

Abstracts

EDITOR: F.A. Kummerow

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

Fats and oils

CALCULATION OF CRITICAL SOLUTION TEMPERATURES. I. Saturated and unsaturated hydrocarbons. G.M. El-Taliawi (Dept. of Pharmaceutical Chem., Faculty of Pharm., Cairo Univ., Cairo Egypt) *Chem. and Phys. Lipids* 27(3),233-5 (1980). Critical solution temperatures (CST) of saturated and unsaturated hydrocarbons are analyzed and individual contributions of molecular moieties estimated. Equations are presented that sum these contributions and permit the calculation of CST-values which agree with those determined experimentally.

THE SEPARATION OF STEROL INTERMEDIATES IN CHOLESTEROL BIOSYNTHESIS BY HIGH PRESSURE LIQUID CHROMATOGRAPHY. E. Hansbury and T.J. Scallen (Dept. of Biochem., Schl. of Med., Univ. of New Mexico, Albuquerque, NM 87131) *J. Lipid Res.* 21(20),921-929 (1980). A three-step procedure has been developed for the separation of complex mixtures of sterol intermediates in cholesterol biosynthesis. The method has been applied to the separation of sterol intermediates formed from [¹⁴C]mevalonate by normal rat hepatocyte culture cells. Relative retention time factors for several functional groups encountered in sterol intermediates in cholesterol biosynthesis have been determined for both reverse-phase and silicic acid HPLC systems. The use of these functional group factors allows one to calculate a predicted relative retention time for a variety of structural possibilities. The HPLC techniques described utilize single columns, isocratic solvent systems, and comparatively short (<30 min) elution times, and the three-step procedure is capable of resolving complex mixtures of sterol intermediates.

MONOALKYL PHOSPHODIESTERS: SYNTHESIS AND DIELECTRIC RELAXATION OF SOLUTIONS. U. Kaatz, S.C. Müller and H. Eibl (Drittes Physikalisches Institut, Universität Göttingen, Burdenstrasse 42-44, D-3400 Göttingen, F.R.G.) *Chem. Phys. Lipids* 27(3),263-80 (1980). Chromatographically pure hexadecylphosphocholine, -(N,N-dimethyl-ethanolamine), -(N-methyl)-ethanolamine and -ethanolamine have been synthesized. Aqueous solutions of these phospholipids have been prepared for the purpose of measuring their dielectric spectra. Micellar phospholipids have been prepared for the purpose of measuring their dielectric spectra. Micellar solutions appropriate for the dielectric studies were obtained with the choline and the (N,N-dimethyl)-ethanolamine head groups. The dielectric spectra of these phospholipid/water systems are evaluated in terms of phenomenologically introduced sum of Cole-Cole relaxation functions and also on the basis of a model relaxation function which has regard to internal depolarizing fields of the colloidal solutions. Parameters reflecting the motions of the dipolar head groups and of the hydration water molecules of the synthetic monoalkyl phosphodiester are discussed and are compared with those for egg lysolecithin. The mobility of the dipolar phospholipid head groups and the number of influenced water molecules per zwitterion decreases when changing from lysolecithin to hexadecylphosphocholine and further to hexadecylphospho-(N,N-dimethyl)-ethanolamine, while the relaxation time of the hydration water increases. These results indicate the micellar surface to get less porous within the above series of lipids.

AUTOXIDATION OF A MODEL MEMBRANE. A COMPARISON OF THE AUTOXIDATION OF EGG LECITHIN PHOSPHATIDYLCHOLINE IN WATER AND IN CHLOROBENZENE. L.R.C. Barelay, K.U. Ingold (Division of Chemistry, National Research Council of Canada, Ottawa, Ontario, Canada K1A 0R6) *J. Am. Chem. Soc.* 102, 7792-4 (1980). The autoxidation of biological membranes, though known to occur readily and to be associated with many important pathological events, is totally lacking in quantitative kinetic data. In this communication we report some results from a kinetic study of the thermally initiated autoxidation of egg lecithin phosphatidylcholine at 30°C in homogeneous solution in chlorobenzene and as bilayer dispersions (vesicles or model membranes) in 0.1 M aqueous NaCl. Our results provide answers to three simple, but extremely important, questions concerning the autoxidation of lecithin bilayers,

answers which we hope will prove relevant to the autoxidation of biomembranes. (1) Is there a large cage-effect in a lecithin bilayer? (2) Is the kinetic rate law for autoxidation the same for biomembranes as for homogeneous systems? (3) Is the oxidizability of egg lecithin the same in homogeneous solution as in an aqueous dispersion? In summary, our results suggest that the physical structure of lecithin bilayers makes them more resistant to autoxidation than would be expected on the basis of their chemical composition. Thus, although these bilayers appear to follow the normal kinetic law for autoxidation, the initiation process appears to be rather inefficient, and oxidizability appears to be reduced.

ANTIOXIDANT ACTIVITY OF VITAMIN E AND RELATED PHENOLS. IMPORTANCE OF STEREOELECTRONIC FACTORS. G.W. Burton, Y. Le Page, E.J. Gabe and K.U. Ingold (Division of Chemistry, National Research Council of Canada, Ottawa, Ontario, Canada K1A 0R6) *J. Am. Chem. Soc.* 102, 7791-2 (1980). There is now a rather general agreement that α -tocopherol(1), the major component of vitamin E, functions as an efficient inhibitor of lipid peroxidation in vivo, but there is widespread confusion regarding its absolute antioxidant effectiveness in vitro. Comparisons of 1 with other natural and synthetic phenols have usually led to the conclusion that it has only a rather modest antioxidant activity in vitro. The apparent "discrepancy" between the high in vivo vitamin E activity of 1 and its apparently low in vitro antioxidant activity has generally been accepted uncritically. This is surprising because 1 has just those structural features in its phenolic moiety which would lead one to predict that it would be a highly efficient chain-breaking (peroxyl radical trapping) antioxidant. In an attempt to reconcile the structure of 1 with its purported low in vitro antioxidant activity, we have measured k_{inh} for 1 in the well-proven autoxidation system of styrene under 760 torr of O₂, thermally initiated with azobis(isobutyronitrile). In summary, the chroman ring system maintains a near-optimal orientation of the etheral oxygen p-type lone pair with respect to the aromatic ring which, in combination with alkyl substitution at the other four ring positions, explains the superior chain-breaking antioxidant properties of α -tocopherol and CH₃. Full details of the X-ray analyses will be published elsewhere.

CARDIOLIPIN, A MAJOR PHOSPHOLIPID OF GRAM-POSITIVE BACTERIA THAT IS NOT READILY EXTRACTABLE. M.H. Filgueiras and J.A.F. Op Den Kamp (Biochemisch Laboratorium, Rijksuniversiteit Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands) *Biochim. Biophys. Acta* 620, 332-7 (1980). Extraction of phospholipids from stationary phase grown cells of the Gram⁺ bacteria, *Bacillus megaterium*, *Bacillus subtilis*, *Bacillus cereus* and *Micrococcus lysodeikticus* was found to be incomplete with various commonly used extraction methods. Phosphatidylglycerol and phosphatidylethanolamine were readily extracted but up to 95% of the cardiolipin appeared to be retained within the cell residue. Extraction of the cardiolipin could be slightly enhanced by increasing the temperature or the acidity of the extraction solutions but complete extraction was obtained only after lysozyme treatment of intact cells or cell residues remaining after extraction. In addition complete extraction could be observed in the case of cells harvested in the early logarithmic phase. Freeze-fracture electron microscopy was carried out on the cell residue remaining after extraction of all phospholipids except cardiolipin. A fracture plane through the plasma membrane could not be observed anymore. Instead fracture planes through lipid vesicles were observed. These vesicles reside within the remnants of the cytoplasm and consist most likely of the non-extracted cardiolipin.

TRANSPORT OF Pr³⁺ ACROSS PHOSPHOLIPID MEMBRANES BY LIPO-

PHILIC β -DIKETONES. G.R.A. Hunt (Dept. of Science, The Polytechnic of Wales, Pontypridd, Mid Glamorgan, CF37 1DL, U.K.) *Chem. Phys. Lipids* 27, 353-64 (1980). Several β -diketones (R.CO.CH₂.CO.R.) with R groups similar to those used in NMR shift reagents have been investigated as carriers for the transport of Pr³⁺ ions across phospholipid vesicular membranes. Only the fluorinated diketone fod (1,1,1,2,2,3,3-heptafluoro-7,7-dimethyloctane-4,6 dione) gave a transport rate which approached that obtained using the calcium ionophore A23187. It was established that the NMR method used to follow the kinetics gave an experimental stoichiometry of Pr(fod)_{2.5} for an expected transported complex Pr(fod)₂. The advantages of using an NMR method for studying facilitated transport in vesicular membrane systems are discussed, in particular their use in determining stoichiometries of transported species.

SELECTIVITY OF FLUORESCENT LIPID ANALOGUES FOR LIPID DOMAINS. R.D. Klausner and D.E. Wolf (Dept. of Biol., Johns Hopkins Univ., Baltimore, MD 21218) *Biochemistry* 19, 6199-203 (1980). We have examined the phase partition preferences of the even chain length ($n=10-22$) diacyl-3,3'-indocarbocyanine iodides (C_ndiI) incorporated in disaturated lecithin (PC) vesicles. Two parameters were used to determine this phase preference: the direction of shift of the phase transition temperature (T_m) induced by the dyes and the self-quenching of fluorescence due to aggregation in the gel phase of those dyes which preferentially partition into the fluid. Dyes that lower T_m preferentially partition into the fluid phase; those that raise T_m preferentially partition into the gel. By these criteria in dimyristoyl-PC, C₁₀ diI and C₁₂ diI preferentially partition into the fluid phase, C₁₄ diI and C₁₆ diI show no preferential partition, C₁₈ diI preferentially partitions into the gel, and C₂₀ diI and C₂₂ diI preferentially partition into the fluid. In dipalmitoyl-PC, the pattern of preference is identical with that observed in dimyristoyl-PC, only shifted to longer chain length diI's by two carbons. Diffusion measurements by fluorescence photobleaching recovery of these dyes in gel-phase multilayers showed them all to be immobile, $D < 10^{-10}$ cm²/s, while in fluid-phase multilayers they all had diffusion coefficients of $D 10^{-8}$ cm²/s independent of chain length. In mixed-phase multilayers, however, each C_n diI showed mobile fractions which reflected its phase-partition preference.

LIPID PEROXIDATION OF RAT LIVER MICROSOMES. J.F. Koster and R.G. Slee (Dept. of Biochem. I, Medical Faculty, Erasmus Univ. Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands) *Biochim. Biophys. Acta* 620, 489-99 (1980). The NADPH-dependent lipid peroxidation process was studied with microsomes and also the effects of addition of superoxide dismutase, catalase and thiourea. Only catalase and thiourea were able to inhibit lipid peroxidation. It seems that the initiating radical is the OH radical formed by the Fenton reaction. During lipid peroxidation glucose-6-phosphatase is inactivated, whilst the microsomal enzyme palmitoyl-CoA hydrolase is practically not affected. Because glucose-6-phosphatase activity decreases during aging and palmitoyl-CoA hydrolase does not, a possible relationship with the aging process is thought to exist. Chromolipids are formed by the NADPH-dependent lipid peroxidation. These chromolipids have the same excitation-emission spectra as described for lipofuscin. The formation of these chromolipids is blocked by the addition of catalase and thiourea. High-molecular weight proteins are formed during the NADPH-dependent lipid peroxidation. This process can be associated with the inactivation of enzymes. Also polymerisation is prevented by catalase and thiourea.

DYNAMIC SURFACE PROPERTIES OF PHOSPHATIDYLGLYCEROL-DIPALMITOYL PHOSPHATIDYLCHOLINE MIXED FILMS. R.H. Notter, S. Holemb and R.D. Mavis (Dept. of Pediatrics and Dept. of Biology and Biophysics, University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642) *Chem. and Physics of Lipids* 27, 305-19 (1980). This paper studies the dynamic surface pressure-area (μ -A) behavior of phosphatidylglycerol (PG) with a mixed fatty acid distribution (bacterial) in pure and binary mixed films with dipalmitoyl phosphatidylcholine (DPL). At 23°C, bacterial PG films generate maximum dynamic surface pressures of only 48-49 dyn/cm on a 0.15 M sodium chloride subphase for both dilute and surface excess initial conditions. By contrast, binary mixed films of 90:10 DPL/PG reach maximum μ values of the order of 70 dyn/cm at similar conditions, the same as for pure DPL films. Differential Scanning Calorimetry (DSC) measurements

on DPL/PG mixtures show decreasing T_m with peak broadening as the percentage of bacterial PG is increased. The experiments here do not establish a clearly required functional role for 10% PG in pulmonary surfactant surface behavior. Further surface studies are suggested before long-term clinical trials of PG containing mixtures for exogenous replacement therapy in Neonatal Respiratory Distress Syndrome (RDS) are initiated on a wide-spread basis.

