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Fats and oils

STUDIES ON THE CATALYTIC HYDROGENATION OF BIOMEMBRANES. HYDROGENATION OF PHOSPHATIDYLCHOLINE LIPOSOMES BY TWO WATER-SOLUBLE RHODIUM-PHOSPHINE CATALYSTS. F. Farin, H.L.M. Van Gaal, S.L. Bonting, and F.J.M. Daemen (Depts. of Biochem. and Inorganic Chem., Univ. of Nijmegen, Nijmegen The Netherlands) *Biochim. Biophys. Acta 711*(2):336-344 (1982). The reactions of two water-soluble amphiphilic rhodium-phosphine hydrogenation catalysts, chlorotris (sodium diphenylphosphinobenzene-*m*-sulfonate) rhodium(I) and chlorotris(bissodium diphenylphosphinoundecylphosphate)rhodium(I), with aqueous dispersions of unsaturated phosphatidylcholines have been studied. Under mild reaction conditions (p_{H_2} =1.2 atm, 37°C, pH 6.9) hydrogenation of aqueous dioleoylphosphatidylcholine dispersions, prepared by sonication, was achieved. This reaction was not affected by the presence of high salt concentrations. The rate of hydrogenation was independent of catalyst concentration. The reaction with dioleoylphosphatidylcholine dispersions, prepared by the ethanolic injection method, was preceded by a lag period of about 5 h. The reaction with dioleoylphosphatidylcholine dispersions, obtained by vigorous shaking, was rather slow, suggesting the presence of a penetration barrier. The reaction with dioleoylphosphatidylcholine proceeds via the isomerisation of the oleoyl to the elaidoyl moiety, followed by hydrogenation of the elaidoyl moiety. The possible interactions of the catalysts with the bilayer are considered and the implications of these findings are discussed.

STEROL COMPOSITION OF BOVINE RETINAL ROD OUTER SEGMENT MEMBRANES AND WHOLE RETINAS. S.J. Fliesler and G.J. Schroepfer, Jr. (Depts. of Biochem. and Chem., Rice Univ., Houston, TX 77001) *Biochim. Biophys. Acta 711* (1):138-148 (1982). The sterol composition of bovine retinal rod outer segment membranes and whole retinas was studied by detailed chromatographic analyses. Cholesterol represented at least 98% of the total 3β-monohydroxy sterols of rod outer segment membranes, accounting for 1.68 ± 0.15% of the dry weight. Whole retinas contained 1.76 ± 0.29% cholesterol by dry weight, representing at least 99% of the total 3β-monohydroxy sterols. Trace amounts of a component having the chromatographic properties of 5α-cholestan-3β-ol were found in rod outer segment membranes and whole retinas. Very small amounts of a component having the chromatographic properties of 5α-cholest-7-en-3β-ol were found in whole retinas, but not in rod outer segment membranes. The molar ratio of cholesterol to rhodopsin in bovine rod outer segment membranes was approximately 4.7. Cholesterol accounted for only 5-7 mol% to total rod outer segment membrane lipids.

FATE OF MILK ^{125}I-LABELED LIPOPROTEIN LIPASE IN CELLS IN CULTURE. COMPARISON OF LIPOPROTEIN LIPASE- AND NON-LIPOPROTEIN LIPASE-SYNTHESIZING CELLS. G. Friedman, T. Chajek, T. Olivecrona, O. Stein, and Y. Stein (Lipid Res. Lab., Dept. of Med. B, Hadassah Univ. Hospt., Dept. of Experimental Med. and Cancer Res., Hebrew Univ.-Hadassah Med. Schl., Jerusalem, Israel) *Biochim. Biophys. Acta 711* (1):114-122 (1982). Radioiodinated lipoprotein lipase, isolated from bovine milk (^{125}I-labeled milk lipoprotein lipase) was shown to retain full hydrolytic activity towards its native substrate, i.e., chylomicron triaclglycerol. The ^{125}I-labeled enzyme interacted with various cells in culture by being bound to the cellular surface, internalized and degraded. Cellular binding of the labeled enzyme occurred in the presence or absence of substrate and was related to enzyme concentration. Heparin reduced cellular binding by 50% but inhibited uptake and degradation more extensively. Cellular uptake was not affected by chloroquine or NH_4Cl, but degradation of the labeled enzyme was blocked. Uptake and degradation were not inhibited by mannose 6-phosphate. The interaction between the exogenous enzyme and cells which do not synthesize lipoprotein lipase, i.e., fibroblasts and endothelial cells, resulted in a high ratio of surface binding to degradation. In heart cell cultures and preadipocyte cultures, which produce lipoprotein lipase, the ratio of enzyme catabolized to that bound was high at all time points examined. Since in the intact organism lipoprotein lipase acts at the luminal surface of vascular endothelium, it seems expedient that these cells are able to bind the enzyme, but will catabolize it only slowly. The rapid and extensive degradation of the ^{125}I-labeled lipoprotein lipase in heart cells and preadipocytes may be related to the metabolism of the endogenously produced lipoprotein lipase.

MICROEMULSIONS OF PHOSPHOLIPIDS AND CHOLESTEROL ESTERS. PROTEIN-FREE MODELS OF LOW DENSITY LIPOPROTEIN. G.S. Ginsburg, D.M. Small, and D. Atkinson (Biophys. Inst., Boston Univ. Med. Center, Boston, MA 02118) *J. Biol. Chem. 257*(14):8216-8227 (1982). Low density lipoproteins (LDL) are microemulsions consisting of an apolar core of cholesterol esters surface stabilized by phospholipid and protein. As models for the lipid organization of LDL, protein-free homogeneous microemulsions have been prepared from specific phospholipids and cholesterol esters. Aqueous dispersions of cholesteryl oleate (CO) or cholesteryl nervonate (CN) with egg yolk (EYPC), dimyristoyl (DMPC), or dipalmitoyl (DPPC) phosphatidylcholine were sonicated. Fractionation by ultracentrifugation and agarose gel column chromatography yielded homogeneous particles. Electron microscopy showed the particles to be circular. Differential scanning calorimetry, x-ray scattering/diffraction, and ^{1}H NMR spectroscopy studies showed that the lipids in the microemulsions could undergo at least *two* specific thermal transitions depending on composition. EYPC/CO and EYPC/CN microemulsions exhibit an order-disorder transition of the core-located cholesterol esters in the particle core compared to the temperatures for the analogous transition in the neat cholesterol esters suggest that the core cholesterol esters are stabilized with respect to temperature. Microemulsions formed with DPPC exhibit *concomitant* melting of surface phospholipids and core cholesterol esters indicative of coupling between the core and the surface. The results show that stable microemulsions with the size and general organization of LDL can be made from phospholipids and cholesterol esters without protein. These results may be extended to native lipoproteins and suggest that interactions between core and surface phases take place dependent on their lipid composition.

BIOSYNTHESIS OF HYDROCARBON IN *ANABAENA VARIABILIS* IN VIVO INCORPORATION OF [18-^{14}C] STEARATE. R.S. Goodloe and R.J. Light (Dept. of Chem., Florida State Univ., Tallahassee, FL 32306) *Biochim. Biophys. Acta 711*(2):261-265 (1982). Cell-free extracts of *Anabaena variabilis* which are capable of incorporating the methyl group of S-[*methyl*-^{14}C] adenosylmethionine into branch methylheptadecanes (Fehler, S.W.G. and Light, R.J. (1972) Biochemistry 11, 2411-2416) have now been shown to incorporate [18-^{14}C] stearate into alkane. The rate of stearate incorporation is only about 0.1% that of the methyl group incorporation, and only about 10% the rate of stearate decarboxylation, as measured by conversion of [1-^{14}C] stearate to carbon dioxide. Oxygen stimulates the stearate incorporation into alkanes but inhibits the methyl group incorporation.

CEREBROTENDINOUS XANTHOMATOSIS: REDUCED SERUM 26-HYDROXYCHOLESTEROL. N.B. Javitt, E. Kok, B. Cohen, S. Burstein (Div. of Hepatic Diseases, New York Hospital-Cornell Med. Center, New York, NY 10021) *J. Lipid Res. 23*(4):627-630 (1982). Serum 26-hydroxycholesterol was quantitated by isotope dilution-mass spectrometry in normal individuals and in patients with cerebrotendinous xanthomatosis. In the normal individuals, the concentration of 26-hydroxycholesterol in serum ranged from 4.3 to 13.0 μg/100 ml. In five patients with CTX, 26-hydroxycholesterol in serum ranged from 0 to 0.6 μg/100 ml. The findings can be explained by reduced or absent mitochondrial C_{27} steroid 26-hydroxylase activity. The method is useful for detection of CTX and perhaps other disturbances of sterol and bile acid metabolism.

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STEREOSPECIFICITY OF PREMATURE HUMAN INFANT LINGUAL LIPASE. R.G. Jensen, F.A. Dejong, R.M. Clark, L.G. Palmgren, T.H. Liao, and M. Hamosh (Dept. of Nutr. Sci., Univ. of Connecticut, Storrs, CT 06268) *Lipids* 17(8):570-572 (1982). The lingual lipase in gastric aspirates from premature infants was found to be partially stereospecific for *sn*-3 esters of synthetic enantiometric triacylglycerols containing 18:1 and 16:0. The *sn*-3 ester was hydrolyzed about 4 times faster than the acid at the *sn*-1 position with no difference in rates between 18:1 and 16:0. The *sn*-2 was also hydrolyzed to some extent.

SIMULTANEOUS DETERMINATION OF TRIGLYCERIDES, PHOSPHOLIPIDS, AND CHOLESTERYL ESTERS BY INFRARED SPECTROMETRY. H.J. Kisner, C.W. Brown, G.J. Kavarnos (Dept. of Chem., Univ. of Rhode Island, Kingston, RI 02881) *Anal. Chem. 54*(9):1479-1485 (1982). A simultaneous infrared analysis of the predominant serum lipids has been developed utilizing the difference in their ester carbonyl absorbance peaks. Band overlap and resultant spectral interference have been minimized by using analytical wavelengths on the band shoulders. Elements of the calibration or *K* matrix were generated for one component at a time by redefining the solvent to include all components except the one being analyzed. In this fashion, intermolecular interactions were incorporated into the calibration and matrix development. Relative concentrations corresponded to biological concentrations in serum. All spectrometric procedures and calculations were handled on-line, which greatly reduced the analysis time.

PARTIAL MOLAR VOLUMES OF SOME 1-ALKANOLS IN ERYTHROCYTE GHOSTS AND LIPID BILAYERS. Y. Kita and K.W. Miller (Depts. of Pharmac. and Anesthesia, Harvard Med. Schl. and Massachusetts Gen. Hosp., Boston, MA 02114) *Biochemistry 21*(12):2840-2847 (1982). The partial molar volumes of 1-heptanol and 1-octanol in red cell ghosts, in egg phosphatidylcholine bilayers, and in water and phosphate buffer have been measured to a precision of better than 4% by using a density meter. In every case, the partial molar volume was independent of concentration in the range studied. In both membranes, the partial molar volume of each alcohol was close to its molar volume, whereas in aqueous solution it was considerably less. Comparison of the two membranes suggests that the major contribution to the partial molar volume arises from alcohol-lipid interactions in each case. Further comparison with partial molar volumes in bulk solvents suggests that on average the alcohols retain a hydrogen bond in the lipid bilayer. The magnitude of the volume change in ghosts in some 5 times less than the corresponding area changes previously reported by others. These two observations can only be resolved by assuming either that the bilayer expands anisotropically, experiencing a decrease in thickness with increasing volume, or that conformational changes in membrane-associated proteins can occur at constant volume to increase membrane area. Finally, these data are used to test the critical volume hypothesis of general anesthetic action. A volume change of 0.15% in red cell ghost membranes is found to be associated with anesthesia, which compares with a value of 0.2% predicted previously from pressure reversal of anesthesia studies. In egg phosphatidylcholine bilayers, a volume change of 0.36% is associated with anesthesia. The larger change in the lipid bilayer compared to the biomembrane originated solely in their different membrane/buffer partition coefficients.

HEPATIC TRIACYLGLYCEROL LIPASE ACTIVITIES AFTER INDUCTION OF DIABETES AND ADMINISTRATION OF INSULIN OR GLUCAGON. T.E. Knauer, J.A. Woods, R.G. Lamb, and H.J. Fallon (Depts. of Biophys., Med., and Pharmacology, Med. College of Virginia, Richmond, VA 23298) *J. Lipid Res. 23*(4):631-637 (1982). Triacylglycerol lipase activities of homogenates and subcellular fractions of rat liver were measured under optimal conditions at pH 7.5 using emulsified tri[1-^{14}C] oleoylglycerol as substrate. Twenty-four hr after administration of streptozotocin, hepatic alkaline lipase activity was 39% of normal, and this lower level of activity was observed at 72 hr and 7 days, after streptozotocin injection. After 24 hr of starvation, lipase activity was also significantly lower (35%) than normal. Insulin (35 U regular/kg body weight) had no acute (90 min) effect on the hepatic lipase activity of either normal or diabetic rats. Chronic insulin administration (4 subcutaneous injections of 10 U protamine zinc insulin/kg at 16-hr intervals) to normal rats provoked a 40% increase in hepatic lipase activity. Diabetic rats given the same insulin treatment showed lipase activity that was significantly higher (155%) than normal. Lipase activity fell to 65% of normal when insulin was withheld (32 hr) from diabetic rats given chronic insulin therapy. Intracardial injection of glucagon (1 mg/kg) into normal rats had no acute (30 min) effect on hepatic alkaline lipase activity. Hepatic alkaline lipase activity varied independently from the concentrations of either glucose or triacylglycerol in the plasma. However, there was an apparent negative correlation between this lipase activity and the concentration of fatty acids in the plasma; lipase activity was highest when fatty acid concentrations were lowest, and lowest when fatty acid concentrations were elevated.

CHARACTERISTICS OF THE ISOLATED PURINE NUCLEOTIDE BINDING PROTEIN FROM BROWN FAT MITOCHONDRIA. C.-S. Lin and M. Klingenberg (Inst. fur Physiol. Chem., Physikalixche Biochem und Zellbiologie der Univ. Muchen, 8000 Muchen 2, Federal Republic of Germany) *Biochemistry 21*(12):2950-2956 (1982). The isolation of a purine nucleotide binding protein (NbP), the putative uncoupling protein, from hamster brown adipose tissue mitochondria and some of its functional characteristics are described. (1) Among various detergents tested, Triton is the most suitable; the total GDP binding capacity can be recovered after solubilization by Triton and is rather stable in this extract. (2) For separation of NbP from the ADP--ATP carrier, differences in the solubilizing conditions and the stability at room temperature between both proteins are exploited. The preparation is substantially free of ADP/ATP carrier. (3) The purified NbP has a binding capacity for 16 μmol of GDP/g of protein, corresponding to a 16-fold purification from mitochondria. (4) In sodium dodecyl sulfate-polyacrylamide gel electrophoresis a single band of M_r 32 000 is found. A dimer structure is suggested from chemical crosslinking, from the binding capacity for GDP, and from the previously reported centrifugation equilibrium. (5) The isolated NbP preparation consists of Triton-protein-phospholipid mixed micelles with a Stokes radius of 60.5 Å as determined by gel filtration. The Triton binding is 1.9 g/g of protein, and the phospholipid binding is 0.2 g/g of protein. (6) The amino acid composition has a polarity index of 43.5%, the N-terminal peptide has the sequence Val-ASp-Pro-Thr-Thr-Ser-Glu-Val. (7) The affinity of NbP for different purine nucleotides decreases in the order GTP>GDP>ATP>ITP>ADP>IDP. The affinity for the monophosphates is 100 times lower. (8) Photooxidation and the lysine reagent 2,4,6-trinitrobenzenesulfonic acid decrease the binding capacity.

