Abstracts

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Biochemistry and nutrition

IN VITRO CONVERSION OF SATURATED TO MONOUNSATURATED FATTY ACID BY EHRLICH ASCITES CELLS. O. Mercure, M. De Tomas, R. Antueno (Catedra de Bioquimica, Facultad de Ciencias Medicas, Univ. Nacional de La Plata, 1900-La Plata, Argentina) *Lipids 16*(12):893-896 (1981). In this paper, evidence is presented on the capacity of the Ehrlich ascites cells to synthesize in vitro monounsaturated fatty acids from radioactive palmitate. Localization of the double bond was determined by ozonolysis and subsequent reduction of the ozonides to aldesters followed by gas liquid chromatography. These results proved that Ehrlich ascites cells have a $\Delta 9$ desaturase that catalyzes the biosynthesis of palmitoleic acid from palmitic acid and oleic and vaccenic acid as substrate. Furthermore, it is shown that, as in the hepatic cells, $\Delta 9$ desaturase enzyme activity of the tumoral cells is associated with the endoplasmic reticulum. The electron transport components involved in the desaturase system, i.e., NADH-cytochrome b_5 reductase and NADH-cytochrome c reductase, were also measured. The activities of these enzymes do not appear to be rate-limiting in the desaturase activity of these tumoral cells.

PARAQUAT AND NADPH-DEPENDENT LIPID PEROXIDATION IN LUNG MICROSOMES. H.P. Mixra and L.D. Gorsky (Laboratory for Energy-Related Health Research, University of California, Davis, CA 95616) *J. Biol. Chem. 256*(19):9994-9998 (1981). Since there exists some controversy in the literature as to whether paraquat augments microsomal lipid peroxidation via superoxide anion (O_2^-) the role of paraquat and active oxygen species in NADPH-dependent lung microsomal lipid peroxidation was investigated. Incubation of buffered aerobic mixture of bovine lung microsome and NADPH, in the presence or absence of exogenously added iron, resulted in a progressive formation of lipid peroxides whose accumulation could be followed at 535 nm as malondialdehyde. Paraquat strongly inhibited this lipid peroxidation. Thus, malondialydehyde formation was 50% inhibited by 4×10^{-5} M Paraquat in the reaction mixture. The malondialdehyde color development by lipid peroxides was not affected by this concentration of paraquat. Lipid peroxidation was also strongly inhibited by singlet oxygen scavengers, e.g. dimethylfuran and diphenylfuran, and by catalase. Hydroxyl radical scavengers, e.g. mannitol, benzoate, and ethanol, had little effect in malondialydehyde productions. Superoxide dismutase, which removes O_2^- efficiently, did not inhibit malondialdehyde production by lung microsomes and rather enhanced its formation. A scheme in which paraquat and active O_2 species may be involved with microsomal lipid peroxidation is presented.

ETHANOLAMINE PLASMALOGEN METHYLATION BY RABBIT MYOCARDIAL MEMBRANES. S. Mogelson and B.E. Sobel (Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO 63110) *Biochim. Biophys. Acta 666*(2):205-211 (1981). Enzymatic methylation of alkenylacylglycerophosphoethanolmine to form alkenylacylglycerophosphocholine was observed in rabbit myocardial membranes, and was compared to the corresponding methylation sequence for diacyl substrates. Membranes were incubated with *S*-adenosyl-L-(*methyl*-^{3}H)methionine and assayed for incorporation of radioactivity into selected lipids. The rate of incorporation of methyl groups into diacylglycerophosphocholine exceeded that for alkenylacylglycerophosphocholine, 12.0 ± 3.6 vs. 3.9 ± 0.7 pmol product formed/mg per h (mean ± S.D.), even when normalized for ethanolamine substrate concentration (5.7 ± 1.6 vs. 1.8 ± 0.4 pmol n CH_3 incorporated/umol diradylglycerophosphoethanolamine). Rabbit myocardial phospholipid methyltransferase activity is optimal at basic pH for each substrate, is moderately stimulated by added Ca^{2+} or Mg^{2+}, and is completely inhibited by *S*-adenosylhomocysteine. An apparent K_m of 0.2 mM for *S*-adenosylmethionine applies to diacyl- and alkenylacylglycerophosphocholine formation; at low concentrations of methyl donor (0.003 mM), the monomethylated products accumulate.

ENZYMATIC FORMATION OF CHOLESTERYL ESTER FROM CHOLESTEROL BY GALLBLADDER MUCOSA. D.H. Neiderhiser (Medical Research Service, Veterans Administration Medical Center and The Department of Medicine, Case Western Reserve University, School of Medicine, Cleveland, OH 44106) *Lipids 16*(12):930-933 (1981). The formation of cholesteryl ester from cholesterol and acyl CoA catalyzed by the enzyme acyl CoA:cholesterol acyltransferase (EC 2.3.1.26) was studied in guinea pig gallbladder mucosa homogenate and the subcellular fractions. The enzymatic activity was enriched in the microsomal fractions. Highest activity was observed with linoleoyl CoA. These data elucidate one mechanism for the formation of cholesteryl ester from cholesterol by the gallbladder wall.

HYPOLIPIDEMIC EFFECTS OF CLOFIBRATE AND SELECTED CHROMAN ANALOGS IN FASTED RATS: I. CHOW-FED ANIMALS. M. O'Brien, S.T. Patel, A. Mukhopadhyay, H.A.I. Newman, D.R. Feller, S.S. Kokrady, D.T. Witiak, R.R. Lanese, and J.C. Rice (Div. of Clinical Che., Dept. of Pathology, College of Med., The Ohio State Univ., Columbus, OH 43210) *Lipids 16*(12):903-911 (1981). The hypolipidemic properties of ethyl 6-chlorochroman-2-carboxylate (II), ethyl 6-phenylchroman-2-carboxylate (III) and ethyl 6-cyclohexylchroman-2-carboxylate (IV) were compared to clofibrate (I) in fasted normolipidemic rats. The chroman analog II, like its parent compound, clofibrate, reduced serum and α-lipoprotein cholesterol concentrations. Although analog III had no effect on serum cholesterol, it caused a slight elevation of α-lipoprotein cholesterol concentration. Serum free cholesterol was increased and LCAT activity was reduced in clofibrate-treated rats. The hypolipidemic agents had no consistent effect on liver lipid concentrations and liver microsomal HMG-CoA reductase activity. In addition, we have shown that drug efficacies varied directly with seasonal variations in serum lipid concentrations.

ARACHIDONIC ACID METABOLISM IN RABBIT RENAL CORTEX. FORMATION OF TWO NOVEL DIHYDROXYEICOSATRIENOIC ACIDS. E.H. Oliw, J.A. Lawson, A.R. Brash and J.A. Oates (Dept. of Pharmacology, Vanderbilt University, School of Medicine, Nashville, TN 37232) *J. Biol. Chem. 256*(19):9924-9931 (1981). [1-^{14}C]Eicosatetraenoic (arachidonic) acid was incubated with a low speed (17,000 × g) rabbit renal cortical supernatant or with a cortical microsomal suspension fortified with NADPH for 15 min at 37 C. The products which were less polar than prostaglandins on reversed phase high performance liquid chromatography were identified by gas chromatography-mass spectrometry. Both the fortified microsomes and the low speed supernatant formed significant amounts of two novel metabolites, 11,12-dihydroxy-5,8,14-eicosatrienoic acid and 14,15-dihydroxy-5,8,11-eicosatrienoic acid. Other identified products were 19- and 20-hydroxyeicosatetraenoic acid, 19-oxoeicosatetraenoic acid, and in the low speed supernatant, eicosatetraen,1,20-dioic acid. The metabolites were not formed in significant amounts by high speed cortical supernatant or by non-fortified cortical microsomes. Carbon monoxide inhibited formation of these compounds, indicating that they may be formed by the cytochrome P-450-linked renal monooxygenase systems.

THE EFFECTS OF SUBFRACTIONS OF HIGH DENSITY LIPOPROTEIN ON CHOLESTEROL EFFLUX FROM CULTURED FIBROBLASTS. REGULATION OF LOW DENSITY LIPOPROTEIN RECEPTOR ACTIVITY. J.F. Oram, J.J. Albers, M.C. Cheung, and E.L. Bierman (Division of Metabolism and Endocrinology and the Northwest Lipid Research Clinic, Dept. of Medicine, University of Washington School of Medicine, Seattle, WA 98195) *J. Biol. Chem. 256*(16):8348-8356 (1981). When cultured human fibroblasts were incubated with medium of varying composition, the activity of the low density lipoprotein (LDL) receptor was strongly correlated with the amount of cholesterol that egressed from the cell. Particles isolated from lipoprotein-deficient serum ($d > 1.25$) by anti-apoprotein (apo) A-I affinity chromatography were as effective as the $d > 1.25$ serum in activating the LDL receptor, suggesting that the acceptors for cellular cholesterol in human lipoprotein-

deficient serum are apo A-I-containing particles. Incubations with high density lipoprotein (HDL) subfractions designated as "very high" density lipoprotein (VHDL, d = 1.21-1.25) and HDL_3 (d = 1.100-1.21) enhanced LDL receptor activity while incubations with HDL_2 (d = 1.063-1.100) had a slight inhibitory effect. Based on apo A-I content, VHDL was a more potent activator than HDL_3. Incubations with HDL_3 also enhanced the rate of sterol synthesis, inhibited cholesterol esterification and decreased cell cholesterol, while incubations with HDL_2 had the opposite effect. These results suggest that net efflux occurs only in the presence of HDL particles from d > 1.100 serum fractions. The greater the density of the apo A-I-containing particles, the greater the ability to remove cholesterol from the cells.

THERMODYNAMICS OF LIPID-PROTEIN ASSOCIATION. THE FREE ENERGY OF ASSOCIATION OF LECITHIN WITH REDUCED AND CARBOXYMETHYLATED APOLIPOPROTEIN A-II FROM HUMAN PLASMA HIGH DENSITY LIPOPROTEIN. J. Pownall, D. Hickson and A.M. Gotto, Jr. (Dept. of Medicine, Baylor College of Medicine, and the Methodist Hospital, Houston, TX 77030) *J. Biol. Chem. 256*(19):9849-9854 (1981). Apolipoprotein A-II is an apoprotein which in human plasma high density lipoproteins exists as a disulfide-linked dimer of 17,400 molecular weight. It is known to spontaneously bind phospholipids with an affinity that has so far eluded measurement. We have prepared and isolated ^{3}H-reduced and carboxymethylated apoA-II ([^{3}H] RCM-A-II, M_r=8700) and measured its free energy of association, ΔG_a, by equilibrium methods. The results represent the first quantitative measurement of the affinity of an apolipoprotein for a lipid or lipoprotein; the measured values are much less than those based upon the sum of the free energies of transfer of the individual amino acid side chains of RCM-A-II. The results suggest that the free energy of association of RCM-A-II with lipids and lipoproteins is entropy-driven, largely due to the hydrophobic effect; that the enthalpy of association is compensated by an additional entropy term; and that the hydrophobic amino acid residues of RCM-A-II associate with a region of the lipid or lipoprotein that is much more polar than the interior of a lipid bilayer. Our results show that RCM-A—II transfers from the phospholipid phase to the aqueous phase, and suggests that native apoA-II probably does not, or does so at a much lower rate.

SYNTHESIS OF PHOSPHATIDYLCHOLINE FROM PHOSPHATIDYLETHANOLAMINE BY AT LEAST TWO METHYLTRANSFERASES IN RAT PITUITARY EXTRACTS. C. Prasad and R.M. Edwards (Section of Endocrinology, Dept. of Med., Louisiana State Univ. Med. Ctr., New Orleans, LA 70112) *J. Bio. Chem. 256*(24): 13000-13003 (1981). Rat pituitary extracts contain at least two methyltransferases that methylated phosphatidylethanolamine to phosphatidylcholine using S-adenosylmethionine as the methyl donor. The first enzyme methylates phosphatidylethanolamine to phosphatidyl-N-monomethylethanolamine and has a high K_m (40-42μM) for S-adenyl-L-methionine. The first enzyme is loosely bound to the membrane fractions; therefore it appears in both particulate (20,000 X g) and supernatant (20,000 X g) fractions, whereas the second enzyme(s) is tightly bound to the membrane and thus appears only in the particulate fraction. Both methyltransferases have two pH optima of 6.5 and 9.5 (9.5 activity > 6.5 activity) and they do not require Mg^{2+}.

ANALOGUES OF CYCLIC AMP INHIBIT PHOSPHATIDYLETHANOLAMINE N-METHYLATION BY CULTURED RAT HEPATOCYTES. P.H. Pritchard, S.L. Pelech, and D.E. Vance (Dept. of Biochem., Univ. of Brit. Columbia, Vancouver, B.C. 1W5, Canada) *Biochim. Biophys. Acta 666*(2):301-306 (1981). The effect of cyclic AMP analogues on the methylation of phosphatidylethanolamine has been examined. Chrlorophenylthio-cyclic AMP (0.5mM) in the culture medium of rat hepatocytes inhibited by approximately 50% the conversion of phosphatidylethanolamine to phosphatidylcholine in the intact cells. In contrast, the microsomal activity of phosphatidylethanolamine-N-methyltransferase was stimulated 2-fold. The mechanisms of these cyclic AMP effects are unknown.

LIPOPROTEIN APOLIPOPROTEIN SYNTHESIS BY HUMAN HEPATOMA CELLS IN CULTURE. J.M. Rash, G.H. Rothblat, and C.E. Sparks (Dept. of Physio. and Biochem., The Med. College of PA, Philadelphia, PA 19129) *Biochim. Biophys. Acta 666*(2)294-298 (1981). Lipoprotein synthesis was demonstrated by double diffusion with low density lipoprotein antibody, and by ^{3}H-labeled amino acid incorporation into proteins of the d <1.063 g/ml centrifugally isolated lipoprotein fraction. Radioactive label was incorporated predominantly into apolipoprotein B (60%), apolipoprotein A-I (20%) and apolipoprotein C (12%), as determined by Sepharose column chromatography and polyacrylamide gel electrophoresis. Incorporation of radioactive label into apolipoprotein B was inhibited by the presence of albumin in the medium, and was restored to apolipoproteins could be modified by culture conditions. The human hepatoma cell line, Hep G2, provides a potential in vitro model for the study of regulation of human hepatic lipoprotein and apolipoprotein synthesis.

IN VIVO SYNTHESIS OF LIPID-LINKED OLIGOSACCHARIDES IN THE LIVERS OF NORMAL AND VITAMIN A-DEFICIENT RATS. G.C. Rosso, C.J. Bendrick and G. Wolf (Dept. of Nutrition and Good Science, Massachusetts institute of Technology, Cambridge, MA 02139) *J. Biol. Chem. 256*(16):8341-8347 (1981). [1-^{14}C] Glucosamine, [2-^{3}H] mannose, or [^{14}C] galactose were injected into vitamin A-deficient and pair-fed control rats at an early stage of deficiency. The livers were homogenized, centrifuged, and the oligosaccharide-lipids extracted into chloroform:methanol:water (10:10:3). This fraction, when labeled with glucosamine, reached a maximum at 35 min after injection, and remained constant to 110 min. The fraction was chromatographed on a DEAE-cellulose-acetate column and was eluted at an ammomium acetate concentration of 20 mM, corresponding to oligosaccharide bound to dolichylpyrophosphate. Fractionation on Bio-Gel P-4 of the oligosaccharide produced by mild acid hydrolysis of the oligosaccharide-lipid, gave a major peak (I) followed by a broad minor peak (II) of smaller molecular weight. In a recovery experiment, vitamin A was given intragastrically to deficient rats. It caused virtual disappearance of peak II between 4 and 8 hr after administration. We conclude that normal rat liver accumulates an oligosaccharide linked to dolichylpyrophosphate consistent with the structure $(glucose)_3(mannose)_9(N\text{-acetylglucosamine})_2$, whereas vitamin A deficiency causes an increased pool of smaller molecular weight oligosaccharide-lipids, the principal one being consistent with $(mannose)_5(N\text{-actyl-glucosamine})_2$-dolichylpyrophosphate.

ABSENCE OF CHOLESTEROGENESIS REGULATION IN THE LIVER AND PROSTATE OF THE BIO 87.20 HAMSTER. C.P. Schaffner, D.R. Brill, A.K. Singhal, D.P. Bonner, N.I. Goldstein, and G.M. Wang (Waksman Inst. of Microgio., Rutgers—The State Univ. of New Jersey, P.O. Box 759, Piscataway, NJ 08854) *Lipids 16*(11):835-840 (1981). Normal, adult golden Syrian hamsters and the inbred stain BIO 87.20 Syrian hamsters were maintained on either control, cholesterol, candicidin or clofibrate diets for time periods of up to 4 months. The ventral prostate gland in both species was found to synthesize cholesterol at a greater rate than the liver. Also, our results show that, while hepatic cholesterol synthesis in the normal Syrian hamster is underfeedback control with dietary cholesterol, hepatic cholesterol synthesis in the BIO 87.20 hamster, and prostatic cholesterol synthesis in either species, is under no such control. This apparent regulatory defect in the BIO 87.20 hamster, which results in a dramatic accumulation of cholesterol in the liver and serum, renders this animal a potentially valuable in vivo model for the study of cholesterol-related disorders.

INHIBITORS OF STEROL SYNTHESIS: CHEMICAL SYNTHESIS AND ACTIVITIES OF NEW DERIVATIVES OF 15-OXYGENATED STEROLS. G.J. Schroepfer, Jr., E.J. Parish, A. Kisic, D.M. Frome and A.A. Kandutsch (Depts. of Biochemistry and Chemistry, Rice University, Houston, TX 77001) *Chem. Phys. Lipids 29*(3): 201-211 (1981). The chemical syntheses of 5α-cholestane-3β, 14α-15β-triol, 5α-cholestane-14α-ol-3,15-dione, 5α-cholestane-3β, 14α-diol-15-one, 14α,15α-epoxy-5α-cholestan-3β-ol, and 5α-cholest-8(14)-en-3βol-15-one oxime are described. All of these compounds were found to be potent inhibitors of sterol synthesis in cultured mouse L cells. However, the former three compounds had little or no effect on the levels of 3-hydroxy-3-methylgutaryl (HMG)-CoA reductase in the same cells. In contrast, in the case of the latter two compounds, the concentrations required to cause a 50% inhibition of the synthesis of digitonin-precipitable sterols were comparable to those required to cause a 50% reduction in the levels of HMG-CoA reductase in the same cells. 5α-Cholest-8(14)-en-3β-ol-15-one oxime had no effect on serum cholesterol levels when administered to male rats at a level of 0.15% in a cholesterol-free diet.