SELECTIVE OXIDATION OF STEROIDAL ALLYLIC ALCOHOLS. E.J. Parish and G.J. Schroepfer, Jr. (Depts. of Biochem. and Chem., Rice Univ., Houston, TX 77001) *Chem. Phys. Lipids* 27, 281-8 (1980). Pyridinium chlorochromate in CH₂Cl₂ containing pyridine (2%) at 2-3°C has been found to effect the high yield selective oxidation of the hydroxyl function of a number of steroidal allylic alcohols. Under these conditions the oxidation of cholest-4-en-3 β -ol to the corresponding ketone was effected in 92% yield. Only the allylic hydroxyl function of 5 α -cholest-8(14)-ene-3 β ,15 α -diol, 5 α -cholest-8(14)-ene-3 β ,15 β -diol and 5 α -cholest-8(14)-ene-3 β ,7 β -diol was oxidized under these conditions to give the corresponding α,β -unsaturated ketones in high yields. 5 α -cholest-8(14)-ene-3 β ,7 α ,15 α -triol gave 5 α -cholest-8(14)-ene-3 β ,7 α -diol-15-one in 82% yield. Attempted oxidation of the 5 α -cholestan-3 β ,15 α -diol and 5 α -cholest-7-ene-3 β ,15 α -diol, both lacking an allylic hydroxyl function, under these conditions, were unsuccessful. Selective oxidation of the allylic alcohol function of 5 α -cholest-8(14)-ene-3 β ,15 β -diol using activated manganese dioxide gave 5 α -cholest-8(14)-en-3 β -ol-15-one in high yield while oxidation of the corresponding 15 α -hydroxy epimer using manganese dioxide was unsuccessful.

THE STABILITY OF LIPOSOMES IN VITRO TO pH, BILE SALTS AND PANCREATIC LIPASE. R.N. Rowland and J.F. Woodley (Biochem. Res. Lab., Dept. of Biological Sciences, Univ. of Keele, Keele, Staffordshire, ST5 5BG, U.K.) *Biochim. Biophys. Acta* 620, 400-9 (1980). If liposomes are to be effective as carriers for the oral administration of insulin they must be able to withstand passage through the stomach and small intestine. Multilamellar liposomes, some identical in composition to those used in reported in vivo studies on the uptake or orally administered insulin, were tested in vitro for their stability in the presence of bile salts, pancreatic lipase, and variations in pH. While low or high pH had little effect on most liposomes, 10 mM bile salts caused the release of over 80% of entrapped marker from all liposomes tested except those composed of distearoyl phosphatidylethanolamine/cholesterol and those composed of dipalmitoyl phosphatidylethanolamine/cholesterol/diethylphosphate. However, the latter were unstable at low pH. The distearoyl phosphatidylethanolamine/cholesterol liposomes were also resistant to pancreatic lipase, and therefore may be suitable for use in the oral administration of therapeutic agents.

PREPARATION AND SPECTROSCOPIC CHARACTERIZATION OF MOLECULAR SPECIES OF BRAIN PHOSPHATIDYL SERINES. N. Salem, Jr., P. Serpentino, J.S. Puskin, and L.G. Abood (Center for Brain Res., Dept. of Chem., and Dept. of Radiation Bio. and Biophys., Univ. of Rochester, Rochester, NY) *Chem. Phys. Lipids* 27, 289-304 (1980). This study describes the first preparation and spectroscopic characterization of naturally occurring phospholipids separated according to degree of unsaturation. Phosphatidylserines (PS) have been prepared from bovine brain and shown to be pure by extensive thin layer chromatographic analysis as well as infrared spectroscopy and fatty acid analysis. The PS has been separated according to degree of unsaturation and prepared using AgNO₃-impregnated silica gel H thin-layer chromatography. With the use of a 100 MHz Fourier transform nuclear magnetic resonance (NMR) spectrometer, the spectra of bovine whole brain, white matter, gray matter, monoenoic, and hexaenoic PS were obtained. The method provides a convenient, non-destructive spectroscopic method for distinguishing monoenoic and polyunsaturated species of intact phospholipids. Electron spin resonance studies of nitroxide-labelled cholestane in sonicated PS vesicles showed greater probe motion as the unsaturation of the acyl chains was increased. The hexaenoic PS vesicles were more fluid than monoenoic PS vesicles at all temperatures in the range 10-55°C. These results suggest that neuronal membranes are more fluid than myelin membranes as neuronal membranes contain more hexaenoic phospholipids.

DETERMINATION BY CAPILLARY GAS-LIQUID CHROMATOGRAPHY OF THE ABSOLUTE CONFIGURATIONS OF UNSATURATED FATTY ACID HYDROPEROXIDES FORMED BY LIPOXYGENASES. C.P.A. Van Os,

G.P.M. Rijke-Schilder, J.P. Kamerling, G.J. Gerwig and J.F.G. Vliegthart (Dept. of Bio-Organic Chem., Univ. of Utrecht, Croesestraat 79, 3522 AD Utrecht, The Netherlands) *Biochim. Biophys. Acta* 620, 326-31 (1980). The absolute configurations of a number of unsaturated hydroperoxy fatty acids obtained by lipoxygenase catalysis were investigated by capillary gas-liquid chromatography after proper derivatization. To this end the hydroperoxy groups were reduced and the resulting hydroxyl groups acetylated. Oxidative ozonolysis of the acetylated methyl esters yielded acetylated 2-hydroxy-carboxylic acids, which were converted into R-(-)-2-butyl esters and then reacylated. The ratio of the resulting diastereomers, which reflect the optical purity of the chiral centers in the parent hydroperoxy fatty acids, was determined by capillary gas-liquid chromatography. Application of this simple method to a number of mono- and di-hydroperoxy fatty acids obtained by incubation with soybean lipoxygenase-1 or-2, or by corn-germ lipoxygenase yields enantiometric compositions which are in good agreement with results obtained by other methods.

THEMAL OXIDATION OF A SERIES OF SATURATED TRIACYLGLYCEROLS. E.D. Crnjar, A. Witchwood and W.W. Nawar (Dept. of Food Sci. and Nutr., Univ. of Massachusetts, Amherst, MA 01003) *J. Agric. Food Chem.* 29, 39-42 (1981). The products from a series of triacylglycerols containing the even-numbered saturated fatty acid chains C_6 to C_{18} , after heating in air for 1 hr at 180 and 250° C, were studied qualitatively and quantitatively. The major decomposition products were alkanes, methyl ketones, alkanals, and γ - and δ -lactones. Among the hydrocarbons and particularly at the higher temperature, the C_{n-1} n-alkane was the most abundant. At the lower temperature, samples containing the longer fatty acid chains produced the C_{n-4} alkane in greater amounts than the decarboxylation product. In all cases the C_{n-1} methyl ketone was the major carbonyl compound, and the most abundant γ -lactone was the compound having a carbon number equal to that of the parent fatty acid. Only C_n δ -lactones were produced from each fatty acid. Mechanisms of thermal decomposition are discussed.

FREE RADICAL LABEL; NEW APPROACH TO THE STUDY OF SUPER-SLOW MOLECULAR DYNAMICS OF LIPID SYSTEMS. V.I. Gol'danski, A.I. Mikhailov, V.G. Omelyanenko, V.N. Smirnov, and V.P. Torchilin (Instit. of Chem. Physics, USSR Acad. of Sciences, Chernogolovka, the Moscow Region and Nat'l Cardiology Res. Center, USSR Academy of Med. Sciences, Moscow Center, USSR) *J. Lipid Res.* 22, 131-7 (1981). A new method of EPR-spectroscopy, the recombination of free radicals appearing as a result of indirect radiolysis of biological molecules after a low temperature irradiation, was applied to the study of molecular dynamics of dimyristoyl phosphatidylcholine in mass and in the structure of liposomes above and below the transition temperature. It was shown that the mobility of lipid molecules in crystalline liposomes was lower than in the structure of liquid-crystalline liposomes. The addition of cholesterol in liposome membranes decreased the lateral molecular motion of lipids in crystalline and liquid-crystalline states; in the latter case, the effect of cholesterol addition was more pronounced. The activation energy for the displacement of fragments of lipid molecules and the lipid molecule as a whole was estimated, and it was shown that the lipid matrix possesses a high degree of heterogeneity. The solubility of oxygen in the lipid bilayer and the mechanism of lipid diffusion are discussed.

IDENTIFICATION OF DEOXY- α -TOCOPHEROLQUINOL AS ANOTHER ENDOGENOUS ELECTRON DONOR FOR BIOHYDROGENATION. P.E. Hughes and S.B. Tove (Dept. of Biochem., North Carolina St. Univ., Raleigh, NC 27650) *J. Biol. Chem.* 255, 11802-6 (1980). Solvent extracts of *Butyrivibrio fibrisolvens* contain 2-[3,7,11,15-tetramethylhexadecyl]-3,5,6-trimethylbenzoquinol (deoxy- α -tocopherolquinol) that can serve as an alternate electron donor for α -tocopherolquinol for the biohydrogenation of *cis*-9,*trans*-11-octadecadienoate. In addition, the cell extracts contain deoxy- α -tocopherolquinone. This compound arises metabolically from α -tocopherolquinone via dehydration to trimethylphytylbenzoquinone followed by hydrogenation to deoxy- α -tocopherolquinone. Although the hydrogenation of the isoprene double bond and the conjugated fatty acid both use NADH as the primary reductant, the two reactions appear to be catalyzed by different enzymes.

VITAMIN E AND FATTY ACID COMPOSITION OF HUMAN MILK. L. Jansson, B. Akesson and L. Holmberg (Dept. of Pediatrics, Univ. of Lund, Malmo General Hosp., and the Dept. of Physiological Chemistry, Univ. of Lund, Sweden) *Am. J. Clin. Nutr.*

34, 8-13 (1981). The vitamin E and fatty acid composition of human milk was determined in 40 milk samples (six colostrum, 10 transitional, and 24 mature) obtained at different stages of lactation. Vitamin E was determined by high performance liquid chromatography with fluorescence detection of the various tocopherols. The total tocopherol level was significantly higher in early milk than in mature milk. The difference was due to a high content of α -tocopherol, as the content of β - and γ -tocopherol was similar in the three milk types. The total tocopherol content in mature milk correlated significantly with both the total lipid and the linoleic acid content. Significantly higher tocopherol/linoleic acid ratios were found in both colostrum and transitional milk than in mature milk. The colostrum milk differed from the other milk types in fatty acid composition, as it had a lower content of lauric acid and a higher content of arachidonic acid and docosahexaenoic acid. The linoleic acid levels reported here are considerably higher than those reported previously in Sweden. Still, the ratio of α -tocopherol equivalent/linoleic acid exceeded 0.5 mg/g in all but three milk samples.

PROTEIN-CATALYZED PHOSPHOLIPID EXCHANGE BETWEEN GEL AND LIQUID-CRYSTALLINE PHOSPHOLIPID VESICLES. A.M. Kasper and G.M. Helmkamp (Dept. of Biochem., Univ. of Kansas Med. Center, Kansas City, KS 66103) *Biochemistry* 20, 146-51 (1981). Bovine liver phospholipid exchange protein catalyzes the transfer of phosphatidylcholine between two populations of single bilayer phospholipid vesicles. Donor vesicles are prepared from egg phosphatidylcholine—phosphatidic acid—lactosylceramide (90:2:8 mol %); acceptor vesicles are prepared from phosphatidylcholine—phosphatidic acid (98:2 mol %). When egg phosphatidylcholine acceptor vesicles over the temperature range 11-45° C are used, a linear Arrhenius plot is obtained. When dimyristoylphosphatidylcholine acceptor vesicles under the same conditions are used, however, a biphasic plot is seen with decreasing transfer activity at lower temperatures. The incorporation of cholesterol into dimyristoylphosphatidylcholine vesicles at a concentration sufficient to abolish the thermotropic phase transition yields a monophasic Arrhenius plot of transfer activity. The results indicate that bovine liver phospholipid exchange protein interacts catalytically with phospholipid bilayer vesicles composed of saturated or unsaturated phosphatidylcholines but preferentially with liquid-crystalline membranes.