KINETICS AND MECHANISM OF FREE CHOLESTEROL EXCHANGE BETWEEN HUMAN SERUM HIGH- AND LOW-DENSITY LIPOPROTEINS. S. Lund-Katz, B. Hammerschlag, and M.C. Phillips (Dept. of Physiol. and Biochem., Med. Coll. of Pennsylvania, Philadelphia, PA 19129) *Biochemistry 21*(12):2964-1969 (1982). The mechanism of cholesterol and phosphatidylcholine (PC) exchange between human serum lipoproteins has been investigated by following the transfer of radiolabeled cholesterol and PC between high-density lipoprotein (HDL) and low-density lipoprotein (LDL). The general characteristics of the process of exchange of cholesterol between lipoproteins resemble those for exchange between smaller unilamellar vesicles. The results are only consistent with a mechanism of exchange in which cholesterol molecules diffuse through the aqueous phase; the experimental activation energy is associated with desorption of lipid from the donor lipoprotein into the aqueous phase.

ESTRADIOL FATTY ACID ESTERS. S.H. Mellon-Nussbaum, L. Ponticorvo, F. Schatz, R.B. Hochberg (Dept. of Med., Roosevelt Hosp. and the Dept. of Biochem., Obstetrics and Gynecology and the Inst. for Human Reproduction, College of Physicians and Surgeons, Columbia Univ., New York, NY 10019 and the Depts. of Obstetrics and Gynecology, Molecular Biophys and Biochem., Yale Univ. Schl. of Med., New Haven, CT 06473) *J. Biol. Chem. 257*(10):5678-5684 (1982). The bovine uterus, like other estrogen-responsive organs of man rat synthesizes an ususual nonpolar metabolite of estradiol (Schatz, F., and Hochberg, R.B. (1981) *Endocrinology* 109, 697-703). This compound, the lipoidal derivative of estradiol (LE_2), was synthesized from estradiol by bovine endometrial tissue *in vitro* and was purified by extensive chromatography on two adsorption columns, a reversed phase partition

celite column and three different high pressure liquid chromatography columns. LE_2 was separated into nine fractions which were analyzed by direct probe mass spectroscopy and by gas chromatography mass spectroscopy after cleavage of the lipoidal moieties. In this manner, 10 fatty acid esters of estradiol, exclusively esterified at C-17 of the steroid nucleus, were identified. The unsaturated fatty acid esters of estradiol comprise more than 85% of the total LE_2 and estradiol 17β-arachidonate is the most abundant component. The cholesterol esters and phospholipids of the bovine endometrium were also purified and it was found that the distribution of fatty acids in these lipids is far different from that of LE_2.

BIOSYNTHETIC CONTROL OF THE NATURAL ABUNDANCE OF CARBON 13 AT SPECIFIC POSITIONS WITHIN FATTY ACIDS IN *SACCHAROMYCES CEREVISIAE*. K.D. Monson, J.M. Hayes (Biogeochemical Lab., Depts. of Chem. and of Geology, Chem. Building, Indiana Univ., Bloomington, IN 47405) *J. Biol. Chem. 257*(10):5568-5575 (1982). Measurements of the natural abundance of ^{13}C at C-1, C-9, and C-10 in fatty acids synthesized by *Saccharomyces cerevisiae* grown aerobically at 30°C show that alkyl chain positions derived from the carboxyl group of the acetate precursor must be enriched in ^{13}C by 2.5 ± 0.6 parts per thousand while those derived from the methyl group in acetate must be depleted in ^{13}C by an equal amount. Selective depletions of ^{13}C observed at the C-9 and C-10 positions of palmitoleate and oleate require that (*i*) the carbon kinetic isotope effect associated with the action of desaturase at C-9 must be between 1.2 and 1.6% *in vivo*, (*ii*) at C-10 the effect must be between 0.9 and 1.3%, and (*iii*) less than 20% of the C_{18} carbon skeletons synthesized are preserved within the cell, the remainder apparently being degraded. It is shown that *de novo* synthesis (*i.e.* by fatty acid synthetase) is responsible for the production of more than 95% of the supply of 18-carbon acyl groups, the remainder being provided by all other elongation pathways. In an ancillary study designed to test the accuracy and generality of these results, it was observed that still larger specific depletions occurred at olefinic carbon positions in fatty acids from soybeans, thus suggesting that the degradation of substantial quantities of C_{18} carbon skeletons may be a widespread feature of fatty acid metabolism in eukaryotes. It is suggested that the required degradation is associated with the action of peroxisomes.

EARLY INCREASES IN PHOSPHOLIPID METHYLATION ARE NOT NECESSARY FOR THE MITOGENIC STIMULATION OF LYMPHOCYTES. J.P. Moore, G.A. Smith, T.R. Hesketh, and J.C. Metcalfe (Dept. of Biochem., Univ. of Cambridge, Cambridge CB2 1QW, United Kingdom) *J. Biol. Chem. 257*(14):8183-8189 (1982). It has been reported that when mouse splenic lymphocytes are mitogenically stimulated by concanavalin A (Con A) there is a transient increase in phospholipid methylation via methionine which is maximal within 10 min of addition of the mitogen, and subsequently decreases to the level in unstimulated cells over 60 min. Here we have examined the effect of Con A at optimal concentrations for mitogenic stimulation on the uptake of L-[*methyl*-^{3}H] methionine and on the incorporation of [^{3}H] methyl groups into phospholipid, in three types of lymphocytes. Our results indicated that normal responses to stimulation by mitogens were occurring under conditions where no change in phospholipid methylation was detected. We concluded that no early change in the net methylation of phospholipid occurs in lymphocytes which are mitogenically stimulated by Con A, and that the effect previously reported is therefore not an obligatory component of the mitogenic signal in these cells.

EFFECT OF CYTOCHROME B_5 ON THE TRANSBILAYER DISTRIBUTION OF PHOSPHOLIPIDS IN MODEL MEMBRANES. J.R. Nordlund, C.F. Schmidt, P.W. Holloway, and T.E. Thompson (Dept. of Biochem., Univ of Virginia Schl. of Med., Charlottesville, VA 22908) *Biochemistry 21*(12):2820-2825 (1982). The transbilayer distribution of phosphatidylethanolamine was assessed in phosphatidylcholine-phosphatidylethanolamine vesicles that contained various amounts of cytochrome b_5. The small vesicles, made by sonication, and the large vesicles, made by ethanol injection, were fractionated by centrifugation before cytochrome b_5 was asymmetrically incorporated into the bilayer. The mole ratio of phospholipid to protein ranged from 280 to 560 in the small vesicles and from 100 to 500 in the large vesicles. The phosphatidylethanolamine distribution, determined by chemical labeling with trinitrobenzenesulfonic acid, was assessed in vesicles that contained intact cytochrome b_5 molecules and in vesicles where only the hydrophobic tail remained associated with the bilayer. At every phospholipid to protein ratio examined, the transbilayer distribution of phosphatidylethanolamine in either the small or large unilamellar vesicles was not significantly different from the distribution in control vesicles that contained no protein. Ethanol was added to some cytochrome b_5-vesicle preparations (20% v/v) in an attempt to facilitate rearrangement of the phospholipids. No differences in the transbilayer distribution were observed. These results are discussed in terms of transbilayer equilibrium and the perturbation induced by the protein.

INTERACTION OF α-TOCOPHEROL WITH DIPHENYLPICRYL HYDRAZYL. A MEANS TO DETERMINE THE POLARITY OF THE ENVIRONMENT AROUND α-TOCOPHEROL AND ITS BINDING WITH LIPIDS. A.M. Rao, U.C. Singh, and C.N.R. Rao (Solid State and Structural Chem. Unit, Indian Inst. of Sci., Bangalore-560012, India) *Biochim. Biophys. Acta 711* (1):134-137 (1982). α-Tocopherol is found to interact with the stable free radical DPPH orders of magnitude faster than ordinary phenols. It is suggested that the high reactivity arises from the coplanarity of the C-O-C framework with the aromatic ring. The rate of the reaction of α-tocopherol with DPPH increases progressively with solvent polarity and can be quantitatively related to Kosower's Z parameter. Fatty acid derivatives slow down the reaction with DPPH due to binding with α-tocopherol.

LIPID-PROTEIN INTERACTIONS. EFFECT OF APOLIPOPROTEIN A-I ON PHOSPHATIDYLCHOLINE POLAR GROUP CONFORMATION AS STUDIED BY ^{1}H NMR. D.-J. Rejingoud, et. al. (Dept. of Physiology and Biochem., Med. Coll. of Pennsylvania, Philadelphia, PA 19129) *Biochemistry 21*(12):2977-2983 (1982). Spin-spin coupling constants derived from high-resolution ^{1}H NMR spectra of pure 1-myristoyl-*sn*-glycero-3-phosphocholine (MLPC) micelles and 60:1 mol/mol MLPC-human apolipoprotein A-I (apo A-I) complexes have been analyzed in order to determine the effects of apoprotein on phosphatidylcholine (PC) polar group conformation. The shift ratios of the polar group proton resonances after addition of the paramagnetic shift reagent $Fe(CN)_6^{3-}$ to the above MLPC systems, egg PC small unilamellar vesicles, and human HDL_3 have been used to compare the PC polar group conformations in all systems. The location of the largely α-helical apo A-I molecules in the complex with MLPC was deduced from its effects on the chemical shifts and spin-lattice relaxation times (T_1) of the well-resolved ^{1}H resonances from the various parts of the lipid molecules. The data are consistent with the apo A-I molecules lying in the surface of the MLPC micelle with their amphipathic, α-helical segments intercalated among the glycerophosphocholine groups of the lipid molecules so that aromatic amino acid side chains are interspersed among the lipid hydrocarbon chains. This leads to a spacing out of the glycerol backbones and immediately adjacent methylene groups of the MLPC molecules, thereby causing an enhancement of the motions affecting T_1. The presence of apo A-I at the lipid-water interface apparently does not perturb the PC polar group conformation, indicating that this conformation is determined by intramolecular effects. The preferred conformation of the phosphocholine groups is characterized by an almost gauche conformation of the choline group and predominantly antiperiplanar conformations.

INTERACTION OF LIVER CLATHRIN COAT PROTEIN WITH LIPID MODEL MEMBRANES. C.J. Steer, R.D. Klausner, and R. Blumenthal (Lab. of Biochem. and Metabolism, Natl. Inst. of Arthritis, Diabetes, and Digestive and Kidney Diseases and the Lab. of Theoretical Biol., Natl. Cancer Inst., Natl. Insts. of Health, Bethesda, MD 20205) *J. Biol. Chem. 257*(14):8533-8540 (1982). Coated vesicles were prepared from rat liver homogenates by sucrose density gradient centrifugation and the clathrin coat, dissociated by mild treatment with Tris-Cl, was characterized by sodium dodecyl sulfate gel electrophoresis. At pH 6.5 and below, the soluble clathrin preparation induced a voltage-dependent increase in ion conductance across a black lipid membrane of oxidized cholesterol under conditions where the intact coated vesicles were inert. Further evidence for the interaction of clathrin with the lipid bilayer was provided by the demonstration that the fluorescent dye, carboxyfluroescein, was released from the internal aqueous space of small

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unilamellar dioleoyl and dipalmitoyl phosphatidylcholine vesicles upon the addition of the soluble protein. Dye release was shown to be a function of both calcium and pH. In the presence of calcium, release was most prominent at pH 5.0-6.5. Formation of stable protein-phospholipid complexes was shown by KBr density gradient centrifugation. Stability of the complexes in high salt concentration suggested that electrostatic interactions were not essential to maintain the recombinants. Here, as with the black lipid membrane, incubation of the phospholipid vesicles with intact coated vesicles failed to induce release of the dye at pH 6.5 or below. Interaction of the clathrin coat protein with unilamellar vesicles at low pH resulted in a change in vesicle size and morphology as shown by electron microscopy.

MEASUREMENT OF EICOSAPOLYENOIC ACIDS IN THE SERUM BY GAS-LIQUID CHROMATOGRAPHY-CHEMICAL IONIZATION MASS SPECTROMETRY. M. Suzuki, M. Nishizawa, T. Miyatake, and Y. Kagawa (Tokyo Metropolitan Institute of Medical Science, 3-18-22 Honkomagome, Tokyo, Japan 113) *J. Lipid Res. 23*:363-366 (1982). The use of octadeutero eicosatetraenoic acid as an internal standard for the reproducible measurement of serum eicosapolyenoic acids ($C_{20:3}$, $C_{20:4}$, and $C_{20:5}$) by gas-liquid chromatography-chemical ionization mass spectrometry is described. The method has the following advantages. The physiochemical properties of the internal standard and the eicosapolyenoic acids are similar. The acids are easily separated from compounds of similar retention times by means of selected ion monitoring. The measurements can be made more rapidly, (10 min per sample) than with previous techniques.

UTILIZATION OF ASCORBYL PALMITATE, α-TOCOPHEROL AND PHOSPHOLIPIDS AS ANTIOXIDANTS IN LARD. C.F. Bourgeois and A.M. Czornomaz, *Rev. Franç. Corps Gras 29*(3):111-116, french. RFCG 82-09 (1982). Lard contains only a small amount of natural antioxidants, so that it is very sensitive to oxidation. In this article, it is shown that a mixture of ascorbyl palmitate, α-tocopherol, and phospholipids is able to efficiently preserve it. This mixture is made liquid by adding some ethanol, which is stripped just after introduction in the fat. Depending on the phospholipids extract used, the mixture is a solution at room temperature, or a suspension.

ON THE REFINABILITY OF OILS. VII. RELATIONS BETWEEN MINERAL ELEMENTS OF REFINED OILS AND ANALYTICAL CHARACTERISTICS OF CORRESPONDING RAW OILS. A. RESEARCH OF NUMERICAL RELATIONS. E. Sambuc, M. Devinat and M. Naudet, *Rev. Franç. Corps Gras 29*(3):117-123, french. RFCG 82-10 (1982). Iron and phosphorus contents of 33 soybean oils and 35 rapeseed oils refined in laboratory in standard conditions have been compared with analytical characteristics of corresponding raw oils by means of polynomial relations concerning only three variables. It is impossible to obtain such numerical relations from which iron or phosphorus contents of refined oils would be previsible with the precision required for significative calculated values. Explanation of this impossibility is attempted.

RAPID DETERMINATION OF MONO-, DI- AND TRIGLYCERIDE DISTRIBUTION IN FATS AND OILS BY THIN-LAYER CHROMATOGRAPHY AND ENZYMATIC ESTIMATION. S. Chemin Douaud and A. Karleskind, *Rev. Franç. Corps Gras 29*(3):125-128, french. RFCG 82-11 (1982). Triglycerides and partial glycerides have been separated by thin layer chromatography. Every glyceride type has been determinated colorimetrically in proximal ultraviolet: enzymatic conversion of glycerol into glycerophosphate by adenosine triphosphate and determination of obtained adenosine diphosphate.

DETERMINATION OF ISOTHIOCYANATES AND OXAZOLIDINETHIONE IN OIL-SEEDS AND MEALS. RESULTS OF TWO INTERNATIONAL COLLABORATIVE ANALYSES FROM AFNOR. E. Nouat, *Rev. Franç. Corps Gras 29*(3):129-132, french. RFCG 82-12 (1982). The studies of french and international standardization on ITC and VTO determination are reviewed. Results of international collaborative analyses from AFNOR are given. They led to a french standard and a draft of international standard.

STATUS OF RESEARCH CONCERNING THE ANALYSIS OF SURFACTANT MIXTURE. FIRST PART. C. Demanze, *Rev. Franç. Corps Gras 29*(3):157-163, french. RFCG 82-13 (1982). The analytical scheme for a full and accurate study of a surfactant complex mixture, for instance in a few sanitary or cleaning products, is described from analytical processes developed during the past decade. Every phase of this analytical scheme is applicable to any active material whatever the composition may be; it gathers the usualest and/or the latest methods by means of a hunder bibliographical references and twenty figures.

STUDY ON LIPOLYTIC ACTIVITY OF MICROCOCCACEAE. C. Delarras, *Rev. Franç. Corps Gras 29*(4):165-167, french. RFCG 82-14 (1982). The modified Rath's technique has been applied to 230 Micrococcaceae strains (73 collections strains and 157 isolated from various foods) for the detection of butter and lard lipases. The collection Staphylococci are more lipolytic (29 and 37%) than collection Micrococci (11 and 3%). 47% of Staphylococcus food strains have a "lipase butter", so 54% a "lipase lard". These lipases are also found in Staphulococci isolated from raw milk or french dry sausage; but, 75% first strains have the "butter lipase" and only 34% for second strains. 32% of dry sausage Staphylococci have the "lard lipase" and so 47% of raw milk Staphylococci. The different Staphylococci species: S. aureus coagulase + and −, S. epidermidis, S. saprophyticus, S. xylosus 1 and 2, S. hominis 1, previously isolated and identified in foods, have these lipases.