METABOLISM OF EPOXIDIZED PHOSPHATIDYLCHOLINE BY PHOSPHOLIPASE A_2 AND EPOXIDE HYDROLASE. A. Sevanian, R. Stein, J. Mead (Laboratory of Nuclear Medicine and Radiation Biology, Univ. of Cal., L.A., CA 90024) *Lipids 16*(11):781-789 (1981). The isolation and measurement of phospholipid epoxides as major peroxidation products in biomembrane preparations prompted an investigation of enzymatic mechanisms which may be responsible for their elimination. Analysis of microsomal epoxide hydrolase and phospholipase A_2 activity against a phospholipid epoxide commonly encountered in tissues indicated it to be a poor substrate for epoxide hydrolase, but rapidly hydrolyzed by phospholipase A_2. Microsomal and purified phospholipase A_2 preparations hydrolyzed the phospholipid epoxide at rate 2-fold

greater than were observed with a monoenoic phospholipid from which the epoxide would be derived. The product fatty acid epoxide, cis-9,10 epoxystearic acid, was rapidly hydrated by microsomal and cytosolic epoxide hydrolase. On the basis of earlier reports demonstrating increased phospholipase activity against oxidized phospholipids, and on the results of the present study, a model for the metabolism of oxidized membrane phospholipids is proposed.

ENZYMATIC SULFATION OF STEROIDS. XVI. VERY RAPID EFFECTS OF STEROID HORMONES ON HEPATIC CORTISOL SULFATION IN INTACT MALE RATS. S.S. Singer and A. Moshtaghie (Chemistry Department, University of Dayton, Dayton, OH 45469) *Biochim. Biophys. Acta 666*(2):212-215 (1981). Hepatic glucocorticoid sulfotransferase activity in male rats was elevated approximately 200, 100 or 60%, respectively, by administration of 0.20 mg estradiol, 1.0 mg testosterone or 12 mg progesterone daily for 2 days. Administration of 3.0 mg corticosterone daily, for 2 days, was without effect. All observed hormone effects were due to elevation of the concentration of sulfotransferase III, the glucocorticoid-preferring steroid sulfotransferase of rat liver. The response of the enzyme activity to the estrogen was blocked by puromycin or actinomycin D. The relationship between these studies and general endocrine control of sulfotransferase production is discussed.

EXCHANGE OF PHOSPHATIDYLCHOLINE BETWEEN RABBIT ERYTHROCYTES AND PLASMA IN VIVO. N.B. Smith and D. Rubinstein (Department of Biophysics, Health Sciences Centre, University of Western Ontario, London, Ontario, Canada N6A 5C1) *Lipids 16*(12):937-939 (1981). Phosphatidylcholine exchange between rabbit erythrocytes and plasma was studied in vivo. The erythrocyte phosphatidylcholine was labeled by exchange in vivo with [^{32}P] phosphatidylcholine and in vitro by acylation with [^{3}H] 16:0 and [^{14}C] 18:2. The erythrocytes were then injected into rabbits and the loss of labeled phosphatidylcholine from the cells by exchange was followed. The rate constants for the exchange of [^{32}P]-, [^{3}H] 16:0-, and [^{14}C] 18:2-phosphatidylcholine were .0131 ± .0010, .0093 ± .0014 and .0074 ± .0013 h^{-1}, and the exchange rates of the labels relative to that of [^{32}P] were 1.0, 0.71 ± .16, and 0.56 ± .14, respectively. These results confirm our earlier in vitro findings and represent the first in vivo demonstration of the dependency of the exchange rate of erythrocyte phosphatdylcholines on their metabolic prehistory.

EFFECT OF BUFFER CONSTITUENTS ON RAT LIVER 3-HYDROXY-3-METHYL GLUTARYL COENZYME A REDUCTASE. M.V. Srikantaiah, N. Noble, L. Orenstein, and R.J. Morin (Departments of Pathology and Medicine, Harbor-UCLA Medical Center, 1000 West Carson St., Torrance, CA 90509) *Lipids 16*(12): 934-939 (1981). Rat liver microsomes prepared in Tris buffer exhibited 3 to 10 times higher 3-hydroxy-3-methyl glutaryl CoA reductase specific activity than microsomes prepared with potassium phosphate buffer. This higher activity was observed in rats killed during mid-light cycle, but microsomes from rats killed during mid-dark cycle showed no significant difference in enzyme activity between buffers. When microsomes prepared in the 2 different buffers were preincubated with ATP and MG^{++}, enzyme activity was inhibited to the same extent. The cytosol fraction in each of the 2 different buffer preparations possessed similar phosphatase activity. The higher 3-hydroxy-3-methyl reductase activity in Tris buffer, therefore, does not appear to be due to differences in phosphorylation or dephosphorylation activity.

BILE ACID INDUCED INTERCONVERSION OF 3-HYDROXY-3METHYLGLUTARYL COENZYME A REDUCTASE IN CULTURED INTESTINE. E.F. Stange, A. Schneider, G. Preclik, and H. Ditschuneit (Div. Metabolism, Nutr., and Gastro., Dept. of Internal Med., Univ. of Ulm, Ulm, F.R.G.) *Biochim. Biophys. Acta 666*(2): 291-293 (1981). The effect of bile acids and bile acid/cholesterol micelles on 3-hydroxy-3methylglutaryl coenzyme A reductase, the key enzyme of cholesterol synthesis, was investigated in cultured intestine. Glycocholic and glycodeoxycholic acid both supressed total (fully activated) reductase activity after 3 h culture. The portion of expressed reductase, determined in the presence of NaF, was unaffected at 3 h, but decreased after 24 h of bile acid treatment. In contrast, total enzyme activity was stimulated up to 2.5-fold at 24 h; this bile acid effect was blocked by additional cholesterol. These results suggest that bile acids modulate both total reductase activity and the activation state of the enzyme in cultured intestine.

THE YEAST FATTY ACID SYNTHETASE. STRUCTURE-FUNCTION RELATIONSHIP AND THE ROLE OF THE ACTIVE CYSTEINE-SH AND PANTETHEINE-SH. J.K. Stoops and S.J. Wakil (Marrs McLean Dept. of Biochemistry, Baylor College of Medicine, Houston, TX 77030) *J. Biol. Chem. 256*(6):8364-8370 (1981). The yeast fatty acid synthetase is a complex of two multifunctional proteins, α and β, and is active only in the hexamer form $\alpha_6\beta_6$. Electron microscopic studies of this complex led to a model for the synthetase as an ovate structure consisting of an equatorial plate-like structure (the α subunit) to which six arches (the β subunit) are equally distributed on either side. Studies involving the bifunctional reagent 1,3-dibromo-2-propanone have shown that this arrangement is necessary for the β-ketoacyl synthetase activity, since its active center requires the juxtaposing of an acyl group attached to an active cystein-SH of one α subunit and a malonyl group attached to a pantetheine-SH of an adjacent α subunit. This conclusion was based in part on the following facts. 1) Iodoacetamide and dibromopropanone inhibit fatty acid synthesis by inhibiting only the β-ketoacyl synthetase activity; acetyl-CoA, but not malonyl-CoA, protected the synthetase against these inhibitors, suggesting that these reagents react with the site of acetyl binding on the enzyme. 2) Dibromopropanone cross-links the α subunits, yielding oligomers of higher molecular weights. 3) The residues involved at the β-ketoacyl synthetase sites were identified as the active cysteine-SH of one α subunit and the pantetheine-SH of the adjacent α subunit. These observations led us to postulate a mechanism of fatty acid synthesis where an active center involves two complementary halves of two α subunits and the arch β subunit. In an $\alpha_6\beta_6$ structure, there exist six sites for fatty acid synthesis, all of which function simultaneously.

MODULATION OF ENZYME ACTIVITIES IN ISOLATED LYMPHOCYTE PLASMA MEMBRANES BY ENZYMATIC MODIFICATION OF PHOSPHOLIPID FATTY ACIDS. M. Szamel and K. Resch (Institute for Virus Research, German Cancer Research Center, D-6900 Heidelberg, Federal Republic of Germany) *J. Biol. Chem. 256*(22):11618-11623 (1981). Plasma membranes were purified from calf thymus lymphocytes after disrupting the cells by nitrogen cavitation. The phospholipids were modified by incubation of the plasma membranes in the presence of lysophosphatidylcholine with increasing concentrations of the coenzyme A derivatives of long chain fatty acids. Incorporation of linoleic acid (18:2) or arachidonic acid (20:4) markedly modulated plasma membrane-associated enzymes with a peak effect at the same degree of fatty acid substitution. The activity of (Na^+ + K^+)- ATPase was increased, which was reversed to ground levels at higher incorporation rates. The activity of lysolecithin acyltransferase itself was also increased, more pronounced towards arachidonoyl coenzyme A, compared to oleoyl coenzyme A. The data show that enzyme-driven phospholipid modification offers a physiological tool for the study of the interrelation of membrane proteins with the structure of phospholipids. Moreover, they suggest a crucial role of lysolecithin acyltransferase in the initiation of lymphocyte activation.

AN ASSESSMENT OF THE SPECIFICITY OF STEROL UPTAKE AND ESTERIFICATION IN *SACCHAROMYCES CEREVISIAE*. F.R. Taylor and L.W. Parks (Dept. of Microbio., Oregon State Univ., Corvallis, OR 97331) *J. Bio. Chem. 256*(24):13048-13054 (1981). By growing a sterol-requiring strain of *Saccharomyces cerevisiae* in the presence of pairs of sterols differing by a single structural change, the *in vivo* specificity of sterol uptake and esterification was measured. Uptake specificity was demonstrated for the Δ^5-, Δ^7-, and Δ^{22}-bonds as well as the 24β-methyl. Sterol uptake was shown to depend on the metabolic state of the cell, and the apparent K_m of uptake for ergosterol (11.1μM) was lower than that of cholesterol (66.7μM). This difference in apparent K_m can explain the preferential utilization of ergosterol. The selectivity for esterification showed that sterols lacking the Δ^7 - or Δ^{22} - bond or the 24β-methyl were preferentially esterified. This specificity of uptake and esterification did not change significantly with alterations in the fatty aicd source. These results suggest that both uptake and esterification are used to control the types of sterols in the free sterol fraction, resulting in the enrichment of ergosterol-like sterols in cellular membranes. An additional finding was that cells supplemented with sterols which have a $\Delta^{5,7}$-diene (7-dehydrocholesterol and ergosterol) had much reduced levels of steryl ester. This may be attributable to inhibition by a breakdown product(s) of these sterols.

THE EFFECTS OF ENERGETIC STEADY STATE, PYRUVATED CONCENTRATION, AND OCTANOYL-(-)-CARNITINE ON THE RELATIVE RATES OF CARBOXYLATION AND DECARBOXYLATION OF PYRUVATE BY RAT LIVER MITOCHONDRIA. W.D. Thienen and E.J. Davis (Indiana University School of Medicine, Dept. of Biochemistry, Indianapolis, IN 46223) *J. Biol. Chem. 256*(16):8371-8378 (1981). Rat liver mitochondria were incubated with controlled concentrations of pyruvate over a range of energetic and respiratory steady states, in the presence and absence of octanoyl-(-)-carnitine in order to evaluate conditions and effectors which perturb the absolute and relative rates of flux through pyruvate carboxylase and pyruvate dehydrogenase. Control experiments using saturating concentrations of pyruvate are also reported. It is concluded that the energetic state of the liver cell, and especially the availability of fatty acids, can trigger a very effective coordinated switch in gating pyruvate carbon to oxaloacetate or acetyl-

CoA. Endocrine signals may initiate this gating by altering these parameters.

INTESTINAL CHOLESTEROL UPTAKE FROM PHOSPHOLIPID VESICLES AND FROM SIMPLE AND MIXED MICELLES. A.B.R. Thomson and L. Cleland, Division of Gastroenterology, Dept. of Med., Univ. of Alberta, Edmonton, Canada T6G 2G3. *Lipids 16* (12):881-887 (1981). This study was undertaken in vitro to examine the rat jejunal uptake of cholesterol from phospholipid vesicles and from mixed bile salt micelles, under conditions of low effective resistance of the intestinal unstirred water layer. Cholesterol uptake, J_d, occured from vesicles only when the cholesterol: phospholipid ratio was high. The addition of phospholipid (PL) to micelles comprising 20 mM taurodeoxycholic acid (TDC) extended the concentration of cholesterol, beyond which the relationship between cholesterol concentration and uptake remained linear. When the concentration of cholesterol in the bulk phase was kept constant and the concentration of TDC or of PL added to the TDC was increased, there was a decline in cholesterol uptake; this effect was masked when the concentration of TDC was high, or when higher concentrations of PL were added to mixed micelles composed of cholesterol, TDC and PL, the uptake of cholesterol decreased; in contrast, cholesterol uptake progressively increased when palmitic acid was added to simple TDC micelles. The results suggest that the mechanism responsible for cholesterol uptake may vary, depending on the nature of the constituents of the micelle, and it is proposed that PL inhibits the intestinal uptake of cholesterol by altering the partitioning of cholesterol out of the micelle.

MITOCHONDRIAL HYDROXYLATION OF THE CYCLOHEXANE RING AS A RESULT OF β-OXIDATION BLOCKADE OF A CYCLOHEXYL SUBSTITUTED FATTY ACID. J. Tulliez, E. Durand, and JPeleran (Laboratoire de Recherches sur les Additifs Alimentares, I.N.R.A.-180, chemin de tournefeulle, 31300 Toulouse, France) *Lipids 16*(12):888-892 (1981). Among the urinary metabolites of dodecylclohexane or cycloexyldecanoic acid produced by terminal oxidation of the alkyl chain of the cylcoparaffin. Three hypotheses were tested: (a) hydroxylation by the liver microsomal mixed-function oxidases involved in detoxication mechanisms; (b) hydroxylation by a cyt. P_{450}-containing mitochondrial hydroxylase; and (c) β-oxidation blockade after the reaction catalyzed by enoyl-CoA-hydratase. Liver microsomal or mitochondrial fractions were prepared and incubated in the presence of ^{14}(C) cyclohexylacetic. acid, glucose-6-phosphate dehydrogenase and a NADPH-producing system. On the other hand, mitochondria were incubated in a suitable respiratory medium with or without cofactors required for ATP production. The reaction products were extracted and analyzed by thin layer radiochromatography and radio gas chromatography. Evidence is given that hydroxylation of cyclohexylacetic acid in position 1 is a mitochondrial step requiring activation in the acyl-CoA form and results from β-oxidation blockade, the cyclohexane ring hidering hydroxyacyl-CoA-dehydrogenase action.

FATTY ACYL COENZYME A-SENSITIVE ADENINE NUCLEOTIDE TRANSPORT IN A RECONSTITUTED LIPOSOME SYSTEM. G. Woldegiorgis, E. Shrago, J. Gipp and M. Yatvin (Depts. of Nutritional Sciences, Medicine, and Human Oncology, University of Wisconsin, Madison, WI 53706) *J. Biol. Chem. 256*(23):12297-12300 (1981). The adenine nucleotide translocase was purified from bovine heart mitochondria and incorporated into membranes of phospholipid liposomes. The rate of transport of the adenine nucleotides was competitively inhibited by oleoyl coenzyme A with an approximate K_i of 1.0 μM. Significant inhibition was limited to those fatty acyl coenzyme A esters which are carnitine dependent for their oxidation in isolated mitochondria. Octanoyl coenzyme A was also most completely inactive as was palmitic acid and palmitoyl carnitine. By comparing the inhibitory characteristics of carboxyatractylate and bongkrekic acid with those of oleoyl-CoA, it was determined that the fatty acyl-CoA esters could produce inhibition whether the carrier was inserted into the liposome in either the conventional (65%) or reverse (30%) orientation. The results demonstrate that the interaction of long chain fatty acyl-CoA esters with the ADP/ATP carrier in a purified reconstituted system mimics their effects with isolated mitochondria and inverted submitochondrial particles. In general, these findings are consistent with the role of acyl-CoA esters acting as natural ligands and biological effectors of the translocator.

METABOLISM OF 2-HEXADECYNOATE AND INHIBITION OF FATTY ACID ELONGATION. R. Wood and T. Lee (Lipid Research Laboratory, Dept. of Biochemistry and Biophysics, and the Texas Agriculture Experiment Station, Texas A & M University, College Station, Texas 77843) *J. Biol. Chem. 256*(23): 12379-12386 (1981). Dietary methyl-2-hexadecynoate appeared to inhibit fatty acid elongation in intact animals. Data from the present *in vitro* studies indicate that the microsomal elongation system is inhibited preferentially to the mitochondrial system. A series of metabolic acyl-CoA thioester intermediates has been isolated, characterized, and identified from microsomal and mitochondrial incubations with the 2-hexadecynoic acid (16≡1Δ2). The data support the following conclusions: 1) 16≡1Δ2 is activated to the CoA ester; 2) 16≡1Δ2 is acted on by an isomerase to produce a 2,3-allene; 3) either 16≡1Δ2 or the allene, or both, are hydrated to yield a β-keto-CoA thioester after rearrangement; 4) the B-keto ester is reduced to the β-hydroxylacyl-CoA; 5) dehydration of the β-hydroxy ester gives rise to *trans*-Δ2-hexadecenoate which accumulates; and 6) accumulation of the latter results from the inhibition of enoyl-CoA reductase by the 2,3-allene. The occurrence of *cis* and *trans* Δ3-hexadecenoates indicates the allene is reduced, after which the Δ3 monoene isomer may be isomerized to the Δ2 monoene by the acetylene isomerase or a different enzyme. Indirect evidence suggests that the fatty acid elongation systems may also be inhibited at another site.

NONPOLAR LIPID METHYLATION. BIOSYNTHESIS OF FATTY ACID METHYL ESTERS BY RAT LUNG MEMBRANES USING S-ADENOSYLMETHIONINE. M. Zatz, P.A. Dudley, Y. Kloog and P. Markey (Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, MD 20205) *J. Biol. Chem. 256*(19): 10028-10032 (1981). Fatty acid methyl esters are the major radioactive lipid products obtained after incubation of rat lung membranes with [methyl-^{3}H or ^{14}C] S-adenosylmethionine. Evidence which suggests an enzymatic transmethylation includes: time and protein dependence, lack of reaction at 0 C or with heat-denatured membranes, an apparent affinity for S-adenosylmethionine of about 1 μM, inhibition by S-adenosylhomocystein, and lack of inhibition by 0.1% methanol. Activity was highest in microsomes but present in other membranous fractions. Endogenous activity was highest in membranes from parotid, lung, and pancreas. Products were analyzed by organic solvent extraction, thin layer chromatography, column chromatography, high performance liquid chromatography, and gas chromatography. Identification of methylpalmitate, methylstearate, methyloleate, and methyllinoleate was confirmed by mass spectrometry. Presence of the radioactive methyl group was demonstrated by the variation of isotopic ratios with specific activity. Addition of oleate to incubation mixture increased the rate of product formation and preincubation experiments suggested the absence of long lived intermediates. The data suggest an enzymatic transfer of methyl groups from S-adenosylmethionine to free fatty acids.