RAMAN SPECTROSCOPIC STUDY OF THE INTERACTIONS OF DIMYRISTOYL- AND 1-PALMITOYL-2-OLEOYLPHOSPHATIDYLCHOLINE LIPOSOMES WITH MYELIN PROTEOLIPID APOPROTEIN. F. Lavielle and I.W. Levin (Lab. of Chemical Physics, National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20205) *Biochem.* 19, 6044-50 (1980). Recombinants of dimyristoylphosphatidylcholine (DMPC) and 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) with myeline proteolipid apoprotein prepared in an aqueous medium were investigated by vibrational Raman spectroscopy. On completion of the phase transition at 15° C, the intramolecular chain disorder is substantially greater compared to that of the pure bilayer form. In addition, no further development of gauche conformers along the chain is apparent as the temperature increases. For the temperature profile derived from the C-H stretching region parameters of DMPC, the phase transition temperature is shifted from 23 to 11° C. The intermolecular disorder in both the gel and liquid-crystalline states is significantly greater in the recombinant systems in comparison to that in the pure liposomes. Temperature profiles obtained for recombinants prepared with unsaturated phospholipid bilayers (POPC) indicate that the apoprotein only slightly perturbs the inter- and intra-molecular parameters describing the liquid matrix. The increased intermolecular disorder exhibited by the pure and reconstituted DMPC systems is discussed in terms of the diffusion properties exhibited by saturated and unsaturated lipid matrices. Within the precision of the Raman experiment, no evidence on the vibrational time scale exists for a boundary lipid.

INFLUENCE OF LECITHIN ON THE CHROMATOGRAPHY OF STEROIDAL GLUCOSIDURONATES IN CHLOROFORM/FORMAMIDE. V.R. Mattox and R.D. Litwiller (Mayo Fnd. and Mayo Graduate Schl., Rochester, MN 55901) *Lipids* 15, 999-1008 (1980). The interaction between lecithin and steroidal glucosiduronates was investigated by use of partition chromatography in chloroform/formamide and infrared spectroscopy. It was observed that lecithin increases the solubility of both glucosiduronic acids and esters in chloroform and concluded that this phenomenon occurs because of the formation of hydrogen bonds between

the phosphodiester group of lecithin and hydroxyl groups of the steroid conjugates.

MEMBRANE FATTY ACID MODIFICATION OF THE NEUROBLASTOMA X GLIOMA HYBRID, NG108-15. R. McGee, Jr. (Dept. of Pharmacology, Georgetown Univ., 3900 Reservoir Rd., Washington, DC 20007) *Biochim. Biophys. Acta* **663**, 314-28 (1981). As a first step in studying the effects of membrane lipid modification on complex cellular functions we have modified the membrane fatty acid composition of the neuroblastoma X glioma hybrid clone, NG108-15. These cultured cells were chosen because they exhibit many complex neuronal functions in vitro. Unsaturated fatty acids were accumulated, metabolized and esterified by the cells. These unsaturated fatty acids stimulated cell growth, whereas saturated fatty acids were toxic to the cells. Changes as large as 40-fold in the ratio of monounsaturated/polyunsaturated fatty acids in the membrane phospholipids were produced by addition of fatty acids directly to serum-containing culture medium. As a result of the exposure of NG108-15 cells to unsaturated fatty acids the amount of phosphatidylethanolamine in the cells was increased by as much as 60%. Polyunsaturated fatty acids also caused a small decrease in the membrane cholesterol/phospholipid molar ratio. These experiments demonstrate that large changes in membrane fatty acid composition can be created in clonal cells capable of differentiated neuronal activities. Additional changes in membrane lipid composition also appear to be induced by these manipulations. The question of the importance of specific membrane lipid composition to neuronal cellular function now can be addressed.

ANALYSIS OF VOLATILE COMPOUNDS IN WHEAT GERM OIL RESPONSIBLE FOR AN AGGREGATION RESPONSE IN TROGODERMA GLABRUM LARVAE. J.M. Nara, R.C. Lindsay and W.E. Burkholder (Dept. of Food Sci. (R.C.L.), Stored-Product and Household Insects Lab, U.S.D.A., Madison, WI 53706) *J. Agric. Food Chem.* **29**, 68-72 (1981). The volatile components of wheat germ oil were demonstrated by bioassay to be responsible for initiating aggregating activity of *Trogoderma glabrum* larvae. A strong aggregation response was induced by the neutral plus basic compound fraction of wheat germ oil but only a small one by the acidic fraction. Fractions collected from Carbowax 20M gas chromatography revealed highly active aggregation-inducing substances in the I_E regions of 7.8-8.1 and 14.1-14.6, but other fractions also showed activity. Principal compounds in the earlier I_E region were C₁₃-C₁₆ saturated and unsaturated hydrocarbons, and a branched hexylbenzene. Major compounds in the latter region were octanoic acid, γ -nonalactone, an ethyl-naphthalene, a methylethyl-naphthalene, and a cyclic branched ketone. Synthetic octanoic acid was an active aggregating stimulant for *Trogoderma glabrum* larvae at some dilutions in mineral oil. Synthetic *cis*-3-hexenal, octanal, and γ -octalactone also induced aggregation when tested alone or in combinations. However, none of the synthetic or natural fractions yielded responses equivalent to that of intact wheat germ oil.

ANTIOXIDANT CONTROL OF RANCIDITY DEVELOPMENT IN GROUND TURKEY MEAT. V.M. Olson and W.J. Stadelman (Dept. of Animal Sciences, Purdue Univ., West Lafayette, IN 47907) *Poultry Sci.* **59**, 2733-7 (1980). Refrigerated raw ground turkey meat using natural proportions (2:1 breast:thigh) undergoes oxidative rancidity rapidly. Various antioxidants were used in different combinations to delay rancidity development and increase shelflife. The antioxidants used were butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and silicone. The percentages of each antioxidant added to the ground meat were based on the fat content. A .01% level was used for BHA and BHT and 1 ppm was used for silicone. Standard plate counts were also studied to determine when the stored meat became unacceptable due to microbiological growth. The ground meat was stored at 4 C up to 14 days. The 2-thiobarbituric acid (TBA) values indicated that the combinations of BHA + BHT and BHA + BHT + silicone retarded development of oxidative rancidity to a greater extent than addition of silicone alone when compared to the control. Microbiological spoilage was assumed to take place after approximately eight days of refrigerated storage when counts reached 10⁷ numbers. However, the sensory panel members rated the ground turkey meat containing BHA + BHT and BHA + BHT + silicone acceptable through 10 days of storage.

AN IMPROVED PROCEDURE FOR THE SYNTHESIS OF ¹⁴C-LABELED PHOSPHATIDYLSERINE FROM CEREBRAL PHOSPHATIDIC ACID. P. Orlando, G. Ippolito, L. Binaglia, C. Giordano, and G. Porcellati

(Dept. of Pharmacology, Università Cattolica del Sacro Cuore, Rome, Italy) *J. Lipid Res.* **21**, 1053-7 (1980). A complete procedure to prepare a highly labeled phosphatidyl-L-[U-¹⁴C]serine possessing the same fatty acid composition of brain phospholipids is reported. CDP-diglyceride was synthesized by reaction between phosphatidic acid and CMP-morpholidate as the dicyclohexylcarboxamidium salt. The reaction between CDP-diglyceride and L-[U-¹⁴C]serine to produce the labeled phosphatidylserine was catalyzed by the CDP-diglyceride: L-serine phosphatidyl transferase (EC 2.7.8.8) from *E.coli*. A selective inhibition of phosphatidylserine decarboxylase activity, present as contaminant in the enzyme extract, was introduced in order to avoid a low yield of product. Traces of phosphatidylethanolamine (about 1%) were easily removed by preparative thin-layer chromatography. The yield of the labeled product was as high as 87% and its specific radioactivity was 170 mCi/mmol.

THE ENZYMES OF PHOSPHOLIPID SYNTHESIS IN CLOSTRIDIUM BUTYRICUM. P. Silber, R.P. Borie, and H. Goldfine (Dept. of Microbiology, School of Medicine, Univ. of Pennsylvania, Philadelphia, PA 19104) *J. of Lipid Res.* **21**, 1022-31 (1980). We have examined extracts of *Clostridium butyricum* for several enzymes of phospholipid synthesis. Membrane particles were shown to catalyze the formation of CDP-diglyceride from [³H]CTP and phosphatidic acid. The reaction was dependent on Mg²⁺ and stimulated by monovalent cations. CDP-diglyceride formed in vitro was found to be a substrate for both phosphatidylglycerophosphate synthetase and phosphatidylserine synthetase. Phosphatidylserine decarboxylase activity was barely detectable in membrane particles from *C. butyricum*. The addition of *E. coli* membrane particles provided efficient phosphatidylserine decarboxylase activity in this system. Although plasmalogens are the principal lipids of *C. butyricum*, none of the products of phospholipid synthesis formed in vitro contained measurable amounts of plasmalogens. The subcellular distribution of both phosphatidylglycerophosphate synthetase and phosphatidylserine synthetase in *C. butyricum* was also studied. Both were found to be membrane-associated.

DETECTION OF HYDROXY FATTY ACIDS IN BIOLOGICAL SAMPLES USING CAPILLARY GAS CHROMATOGRAPHY IN COMBINATION WITH POSITIVE AND NEGATIVE CHEMICAL IONIZATION MASS SPECTROMETRY. H.-J. Stan and M. Scheutwinkel-Reich (Institut für Lebensmittelchemie, Technische Universität, Müller-Breslau-Strasse 10, 1000 Berlin 12, Germany) *Lipids* **15**, 1044-50 (1980). The most common method for use in the structural analysis of hydroxy fatty acids in biological samples is the gas chromatography-mass spectrometry (GC-MS) analysis of trimethylsilyl ethers of the methyl esters using electron impact ionization. A comparison of electron impact (EI) with chemical ionization mass spectrometry (CI-MS) shows that CI-MS is the superior technique. Heptafluorobutyrate exhibit useful mass fragmentation patterns in the positive as well as in the negative CI mode. With methane as the reactant gas, M+H usually is base peak in positive mass spectra. The ionic series M+H-n \times 214 leads to the number of hydroxy groups in the molecule. In the negative mass spectra, M and M-20 are indicative for the molecular weight. The ion group m/z 213, 194 and 178 at high levels of intensity is typical for heptafluorobutyrate. The advantage of the application of heptafluorobutyrate is the high sensitivity which can be obtained in trace analysis using negative MS. Heptafluorobutyrate of hydroxy fatty acids gave a 20-fold higher response in the negative scan mode compared to that of the positive. The detection limit for heptafluorobutyrate in negative CI-MS was on the order of 1 fg (10⁻¹⁵ g).

AN ABNORMAL TRIGLYCERIDE-RICH LIPOPROTEIN CARRYING EXCESS APOLIPOPROTEIN C-II. J. Stocks, G. Holdsworth, P. Dodson and D.J. Galton (Diabetes and Lipid Res. Lab., St. Bartholomew's Hosp., London EC1A 7BE, U.K.) *Atherosclerosis* **38**, 1-9 (1981). The polypeptide composition of a variant lipoprotein (d < 1.006) carrying a relative excess of apolipoprotein C-II has been characterized by polyacrylamide gel electrophoresis and isoelectric focussing. The apo-C peptides of the variant lipoprotein contained 45.2 \pm 1.3 (n=9) % of apo C-II compared with 21.5 \pm 5.4 (n=30) % for hypertriglyceridaemic controls. The variant lipoprotein activated purified bovine milk lipoprotein lipase normally, but was an inefficient substrate for this enzyme as assessed by direct release of fatty acids from the lipoprotein or by a substrate competition assay. Electron microscopy revealed the variant lipoprotein as non-spherical flattened particles compared with the more spherical appearance of control triglyceride-rich lipoproteins. We sug-

gest that the relative proportion of apo C peptides associated with the lipoprotein particle may be critical for optimal enzyme-substrate interaction.

EFFECT OF LIGAND BINDING ON THE CONFORMATION OF HUMAN PLASMA VITAMIN D BINDING PROTEIN (GROUP-SPECIFIC COMPONENT). R. Surarit and J. Svasti (Dept. of Biochem., Faculty of Sci., Mahidol Univ., Rama VI Road, Bangkok 4, Thailand) *Biochem. J.* 191, 401-10 (1980). Spectrofluorimetric studies showed that 25-hydroxycholecalciferol causes a saturable enhancement of intrinsic fluorescence of human vitamin D binding protein and alters the pH profile of protein fluorescence, suggesting that there are alterations in the local environment of tryptophan residue(s) after ligand binding. Furthermore, in the presence of 25-hydroxycholecalciferol, the rate of chemical modification of the amino groups in human vitamin D binding protein is decreased and the susceptibility of intact vitamin D binding protein to proteolytic degradation is reduced, suggesting that some surface sites in the vitamin D binding protein molecule are less accessible to external agents. In addition, although the absorbance of vitamin made it difficult to interpret the ultraviolet spectra of holoprotein and apoprotein, the presence of vitamin D binding protein appears to stabilize the vitamin in an aqueous environment, a phenomenon that may be of physiological importance.

