of deuteration and from that the molecular volume as a function of temperature.

BILE ACID SYNTHESIS, METABOLISM OF 3 $\beta$ -HYDROXY-5-CHOLENOIC ACID IN THE HAMSTER. E. Kok, S. Burstein, N.B. Javitt, M. Gut and C.Y. Byon (Div. of Hepatic Dis., New York Hosp-Cornell Med. Center, New York, NY) *J. Bio. Chem.* 256(12): 6155-6159 (1981). Synthesis of 3 $\beta$ -hydroxy-5-(1,2-<sup>3</sup>H)cholenic acid has permitted a study of its metabolism in bile-fistula hamsters that received the compound by intravenous infusion. Metabolites in bile were identified by reverse isotope dilution after their complete resolution by high pressure liquid chromatography using  $\mu$ Porasil. Recovery of administered radioactivity ranged from 21-60% in three animals. In each study, lithocholic acid (0.8-4.4%) and chenodeoxycholic acid (7.8-11.3%) were identified as metabolites of 3 $\beta$ -hydroxy-5-cholenic acid and can be considered primary bile acids in the side-chain pathway of bile acid synthesis beginning with the oxidation of cholesterol to 26-hydroxycholesterol.

LIPID CONTENT AND FATTY ACID COMPOSITION IN BAMBOO SHOOTS. E. Kozukue and N. Kozukue (Dept. of Home Ec., Kenmei Junior College, Himeji City, Hyogo Prefecture, Japan) *J. Food Sci.* 46:751-755 (1981). Lipids from bamboo shoots (*phyllostachys pubescens*), peeled and divided from top to base, were extracted and fractionated into three classes, and each class separated into constituent components by thin-layer chromatography (TLC). Fatty acid composition and amount of separated lipids were determined. The main fatty acids of the three lipid classes were palmitic, linoleic and linolenic acids, but composition was remarkable different among these fractions. Bamboo shoots contained 9 PL fractions, the major being phosphatidylcholine (PC) and phosphatidylethanolamine (PE). PC contained about 48% linoleic, 31% palmitic and 11% linolenic acids, and PE also had the similar tendency as PC.

EFFECT OF SURFACE CURVATURE ON STABILITY, THERMODYNAMIC BEHAVIOR AND OSMOTIC ACTIVITY OF DIPALMITOYLPHOSPHATIDYLCHOLINE SINGLE LAMELLAR VESICLES. D. Lechtenberg, E. Freire, C.F. Schmidt, Y. Barenholz, P.L. Felgner and T.E. Thompson (Dept. of Biochem., Univ. of Virginia Sch. of Med., Charlottesville, Virginia) *Biochem.* 20:3562-3467 (1981). The size and surface curvature dependence of the properties and stability of single lamellar vesicles have been investigated by using a variety of physicochemical techniques. Dipalmitoylphosphatidylcholine single lamellar vesicles of sizes ranging between 200 and 900 Å in diameter have been prepared by the French press method and characterized with respect to their size distribution, stability, and thermotropic behavior by negative stain electron microscopy, molecular sieve chromatography, nuclear magnetic resonance spectroscopy, and differential scanning calorimetry. Vesicles with a diameter smaller than 400 Å are unstable below their transition temperature and fuse spontaneously to form larger single lamellar vesicles. Changes in the fractions degree of self-quenching of trapped 6-carboxyfluorescein induced by osmotic stress indicate that large single lamellar vesicles are not spherical under isoosmotic conditions. These vesicles are relatively flexible and can sustain almost a 2-fold increase in their internal aqueous volume without any leakage of the internal content.