PLASMA EXTRADIOL, THYROID HORMONES, AND LIVER LIPID CONTENT IN LAYING HENS FED DIFFERENT ISOCALORIC DIETS. Y. Akiba, L.S. Jensen, C.R. Barb, and R.R. Kraeling (Department of Poultry Science, University of Georgia) *J. Nutr. 112* (2):299-308 (1982). Two experiments were conducted with laying hens fed a corn-soy basal diet or diets containing fish meal, distillers dried grains with solubles (DDGS) or torula yeast formulated to be isocaloric and isonitrogenous with the basal diet. One half of the hens were kept at a temperature range of 13-24° and the other half at 24-35° for 49 days in experiment 2. Liver lipid content was significantly lower in hens fed DDGS (experiment 1) and DDGS or fish meal (experiment 2) than in hens fed the corn-soy basal diet, but it was not influenced by environmental temperature. Feeding DDGS or fish meal reduced lipoprotein lipase activity in adipose tissue. High temperature reduced plasma estradiol level but not thyroid hormone levels. Plasma estradiol in the hens fed DDGS or fish meal (experiment 1) and DDGS, fish meal or torula yeast (experiment 2) and plasma thyroxine and triiodothyronine in hens fed the DDGS or fish meal at 24-35° in experiment 2 were significantly lower than that of hens fed the corn-soy basal diet. Significant correlations were observed between liver lipid content and plasma estradiol or thyroxine concentrations. These findings show that plasma estrogen and thyroxine levels were influenced by diet composition and that these hormones have a close relation to induction of fatty livers in laying hens.

HERBAL REMEDIES OF THE MARITIME INDIANS: STEROLS AND TRITERPENES OF TANACETUM VULGARE L. (TANSY). R.F. Chandler, S.N. Hooper, D.L. Hooper, W.D. Hamieson, and E. Lewis (College of Pharmancy, Department of Chemistry, Dalhousie University, Halifax, Nova Scotia, Canada; and Atlantic Research Laboratory, National Research Council, Halifax, Nova Scotia, Canada) *Lipids 17*(2):102-106 (1982). Plant sterols and triterpenes exhibit a wide range of pharmacological activites. As part of our ongoing studies of the medicinal aspects of Maritime flora, particularly the herbal remedies of the Micmac and Malecite Indians, we determined the nature of the sterols and triterpenes of Tanacetum vulgare L. (Compositae) - a widely used herbal remedy usually referred to as tansy. By using thin layer and gas chromatographics, nuclear magnetic resonance (NMR) spectroscopy and combined gas chromatography-mass spectrometry, we were able to identify β-sitosterol as

the major sterol and α-amyrin as the triterpene of tansy. We also identified the sterols stigmasterol, campesterol and cholesterol, and the triterpenes α-amyrin and taraxasterol. A fourth triterpene was tentatively identified as pseudo-taraxasterol. The successful therapeutic application of this herb may be due partly to the presence of one or more of these compounds. The sterols and triterpenes of tansy have not been previously reported; neither, to our knowledge, have the NMR spectra of the amyrins and the NMR and mass spectra of taraxasterol.

EFFECTS OF DIETARY SELENIUM AND VITAMIN E ON COVALENT BINDING OF AFLATOXIN TO CHICK LIVER CELL MACROMOLECULES. J. Chen, M.P. Goetchius, G.F. Combs Jr., and T.C. Campbell (Dept. of Poultry and Avian Sciences, Cornell University, Ithaca, NY 14853) *J. Nutr. 112*(2):350-355 (1982). Day-old single comb white Leghorn chicks of both sexes maternally depleted in selenium (Se) and vitamin E (VE) were fed a low Se and VE-free semipurified basal diet or that diet supplemented with graded levels of Se (0.2-20.0 ppm as Na_2SeO_3) or VE (100 IU/g as all-rac-α-tocopheryl acetate), or both. At 14 days of age, chicks were given 1 mg/kg[^{3}H] aflatoxin B_1 (AFB_1) i.p. and killed either 2 or 24 hours later. Covalent binding of AFB_1 to liver DNA and RNA in chicks fed the basal diet was significantly greater than in chicks supplemented with Se or VE, or both. Phenobarbital treatment prior to administration of AFB decreased adduct formation in most groups, and abolished differences in adduct formation due to diet. These results suggest that combined Se-VE deficiency enhances activation or inhibits detoxification of AFB_1 in vivo.

NONCALORIC EFFECTS OF DIETARY FAT AND CELLULOSE ON THE VOLUNTARY FEED CONSUMPTION OF WHITE LEGHORN CHICKENS. J.A. Cherry (Poultry Science Department, Virgini Polytechnic Institute and State University, Blacksburg, Virginia 24061) *Poultry Sci. 61*:345-350 (1982). Prior to adjusting caloric intake, Single Comb White Leghorn pullets changed to diets with 10% cellulose decreased feed consumption, while those changed to diets supplemented with 10% hydrolyzed fat increased feed consumption. The feeding of diets supplemented with both 10% fat and 10% cellulose suggested that the feed consumption responses observed were not due either to metabolizable energy concentrations or feed density. It was concluded that palatability was an important factor contributing to the voluntary feed intake of chickens on a relatively short term basis.

EFFECTS OF GARLIC ON LIPID METABOLISM IN RATS FED CHOLESTEROL OR LARD. M.S. Chi, E.T. Koh, T.J. Stewart (Departments of Home Economics and Chemistry, Alcorn State University, Lorman, MS) *J. Nutr. 112*(2):241-248 (1982). Effects of garlic on lipid metabolism were studied in three experiments using different aged male rats fed a diet containing 1% cholesterol or 15% lard. Lyophilized garlic was supplemented at 2% and 4% of the diet. Plasma glucose was not changed by dietary treatments. Rats fed cholesterol and lard diets increased plasma cholesterol and triglycerides compared to controls. Garlic decreased plasma cholesterol in cholesterol- and lard-fed rats, but decreased plasma triglycerides only in the lard-fed group. Garlic supplementation decreased very low density lipoprotein cholesterol and increased high density lipoprotein cholesterol. The liver weight, total liver lipid and cholesterol were increased in rats fed the cholesterol diet but a supplementation of garlic decreased those parameters by about 30%. Dietary cholesterol and lard decreased hepatic glucose-6-phosphate dehydrogenase and malic enzyme activities: the garlic supplementation further decreased these enzyme activities. Garlic feeding increased the excretion of the neutral steroids in both 16 week and 10-week-old rats and bile acids in only 16-week-old pair-fed rats. Garlic at the 2% level was similarly effective on lipid metabolism as at 4%. These results demonstrate that garlic increase the excretion of neutral and acidic steroids and exerts hypocholesterolemic effects in cholesterol-fed rats.

MYO-INOSITOL ACTION ON GERBIL INTESTINE ASSOCIATION OF PHOSPHATIDYLINOSITOL METABOLISM WITH LIPID CLEARANCE. S-H.W. Chu and R.P. Geyer (Dept. of Nutrition, Harvard School of Public Health, Boston, MA 02115) *Biochim. Biophys. Acta 710*(1):63-70 (1982). The synthesis and turnover of phosphatidylinositol as well as lipid clearance were studied in the intestines of lipodystrophic gerbils treated with or without an intraperitoneal dose of *myo*-inositol by monitoring the incorporation of $^{32}P_i$ and the retention of absorbed [1-^{14}C] palmitic acid. *myo*-Inositol deficiency produced an intestinal lipodystrophy with a large lipid accumulation and a decreased level of phosphatidylinositol. Upon *myo*-inositol repletion, the intestinal phosphatidylinositol rapidly returned to the control level by 2 hr, at which time the removal of excess lipid still remained in a lag phase. *myo*-Inositol injection caused an increase in the incorporation of $^{32}P_i$ into phosphatidylinositol mainly due to an increased phosphatidylinositol synthesis de novo. As a result, the turnover of phosphatidylinositol molecules might increase because of an expanded pool size. The stimulation of phosphatidylinositol synthesis was then followed by an enhanced clearance of absorbed [^{14}C] palmitate and by an intestinal recovery which was monitored by the loss of accumulated triacylglycerol. This study indicates that *myo*-inositol availability appears to regulate the in vivo biosynthesis of phosphatidylinositol which, in turn, may play a crucial role in normal lipid transport across gerbil intestine.

ORAL CONTRACEPTIVE AND PLATELET LIPID BIOSYNTHESIS IN FEMALE RATS: DOSE-RESPONSE RELATIONSHIP. M. Ciavatti, E. Davenas, G. Michel, and S. Renaud (INSERM, Unit 63, 22 avenue du Doyen Lépine, 69500 Bron, France; and Laboratoire de Biochimie Microbienne, Université Claude Bernard, 69622 Villeurbanne Cedex, France) *Lipids 17*(2):111-114 (1982). Female rats were treated with different doses of an oral contraceptive (ethinyl estradiol + lynestrenol) and lipid biosynthesis was studied in blood platelets by acetate incorporation into different fractions separated by thin layer chromatography. A marked increase in lipid biosynthesis was observed, especially in the sterol fractions (cholesterol and lanosterol-dihydrolanosterol). It was dose-dependent, observed after a lag-phase, maximal in 3 days and normalized in 8 days. Thus, the oral contraceptive studied here appears to modify platelet biosynthesis for the entire life of the platelets.

CORRELATION OF PERCENT BODY FAT WITH BODY SPECIFIC GRAVITY IN RATS. W.T. Dahms and A.R. Glass (Depts. of Pediatrics and Medicine, University of California at Los Angeles, Harbor General Hospital Campus, Torrance, CA 90509) *J. Nutr. 112*(2):398-400 (1982). We determined the body specific gravity of young rats from their submerged weight. Trapped air in the carcass was eliminated by removing the respiratory and gastrointestinal tract and by dissolving body hair with a depilatory. Body specific gravity was highly correlated with percent fat determined by an extraction method (r=.977) over a wide range of body fat content produced by dietary alterations.

ARACHIDONIC ACID METABOLISM IN ISOLATED RAT AORTA. R. Dücing, R. Shcerhag, R. Tippelmann, U. Budde, K. Glänzer, and H.J. Kramer (Medizinische Universitäts-Poliklinik and Institut für Experimentelle Hämatologie and Bluttransfusionswesen der Universität, Bonn, West Germany) *J. Bio. Chem. 257*(4):1993-1996 (1982). Slices of rat aorta were incubated in Krebs-Ringer bicarbonate buffer for measurements of immunoreactive 6-keto-prostaglandin $F_{1\alpha}$, thromboxane (TX) B_2, prostaglandin (PG)E_2, $PGF_{2\alpha}$, and in Tris buffer (pH 9.3) for determination of prostacyclin (PGI_2)-like activity. No significant generation of TXB_2, PGE_2, or $PGF_{2\alpha}$ by rat aortic tissue could be detected. The time dependent release of 6-keto-$PGF_{1\alpha}$ into Krebs-Ringer bicarbonate buffer closely correlated with PGI_2 generation in alkaline Tris buffer. During a 30-min incubation period, 6-keto-$PGF_{1\alpha}$ release was 79.8 ± 3.3 pmol/mg at a buffer potassium concentration of 3.9 mmol/liter and significantly increased by 23% to 98.3 ± 8.5 pmol/mg ($P<0.025$) in the absence of potassium in the incubation medium. A smaller decrease in buffer potassium concentration to 2.1 mmol/liter and an increase to 8.8 mmol/liter did not significantly alter aortic 6-keto-$PGF_{1\alpha}$ release. Changes in the incubation buffer sodium concentration from 144 mmol/liter to either 138 or 150 mmol/liter at a constant potassium concentration of 3.9 mmol/liter did not alter the recovery of 6-keto-$PFG_{1\alpha}$. Our results support the concept that PGI_2 is the predominant product of arachidonic acid metabolism in rat aorta. They further show that PGI_2 can be recovered quantitatively as 6-keto-$PGF_{1\alpha}$ under the present in vitro conditions. In addition, this invitro study points to the potassium ion as a modulator of vascular PGI_2 synthesis with a stimulation to low potassium concentrations.

ROLE OF DIETARY SATURATED FATTY ACIDS ON LOWERING THE INCIDENCE OF HEART LESIONS IN MALE RATS. E.R. Farnworth, J.K. G. Kramer, B.K. Thompson, and A.H. Corner (Animal Research Centre, Agriculture Canada, Ottawa, Canada) *J. Nutr. 112*(2):231-240 (1982). Male weanling rats were fed soybean or low erucic acid rapeseed oils alone or in combination with cocoa butter (a source high in saturates) or triolein for 16 weeks. All diets contained 20% by weight of the test oils. The apparent digestibility of all diets and test oils increased with the age of the rat. The apparent digestibility of the saturated fatty acids was lower in rats fed the diets containing cocoa butter. The relative organ weights, however, were not affected by diet, but growth was improved by supplementing the vegetable oils with cocoa butter. This growth difference was significant for the addition of cocoa butter to low erucic acid rapeseed oil. After 16 weeks all groups of rats developed myocardial necrosis. A dramatic lowering of myocardial lesion incidence was observed in rats fed diets enriched with saturated fatty acids. The

results of the present experiment suggest that enriching a vegetable oil with saturated fatty acids affects both nutritional and cardiopathological properties of the oil.

WITHDRAWAL TIME REQUIRED FOR CLEARANCE OF AFLATOXINS FROM PIG TISSUES. R.M. Furtado, A.M. Pearson, M.G. Hogberg, E.R. Miller, J.I. Gray, and S.D. Aust (Department of Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan 48824) *J. Agric. Food Chem. 30*:101-106 (1982). The time necessary to obtain clearance of aflatoxins from the tissues of the pig after removal from a contaminated diet was determined in two trials involving 20 pigs in each. There was a significant reduction in aflatoxin levels in all organs and tissues 1 day after placing the pigs on an aflatoxin-free ration. After 2 days, only one pig contained trace amounts (<0.50 μg/kg) of aflatoxins in the tissues. Four days after removal from the contaminated diet, there were no detectable levels of aflatoxins in any of the tissues. It was also found that a naturally contaminated diet containing only 20 and 31 μg/kg aflatoxins B_1 and B_2, respectively, resulted in traces of aflatoxins B_1, B_2, M_1, and M_2 in the livers and kidneys after 13-14 h withdrawal from the contaminated diet, although none were detected in any other tissues.

LATERAL MOBILITY OF PHOSPHOLIPIDS IN TURKEY ERYTHROCYTES. Y.I. Henis, G. Rimon, and S. Felder (Department of Biochemistry, The George S. Wise Faculty of Live Sciences, Tel-Aviv University, Tel-Aviv, Israel) *J. Biol. Chem 256*(3):1407-1411 (1982). Hormone activation of turkey erythrocyte adenylate cyclase is affected by temperature and by *cis*-vaccenic acid incorporation. This was correlated with changes in membrane "fluidity" which were previously assumed to affect lateral diffusion of the β-adrenergic receptor. To test this hypothesis, we measured the lateral mobility of the fluorescent phospholipid *N*-4-nitrobenzo-2-oxa-1,3-diazoloyl-phosphatidylethanolamine (NBD-PE) in turkey erythrocyte ghosts, using fluorescence photobleaching recovery. Ghosts labeled only the external leaflet exhibited a linear Arrhenuis plot of the lateral diffusion coefficient (d) with little change in the mobile fraction of NBD-PE between 45 and 5°C. Labeling from both sides, however, yielded a break point (~30°C) in the Arrhenius plot and a 30% decrease in the mobile fraction of NBD-PE from 24 to 10°C, indicating differences between the external and inner monolayers. The observed discontinuities are not due to gel-phase formation, since fluorescence depolarization of *cis*- and *trans*-parinaric acid did not reveal discontinuities at this temperature range. *cis*-Vaccenic acid incorporation did not markedly affect either D or the mobile fraction of NBD-PE at 25°C. These results are discussed in view of the effects of temperature and *cis*-vaccenic acid incorporation on adenylate cyclase activation.

HYPERCHOLESTEROLEMIA IN EXHC RATS AND LIPID-LOWERING DRUG: A SCREENING METHOD FOR NEW HYPOCHOLESTEROLEMIC AGENTS. T. Kitazaki, M. Tsuda, H. Matsumura, T. Ohtagaki, and Y. Imai (Biological Research Laboratories, Central Research Division, Takeda Chemical Ind., Ltd. Jusohonmachi, Yodogawa-ku, Osaka, 532, Japan) *Artery 9*(6):414424 (1981). The ExHC rat strain which was selected and bred from the Sprague-Dawley strain, develops severe hypocholesterolemia and is liable to aortic lipid deposition when the rats are fed an atherogenic diet. After only 4 days on the atherogenic diet, hypercholesterolemia was induced in these ExHC rats. Following a study of gelfiltration and SDS-disc electrophoresis, significant increases of broad-LDL (β-VLDL) and of apo E and apo B were noted. On the other hand, plasma HDL and its major apolipoprotein, apo A-I, showed decreases. All these changes might be responsible for the future induction of aortic lipid deposition. A screening method for new hypocholesterolemic agents which takes advantage of these ExHC rat characteristics has been set up; it involves the evaluation of cholesterol lowering and HDL increasing effects by the determination of plasma cholesterol and by radioimmunoassay of apo A-I, respectively. Although clofibrate (100 mg/kg body weight) reduced plasma cholesterol levels significantly, there was no apo A-I increasing effect;

IN VIVO AND IN VITRO ANTILIPOLYTIC EFFECTS OF SOME VARIOUS SUBSTITUTED HOMOCYSTEINE-THIOLACTONE-NICOTINAMIDES: STRUCTURE-ACTIVITY STUDY. F. Maccari, O. Ghirardi, P. DeWitt, and M.T. Ramacci (Research Laboratories, Sigma-Tau S.p.A., Pomezia, Italy) *Lipids 17*(2):78-83 (1982). The antilipolytic activity of homocysteine-thiolactone-nicotinamide (ST22) and its 2-chloro (ST71), 6-chloro (ST82) and 6-hydroxy (ST90) derivatives was investigated by evaluation of serum free fatty acids (FFA) and triglycerides (TG) (in vivo) and FFA release from adipose tissue (in vitro). Increased FFA levels in 17-hr fasted rats at 60 min following treatment with $7 \cdot 10^{-4}$ mol kg^{-1} p.o. were reduced by 70% (ST22), 60% (ST82) and 18% (ST71), whereas ST90 provoked no change; TG levels showed similar changes. Basal FFA release from epididymal rat adipose tissue at 60 min following treatment with $7 \cdot 10^{-4}$ mol kg^{-1} p.o. of ST22 and ST82 was reduced by 79 and 45%, respectively. Lipid mobilization induced by noradrenaline (NA) was diversely affected by the compounds according to the tests employed: with in vivo experiments, serum FFA levels were reduced by 60, 70, 10 and 5% at 60 min following treatment with ST22, ST82, ST71 and ST90, respectively ($7 \cdot 10^{-4}$ mol kg^{-1} p.o.; NA bitartrate, 2 mg kg^{-1} s.c.); in vitro ST22 produced no change, whereas the other compounds induced a significant mobilization of FFA. The results suggest that: (a) antilipolytic activity can be greatly modified when various substituents capable of influencing either the inductive (-I) or the resonance (+M) effect are introduced into the different positions of the pyridine ring; and (b) the lipolysis experiments did not evince any direct relationship between the effects obtained by the in vivo tests and those obtained by the in vitro tests.