ON THE REFINABILITY OF OILS. VII. RELATIONS BETWEEN MINERAL ELEMENTS OF REFINED OILS AND ANALYTICAL CHARACTERISTICS OF CORRESPONDING RAW OILS. B. DISPLAY AND UTILIZATION OF "INDICATOR TESTS". M. Naudet and E. Sambuc, *Rev. Franç. Corps Gras 29*(4):169-176, french. RFCG 82-15 (1982). 35 raw new rapeseed oils and 33 raw soybean oils have been refined in laboratory in precisely definite conditions. The contents in a few mineral elements participate in the prevision of immediate flavor score. Although these mineral element contents of refined oils are not previsible from characteristics of raw oils by means of numerical relations, it is shown, on qualitative plan, that some characteristics of raw oils are "indicator tests" of as well iron as phosphorus content of refined oils. These characteristics of raw oils are, in the case of new rapeseed oils the carbonyl value, sodium content, not glyceride content (refining loss) and absorption at 270 nm; in the case of soybean oils, the absorption at 315 nm, phosphorus content, p. anisidine value and trichromatic color. So, it is possible to suggest the required characteristics of raw oils in order to, after refining in the used conditions, the mineral element contents of the freshly deodorized oil be compatible with the immediate sensorial acceptability threshold.

VARIATION IN *CERCOSPORIDIUM PERSONATUM* SYMPTOMS ON CERTAIN CULTIVARS OF *ARACHIS HYPOGAEA*. P. Subrahmanyam, D. McDonald and R.W. Gibbons, *Oleagineux 37*(2):63-68 (1982). Symptoms of the leafspot diseases of groundnut caused by *Cercosporidium personatum* (Berk. & Curt.) Deighton and *Cercospora arachidicola* Hori are superficially similar. The two diseases commonly occur together and in some research reports are not differentiated. This could cause confusion as a cultivar could be susceptible to one disease but resistant to the other. Ten cultivars with characteristic reactions to *C. personatum* at Hyderabad were inoculated with the pathogen in a glasshouse trial and disease symptoms studied. Symptoms varied some closely resembling those of *C. arachidicola*. Detailed observations were necessary for accurate diagnosis. This is of obvious importance when screening for disease resistance.

TOLERANCE TO DROUGHT OF SOME COCONUT HYBRIDS. M. Pomier and G. de Taffin, *Oléagineux 37*(2):55-62 (1982). The extension of coconut development programmes to zones which are marginal for this species has become a necessity for many countries. It is in pursuance of this policy that supporting research has been set up in the mid-Ivory Coast, which suffers from a long dry season. Several types of hybrids were tested in this region, and compared to the local variety, West African Tall. Current results show that the hybrid PB 121 (Malayan Yellow Dwarf X West African Tall) is the most tolerant to drought as regards both the reduction in the number of leaves and the loss of nuts, two particularly representative factors. As for West African Tall, it is distinctly sensitive, and so is its hybrid with Rennell Tall. The other Dwarf X Tall hybrids behave differently according to the factor observed. At present, therefore, PB 121 is recommended for regions with marginal rainfall.

POSSIBLE INCIDENCE OF INFESTATION BY *RECILIA MICA*, VECTOR OF OIL PALM BLAST, ON AMINO-ACID METABOLISM IN THE PLANT. J.L. Renard, G. Quillec and M. Ollagnier,

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Oléagineux 37(2):43-48 (1982). The blast vector agent *Recilia mica* Kramer, when introduced in the oil palm seedling, can cause two types of metabolic disturbances: precocious ones linked to the insects' presence alone, and later ones linked to the infectious process.

STUDY OF THE GLYCERIDE STRUCTURE OF SHEA BUTTER. K. Sawadogo and J. Bezard, *Oléagineux 37*(2):69-74 (1982). This work gives analytical data on the triacylglycerol structure of shea butter from Upper Volta (Africa). This concrete fat exhibited a high proportion of oleic acid (45.6%) and of stearic acid (44.3%). Oleic acid was found preferentially esterified in the 2-position (60%), less than linoleic acid (85%) in low proportion in shea butter, but much more than stearic acid (2.4%). The total triacylglycerols were fractionated according to unsaturation and the fractions were analyzed for fatty acid and triglyceride compositions. The monounsaturated fraction accounted for 50% and the di-monounsaturated fraction for 27.3%, together representing more than two thirds of the fat. A mathematical method using data brought about by the fatty acid and triacylglycerol composition permitted the proportion of 21 triglyceride types to be determined (the 3 constituent fatty acids are known but not their positioning). Two types represented respectively 42.4 and 24.2%, that is together 2/3 of the total triacylglycerols. These triacylglycerol fractions were hydrolyzed with pancreatic lipase and fatty acid compositions of the monoacylglycerols formed and more often of one class of diacylglycerols permitted, using a mathematical method, the proportion of 30 isomers to be determined (the 3 constituent fatty acids and the acid in the 2-position are known). Only 11 isomers were found in appreciable amount (>1%). Out of them, two isomers were found in high proportion, namely 18:0-18:1-18:0 (42.1%) and 18:0-18:1-18:1 (23.9%) together accounting for 60% of shea butter. Trioleoylglycerol was also found in appreciable amount (5.8%), as compared with tristearoylglycerol (0.8%). The percentages so determined generally deviated markedly from a random distribution between respectively the internal and external positions. This probably could be explained by the high proportion of stearic acid in shea butter.

CYCLIC FATTY ESTERS: SYNTHESIS AND CHARACTERIZATION OF METHYL ω-(6-ALKYL-3-CYCLOHEXENYL) ALKENOATES. R.A. Awl and E.N. Frankel (Northern Regional Res. Center, Agric. Res. Service, U.S. Dept. of Agric., Peoria, IL 61604) *Lipids 17*(6):414-426 (1982). Diunsaturated C_{18} cyclic fatty acid methyl esters of known structure and configuration were synthesized as model derivatives of cyclic fatty acids formed in heat-abused vegetable oils for characterization and further biological evaluation. The Wittig reaction was used to prepare 5 pure methyl esters: (a) 12-(3-cyclohexenyl)-11-dodecenoate, (b) 11-(6-methyl-3-cyclohexenyl)-10-undecenoate, (c) 10-(6-ethyl-3-cyclohexenyl)-11-decenoate, (d) 9-(6-propyl-3-cyclohexenyl)-8-nonenoate and (e) 8-(6-butyl-3-cyclohexenyl)-7-octenoate. Diels-Alder cycloaddition reactions between 1,3-butadiene and appropriate (E)-2-alkenals produced 3-cyclohexenal intermediates. The appropriate methyl ω-bromoesters and their triphenylphosphonium bromides were made and converted to their respective ylids with $NaOCH_3$ in DMF. The appropriate 3-cyclohexenals and phospho-ylids were reacted, and the desired cyclic ester products were isolated in crude yields of 30-83% as liquids and fractionally distilled. The crude cyclic esters were purified either by preparative TLC or by saponification-esterification. Double bonds in purified cyclic esters were *trans*-isomerized and hydrogenated. Each derivative was characterized by IR, ^{1}H-NMR, ^{13}C-NMR, capillary GLC and GC-MS. On the basis of these analyses, no positional isomers were detected, Z-unsaturated isomers were produced in better than 90% purity, and the alkyl and ester ring substituents were predominantly *trans* to each other.

1,1,2-TRICHLORO-1,2,2-TRIFLUOROETHANE AS A CARRIER SOLVENT IN LIQUID CHROMATOGRAPHY. J.L. Glajch, J.J. Kirkland, W.G. Schindel (Central Res. and Development Dept., E.I. du Pont de Nemours and Co., Experimental Station, Wilmington, Delaware 19898) *Anal. Chem. 54*:1276-1279 (1982). 1,1,2-Trichloro-1,2,2-trifluoroethane (FC-113) is demonstrated to be a superior mobile phase carrier solvent for normal-phase liquid chromatography. Low flammability, low toxicity, high purity, and good ultraviolet transparency are all desirable properties of FC-113. Complete miscibility of most desirable organic solvent modifiers, along with the significantly higher solubility of many organic compounds, gives FC-113 distinct advantage over commonly used *n*-alkalane or cycloalkane hydrocarbon carriers (e.g., *n*-hexane, cyclopentane), particularly in preparative applications. Studies with mobile phase systems optimized by statistical technique show that FC-113 can be substituted for hydrocarbon as the base carrier solvent in normal-phase applications without significant differences in solvent stength and selectivity characteristics. An ϵ° value (solvent strength) of 0.02 has been determined for FC-113 compared to 0.01 reported for *n*-hexane.

AUTOXIDIZED AND LIPOXIDASE-TREATED POLYUNSATURATED FATTY ACIDS. AUTOFLUORESCENCE ASSOCIATED WITH THE DECOMPOSITION OF LIPID PEROXIDES. J.M.C. Gutteridge, P.J. Kerry and D. Armstrong (Natl. Inst. for Biol. Standards and Control, Holly Hill, Hampstead, London, NW3 6RB and Dept. of Ophthalmology, Univ. of Florida, Col. of Med., Gainesville, FL 32611) *Biochim. Biophys. Acta 711*(3):460-465 (1982). Autoxidized and lipoxidase-catalyzed oxidation of polyunsaturated fatty acids in aqueous dispersion produced lipid peroxides which were extractable into diethyl ether. These lipid peroxides had little fluorescence associated with them, most remaining behind in the aqueous phase. When lipid peroxide decomposition was accelerated by heating, substantial amounts of autofluorescent material then formed. These products had excitation and emission spectra similar to those of polymerized malondialdehyde.

AN IMPROVED PROCEDURE FOR THE SYNTHESIS OF CHOLINE PHOSPHOLIPIDS VIA 2-BROMOETHYL DICHLOROPHOSPHATE. W.J. Hansen, R. Murari, Y. Wedmid, and W.J. Baumann (Section of Bio-Organic Chem., Hormel Inst., Univ. of Minnesota, Austin, MN 55912) *Lipids 17*(6):453-459 (1982). Choline phospholipids can be conveniently synthesized by reaction of a lipophilic alcohol, such as diacylglycerol, with 2-bromoethyl dichlorophosphate followed by nucleophilic displacement of the bromine with trimethylamine. We found that the low yields often encountered in the initial phosphorylation step are particularly due to exchange of both chlorines for alkoxy functions (triester formation) and to chlorination of the alcohol by 2-bromoethyl dichlorophosphate. However, these drawbacks can be overcome by proper choice of the reaction medium and by optimizing other reaction conditions. The procedure described is efficient and most versatile, and it lends itself to the preparation of a wide range of choline phospholipids containing a glycerol, diol, or long-chain alkyl backbone and bearing various aliphatic functions. Proton and carbon-13 nuclear magnetic resonance spectroscopy proved useful in establishing the homogeneity and structures of the synthetic intermediates and byproducts and of the choline phospholipids synthesized.

ADSORPTION OF Mn(II) IONS TO HUMAN LOW DENSITY LIPOPROTEINS. MAGNETIC RESONANCE STUDIES. J.N. Herak, G. Pifat, J. Brnjas-Kraljević, G. Jürgens (Faculty of Pharmacy and Biochem., Ruder Bosković Inst. and Faculty of Med., Univ. of Zagreb, Zagreb, Croatia, Yugoslavia) *Biochim. Biophys. Acta 170*(3):324-331 (1982). Mn(II) ions were used to study ion-binding properties of human low density lipoproteins (LDL). From the intensity of the EPR lines corresponding to the unbound Mn(II) ions the percentage of the ions bound to LDL is determined. By the titration of LDL with Mn(II) the binding parameters, dissociation constant, K_d, and the number of binding sites, n, could be derived. It has been found that there are at least two types of binding sites on the LDL surface: 'strong' sites characterized by n=6, K_d=1.5·10^{-5}M·1^{-1}, and 'weak' sites characterized by n=145 and K_d= 6.6·10^{-3} M·1^{-1} for the sample in 0.01 M Tris-HCl buffer at 10 C. At very low Mn(II) concentrations binding to the 'strong' sites exhibits a cooperative behavior. In the 0.1 M buffer the 'strong' sites are almost completely occupied or blocked by the monovalent buffer cations. The number of the 'weak' sites remains unaltered and K_d is decreased slightly (K_d=4.9·10^{-3} M·1^{-1}). The location, chemical nature and the structural and functional relevance of the binding sites are discussed.

CONNECTIVE (C-C) ROUTE TO HINDERED EPOXIDES AND OLEFINS FROM HINDERED KETONES. D. Labar and A. Krief (Facultés Universitaires N.S. de la Paix, Département de Chimie, 61, rue de Bruxelles, 5000 – Namur, Belgium) *J. Chem. Soc., Chem. Commun. 10*:564-566 (1982). Hindered ketones have been transformed into hindered epoxides (5) and olefins (6) with the concomitant formation of new carbon-carbon bonds using β-hydroxyselenides as key intermediates; the unusual reactivity of such crowded molecules is reported.

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SUBCELLULAR DISTRIBUTION OF PHOSPHOLIPIDS AND OF POLYPRENYL PHOSPHATE IN *ASPERGILLUS NIGER* VAN TIGHEM. R. Letoublon, B. Mayet, J. Frot-Coutaz, C. Nicolau, and R. Got (Lab. De Biol. et Tech. des Membranes, Univ. Lyon I, 43 Boulevard du 11 Novembre 1918, 69622 Villeurbanne, France) *Biochim. Biophys. Acta 711*(3):509-514 (1982). Growth of *Aspergillus niger* Van Tieghem in presence of $^{32}P_i$ was followed by cell fractionation; the subcellular fractions were identified using marker enzymes and their phospholipid content was analyzed. Microsomes are characterized by high PC and PS values, whereas the largest amount of cardiolipin is recovered in the mitochondria. Polyprenyl phosphate was found mainly in mitochondria (46%) (microsomal content: 38%) and, more precisely, the highest ratio was in the outer membrane. This situation raises the problem of polyprenyl phosphate transfer from mitochondria to microsomes where the enzyme responsible for polyprenyl phosphate mannosylation is located.

STRUCTURAL STUDIES ON GLYCOLIPID OF SHELLFISH. IV. A NOVEL PENTAGLYCOSYLCERAMIDE FROM ABALONE, *HALIOTIS JAPONICA*. T. Matsubara and A. Hayashi (Dept. of Chem., Faculty of Sci. and Tech., Kiniki Univ., Kowakae, Higashiosaka, Osaka 577, Japan) *Biochim. Biophys. Acta 711*(3):551-553 (1982). A novel pentaglycosylceramide containing 2 mol fucose has been isolated from abolone, *Haliotis japonica*. The structure of this lipid was established by degradative studies, permethylation analysis and ^{1}H-NMR spectroscopy to Fucα1→3GalNAcα1→3(Fucα1→2)Galβ1→4Glcβ1→1 ceramide. The ceramide was composed mainly of hexadescasphing-4-enine and hexadecanoic acid.

POSITIONAL DISTRIBUTION OF FATTY ACIDS IN TRIGLYCERIDES FROM MILK OF SEVERAL SPECIES OF MAMMALS. P.W. Parodi (Fats Res. Lab., The Butter Marketing Board, Hamilton Central, 4007, Queensland, Australia) *Lipids 17*(6):437-442 (1982). Milk triglycerides from the echidna, koala, Tammar wallaby, guinea pig, dog, cat, Weddell seal, horse, pig, and cow were subjected to fatty acid and stereospecific analysis to determine the positional distribution of the fatty acids in the triglycerides. The samples presented a wide range of fatty acids, most of which varied in content among species. The compositions of the acids at the 3 positions also varied among species, reflecting the content of these acids in the triglycerides. However, there was a general similarity in fatty acid positional distribution patterns for all the species with the exception of the echidna. The echidna exhibited a completely different fatty acid positional distribution pattern. The saturated acids were preferentially esterified at the *sn*-2-position. The triglyceride carbon number distribution of milk from the above species (with the exception of the Weddell seal) was determined by gas liquid chromatography and compared to that predicted by the 1-random-2-random-3-random fatty acid distribution hypothesis. Agreement was excellent between observed and predicted composition for echidna, koala, Tammar wallaby, guinea pig, and pig milk, and agreement was reasonable for dog, cat, horse, and cow milk. Results are discussed in relation to biochemical mechanisms.