EFFECT OF CHOLESTEROL IN MEMBRANES. PULSED NUCLEAR MAGNETIC RESONANCE MEASUREMENT OF LIPID LATERAL DIFFUSION. G. Lindblom, L.B.-A. Johansson, and G. Arvidson (Div. of Phys. Chem. 2, Chem. Centre, Univ. of Lund, Lund Sweden) *Biochemistry* 20:2204-2207 (1981). Lateral diffusion coefficients of lipids in a bilayer can be measured directly in a macroscopically aligned sample by use of a pulsed NMR method with pulsed magnetic field gradients. This technique has been utilized to investigate the influence of field gradients. This technique has been utilized to investigate the influence of cholesterol on the lipid diffusion of egg yolk lecithin, palmitoyllecithin, and dioleoyllecithin. It is found that cholesterol has a very small effect on the phospholipid diffusion. On the other hand, cholesterol has a great influence on the molecular ordering in the bilayer and

on the lipid phase structure. It is therefore suggested that cholesterol exerts its dominant effect on the lipid membrane stability.

CHARACTERIZATION, CELL-FREE SYNTHESIS, AND PROCESSING OF APOLIPOPROTEIN A-I OF RAT HIGH-DENSITY LIPOPROTEINS. M.-H. Lin-Su, Y.-C. Lin-Lee, W.A. Bradley, and L. Chan (Depts. of Cell Bio. and Med. and the Methodist Hosp., Houston, Texas) *Biochem.* 20:2470-2475 (1981). Rat apolipoprotein A-I (apoA-I) was isolated from delipidated high-density lipoproteins by sequential chromatography on Sephacryl S-200 and Sephadex G-150 columns in guanidine buffer. The purified protein was homogenous by NaDodSO<sub>4</sub> and urea gel electrophoresis. When translation was performed in the presence of dog pancreatic microsomal membranes, the iminoprecipitable material was cotranslationally cleaved to a product identical in size with plasma apoA-I. Thus, we have synthesized in vitro a putative precursor to rat apoA-I, designated preapoA-I. The preapoA-I has been processed in a cell-free system to its mature plasma counterpart by the addition of exogenous microsomal membranes.

COMMENTS ON THE USE OF THE ORDER PARAMETERS OBTAINED FROM <sup>2</sup>H-NMR TO DESCRIBE THE ANISOTROPIC MOTIONS OF THE METHYLENE GROUPS OF THE FATTY ACYL CHAINS IN LIPID BILAYER MEMBRANES. J.-P. Merald (Biocenter of the Univ. of Basel, Dept. of Biophys. Chem., Klingelbergstrasse 70, Basel, Switzerland) *Chem. and Phys. Lipids* 28:227-239 (1981). The informational content of a deuteron quadrupole splitting obtained from the methylene group undergoing anisotropic motion is inversely proportional to the degree of symmetry underlying this motion. To accurately assess the power and the limitation of <sup>2</sup>H-NMR, the motional symmetry of the methylene groups was strictly examined. From the two orthogonal and geometrical planes of symmetry of the methylene groups was strictly examined. From the two orthogonal and geometrical planes of symmetry of a methylene group one was found to remain a plane of symmetry property only in first approximation. The direction defined by the intersection of the two orthogonal planes was not found to be a motional axis of axial symmetry (even as a poor assumption). However, to a good approximation the motion of this direction with respect to the bilayer normal can be specified by the deuteron quadrupole splitting arising from the particular geometry of a given methylene group.

THE ADSORPTION TO AND HYDROLYSIS OF 1,3-DIDECANOYL GLYCEROL MONOLAYERS BY PANCREATIC LIPASE. W.E. Momsen and H.L. Brockman (Hormel Inst., Univ. of Minn., Austin, MN) *J. Biol. Chem.* 256(13):6913-6916 (1981). The kinetics of hydrolysis of 1,3-didecanoyl glycerol by pancreatic lipase were measured in monomolecular films at the air-water interface. Determination of the quantity of active enzyme associated with the film under conditions of constant reaction velocity and surface pressure showed that both the extent of enzyme adsorption and the catalytic rate constant of the adsorbed enzyme were substrate packing dependent. If this rate constant was corrected for the concentration of reactive substrate predicted from kinetic and physical studies, a constant value of  $2.5 \pm 0.5 \times 10^{12}$  (S.E.) cm<sup>2</sup>/(mol·s) was obtained for the second order rate constant. This constancy supports the hypothesis that a packing dependent conformational distribution of substrate head groups is a factor in the regulation of lipolysis.