DILATOMETRY AND CALORIMETRY OF SATURATED PHOSPHATIDYLETHANOLAMINE DISPERSIONS. D.A. Wilkinson and J.F. Nagle (Depts. of Physics and Biological Sciences, Carnegie-Mellon Univ., Pittsburgh, PA 15213) *Biochemistry* 20, 187-92 (1981). The specific volumes of a series of saturated phosphatidylethanolamine dispersions with 12, 14, and 16 carbon atoms per chain have been measured in the region of the chain melting transition, T_m . The change in specific volume at T_m for the 12 and 14 carbon compounds are 0.0160 and 0.0204 mL/g, respectively. Comparisons are drawn between this class of lipids and phosphatidylcholines. In both cases, T_m extrapolates with increasing chain length to the melting point of polyethylene. Both types of lipids appear to be packed in a similar way below T_m . One major difference is that dilauryl-

phosphatidylethanolamine undergoes a second transition above T_m .

STRUCTURAL ROLE OF PHOSPHOLIPIDS IN UBIQUINOL-CYTOCHROME C REDUCTASE. C. Yu and L. Yu (Dept. of Chemistry, State University of New York at Albany, Albany, New York 12222) *Biochem.* 19, 5715-20 (1980). The role of phospholipids in ubiquinol-cytochrome c reductase has been studied by the following methods: (1) removal and restoration of phospholipids, (2) circular dichroism measurements, and (3) phospholipase A_2 treatment. Over 90% of the phospholipids in the cytochrome b-c₁ III complex (a highly purified ubiquinol-cytochrome c reductase) can be removed by repeated precipitation with ammonium sulfate in the presence of 0.5% sodium cholate. The delipidated enzyme complex is inactive. Full restoration of enzymatic activity can only be achieved with a freshly prepared delipidated enzyme complex, made in the presence of 20% glycerol. Removal of phospholipids from the cytochrome b-c₁ III complex resulted in an immediate decrease of ~15% in molar ellipticities in both the far-UV and the Soret regions. The absolute requirement for phospholipids in the cytochrome b-c₁ III complex was demonstrated by treatment of the enzyme with purified phospholipase A_2 . The inactivation of the cytochrome b-c₁ III complex by phospholipase A_2 was not prevented by the presence of excess exogenous ubiquinone but was prevented by the presence of ethylenediaminetetraacetic acid (EDTA). The enzymatic activity of the phospholipase A_2 treated complex is fully restorable upon the addition of EDTA and phospholipids.

STEROL ANALYSIS OF THE INNER AND OUTER MITOCHONDRIAL MEMBRANES IN YEAST. C.K. Bottema and L.W. Parks (Dept. of Microbiol., Oregon St. Univ., Corvallis, OR 97331) *Lipids* 15, 987-92 (1980). The membranes of yeast mitochondria were separated and analyzed for lipid content. The sterol-to-phospholipid molar ratio was found to be very similar between the inner and outer membranes (1:30). These observed ratios could be substantially altered by using a crude mitochondrial pellet contaminated with a "floating lipid layer." In this case, the sterol-to-phospholipid molar ratios were 1:8 to 1:26 for the outer and inner mitochondrial membranes, respectively.

Biochemistry and nutrition

A QUANTITATIVE EVALUATION OF THE CONVERSION OF 25-HYDROXYCHOLESTEROL TO BILE ACIDS IN MAN. L. Swell, C.C. Schwartz, J. Gustafsson, H. Danielsson and Z.R. Vlahcevic (Div. of Gastroenterology, Depts. of Med. and Surgery, Veterans Admin. Med. Center and Medical College of Virginia, Richmond, VA 23249) *Biochim. Biophys. Acta* 663, 163-8 (1981). The present study was directed toward providing additional information in man on the nature of a potential alternative pathway to cholic acid not involving an initial 7 α -hydroxylation of cholesterol. Two bile fistula patients and one normal subject each received 25-hydroxy[G-³H]cholesterol; [¹⁴C]cholic and [¹⁴C]chenodeoxycholic acids were also simultaneously administered to one bile fistula patient and normal subject. The labeled 25-hydroxycholesterol was found to be poorly converted to primary bile acids by all three patients; the range of conversion was 9.7 to 18.9%. Cholic acid was favored over chenodeoxycholic acid by a margin of about 1.4/1. It is concluded that a pathway to primary bile acid via the 25-hydroxylation of cholesterol is of minor importance under conditions of normal or accelerated synthesis in man.

BIOLOGICAL LABELING OF VERY LOW DENSITY LIPOPROTEINS WITH CHOLESTERYL LINOLEYL ETHER AND ITS FATE IN THE INTACT RAT. O. Stein, G. Halperin and Y. Stein (Dept. of Experimental Medicine and Cancer Res., Hebrew Univ.—Hadassah Med. Schl., Jerusalem, Israel) *Biochim. Biophys. Acta* 620, 247-60 (1980). In vitro labeling of very low density lipoproteins (VLDL) with radioactive cholesteryl linoleyl ether, an analog of cholesteryl linoleate, was studied. The protocol which gave the highest efficiency and seemed least injurious to the final product included: sonication of the labeled cholesteryl ether with partially delipidated high density lipoproteins (HDL); transfer of the labeled lipids to VLDL in the presence of lipoprotein-deficient human serum; reisolation of the VLDL by ultracentrifugation. Under optimal conditions 70% of the added labeled lipid was recovered with HDL and 60% were trans-

ferred from HDL to VLDL. The labeled cholesteryl linoleyl ether was shown to comigrate with the protein of VLDL on agarose gel electrophoresis. The main advantage of the presently described approach in which a nondegradable analog of cholesteryl ester was introduced into VLDL by a biological procedure is the possibility to study the role of various organs to take up circulating cholesteryl ester, especially in species in which LDL is produced from VLDL.

MORPHOLOGICAL EVIDENCE OF ENDOGENOUS LIPID PRODUCTION IN SWINE DUCTUS VASCULATURE. T. Toda, D.E. Leszczynski and F.A. Kummerow (Harlan E. Moore Heart Res. Foundation, Champaign, IL 61820) *Atherosclerosis* 37, 325-30 (1980). Specimens of ductus arteriosus and venosus from 2-month-old and 6-month-old swine were examined by electron microscope. The purpose of the study was to observe the effects of vessel closure and hypoxia on medial vascular smooth muscle cells. At 6 months of age, smooth muscle cells from the medial layer of ductus arteriosus showed signs of considerable cellular degeneration and contained lipid vacuoles which were often surrounded by granular endoplasmic reticulum. Specimens from the portal side of ductus venosus showed initial stages of smooth muscle cell degeneration and lipid vacuolization, while samples from the hepatic side of ductus venosus were nearly normal. This study contains the first reported morphological evidence of organelles in vascular smooth muscle cells which are responsible for endogenous lipid droplet production.

EPIDEMIOLOGY OF PLASMA HIGH-DENSITY LIPOPROTEIN CHOLESTEROL LEVELS. THE LIPID RESEARCH CLINICS PROGRAM PREVALENCE STUDY. INTRODUCTION. H.A. Tyroler (Dept. of Epidemiology, Rosenau Hall, Room 201-H, Schl. of Public Health, Univ. of North Carolina, Chapel Hill, NC 27514) *Circulation* 62, IV-1-3 (1980). A summary chapter reviews the empirical findings reported in the monograph and examines the impact of the attributes studied on HDL cholesterol distributions in

populations. The summary also provides a systematic overview of the literature on the known determinants and consequences of HDL cholesterol levels.

PHOSPHATIDYLCHOLINE BIOSYNTHESIS IN ISOLATED HAMSTER HEART. T.A. Zelinski, J.D. Savard, R.Y.K. Man and P.C. Choy (Dept. of Biochem. and Dept. of Phys., Univ. of Manitoba, Winnipeg, Manitoba, R3E 0W3, Canada) *J. Biol. Chem.* **255**, 11423-8 (1980). The pathways for the new formation of phosphatidylcholine in hamster hearts were investigated. The rate-limiting step of the CDP-choline pathway was determined in the isolated heart by pulse-chase studies. Based on the specific radioactivities of choline, phosphocholine, and CDP-choline in the pulse-chase studies, we conclude that the rate-limiting step for phosphatidylcholine biosynthesis in the hamster heart is catalyzed by phosphocholine cytidyltransferase.

THE EFFECT OF VITAMIN A FORTIFICATION OF SUGAR ON THE SERUM VITAMIN A LEVELS OF PRESCHOOL GUATEMALAN CHILDREN: A LONGITUDINAL EVALUATION. G. Arroyave, L.A. Mejia, and J.R. Aguilar (Div. of Biol. and Human Nutr., Inst. of Nutr. of Central America and Panama, INCAP, Guatemala, Central America) *Am. J. Clin. Nutr.* **34**, 41-9 (1981). Based on the Guatemalan program of vitamin A fortification of sugar, a longitudinal evaluation on serum retinol levels of preschool-aged children was performed. Five consecutive surveys executed every 6 months were examined, considering only children who were surveyed more than once. Thus, the changes in their serum retinol after the intervention were evaluated. Natural dietary vitamin A remained unchanged throughout. Addition of retinyl palmitate to sugar increased significantly the intake ($p < 0.001$). After 1 yr of fortification 76% of the children experienced an elevation of retinol. All those with initial values $< 20 \mu\text{g}/\text{dl}$ showed an increase. Mean values increased significantly, particularly for children below $20 \mu\text{g}/\text{dl}$ whose levels changed from 16.2 ± 2.9 to 30.2 ± 9.7 ($P < 0.00001$). Those between 20 to $29 \mu\text{g}/\text{dl}$ increased from 24.9 ± 3.2 to 30.1 ± 8.1 ($p < 0.0003$). Similar results were obtained after 2 yr. The results indicated the effectiveness of the program in raising serum retinol levels.

THE PRESENCE OF LYSOPHOSPHATIDYLCHOLINE IN CHROMAFFIN GRANULES. G. Arthur and A. Sheltawy (Dept. of Biochem., Univ. of Leeds, 9 Hyde Terrace, Leeds LS2 9LS, U.K.) *Biochem. J.* **191**, 523-32 (1980). Lysophosphatidylcholine is thought to be a characteristic component of the chromaffin granules in adrenal glands. By the use of a t.l.c. system that resolved minor phospholipids satisfactorily, this subcellular location was demonstrated in homogenates of the adrenal medulla and cortex under conditions similar to those of subcellular fractionation (incubation at 4°C for 90 min). In a control experiment, the glands from rabbit were dissected and treated in the same manner as with those of ox, and then the lipids were extracted. No lysophosphatidylcholine was detected in the extracts from glands frozen in liquid N_2 but lysophosphatidylcholine was observed in the controls. These results suggest that lysophosphatidylcholine is not a component of chromaffin granules, but is produced if the period between death of the animal and lipid extraction is unduly prolonged. To discover whether lysophosphatidylcholine affected the permeability barrier properties of chromaffin granules, sonicated liposomes of egg phosphatidylcholine alone or with lysophosphatidylcholine ($15 \text{ mol}/100 \text{ mol}$) were prepared. These results indicate that the presence of this proportion of lysophosphatidylcholine in chromaffin-granule membranes is not likely to influence their barrier properties towards catecholamines.

EFFECT OF DIETARY LIPIDS ON COMPOSITION AND GLUCOSE UTILIZATION BY RAT ADIPOSE TISSUE. A.B. Awad (Dept. of Biochem., Kirksville College of Osteopathic Med., Kirksville, MO 63501) *J. Nutr.* **111**, 34-9 (1981). Feeding rats diets rich in either safflower oil or coconut oil resulted in a significant change in the lipid composition of epididymal fat pads as compared with those obtained from rats fed a commercial stock diet. A safflower oil diet resulted in an increase in tissue cholesterol and a decrease in phospholipid concentration as compared with the stock diet. A coconut oil diet resulted in a decrease in both tissue cholesterol and phospholipid concentrations as compared with the stock diet. Adipose tissue fatty acid composition was also altered due to these dietary manipulations. Glucose utilization by adipose tissue from animals fed the safflower oil diet was 2 and 10 times greater than glucose utilization by adipose tissue from animals fed the stock and coconut oil diets, respectively. The coconut oil diet resulted in an increase in the percentage of glucose incorporated into

triglycerides as compared with animals fed the stock or safflower oil diet. The incorporation of glucose into adipose tissue fatty acids was depressed by a saturated fatty acid diet as compared with either a polyunsaturated fatty acid diet or the stock diet.

PECTIN: ITS INTERACTION WITH SERUM LIPOPROTEINS. M.M. Baig and J.J. Cerda (Div. of Gastroent. and Nutr., Dept. of Med., Univ. of Florida College of Med., Gainesville, FL 32610) *Am. J. Clin. Nutr.* **34**, 50-3 (1981). In vitro studies of interaction between grapefruit (*Citrus paradisi*) pectin and various human serum lipoproteins indicated that pectin interacts specifically with low-density lipoprotein. Examination of observed interaction between the pectin and low-density lipoprotein under variable experimental conditions revealed the electrostatic nature of this interaction. The results obtained from these studies suggest a possible biochemical basis by which dietary pectin may cause lowering of serum and/or tissue cholesterol levels.