HIGH PERFORMANCE REVERSED-PHASE CHROMATOGRAPHY OF THE TRIGLYCERIDES FROM HUMAN PLASMA LIPOPROTEINS. E.G. Perkins, D.J. Hendren, N. Pelick, and J.E. Bauer (Dept. of Food Sci., Burnsides Res. Lab., 1208 W. Pennsylvania, Univ. of Illinois, Urbana, IL 61801) *Lipids 17*(6):460-463 (1982). The triglycerides of human plasma lipoproteins were separated with high performance reversed-phase liquid chromatography. An octadecyl bonded 5-μ silica column was used with a mobile phase of acetonitrile/acetone. Individual triglyceride types and critical pairs may be easily separated and identified.

USE OF RADIOLABELED HEXADECYL CHOLESTERYL ETHER AS A LIPOSOME MARKER. G.L. Pool, M.E. French, R.A. Edwards, L. Huang, and R.H. Lumb (Biochem. Group, Western Caroline Univ., Cullowhee, NC 28723) *Lipids 17*(6):448-452 (1982). Radiolabeled hexadecyl cholesteryl ether can serve as an effective marker for liposomes in a variety of studies. This paper demonstrates the use of cholesteryl ether marker in the assay of phospholipid transfer protein activity and in assessing phospholipid vesicle-cell interactions. The cholesteryl ether has the advantages of ease of synthesis, metabolic inertness, lipid solubility and nonexchangeability.

GANGLIOSIDES IN NORMAL AND CATARACTOUS LENSES OF SEVERAL SPECIES. C.P. Sarkar and R.J. Cenedella (Dept. of Biochem., Kirksville Coll. of Osteopathic Med., Kirksville, MO 63501) *Biochim. Biophys. Acta 711*(3):503-508 (1982). The present study was undertaken to compare the content, composition and distribution of gangliosides in ocular lenses of different species, both normal and cataractous. Gangliosides were extracted from cow, pig, rat and human lens by either the Folch-Suzuki partition procedure or by the modified tetrahydrofuran procedure. Between 20 and 45% more ganglioside was recovered from lenses by the tetrahydrofuran than the Folch-Suzuki partition procedure. Cow, pig, rat and human normal lens extracted by the tetrahydrofuran procedure contained, respectively, 24.8, 22.3, 71.2 and 272.5 μg of ganglioside sialic acid/g lens (wet wt). No protein-bound sialic was detected in any lens. No significant differences were observed in the content or composition of gangliosides from normal and cataractous human lens and from cataractous rat lens induced by the drug U18666A. Although hematoside (G_{M3}) was the predominant ganglioside in bovine (73%) and pig (68%) lens, small amounts of what appeared to be G_{M1}, G_{D1a}, and G_{T1} were also detected. G_{M1} predominated (95%) in rat lens, with the remainder being G_{M3}. This is apparently the highest relative content of G_{M1} reported in a non-neural tissue. Human lens contains two gangliosides, G_{M1} (53%) and G_{M3} (47%). Examination of the distribution of gangliosides between various regions of the bovine lens revealed that the epithelial cell fraction contained an about 10 times higher concentration of total gangliosides than either the lens cortex or nucleus.

IMPROVED METHODS FOR THE ISOLATION AND STUDY OF THE C_{18}, C_{20} AND C_{22} MONOETHYLENIC FATTY ACID ISOMERS OF BIOLOGICAL SAMPLES: Hg ADDUCTS, HPLC, $AgNO_3$-TLC/FID, AND OZONOLYSIS. J-L. Sebedio, T.E. Farquharson, and R.G. Ackman (Technical Univ. of Nova Scotia, Fisheries Res. and Technology Lab., PO Box 1000, Halifax, Nova Scotia, B3J 2X4) *Lipids 17*(6):469-475 (1982). The monoethylenic isomers of C_{18}, C_{20} and C_{22} chain lengths of the depot fat of a nonhominid primate (cynomolgus monkeys, *Macaca fasicicularis*), fed a partially hydrogenated herring oil (IV=76.0) for 30 months, were examined by 2 different approaches. The first isolation method involved preparative gas liquid chromatography and argentation thin layer chromatography (TLC). The second sequence involved a chain-length fractionation system based on the TLC of the methoxy-bromomercuri adducts of the total methyl esters to isolate groups of acids of common degrees of unsaturation, and then high performance liquid chromatography on a reverse-phase column. In both cases, the monethylenic isomer distribution was determined by ozonolysis in BF_3MeOH. Comparable results were obtained with the 2 methods. The second approach is recommended for small biological samples, especially for those containing a relatively high proportion of di- and other polyethylenic isomers which might interfere.

RETINOIC ACID 5,6-EPOXIDASE. W.K. Sietsema, and H.F. DeLuca (Dept. of Biochem., College of Agricultural and Life Sciences, Univ. of Wisconsin-Madison, Madison, WI 53706) *J. Biol. Chem. 257*(8):4265-4270 (1982). A retinoic acid epoxidase is present in rat kidney homogenates. It is found in the particulate fraction, and is dependent on ATP, NADPH, and oxygen. It is stimulated by Fe^{2+} and inhibited by Mn^{2+}, Zn^{2+}, Cu^{2+}, EDTA, and N,N′-diphenyl-para-phenylenediamine. Its properties are closest to those of microsomal lipid peroxidases previously studied. Epoxidase activity is not affected by the rat's vitamin A status, nor is it inducible by retinoic acid. In N,N′-diphenyl-*para*-phenylenediamine-feeding experiments, it was shown that blocking epoxidation in vivo does not inhibit the function of retinoic acid. It is concluded that retinoic acid epoxidation is not required for retinoic acid function.

A MODEL SYSTEM FOR STUDYING THE MECHANISM OF MEDIUM-CHAIN FATTY ACID BIOSYNTHESIS IN PLANTS. A.R. Slabas, P.A. Roberts, J. Ormesher and E.W. Hammond (Biosciences Div., Unilever Res., Colworth House, Sharnbrook, Bedford, MK44 1LQ) *Biochim. Biophys. Acta 711*(3):411-420 (1982). Seeds of *Cuphea procumbens* are rich in medium-chain fatty acids. In mature seeds, C10:0 constitutes over 80% of the fatty acid of the triacylglycerol fraction but is only a very minor constituent of the polar lipid fraction. C10:0 was found to a small extent in the neutral lipid fraction of other parts of the *Cuphea* plant but was absent from the polar lipid fraction—C10:0 thus acts as a marker for seed lipid biosynthesis. The fatty acid composition of maturing *Cuphea* seeds was analyzed. Between 11-14 days after flowering

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C10:0 biosynthesis increases dramatically. Radioactive incorporation experiments using mature desiccated seeds demonstrate that they still have the capacity to synthesize C10:0. In vitro incorporation experiments demonstrate that 13-16-day-old seeds synthesize almost exclusively C10:0. These observations indicate the *C. procumbens* is a good experimental system for studying the mechanism of medium-chain fatty acid biosynthesis in plants.

DETERMINATION OF CHOLESTERYL ESTERS AND OF CHOLESTERYL AND EPICHOLESTERYL SILYL ETHERS BY CAPILLARY GAS CHROMATOGRAPHY. N.B. Smith (Biophysics Dept., Health Sci. Centre, Univ. of Western Ontario, London, Ontario, Canada N6A 5C1) *Lipids 17*(6):464-468 (1982). The capillary gas chromatography of cholesteryl esters after splitless injection into a 25-m, OV-1-coated, fused silica WCOT column and a 7-m Silar 10C-coated glass WCOT column is reported. The nonpolar OV-L column separated the cholesteryl esters principally on the basis of carbon number, but separation of the saturated esters from the unsaturated esters was also achieved. The polar Silar 10C column separated the esters mainly according to the degree of unsaturation. Thus, the 2 column types complement each other in the analysis of nanogram quantities of cholesteryl esters from small samples, such as those from plasma or single arterial atherosclerosis lesions. This technique therefore obviates some of the difficulties of analyzing such cholesteryl ester samples in the form of methyl esters (incomplete transmethylation, and contamination by solvent impurities and/or plasticizer esters). Both columns were also found to be useful for the separation and quantitation of the *t*-BDMS ethers of cholesterol and epicholesterol in mixtures containing various proportions of these epimers.

EFFECTS OF PHOSPHATIDYLCHOLINES ON DE NOVO SYNTHESIS AND EXCRETION OF STEROL BY L-929 FIBROBLASTS. G. Thomaidis, C.E. Holmlund (Dept. of Chem., Univ. of Maryland, College Park, MD 20742) *Lipids 17*(6):427-433 (1982). The effects of [^{14}C] sterol synthesis and excretion by exposure of L-929 cells to several phosphatidylcholines (PC) has been examined. No significant effects were noted on either parameter during a 6-hr period if exposure of cells to the phospholipid preceded the addition of [1-^{14}C] acetate by just 30 min. If cultures were grown in media containing delipidized serum and 2×10^{-5}M PC, the amount of [^{14}C] sterol increased in both cells and medium by 70-200% when saturated or monounsaturated PC were used. Dilinoleylphosphatidylcholine at the same concentration did not stimulate synthesis or excretion of newly synthesized sterol. Total cellular sterol was determined by gas chromatography, and was only marginally affected by long-term exposure to dipalmitylphosphatidylcholin; the total sterol of the medium increased by 4-fold. Cultures which had been exposed to 16:0 PC continued to display enhanced de novo sterol synthesis, but not excretion. The disparity in response to 2×10^{-5}M levels of 16:0 PC and 18:2 PC may relate to differences in metabolism of the PC. Exposure to 18:2 PC resulted in about 3-fold increases in the 18:2 to 18:0 plus 18:1 ratio of cellular fatty acids. Relatively small changes in the cellular fatty acid composition were noted with 16:0 PC-treated cells. The results indicate that extracellular PC can promote sterol synthesis and excretion by L-929 cells, and that the magnitude of this response is influenced by the time of exposure to the phospholipid and by its fatty acid composition.

INHIBITION OF LIPOPROTEIN LIPASE BY BENZENE BORONIC ACID EFFECT OF APOLIPOPROTEIN C-II. P. Vainio, J.A. Virtanen and P.K.J. Kinnunen (Dept. of Med. Chem. and Dept. of Chem., Univ. of Helsinki, SF-00170 Helsinki 17 (Finland)) *Biochim. Biophys. Acta 711*(3):386-390 (1982). The catalytic mechanism of triacylglycerol hydrolysis by lipoprotein lipase was studied. We found lipoprotein lipase to be inhibited by benzene boronic acid, with an apparent K_i of 8.9 μM at pH 7.4. This indicates the presence of serine and histidine in the active site of the enzyme. Inhibition of lipoprotein lipase by benzene boronic acid is likely to be due to the formation of an inhibitor-enzyme complex having analogous bonding to the active site histidine and serine as the transition-state complex which precedes the formation of an obligatory acyl-enzyme intermediate. The presence of apolipoprotein C-II, the apolipoprotein activator of lipoprotein lipase, partly reverses the inhibition of lipoprotein lipase by benzene boronic acid. This reversal by apolipoprotein C-II has a distinct pH optimum in the range of 8-9.

CALMODULIN BINDS TO CHICK LENS GAP JUNCTION PROTEIN IN A CALCIUM-INDEPENDENT MANNER. M.J. Welsh, J.C. Aster, M. Ireland, J. Alcala, and H. Maisel (Department of Anatomy and Cell Biology and Department of Genetics, University of Michigan Medical School, Ann Arbor, MI 48109) *Science 216*:642-643 (1982). A biochemically active conjugate of calmodulin and tetramethylrhodamine isothiocyanate (CaM-RITC) was synthesized. When incubated with sections of chick lens, this conjugate bound to the surface membranes of lens fiber cells in the presence or absence of calcium. Incubation of lens sections with antibodies to gap junction protein of lens completely blocked the binding of the conjugate to cell membranes, whereas serum from nonimmunized animals or antibodies to other lens proteins reduced the binding only slightly. By means of a gel overlay procedure, ^{125}I-labeled calmodulin was found to bind the gap junction protein to lens, also in a calcium-independent manner. These results support the concept that calmodulin may interact with and regulate gap junctions in living cells.

EFFECT OF STEROL STRUCTURE ON THE TRANSFER OF STEROLS AND PHOSPHOLIPIDS FROM LIPOSOMES TO ERYTHROCYTES IN VITRO. S.A. Wharton and C. Green (Dept. of Biochem., Univ. of Liverpool, P.O. Box 147, Liverpool L69 3BX) *Biochim. Biophys. Acta 711*(3):398-402 (1982). Human erythrocytes were incubated for 24 hr with liposomes containing [^{3}H] phosphatidylcholine, [^{14}C] cholesterol and one of several other sterols. Of the other sterols, 3-hydroxycholest-3-en-2-one did not appear to be taken up by the cells, sterophenol was taken up at about the same rate as cholesterol, and cholesta-4,6-dien-3-one and 7-oxocholesterol were taken up much more rapidly than cholesterol. Each component of the liposomes was incorporated into the cells independently of the others and the rate of incorporation of the test sterol had little, if any, effect on the rate of incorporation of phospholipid or cholesterol. The results support the proposal that sterol exchange is mediated via the pool of monomers present in the medium rather than by a collision mechanism.

PROSTAGLANDINS. 3. SYNTHETIC APPROACHES TO 11-DEOXYPROSTAGLANDINS. W.L. White, P.B. Anzeveno, and F. Johnson (Dow Chemical U.S.A. Central Res., New England Lab., Wayland, MA 01778) *J. Org. Chem. 47*(12):2379-2387 (1982). A straightforward synthesis is described of *dl-trans*-2[6-(methoxycarbonyl)hex-2(*Z*)-enyl]-3-formylcyclopentanone (5), a versatile intermediate for the synthesis of 11-deoxyprostaglandins and their analogues. Base-catalyzed cyclization of 1,2,4-tris(methoxycarbonyl)butane under kinetically controlled conditions led to (70%) *trans*-2,3-bis(methoxycarbonyl)cyclopentanone (3). Treatment of 3 with hot benzyl alcohol gave (80%) *trans*-2-(benzyloxycarbonyl)-3-(methoxycarbonyl)cyclopentanone (25), which was purified as its copper chelate. Alkylation of 25 with 6-cyanohex-2-ynol mesylate (20) with NaH/dimethoxyethane afforded 82% 2-(6-cyanohex-2-ynyl)-2-(benzyloxycarbonyl)-3-(methoxycarbonyl)cyclopentanone (26). Catalytic reduction of 26 in ethanol/pyridine, using a Pd/$BaSO_4$ catalyst followed by warming, gave (91.5%) a 9:1 mixture of *trans*-2-(6-cyanohex-2(*Z*)-enyl)-3-(methoxycarbonyl)cyclopentanone (27) and its 2,3-dihydro derivative 29. Chromatography of the corresponding carboxylic acids gave the pure acid 30 (69% from 26). Reduction ($NaBH_4$) of the acid chloride (31) of acid 30 led to a mixture of diols 32. Saponification of the nitrile group of 32 afforded the C-1 epimeric mixture of the diol acids 33, which on CH_2N_2 esterification (69% of 31) and Collins oxidation afforded (92%) the desired 5. A second route to 5 also was explored. Cyclization (*t*-BuOK/*t*-BuOH) of 4-[2-(methoxycarbonyl)ethyl]-tetrahydrofuran-2-one (12) led to *cis*-hexahydro-1*H*-cyclopental[*c*]furan-1,6-dione (4), which when alkylated (*t*-BuOK/*t*-BuOH) with 20 gave (90%) the C-6a alkylation product 35. Alkaline saponification of 35 led to *trans*-2-(6-cyanohex-2-ynyl)-3-(hydroxymethyl)cyclopentanone (36) in only modest yield (15%). A four-step sequence of catalytic reduction, nitrile hydrolysis, acid esterification, and oxidation then gave 5 in yield from 36.