RATE OF FOOD PASSAGE (TRANSIT TIME) AS INFLUENCED BY LEVEL OF SUPPLEMENTAL FAT. G.G. Mateos, J.L. Sell and J.A. Eastwood (Dept. of Animal Science, Iowa State University, Ames, IA 50011) *Poultry Sci. 61*(1):94-100. An experiment involving 35 White Leghorn hens was conducted to study the influence of graded levels of supplemental yellow grease on rate of food passage (transit time). Seven experimental diets (0,5,10,15,20,25, and 30% supplemental fat) were formulated. Transit time was determined by utilizing either Cr_2O_3 or ^{144}Ce as indicators. First appearance of the markers in the excreta and percentages of the markers ingested that were recovered in excreta 10 hr after feeding were criteria used to determine transit time. The time required for Cr_2O_3 to appear in the excreta increased linearly with increments of supplemental fat. Average first appearance time of Cr_2O_3 was 193, 219, 214, 227, 251, 250 and 270 min for the diets containing 0, 5, 10, 15, 20, 25, and 30% supplemental fat, respectively. Transit time, measured as percentage of market recovered in excreta 10 hr after feeding, was faster for the control than for the fat-supplemented diets, although the linear effects of fat were not statistically significant. The results show that supplemental fat increased transit time of ingesta in chickens. By increasing transit time, supplemental fats may improve digestibility of other dietary constituents and thereby increase the utilization of dietary energy.

STEROID-PROTEIN INTERACTIONS. HUMAN CORTICOSTEROID-BINDING GLOBULIN: CHARACTERIZATION OF DIMER AND ELECTROPHORETIC VARIANTS. K.E. Mickelson, G.B. Harding, M. Forsthoefel, and U. Westphal (Department of Biochemistry, University of Louisville School of Medicine, Health Sciences Center, Louisville, Kentucky). *Biochem. 21*(4): 654-660 (1982). Human corticosteroid-binding globulin (CBG) forms a dimer that was isolated by gel fitration, has full binding affinity and capacity, and can be dissociated to the monomer. Monomeric CBG consists of two distinct molecular variants, which were detected by polyacrylamide gel electrophoresis in the presence and absence of sodium dodecyl sulfate. The two monomeric CBG species were separated by preparative gel electrophoresis and were found to bind cortisol, as well as progesterone, with equal affinity. They have one steroid binding site per CBG molecule. Amino acid and carbohydrate analyses are essentially the same for both of the CBG variants. Removal of sialic acid or 90% of the carbohydrate did not affect the existence of the two molecular forms. The two CBG species were isolated from each of the sera from five individual donors, indicating that the observed heterogeniety does not result from pooling genetic variants. The two species are immunologically identical. A possible explanation for the existence of the two electrophoretic variants is a difference in amidation.

VITAMIN A REQUIREMENT OF BROILER CHICKS IN NIGERIA. B.K. Ogunmodede (Department of Animal Science, University of Ibadan, Ibadan, Nigeria) *Poultry Sci. 60*(12):2622-2627 (1981). Feeding of a vitamin A deficient diet resulted in cessation of growth of RIR chicks at between 19 and 24 days of age. When depleted chicks were fed graded levels of vitamin A acetate it was observed that the minimum requirement was 90 IU/100 g of diet. Trials with four commercial strains of broiler chicks revealed strain differences with respect to the requirement when body weight gain, feed conversion, liver storage of the vitamin, and blood uric acid were used as indicators of vitamin A status. Coccidiosis was induced and the results showed that under practical conditions at least 150 IU of vitamin A per 100 g of diet should be fed in Nigeria.

REGULATION VOLATILE FATTY ACID UPTAKE BY MITOCHONDRIAL ACYL COA SYNTHETASES OF BOVINE HEART. C.A. Ricks and R.M. Cook (Department of Dairy Science, Michigan State University, East Lansing) *J. Dairy Sci. 64*(12):2336-2343 (1982). Purification of components of heart mitochondria activating

short chain fatty acids prepared from tissue of lactating Holstein cows demonstrated predominantly one acyl CoA synthetase, acetyl CoA synthetase activating acetate, and propionate. Activity of butyryl CoA synthetase was low. Propionyl CoA synthetase characteristically in bovine liver and kidney tissue could not be demonstrated in heart mitochondria. Thus, of the ruminally derived volatile fatty acids only acetate can be used by heart mitochondria as a primary energy source because of small quantities of propionate in peripheral blood. Acetyl CoA synthetase was a glycoprotein composed of a single polypeptide chain of apparent molecular weight 67,500. The Michaelis-Menten constant for acetate was 1.8×10^{-4} M. By comparison with literature for blood acetate concentration we conclude that enzyme is saturated with substrate at all physiological concentrations of acetate. These kinetic properties ensure a constant supply of acetate as an energy source for maintaining heart function in ruminants.

EFFECTS ON CA AND P METABOLISM IN HUMANS BY ADDING MEAT, MEAT PLUS MILK, OR PURIFIED PROTEINS PLUS CA AND P TO A LOW PROTEIN DIET. S.A. Schuette and H.M. Linkswiler (Dept. of Nutri. Sci., University of Wisconsin, Madison, WI 53706) *J. Nutr. 112*(2):338-349 (1982). The effects on calcium and phosphorus metabolism of adult man by adding meat or meat plus dairy products to a diet low in protein (55 g), calcium (590 mg), and phosphorus (890 mg) were determined. When the low protein diet was consumed, the subjects retained a mean of 20 mg calcium daily but lost 106 mg phosphorus. The addition of meat which increased protein and phosphorus to 146 g and 1660 mg, respectively, caused calcium retention to decrease from 19 to -17 mg but phosphorus retention to increase from 106 to 55 mg. When the meat plus dairy diet high in protein (146 g), calcium (1370 mg), and phosphorus (2060 mg) was consumed the subjects retained substantial amounts of calcium (101 mg) and phosphorus (177 mg). The s simulated diets high in purified proteins and supplemented with calcium and phosphorus but they had a marked negative effect on phosphorus retention; this indicates that supplements of calcium gluconate were well utilized but that those of monopotassium phosphate were not. The results obtained on urinary sulfate, acid, cyclic AMP and hydroxyproline support the conclusions made from the calcium and phosphorus data.

BRAIN TARGET SITES FOR 1,25-DIHYDROXYVITAMIN D_3. W.E. Stumpf, M. Sar, and S.A. Clark (Depts. of Anatomy and Pharmacology, University of North Carolina, Chapel Hill) *Science 215 (4538)*:1403-1405 (1982). Autoradiographic studies with H-labeled 1,25 dihydroxyvitamin D_3 [1,25$(OH)_2 D_3$] demonstrate, in certain neurons of rat forebrain, hindbrain, and spinal cord, a nuclear retention and concentration of radioactivity, which can be prevented by treatment with 1,25(OH) D_3, but not with 25-hydroxyvitamin D_3. These results indicate the presence of brain receptors for 1,25 $(OH)_2 D_3$ and suggest a central modulation of calcium homeostasis and other central effects for this hormone. The existence of a brain-putuitary axis for certain 1,25(OH) D_3-mediated endocrine-autonomic effects is postulated.

TISSUE FATTY ACID CHANGES AND TUMOR INCIDENCE IN C3H MICE INGESTING COTTONSEED OIL. I.J. Tinsley, G. Wilson, and R.R. Lowry (Department of Agricultural Chemistry, Oregon State University, Corvallis, OR) *Lipids 17*(2):115-117 (1982). The incidence of spontaneous mammary tumors in C3H mice at 35 wk was higher in mice fed rations containing cottonseed oil than in mice fats of comparable fatty acid composition. The time to 50% (T_{50}) incidence was also shorter in the first group. The fatty acid composition of tissue lipids from mice fed the cottonseed oil showed changes indicating the presence of cyclopropene fatty acids-higher levels of saturates and lower oleate/stearate and palmitoleate/palmitate ratios. A possible association between the development of a spontaneous mammary tumor in the C3H mouse and the presence of cyclopropene fatty acids in the cottonseed oil is indicated.

REGULATION OF SPHINGOMYELIN LONG CHAIN BASE SYNTHESIS IN HUMAN FIBROBLASTS IN CULTURE: ROLE OF LIPOPROTEINS AND THE LOW DENSITY LIPOPROTEIN RECEPTOR. R.B. Verdery, III and R. Theolis, Jr. (Clinical Research Institute of Montreal, Laboratory of Lipoprotein Metabolism, Montreal H2W 1R7, Quebec, Canada) *J. Biol. Chem. 257*(3):1412-1417 (1982). We have studied regulation of synthesis of long chain bases in human fibroblasts using 3 different radioactive precursors and 2 different hydrolysis and separation procedures. Serum and low density lipoproteins inhibited synthesis. Inhibition of long chain base synthesis by various concentrations of low density lipoproteins paralleled inhibition of cholesterol synthesis. This inhibition was dependent on the low density lipoprotein receptor pathway since fibroblasts from homozygous familial hypercholesterolemic patients did not show the inhibition observed with normal fibroblasts. Incorporation of precursor palmitate into free or total long chain bases was inhibited by low density lipoproteins to the same extent as incorporation into sphingomyelin long chain bases. We thus propose that an enzyme in the pathway leading to sphinganine synthesis, probably palmitoyl-CoA:L-serine *C*-palmitoyltransferase (decarboxylating) EC 2.3.1.50, is regulated by low density lipoproteins.

DISORDERS OF CHOLECALCIFEROL METABOLISM IN OLD EGG-LAYING HENS. E. Abe, H. Horikawa, T. Masumura, M. Sugahara, M. Kubota, and T. Suda (Dept. of Biochem., School of Dentistry, Showa Univ., 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142) *J. Nutr. 112*(3):436-446 (1982). It has been reported that the rate of cracked or soft-shelled eggs markedly increases in old laying hens. We investigated the effect of age on cholecalciferol metabolism in different age groups of laying hens. The egg production rate in hens more than 500 days old was maintained within a range of about 70% of that in young hens (230-320 days old), whereas the rate of cracked or soft-shelled eggs increased markedly with age. When kidney homogenates from the different age groups were incubated with [^{3}H]-25-hydroxyvitamin D-3, renal 25-hydroxyvitamin D-3-1α-hydroxylase activity was found to decrease markedly with age. When birds were given intravenously either [^{3}H]-25-hydroxyvitamin D-3 or [^{3}H]-1α,25-dihydroxyvitamin D-3, the accumulation of [^{3}H]-1α,25-dihydroxyvitamin D-3 in plasma and large tissues also decreased with age. Forced molting performed in old hens restored eggshell quality. The treatment also restored, though partially, the in vivo accumulation of [^{3}H]-1α,25-dihydroxyvitamin D-3 in the target tissues. These results suggest that the increased rate of cracked or soft-shelled eggs seen in older birds is associated with disorders of vitamin D-3 metabolism.

DIGESTION AND ABSORPTION OF A SULPHOXIDE ANALOGUE OF TRIACYLGLYCEROL IN THE RAT. B. Åkesson and Peter Michelsen (Dept. of Clinical Chem. and Div. of Organic Chem. 1, Chem. Center, Univ. of Lund, S-221 85 Lund (Sweden)) *Chem. and Physics of Lipids 29*(4):341-349 (1981). The stereochemistry of fat digestion and absorption was studied by feeding a triacylglycerol analogue to rats with a thoracic duct annula. The analogue, *rac*-1,2-dioleoyl-3-S-tetradecyl-3-thioglycerol-*S*-oxide was chosen since its enantiomers exhibited high rotation in optical rotatory dispersion (ORD) and circular dichroism (CD). In the chyle, triacylglycerol was the major lipid but *X*-1,2-diacyl-3-S-tetradecyl-3-thioglycerol-*S*-oxide constituted 8% of lipid weight. It was resolved by thin-layer chromatography (TLC) into two diastereomers. Each of the diastereomers were analyzed for the proportions of 1-thio-*sn*-glycerol/3-thio-*sn*-glycerol isomers by ORD and CD. The 1-thio-*sn*-glycerol isomers dominated for both compounds indicating that they were enriched during the absorption processes, since a racemic compound was fed. The stereospecificities are probably exerted by acyltransferase(s) during chyle lipid synthesis. The methods used will be valuable tools in studies on the metabolism of enantiomeric glycerides and also for characterization of naturally occurring sulphur-containing lipids.

DIRECT DETERMINATION BY RAMAN SCATTERING OF THE CONFORMATION OF THE CHOLINE GROUP IN PHOSPHOLIPID BILAYERS. H. Akutsu (Dept. of Biophysical Chemistry, Biocenter of the University of Basel, CH-4056 Basel, Switzerland) *Biochemistry 12*(26):7359-7566 (1981). For clarification of the assignments of the vibrational modes of the choline group, Raman spectra of choline iodides selectively deuterated at three different positions were investigated. The isotope shifts of the C-N stretching vibrations suggested that they are conformation sensitive. When the Raman spectra of choline chloride, carbamoylcholine iodide, carbamoylcholine choride, and methoxy-carbonycholine iodide are compared with the crystal structures of these compounds, a correlation between the vibrational frequency and the conformation of the O-C-C-N$^+$ backbone could be established. The Raman bands attributed to the "totally" symmetric stretching (υ_1) and symmetric stretching vibrations (υ_2) of the C-N bonds of the quaternary ammonium group appeared at about 720 cm^{-1} and about 870 cm^{-1}, respectively, for the gauche conformation of the O-C-C-N$^+$ backbone, and in the trans conformation, they shifted to about 770 cm^{-1} (υ_1) and about 910 cm^{-1} (υ_2), respectively. On the basis of this correlation and from measurements of phosphatidylcholine and sphingomyelin bilayers, it was concluded that most of the choline groups in both biolayers take the gauche conformation not only in solid state but also in the gel and liquid-crystalline states. These data represent the first direct evidence that a gauche conformation for the O-C-C-N$^+$ bond is preferred in the gel and liquid-crystalline states. These key bands, especially the υ_1 band, are a powerful tool to study the conformation of the choline group in situ not only in the membrane field but also in the neuroscience in connection with acetylcholine.

ABNORMAL HORMONE LEVELS IN MEN WITH CORONARY ARTERY DISEASE. B. Aumoff, R.G. Troxler, J. O'Conner, R.S. Rosenfeld, J. Kream, J. Levin, J.R. Hickman, A.M. Sloan, W. Walker, R.L. Cook, and D.K. Fukushima (Dept. of Medicine, Beth Israel Medical Center, New York, NY 10003) *Arteriosclerosis 2*(1):

58-67 (1982). Plasma concentrations and urinary excretions of various hormones and hormone metabolites were measured in four groups. Group 1 was composed of 13 men with prior myocardial infarction; Group 2 contained 35 clinically normal men; Group 3 consisted of 44 men with normal coronary arteriograms; and Group 4 was composed of 25 men with severe coronary artery disease shown on arteriogram but no infarction. There were four major findings: Group 1 had significantly higher 24-hour mean plasma concentrations of estrone (E1), dehydro-isoandrosterone (DHA), and dehydroisoandrosterone sulfate (DHAS) than Group 2, while Group 3 had the same levels as Group 4; Group 4 had significantly lower urinary excretion of androsterone glucurnide (AG) than Group 3, while Group 1 excreted normal amounts. There are three possible explanations for these findings: 1) myocardial infarction occurring in men with coronary artery disease may elevate the plasma levels of E1, DHA, and DHAS and eliminate the preinfarction depression of urinary AG levels; 2) higher than average levels of E1, DHA, DHAS, and AG may favor the development of infarction in men with coronary artery disease; 3) higher than average levels of E1, DHA, DHAS, and AG may favor survival from any infarction that occurs in men with coronary artery disease. Experimental and epidemiological evidence seems to favor the third possibility.

25-HYDROXYCHOLECALCIFEROL-1-HYDROXYLASE ACTIVITY IN HEAT STRESSED LAYING HENS. C.A. Bailey and C.R. Creger (Dept. of Poultry Sci, Texas Agr. Exp. Stn. Texas A & M Univ., College Stn., Texas 77843) *Poultry Sci. 61*(3):586-588 (1982). The activity of 25-hydroxycholecalciferol-1-hydroxylase was assayed in heat stressed laying hens fed a 3.5% calcium diet with either .625% or .925% phosphorus. The activity of this enzyme in the heat stressed birds was higher than in the control birds ($P \leq .07$). The phosphorus content of the diets had no effect on enzyme activity.

INFLUENCE OF PHOSPHOLIPID STRUCTURE ON STEROL EFFLUX INDUCED BY ALBUMIN-PHOSPHOLIPID COMPLEXES. L.C. Bartholow and R.P. Geyer (Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts 02115) *Biochemistry 21*:1271-1273 (1982). Sterol release from strain L fibroblasts was measured in serum-free medium supplemented with delipidated human serum albumin and various phospholipids. The sterol molecule appears to preferentially interact with the *sn*-2 acyl chain of the phospholipid. The carbonyl oxygen of the phospholipid acyl ester linkage is not required for sterolphospholipid interactions, while the phosphate and choline groups are required. In the presence of the human serum albumin-phospholipid complex, phospholipids containing *trans*-acyl groups are significantly more effective at removing cellular sterol than the corresponding *cis*-acyl group.

FURTHER CHARACTERIZATION OF A CHINESE HAMSTER OVARY CELL MUTANT DEFECTIVE IN LANOSTEROL DEMETHYLATION. D.J. Berry and T. Chang (Dept. of Biochem., Dartmouth Medical School, Hanover, NH 03755) *Biochem. 21*(3): 573-580 (1982). Sensitive in vitro lanosterol 14 - methylsterol oxidase assays, particularly suitable for cell extracts of tissue culture cells, were developed and validated. Using these assays, we showed that the biochemical lesion of mutant 215, a cholesterol-requiring Chinese hamster ovary cell auxotroph isolated and partially characterized previously [Chang, T.Y., Telakowski, C., Vanden Heuvel, W., Alberts, A.W., and Vagelos, P.R. (1977) *Proc. Natl. Acad. Sci.* USA 74, 832-836], was localized at the 4α-methylsterol oxidase enzyme system. The defect in 4α-methylcholesterol oxidase activity in mutant 215 cells could be demonstrated by using either 4,4-dimethylcholestanol as the substrate, suggesting that the enzyme systems responsible for 4α-methyl- and 4,4-dimethylsterols may share a common component. However, demethylation of the C-14α methyl group was found to occur at identical rates in wild-type and mutant 215, suggesting that C-14α demethylation and C-4α demethylation may occur by separate enzyme systems. A [^{3}H] dihydrolanosterol incorporation experiment in intact cells of wild-type and mutant 215 supported these conclusions. Despite these results, a [^{14}C] acetate pulse experiment indicated that [^{14}C] lanosterol, instead of ^{14}C-labeled 14-demethylated sterol derivative(s), accumulated in intact cells of mutant 215. Possible implications of these findings for the mechanisms of lanosterol demethylation reactions are discussed.

REVERSAL BY BILE ACID ON THE INHIBITION OF α-TOCOPHEROL ABSORPTION BY RETINOIC ACID. J.G. Bieri and T.J. Tolliver (Laboratory of Nutrition and Endocrinology National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases National Institutes of Health, Bethesda, MD 20205) *J. Nutr. 112*(2):401-403 (1982). This study explores several possible mechanisms by which dietary retinoic acid may cause the previously described reduced intestinal absorption of α-tocoperol. Measurement of fecal excretion of rats showed that dietary retinoic acid caused twice as much α-tocopherol to be excreted as when retinol was the source of vitamin A. Excretion was the same for free and esterified α-tocopherol, thus, the retinoic acid effect originally observed was not due to impaired hydrolysis of the ester. There was no effect of retinoic acid on triglyceride absorption. Collection and analysis of bile from rats fed either form of vitamin A showed no difference in bile volume or bile acid composition. The addition of 0.2% taurocholic acid to the diet, however, reversed the effect of retinoic acid on tocopherol absorption. In vitro studies of mixed micelles containing H-α-tocopherol and retinoic acid or retinol showed no difference in size due to the form of vitamin A in the micelles.