HYDROXYLATION OF CHOLIC, CHENODEOXYCHOLIC, AND DEOXYCHOLIC ACIDS IN PATIENTS WITH INTRAHEPATIC CHOLESTASIS. A. Bremmelgaard and J. Sjøvall (Medical Department A, Division of Hepatology, Rigshospitalet, Copenhagen, Denmark) *J. Lipid Res.* **21**, 1072-81 (1980). The metabolism of ^{14}C -labeled chenodeoxycholic, cholic, and deoxycholic acids was studied in patients with intrahepatic cholestasis. Radioactively labeled metabolites were isolated from urine and were identified by gas-liquid chromatography-mass spectrometry. About 5% of the radioactivity was recovered in urine after administration of labeled chenodeoxycholic acid to a patient with mild intrahepatic cholestasis. In urine collected 0-24 hr after the injection, 20% of the radioactivity appeared in the combined glycine and taurine conjugate fractions, and the predominant metabolite in these fractions was identified as hyocholic acid. Eighty percent of the activity was eluted in the sulfate fraction presumably representing mainly sulfated chenodeoxycholic acid conjugates. Twenty percent of the radioactivity was recovered in urine following administration of labeled cholic acid to a patient with biliary cirrhosis and severe cholestasis. In urine collected on the fifth day, half of this radioactivity appeared in the glycine and taurine conjugate fractions, and 10% of this activity was present as tetrahydroxycholanoates. About 5% of the radioactivity appeared in urine after oral administration of labeled deoxycholic acid to a patient with mild intrahepatic cholestasis. Twenty-two percent of the activity appeared in the glycine and taurine conjugate fractions isolated from urine collected on the second day after the administration.

DEHYDROXYLATION OF 16α -HYDROXYPROGESTERONE BY FECAL FLORA OF MAN AND RAT. V.D. Bokkenheuser, J. Winter, P.B. Hylemon, N.K.N. Ayengar, and E.H. Mosbach (Dept. of Path., St. Luke's-Roosevelt Hospital Center, New York, NY 10025; Dept. of Micro., Med. College of Virginia V.C.U., Richmond, VA 23298; and Lipid Res. Lab., Dept. of Surgery, Beth Israel Med. Center, New York, NY 10003) *J. Lipid Res.* **22**, 95-102 (1981). 16α -Hydroxyprogesterone, precursor of biliary 16α -hydroxypregnanolone, was incubated with mixed fecal flora of humans and rats. The major steroid metabolite formed in both systems was 3α -hydroxy- 17α -pregnan-20-one. These results demonstrated that the fecal flora reduced the $\Delta^4,3$ keto structure, removed the hydroxy group at C-16 and isomerized the side chain from the β to the α configuration. Ring-A reduction of the substrate resulted in a 5β -compound with human flora and a 5α -product with rat bacteria. The prevalence of 16α -dehydroxylating organisms varied considerably in human fecal flora and was approximately $10^5/\text{g}$ of feces in the three rats tested. Rat fecal flora dehydroxylated 16α -hydroxyprogesterone after 4-5 days incubation at 37°C , at pH 6.5-7.5, and with a substrate concentration of 20-80 $\mu\text{g}/\text{ml}$ (optimal condition). Preliminary evidence suggests that 16α -dehydroxylase is exclusively of bacterial origin and is synthesized by an obligate anaerobe.

STUDIES OF ESTERIFIED CHOLESTEROL IN SUB-FRACTIONS OF PLASMA HIGH DENSITY LIPOPROTEINS. P.J. Barter, Y.C. Ha and G.D. Calvert (Unit of Clin. Biochem., Schl. of Med., Flinders Univ. of South Australia, Bedford Park 5042, South Australia, Australia) *Atherosclerosis* **38**, 165-75 (1981). With human lipoproteins and lipoprotein-free plasma incubated at 37°C as a source of esterified cholesterol transfer activity, there was a molecular exchange of esterified cholesterol between the high density lipoprotein (HDL) subfractions, HDL₂ and HDL₃. A transfer of esterified cholesterol from both

HDL₂ and HDL₃ to very low density lipoproteins (VLDL) was also observed. When human plasma was incubated with [³H]cholesterol at 37° C, the newly formed esterified [³H]cholesterol became distributed among all plasma lipoprotein fractions. The addition of rabbit lipoprotein-free plasma as a source of exogenous esterified cholesterol transfer activity to incubations of pig lipoproteins resulted in a distribution of the newly formed esterified [³H]cholesterol which was very similar to that in incubated human plasma. It has been concluded that the formation of plasma esterified cholesterol occurs in the HDL₃ subfraction. In man, who possesses adequate levels of esterified cholesterol transfer activity, the esterified cholesterol so formed becomes distributed among all plasma lipoprotein fractions. In pigs, however, which lack transfer activity, the esterified cholesterol formed in HDL₃ is only minimally transferred to other fractions and remains predominantly in HDL, resulting in an increase in the HDL particle size with a consequent conversion of HDL₃ to the larger HDL₂.

EFFECT OF HDL ON THE INTERACTION OF HYPERLIPEMIC LDL WITH MONKEY SMOOTH MUSCLE CELLS. S.R. Bates (Specialized Center of Res. in Atherosclerosis, Dept. of Path., Box 414, The Univ. of Chicago, 950 E. 59th St., Chicago, IL 60637) *Artery* 7, 303-15 (1980). The stimulation of the esterification of cholesterol and the subsequent enrichment of cholesterol esters in arterial smooth muscle cells produced by low density lipoproteins (LDL) from hyperlipemic monkeys was altered by the presence of high density lipoproteins (HDL) isolated from normolipemic monkeys. Increasing concentrations of HDL decreased the rate of cellular cholesterol esterification and cholesterol ester accumulation. This was accompanied by a reduction in the cellular uptake of radioiodinated LDL. At physiological levels of lipoproteins, HDL interfered with the interaction of smooth muscle cells and LDL from hyperlipemic serum.

FEEDBACK REGULATION OF CHOLESTEROL BIOSYNTHESIS IN RHEBUS MONKEYS WITH VARIABLE HYPERCHOLESTEROLEMIC RESPONSE TO DIETARY CHOLESTEROL. A.K. Bhattacharyya and D.A. Eggen (Depts. of Pathology, Physiology, and Biometry, Louisiana State University Medical Center, New Orleans, LA 70112) *J. Lipid Res.* 22, 16-23 (1981). To test the hypothesis that high-responding rhesus monkeys should have a greater degree of feedback inhibition of hepatic cholesterol biosynthesis than the low-responding monkeys because the former group absorbs a higher percentage of cholesterol than the latter group, we determined the relative rates of cholesterol biosynthesis by measuring plasma desmosterol levels while feeding triparanol along with diets high and low in cholesterol and with or without 2% plant sterols. The build-up of plasma desmosterol was more rapid in low-responders than in high-responders on all diets. The mean percent cholesterol absorption in high responders was significantly higher than in low-responders on high and low cholesterol diets with low levels of plant sterols. On adding 2% plant sterols to both diets, the percent cholesterol absorption decreased significantly and became essentially the same in both groups. Triparanol feeding decreased plasma cholesterol significantly in both groups on both diets. The study demonstrates that high-responders have a greater degree of feedback inhibition of cholesterol biosynthesis than low-responders probably because of higher absorption of cholesterol. The results also indicate that both endogenous and exogenous cholesterol are effective mediators of the feedback inhibition mechanism.

THE ASSOCIATION BETWEEN SARTORIAL FAT AND FAT DEPOSITION IN MEAT-TYPE CHICKENS. J.A. Burgener, J.A. Cherry and P.B. Siegel (Poultry Sci. Dept., Virginia Polytech. Inst. and St. Univ., Blacksburg, VA 24061) *Poultry Sci.* 60, 54-62 (1981). The potential of using the weight of the sartorial (*M. sartorius*) fat depot as a measurement of abdominal and/or total body fat was investigated. A preliminary experiment using populations of chickens exhibiting wide differences in growth rate revealed correlations, at 63 days of age, of .40 between abdominal and percent carcass fat and .71 between abdominal and sartorial fat. The relationships among these traits were further explored using two commercial broiler stocks chosen on the basis of known differences in adiposity. In addition, dietary effects were investigated by feeding diets containing different calorie to protein ratios. Sartorial fat was highly correlated with abdominal and total carcass fat. Genetic and nutritional effects on the weight of abdominal fat were consistent with corresponding weight changes in sartorial fat. Thus, a final experiment was conducted to study the longitu-

dinal development of these adipose depots in commercial broilers with the objective of determining the association between abdominal fat deposition and sartorial fat weights over time. Although sartorial fat was not highly associated with abdominal fat at 14 days, correlations were highly significant at 28 and 42 days of age and significant at 56 days of age. Differences among genetic populations in fat deposition were particularly evident at 28 and 42 days of age.

STIMULATION OF PROSTAGLANDIN CYCLOOXYGENASE AND PROSTACYCLIN SYNTHETASE ACTIVITIES BY ESTRADIOL IN RAT AORTIC SMOOTH MUSCLE CELLS. W.-C. Chang, J. Nakao, H. Orimo and S.-I. Murota (Dept. of Pharmacol., Tokyo Metropolitan Inst. of Geront., Itabashi-ku, Tokyo-173, Japan) *Biochim. Biophys. Acta* 620, 472-82 (1980). The effects of estradiol on the arachidonic acid pool and prostacyclin biosynthetic activity in rat aortic smooth muscle cells were studied. Estradiol has no significant effect on the distribution of [¹⁴C]arachidonic acid in cells with respect to prostacyclin production assay, the endogenous fatty acid (specifically, arachidonic acid) composition of cellular phospholipid fractions and cellular phospholipase (or/and lipase) activities. However, estradiol significantly stimulates both prostaglandin cyclooxygenase and prostacyclin synthetase activities of cells, and induction of new protein biosynthesis is involved in the effect of estradiol on the stimulation of prostacyclin biosynthetic activity.

STEREOSPECIFIC DISTRIBUTION OF PALMITIC ACID IN THE TRIACYLGLYCEROLS OF RAT ADIPOCYTES: EFFECTS OF VARYING THE COMPOSITION OF THE SUBSTRATE FATTY ACID IN VITRO. W.W. Christie and M.L. Hunter (The Hannah Res. Inst., Ayr, Scotland KA6 5HL, U.K.) *Biochem. J.* 191, 637-43 (1980). The effects of inclusion of different fatty acids in the medium on the rate of esterification of palmitic acid and its stereospecific distribution among the three positions of the triacyl-*sn*-glycerols by preparations of rat adipocytes *in vitro* have been determined. Myristic acid, stearic acid, oleic acid and linoleic acid were used as diluents and the concentration of the combined unesterified fatty acids in the medium was held constant; only the proportion of palmitic acid esterified was always linearly related to its relative concentration in the medium and was not significantly affected by the nature or the diluent fatty acid chosen. Constant relative proportions were recovered in triacylglycerols and in intermediates in each instance. The results are discussed in terms of changes in the relative affinities of the acyltransferases for palmitic acid. Palmitic acid was esterified into various molecular species in proportions that indicated acylation with non-correlative specificity at higher relative concentrations but not at lower.

RED CELL CHOLESTEROL ENRICHMENT AND SPUR CELL ANEMIA IN DOGS FED A CHOLESTEROL-ENRICHED, ATHEROGENIC DIET. R.A. Cooper, M.H. Leslie, D. Knight, and D.K. Detweiler (Hematology-Oncology Section, Dept. of Medicine, School of Medicine and Depart. of Animal Biology, School of Veterinary Med., Univ. of Pennsylvania, Philadelphia, PA 19104) *J. Lipid Res.* 21, 1082-9 (1980). A diet supplemented with cholesterol and coconut oil is atherogenic in dogs. The purpose of the present study was to examine the effects of this diet on red cells in purebred beagles and greyhounds. Within 3 days after the initiation of this diet red cell cholesterol/phospholipid increased and membrane fluidity decreased, with maximum changes attained by 12 weeks. Serum lipoprotein cholesterol/phospholipid also increased, and serum from cholesterol-fed dogs transferred cholesterol to normal red cells. Significant abnormalities of liver function developed in all cholesterol-fed dogs. Hematocrit declined beginning at 6 weeks, with a parallel increase in osmotic fragility. Reticulocytes were elevated in beagles but normal in greyhounds. Red cell morphology resembled acanthocytes or spur cells. All red cell parameters returned to normal within 4 weeks after stopping the diet. These studies demonstrate that a cholesterol-enriched, atherogenic diet causes profound and reversible changes in the lipid composition, membrane fluidity, and morphology of red cells in dogs.