PURIFICATION AND PROPERTIES OF A PHOSPHOLIPID ACYL HYDROLASE FROM PLASMA MEMBRANES OF *SACCHAROMYCES CEREVISIAE*. W. Witt, H.J. Brüller, G. Falker, and G.F. Fuhrmann (Dept. of Pharmacology, Schl. of Med., Philipps Univ., Lahnberge, D-3550 Marburg/Lahn) *Biochim. Biophys. Acta 711*(3):403-410 (1982). The properties of a phospholipid acyl hydrolase bound to yeast plasma membranes are described in detail. The enzyme is capable of splitting all phospholipids which can be extracted from yeast cells. The specific activity with lysophosphatidylcholine as substrate was much higher than with phosphatidylcholine.

With dipalmitoyl phosphatidylcholine as substrate a broad pH optimum was measured between pH 3.0 and 4.5. The membrane-bound enzyme was activated strongly by the anionic detergents SDS, deoxycholate and, to a lesser extent, by cholate. The uncharged detergent Triton X-100 and the zwitterionic detergent SB12 exerted an only slightly activating effect, KCl, NaCl, $MgCl_2$, and $CaCl_2$ were inhibitory in the presence of glycine/acetic acid buffer at pH 4.0. The enzyme was solubilized by cholate or by SB12 in an active form from the plasma membrane purified by acetone and $(NH_4)_2SO_4$ precipitation or gel filtration with Sephadex G-200. The phospholipid acyl hydrolase was identified as a glycoprotein with an apparent molecular weight of 145 000 by SDS slab gel electrophoresis.

AUTOXIDATION OF PHOSPHATIDYLCHOLINE LIPOSOMES. G.S. Wu, R.A. Stein and J.F. Mead (Lab. of Biomedical and Environmental Sci., 900 Veteran Ave., Univ. of California, Los Angeles, CA 90024; and Dept. of Biological Chem., UCLA Schl. of Med., Los Angeles, CA 90024) *Lipids 17*(6):403-413 (1982). Autoxidation of pure soybean phosphatidylcholine liposomes at 40 C was found to proceed without an observed induction period, but otherwise, the rates of disappearance of the linoleic acid (70% of total) and linolenic acid (6% of total) followed typical autocatalytic kinetics. Incorporation of 0.05 mol % of tocopherol into the liposomes produced an induction period of about 7 hr under the condition used for incubation. The products formed from the autoxidation of pure soybean phosphatidylcholine liposomes were mostly 9- and 13-hydroperoxyoctadecadienoates (isolated as hydroxy esters). The yield of hydroperoxides with *cis,trans* configuration was about the same as those with *trans,trans* configuration throughout incubation period. After extensive autoxidation, a large quantity of trihydroxyoctadecenoate was also produced. When a large quantity of dipalmitoyl phosphatidylcholine was incorporated into soybean phosphatidylcholine liposomes, the rate of autoxidation decreased and was found to conform to apparent first-order kinetics. In this system, the yield of *trans,trans* hydroperoxides was much greater than that of *cis,trans* isomers at all stages of autoxidation. Late in the autoxidation of the mixed liposomes, both trihydroxyoctadecenoate and hydroxyepoxyoctadecenoate were produced in substantial quantities.

PROSTAGLANDIN ENDOPEROXIDES. 12. CARBOXYLATE CATALYSIS AND THE EFFECTS OF PROTON DONORS ON THE DECOMPOSITION OF 2,3-DIOXABICYCLO[2.2.1]HEPTANE. M.G. Zagorski and R.G. Salomon (Dept. of Chem., Case Western Reserve Univ., Cleveland, OH 44106) *J. Am. Chem. Soc. 104*(12):3498-3503 (1982). Tri- and tetraalkylammonium acetates are potent catalysts for decomposition of the prostaglandin endoperoxide nucleus, 2,3-dioxabicyclo[2.2.]heptane. The effectiveness of these catalysts is comparable to that of tertiary amines such as 1,4-diazabicyclo[2.2.2]octane and triethylamine. The decomposition yields both fragmentation product, levulinaldehyde, and disproportionation products, 3-hydroxycyclopentanone and cyclopent-2-en-1-one. The fragmentation reaction exhibits a deuterium isotope effect $k_H/k_D = 7.6$. Most significantly, the decomposition can be channeled to favor disproportionation by avoiding fragmentation of an intermediate keto alkoxide by conducting the acetate-catalyzed reactions in the presence of excess acetic acid.

Biochemistry and nutrition

STIMULATION OF FATTY ACID METHYLATION IN HUMAN RED CELL MEMBRANES BY PHOSPHOLIPASE A_2 ACTIVATION. S.J. Engelsen and M. Zatz (Section on Pharmacology, Lab of Clinical Sci., Natl. Inst. of Mental Health, Bethesda, MD 20205) *Biochim. Biophys. Acta 711*(3):515-520 (1982). Nonpolar methylated products comprise approximately 50% of the radioactive material extractable into chloroform/methanol after incubation of human red cell membranes with *S*-[*methyl*-^{3}H]adenosylmethionine. One of these nonpolar products is fatty acid methyl ester. The enzyme which synthesizes fatty acid methyl ester had an apparent K_m for *S*-adenosylmethionine of about 0.6 μM and a V_{max} of about 0.6 pmol/mg protein per 30 min. Half-maximal activity was achieved upon addition of about 20 μM sodium oleate. Of the fatty acids tested, sodium oleate increased activity most effectively (6-fold) and arachidonic acid was ineffective. Evidence indicated that fatty acid methylation takes place on the cytoplasmic side of the plasma membrane. The reaction was demonstrable in intact cells incubated with [*methyl*-^{3}H]methionine, and increased upon addition of sodium oleate. Incubation of intact cells with melittin, a potent membrane phospholipase A_2 activator from bee venom, increased fatty acid methylation several-fold. Fatty acid methylation appears to be one of the consequences of phospholipase A_2 action in plasma membranes.

LIPID METABOLISM IN CULTURED CELLS. S.J. Feinmark and J.M. Bailey (Dept. of Biochem., George Washington Univ. Schl. of Med., Washington, D.C. 20037) *J. Biol. Chem. 257*(6)2816-2821 (1982). Thromboxane A_2 (rabbit aorta-contracting substance) is a proaggregatory vasoconstrictive, oxygenated metabolite of arachidonic acid which was originally discovered in guinea pig lung perfusates during antigen-induced anaphylaxis. The specific stimuli which activate synthesis and the cellular source in the lung remained undefined. In order to study pulmonary thromboxane A_2 (TXA_2) synthesis, a cultured lung cell model has been used. Monolayer cultures of human diploid embryonic lung fibroblast (WI-38) metabolized exogenously supplied [^{14}C]arachidonic acid to TXA_2 as well as prostaglandin E_2. Both were unequivocally identified by gas chromatography/mass spectrometry. Cellular phospholipids were labeled by preincubating cultures overnight with [^{14}C]arachidonic acid. Release of thromboxane A_2 into the culture fluid from these prelabeled cultures was stimulated by two phospholipase activating agents, melitten and the calcium ionophore A23187. The lung cells also released TXA_2 and prostaglandin in a dose-dependent fashion when treated with thrombin but not when exposed to trypsin. Bradykinin, an anaphylactic mediator in vivo, was a potent TXA_2 releasing agent in this in vitro system whereas histamine was inactive. In addition, anaphylactic shock perfusates from guinea pig lung were shown to contain a factor (other than bradykinin) which activates fibroblast TXA_2 synthesis in these cultured lung cells. These experiments indicate that the lung fibroblast is probably a source of pulmonary thromboxane in vivo and that the cultured lung cell system described here is a useful model for defining the complex interactions of mediators of anaphylaxis and asthma.

PROSTACYCLIN METABOLITES, IN URINE OF ADULTS AND NEONATES, STUDIED BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY AND RADIOIMMUNOASSAY. S. Fischer, B. Scherer and P.C. Weber (Medizinische Klinik Innenstadt der Univ. München, Ziemssenstrasse 1, 8000 Munich 2, F.R.G.) *Biochim. Biophys. Acta 710*(3):493-501 (1982). Endogenous levels of two metabolites of prostacyclin, 6-keto-prostaglandin $F_{1\alpha}$ (spontaneous hydrolysis product) and 6,15-diketo-13,14-dihydroprostaglandin $F_{1\alpha}$ and 6,15-diketo-13,14-dihydroprostaglandin $F_{1\alpha}$ added to urine were recovered quantitatively by gas chromatography-mass spectrometry. Endogenous levels of 6-keto-prostaglandin $F_{1\alpha}$ in urine of adults were 0.11 ± 0.05 (S.D.) ng/ml (n=12), whereas in urine of neonates the levels were much higher: 1.41 ± 0.36 (S.D.) ng/ml (n=5) on the 3rd day of life declining to 0.51 ± 0.21 (S.D.) ng/ml (n=5) on the 5th day. 6-keto-prostaglandin $F_{1\alpha}$ was also estimated in both age groups by radioimmunoassay. Urinary levels of 6,15-diketo-13,14-dihydroprostaglandin $F_{1\alpha}$ in neonates on the 3rd day of life were 2.12 ± 0.70 (S.D.) ng/ml (n=4) and declined until the 5th day. In adult urine this metabolite was below the limit of detection (0.20 ng/ml).

CHRONIC AND ACUTE EFFECTS OF THYROTROPIN ON PHOSPHATIDYLINOSITOL TURNOVER IN CULTURED PORCINE THYROID CELLS. C. Gerard, B. Haye, C. Jacquemin, J. Mauchamp (Lab. de Biochimie, U.E.R. Sciences BP 347, 51062 Reims cedex, France) *Biochim. Biophys. Acta 710*(3):359-369 (1982). The ^{32}P incorporation into phospholipids of isolated porcine thyroid cells, cultured for 1-4 days, has been studied in subsequent 2-hr incubations. Along with culture ageing, decreased ^{32}P incorporation into total phospholipid of control cells was observed. The presence of 40 munits/ml TSH during the 2 hr incubation yielded a relative increase in labelling of phosphatidylinositol, named "acute phospholipid effect." A chronic treatment of the cells with TSH concentrations ranging from 0.1 to 10 munits/ml ensured the maintenance of a high turnover rate of total phospholipids. The analysis of individual phospholipids revealed that 1-day culture cells in the presence of 0.1 munits/ml TSH presented a strong increase of phosphatidylinositol labelling. This "chronic phospholipid effect" of TSH can be reproduced by a chronic treatment with dibutyryl cyclic AMP (10^{-3} M) or prostaglandin E_2 (10^{-6} M), which did not evoke a classical phospholipid effect in a 2 hr incubation. If TSH (40 munits/ml) is added to the cells in a 2 hr incubation, control cells show the classical

phospholipid effect whereas cells chronically treated with TSH, dibutyryl cyclic AMP or prostaglandin E_2 presented a "reverse phospholipid effect," i.e. a relative decrease in phosphatidylinositol labelling. 10^{-4}M cycloheximide presence during the last 12 hr of culture prevented the establishment of the "chronic phospholipid effect" and of its consequence, "the reverse phospholipid effect." On the basis of these results a scheme is proposed in keeping with current hypotheses concerning phosphatidylinositol metabolism.

SUCROSE POLYESTER AND COVERT CALORIC DILUTION. C.J. Glueck, M.M. Hastings, C. Allen, E. Hogg, L. Baehler, P.S. Gartside D. Philips, M. Jones, E.J. Hollenbach, B. Braun, and J.V. Anastasia (Lipid Res. Clinic and Gen. Clinical Res. Center, Univ. of Cincinnati, Coll. of Med., Cincinnati, OH 45267) *Amer. J. Clin. Nutr. 35*(6):1352-1359 (1982). Total daily caloric intake was measured in 10 obese subjects when sucrose polyester (SPE), a non-absorbable synthetic fat, covertly replaced conventional fats in a single crossover study consisting of the three periods: a period of 7 to 14 days to determine baseline caloric intake and two 20-day study periods. An average of 60 g SPE/day replaced conventional fat in one of the two study periods. During both study periods, 60% of the base line caloric intake was "required intake" at mealtime; an additional 60% of base line caloric intake was allowed as "free choice" foods at a specified snacktime. It was thus possible during both study periods to consume more than 100% of the base line caloric intake. In the SPE study period, 40 g SPE replaced 40 g conventional fat for every 1200 kcal of required intake, resulting in a 30% reduction in mealtime caloric intake. Mean total caloric intake (meal and snack) fell 23% during the SPE period ($p<0.05$), despite an average daily weight loss of 0.18 kg. Snack caloric intake did not increase significantly to compensate for caloric dilution of the meals during the SPE period. These results indicate that the obese may not detect or may not compensate for covert dilution of fat calories with SPE. In addition, during the SPE period, there was a 10% reduction in total plasma cholesterol, a 14% reduction in low-density lipoprotein cholesterol, and a 10% reduction in triglyceride concentration. Thus, fat replacement with SPE may benefit weight reduction regimens in obese subjects by facilitating decreased caloric intake by improving the circulating lipoprotein profile.

RAT LIVER CHOLESTEROL 7α-HYDROXYLASE. C.D. Goodwin, B.W. Cooper, and S. Margolis (Dept. of Med., John Hopkins Univ. School of Med., Baltimore, MD 21205 and The Howard Hughes Med. Inst., Coconut Grove, FL 33133) *J. Biol. Chem. 257*(8):4469-4472 (1982). Four lines of evidence presented here suggest that the activity of cholesterol 7α-hydroxylase in rat liver is modulated by changes in its phosphorylation state. 1) livers were homogenized and microsomes were isolated and washed in the presence of either 50 mM NaCl or 50 mM NaF, the latter an inhibitor of phosphoprotein phosphatases. The 7α-hydroxylase activity of microsomes prepared with NaF was 80% greater than that of microsomes prepared with NaCl. 2) Incubation of 10,000 × g supernatants from rat liver for 20 min at 37 C in the absence of 50 mM KF decreased the activity of microsomal cholesterol 7α-hydroxylase by 52%. No significant change was seen in the presence of KF. 3) 7α-Hydroxylase activity fell by 40% when microsomes were incubated with bacterial alkaline phosphatase compared to incubation of microsomes with phosphatase that was inhibited by phosphate and EDTA. 4) 7α-Hydroxylase activity increased by 22% when phosphatase-treated microsomes were incubated for 40 min at 37 C with 1 mM MgATP, 50 μM cAMP, and 200 units of cAMP-dependent protein kinase.

PHOSPHATIDYLCHOLINE OF BLOOD LIPOPROTEIN IS THE PRECURSOR OF GLYCEROPHOSPHORYLCHOLINE FOUND IN SEMINAL PLASMA. R.H. Hammerstedt, W.A. Rowan (Biochem. Program, The Pennsylvania State Univ., Univ. Park, PA 16802, USA) *Biochim. Biophys. Acta 710*(3):370-376 (1982). Glycerophosphorylcholine (GPC) concentration of the male reproductive tract increases from under 0.1 mM in the efferent ducts of the testis to 10-50 mM in the cauda epididymidis. It is reasonable to assume that choline-containing phospholipids serve as precursor(s) of GPC but the identity and source of these precursors had not been established. We performed in vivo labeling experiments in rabbits to distinguish between two types of phospholipid metabolism that might account for epididymal GPC formation: (a) addition of phospholipids to sperm during epididymal transit with degradation of these phospholipids by sperm to yield GPC or (b) degradation of phospholipids by epididymal epithelial cells with excretion of GPC into the duct lumen. We concluded that the latter route accounted for most of the GPC accumulation and that the primary source of the choline moiety of GPC was phosphatidylcholine of blood lipoprotein. We believe that these observations establish a specific, vectorial metabolism of phospholipids whereby circulating lipoproteins on one side of the epididymal epithelial cell are transferred to the cell, the lipoproteins degraded by the epithelial cell to GPC, and the GPC moved into the lumen.