EFFECT OF pH AND FATTY ACID CHAIN LENGTH ON THE INTERACTION OF MYELIN BASIC PROTEIN WITH PHOSPHATIDYLGLYCEROL. J.M. Boggs, D. Stamp, and M.A. Moscarello (Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada M5G 1X8) *Biochemistry 21*:1208-1214 (1982). The basic protein of myelin binds electrostatically to acidic lipids but has several hydrophobic segments which may penetrate into the lipid bilayer. Calorimetric and spin-label evidence suggests that below the phase transition temperature, T_c, several phase states occur in the complex of phosphatidylglycerol with basic protein, possibly due to differences in the degree of penetration of the protein and/or interdigitation of the lipid acyl chains. One of these states is a metastable state which starts to melt 10 C below the T_c of the pure lipid but restricts the motion of a fatty acid spin-labeled near the terminal methyl much more than does the pure lipid. The relationship between the rate of conversion to the stable state and the degree of penetration of the protein at varying pH, in the range 4-8, and the lipid acyl chain length, in the range 14 to 18 carbons, was investigated. Altering the pH in this range affects protonation of the histidines of the protein but has no effect on the lipid at pH 4 and above. The rate of conversion of the sample to both the metastable state and the stable state decreased with increase in pH for phosphatidylglycerol with all lipid chain lengths. It also decreased with decreasing chain length at constant pH. This suggested that the lipid could refreeze into the stable state more readily if a smaller proportion of the total bilayer thickness was occupied by the hydrophobic segments of the protein. The consistency of these results with the concept of penetration of portions of the protein partway into the bilayer lends support to this hypothesis.

CHANGES IN PLASMA LIPID AND LIPOPROTEIN LEVELS IN MEN AND WOMEN AFTER A PROGRAM OF MODERATE EXERCISE. K.D. Brownell, P.S. Bachorik, and R.S. Ayerle (Dept. of Psych., Univ. of Pennsylvania, 205 Piersol Bldg. Philadelphia, PA 19104) *Circulation 65*(3):477-484 (1982). Levels of high-density lipoprotein (HDL) cholesterol and other lipids and lipoproteins of 24 men and 37 women were measured before and after a 10-week exercise program. The program involved three sessions of aerobic exercise each week, with 15-20 minutes of activity at 70% of maximal heart rate. Men and women had significantly different lipid patterns in response to exercise, despite equivalent increases in maximal oxygen uptake. Men showed a 5.1% increase in HDL cholesterol, a 6% decrease in low-density lipoprotein (LDL) cholesterol, and 12.4% increase in the HLD/LDL ratio. In contrast, women showed a 1% decrease in HDL cholesterol, a 4.3% decrease in LDL cholesterol, and no significant change in the HDL/LDL ratio. The number of sessions attended correlated positively with HDL/LDL changes in men and correlated negatively with HDL/LDL changes in women. These findings suggest that moderate exercise may have different effects on men and women.

NEONATAL UMBILICAL CORD BLOOD LIPOPROTEINS. ISOLATION AND CHARACTERIZATION OF INTERMEDIATE DENSITY AND LOW DENSITY LIPOPROTEINS. P.A. Davis and T.M. Forte (265 Donner Laboratory, University of California, Berkeley, Berkeley, CA 94720) *Arterioscelerosis 2*(1):37-43 (1982). Intermediate density lipoproteins (IDL) (d=1.006 to 1.019 g/ml) and low density lipoproteins (LDL) (d=1.019 to 1.063 g/ml) were isolated from human umbilical cord blood plasma by sequential ultracetrifugation. The concentration, chemical and apolipoprotein composition, size and size distribution of the neonatal IDL and LDL for both sexes were determined. The IDL and LDL from the neonates showed no sex-related differences in composition or concentration. The IDL and LDL were lower in concentration and differed in composition with regard to each other and with regard to the comparable adult fractions. The apolipoprotein (apo) composition showed only the high molecular weight form of apo B present in the IDL, while the LDL showed the presence of two lower molecular weight forms of apo B in addition to the high molecular weight form, along with appreciable amounts of apo E and apo A-I. The size distribution of the neonatal IDL and LDL showed a constant pattern, with peaks at approximately 300 Å for IDL and 257 and 244 Å for neonatal LDL. The alterations in composition, size and size distribution as well as the lower concentrations present in the neonate, point to differences between the neonate and the adult in the metabolism of

lipoproteins with a density of 1.006 to 1.063 g/ml.

COMPARISON OF SUBCUTANEOUS FAT THICKNESS, MARBLING AND QUALITY GRADE FOR PREDICTING PALATABILITY OF BEEF. H.G. Dolezal, G.C. Smith, J.W. Savell, and Z.L. Carpenter (Meats and Muscle Biol. Section, Dept. of Animal Sci., Texas Agr. Exp. Stn., Texas A & M Univ., College Stn., TX 77843) *J. of Food Sci. 47*(2):397-401 (1982). Beef from yearling steers (n= 254) which were fed either grass only or high-concentrate diets was used to study subcutaneous fat thickness as an alternative method for grading beef carcasses. Assigning carcasses to three expected-palatability groups based on fat thickness was at least equivalent to, and perhaps slightly more precise than, the use of USDA quality grades for grouping the carcasses according to expected palatability. There were progressive increases in palatability of cooked beef as fat thickness of carcasses from cattle fed 90-160 days increased from less than 2.53 mm up to 7.61 mm, but quantitites greater than 7.61 mm did not further improve palatability.

EFFECTS OF VITAMIN E AND SELENIUM ON COPPER-INDUCED LIPID PEROXIDATION *IN VIVO* AND ON ACUTE COPPER TOXICITY. J.J. Dougherty and W.G. Hoekstra (Dept. of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin-Madison, Madison, WI 53706) *Proc. Soc. Exp. Biol. Med. 169*(2):201-208 (1982). Copper sulfate injected intraperitoneally at a dose of 2 mg Cu/kg into vitamin E- and selenium-deficient rats caused a sixfold increase in the formation *in vivo* of the lipid peroxidation product ethane, and caused acute mortality in 4/5 rats. Selenium supplementation of the diet at 0.5 ppm Se largely prevented the increase in ethane production caused by copper injection and reduced mortality to 1/5 rats. Vitamin E supplementation of the diet at 200 IU/kg fully eliminated the increase in ethane production caused by copper injection, and completely prevented mortaltiy. Vitamin E-deficient rats injected with copper sulfate at 5 mg Cu/kg produced over 10 times the ethane produced by rats injected with sodium sulfate or left uninjected. The ethylene produced by the rats injected with copper sulfate was 5% of the ethane produced, and did not differ significantly from the ethylene produced by the controls. Adding copper sulfate at 5 ppm Cu to a liver homogenate stimulated the production of ethane but not of ethylene. The correlation of increased ethane production with increased mortality suggests that lipid peroxidation may be important in the increased toxicity of copper in vitamin E- and selenium-deficient rats.

INTESTINAL ABSORPTION OF BILIARY AND EXOGENOUS CHOLESTEROL IN THE RAT. C. Dulery and D. Reisser (Laboratoire de Physiologie animale et de Nutrition (LA 273 CNRS), UER Nutrition, BP 138, 21004 Dijon Cedex (France)) *Biochim. Biophys. Acta 710*(2):164-171 (1982). Non-starved rats (fed a cholesterol-free diet prior to the experiments) with common bile fistula were infused intraduodenally with rat bile labelled with [1,2-^{3}H] cholesterol at a constant rate (0.6 ml/h) and a nutritive mixture containing, in particular, olive oil and 1 μmol (4-^{14}C] cholesterol per ml at rates of 1 ml/hr (group B) or 2.3 ml/hr (group A) for 5 hr control rats (group C) were prepared as group B rats but the nutritive mixture was free of cholesterol. 1 hr after the end of infusions, the animals were killed. Biliary and exogenous cholesterol were absorbed in the upper two-thirds of the small intestine; a large proportion of ^{3}H and ^{14}C radioactivity was present in the mucosa, but cholesterol from exogenous origin went across the mucosa more rapidly than cholesterol from biliary source. These observations suggest the existence of a non-homogeneous luminal mixture of molecules of cholesterol from different sources. The luminal dilution of [^{3}H]- and [^{14}C] sterols by non-labelled sterols increased from the proximal to the distal part of the small intestine. Precursor sterols and coprosterol were present in the stomach contents and in the lumen of caecum, colon and feces.

EFFECT OF INTERFERON ON PLASMA LIPOPROTEINS AND ON THE ACTIVITY OF POSTHERPARIN PLASMA LIPASES. C. Ehnholm, K. Aho, J.K. Huttunen, E. Kostiainen, K. Mattila, J. Pikkarainen, K. Cantell (Dept. of Immunology, National Public Health Institute, Mannerheimintie 166, SF-00280 Helsinki 28, Finland) *Arteriosclerosis 2*(1):68-73 (1982). The effect of interferon administration on the concentration of plasma lipoproteins and on the activity of postheparin plasma lipoprotein lipase and hepatic lipase was studied in six healthy men. Daily injection of human leukocyte interferon for 1 week lowered the plasma level of total cholesterol, very low density lipoprotein + low density lipoprotein cholesterol, hgih density lipoprotein cholesterol, and apo-lipoprotein A-I in all subjects. Simultaneously, the activity of postheparin plasma hepatic lipase and lipoprotein lipase decreased by 20% to 50%. These observations may be of importance in the interpretation of lipoprotein changes seen in acute and chronic infections and should be borne in mind when prolonged treatment with interferon is considered.

INCORPORATION KINETICS OF LYSOLECITHIN INTO LECITHIN VESICLES. KINETICS OF LYSOLECITHIN INDUCED VESICLE FUSION. K. Elamrani and A. Blume (Institut fur Physikalische Chemie II, D-7800 Freiburg, Federal Republic of Germany) *Biochem. 21*(3):521-526 (1982). The incorporation kinetics of 1-palmitoylphosphatidylcholine (lysolecithin) into dimyristoyl- and dipalmitoylphosphatidylcholine vesicles and the subsequent aggregation and fusion of the vesicles into larger aggregates were studied by using stopped-flow rapid-mixing techniques. The half-times for the lysolecithin incorporation vary between 50 and 500 ms. The incorporation rate has a maximum in the temperature range of the vesicle phase transition. This process is not diffusion controlled. The rate-limiting step is the incorporation of the lysolecithin monomer into the lipid bilayer. After this fast process, a slow reaction in the 10-50-min time range is observed. The large irreversible increase in terbidty indicates aggregation and fusion of the vesicles. The initial step is a second-order reaction with respect to the vesicle concentration, indicating aggregation or fusion of two vesicles. The aggregation rate passes through a maximum at the phase transition temperature.

EFFECTS OF LIPID PEROXIDATION ON PROSTAGLANDIN SYNTHESIS IN RABBIT KIDNEY MEDULLA SLICES. Y. Fujimoto and T. Fujita (Osaka College of Pharmacy, Kawai, Matsubara, Osaka 580 (Japan)) *Biochim. Biophys. Acta 710*(1):82-86 (1982). Ascorbic acid and FE^{2+} had a more powerful stimulatory effect on the lipid peroxidation of rabbit kidney medulla slices in 0.15 M KCl/0.02 M Tris-HCl buffer than in Krebs-Henseleit buffer. The lipid peroxidation induced by ascorbic acid and FE^{2+} enhanced the release of unsaturated fatty acids from tissue lipids, but inhibited medullary generation of prostaglandin E. These results suggest that the lipid peroxidation induced by ascorbic acid and FE^{2+} may inhibit the formation of prostaglandin E and that this inhibitory effect may be mediated by lipid peroxides via the inhibition of prostaglandin synthetase.

WITHDRAWAL TIME REQUIRED FOR CLEARANCE OF AFLATOXINS FROM PIG TISSUES. R.M. Furtado, A.M. Pearson, M.G. Hogberg, E.R. Miller, J.I. Gray, and S.D. Aust (Dept. of Food Sci, and Human Nutr., Dept. of Anim. Sci., and Dept. of Biochem., Michigan State Univ., East Lansing, Michigan 48824) *J. Agr. Food Chem. 30*(1):101-106 (1982). The time necessary to obtain clearance of aflatoxins from the tissues of the pig after removal from a contaminated diet was determined in two trials involving 20 pigs in each. There was a significant reduction in aflatoxin levels in all organs and tissues 1 day after placing the pigs on a aflatoxin-free ration. After 2 days, only one pig contained trace amount (0.50 μg/kg) of aflatoxins in the tissues. Four days after removal from the contaminated diet, there were no detectable levels of aflatoxins in any of the tissues. It was also found that a naturally contaminated diet containing only 20 and 31 μg/kg aflatoxins B_1 and B_2, respectively, resulted in traces of aflatoxins B_1, B_3, M_1, and M_2 in the livers and kidneys after 13-14 hr withdrawal from the contaminated diet, although none were detected in any other tissues.

DEMONSTRATION OF THE OCCURRENCE OF INACTIVE FATTY ACID SYNTHETASE IN RAT LIVER BY IMMUNOTITRATION AND ITS IN VITRO PARTIAL ACTIVATION. F.A. Lornitzo, S.A. Katiyar, R.N. Puri and J.W. Porter (Lipid Metabolism Laboratory, William S. Middleton Memorial Veterans Hospital and the Dept. of Physiological Chemistry, University of Wisconsin, Madison, WI 53706) *J. Biol. Chem. 256*(16):8498-8505 (1981). Direct immunotitrations of rat liver fatty acid synthetase in crude tissue homogenates with monospecific rabbit anti-rat liver fatty acid synthetase antibody enabled us to make a comparison of fatty acid synthetase protein and activity (percentage of maximal activity) as a function of the nutritional state in normal, diabetic, and insulin- and glucagon-insulin treated animals. Previous results, in which large changes in fatty acid synthetase activity were related to protein synthesis and degradation rather than to enzyme activation, were confirmed. It was also shown that fatty acid activation does not occur immediately on synthesis but follows the synthesis of fatty acid synthetase protein. Conditions were sought to obtain large amounts of fatty acid synthetase protein free from, or low in, activity. It was found that treatment of hypophysectomized rats with triiodothyronine meets these requirements, yielding milligram quantities of inactive fatty acid synthetase protein with less than 2% of maximal activity.

ADENYLATE KINASE OF *ESCHERICHIA COLI*: EVIDENCE FOR A FUNCTIONAL INTERACTION IN PHOSPHOLIPID SYNTHESIS. S.E. Goelz and J.E. Cronan, Jr. (Dept. of Molecular Biophys. and Biochem., Yale Univ., New Haven, CT 06510) *Biochemistry 21*(1):189-195 (1982). Previous genetic and biochemical experiments have suggested that the adenylate kinase of *Escherichia coli* may be directly involved in phospholipid synthesis through formation of a complex with *sn*-glycerol-3-phosphate acyltransferase, the

membrane-bound enzyme that catalyzes the first step in phospholipid synthesis. In this paper we report direct experiments to test this hypothesis. A mutation within the adenylate kinase structural gene is described that results in a temperature-sensitive phospholipid synthesis (assayed in vivo) and a temperature-sensitive acyltransferase. The adenylate kinase activity of this strain is only minimally altered either in vitro or [as assayed by adenosine 5'-triphosphate (ATP) levels] in vivo. This result demonstrates that the inhibition of phospholipid synthesis is not the result of reduced ATP levels. We report the purification of *E. coli* adenylate kinase to homogeneity and find that the addition of homogeneous wild-type adenylate kinase to membranes containing a mutationally altered temperature-sensitive acyltransferase results in thermal stabilization of the acyltransferase activity. Ovalbumin has no such protective effect. Purified *E. coli* inner membranes contain several proteins that are precipitated by addition of anti adenylate kinase antibody to detergent-solubilized membranes.

STUDIES ON SERUM LIPOPROTEINS OF RATS DEVELOPING SPONTANEOUS HYPERLIPIDEMIA. K. Gustafsson and H. Kiessling (Astra Läkemedel AB, Research and Development Laboratories S-151 85 Södertälje, Sweden) *Artery 9*(6):456-476 (1981); The lipid composition of the serum lipoproteins of Sprague-Dawley rats with varying degrees of hyperlipidemia was investigated. The concentrations of all the lipoproteins were increased with increasing hyperlipidemia. The elevation was found to be more pronounced for very low density (VLDL) and low density (LDL) than for high density lipoproteins (HDL). A linear relationship exists between triglyceride and cholesterol contents of VLDL fractions independent of the VLDL level. Eighty to ninety percent of serum triglycerides are located in the VLDL fraction. Linear relationships were found between the levels of serum triglycerides or VLDL triglycerides and the levels of LDL and HDL exist between total cholesterol, respectively. Furthermore, linear relationships exist between total cholesterol content in serum and cholesterol concentration in VLDL, LDL and HDL, respectively. Thus, lipotriglyceride concentrations. Disk electrophoresis and ultracentrifugation revealed that the LDL fraction (d. 1.006-1.063) could be divided into two fractions, which both increase with age. The spontaneous hyperlipoproteinemia of the old rats make these animals suitable as a model for evaluation of drugs against human hyperlipoproteinemia.

STUDIES OF LIVER LIPIDS IN NORMAL, ALLOXAN-DIABETIC AND PREGNANCY-TOXAEMIC SHEEP. G.D. Henderson, L.C. Read, and A.M. Snoswell (Dept. of Agri. Biochem., Waite Agri. Res. Inst., Univ. of Adelaide, Glen Osmond, South Australia, 5064 (Australia)) *Biochim. Biophys. Acta 710*(2):236-241. Triacylglycerols were the major lipid class in the fatty livers from alloxan-diabetic sheep and those suffering from pregnancy toxaemia, with the concentrations increased by 15- and 25-fold, respectively, compared with the normal state. Analysis of the fatty acid composition of total liver triacylglycerols in these animals showed a significant decrease in the proportion of saturated fatty acids, 16:0 and 18:0, and increase in the proportion of polyunsaturated fatty acids (18:2ω6, 18:3ω3 and 20:4ω6), particularly in those with pregnancy toxaemia. In contrast, total liver phospholipids showed a significant increase in the proportion of 18:0 in ewes with pregnancy toxaemia and a significant decrease in a range of polyunsaturated fatty acids in both the diabetic and toxaemic animals. Also, although the concentration of both phosphatidylcholine and phosphatidylethanolamine increased in the diabetic livers the ratio of phosphatidylcholine/phosphatidylethanolamine fell significantly, from 2.22 in the control animals to 1.59. The data suggest that, following the large influx of plasma fatty acids into the ovine liver in diabetes and pregnancy toxaemia, there is a diversion of polyunsaturated fatty acids from phospholipids to triaclglycerols. In diabetic sheep these changes may in turn affect phosphatidylcholine synthesis via the methylation pathway in liver. These changes in lipid composition may, in part explain the degenerative changes in membrane and subcellular organelle structure and the failure of liver function observed both in advanced diabetes and in severe pregnancy.