STRUCTURAL ANALYSIS OF HYDROPEROXIDES FORMED BY OXIDATION OF PHOSPHATIDYLCHOLINE WITH SINGLET OXYGEN. J. Terao, Y. Hirota, M. Kawakatsu and S. Matsushita (Res. Inst. for Food Sci., Kyoto Univ., Kyoto, Japan) *Lipids* 16(6): 427-432 (1981). Soybean phosphatidylcholine (PC) and di-18:2 PC (di-18:2 PC) were oxidized with singlet molecular oxygen using methylene blue as the photosensitizer. The oxidation products, PC monohydroperoxides (PC-MHP) and PC dihydroperoxides (PC-DHP), were isolated by reverse phase liquid chromatography, and their structures were analyzed by nuclear magnetic resonance (NMR) and gas chromatography-mass spectrometry (GC-MS). The isomeric composition of hydroperoxylinoleate component in di-18:2 PC-MHP was determined by methanolysis of the hydrogenated diglyceride and mass chromatographic analysis of the resulting isomeric hydroxy octadecanoates.

COMPARATIVE PHYSICAL STUDIES OF PHOSPHATIDYLSULFOCHOLINE AND PHOSPHATIDYLCHOLINE. CALORIMETRY, FLUORESCENCE POLARIZATION AND ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY. P.-A. Tremblay and M. Kates (Dept. of Biochem., Univ. of Ottawa, Ottawa, Canada) *Chem. Phys. Lipids* 28:307-322 (1981). Transition temperatures of phosphatidylsulfocholines (PSCs; di-14:0-, di-16:0-, di-18:0- and di-18:1-) were compared with those of the corresponding phosphatidylcholines (PCs) using the techniques of differential scanning calorimetry, fluorescent polarization with diphenylhexa-



triene (DPH) and *cis*- and *trans*-parinaric acids as probes, and electron paramagnetic resonance (EPR) with 5-doxy stearic acid as probe. These results provide a rationale for the observed ability of the sulfonium analogs to substitute for PC in some natural membranes.

**PURIFICATION OF STABILIZED BAND 3 PROTEIN OF THE HUMAN ERYTHROCYTE MEMBRANE AND ITS RECONSTITUTION INTO LIPOSOMES.** M.F. Lukacovic, M.B. Feinstein, R.I. Sha'afi, and S. Perrie (Depts. of Pharmacology and Physiology, Univ. of Connecticut Health Center, Farmington, CT 06032) *Biochemistry* 20(11):3145-3151 (1981). The red cell membrane protein, identified on gel electrophoresis as band 3, has been implicated with anion transport. We report here a new rapid procedure for obtaining a stable, functional band 3, essentially free from all other membrane proteins. Red cell ghosts were washed with isotonic saline and solubilized overnight in 0.5% Triton X-100. This extract was applied to a DEAE-cellulose column, and bands 3 and 4.2 and glycophorin (PAS-1) were eluted with high salt concentration. This high-salt fraction was applied to a [[p-(chloromercuri)benzamido]-ethyl] agarose 4B gel (synthesized according to a modification of the method of Cuatrecasas), which removed glycophorin and some band 4.2. Pure band 3 was eluted with 0.1 mM cysteine after a low-salt wash. Addition of 15 mM mercaptoethanol immediately after elution was found to prevent protein aggregation. It should also be noted that this affinity gel can be used to isolate pure glycophorin. When transport was carried out with no external transportable anions, sulfate efflux was markedly reduced. Influx into liposomes containing band 3 was measured, and a turnover number was calculated which was 24% of the value found for the intact red cell. When stored in 15 mM mercaptoethanol, band 3 remained functional and monomeric for at least a week.