LIPOPROTEINS CONTAINING APOLIPOPROTEIN A-I EXTRACTED FROM HUMAN AORTAS. C.L. Heideman and H.F. Hoff (Baylor Col. of Med., Houston, TX 77030) *Biochim. Biophys. Acta 711*(3):431-444 (1982). Apolipoprotein A-I was quantitated by electroimmunoassay in buffer-soluble fractions of both grossly normal intima and raised atherosclerotic lesions of the human aorta. The mean value for apolipoprotein A-I content in μ g/mg tissue dry weight of normal intima (12 cases) was 0.71 ± 0.10 S.E. and of aortic plaques (19 cases) was 0.64 ± 0.40 S.E. When compared to the buffer-extractable apolipoprotein B content measured in these same cases from both regions, the ratio of apolipoprotein B to apolipoprotein A-I was approximately 6. No apolipoprotein A-I was measurable in tunica media. Following differential ultracentrifugation into d<1.063, d 1.063-1.21 and d>1.21 fractions, the distributions of recovered apolipoprotein A-I were, respectively: 1, 94 and 5% for normal intima, 19, 31 and 50% for plaques and 1, 89 and 10% for plasma. Characterization of a chromatographically purified d 1.063-1.21 or HDL density fraction from fatty-fibrous plaques demonstrated particles of between 60 and 120 Å diameter, a characteristic apolipoprotein A-I band by SDS-polyacrylamide gel electrophoresis, and a precipitin peak closely migrating with that for plasma HDL by two-dimensional immunoelectrophoresis. The d>1.21 density fraction from plaques isolated by affinity chromatography on a Sepharose-anti-apolipoprotein A-I column contained small amounts of phospholipid but no measurable cholesterol. The d 1.063-1.21 density fraction from plaques showed a significant increase in percent free cholesterol and phospholipid contents and decrease in cholesteryl ester content relative to plasma HDL. This increase in free cholesterol could represent evidence for an anti-atherogenic mechanism wherein infiltrated HDL removes cholesterol together with phospholipid from the arterial wall.

MECHANISM OF COUMARIN ACTION: SENSITIVITY OF VITAMIN K METABOLIZING ENZYMES OF NORMAL AND WARFARIN-RESISTANT RAT LIVER. E.F. Hildebrandt and J.W. Suttie (Dept. of Biochem., College of Agricultural and Life Sciences, Univ. of Wisconsin-Madison, Madison, WI 53706) *J. Biochem. 21*(10):2406-2411, (1982). The in vitro effects of two coumarin anticoagulants, warfarin and difenacoum, on rat liver microsomal vitamin K dependent carboxylase, vitamin K epoxidase, vitamin K epoxide reductase, and cytosolic vitamin K reductase (DT-diaphorase) from the livers of normal and warfarin-resistant strain of rats have been determined. Millimolar concentrations of both coumarins are required to inhibit the carboxylase and epoxidase activities in both strains of rats. Sensitivity of DT-diaphorase to coumarin inhibition differs when a soluble of liposomal-associated substrate is used, but the diaphorases isolated from both strains of rats have comparable sensitivity. The anticoagulant difenacoum is an effective rodenticide in the warfarin-resistant strain of rats, and the only enzyme studied from warfarin-resistant rat liver that demonstrated a significant differential inhibition by the two coumarins used was the vitamin K epoxide reductase. This enzyme also showed the greatest sensitivity to coumarin inhibition among the enzymes studied. These results support the hypothesis that the physiologically important site of action of coumarin anticougulants is the vitamin K epoxide reductase.

IN VITRO AND IN VIVO EFFECTS OF VITAMIN E ON ARACHIDONIC ACID METABOLISM IN RAT PLATELETS. D.H. Hwang and J. Donovan (Louisiana Agric. Exp. Stn., Human Nutr., Home Econ. Bldg., Louisiana State Univ., Baton Rouge, LA 70803-4300) *J. Nutr. 112*(6):1233-1237 (1982). Preincubating platelet-rich plasma (PRP) of vitamin E-deficient rats with *RRR*-α-tocopherol prior to the aggregation induced by collagen suspension resulted in inhibition of the formation of endoperoxide metabolites derived from endogenous arachidonic acid (AA). This inhibition was not dose dependent at concentrations above the plasma level of *RRR*-α-tocopherol of vitamin E-supplemented rats. Preincubating the vitamin E-deficient PRP with *RRR*-α-tocopherol did not affect the formation of 12-hydroxyeicosatetraenoic acid, the platelet lipoxygenase product. Concentrations of endoperoxide metabolites in diluted whole blood challenged with collagen suspension were significantly greater in the vitamin E-deficient group than the supplemented

group. The level of AA in platelet or plasma phospholipids was not different between the two groups. However, blood platelet counts in the deficient group were significantly greater than those of the supplemented group. Concentration of endoperoxide metabolites in PRP samples in which platelet concentrations were equalized were still greater in the vitamin E-deficient group; however, the difference was not statistically significant. There was also no difference in the degree of maximal platelet aggregation between the two groups. These results indicated that vitamin E deficiency can slightly stimulate the formation of cyclooxygenase products derived from endogenous AA, but it did not affect the formation of lipoxygenase product in rat platelets.

MODULATING EFFECTS OF CANINE HIGH DENSITY LIPOPROTEINS ON CHOLESTERYL ESTER SYNTHESIS INDUCED BY β-VERY LOW DENSITY LIPOPROTEINS IN MACROPHAGES. T.L. Innerarity, R.E. Pitas, and R.W. Mahley (Gladstone Foundation Lab. for Cardiovascular Disease, Cardiovascular Res. Inst. Depts. of Pathology and Med., Univ. of California, San Francisco, CA) *Arteriosclerosis 2*(2):114-124 (1982). We have previously observed that cholesterol-fed dogs with plasma cholesterol levels of 350 to 750 mg/dl failed to develop atherosclerosis (hyporesponders), whereas cholesterol-fed dogs with cholesterol levels greater than 750 mg/dl developed markedly accelerated atherosclerosis (hyperresponders). Two striking features of the hypercholesterolemia of the hyperresponders were the occurrence of cholesteryl ester-rich, β-migrating very low density lipoproteins (β-VLDL) in the d<1.006 fraction and a decrease in plasma concentration of typical high density lipoproteins (HDL). Cholesterol-induced β-VLDL have been shown to cause massive accumulations of cholesteryl esters in mouse peritoneal macrophages in vitro, and HDL have been shown to remove cholesterol from these cells. In the present study, the mouse peritoneal macrophage system was used to explore the effects of high levels of cholesterol-induced d<1.006 lipoproteins and low levels of HDL in mediating cholesteryl ester synthesis and accumulation in these cells. In the present study, the mouse peritoneal macrophage system was used to explore the effects of high levels of cholesterol-induced d< 1.006 lipoproteins and low levels of HDL in mediating cholesteryl ester synthesis and accumulation in these cells. It is reasonable to speculate that an important determinant of whether cholesteryl esters accumulate in the macrophage model system, and possibly in the arterial wall, is the ratio of those lipoproteins capable of delivering cholesterol to the cells (e.g., β- VLDL, cholesterol-enriched d< 1.006 lipoproteins) to those lipoproteins capable of removing cholesterol from the cells (e.g., HDL). More direct parallels between the in vivo and in vitro observations remain to be determined.

REDUCED PLASMA CHOLESTEROL ESTERIFYING ACTIVITY IN IRON-DEFICIENT RATS: ITS POSSIBLE ROLE IN THE LIPEMIA OF IRON DEFICIENCY. S.K. Jain, R. Yip, A.K. Pramanik, P.R. Dallman, and S.B. Shohet (Cancer Res. Inst. and Dept. of Pediatrics, Univ. of California Schl. of Med., San Francisco, CA 94143) *J. Nutr. 112*(6):1230-1232 (1982). The association of iron deficiency and lipemia has been described by many investigators. However, the mechanism involved in the etiology of hyperlipidemia in iron deficiency is not known. The present communication reports that the plasma activity of lecithin:cholesterol acyltransferase, the enzyme involved in the esterification of cholesterol to cholesterol ester, is markedly diminished in iron-deficient rats, providing a possible basis for the hyperlipidemia associated with iron deficiency.

TRANSPORT OF HYDROCARBONS BY THE LIPOPHORIN OF INSECT HEMOLYMPH. H. Katase and H. Chino (Biochemical Lab., Inst. of Low Temperature Sci., Hokkaido Univ., Sapporo, Japan) *Biochim. Biophys. Acta 710*(3):341-348 (1982). When [1-^{14}C]acetate was injected into the American cockroach, the labeled acetate was incorporated preferentially into the hydrocarbon fraction and, subsequently, the labeled hydrocarbon was released into the hemolymph where it was associated with the lipophorin (formerly called diacylglycerol-carrying lipoprotein). The label was traced to the three hydrocarbons, *n*-pentacosane, 3-methylpentacosane and 6,9-heptacosadiene that had been shown previously to be associated with the lipophorin. The specific capacity of lipophorin to accept hydrocarbons from oenocytes, which are believed to be the site of hydrocarbon synthesis, was demonstrated in vitro, and the uptake of hydrocarbon by lipophorin was retarded by respiratory poisoning. When lipophorin containing ^{14}C-labeled hydrocarbon was injected into the hemocoele of cockroach, the labeled hydrocarbon soon appeared at the cuticular surface where it was deposited specifically. The above observations and our previous data support the postulate that insect lipophorin serves as the true carrier molecule for the transport of hydrocarbons from the site of synthesis (oenocyte) to the site of deposition (cuticle), in addition to its function of transporting diacylglycerol and cholesterol from the fat body and intestine.

THE HYDROLYSIS OF DOLICHYL PALMITATE BY INTESTINAL MUCOSA. R.W. Keenan, N. Rice and G.S. Adrian (Dept. of Biochem., The Univ. of Texas Health Sci. Center, San Antonio, TX 78284) *Biochim. Biophys. Acta 711*(3):490-493 (1982). A dolichyl palmitate esterase was found in cell-free extracts of both pancreas and intestinal mucosa. The substrate for the reaction was dolichyl palmitate that was synthesized with labeled fatty acid. The reaction was monitored by the liberation of the free fatty acid and HPLC. All polyprenol esters studied were hydrolyzed despite differences in chain length. The role of this enzyme might be to promote the absorption of dolichol from the diet.

TESTOSTERONE-MEDIATED SEXUAL DIMORPHISM OF THE RODENT HEART. VENTRICULAR LYSOSOMES, MITOCHONDRIA, AND CELL GROWTH ARE MODULATED BY ANDROGENS. H. Koenig, A. Goldstone, and C.Y. Lu (Neurology Service, Veterans Admin. Lakeside Med. Center, and Depts. of Neurology and Biochem., Northwestern Univ. Med. Schl., Chicago, IL) *Circ. Res. 50*(6):782-787 (1982). The ventricular myocardium was studied in A/J mice and in Sprague-Dawley rats. In male mice, the ventricles were slightly larger and the specific activities of the lysosomal hydrolases, β-glucuronidase, hexosaminidase, β-galactosidase, and arylsulphatase, and the inner mitochondrial enzyme cytochrome *c* oxidase were substantially higher than in female mice. Orchiectomy abolished this sex difference. Testosterone administration induced myocardial hypertrophy and accretion of RNA and protein without altering the DNA, and substantial increases in the activities of the lysosomal hydrolases and cytochrome *c* oxidase. However, the mitochondrial membrane enzyme monoamine oxidase was unaffected by sex, orchiectomy, and testosterone administration. Heart lysosomes from male mice showed a smaller structure-linked latency of the lysosomal enzymes and a greater fraagility of the lysosomal membrane to osmotic and mechanical stress than those from female mice. This sex difference was also abolished by orchiectomy and restored by testosterone replacement. Similar sex differences were observed in the rat with respect to heart size, acid hydrolase activities, and lysosomal enzyme latency and membrane stability. These findings indicate that endogenous androgens regulate myocardial cell growth, the activity of enzymes associated with lysosomes and the inner mitochondrial membrane, and some physicochemical properties of lysosomes.

THE PHOSPHOLIPID AND FATTY ACID COMPOSITION OF HUMAN PLATELET SURFACE AND INTRACELLULAR MEMBRANES ISOLATED BY HIGH VOLTAGE FREE FLOW ELECTROPHORESIS. M. Lagarde, M. Guichardant, S. Menashi, and N. Crawford (Inst. National de la Sante et de la Recherche Med., Inst. Pasteur, Faculte Alexis Carrel 69372, Lyon Cedex 2, France) *J. Biol. Chem. 257*(6):3100-3104 (1982). Human platelets were labeled with tracer doses of [^{14}C]arachidonic acid, then fractionated into mixed membranes which are separated into intracellular membranes and two different domains of surface membranes by high voltage free flow electrophoresis. Each subfraction was analyzed for its phospholipid content. Glycerophospholipids were separated by high performance liquid chromatography and their fatty acids analyzed by glass capillary gas chromatography. Intracellular membranes appeared substantially depleted in sphingomyelin, while enrichment of this phospholipid was seen in surface membranes. PC and PI were more enriched in intracellular membranes than in the surface membranes and the contrary was observed for PE. On the other hand, the pattern of the phospholipid labeling by [^{14}C]arachidonate followed closely the glycerophospholipid profiles of the membrane subfractions, but the specific radioactivity of PI was higher than of PC, which itself was higher than that of PE. Moreover, the endogenous content of arachidonic acid accentuates these tendencies. The percentage of arachidonate in PE was higher in the surface membranes than in the intracellular membranes and the contrary was observed for arachidonyl-PC and PI. These differences were compensated for by certain saturated and monounsaturated fatty acids present in the composition profiles. These findings are discussed in relation to the membrane localization of lipases involved in the liberation of arachidonic acid for prostanoid synthesis.

CYTOCHROME P-450$_{scc}$-SUBSTRATE INTERACTIONS. BIN-

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DING AND CATALYTIC ACTIVITY USING HYDROXYCHOLESTEROLS. J. Lambeth, S. Kitchen, A. Farooqui (Dept. of Biochem., Emory Univ. Schl. of Med., Atlanta, GA 30322) *J. Biol. Chem. 257* (4):1876-1884 (1982). Catalysis of cholesterol side chain cleavage by cytochrome P-450$_{scc}$ proceeds via two hydroxycholesterol intermediates: 22R-hydroxycholesterol and 20α,22R-dihydroxycholesterol. The steroid-binding and catalytic activity of phospholipid vesicle-reconstituted cytochrome P-450$_{scc}$ was measured using both cholesterol and hydroxycholesterols. Cholesterol binding was relatively independent of ionic strength, but highly dependent upon the phospholipid used, and cardiolipin most promoted binding. Hydroxycholesterol binding was nearly independent of phospholipid. Binding of both cholesterol and 20α,22R-dihydroxycholesterol was accompanied by uptake of a proton by a group with a pK_a of 6.8 on substrate-free cytochrome. Binding of all hydroxysteroids was tighter than binding of cholesterol, preventing competition by substrate or product with consequent accumulation of hydroxysteroid intermediates. Relative specificity of binding was observed depending upon the position of the hydroxyl group on the substrate side chain; binding was strongest when the hydroxyl group was at position 22 suggesting a specific interaction, perhaps hydrogen bonding, with an active site grouping near position 22. The first hydroxylation has a high activation energy while subsequent oxidative steps to yield pregnenolone have low activation energies; thus, below 37 C, the first hydroxylation is rate-limiting in the overall side chain cleavage of cholesterol. At 37 C or higher, another rate-limiting step in the reaction becomes important, and each oxidation occurs at nearly the same rate.