EFFECT OF CHOLESTEROL ON MACROMOLECULAR SYNTHESIS AND FATTY ACID UPTAKE BY MYCOPLASMA CAPRICOLUM. J.S. Dahl, C.E. Dahl, and K. Bloch (James Bryant Conant Laboratories, Dept. of Chemistry, Harvard Univ., Cambridge, MA 02138) *J. Biol. Chem.* 256, 87-91 (1981). The rates of protein and lipid synthesis of *Mycoplasma capricolum* were essentially synchronous during growth and depended on the sterol supplement in the media increasing in the order cholesterol (0.5 µg/ml) < lanosterol (10 µg/ml) < lanosterol (10 µg/ml) +

cholesterol (0.5 $\mu\text{g/ml}$) < cholesterol (10 $\mu\text{g/ml}$). The effect of lanosterol plus low cholesterol on macromolecular synthesis was synergistic. Whereas protein and lipid synthesis were brought virtually to a halt by cholesterol starvation, DNA synthesis continued for about 8 h. Increasing the palmitate and elaidate concentrations 4-fold in the lanosterol-supplemented media raised the growth rate even in the absence of the small amount of cholesterol (0.5 $\mu\text{g/ml}$) needed otherwise for the synergistic effect on growth. Studies of the kinetics of fatty acid uptake by resting cells showed that the apparent K_m (17 μM) of oleate uptake in lanosterol-grown cells was specifically lowered to 3 μM , a value equal to that seen in cholesterol-grown cells, by the inclusion of a synergistic amount of cholesterol in the growth media. By contrast, the apparent K_m for palmitate uptake was the same (2 μM) for all three cell types. The results are consistent with the membrane cholesterol serving in a dual role, one as a bulk component and another more specific function involving the regulation of unsaturated fatty acid uptake and thereby phospholipid biosynthesis.

UPTAKE AND METABOLISM OF FATTY ACIDS BY DISPERSED ADULT RAT HEART MYOCYTES. I. KINETICS OF HOMOLOGOUS FATTY ACIDS. R.F. DeGrella and R.J. Light (Dept. of Chemistry, Florida State University, Tallahassee, Florida 32306) *J. Biol. Chem.* 255, 9731-8 (1980). An adult rat heart myocyte preparation was used to study the uptake and metabolism of the $1\text{-}^{14}\text{C}$ -labeled free fatty acids decanoate, laurate, myristate, palmitate, and oleate at 37° C in the absence of serum albumin. The rate of total uptake consisted of both a nonsaturable and a saturable component. The relative product distribution did vary with chain length, however, ranging from primarily carbon dioxide for decanoate to approximately equal quantities of carbon dioxide, triglyceride, and polar lipid for palmitate. Two internal pools of free fatty acid are postulated: a minor pool that equilibrates rapidly with external fatty acid and serves as the precursor for fatty acid activation, and a major pool containing most of the accumulated free acid. The data support a simple diffusion or membrane-partitioning process for the accumulation of fatty acid in the second pool. The data presented in this paper are not sufficient to distinguish between a simple diffusion or a carrier-mediated process for uptake into the first pool. The saturation kinetics observed appear to represent a metabolic step such as fatty acid activation, rather than a transport carrier. Evidence of toxicity at a higher concentration of the longer chain fatty acids limits the concentration range that can be studied in the absence of albumin. Decanoate did not appear to be toxic at concentrations up to 300 μM , but laurate and myristate uncoupled respiratory control.

VITAMIN E STATUS OF AGRICULTURAL MIGRANT WORKERS IN SOUTHERN BRAZIL. I.D. Desai, M.A. Swann, M.L.G. Tavares, B.S. Dutra de Oliveira, F.A.M. Duarte and J.E. Dutra de Oliveira (Div. of Human Nutr., Schl. of Home Econ., Univ. of British Columbia, Vancouver, BC, Canada V6T 1W5) *Am. J. Clin. Nutr.* 33, 2669-73 (1980). Vitamin E status of agricultural migrant workers representing low socioeconomic population of Southern Brazil was evaluated by determining dietary intake and plasma levels of vitamin E. The mean plasma vitamin E level of 85 female and 39 male subjects was 1.14 ± 0.33 mg/100 ml or 2.27 ± 0.53 mg/g of total lipids in plasma. The difference between the plasma vitamin E values of male and female subjects was insignificant. Using various criteria for the assessment of plasma vitamin E levels, it was established that plasma vitamin E expressed in terms of plasma total lipids is a better indicator of vitamin E status. The actual mean α -tocopherol intake of this population was 5.51 ± 3.30 mg/person from a typical diet supplying about 1500 kcal/day. On a 2500 kcal basis, the estimated mean α -tocopherol intake would be about 9 mg/day which compares favorably with the intake values reported for well-nourished populations. The main dietary source of vitamin E in this population is the traditional rice and beans diet with increased use of soybean oil and vegetable oil products in recent years. On the whole the vitamin E status of this Brazilian population is quite satisfactory despite inadequacies in their intake of dietary calories and other essential nutrients. The plasma vitamin E status of these subjects supports the dietary data for the intake of vitamin E in this population.

SECRETION OF VERY LOW DENSITY LIPOPROTEINS ENRICHED IN CHOLESTERYL ESTERS BY CULTURED RAT HEPATOCYTES DURING SIMULATION OF INTRACELLULAR CHOLESTEROL ESTERIFICATION. C.A. Drevon, S.C. Engelhorn and D. Steinberg (Division of

Metabolic Disease, Department of Medicine, University of California, San Diego, School of Medicine, La Jolla, CA 92093) *J. Lipid Res.* 21, 1065-71 (1980). Very low density lipoproteins, newly secreted by cultured rat hepatocytes into a serum-free medium, contain some cholesteryl esters although the percentage of total cholesteryl in ester form is less than that in plasma very low density lipoproteins. When acyl CoA:cholesterol acyltransferase activity in hepatocytes was stimulated by the addition of 25-hydroxycholesterol (10 $\mu\text{g/ml}$) or mevalonolactone (1 mM), the absolute amount of esterified cholesterol secreted in very low density lipoproteins increased significantly, but the amount of free cholesterol decreased or showed no change. Thus the percentage of very low density lipoprotein cholesterol in ester form increased, in some experiments to as much as 50% of the total. These results provide additional evidence that hepatic acyl CoA:cholesterol acyltransferase plays a role in the generation of some of the cholesteryl ester in newly-secreted lipoproteins. They further suggest that changes in the activity of the enzyme can potentially regulate the fraction of cholesterol secreted in esterified form.

THE EFFECT OF ARACHIDONIC- AND EICOSAPENTAENOIC ACID ON THE SYNTHESIS OF PROSTACYCLIN-LIKE MATERIAL IN HUMAN UMBILICAL VASCULATURE. J. Dyerberg and K.A. Jorgensen (Dept. of Clin. Chem., Aalborg Hosp., Section North DK-9000, Aalborg, Denmark) *Artery* 8, 12-7 (1980). All cis-5,8,11,14,17 eicosapentaenoic acid (EPA) inhibits platelet aggregation. The mechanisms for this inhibition are not known in detail. One of them might be a competitive inhibition of TXA₂ production. Even if rat and human vasculature in pure systems convert EPA to PGL₂ with the same properties as PGL₂, it is essential to know if EPA influences the conversion of arachidonic acid (AA) to PGI₂ in human vasculature. This problem was investigated in human umbilical vascular tissue deprived of substrate for PGI synthesis. After incubation with AA or EPA alone and in combinations, prostacyclin synthesis was measured as PGI₂. Prostacyclin production in assays with AA and EPA in combinations in the incubation mixture, was found additive as calculated from assays with pure substrates. Thus, EPA did not influence the conversion of AA to PGI₂ but gave obviously rise to additional synthesis of PGI-like material.

EFFECTS OF THE SOURCE OF DIETARY PROTEIN ON SERUM LOWER DENSITY LIPOPROTEIN (VLDL + LDL) AND TOCOPHEROL LEVELS IN FEMALE RATS. A. Eklund and L. Sjöblom (Institute of Medical and Physiological Chemistry, University of Uppsala, P.O. Box 575, 751 23 Uppsala, Sweden) *The J. of Nutr.* 110, 2321-35 (1980). The effect of dietary protein on lipid levels of serum and liver and mineral contents of bone tissue was studied in female rats by using 13 semipurified diets differing with respect to protein source. The diets were characterized with regard to contents of protein, amino acids, fat, and metabolizable energy. Six of the proteins tested were of animal origin and six proteins used were plant proteins. Each diet was fed at a 20% protein, 10% fat level. There was a large variation between groups in the serum content of lower density lipoproteins (VLDL + LDL). Rats fed plant protein diets showed values in a lower range than rats consuming animal protein diets.

THE EFFECTS OF ALTERED THYROID STATUS ON LIPID METABOLISM IN THE GENETIC HYPERLIPEMIC ZUCKER RAT. S.F. Egleken and R.P. Eaton (Univ. of New Mexico Schl. of Med., Div. of Endoc. and Metabolism, Albuquerque, NM 87131) *Atherosclerosis* 38, 177-88 (1981). Exposure to thyroid hormone (T₄) has been known to affect the plasma triglyceride (TG) as well as the plasma cholesterol level, but the mechanisms and degree of response in genetic hyperlipidemic states have not been defined. In the present study, we examined TG secretion and removal in vivo in genetically hyperlipemic Zucker rats maintained in hypothyroid, euthyroid, and hyperthyroid states for 6 weeks. The induction of the hypothyroid state resulted in marked weight loss with reduced food intake, and a parallel reduction in plasma TG concentration, hepatic TG production, and peripheral TG removal. The changes in plasma cholesterol concentration in the hyperthyroid state were striking, with a 94% reduction in LDL cholesterol, but only a minimal reduction in the HDL cholesterol level. The results suggest that the very low density lipoprotein TG metabolism is influenced by hypothyroid but not the hyperthyroid state in this model of human genetic Type IV hyperlipemia. The primary reduction in LDL relative to HDL in response to thyroxine excess, suggests a therapeutic potential in disorders of genetic hyperlipidemia.

SYNTHESIS OF CALCITROIC ACID, A METABOLITE OF 1 α ,25-DIHYDROXYCHOLECALCIFEROL. R.P. Esvelt, M.A. Fivizzani, H.E. Paaren, H.K. Schnoes and H.F. DeLuca (Dept. of Biochem., College of Agricultural and Life Sciences, Univ. of Wisconsin—Madison, Madison, WI 53706) *J. Org. Chem.* **46**, 456–8 (1981). 1 α ,25-dihydroxyvitamin D₃ (1 α ,25-(OH)₂D₃) is the most potent known metabolite in the vitamin D series for the regulation of calcium and phosphate homeostasis. Recently it was discovered that rats rapidly metabolize 1,25-(OH)₂D₃ to a compound having an acid function on the side chain. This metabolite was isolated as the methyl ester and identified as methyl 1 α ,3 β -hydroxy-24-nor-9,10-secochola-5,7,10(19)-trien-23-oate or calcitroic acid methyl ester. The synthesis of 1a was of interest to confirm the structure of the biologically generated compound and to provide a route for obtaining a sufficient quantity of the metabolite for examining its biological activity. A convenient synthetic route yielding 1 and a comparison of spectral and chromatographic properties of synthetic and biologically generated 1b are presented herein. By use of the general method of Ryer and Gebert, an Arndt-Eistert homologation sequence provided the side chain desired in the final product. Synthetic 1b demonstrated UV and mass spectra identical with those found for the methyl ester of the isolated metabolite and was found to comigrate with the biological material in analytical high-pressure LC. These findings confirm the structure proposed by Esvelt et al. and establish the hydroxyl configurations at 1 α and 3 β . Thus the structure of this major metabolite of 1,25-(OH)₂D₃ was confirmed to be 1 α ,3 β -dihydroxy-24-nor-9,10-sec-5,7,10-(19)-cholatrien-23-oic acid, 1a.

CHARACTERISTICS OF TRIACYLGLYCEROL AND PARTIAL ACYLGLYCEROL HYDROLYSIS BY HUMAN PLASMA LIPOPROTEIN LIPASE. C.J. Fielding and P.E. Fielding (Cardiovascular Res. Inst. and Dept. of Physiol., Univ. of California at San Francisco, San Francisco, CA 94143) *Biochim. Biophys. Acta* **620**, 440–6 (1980). The rates of reaction of human lipoprotein lipase (EC 3.1.1.34) with triacylglycerol and partial acylglycerol substrates have been compared as a function of the concentration of lipase cofactor protein (apolipoprotein C-II). The data indicate that the dissociation constant for monoacylglycerol is approximately three orders of magnitude greater than for diacylglycerols, indicating that only when the concentrations of higher acylglycerols become vanishingly small will significant monoacylglycerol hydrolysis (from 1-monoacylglycerol generated by isomerization of the 2-substituted primary product) be mediated by the lipase. This is in spite of the fact that maximal reaction velocities with each of the potential substrates are similar. A 'lipolytic cycle' is proposed to explain binding and dissociation of substrates with cofactor-lipase complex during catabolism of triacylglycerols.