**CHARACTERIZATION BY INFRARED SPECTROSCOPY OF THE BILAYER TO NONBILAYER PHASE TRANSITION OF PHOSPHATIDYLETHANOLAMINES.** H.H. Mantsch, A. Martin and D.G. Cameron (Div. of Chem., Nat. Res. Coun. of Canada, Ottawa, Canada) *Biochemistry* 20:3138-3145 (1981). A Fourier-transform infrared spectroscopic study of the thermotropic behavior of egg yolk phosphatidylethanolamines is reported. Two phase changes were monitored, the gel to liquid-crystalline acyl chain melting transition, centered at 12 C, and a transition from the liquid-crystalline to the inverted hexagonal phase, centered at 28 C. A model is developed for the bilayer to nonbilayer phase transition in which it is proposed that the driving force which triggers this phase transition is the introduction of a degree of conformational disorder so high that the integrity of the bilayer surface can no longer be maintained, due to the volume requirements of the acyl chains. A number of previously reported data are rationalized in terms of this hypothesis.

**EFFECTS OF MOLECULAR STRUCTURE ON TWO-DIMENSIONAL PHASE DIAGRAM AND THERMODYNAMIC QUANTITIES OF MIXED MONOLAYERS.** H. Matuo, K. Motomura and R. Matuura (Dept. of Chem., Faculty of Sci., Kyushu Univ., Fukuoka, Japan) *Chem. Phys. Lipids* 28:385-397 (1981). Many kinds of two-component mixed monolayer systems were investigated to clarify the effect of molecular structure on the monolayer state. Two-dimensional phase diagrams and thermodynamic quantities were evaluated by correct thermodynamic analysis. The diagrams were classified into the following six types (1) cigar type; (2) modified cigar type; (3) positive azeotropic type; (4) negative azeotropic type; (5) eutectic type; (6) complicated type. Phase diagram analysis and thermodynamic quantities, such as entropy, enthalpy and energy changes, were in good agreement with each other.

**DEGREE OF HYDRATION AND LATERAL DIFFUSION IN PHOSPHOLIPID MULTIBILAYERS.** J.T. McCown, E. Evans, S. Diehl, and H.C. Wiles (Dept. of Biomed. Engineering, Duke Univ., Durham, NC) *Biochemistry* 20:3134-3138 (1981). The fluorescence recovery after photobleaching technique (FRAP) was used to measure the lateral diffusion coefficient of the fluorescent lipid analogue *N*-(7-nitro-2,1,2-benzoxadiazol-4-yl)phosphatidylethanolamine (NBD-PE) in egg yolk phosphatidylcholine (PEC) multibilayers as a function of water content. Diffusion coefficients were determined relative to a reference state of constant degree of hydration. The use of relative measurements allows changes in diffusivity to be determined accurately without the need for precise measurement of laser beam intensity profiles.

**SYNTHESIS OF ESTERS OF SATURATED AND UNSATURATED SIXTEEN-CARBON ACIDS DEUTERATED AT THE TERMINAL OR PENULTIMATE CARBONS.** A.P. Tulloch and L. Berger (Nat. Res. Coun. of Canada, Prairie Regional Lab., Saskatoon, Saskatchewan, Canada) *Chem. Phys. Lipids* 28:347-355 (1981). General synthesis of saturated and unsaturated fatty acids, specifically trideuterated at the terminal carbon or dideuterated at the penulti-

mate carbon, from  $\omega$ -hydroxy esters, have been developed. Methyl (16-<sup>2</sup>H<sub>3</sub>)hexadecanoate was synthesized from methyl 16-hydroxyhexadecanoate. Overall yields ranged from 30% to 38%.