Fats and oils

POSSIBLE MECHANISM FOR THE CHEMICAL CARCINOGENESIS WITH POLYCYCLIC ALTERNANT HYDROCARBONS IN ACCORDANCE WITH LIPOPEROXIDE THEORY. K. Fukuzumi, *Oleagineux 36*(11):563-566 (1981). It was found that *trans,trans*- or *cis,trans*-conjugated diene hydroperoxides (a kind of lipoperoxides) exist in the lipids of cancerous tissues, in 1961 and 1963 by this author et al. To elucidate many phenomena about cancer, this author first proposed in the world in 1965 and 1969 so-called « lipoperoxide theory », and in 1972, 1974 and then 1978 the theory that cancer itself might be DNA radicals. No mechanism actually explaining the differences between the strengths for the chemcial carcinogenesis with polycyclic alternant hydrocarbons, such as 3,4-benzpyrene, 1,2;5,6-dibenzphenanthrene, triphenylene, and 1',2'-naphtha-2,3-anthracene, has been presented. However, the relationship between the chemical carcinogenesis with these substances and the reactivities in « K-region » and « L-region » of the substances has been given. The possible mechanism actually explaining the differences in accordance with « lipoperoxide theory » is first proposed in the world by the author.

A PRECOCIOUS TEST FOR PRODUCTIVITY IN THE OIL PALM (*ELAEIS GUINEENSIS* JACQ.) BY MEASUREMENT OF MITOCHONDRIAL ACTIVITIES. B. Kouamé, J.M. Noiret, *Oléagineux 36*(11):533-542 (1981). Crosses in four comparative trials of which the results are known were made again. The oxidative phosphorylation of the mitochondria isolated from germinated seeds and seedlings of these crosses was measured by polarography. The mitochondrial parameters obtained on seedlings are in good correlation with bunch production of the crosses in the field trials, but no correlation was found with oil yield and vegetative characters. These results allow the crosses most productive in bunches to be sorted out at the seedling stage, and an application is contemplated for the improvement and seed production programmes as well as for the choice of trees to be cloned by in vitro culture.

RIPENESS OF COCONUT SEEDS AND GERMINATION. W. Wuidart, M. de Nucé de Lamothe, *Oléagineux 36*(11):549-554 (1981). The time taken to convey coconut seeds from their place of production to the nurseries is often too long for this seed which lack dormancy. To avoid precocious germination, there is sometimes a tendency to collect slightly unripe nuts. In this article, the authors point out the dangers of such practices. Malayan Yellow Dwarf hybrid seeds must not be collected before the age of 11 months (pollination–collection). At 10 months, the germination percentage is sharply reduced, and disappears at 8 months. All sowing with unripe nuts include a high proportion of abnormal sprouts. In order to reduce the cost price of the plants and cull the plants properly in the nursery, only ripe nuts must be collected: 11-12 months for Yellow Dwarf × WAT hybrids. In practice, only bunches bearing at least one healthy nut with a brown epidermis should be collected.

CONTROL OF MILLIPEDES (DIPLOPODES, SPIROSTREPTOIDEA) IN GROUNDNUT CROPS IN SENEGAL. H. Masses, *Oléagineux 36*(11):555-562 (1981). Millipedes are amongst the chief groundnut parasites in Senegal. Attacking seedlings and the developing pods, they are responsible for poor sprouting and a reduction of 10-35 p. 100 in yields. In other respects, the holes they make in the pods considerably depreciate harvest quality. Three methods of control have been chosen: dusting of seed with insecticide, spreading of poisoned bait just after sowing and insecticide treatment of the soil on the 45th day of growth. Laboratory tests have allowed the selection of a large number of insecticide molecules likely to be of value in one or other method of control. Carbofuran, propoxur, bendiocarb and methiocarb have shown good iulicides action by ingestion; fonofos, ethoprophos, phoxime and chlorpyriphos ethyl have proved to have high contact toxicity. For dusting the seeds, carbo furan has been found to keep up a high death rate amongst the millipedes for 15-18 days after sowing. In the field, soil treatments on the 45th day of growth with carbofuran, diazinon and fonofos (at rates of 0.75, 2 and 2 kg a.i./ha) have led to an improvement in pod yield (+ 30 p. 100) as well as in harvest quality. Dusted on the seeds, carbofuran (at 0.2 p 1000 of the weight of seeds) has shown good ability to protect sprouting while allowing an added value of 8 p. 100 on density. Propoxur and carbofuran incorporated in baits have caused a very high death toll amongst the millipedes, but their impact on groundnut productivity has yet to be determined.

ON THE REFINABILITY OF OILS. VI. RELATIONS BETWEEN SPECTROMETRIC CHARACTERISTICS OF REFINED OILS AND ANALYTICAL CHARACTERISTICS OF CORRESPONDING RAW OILS. A. ABOUT NEW RAPESEED OILS. E. Sambuc, G. Devinat and M. Naudet, *Rev. Franç. Corps Gras. 29*(1):25-29, french. RFCG 82-04 (1982). 35 raw new rapeseed oils have been refined in laboratory in the same conditions. A few simple spectrometric characteristics of refined oils which participate in the prevision of immediate or ending flavor scores have been compared with different analytical characteristics of raw oils by means of statistical analysis. The absorption at 420, 270 and 230 nm of refined oils are previsible, for the used refining process, by polynomial first degree relations, from respectively: carbonyl value, copper content and absorption at 270 nm–peroxide value and

absorption at 270 nm—and, at last, peroxide value, copper content and absorption at 270 nm—of raw oils. The analytical retained test for every raw oils is compared with the previsible photonic absorption of refined oil; this study suggests in the used conditions, the required characteristics of raw oils in order to, after refining, the spectrometric characteristics of the freshly deodorized oil be compatible with the immediate sensorial acceptability threshold.

PHYSICOCHEMISTRY OF LIPIDS AND DERIVATIVES. R. Perron, *Rev. Franc. Corps Gras 29*(1):3-9, French. RFCG 82-01 (1982). The main structure characteristics of lipids: fatty chains, amphiphily, complexity of natural products, have been reviewed, then studied separately. About amphiphily, the competition between the molecule distinct parts is shown; the hypothesis in which two main macrostructures are considered from which the others are derived by undulatory phenomena in these phases has been recalled. The length of natural hydrocarbon chains has been confronted with an equation relating the melting temperature, carbon atoms and unsaturation while the conformation state is evoked. At last, the complexity of biological lipid mixtures has been succinctly studied in the case of a method foreseeing the fat thermic behavior from the glyceride structure.

THE TRIGLYCERIDES OF COTTONSEED OIL. M.A. Ouedraogo and J.A. Bezard, *Rev. Franç. Corps Gras 29*(1):11-16, french. RFCG 82-02 (1982). The triglyceride structure of a cottonseed oil from seed harvested in Upper Volta has been studied. The contents in linoleic, palmitic and oleic acids are respectively 52.2, 24.5 and 19.5%. The two unsaturated acids, especially linoleic acid, are preferentially esterified in the 2-position of glycerol. The triglycerides have been fractionated into nine classes according to their unsaturation, then analyzed by gas-liquid chromatography. From these data, it has been possible to calculate the proportion of 31 triglycerides types. The proportion of 51 triglyceride isomers has also been determined; their composition is given.

DETERMINATION OF MONOGLYCERIDES BY GAS CHROMATOGRAPHY. A. Prevot and J.L. Coustille, *Rev. Franç. Corps Gras 29*(1):17-24, french. RFCG 82-03 (1982). The proportions of mono-, di- and triglycerides in a mixture are determined by means of TMS derivatives using pyrex short capillary columns deactivated by persilylation and SE-30 impregnated. The influence of: injector type, column length and inlet pressure on the repeatability and relative response factors is studied thanks to standards. The most short is the stay of the sample in the column, the next of 1 are the response factors. In the best operational conditions, response factors equal to 1 are obtained with "cold on column" injector, but not with glass needle injector.

COMPONENTS OF MEYER LEMON LEAF OIL. E.D. Lund, P.E. Shaw, and C.L. Kirkland (U.S. Citrus and Subtropical Products Lab., Southern Region, Agr. Res. Service, U.S. Dept. of Agr., Winter Haven, FL 33880) *J. Agr. and Food Chem. 30*(1):95-97 (1982). Twenty-two compounds were isolated by gas chromatography from steam-distilled leaf oil of Meyer lemon (*Citrus limon* × *Citrus sinensis*) and identified by infrared spectroscopy, gas chromatographic retention times, and, in some instances, mass spectroscopy. The identified components and relative amounts were as follows (peak area percent): limonene, 73; 1,8-cineole, 7; isopulegol, 4; linalool, 1.7; myrcene, 1.3; citronellal, 1.2; α-terpineol, 1.1; terpinen-4-ol, 1.0; ocimene, 1.0; geranial, 0.9; γ-terpinene, 0.7; neral, 0.7; methylheptenone, 0.6; geranylacetate, 0.3; sabinene, 0.27; α-pinene, 0.2; isoisopulegol, 0.16; thymol, 0.04; β-caryophyllene, 0.0010; neryl acetate, 0.004; citronellol, 0.002; caryophyllene oxide, 8×10^{-5}. A number of additional compounds were isolated but not positively identified.

A CONVENIENT METHOD FOR THE PREPARATION OF ASIALO-G_{M1}. N. Kasai, L.O. Sillerud, and R.K. Yu (Neurology and Molecular Biophysics and Biochemistry, Yale University, School of Medicine, New Haven, CT) *Lipids 17*(2):107-110 (1982). A convenient and efficient procedure has been devised for the large-scale preparation of asialo-G_{M1} from bovine brain gangliosides. The procedure relies on the complete desialylation of brain gangliosides, consisting primarily of G_{M1}, G_{D1a}, G_{D1a}, G_{D1b} and G_{T1b}, by mild formic acid hydrolysis (0.1 N, 100 C; 2 hr). Following the hydrolysis step, asialo-G_{M1} can be isolated and purified by Folch partitioning and Iatrobeads column chromatography, with an overall yield of more than 50%.

GAS CHROMATOGRAPHIC ASSAY OF THE DIASTEREOMERIC COMPOSITION OF ALL-RAC-α-TOCOPHERYL ACETATE. C.B. Scott, N. Cohen, P.P. Riggio, and G. Weber (Chemical Research Department, Hoffmann-La Roche Inc., Nutley, NJ) *Lipids 17*(4):97-101 (1982). It has been established by an evaluation of 6 production samples of all-rac-α-tocopheryl acetate that all 4 racemates (RRS/SSR, RRR/SSS) are consistently present in equimolar amounts (SD < 0.3, RSD < 1.2%). An analysis of variance indicated variance due to signal noise to be consistent within a sample run but to vary from day to day. Variance due to area measurement was greater for the first and last eluted racemates than for the second and third. Peak width and asymmetry were found to be extremely sensitive to sample loading and, even within acceptable limits for good quantitation, the distortion was sufficient to give the elution profile the appearance of a sample composed of 4 components in unequal proportions increasing according to the order of elution.

DETERMINATION OF BHA AND BHT IN DEHYDRATED MASHED POTATOES. F. Beaulieu and D. Hadziyev (Dept. of Food Sci., Univ. of Alberta, Edmonton, Alberta, Canada T6G 2P5) *J. Food Sci. 47*(2):589-592 (1982). The extraction step in BHA (butylated hydroxyanisole) or BHT (butylated hydroxytoluene) analysis of potato granules, unless precautions are taken, leads to a recovery of only 10-50%. This study showed that BHA (BHT) is retained in granules by retrograded starch and, mostly, its amylose moiety. No satisfactory recovery was obtained using solvents of increasing dielectric constant unless the granules were first hydrated with water. A rapid antioxidant extraction procedure, based on the hydration principle and suitable for quality control labs, is described. Comparative data were acquired for the content of BHA (BHT) in potato granules analyzed by differential pulse voltammetry (using a glassy carbon electrode) and gas-liquid chromatography.

GLUCOCEREBROSIDE TRANSFER BETWEEN PHOSPHATIDYLCHOLINE BILAYERS. M.C. Correa-Freire, Y. Barenholz, and T.E. Thompson (Department of Biochemistry, University of Virginia School of Medicine, Charlottesville, VA 22908) *Biochemistry 21*:1244-1248 (1982). We have studied the kinetics of transfer of glucocerebroside between phospholipid bilayers by using pyrene and ^{3}H-labeled glucocerebroside incorporated into dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC) bilayers. Pyrene-labled glucocerebroisde (PyrCer) molecules are able to form an excited complex (eximer, E) between a PyrCer in the ground state and an excited monomer (M). When vesicles containing a known amount of PyrCer (donors) are incubated with unlabeled vesicles (acceptors), transfer of PyrCer from donor to acceptor populations is reflected in a decrease of the observed *E/M* intensity ratio. The results obtained from these studies show that the half-time of transfer from donor DMPC-PyrCer vesicles to acceptor DMPC vesicles is greater than 30 days at 37 C. This very slow transfer of glucocerebroside was confirmed by using tritiated glucocerebroside incorporated into small unilamellar DPPC donor vesicles incubated with large unilamellar DPPC acceptor vesicles above the phase transition. Separation of the two vesicle populations by molecular sieve chromatography at 45 C shows a half-time for transfer of approximately 32 days. We conclude that, in contrast to the results obtained for phosphatidylcholines [Roseman, M., and Thompson, T.E. (1980) *Biochemistry 19*, 439], glucocerebroside does not rapidly transfer between bilayers under these conditions.

EFFECT OF 1-*O*-OCTADECYL-2-*O*-ACETYL-*SN*-GLYCERO-3-PHOSPHOCHOLINE (PAF-ACETHER) ON LEUKOCYTES: I. ANALYSIS OF THE IN VITRO MIGRATION OF HUMAN NEUTROPHILS. B.M. Czarnetzki and J. Benveniste (Dept. of Dermatology, University of Munster, D-4400 Munster (Federal Republic of Germany)) *Chem. and Physics of Lipids 29*(4):317-326 (1981). The effect of synthetic 1-*O*-octadecyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine (PAF-acether) and of 1-*O*-octadecyl-*sn*-glycero-3-phosphocholine (lyso-PAF-acether) on human neutrophil migration was studied in modified Boyden chambers, with the following results: (1) By checkerboard analysis and deactivation experiments, the factors are chemokinetic at low (10^{-8} M) and chemotactic at higher concentrations (10^{-6}), with lyso-PAF-acether being less potent at all concentrations. (2) Cross-deactivation occurs between the two PAF compounds, but not with two other chemotactic factors, suggesting a specific, common receptor for the PAFs on the neutrophil membrane. (3) Other chemotactic substances may act as potentiating or additive factors to the PAF compounds. (4) Inhibition of arachidonic acid turnover during chemotaxis by compound BW 755 C enhances leukocyte chemotaxis towards the PAF compounds and towards other chemotactic factors. The data suggest that PAF and its lyso-derivate may contribute in a unique and potent fashion to leukocytes accumulation at inflammatory sites.

SATURATED AND UNSATURATED 1-*O*-ALKYL-2-*O*-ACETOYL-*SN*-GLYCERO-3-PHOSPHOCHOLINES DERIVED FROM RATFISH LIVER OIL: EFFECT ON HUMAN LEUKOCYTE MIGRATION. B.M. Czarnetzki and T. Muramatsu (Dept. of Dermatology, Univ. of Munster, and Federal Center for Lipid Research, Inst. for

Biochem. and Technology—H.P. Kaufmann Inst., D-4400 Munser (Federal Republic of Germany)) *Chem. and Physics of Lipids 29*(4): 309-315 (1981). A mixture of 1-*O*-alkyl-2-*O*-acetoyl-*sn*-glycero-3-phosphocholines containing saturated alcyl moieties and a mixture of such compounds with saturated and unsaturated alkyl moieties, prepared from ratfish (*Hydrolagus colliei*) liver oil, were studied for their in vitro effect on human neutrophil migration. The mixture containing unsaturated compounds (*II*) was more active compared to the saturated (*I*) ones at a range from 10^{-6} M to 10^{-10} M concentrations. At 10^{-4} M, *II* was cytotoxic. Both *I* and *II* were more potent than synthetic 'PAF-acether' (*II*) and the material prepared from beef heart plasmalogens (*IV*). Preincubation and checkerboard titration experiments showed that the ether phospholipids derived from ratfish liver oil have primarily chemokinetic, but also chemotactic effects on neutrophils, as has been reported for compound *III*. These compounds are therefore highly potent stimulants of human neutrophils with potentially unique membrane-activating properties.

HYDROLYSIS OF PHOSPHATIDYLINOSITOL BY PANCREAS AND PANCREATIC SECRETION. R.M.C. Dawson, R.F. Irvine, K. Hirasawa, and N.L. Hemington (Biochem. Dept. ARC Institute of Animal Physiology, Babraham, Cambridge CB2 4AT (U.K.)) *Biochim. Biophys. Acta 710*(2):212-220 (1982). 1. The secretion from sheep pancreas and a supernatant fraction prepared from the gland contained an EDTA-insensitive acid phospholipase A_1 which readily deacylated phosphatidylinositol (pH optimum, 5.3), 1-acylglycerophosphoinositol and phosphatidic acid, but had limited action on phosphatidylcholine and phosphatidylethanolamine even with deoxycholate present. The enzyme was not a triacylglycerol lipase. 2. The action of the phospholipase A_1 on phosphatidylinositol was inhibited effectively by Ca^{2+} and Mg^{2+}, probably by interaction of those ions with the substrate. 3. In the presence of calcium the decomposition of phosphatidylinositol and lysophosphatidylinositol by the supernatant fraction was overwhelmingly by phosphodiesterase action (EC 3.1.4.10), producing inositol monophosphate and its cyclic derivative. Its pH optimum was about 6.0 but with considerable activity extending to pH 8.5. 4. The phosphodiesterase was not secreted in the pancreatic juice.

TOCOPHEROLS OF WINGED BEAN (*PSOPHOCARPUS TETRAGONOLOBUS*) OIL. B.O. de Lumen and S. Fiad (Department of Nutritional Sciences, University of California, Berkeley, Berkeley, CA) *J. Agr. and Food Chem. 30*(1):50-53 (1982). Oil samples extracted from 27 varieties of winged bean seeds were analyzed directly for individual tocopherols after dissolution in the mobile phase by high performance liquid chromatography (HPLC) and ultraviolet detection at 295 nm. γ-Tocopherol was found to be the dominant form of tocopherol with traces of α-, β-, and δ-tocopherols. The samples showed a low of 8 and a high of 130 mg of γ-tocopherol/100 g of oil while most of the samples fell in the range of 23-44 mg/100 g of oil. Less variation was observed in the oil content of the different varieties which averaged 14.7%. On the basis of published data on fatty acid composition of winged bean oil, the tocopherol to polyunsaturated fatty acid ratio was calculated to be 0.2 mg of δ-α-tocopherol equiv/g of polyunsaturated fatty acids, a value similar to that of soybean and less than that of a number of vegetable oils. The nutritional and functional significance of the predominance of γ-tocopherol in winged bean oil is discussed.