RECEPTOR-DEPENDENT UPTAKE OF HUMAN CHYLOMICRON REMNANTS BY CULTURED SKIN FIBROBLASTS. C.H. Florén, J.J. Albers, B.J. Kudchodkar, and E.L. Bierman (Division of metabolism and endocrinology, and the Northwest Lipid Research Clinic, Department of Medicine, Univ. of Washington School of Medicine, Seattle, WA 98195) *J. Biol. Chem.* **256**, 425–33 (1981). Human chylomicrons were isolated from plasma from a subject with familial hypertriglyceridemia and converted to chylomicron remnants by incubation with postheparin plasma. The interaction of these apolipoprotein E-containing, cholesterol-rich human chylomicron remnants with cultured skin fibroblasts was studied. Chylomicron remnants were internalized by skin fibroblasts as a unit, mainly via the low density lipoprotein (LDL)-receptor pathway, resulting in increased cell cholesterol content. After entering the fibroblast, chylomicron remnants stimulated cholesterol esterification, suppressed 3-hydroxy-3-methylglutaryl coenzyme A reductase activity, and down-regulated LDL receptor activity similar to the action of LDL. As a function of increasing lipolysis, remnant particles were progressively more effectively taken up by skin fibroblasts, despite a decrease in the apolipoprotein E content per lipoprotein particle. Remnant particles produced after hydrolysis of 70 to 80% of chylomicron triglyceride increased cell cholesterol content to an amount nearly identical to that observed with LDL when the two lipoproteins were incubated at an equal cholesterol concentration. However, when incubated on the basis of equal particle number, chylomicron remnants were 2 to 3 times more effective than LDL in delivering cholesterol to the cells. These results suggest that chylomicron remnants play a role in the regulation of postabsorptive cholesterol homeostasis in nonhepatic cells, and possibly in the pathogenesis of atherosclerosis.

EFFECTS OF DIETARY PROTEIN AND FAT SOURCES ON PLASMA CHOLESTEROL PARAMETERS, LCAT ACTIVITY AND AMINO ACID LEVELS AND ON TISSUE LIPID CONTENT OF GROWING PIGS. W.A. Forsythe, E.R. Miller, G.M. Hill, D.R. Romsos, and R.C. Simpson (Dept. of Food Science and Human Nutr. and Dept. of Animal Sciences, Michigan State Univ., East Lansing, MI 48824 and Standard Brands, Inc., Stamford, CT) *The J. of Nutr.* **110**, 2467–79 (1980). Young male pigs were used to examine effects of dietary protein and fat sources on plasma cholesterol parameters. Diets providing 16 and 42% of metabolizable energy from protein and fat, respectively, were fed for 12–14 weeks. Protein was derived either from plant sources (50% from soybean meal and 25% each from corn and wheat) or from animal sources (90% from casein and 10% from lactalbumin). The polyunsaturated to saturated fat ratio in the diets averaged 3.0 in the polyunsaturated fat diets and 0.3 in the saturated fat diets. Cholesterol content of the four experimental diets (plant protein-polyunsaturated fat; plant protein-saturated fat; animal protein-polyunsaturated fat; and animal protein-saturated fat) was 0.6 mg/kcal. Consumption of diets containing plant protein rather than animal protein reduced total plasma cholesterol levels by 50 mg/dl; high density lipoprotein (HDL) cholesterol levels were also lowered in pigs fed plant protein. Similarly, plasma cholesterol levels were approximately 40 mg/dl lower in pigs fed the polyunsaturated fat diets than in pigs fed the saturated fat diets. HDL cholesterol levels, however, were unaffected by source of fat fed.

OXIDATION OF ARACHIDONIC ACID IN MICELLES BY SUPEROXIDE AND HYDROGEN PEROXIDE. S.E. Fridovich and N.A. Porter (Dept. of Chemistry, Duke Univ., Durham, North Carolina 27706) *J. Biol. Chem.* **256**, 260–5 (1981). Arachidonic acid was co-oxidized by xanthine oxidase. Both superoxide radical and hydrogen peroxide were required for oxidation, as shown by essentially complete inhibition caused by superoxide dismutase or by catalase. Pure arachidonate, free of lipid hydroperoxides, was susceptible to this co-oxidation, and the presence of lipid hydroperoxides did not accelerate the process. The role of trace metals was indicated by the stimulatory effect of EDTA-Fe and by the inhibitory effect of diethylenetriamine pentaacetate. Initiation of arachidonate co-oxidation was due to a potent oxidant generated by the interaction of H₂O₂ and O₂ in the presence of Fe, rather than to either O₂ or H₂O₂ *per se*. Hence, mannitol, a scavenger of OH·, but not of O₂ or H₂O₂, also inhibited oxidation. Arachidonic acid autoxidation, a much slower process than xanthine oxidase co-oxidation, was barely detectable on the time scale of these observations. Unlike the co-oxidation, autoxidation was autocatalytic and therefore accelerated by hydroperoxide products. Marked quantitative differences in the distribution of isomeric hydroperoxide products of the enzymic co-oxidation, as compared to the autoxidation, were noted and their significance was discussed.

POLYUNSATURATED FATTY ACID ACCUMULATION IN THE LIPIDS OF CULTURED FIBROBLASTS AND SMOOTH MUSCLE CELLS. V.C. Gavino, J.S. Miller, J.M. Dillman, G.E. Milo and D.G. Cornwell (Dept. of Physiological Chem., The Ohio State Univ., Columbus, OH 43210) *J. Lipid Res.* **22**, 57–62 (1981). The lipid content per cell of cells in tissue culture depended on the cell type. Fibroblasts derived from human neonatal foreskin contained less triglyceride and phospholipid and more cholesterol ester than smooth muscle cells derived from guinea pig aorta. When fibroblasts and smooth muscle cells were challenged with 120 μ M polyunsaturated fatty acid, the fibroblasts accumulated much less fatty acid than smooth muscle cells. The total fatty acid content of the phospholipid fraction was unchanged in cells challenged with a fatty acid. The polyunsaturated fatty acid and its derivatives exchanged with fatty acyl groups in the cellular phospholipid fraction. These fatty acyl groups were transferred to the triglyceride fraction and the total cellular content of each fatty acid was conserved. The total fatty acid content of the triglyceride fraction was markedly increased in cells challenged with a fatty acid. The polyunsaturated fatty acid and its derivatives accumulated in the triglyceride fraction. The triglyceride fraction contained an unusual triacyl derivative of the polyunsaturated fatty acid. These data support the hypothesis that microsomal fatty acyl-CoA intermediates are shunted into neutral lipid droplets when cells are stimulated to accumulate lipid.

ABNORMALITIES IN LIPOPROTEINS OF d < 1.006 G/ML IN FAMILIAL LECITHIN:CHOLESTEROL ACYLTRANSFERASE DEFICIENCY. J.A.

Glomset, K. Applegate, T. Forte, W.C. King, C.D. Mitchell, K.R. Norum, and E. Gjone (Regional Primate Res. Center, Univ. of Wash., Seattle, WA 98195) *J. Lipid Res.* 21, 1116-27 (1980). Studies of different sized lipoproteins of $d < 1.006$ g/ml from patients with familial lecithin: Cholesterol acyltransferase deficiency have yielded new evidence of abnormalities in this lipoprotein class. Lipoproteins of all sizes contain high amounts of unesterified cholesterol, low amounts of total protein, and particularly low amounts of apolipoproteins C-II and C-III. Lipoprotein 60 nm in diameter or larger include particles that show a notched appearance upon electron microscopy, and contain a) a high combined volume of phospholipid, unesterified cholesterol, and protein; b) high amounts of cholesteryl ester and apolipoproteins C-I and E, and c) two major tetramethylurea-insoluble proteins that can be separated by electrophoresis in the presence of sodium dodecyl-sulfate. In contrast, lipoproteins that are 40 nm in diameter or less appear to contain low amounts of cholesteryl ester, normal amounts of apolipoproteins C-I and E, and a single tetramethylurea-insoluble protein the size of that in control lipoproteins. Since these abnormalities occur in the lipoproteins of four different patients from four different families, they are probably effects of the enzyme deficiency. Most, however, appear to arise indirectly because in vitro experiments published earlier indicate that few are reversed by incubation in the presence of the enzyme and patient high density lipoproteins.

INFLUENCE OF NICOTINIC ACID ON METABOLISM OF CHOLESTEROL AND TRIGLYCERIDES IN MAN. S.M. Grundy, H.Y.I. Mok, L. Zech and M. Berman (Dept. of Med., VA Medical Center and University of California, San Diego, CA 92161) *J. Lipid Res.* 22, 24-36 (1981). The mechanisms for the hypolipidemic action of nicotinic acid were examined in 12 patients with hyperlipidemia. During treatment with nicotinic acid, the triglycerides (TG) decreased in total plasma by an average of 52% and in very low density lipoproteins (VLDL) by 36%. Transport rates of VLDL-TG were determined in multicompartmental analysis following injection of [3 H]-glycerol as a precursor. Nicotinic acid decreased transport (synthesis) of VLDL-TG by an average of 21%. Kinetic modeling of the VLDL-TG data suggested that the TG reduction was due to a decrease in TG content of VLDL and hence a reduction in lipoprotein size more than number. For the whole group, plasma cholesterol fell during nicotinic acid therapy by a mean of 22%. The drug produced no detectable changes in fecal excretions of cholesterol (neutral steroids) or bile acids. However, it induced a small but significant increment in hepatic secretion of biliary cholesterol that might have led to a net loss of cholesterol from the body even though this loss could not be detected by sterol balance. Despite this increase in outputs of biliary cholesterol, there was not a significant increase in molar % cholesterol or in % saturation of gallbladder bile. Therefore, it is doubtful that nicotinic acid enhances the risk for cholesterol gallstones.

INTERACTION OF UNILAMELLAR LIPOSOMES WITH SERUM LIPOPROTEINS AND APOLIPOPROTEINS. L.S.S. Guo, R.L. Hamilton, J. Goerke, J.N. Weinstein, and R.J. Havel (Cardiovascular Res. Instit. and the Dept. of Anatomy, Physiology, and Medicine, Univ. of California, San Francisco, CA 94143 and Lab. or Theoretical Biol., Nat'l Cancer Instit., NIH, Bethesda, MD 20205) *J. of Lipid Res.* 21, 993-1003 (1980). The effect of rat whole blood plasma, serum, serum lipoproteins, and apolipoproteins on the stability of unilamellar liposomes prepared with the French pressure cell was evaluated by measuring the release of entrapped carboxyfluorescein and by electron microscopy. In the absence of serum components, dye escaped very slowly (hours) from egg phosphatidylcholine and phosphatidylcholine-cholesterol vesicles without apparent change in liposomal structure. This slow release was both temperature- and size-dependent. Serum and some of its constituents induced a far more rapid (seconds) loss of entrapped dye from phosphatidylcholine liposomes, associated with structural changes. Substantial activity was found in three preparations of bovine serum albumin. This activity could be attributed to small and variable amounts of contaminating lipoprotein-like particles and apolipoprotein A-I. Induced release of dye from liposomes by apolipoproteins was usually associated with rapid formation of discs although other structures were sometimes formed. Purified rat apolipoproteins A-I and E appeared to interact identically with liposomes to induce dye release. This effect was progressively impaired for both apoproteins by increasing amounts of cholesterol and was completely inhibited

when liposomes contained 37 mol % cholesterol.

LYSOSOMAL ACID CHOLESTERYL ESTERASE ACTIVITY IN NORMAL AND LIPID-LADEN AORTIC CELLS. N.J. Haley, S. Fowler and C. de Duve (Rockefeller Univ., New York, NY 10021) *J. Lipid Res.* 21, 961-9 (1980). We have investigated the kinetic properties of acid cholesteryl esterase in preparations of rabbit aortic cells, with the aim of establishing conditions suitable for the quantitative assay of the enzyme in freshly prepared homogenates and subcellular fractions, whether derived from normal cells or from atheromatous cells heavily laden with cholesteryl and cholesteryl esters. As substrate we used cholesteryl [14 C]oleate incorporated at a 1:100 molar ratio into egg-lecithin liposomes and measured the radioactivity remaining in the alkaline buffer phase after organic solvent extraction of unhydrolyzed substrate. When the liposome substrate was used as such, more than 80% of the enzyme activity was latent in fresh homogenates. The following conditions gave satisfactory linearity with both time of incubation and enzyme concentration, with both normal and atheromatous cell preparations, and were adopted for the assay: 12.7 μ M cholesteryl oleate dispersed in 1.27 mM egg lecithin; 50 mM acetate buffer, pH 3.9; 2.0 mM Na taurocholate; and 0.005% digitonin. A considerable part of the enzyme is localized in lysosomes, both in normal and in either moderately or heavily lipid-laden atheromatous aortic cells. When assayed under optimal conditions, lipid-laden atheromatous arterial cells displayed up to 3.5 times the acid cholesteryl esterase activity of normal aortic cells.