EVIDENCE FOR A MONOGLUCURONIDE OF 1,25-DIHYDROXYVITAMIN D_3 IN RAT BILE. R. Litwiller, V. Mattox, I. Jardine, R. Kumar (Depts. of Biochem., Parmacology, and Internal Med., Mayo Clinic and Mayo Foundation, Rochester, MN 55905) *J. Biol. Chem. 257*(13):7491-7494 (1982). We have isolated and characterized a monoglucuronide fraction of 9,10-secocholesta-5,7,10(19)triene-1α,3β,25-triol, *5,6-cis* isomer (1,25-dihydroxyvitamin D_3) from rat bile. Polar radioactive metabolites of 1,25-dihydroxyvitamin D_3 were purified by a sequence of chromatographic procedures which utilized Amberlite XAD-2, diethylaminohydroxypropyl Sephadex LH-20, liquid-liquid partition on paper, and reverse phase chromatography on C-18 microparticulate columns. A purified radioactive substance showed maximal absorbance at 264 nm, indicating the presence of a triene in the 5,6-*cis* configuration. Mass spectrometry by fast atom bombardment of the product demonstrated an ion at m/z 637 atomic mass units that is consistent with a natriated sodium salt of a monoglucuronide of 1,25-dihydroxyvitamin D_3 ([MNa]Na^+). Following methylation of the carboxylic acid group and formation of trimethylsilyl ethers of the hydroxyl groups, the fragmentation pattern of the product was compatible with that of a monoglucuronide of 1,25-dihydroxyvitamin D_3. The intact metabolite was treated with β-glucuronidase and the aglycon was isolated by chromatography on microparticulate silica. The aglycon co-migrated with authentic 1,25-dihydroxyvitamin D_3 during chromatography and it gave a mass fragmentation pattern consistent with 1,25-dihydroxyvitamin D_3. The aglycon was bound by an intestinal cytosol receptor with essentially the same affinity as 1,25-dihydroxyvitamin D_3. These findings indicate that bile contains a monoglucuronide of 1,25-dihydroxyvitamin D_3.

SELECTIVE STIMULATION OF HUMAN PLATELET LIPOXYGENASE PRODUCT 12-HYDROXY-5,8,10,14-EICOSATETRAENOIC ACID BY CHLORPROMAZINE AND 8-(*N,N*-DIETHYLAMINO)-OCTYL-3,4,5-TRIMETHOXYBENZOATE. J. Maclouf, H. De La Baume, S. Levy-Toledano, J.P. Caen (U. 150 INSERM, ERA 335 CNRS, Hôpital Lariboisière, 6, rue Guy Patin, 75475 Paris, Cedex, France) *Biochim. Biophys. Acta 711*(3):377-385 (1982). Stimulation of human platelets by thrombin and by the Ca^{2+} ionophore A23187 leads to a rapid Ca^{2+}-dependent activation of phospholipases that release membrane-bound arachidonic acid for oxidation by a cyclooxygenase and lipoxygenase enzymes into so-called eicosanoids. Chlorpromazine and the intracellular calcium antagonist 8-(*N,N*-diethylamino)-octyl-3,4,5-trimethoxygenzoate (TMB-8) inhibited the release of eicosanoids, as estimated by a quantitative glass capillary-gas chromatography analysis. TMB-8 was more efficient for thrombin- than for ionophore-induced eicosanoids liberation. Chlorpromazine, the more potent inhibitor, was active at the same concentration against either inducer. The reduction of oxidative metabolism by the cyclooxygenase pathway was more pronounced than reduction in the lipoxygenase pathway. When exogenous arachidonic acid was added to the platelets, both drugs stimulated selectively the production and the formation rate of 12-hydroxy-5,8,10,14-eicosatetraenoic acid by a factor of 2-2.5 in the absence of variation of cyclooxygenase products. The stimulation of the lipoxygenase metabolite by the two drugs was obtained with both endogenous and exogenous arachidonic acid. This selective stimulation by drugs of a lipoxygenase product in the absence of inhibition of cyclooxygenase is the first reported of this type and suggests a differential control for the two oxidation enzymes. These findings emphasize the importance of a simultaneous quantitative analysis of both oxidation pathways.

THE RELATIONSHIP BETWEEN PROTEIN-PROTEIN AND PROTEIN-LIPID INTERACTIONS AND THE IMMUNOLOGICAL PROPERTIES OF BOVINE PROTHROMBIN AND SEVERAL OF ITS FRAGMENTS. D.A. Madar, M.M. Sarasua, H.C. Marsh, L.G. Pedersen, K.E. Gottschalk, R.G. Hiskey, K.A. Koehler (Chem. and Pathology Depts., Univ. of North Carolina at Chapel Hill, Chapel Hill, NC 27514) *J. Biol. Chem. 257*(4):1836-1844 (1982). An investigation of the antigenicity of bovine prothrombin and several of its fragments: fragment 1 and NH_2-terminal residues 1-39, employed a rabbit antibovine prothrombin fragment 1 antibody which had previously been fractionated by affinity chromatography and which shows an absolute specificity for the metal ion-induced conformation of prothrombin and fragment 1. In the presence of calcium ions, 1-39 self-association, the formation of (1-39)/fragment 1 complexes, and prothrombin/phospholipid binding all mask and antigenic site. In the presence of calcium ions a conformational change in the previously metal ion-free protein generates a hydrophobic region which is co-extensive with the antigenic region. This hydrophobic region is involved in the protein/protein and protein/lipid interactions noted above. Implicit in the interpretation of the protein-binding data has been the development of thermodynamic equations consistent with appropriate conceptual model systems. Application of these models has allowed the estimation of several association constants. While the absolute values of these association constants may vary when this work is repeated using homospecific antibodies, the ability of the models to predict the observed relative changes in antigenicity is the important factor in generating the hypotheses presented. The derivation of the equations is independent of the number of antibody populations present and therefore applicable to any similar system.

ASSOCIATION BETWEEN AGE, BLOOD LEAD CONCENTRATION, AND SERUM 1,25-DIHYDROXYCHOLECALICIFEROL LEVELS IN CHILDREN. K.R. Mahaffey, J.F. Rosen, R.W. Chesney, J.T. Peeler, C.M. Smith, and H.F. DeLuca (Food and Drug Administration, Cincinnati, OH 45226; the Dept. of Pediatrics, Montefiore Hospital and Med. Center, Albert Einstein College of Med., Bronx, NY 10467; and the Dept. of Pediatrics and Biochem., Univ. of Wisconsin, Madison, WI 53792) *Am. J. Clin. Nutr. 35*(5):1327-1331 (1982). Serum levels of 1,25-dihydroxycholecaliciferol (1,25-CC), the form of vitamin D active in stimulating intestinal absorption of calcium, phosphorus, and lead, were determined in 177 human subjects ages 1 to 16 yr. Significant negative association (r=-0.88) was observed between serum 1,25-CC levels and blood lead concentrations over the entire range of blood lead levels, 12 to 120 μg/dl. Adolescents ages 11 to 16 yr had serum 1,25-CC levels higher than those observed among children 10 yr old or younger. No effect of sex or season on serum 1,25-CC level was observed. When the 1,25-CC values for children with blood lead concentrations greater than 30 μg/dl were excluded from the analysis, no significant effect of geographic location on 1,25-CC levels was observed.

METABOLISM OF PHOSPHOINOSITIDES IN THE RAT ERYTHROCYTE MEMBRANE. A REAPPRAISAL OF THE EFFECT OF MAGNESIUM ON THE ^{32}P INCORPORATION INTO POLYPHOSPHOINOSITIDES. P. Marche, S. Koutouzov, P. Meyer (INSERM U7/CNRS LA 318 Res. Unit, Dept. of Nephrology, Hopital Necker, 161 rue de Sèvres 75015 Paris, France) *Biochim. Biophys. Acta 710*(3):332-340 (1982). The metabolism of phosphoinositides was investigated in the red blood cell membrane of the rat by measuring ^{32}P-incorporation into phospholipids after incubation of membranes with [γ-^{32}P]ATP in a medium containing magnesium. A new chromatographic procedure has been developed which facilitates the separation of triphosphoinositide, diphosphoinositide and phosphatidylinositol from the phospholipids present in lipid extracts of incubated "ghost" membranes. Under our experimental conditins only two phospholipids, diphosphoinositide and triphospho-

inositide, were ^{32}P-labelled. The results indicate that either di- or triphosphoinositide could be labelled preferentially, depending upon the magnesium concentration of the incubation medium. This clarifies some apparent discrepancies reported in the literature between the ^{32}P labelling of polyphosphoinositides observed in intact erythrocytes and that observed with "ghost" membranes. In addition, the enzymatic pathways involved in the phosphoinositide metabolism are discussed.

TISSUE STORAGE OF VITAMINS A AND E IN RATS DRINKING OR INFUSED WITH TOTAL PARENTERAL NUTRITION SOLUTIONS. M.C. McKenna, J.G. Bieri (Lab. of Nutr. and Endocrinology, Natl. Inst. of Arthritis, Diabetes, and Digestive and Kidney Disease, Bethesda, MD 20205) *Am. J. Clin. Nutr. 35*(5):1010-1017 (1982). The total parenteral nutrition (TPN) rat and its sham-operated control were used as a model to compare the metabolism and storage of vitamin A and E when they are administered intravenously or orally. Male Fisher rats were depleted of both vitamins for several months with a diet free of vitamins A or E, but containing retinoic acid for growth. TPN solutions containing aqueous dispersions of retinol, retinyl palmitate and *dl*-α-tocopheryl acetate were infused at 2.3 ml/hr into the jugular veins of 10 TPN rats. Eight sham-operated control rats drank similar volumes from food cups. TPN rats received 115.3 ± 4.5 (mean ± SEM) μg of retinol equivalents and 2.2 ± 0.2 mg of α-tocopherol equivalents per day; controls received 146.4 ± 16.5 μg and 2.1 ± 0.3 mg, respectively. After 7 days the animals were fasted overnight and killed. Plasma levels of retinol were 27.8 ± 1.5 μg/dl for TPN rats, and 27.4 ± 1.2 for controls. Plasma α-tocopherol was 1909 ± 183 μg/dl for TPN rats and 1063 ± 77 for controls. The only forms of the vitamins found in plasma after overnight fasting were unesterified retinol and unesterified α-tocopherol. Sham-operated control rats stored amounts of vitamins A and E similar to values reported in the literature for fed animals. TPN rats stored more of both vitamins than controls in liver, heart, and spleen, but not in testes. The enhanced liver vitamin storage by TPN rats did not appear to be due to a slight increase in lipid content. The results indicate that both vitamins A and E infused in TPN solutions maintain blood levels and are stored in tissues.

THE EFFECT OF VITAMIN A FORTIFICATION OF SUGAR ON IRON METABOLISM IN PRESCHOOL CHILDREN IN GUATEMALA. L.A. Mejia, G. Arroyave (Div. of Human Biol. and Nutr., Inst. of Nutr. of Central America and Panama (INCAP), P.O. Box 1188, Guatemala, Guatemala, C.A.) *Amer. J. Clin. Nutr. 36*(1):87-93 (1982). The effect of improvement in vitamin A nutriture on biochemical indicators of iron nutrition during national vitamin A fortification of sugar was investigated longitudinally. Four "paired-comparison-subgroups" of preschoolers were studied before fortification (survey I) and, respectively, at 6 months (survey I versus II), at 1 yr (survey I versus III), at 1½ yr (survey I versus IV), and at 2 yr (survey I versus V) after fortification began. Comparing I versus II gave a positive correlation ($p<0.001$) between changes in serum retinol or retinol-binding protein and changes in iron, total iron binding capacity, and percentage transferritin saturation. In contrast, changes in serum ferritin correlated negatively ($p<0.05$). Comparing V with I, retinol, retinol-binding protein, iron, and percentage transferritin saturation increased, but ferritin also increased ($p<0.05$). Consequently, the distribution of serum iron and ferritin values of the children improved ($p<0.05$). Because dietary iron did not change through the study period, the results suggest that vitamin A fortification had a favorable effect on iron metabolism and nutritional status.

REDUCTION OF β-OXIDATION CAPACITY OF RAT LIVER MITOCHONDRIA BY FEEDING OROTIC ACID. S. Miyazawa, S. Furuta, and T. Hashimoto (Dept. of Biochem., Shinshu Univ. Schl. of Med., Matsumoto, Nagano-ken 390, Japan) *Biochim. Biophys. Acta 711*(3):494-502 (1982). Rats were maintained on fat-free high carbohydrate diets either with or without orotic acid (1%, w/w), pantethine (1%, w/w), adenine (0.35%, w/w), and/or *p*-chlorophenoxyisobutyrate (0.25%, w/w). Oxidation of fatty acid by liver mitochondria was inhibited to less than half that of the control after administration of orotic acid. Activities of acyl-CoA dehydrogenases were markedly decreased by orotic acid administration, but the following enzyme activities were not, or only slightly, decreased: acyl-CoA synthetase, carnitine acyltransferases, enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase and 3-ketoacyl-CoA thiolase. Simultaneous addition of pantethine in the orotic acid-containing diet prevented induction of fatty liver. It also prevented decreases in fatty acid oxidation capacity and acyl-CoA dehydrogenase activity. Introduction of adenine or *p*-chlorophenoxyisobutyrate, which reverse orotic acid-induced fatty liver, reversed oxidation and acyl CoA dehydrogenase activities to control levels. The oxidation capacity of the peroxisomal system remained unchanged after administration of orotic acid.

PROPERTIES OF CARNITINE INCORPORATION IN WORKING SWINE HEARTS. EFFECTS OF CORONARY FLOW, ISCHEMIA, AND EXCESS FATTY ACIDS. A.J. Liedtke, T.C. Vary, S.H. Nellis, and C.W. Fultz (Cardiology Div., Pennsylvania State Univ., Hershey Med. Center, Hershey, PA) *Circ. Res. 50*(6):767-774 (1982). Sarcolemmal transport of L-carnitine was determined in three groups of intact working swine hearts under varying conditions of oxygen delivery and fatty acid (FA) availability. Hearts were regionally perfused with controlled flows to the right, left circumflex (LCF), and left anterior descending (LAD) coronary circulations. All hearts were treated with 50 μCi [^{14}C]-L-carnitine. Ischemia was induced in the LAD bed by restricting flow by 59% over 30 minutes. Metabolic data were collected from both the LCF (nonischemic) and LAD (ischemic) perfusion beds, and comparisons were made between the three groups of animals: eight untreated control animals (group 1); eight animals perfused similarly to group 1 hearts but additionally treated with excess FA (>1 μmol/ml, group 2). Labeled L-carnitine was taken up by and distributed into acid soluble and long-chain acyl esters in all groups; athe long-chain fraction contained less absolute counts but higher specific activity. Transport rates of L-carnitine decreased modestly with ischemia in all groups. High FA per se caused only slightly greater suppression of uptake. Conversely, hyperperfusion on conjuction with FA treatments (group 3) increased transport significantly ($P<0.01$) in both LCF(+70Δ%) and LAD (+104Δ%) beds. Thus, carnitine transport at physiological concentrations is in part dependent on rates of coronary flow, even at nonischemic levels.

EFFECT OF DIETARY PROTEIN AND CHOLESTEROL ON CHOLESTEROL CONCENTRATION AND LIPOPROTEIN PATTERN IN THE SERUM OF CHICKENS. M.A.E. Mol., R.C. DeSmet, A.H.M. Terpstra, and C.E. West (Dept. of Human Nutr., Agric. Univ., De Dreijen 12, 6703 BC Wageningen, The Netherlands) *J. Nutr. 112*(6):1029-1037 (1982). The effect of dietary cholesterol and the type of protein in the diet on the cholesterol concentration and the lipoprotein pattern in serum was studied in male chickens. The chickens were divided into six groups each of five animals, each group receiving a different diet for 6 weeks. Two groups received a commercial diet, to one of which cholesterol (1% wt/wt) had been added. The remaining four groups received a semipurified diet containing either casein or soybean protein as protein source as follows: casein, casein plus cholesterol (1% wt/wt), casein plus arginine (0.85% wt/wt) and soybean protein. No change in the concentration of cholesterol in serum was observed in the chickens fed diets without added cholesterol. When cholesterol was added to the diets, there was a significant increase in the cholesterol concentration. After the animals were fed the diets for 6 weeks, blood was taken for the examination of serum lipoproteins. In the experimental groups on the cholesterol-containing diets, a shift in the lipoprotein pattern from the low-density lipoproteins was seen. This was confirmed by determination of the composition of the lipoprotein particles and by their appearance under the electron microscope. However, no differences in the density profile and lipoprotein composition could be observed between the groups receiving a cholesterol-free diet containing casein or soybean protein.