A FLUORESCENCE STUDY OF APOLIPOPROTEIN LOCALIZATION IN RELATION TO LIPIDS IN SERUM LOW DENSITY LIPOPROTEINS. B.E. Dobretsov, M.M. Spirin, O.V. Chekrygin, I.M. Karmansky, V.M. Dmitriev, and Yu.A. Vladimirov (2nd Moscow State Medical College and Inst. of Bio. and Medical Chem., Academy of Medical Sciences, 117437 Moscow (U.S.S.R.)) *Biochim. Biophys. Acta 710*(2):172-180 (1982). Fluoresence energy transfer studies were carried out on low density lipoproteins (LDL) containing pyrene, in order to investigate their structure. The results indicate that almost all of LDL tryptophan residues are located in the same surroundings near the surface of the particle and are immersed in the lipid phase 10-20 Å below the lipid/water interface. The data do not support a model of protein spikes protruding from the particle surface. Such spikes have been observed in LDL preparations only after long-term storage.

SOLID STATE PROPERTIES OF ANOMERIC 1-*O*-*N*-OCTYL-D-GLUCOPYRANOSIDES. D.L. Dorset and J.P. Rosenbusch (Dept. of Microbio., Biozentrum Universitiät Basel, CH-4056 Basel (Switzerland)) *Chem. and Physics of Lipids 29*(4):299-307 (1981). The anomers of 1-*O*-*n*-octyl-D-glucopyranosides exhibit different crystal packing and thermodynamic properties. Crystallization either from solution or by epitaxy of the α-anomer resembles that of other amphiphiles, such as lysolecithin, and isostructural to the decyl homologue. The β-anomer crystallizes into a unique form, independent of conditions, with the longest crystallographic axis parallel to the best developed crystal face. Both compounds exhibit two phase transitions, one near 70 C, the other above 100 C. The latter corresponds to the melting to an isotropic liquid for both forms, but the former is distinctly different for the two anomers. Thus, birefringence is lost only with the β-anomer, while the enthalpy change is two-fold larger for the α-anomer. The crystal packing of the two compounds are thus clearly different.

PURIFICATION OF A BASIC PHOSPHOLIPID TRANSFER PROTEIN FROM MAIZE SEEDLINGS. D. Douady, M. Grosbois, F. Guerbette, and J.-C. Kader (Laboratoire de Physiologie Cellulaire, Universite Pierre et Marie Curie, 4 place Jussieu, 75005 Paris, France) *Biochim. Biophys. Acta 710*:143-153 (1982). A phospholipid transfer protein has been purified 125-fold from maize seedlings. The successive steps of purification comprised gel filtration on Sephadex G-75, DEAE- and CM-chromatography and chromatofocusing. The homogeneity of the protein was determined by polyacrylamide gel electrophoresis with and without SDS and by isoelectric focusing. The protein has an apparent molecular weight of 20000, as estimated from SDS electrophoresis, and an isoelectric point of 8.8 ± 0.2. The amino acid composition of the protein is characterized by a high content of alanine, glycine, cysteine and serine and a small amount of lysine. A molecular weight of 14058 was calculated from this amino acid composition. The protein only loses 25% of its activity after 5 min heating at 95 C. The purified protein is able to transfer phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine between liposomes and mitochondria at the rates of, respectively, 100, 56, and 1.6. After incubation of the purified protein with (^{3}H)phosphatidylcholine, a labeled phosphatidylcholine-protein complex was obtained after chromatofocusing. This suggests that the protein acts by carrying phosphatidylcholine from a membrane toward another one.

VALUES FOR AND SIGNIFICANCE OF ORDER PARAMETERS AND "CONE ANGLES" OF FLUOROPHORE ROTATION IN LIPID BILAYERS. L.W. Engel and F.G. Prendergast (Dept. of Pharm., Mayo Foundation, Rochester, MN 55901) *Biochemistry 20*(26): 7338-7345 (1981). A rigid formalism has been developed for the calculation of the order parameter S for fluorescence probes embedded in environments that hinder the motions of the probes and for calculation of a "cone angle" of fluorophore rotation from the order parameters. The motions of the fluorescence probes 1,6-diphenylhexa-1,3,5-triene (DPH) and 1-[4-(trimethylamino)phenyl]-6-phenylhexa-1,3,5-triene (TMA-DPH) embedded in lipid bilayers were then analyzed in terms of the order parameter and the cone angle. Order parameters for such fluorescence can only be compared to the average order parameter over a segment of a fatty acyl chain or of membrane thickness. Also, because the bilayer may be perturbed by the fluorophore at regions distant from the immediate location of the probe, these "averaged" order parameters cannot be easily compared to those calculated from nuclear magnetic resonance data but are more readily compared to order parameters of electron spin resonance probes. There is no defined mathematical relation between the order parameter and a dynamical parameter which would afford a calculation of membrane "microviscosity". A Gaussian angular freedom parameter or cone angle of fluorophore motion has been calculated from the order parameters and shows in a geometric sense the limitations imposed on the angular displacements of TMA-DPH as compared to DPH.

ALKYLTHIOLATION FOR THE DETERMINATION OF DOUBLE-BOND POSITION IN UNSATURATED FATTY ACID ESTERS. G.W. Francis (Dept. of Chem., Univ. of Bergen, 5000 Bergen (Norway)) *Chem. and Physics of Lipids 29*(4):369-374 (1981). The methyl esters of some mono-unsaturated fatty acids have been methylthiolated by the iodine-catalyzed addition of dimethyl disulfide across the double bond. The resulting derivatives are suitable for gas chromatography. The fragmentation of the derivatives on electron impact yields mass spectra which allow immediate recognition of the position of the original double bond.

CHARACTERIZATION OF SOYBEAN OIL EXTRACTED BY SUPERCRITICAL CARBON DIOXIDE AND HEXANE. J.P. Friedrich and G.R. List (Northern Regional Research Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Peoria, IL 61604) *J. Agric. Food Chem. 30*: 192-193 (1982). Exhaustive extraction of full-fat soybean flakes with supercritical carbon dioxide ($SC\text{-}CO_2$) yields an oil that is comparable to hexane-extracted oil except for significantly lower chromatographic refining loss and phosphorus content. In a long cylindrical batch extractor, the flakes act much like the stationary phase of a chromatography column, which permits the recovery of light-colored, essentially degummed, crude oil fractions.

INTERACTIONS BETWEEN DIACYLGLYCEROPHOSPHOETH-

ANOLAMINES AND *N*-ALKANES IN MONOLAYERS AND BILAYERS. M. Hayashi, T. Kogayashi, and Tsutomu Seimya (Lab. of Chem., College of Arts and Sciences, Chiba Univ., Yayoicho, Chiba) *Chem. and Physics of Lipids 29*(4):289-298 (1981). Surface pressure-area isotherms of L-diacylglycerophosphoethanolamines were measured at the *n*-alkane/water interfaces for alkanes ranging from *n*-hexane to *n*-hexadecane. Transition pressures from the expanded film to the condensed state varied largely depending on the chain length of the *n*-alkane in the oil phase. The phase transition temperature and the entropy were studied by differential scanning calorimetry in presence of *n*-alkanes for the same lipid. The temperatures of gel-to-liquid crystalline phase transitions were changed in the same way as the monolayers reflecting the chain lengths of the *n*-alkanes present. The effects of the *n*-alkanes on monolayers and bilayers were entirely parallel; they are discussed taking the mixing of the lipids and the *n*-alkanes into account in the condensed films for the former and in the gel phase for the latter.

STEROL SYNTHESIS IN *CHLAMYDOMONAYS REINHARDTII* 137^+ CELL-CYCLE VARIATIONS. D.R. Janero and R. Barrnett (Section of Cell Biology, Yale Univ. School of Medicine, New Haven, CT 06510) *Biochim. Biophys. Acta 710*(2):242-247 (1982). Biosynthesis of cellular sterol during the 12 hr light/12 hr dark vegetative cycle of the green alga *Chlamydomonas reinhardtii* 137^+ (wild-type) has been investigated in highly synchronous, axenic cultures. Pulse exposure of algae to saturating concentrations of radioactive acetate and to ^3H_2O demonstrate that sterol is synthesized continuously, but differentially, with respect to cell-cycle time. High rates of sterol synthesis are confined to the light period (mid-to-late G_1), a maximal rate attained by 4.5 hr in the cell cycle. With the onset of the dark period, sterol synthesis drops sharply by about one order of magnitude. Sterol synthesis in the dark (S, M, early-to-mid G_1) does not rise above negligible levels until the dark-to-light transition, which is accompanied by a 30-fold rate increase. The kinetics indicate that sterol synthesis is modulated through the cell cycle both by acute and photocontrols operative at the light/dark transitions and by long-term controls active over the course of the light and dark periods.

ON THE LIPID PEROXIDATION OF RAT LIVER HEPATOCYTES, THE FORMATION OF FLUORESCENT CHROMOLIPIDS AND HIGH MOLECULAR WEIGHT PROTEIN. J.F. Koster, R.G. Slee and Th.J.C. Van Berkel (Dept. of Biochem. I, Medical Faculty, Erasmus Univ. Rotterdam, PO Box 1738, 3000 DR Rotterdam (The Netherlands)) *Biochim. Biophys. Acta 710*(2):230-235 (1982). 1. The formation of malondialdehyde by intact hepatocytes, induced by ADP/Fe^{3+} or cumene hydroperoxide, can be inhibited by the addition of thiourea. This may indicate that hydroxyl radicals are involved in this process. 2. Lipid peroxidation of intact hepatocytes leads to the formation of fluorescent chromolipids. When similar amounts of malondialdehyde are formed by either ADP/Fe^{3+} or cumene hydroperoxide, the lipid peroxidation induced by cumene hydroperoxide generates more fluorescent chromolipids than does the lipid peroxidation induced by ADP/Fe^{3+}. 3. The formation of chromolipids is accompanied by the genesis of high molecular weight protein. With cumene hydroperoxide more high molecular weight protein is formed than with ADP/Fe^{3+}. 4. It can be concluded that the defense system against lipid peroxidation of intact hepatocytes does not prevent the formation of lipofuscin-like chromolipids and high molecular weight protein as found earlier in microsomes. Cumene hydroperoxide, at least in this system, can be considered as an effective inducer of chromolipids.

SYNTHESIS OF STEROLS WITH CYCLOPROPANE-CONTAINING SIDE CHAINS. SPECTROSCOPIC PROPERTIES AND ABSOLUTE CONFIGURATIONS. R.W. Land and C. Djerassi (Dept. of Chem., Stanford University, Stanford, CA 94305) *J. Org. Chem. 47*(4):625-633 (1982). The synthesis of seven Δ^5 sterols with cyclopropane-containing side chains (1-3 and 5-8) by means of dichlorocarbene addition to the appropriate olefinic precursor is described. Separation of the diastereoisomeric mixtures of the primary dichlorinated adducts was accomplished by reverse-phase high-performance liquid chromatography. The effect of stereochemistry upon the NMR and mass spectroscopic properties of the diastereoisomerically pure sterols are reported, and their absolute configurations were determined either by X-ray crystal structure analysis of related precursors and/or by chemical and spectroscopic correlations.

INFLUENCE OF DEEP-FAT FRYER LOAD ON COMPUTED FRY TIME OF BREADED BROILER THIGHS. R.H. Lane and S.W. Jones (Dept. of Nutr. and Food Sci., Univ. of Kentucky, Lexington, KY 40506) *Poultry Sci. 61*(3):610-611 (1982). The validity of a regression equation for computing fry time for raw, breaded broiler thighs was tested with three times the number of thighs (24) used in establishing the equation. No difference ($p > .05$) was found in computed fry times based on mean weight of the load and actual mean fry times obtained from internal temperature measurement at the three frying temperatures (163, 177, and 191 C) under consideration.

STRUCTURE AND DYNAMICS OF PHOSPHOLIPID MEMBRANES: AN ELECTRON SPIN RESONANCE STUDY EMPLOYING BIRADICAL PROBES. P. Meier, A. Blume, E. Ohmes, F.A. Neugebauer, and G. Kothe (Institute of Physical Chem., Univ. of Freiburg, D-7800, Federal Republic of Germany) *Biochemistry 21*: 526-534 (1982). The large zero-field splitting of rigid biradicals makes them important candidates for spin probes of phospholipid membranes. Here we develop an electron spin resonance line-shape model for such probes on the basis of the stochastic Liouville equation. Particular emphasis is given to the slow-diffusional regime, characteristic of bilayers in the gel phase. The theory is employed to study the line shapes of bis(verdazyl) biradicals, incorporated into oriented multibilayers of dimyristoyl-phosphatidylcholine. Computer simulations of the *angular-dependent spectra* provide the orientational distribution functions and rotational correlation times of the spin probes. They occupy two different sites in bilayer membrane. The orientational distribution of the spin probes is related to the structure of the phospholipid phases. In the $L_{\beta'}$ phase the hydrocarbon chains are uniformly tilted by $\delta = 23°$ with respect to the bilayer normal. For the $P_{\beta'}$ phase we observe a random distribution of tilt angles from $\delta = 0°$ to $\delta = 19°$, indicating that the chains orient perpendicular to the local (rippled) bilayer surface. This structure has not been established previously. In agreement with other studies we find no tilt for the L_α phase. The order parameters of the hydrocarbon chains increase with decreasing temperature, jumping for $S \leqslant 0.6$ to $S \geqslant 0.8$ at the main transition. From the rotational correlation times of the spin probes, intrinsic bilayer viscosities of 0.08 $P \leqslant \eta \leqslant 20P$ ($50°C \geqslant T \geqslant 1°C$) are determined. An Arrhenius plot provides activation energies of the viscous flow. The values increase from $E_{visc} \sim 10$ kcal/mol in the L_α phase to $E_{visc} \sim 18$ kcal/mol in the $L_{\beta'}$ phase.

CHARACTERIZATION OF HEXADECANOIC ACID PERDEUTERATED FROM CARBON EIGHT TO CARBON SIXTEEN: A NOVEL CONFORMATIONAL PROBE FOR AMPHIPATHIC SYSTEMS. S.D. Merajver (Dept. of Chem., Polytechnic Inst. of New York, 333 Jay Street, Brooklyn, NY 11201) *Chem. and Physics of Lipids 29*(4):379-384 (1981). The molecule (9,9,10,10, 11,11,12,12,13,13,14,14,15,15,16,16,16)-heptadecadeutrio hexadecanoic acid has been characterized as a useful tool for conformational studies of lipid chains in membranes. Calorimetric and mass spectrometry tests successfully established the sample to be of high purity 99.6±0.2% and to be deuterated as specified above. Detailed Raman spectra of the sample were analyzed in all regions of interest between 50 cm^{-1} and 3100 cm^{-1}. The optical sceletal bands arising from the perdeuterated octyl tail are uncoupled from the bands proceeding from the head portion of the molecule. Likewise, the very strong symmetric and antisymmetric CD_2 stretching bands fall in the region of 2000-2200 cm^{-1} where no other bands are present. Bands associated in the CH_2 twisting, CH_2 bending and CH_2 stretching have also been identified. The relevance of this study to conformational analyses in lipids is discussed.

BOVINE BONE MARROW LIPIDS. G.J. Miller, M.R. Frey, J.E. Kunsman, and R.A. Field (Div. of Anim. Sci., Univ. of Wyoming, Laramie, WY 82971) *J. of Food Sci. 47*(2):657-660 (1982). Characteristics of marrow lipids in bones from three different anatomical locations (cervical, lumbar, femur) in steers and cows on low energy (range) or high energy (feedlot) diets are reported. Cervical marrow contained the least and femur marrow the most total lipid and triglycerides (TG) were the major type of lipid present in all marrows. Phosphatidylcholine was the major PL present in all marrows. The predominant fatty acids were 16:0, 18:0 and *cis* 18:1. There were no consistent effects due to anatomical location of bone, diet or sex upon any of the acids measured. TG structures were similar in all marrows and the fatty acid composition of TG from steer marrow resembled that of steer intramuscular TG.

1-[4-(TRIMETHYLAMINO)PHENYL]-6-PHENYLHEXA-1,3,5-TRIENE: SYNTHESIS, FLUORESCENCE PROPERTIES, AND USE AS A FLUORESCENCE PROBE OF LIPID BILAYERS. F.G. Prendergast, R.P. Haugland, and P.J. Callahan (Dept. of Pharm., Mayo Foundation, Rochester, MN 55901) *Biochemistry 20*(26): 7333-7338 (1981). 1-[4-(Trimethylamino)phenyl]-6-phenylhexa-1,3,5-triene (TMA-DPH), a cationic analogue of diphenylhexatriene (DPH), has photophysical properties that are generally similar to those of DPH. In solution the fluorescence lifetime (τ) of TMA-DPH is short (<1.5ns), but τ increases to ~7ns when the probe is embedded in lipid bilayers at temperatures less than the thermal transition temperature (T_c) of the lipid. The cationic charge ensures

that the probe is anchored at the lipid-water interface, most likely with the DPH moiety intercalated between the upper portions of the fatty acyl chains. The profiles of changes in steady-state anisotropies (r_{ss}) and limiting hindered anisotropies (r_∞) are similar for both TMA-DPH and DPH embedded in lipid bilayers, but r_∞ values for TMA-DPH even at $T>>T_c$ are generally >0.14, e.g., at 35 C in 1,2-dimyristoylglycero-3-phosphocholine (DMPC) (cf. 0.03 for DPH in CMPC at 35 C). Electrostatic interactions of the cationic probe with head groups of phospholipids do not appear to significantly influence the apparent dynamics of the probe. TMA-DPH should prove useful in the study of the dynamics of phospholipid monolayers, e.g., in native or reconstituted lipoproteins.

POLYMERIZED PHOSPHATIDYLCHOLINE VESICLES. SYNTHESIS AND CHARACTERIZATION. S.L. Regen, A. Singh, G. Oehme, and M. Singh (Dept. of Chem., Marquette University, Milwaukee, WI 53233) *J. Am. Chem. Soc. 104*(3):791-795 (1982). The synthesis and characterization of photopolymerized vesicles derived from bis[12-(methacryloyloxy)dodecanoyl] -L-α-phosphatidylcholine (3) 1-[12-(methacryloyloxy)dodecanoyl-2-palmitoyl-L-α-phosphatidylcholine (4), and 1-palmitoyl-2-[12-(methacryloyloxy)dodecanoyl] -L-α-phosphatidylcholine (5) are described. Ultrasonic irradiation of 3,4,5 20% 3+80% 4, and 20% 3+80% 5 in water at 50 C yields opalescent to optically clear dispersions. Electron microscopy, entrapment of [^{14}C] sucrose, and permeability measurements provide strong evidence for closed multilamellar vesicles having diameters ranging between 350 and 1400 Å. Fourier transform ^{1}H NMR spectra of the aqueous dispersions as well as IR spectra of chloroform extracts establish that no significant lipid decomposition occurs during vesicle preparation. Direct UV irradiation (254 nm) produces polymerized analogues of similar size and shape which (1) entrap [^{14}C] sucrose, (2) show reduced permeability, and (3) exhibit enhanced stability.