**RECONSTITUTION OF COLICIN E1 INTO DIMYRISTOYLPHOSPHATIDYLCHOLINE MEMBRANE VESICLES.** Y. Uratami and W.A. Cramer (Dept. of Bio. Sci., Purdue Univ., West Lafayette, Indiana) *J. Bio. Chem.* 256(8):4017-4023 (1981). Colicin E1 has been incorporated in the presence of cholate into large unilamellar membrane vesicles made of the single phospholipid, dimyristoylphosphatidylcholine, that retained labeled insulin and sucrose in the presence and absence of colicin. The average number of colicin molecules incorporated per vesicle was approximately 10. The ability of colicin E1 to conduct ion flow across the vesicle membrane was measured above (32 C) and below (12 C) the measured phase transition ( $T_m = 23.5-24$  C) of the vesicles through dissipation of an ionophore-induced potassium diffusion potential. A channel-like function of colicin E1, with an apparent size discrimination at 10 C against non-electrolytes of the size of glycerol or larger, can thus be defined for colicin E1 incorporated into membrane vesicles made of a single pure lipid.

**A NEW METHOD FOR THE MEASUREMENT OF BILE ACID TURNOVER AND POOL SIZE BY A DOUBLE LABEL, SINGLE INTUBATION TECHNIQUE.** G. Vantrappen, P. Rutgeerts, and Y. Ghos (Dept. of Med. Res., Univ. Hosp. St. Rafael, Univ. of Leuven, Leuven, Belgium) *J. Lipid Res.* 22:528-531 (1981). A new method is described for measuring the cholic acid turnover and pool size by a single duodenal intubation technique. The method is based on determination in a single bile sample of the ratio of the specific activities of (<sup>14</sup>C)cholic acid and (<sup>3</sup>H)cholic acid administered intravenously with an interval of 24 hr. With this ratio the fractional turnover rate (k) of cholic acid can easily be calculated as well as the half-life and pool size. Studies in ten normal subjects indicate that the cholic acid half life and pool size, determined by this single intubation technique, correlate very well ( $r > 0.98$ ) with the results obtained by Lindstedt's method. Unlike the other methods using a single intubation, this method allows a good estimate of the bile acid turnover as well as the bile acid pool size.

**THE OV 275-PACKED STAINLESS STEEL COLUMN IN TRANS-FATTY ACID RESEARCH: A NOTE OF CAUTION.** B.L. Walker (Dept. of Nutr., Col. of Bio. Sci., Univ. of Guelph, Guelph, Ontario, Canada) *Lipids* 16(6):468-471 (1981). Gas chromatographic analysis of fatty acid methyl esters in a 6.1 m stainless steel column packed with 15% OV 275 and operated at 220 C resulted in poor recovery of polyunsaturated esters relative to their saturated or monoenoic counterparts. Relative responses of the esters declined with increasing unsaturation, were concentration-dependent at low concentrations and changed as the column aged. Decomposition of polyunsaturated compounds in the hot stainless steel column appeared to be the major reason for their poor recovery and renders this particular column of doubtful value in analysis of complex ester mixtures.

**SURFACE POTENTIAL OF LIPID MEMBRANE ESTIMATED FROM THE PARTITIONING OF METHYLENE BLUE INTO LIPOSOMES.** M. Nakagaki, I. Katoh and T. Handa (Faculty of Pharm. Sci., Kyoto Univ., Sakyo-ku, Kyoto, Japan) *Biochemistry* 20:2208-2212 (1981). The partition of methylene blue between negatively charged phospholipid membrane and the bulk aqueous phase was measured by using visible spectroscopy in very dilute aqueous membrane suspensions, 0.5-0.3 mg of dried phospholipids in 1 mL of buffer solution. Under these experimental conditions, the turbidities of liposomes systems and the overlapping of the electrical double layers of different liposomes were negligible. The positively charged probe, methylene blue, forms dimers in membrane phase, resulting in a reduction of the absorbance intensity. The surface potential of the membranes (liposomes) was calculated from the partition coefficient of the dye between the membrane and the bulk phase. The effects of charge density of the membrane and of the ionic strength on the surface potential were also studied.