EFFECT OF SALTS ON MEMBRANE BINDING AND ACTIVITY OF ADIPOCYTE PHOSPHATIDATE PHOSPHOHYDROLASE. F. Moller and M.R. Hough (Dept. of Biochem., Queen's Univ., Kingston, Ontario K7L 3N6, Canada) *Biochim. Biophys. Acta 711*(3):521-531 (1982). Isosmotic replacement of sucrose in a low ionic strength homogenizing buffer (0.25 M sucrose/1mM EDTA/1 mM Tris-HCl, pH 7.4) with KCl increased the microsomal and decreased the soluble phosphatidate phosphohydrolase (EC 3.1.3.4) activity of isolated rat fat cells. At 54 mM KCl the microsomal specific activity was increased 6-fold and the soluble activity was decreased to less than one-third. Enzyme binding was promoted by KCl and NaCl when the once isolated soluble and microsomal fractions were recombined and incubated at 37 C. Half-maximal binding occurred at about 17 mM salt and maximal binding at about 50 mM. The pH optimum of binding was 7.8 in 15 mM Hepes. $MgCl_2$, $CaCl_2$ and spermine prevented desorption of microsomal enzyme at μ molar levels and maximal effects were observed at concentrations below the 1 mM level. At maximum, however, the prevention of desorption was less by these salts than it was by KCl. $MgCl_2$ and spermine also interfered

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with the effect of KCl. Moderate salt-induced loading of microsomes with the phosphohydrolase (specific activity increased 3.5-fold) increased their ability to incorporate ^{14}C into triacylglycerol from *sn*-[U-^{14}C]glycerol 3-phosphate while a high loading (specific activity increased 6-fold) had no effect or even suppressed it. The results are discussed in relation to a role of translocation of phosphatidate phosphohydrolase in glyceride biosynthesis and its control.

TRANSPORT OF FLUORESCENT DERIVATIVES OF FATTY ACIDS INTO CULTURED HUMAN LEUKEMIC MYELOID CELLS AND THEIR SUBSEQUENT METABOLIC UTILIZATION. O. Morand, E. Fibach, A. Dagan, and S. Gatt (Dept. of Neurochem., Hebrew Univ.-Hadassah Med. Schl.) *Biochim. Biophys. Acta 711*(3): 539-550 (1982). Transport of fluorescent derivatives of fatty acids across the cell membrane of cultured human leukemic myeloid cells (HL 60) and their subsequent metabolic utilization were studied. The rates of uptake of these derivatives and their incorporation into cellular lipids were compared with that of radioactively labelled palmitic acid. Three groups of fluorescent derivatives were observed: A, those transported into the cells and subsequently incorporated into neutral lipids and phospholipids, B, fatty acids which were taken up by the cells but not utilized metabolically and C, fatty acids which were not transported across the cell membrane. Fatty acids of the latter group, except the hydrophobic probe, also contained functional groups such as hydroxy, acetylamino or sulfonylamino. When observed in fluorescence microscopy, cells incubated with group A fatty acids contained intracellular fluorescent granules, whereas those incubated with group B fatty acids showed diffuse fluorescence. HL 60 cells undergo differentiation into granulocytes or macrophages upon treatment with dimethylsulfoxide or a phorbol ester, respectively. When compared to the uninduced cells, the transport of the fluorescent fatty acids or palmitic acid as well as their subsequent incorporation into lipids were considerably lower in the granulocytes and higher in the macrophages. The use of the fluorescent derivatives as a tool for studying transport of fatty acid across the cell membrane is discussed.

MOLECULAR CLONING OF GENE SEQUENCES FOR AVIAN FATTY ACID SYNTHEASE AND EVIDENCE FOR NUTRITIONAL REGULATION OF FATTY ACID SYNTHASE MRNA CONCENTRATION. S.M. Morris, Jr., J.H. Nilson, R.A. Jenik, L.K. Winberry, M.A. McDevitt, and A.G. Goodridge (Depts. of Pharmacology and Biochem., Case Western Reserve University, Cleveland, OH 44106 and the Dept. of Biochem., Michigan State Univ., East Lansing, MI 48824) *J. Biol. Chem. 257*(6):3225-3229 (1982). A double-stranded cDNA library was constructed using total poly(A)$^+$ RNA from the goose uropygial gland. Clones containing sequences complementary to fatty acid synthase mRNA were initially identified by colony hybridization with a ^{32}P-labeled cDNA transcribed from RNA enriched for fatty acid synthase mRNA. Identity of the fatty acid synthase clones was confirmed by hybrid-selected translation. Mature fatty acid synthase mRNA is approximately 16 kilobases in length. When unfed neonatal goslings were fed for 24 hr, relative synthesis of hepatic fatty acid synthase increased more than 42-fold. Concomitantly, hepatic fatty acid synthase mRNA levels increased 70-fold. Thus, nutritional regulation of the synthesis of hepatic fatty acid synthase probably occurs at the pretranslational level. The availability of a specific probe for fatty acid synthase mRNA should allow us to analyze the regulation of expression of this gene during development, by nutrition and by hormones in both the liver and the uropygial gland.

FAMILIAL ASSOCIATIONS OF LIPIDS AND LIPOPROTEINS IN FAMILIES OF HYPERCHOLESTEROLEMIC PROBANDS. J.A. Morrison, P. Khoury, P.M. Laskarzewski, M.J. Mellies, R. Heinemeyer, and C.J. Glueck (Lipid Res. Clinic, General Clinical Res. Center, and CLINFO Center, Depts. of Med. and Pediatrics, Lipid Res. Div., and Div. of Epidemiology and Biostats, Univ. of Cincinnati Medical Center, Cincinnati, OH) *Arteriosclerosis 2*(2):151-159 (1982). We used the Princeton School Family Study hypercholesterolemic recall group to assess whether, and to what degree, the identification of hypercholesterolemic subjects could be improved though the phenomenon of familial lipid and lipoprotein aggregation. A second aim was to assess whether within-family lipid and lipoprotein correlations outlasted the period of shared family environment. Approximately twice as many (as expected) siblings and offspring of hypercholesterolemic probands had plasma total and low density lipoprotein cholesterol levels greater than the 90th and 75th percentiles respectively, emphasizing how identification of hypercholesterolemic subjects can be facilitated by use of the phenomenon of familial aggregation of plasma total and low density lipoprotein cholesterol. After exclusion of the hypercholesterolemic probands from calculations of within-family correlations, and use of natural log transformations for the probands' first-degree relatives' lipids as required, most father/pediatric offspring correlations for lipids and lipoprotein cholesterols were significant, while most parent/adult offspring correlations in adults who no longer shared a common household environment points to environmental influences on total, high, and low density lipoprotein cholesterol in kindreds with a hypercholesterolemic proband.

INTRAFAMILIAL ASSOCIATIONS OF LIPIDS AND LIPOPROTEINS IN KINDREDS WITH HYPERTRIGLYCERIDEMIC PROBANDS: THE PRINCETON SCHOOL FAMILY STUDY. J.A. Morrison, P. Khoury, P.M. Laskarzewski, M.J. Mellies, K. Kelly, and C.J. Glueck (Lipid Res. Clinic and the General Clinical Res. Center, the CLINFO Centers, Depts. of Med., Lipid Res., Div. and Div. of Epidemiology and Biostatistics, Univ. of Cincinnati Med. Center, Cincinnati, OH) *Circulation 66*(1):67-76 (1982). All lipid and lipoprotein correlations were significant for both pediatric and adult siblings. Whatever accounts for significant correlations among siblings for lipids and lipoproteins in families transcends the period of shared common environment. Although we cannot differentiate between environment and heredity in the observed famial trends, close sibling and parent-offspring lipid and lipoprotein risk factor associations in hypertriglyceridemic family units suggest sharing of coronary heart disease risk. Presumably, shared genetic and environmental factors that elevate triglyceride, cholesterol and LDL cholesterol and depress HDL cholesterol can be specifically identified, allowing for early intervention.

THE FORMATION OF LIPID-LINKED OLIGOSACCHARIDES IN MADIN-DARBY CANINE KIDNEY CELL. Y.T. Pan, and A.D. Elbein (Dept. of Biochem., The Univ. of Texas Health Sci. Center, San Antonio, TX 78284) *J. Biol. Chem. 257*(6):2795-2801 (1982). Glucosamine inhibits the incorporation of [2-^3H] mannose into lipid-linked oligosaccharides and into glycoproteins in influenza virus-infected MDCK cells. Fifty percent inhibition of these components requires about 2mM glucosamine. The oligosaccharide portions of the lipid-linked oligosaccharides in cells inhibited with glucosamine were compared to that of normal cells by chromatography on Bio-Gel P-4 columns. In uninhibited cells, the major oligosaccharide formed from [2-^3H] mannose was the $Glc_3Man_9GlcNac_2$ species as demonstrated by the products of endoglucosaminidase H and α-mannosidase digestion. At low concentrations of glucosamine (~2mM) or in short term incubations (1 to 2 hr), the large oligosaccharide disappeared and was replaced by a $Man_7GlcNAc_2$ species. This was also characterized by various enzymatic treatments as well as its migration rate on Bio-Gel P-4 as compared to known oligosaccharides. At still higher glucosamine concentrations or longer incubation times, the $Man_7GlcNac_2$ species also disappeared and was replaced by a $Man_3GlcNAc_2$ species. The effect of glucosamine was reversible such that when the cells were washed free of this inhibitor, they resumed the synthesis of the $Glc_3Man_9GlcNAc_2$ species and the other two oligosaccharides disappeared. These smaller oligosaccharides were not observed when glucosamine was replaced by either 5 mM galactosamine or 5 mM N-acetylglucosamine.

14α-ETHYL-5α-CHOLEST-7-ENE-3β,15α-DIOL, A POTENT INHIBITOR OF STEROL BIOSYNTHESIS, HAS TWO SITES OF ACTION IN CULTURED MAMMALIAN CELLS. F.D. Pinkerton, A. Izumi, C.M. Anderson, L.R. Miller, A. Kisic, and G.J. Schroepfer, Jr. (Depts of Biochem.and Chem., Rice Univ., Houston, TX 77001) *J. Biol. Chem. 257*(4):1929-1936 (1982). 14α-Ethyl-5α-cholest-7-ene-3β,15α-diol is a very potent inhibitor of sterol synthesis in mouse L-cells and in primary cultures of fetal mouse liver cells. In these cells, this 15-oxygenated sterol showed a discrepancy between the potencies with respect to the inhibition of the synthesis of digitonin-precipitable sterols and to the reduction of 3-hydroxy-3-methylglutaric acid (HMG)-CoA reductase activity. The combined findings presented herein indicate that 14α-ethyl-5α-cholest-7-ene-3β,15α-diol inhibits sterol biosynthesis at two sites, one at the level of HMG-CoA reductase, and one at the level of the metabolism of lanosterol and 24,25-dihydrolanosterol. The actions of the 14α-ethyl-Δ^7-3β,15α-diol at these two sites appear to be independent of each other.

THE EFFECT OF VARIATIONS IN DIETARY FATTY ACIDS ON THE FATTY ACID COMPOSITION OF ERYTHROCYTE PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLETHANOLAMINE

IN HUMAN INFANTS. J.C. Putnam, S.E. Carlson, P.W. DeVoe, and L.A. Barness (Col. of Med., Univ. of South Florida Med. Center, Dept. of Pediatrics, Tampa, FL 33612) *Am. J. Clin. Nutr. 36*(1): 106-114 (1982). Human milk, or one of two formulas that derive their fat from vegetable oil, was fed to infants from birth until 4.5 to 6 months of age. Infants fed human milk received 2% of total fatty acids as 20 to 22 carbon polyunsaturated fatty acids. These fatty acids which are not found in vegetable oils, are synthesized by animals from the essential vegetable-derived fatty acids, linoleic and α-linolenic acids. Enfamil (Mead Johnson, Evansville, IN) contained three times as much linoleic acid as human milk or SMA (Wyeth Laboratories, Philadelphia, PA); however, the ratios of linoleic/α-linolenic acid were 9.0, 18.8, and 11.7 for Enfamil, human milk, and SMA, respectively. Erythrocyte phosphatidylethanolamine and phosphatidylcholine in infants fed human milk had significantly more 20 to 22 carbon polyunsaturated fatty acids than did those of infants consuming only vegetable fat. Concentrations of 20 to 22 carbon polyunsaturated fatty acids in the erythrocyte membrane phosphatidylethanolamine and phosphatidylcholine of SMA and Enfamil-fed infants were similar despite very significant differences in the amount of dietary 18 carbon precursor. The degree of unsaturation of both erythrocyte phosphatidylethanolamine and phosphatidylcholine was highest with the feeding of human milk compared to the formulas, but the relative concentration of the four major erythrocyte phospholipids, and the ratios of membrane phosphorus/cholesterol were not affected by these diets.

LIMITED ELASTASE DIGESTION OF PIGEON LIVER FATTY ACID SYNTHETASE WITH RETENTION OF ALL PARTIAL ENZYME ACTIVITIES. S.S. Rabinowitz, M. LaPorte, and J.W. Porter (Lipid Metabolism Lab., W.S. Middleton Memorial Veterans Hosp.) *J. Biol. Chem. 257*(6):3291-3300 (1982). Pigeon liver fatty acid synthetase which contains two subunits of 240,000 daltons each has been treated with elastase. This treatment yields four protein fragments which can be separated on sodium dodecyl sulfate (SDS) gel-electrophoresis. After the subunit protein has been treated with elastase, all of the partial enzyme activities catalyzed by the complex are present, but enzyme activity for fatty acid synthesis is lost. The formation of protein fragments during proteolysis has been followed by densitometric scanning of the SDS gels. The results of these scans have suggested that there are two peptide components present in the highest molecular weight band, both are rapidly digested to yield the second and third largest peptides, and a further cleavage of the third largest peptide gives rise to the smallest of the four major peptides. Crossed-rocket immunoelectrophoretic analysis of the four protein fragments has confirmed these conclusions and established also that the three smallest peptides are homogeneous. Each of the four peptides has been isolated by preparative SDS-gel electrophoresis, and antibody to one has been prepared. This antibody fraction immunotitrates overall fatty acid synthetase activity and immunoprecipitates the native enzyme. Immunoelectrophoresis of the four elastase-digested synthetase products against this antibody showed some cross-reactivity with a peptide that was neither the precursor nor the product of the immunogen. This cross-reacting antibody was removed by reaction with the nonrelated protein to yield antibody specific for one region of the fatty acid synthetase complex.

HUMAN APOLIPOPROTEIN E. THE COMPLETE AMINO ACID SEQUENCE. S.C. Rall, K.H. Weisgraber, and R.W. Mahley (Gladstone Foundation Labs for Cardiovascular Disease and the Cardiovascular Res. Inst., Univ. of California, San Francisco, CA 94140) *J. Biol. Chem. 257*(8):4171-4178 (1982). The amino acid sequence of human apolipoprotein E (apo-E) has been determined. Apo-E2, one of the major isoforms of apolipoprotein E, is a polypeptide of 299 amino acids having a calculated molecular weight of 34,145. The isoform apo-E3 differs from apo-E2 by a single amino acid substitution of arginine for cysteine at residue 158. Consideration of the primary and secondary structure provides information related to two known functions of apo-E, lipid binding and apo-B,E cell surface receptor interaction. There are a series of amphipathic helices in the carboxyl-terminal third of the polypeptide chain which may represent the lipid-binding site(s) for apo-E. In addition, a sequence enriched in lysine and arginine residues, which includes the site of amino acid substitution in apo-E2 and apo-E3, appears to be the region of the molecule involved in the interaction of E-containing lipoproteins with specific lipoprotein receptors on cell membranes.

DEVELOPMENT OF THE DIURNAL RHYTHM OF CHICK 3-HYDROXY-3-METHYLGLUTARYL-CoA REDUCTASE. H. Ramirez, M.J. Alejandre, and E. Garcia-Peregrin (Dept. of Biochem., Univ. of Granada, Granada, Spain) *Lipids 17*(6):434-436 (1982). Chick liver and intestine 3-hydroxy-3-methylglutaryl-CoA reductase did not show diurnal rhythm at hatching. Differences in activity between light and dark periods appeared during the first week and remained more or less constant between 10-14 days after hatching. Hepatic and intestinal reductase activities were maximal during the light period and minimal during the dark period. Amplitude of the rhythm was practially similar in both tissues (about 3-fold) although specific activities and differences between peak and nadir values were always higher in liver. Chick brain reductase did not show significant diurnal variations in the age range assayed.

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