STABILIZATION OF LIPOPROTEIN LIPASE BY ENDOTHELIAL CELLS. K. Shimada, J.J. Lanzillo, W.H.J. Douglas, and B.L. Fanburg (Dept. of Medicine, New England Medical Center Hospital, Boston, MA) *Biochim. Biophys. Acta 710*:117-121 (1981). Lipoprotein lipase, purified from bovine milk, lost 90% of its activity when incubated in Hanks' balanced salt solution for 5 min at 37 C. Bovine pulmonary artery endothelial cells, maintained in culture, markedly stabilized this enzyme. The stabilizing factor of endothelial cells was non-dialyzable, resistant to heating at 100 C and to changes in pH, and unaffected by treatments of cells with proteolytic enzymes or with heparinase (*Flavobacterium heparinum* enzyme). However, the stabilizing effect on lipoprotein lipase was reduced by 60-70% by the extraction of cells with chloroform/methanol (2:1). The lipid extract of the cells stabilized the enzyme, suggesting that lipid component(s) of the endothelial cells account for their stabilizing effect. Since the endothelial cell is thought to be the site of action of lipoprotein lipase, stabilization of the enzyme by this cell may play a role in its preservation and function in vivo.

PHOSPHATIDYL N-METHYLETHANOLAMINE IN RAT BRAIN. R.R. Shukla, N. Srivastava, A. Bajpai, H.C. Joshi, and U.K. Misra (Dept. of Biochem., V P Chest Inst., Univ. of Delhi, Delhi 110 007) *Indian J. of Biochem. and Biophys. 19*(1):69 (1982). Phosphatidyl monomethylethanolamine has been isolated from 10-day-old rats. It constituted about 4 per cent of total brain phospholipids.

INFLUENCE OF CHOLESTEROL ON WATER PENETRATION INTO BILAYERS. S.A. Simon, T.J. McIntosh, and R. Latorre (Dept. of Anesthesiology and Physiol., Duke Univ. Med. Center, Durham, NC 27710) *Science 216*:65-67 (1982). X-ray diffraction and capacitance measurements have been used to calculate the depth to which water penetrates in fully hydrated bacterial phosphatidylethanolamine bilayers in the presence and absence of cholesterol. The data indicate that cholesterol decreases the depth of water penetration by about 2.5 angstroms.

A RAMAN STUDY OF THE INTERACTION OF Mg^{2+}, Ca^{2+}, AND Ba^{2+} IONS WITH AN ACIDIC MODEL MEMBRANE. H. Susi (Eastern Regional Res. Center, Philadelphia, PA 19118) *Chem. and Physics of Lipids 29*(4):359-368 (1981). The interaction of dipalmitoylphosphatidylglycerol (DPPG) liposomes with divalent ions of magnesium, calcium and barium have been investigated with laser-Raman spectroscopy over the temperature range of 0-60 C. The effect of Ca^{2+} ions was also investigated as a function of concentration. At a Ca^{2+}/DPPG molar ratio of 0.1, the number of *trans* carbon to carbon bonds in the hydrocarbon domain of the phospholipid and the lateral order of the hydrocarbon chains was increased both below and above the gel to liquid crystal transition. At higher Ca^{2+} concentrations the number of *trans* bonds and the lateral order is further increased over the entire temperature range studied, while the transition disappears. Magnesium and barium ions have a much smaller ordering effect on the side-chain packing of DPPG liposomes. At molar ratio of 0.3, the gel to liquid crystal transition is still discernible for DPPG liposomes in the presence of Ba^{2+} ions, but not in the presence of Mg^{2+} ions.

THE MOLECULAR DYNAMICS OF CHOLESTEROL IN BILAYER MEMBRANES: A DEUTERIUM NMR STUDY. M.G. Taylor, T. Akiyama, and I.C.P. Smith (Div. of Biol. Sci., Ntl. Res. Council of Canada, Ottawa, Ontario K1A 0R6 (Canada)) *Chem. and Physics of Lipids 29*(4):327-339 (1981). The ^{2}H-NMR spectra of selectively deuterated cholesterol, intercalated in egg phosphatidylcholine, were examined. The orientation of the axis of motional averaging was calculated using the observed quadrupole splittings and the atomic coordinates. With the known orientation of the rotation axis, quadrupole splittings observed for deuterium labels on cholesterol can be related to the molecular order parameter of the sterol. In addition, knowledge of the axis orientation allows prediction of the magnitudes of quadrupole splittings for deuterium at other positions, which is useful in the choice of labeling for particular applications. Finally, preliminary relaxation time measurements yield information on the rates of anisotropic motion of cholesterol in bilayer membranes.

AROMA, COLOR, AND LIPID OXIDATION OF TURKEY MUSCLE EMULSIONS. C.S. Tellefson, J.A. Bowers, C. Marshall, and A.D. Dayton (Dept. of Foods and Nutr. and Statistics, Kansas State Univ., Manhattan, KS 66506) *J. of Food Sci. 47*(2):393-396 (1982). Turkey emulsions were prepared with (1) no additives, (2) sodium chloride (NaCl), (3) sodium nitrite ($NaNO_2$), (4) sodium ascorbate (NaAsc), or (5) both $NaNO_2$ and NaAsc. Raw and cooked emulsions from each of the five treatments were stored (-18 C) and then evaluated before and after heating. Emulsions with $NaNO_2$ and NaAsc contained less malonaldehyde than those with NaCl or no additive and raw turkey emulsions generally contained less malonaldehyde than cooked. Nitrite was the additive that produced the major effect on color of heated emulsions. Generally, emulsions containing both $NaNO_2$ and NaAsc had the most meaty aroma and the least stale aroma. Emulsions with NaCl tended to have greater stale aroma.

PHOTOINDUCED CALCIUM RELEASE FROM RHODOPSIN–PHOSPHOLIPID MEMBRANE VESICLES. P.N. Tyminski, R.T. Klingbiel, R.A. Ott, and D.F. O'Brien (Research Laboratories, Eastman Kodak Company, Rochester, NY 14650) *Biochemistry 21*:1197-1204 (1982). Brief blue-green light exposure of rhodopsin-phospholipid membrane vesicles that contained divalent cations released the cations from the vesicles. The photoinduced release is due to an increase in permeability of the membrane. The quantity of ions released depends on the initial ionic concentration inside the vesicles. At 37 C and an internal concentration of 30 mM Ca^{2+}, the initial flux for rhodopsin-egg phosphatidylcholine membrane vesicles was 0.25 ± 0.11 Ca^{2+} per bleached rhodopsin per s. Similar fluxes were observed for the release of Co^{2+}, Mn^{2+}, Ni^{2+}, and Mg^{2+}. The addition of proton uncouplers and lipophilic anions accelerated the rate to ~1 Ca^{2+} per bleached rhodopsin per s. The flux was independent of the concentration of rhodopsin in the membranes and sensitive to the head-group composition of the rhodopsin-phospholipid vesicles. Analysis of the fraction of Ca^{2+} released and the fraction of bleached rhodopsin per vesicle showed that a single bleached rhodopsin per vesicle is necessary and sufficient for Ca^{2+} release. Ca^{2+} release was not observed from thermally bleached rhodopsin. These results are discussed with regard to the possible role of Ca^{2+} as an excitatory transmitter in vision.

PURIFICATION AND CHARACTERIZATION OF TWO LIPOXYGENASE ISOENZYMES FROM COWPEA [*VIGNA UNGUICULATA* (L.) WALP.] T. Van Den and E.M.T. Mendoza (Biochem. Lab., Inst. of Plant Breeding, Univ. of the Philippines at Los Baños, College, Laguna, Philippines 3720) *J. Agr. and Food Chem. 30*(1):50-53 (1982). Lipoxygenase specific activity in 89 cultivars of cowpea (*Vigna unguiculata* (L.) Walp.] varied from 30 to 397 units/mg of protein. Two lipoxygenase isoenzymes L-1 and L-2 were purified from a variety UPL Cp2 by extraction with water, followed by dialysis against water, 40-60% ammonium sulfate fractionation, and DEAE-Sephadex A-50 ion-exchange and hydroxylapatite chromatography. L-1 and L-2 were both highly specific for linoleic acid and exhibited a narrow optimal activity at pH 6.2. Apparent K_m values of 0.8×10^{-3} M and 0.55×10^{-4} M linoleic acid were obtained for L-1 and L-2 respectively. L-1 and L-2 had molecular weights of 68,000 and 74,000, respectively, by $NaDodSO_4$ gel electrophoresis and had R_f values of 0.25 and 0.11, respectively, by regular gel electrophoresis. L-1 and L-2 isoenzymes were inhibited to varying degrees by different metal ions although, in general, L-2 was more sensitive than L-2. The lipoxygenase isoenzymes were stable at pH 4-9. The enzyme in situ was highly stable even in seeds soaked in acidic solution at pH 2 for 10 hr. However, blanching of soaked and unsoaked seeds resulted in total loss of activity.

SYNTHESIS OF METHYL ω-DEUTERATED TETRADECANOATE AND HEXADECANOATE. P.W. Westerman and N. Ghrayeb (Northeastern Ohio Universities College of Medicine, State Route 44, Rootstown, OH 44272) *Chem. and Physics of Lipids 29*(4):351-358 (1981). Methyl ω-deuterated, d tetradecanoates have been prepared in high purity by two synthetic routes from methyl hydrogen tetradecanedioate. One method utilizes the selective reducing properties of sodium borodeuteride and sodium cyanoborodeuteride towards, acid chloride, ester, tosyloxy, and iodo groups to introduce the deuterium label at only the carboxyl group of methyl hydrogen tetradecanedioate. The second procedure utilizes a coupling reaction between an organic halide and lithium di-(trideuteriomethyl)cuprate (I). Corresponding ω-methyl hydrogen hexadecanedioate. The two methods should be generally applicable in the synthesis of ω-deuterated alkanoic acids.

23,24,25-TRIHYDROXYVITAMIN D_3, 24,25,26-TRIHYDROXYVITAMIN D_3, 24-KETO-25-HYDROXYVITAMIN D_3, AND 23-DEHYDRO-25-HYDROXYVITAMIN D_3: NEW IN VIVO METABOLITES OF VITAMIN D_3. J.K. Wichmann, H.K. Schnoes, and H.F. DeLuca (Dept. of Biochem., College of Agr. and Life Sci., Univ. of Wisconsin-Madison, Madison, WI 53706) *Biochemistry 20*(26):7385-7391 (1981). Four new in vivo metabolites of vitamin D_3 were isolated from the blood plasma of chicks given large doses of vitamin D_3. The metabolites were isolated by methanol-chloroform extraction and a series of chromatographic procedures. By use of mass spectrometry, ultraviolet absorption spectrophotometry, and specific chemical reactions, the metabolites were identified as 23,24,25-trihydroxyvitamin D_3, 24,25,26-trihydroxyvitamin D_3, 24-keto-25-hydroxyvitamin D_3 and 23-dehydro-25-hydroxyvitamin D_3.

CHOLESTEROL BEHAVIOR IN HUMAN SERUM LIPOPROTEINS. P.L. Yeagle, J. Bensen, M. Greco, and C. Arena (Department of Biochemistry, State University of New York at Buffalo, Buffalo, NY 14214) *Biochemistry 21*:1249-1254 (1982). A derivative of erogsterol, ergosta-5,7,9,22-tetraen-3β-ol, was synthesized and characterized. Its properties in membranes are similar to those of cholesterol as measured by glucose permeability and by order parameters derived from electron spin resonance of spin-labels. Thus, because of the three conjugated double bonds, this molecule can be used as an optical probe of sterol behavior in membranes. Circular dichroism (CD) spectra of sonicated egg phosphatidylcholine vesicles containing the probe exhibited CD transitions whose intensity was dependent on sterol content. CD spectra from those probe in human low-density and high-density lipoproteins indicated distinctly different environments for the sterol in the two lipoproteins.

STEREOSELECTIVE SYNTHESIS OF (22R)- and (22S)-22-METHYLCHOLESTEROL. J. Zielinski, H.T. Li, and C. Djerassi (Dept. of Chem., Stanford University, Stanford, CA 94305) *J. Org. Chem. 47*(4):620-625 (1982). 22-Methylcholesterol, though hitherto unknown, is likely to exist in nature in the marine environment. In order to facilitate its eventual recognition, stereoselective syntheses of the 22R and 22S isomers of 22-methylcholesterol were developed by using the Claisen rearrangement of appropriate precursors of established absolute configuration. An alternate approach involved hydroboration of an appropriate 22-methylene precursor, separation of the isomeric primary alcohols, mesylation, and lithium aluminum hydride reduction. Differentiation of these two isomers between themselves and from the common (24R)- and (24S)-22-methylcholesterols is possible on the basis of proton and ^{13}C NMR measurements as well as chromatographic mobility.

HYDROPEROXIDES FORMED BY FERROUS ION-CATALYZED OXIDATION OF METHYL LINOLENATE. I. Toyoda, J. Terao, and S. Matsushite (Research Institute for Food Science, Kyoto University, Uji, Kyoto 611, Japan) *Lipids 17*(2):84-90 (1982). An emulsion of methyl linolenate was allowed to oxidize with a catalyst of ferrous sulfate and ascorbic acid. Three oxidation products were isolated, and their hydrogenated derivatives were characterized as the isomeric mixture of methyl monohydroxyoctadecanoate (monoOH), methyl 9,16-dihydroxyoctadecanoate (diOH), and the isomeric mixture of methyl trihydroxyoctadecanoate (triOH). The monoOH isomers and diOH apparently were deived from methyl monohydroperoxyoctadecatrienoate (monoHPO) and methyl dihydroperoxyoctadecatrienoate (diHPO), respectively. Two triOH isomers (the 9,10,12- and 13,15,16-isomers) were thought to be derived from the products containing cyclic peroxide-hydroperoxide structure. 9,16-diHPO was produced by the incubation of monoHPO with ferrous sulfate and ascorbic acid. Moreover, the experiment using $^{18}O_2$ demonstrated that monoHPO yielded 9,16-diHPO by reacting with oxygen molecule, 9,10,13- and/or 9,12,13- and 12,13,16- and/or 12,15,16-triOH isomers were also detected in the hydrogenated derivatives of oxidation products from monoHPO.

A NEW PROCEDURE FOR THE ACETYLATION OF LIPIDS. N. Totani and T. Muramatsu (Federal Center for Lipid Research, Piusallee, D-4400 Munser (Federal Republic of Germany)) *Chem. and Physics of Lipids 29*(4):375-377 (1981). Long-chain alcohols and other lipids containing hydroxy groups are acetylated at room temperature by reaction with acetic anhydride in acetonitrile in the presence of an ion interchanger.

Biochemistry and nutrition

INCREASED HEPATIC CHOLESTEROL PRODUCTION DUE TO LIVER HYPERTROPHY IN RAT EXPERIMENTAL NEPHROSIS. A.C.R.K. Goldberg, H.C.F. Oliveira, F.C.R. Quintão, and D.J. McNamara (Lipid Unit, Univ. of São Paulo Medical School, Dept. of Internal Medicine, Av. Dr. Arnaldo 455 3° a/sala 40, 01246 Sao Paulo (Brazil)) *Biochim. Biophys. Acta 710*(1):71-75 (1982). Control and nephrotic rats were compared as to the liver contents of cholesterol, phospholipid and the activity of microsomal 3-hydroxy-3-methylglutaryl-coenzyme A reductase. Whole liver homogenates as well as endoplasmic reticulum membrane samples showed increased free cholesterol and phospholipid mass in the nephrotics. Correction of the values by the protein content indicated membrane expansions, i.e. liver hypertrophy. However, total hepatic cholesterol synthesis as measured by the reductase activity was increased in the nephrotic rat. These results are in accordance with previous studies showing enhanced cholesterol production in experimental nephrosis. In short, enhanced cholesterol mass in the liver coexists with increased hepatic synthesis in the experimental model used.

ON THE SPECIFICITY OF A PHOSPHOLIPASE A_2 PURIFIED FROM THE 106,000 X G PELLET OF BOVINE BRAIN. N.C.C. Gray and K.P. Stirckland (Dept. of Biochemistry, University of Western Ontario, London, N6A 5C1, Ontario, Canada) *Lipids 17*(2):91-96 (1982). Assessment has been made of the specificity of a purified phospholipase A_2 from the 106,000 X g pellet (microsomal fraction) of bovine grey matter which shows strong activity toward phosphatidyl-inositol (PI). In the first series of experiments involving the utilization as substrates of PI with different ^{14}C- or ^{3}H-labeled fatty acids in the 2-position, the purified phospholipase A_2 most readily removed 16:0 palmitic acid, followed by 18:0 stearic acid, 18:1 oleic acid and 20:4 arachidonic acid. In the second series of experiments, the purified phospholipase A_2 showed preferential action toward PI (100%) compared to phosphatidylcholine (PC, 62.5%), phosphatidic acid (PA, 32.6%), phosphatidylethanolamine (PE, 25.1%) and phosphatidylserine (PS, 21.5%), where each phosphoglyceride was labeled in the 2-position with [1-^{14}C] oleic acid. In the third series of experiments, fatty acids were shown to cause inhibition of action of the purified phospholipase A_2 on 1-acyl, 2-[1-^{14}C] oleoyl PI in the order 20:4 18:1 18:0 16:0 which is the reverse order to that just noted. In the final series of experiments, the addition of the phosphoglycerides PC, PE, PS and PA in amounts of 5 or 10 M caused either no inhibition (PE, 2%), slight inhibition (PC, 15%) or reasonably significant inhibition (PA, 20% and PS, 40%) of

action of the purified phospholipase A_2 on 1-acyl, 2-[1-^{14}C]-oleoyl PI.

STUDIES ON THE IN VIVO SYNTHESIS OF TRIACYLGLYCEROL IN MOUSE LIVER. M.D. Greenspan, E.A. Schroeder, and J.B. Yudkovitz (Merck Sharp and Dohme Research Laboratories, Rahway, NJ 07065) *Biochim. Biophys. Acta 710*:15-22 (1982). The in vivo biosynthesis of hepatic glycerolipids was examined by studying the incorporation of [2-^{3}H]glycerol into triacylglycerol in the mouse. The isotope was administered by rapid injection into the portal view. The incorporation of glycerol was linear for about 1 min and maximal rates were seen in the presence of additional oleic acid. At 20 μmol of oleic acid bound to 1 μmol of bovine serum albumin, the highest level tested, glycerol incorporation was still increasing linearly, whereas a plateau was reached at 4 μmol of glycerol. The liver incorporated 200-300 nmol of [2-^{3}H]glycerol into triacylglycerol per min when 20 μmol of albumin-bound oleic acid plus 4 μmol of glycerol were injected in a 0.2 ml volume. Studies on the uptake by the liver after intraportal injection revealed that at 0.5 min the liver retained 2 μmol of glycerol and 5 μmol of oleic acid, indicating that the uptake of substrate was not rate-limiting. The results suggest that the availability of substrate is a major factor in the regulation of triacylglycerol biosynthesis by the liver.