**HIGH PRESSURE LIQUID CHROMATOGRAPHY OF AUTOXIDIZED LIPIDS: II. HYDROPEROXY-CYCLIC PEROXIDES AND OTHER SECONDARY PRODUCTS FROM METHYL LINOLENATE.** W.E. Neff, E.N. Frankel and D. Weisleder (Northern Regional Res. Center, Ag. Res., Sci. and Ed. Admin., U.S. Dept. of Ag., Peoria, IL) *Lipids* 16(6):439-448 (1981). A previous study of autoxidation products by high pressure liquid chromatography (HPLC) of methyl oleate and linoleate was extended to methyl linolenate. Autoxidized methyl linolenate was fractionated by HPLC either after reduction to allylic alcohols on a reverse phase system, or directly on a micro silica column. Isolated oxidation products were characterized by thin layer and gas liquid chromatography and by ultraviolet, infrared, nuclear magnetic resonance and mass spectrometry. Cyclic peroxides and dihydroperoxides are

suggested as important flavor precursors in oxidized fats.

**KINETICS OF SOLUBLE LIPID MONOMER DIFFUSION BETWEEN VESICLES.** J.W. Nichols and R.E. Pagano (Dept. of Embryology, Carnegie Inst. of Washington, Baltimore, MD) *Biochemistry* 20:2783-2789 (1981). The fluorescent phospholipid 1-acyl-2-[12-[7-nitro-2,1,3-benzoxadiazol-4-yl)amino]dodecanoyl]phosphatidylcholine (C<sub>12</sub>-NBD-PC) was used to study the kinetics of lipid transfer between phospholipid vesicles. A model based on lipid transfer resulting from the diffusion of soluble monomers was found to accurately predict the kinetics of this transfer process. From these studies, we conclude that (i) C<sub>12</sub>-NBD-PC transfer between vesicles results from the diffusion of soluble monomers and not from vesicle collision, (ii) the rate at which a lipid molecule enters or leaves a bilayer is dependent upon both its molecular structure and the characteristics of the donor and acceptor bilayers, and (iii) under the appropriate conditions, either the rate of lipid association or dissociation from the bilayer or a combination of both may determine the rate of transfer.

**THE ORDER-DISORDER TRANSITION OF THE CORE CHOLESTERYL ESTERS OF HUMAN PLASMA LOW DENSITY LIPOPROTEIN.** A proton nuclear magnetic resonance study. P.A. Kroon (Dept. of Biochem. Regulation, Merck Sharp and Dohme Res. Lab., Rahway, NJ) *J. Biol. Chem.* 256(11):5332-5339 (1981). We have

used proton NMR spectroscopy to study the order → disorder transition of the cholesteryl ester-rich core of human low density lipoprotein and of cholesteryl oleate and cholesteryl oleate/triolein mixtures. NMR spectra of cholesteryl oleate and cholesteryl oleate/triolein mixtures in the liquid state give well resolved resonances, consistent with freely tumbling cholesteryl ester molecules. We propose a model for the LDL core which is consistent 1) with the existence of a liquid core above the phase transition temperature and 2) with the smaller transition enthalpies observed for core cholesteryl esters compared to extracted cholesteryl esters.

**LIPOSOME ACCUMULATION IN ISCHAEMIC INTESTINE FOLLOWING EXPERIMENTAL MESENTERIC OCCLUSION.** T.N. Palmer, F.J. Caride, L.A. Fernandez and J. Twickler (Dept. of Biochem., Charing Cross Hosp. Med. Sch., Fulham Palace Road, London, England) *Biosci. Reports* 1(4):337-344 (1981). Partial intestinal ischaemia was produced by ligation of selected primary laterals of the mesenteric artery in the rat. Both positively and negatively charged liposomes (multiply labelled with <sup>99m</sup>Tc) diethylenetriamine pentaacetic acid, (<sup>3</sup>H)methoxy-inulin, and (4-<sup>14</sup>C)-cholesterol, administered 24 hr following ligation, were accumulated in ischaemic (necrotic) intestine.

**PUBLICATIONS ABSTRACTED**

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