UNILAMELLAR LIPOSOMES MADE WITH THE FRENCH PRESSURE CELL: A SIMPLE PREPARATIVE AND SEMI-QUANTITATIVE TECHNIQUE. R.L. Hamilton, Jr., J. Goerke, L.S.S. Guo, M.C. Williams, and R.J. Havel (Cardiovascular Research Institute, Univ. of Calif., San Francisco, CA 94143) *J. Lipid Res.* 21, 981-92 (1980). A simple, rapid, and almost quantitative technique is described for the preparation of 1-40 ml of homogeneous unilamellar liposomes from dilute or concentrated aqueous suspensions of egg phosphatidylcholine. The method is especially useful for trapping small molecular weight substances because the concentration of both lipid and solute can be made quite high. Cholesterol up to 45 mole % can be incorporated into larger liposomes of egg phosphatidylcholine (mean diameter 315 Å). Other phospholipids and different lipid mixtures can also be transformed into unilamellar vesicles with this method which has the advantage that additional steps of ultracentrifugation, column chromatography, dialysis, and concentrating procedures are usually unnecessary. Multilayered liposomes of small size (980 Å mean diameter; > 95% between 500-1,500 Å) are produced at lower pressure (3,000 psi). The latter are separated by gel permeation chromatography from a second population of homogeneous vesicles of even smaller size (580 Å mean diameter; > 95% between 300-900 Å) that contain two bilayer shells.

ENDOTHELIAL PROLIFERATION AND ATHEROGENESIS IN RABBITS WITH MODERATE HYPERCHOLESTEROLEMIA. G.K. Hansson and G. Bondjers (Arterial Biology Group, Departments of Histology and Medicine I, University of Göteborg, Box 33031, S-400 33 Göteborg, Sweden) *Artery* 7, 316-29 (1980). The formation and growth of atherosclerotic lesions in experimental hypercholesterolemia has been attributed to endothelial injury. Many injured endothelial cells have been observed in the periphery of the lesions, but few in the central parts. In the present study, we have investigated the distribution of endothelial cells, leucocytes, and smooth muscle cells on the surface of the lesions, as well as the regeneration of the surface cell layer, on dietary induced experimental atherosclerotic lesions. In central areas of the lesions, flat cells with Weibel-Palade bodies and intercellular junctions characteristic of endothelium, were observed on the surface. In peripheral areas of the lesions, surface cells were more bulging and contained many free ribosomes and short cisternae of endoplasmic reticulum, suggesting that these cells were more primitive. Weibel-Palade bodies and typical intercellular junctions suggested that many of the cells should be regarded as endothelial cells. ANAE-positive monocytes were also frequent in these areas. The incorporation of 3 H-thymidine was considerably larger over the lesions than in surrounding normal tissue, suggesting a regeneration of the endothelial cell layer from cells on the lesions. Still, regression does not occur after re-endothelialization in dietary induced atherosclerosis.

PHOSPHORYLASE KINASE DEFICIENCY AND DECREASED FAT ACCUMULATION IN HYBRID MALE MICE (I \times C3H). J. Hoover-

Plow (Dept. of Medicine, Division of Pharmacology, University of California, San Diego, La Jolla, California 92093) *Proc. Soc. Exp. Biol. Med.* 165, 409-12 (1980). I strain mice have a deficiency of muscle phosphorylase b kinase. Their skeletal muscle shows a three- to fivefold elevation in glycogen concentration and a decreased rate of glycogen breakdown. In addition, I strain mice accumulate less fat with age or high fat diets than control mice. The purpose of this study was to determine whether phosphorylase b kinase deficiency and decreased fat accumulation of I strain mice are related. Body weight, epididymal fat pad size, adipose cell number, and size were determined in male I, C3H, and hybrid (I × C3H) mice. Since the phosphorylase kinase deficiency is sex-linked, the male offspring of the I (female) × C3H (male) cross were used. As expected, phosphorylase b kinase activity and glycogen content in I and I × C3H skeletal muscle are similar. Body weight at 16 weeks of age in I × C3H mice resembles the body weight of I mice rather than C3H mice. Epididymal fat pad weight is significantly lower in I and I × C3H mice compared to that in C3H mice. However, adipose cell number is intermediate in I × C3H mice compared to I and C3H mice. These results suggest that the decreased fat accumulation in I mice is genetically expressed with phosphorylase kinase deficiency. The inability of I mice to accumulate fat may be due to decreased adipose cell number.

OCCURRENCE OF BILE ALCOHOL GLUCURONIDES IN BILE OF PATIENTS WITH CEREBROTENDINOUS XANTHOMATOSIS. T. Hoshita, M. Yasuhara, M. Une, A. Kibe, E. Itoga, S. Kito, and T. Kuramoto (Instit. of Pharmaceutical Sciences and 3rd Dept. of Internal Medicine, Hiroshima Univ. School of Medicine, Hiroshima, Japan) *J. of Lipid Res.* 21, 1015-21 (1980). Using thin-layer chromatography, bile alcohol glucuronides were found with taurine- and glycine-conjugated bile acids in the bile of four patients with cerebrotendinous xanthomatosis. The concentration of the bile alcohol glucuronides was 1.7-5.2 times higher than that of the conjugated bile acids. Detectable amounts of unconjugated bile alcohols were not found in the bile of these patients. The bile alcohol glucuronides were isolated from the bile of one of the patients by means of preparative thin-layer chromatography. Treatment with β -glucuronidase of the bile alcohol glucuronides liberated glucuronic acid and a mixture of bile alcohols. The bile alcohol glucuronides were not oxidized by the treatment with 3α -hydroxysteroid dehydrogenase, indicating that the glucuronide moiety was at 3α -hydroxyl position of the bile alcohols. Comparison of the mass spectra of the acetylated and methylated derivatives of the natural glucuronides and the synthetic 7α , 12α , 25-triacetoxy- 5β -cholestan- 3α -O-(methyl 2,3,4-tri-O-acetyl- β -D-glucopyranosyluronate) also indicated that the bile alcohol glucuronides consisted of mainly 5β -cholestan- 3α , 7α , 12α , 25-tetrolglucuronide.

STUDIES OF VERY LOW DENSITY LIPOPROTEIN TRIGLYCERIDE METABOLISM IN AN OBESE POPULATION WITH LOW PLASMA LIPIDS: LACK OF INFLUENCE OF BODY WEIGHT OR PLASMA INSULIN. B.V. Howard, L. Zech, M. Davis, L.J. Bennion, P.J. Savage, M. Nagulesparan, D. Bilheimer, P.H. Bennett and S.M. Grundy (National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, 4212 N. 16th Street, Phoenix, AZ 85016) *J. Lipid Res.* 21, 1032-41 (1980). Pima Indians have a high prevalence of hyperinsulinemia, obesity, and diabetes, but they have low plasma cholesterol levels, reduced low density lipoprotein synthesis, and little arteriosclerotic heart disease. To investigate lipoprotein metabolism further in this group, very low density lipoprotein (VLDL) metabolism was studied, using [3 H]glycerol as an endogenous precursor of triglyceride (TG) synthesis, in 15 obese Pima nondiabetic males and compared to that of 10 obese and 13 normal weight, normolipidemic, nondiabetic Caucasian males. When the relation between VLDL-TG metabolism and plasma insulin was examined, plasma insulin levels in the Pima were not correlated with VLDL-TG synthetic rates, catabolic rates, or plasma pools. On the other hand VLDL-TG synthetic rates were correlated with plasma free fatty acid levels. Thus, in this population with low plasma lipids and reduced arteriosclerotic heart disease, VLDL-TG synthesis is low, VLDL-TG catabolism is accelerated, and VLDL pools appear to be insensitive to the influence of body weight and hyperinsulinemia.

ESSENTIAL ROLE OF GTP IN EPINEPHRINE STIMULATION OF HUMAN FAT CELL ADENYLATE CYCLASE. M.S. Katz, J.S. Partilla, M.A. Pineyro, and R.I. Gregerman (Gerontology Res. Center, Nat'l Instit. on Aging, Nat'l Instit. of Health at Baltimore City Hosp, and Depts. of Med., Baltimore City Hosp. and

Johns Hopkins Univ. School of Med., Baltimore, MD) *J. Lipid Res.* 22, 113-21 (1981). The activity of epinephrine-sensitive adenylate cyclase of human fat cell ghosts is markedly enhanced by the GTP analog 5'-guanylyl-imidodiphosphate (GMP-P(NH)P), but a similar effect of GTP itself has not been heretofore demonstrable. In the present work, comparison of adenylate cyclase activity in the presence of epinephrine alone versus epinephrine plus GTP showed that at 37° C GTP doubled activity (10-min incubation); at 30° C less than half this effect was apparent. However, time course studies at both 30 and 37° C showed that comparisons at a single point in time based on ratios of hormone-stimulated activity to basal or basal plus GTP were misleading, since basal activities were not linear with time and were inhibited by GTP. The time course data showed clearly that epinephrine alone did not stimulate adenylate cyclase activity; rather, the hormone merely prevented fall-off of initial rate of unstimulated (basal) enzyme activity. Only when GTP was added together with epinephrine was an unequivocal stimulation of enzyme activity observed. The GTP effect was not hormone-receptor mediated, since no shift was seen of the epinephrine dose-response curve toward higher sensitivity.

ISOLATION AND PARTIAL CHARACTERIZATION OF THE LIPID PHASES OF HUMAN ATHEROSCLEROTIC PLAQUES. S.S. Katz and D.M. Small (Dept. of Med., Royal Victoria Hospital, Montreal, Quebec H3A 1A1) *J. Biol. Chem.* 255, 9753-9 (1980). Human atherosclerotic plaques have lipid compositions that fall in the three-phase region of the phase diagram of the major lipids of plaques, cholesterol, cholesterol ester, and phospholipid. The top layer of homogenized plaque consisted of lipid droplets composed of 79.2% cholesterol ester, 7.8% triglyceride, 8.5% cholesterol, and 4.5% phospholipid. The top layer of extracted total plaque lipids had a similar composition. The top layer of the extracted lipid system comprised 52% of total lipids closely approximating the 50% calculated from the phase diagram. Homogenate layers were filtered and re-centrifuged on a density gradient to further purify the crystals. The composition of the purified crystals was 88.5% cholesterol, 10.0% cholesterol ester, 1.1% triglyceride, and 0.9% phospholipid. The $d = 1.054$ layer of the extracted lipid system had a similar composition. Thus, two of the lipid phases of plaques, the cholesterol ester phase and the cholesterol crystalline phase, were isolated in relatively pure form. Almost all the cholesterol ester phase of extracted plaque lipids was recovered as floating lipid droplets, while only half of the calculated cholesterol ester phase was isolated a lipid droplets from plaque homogenates. A fraction of plaque lipids is bound to protein or other more dense plaque constituents, and will have to be considered in future phase equilibrium studies of plaque lipids.

IN VITRO EFFECTS OF GLYCOSPHINGOLIPIDS ON HUMAN TUMOR CELL PROLIFERATION. P.M. Kimball, L. Hammonds, J.M. McKibbin, M.G. Brattain, G. Glover and M. Webb (Dept. of Biochemistry, University of Alabama in Birmingham, University Station, Birmingham, Alabama 35294) *Proc. Soc. Exp. Biol. Med.* 166, 107-12 (1981). A drug-resistant subpopulation of cells (HTFU) was previously isolated from the human colonic carcinoma cell line HT29. The cell lines varied significantly in *in vitro* growth patterns with HTFU cells showing contact inhibition while HT29 cells demonstrated uncontrolled growth. The malignant cell lines were grown in the presence of various concentrations of blood group A fucolipid, Lewis_x, Forssman hapten, and ceramide trihexoside for 2 weeks. Although HTFU cells demonstrated an equal or enhanced sensitivity to the lipids relative to HT29 cells, the cell lines responded in similar fashion to each lipid. Treatment with Forssman and ceramide trihexoside induced a temporary inhibition of cell proliferation, however, continued exposure to these lipids became stimulatory. Exposure to Lewis_x and blood group A fucolipids caused a permanent retardation of cell proliferation.

VITAMIN E ACTIVITY OF α -TOCOPHEROL SIDE CHAIN ANALOGS IN SELENIUM-DEFICIENT CHICKS. P.B. Kingsley and G.F. Combs, Jr. (Dept. of Biochem., Molecular and Cell Biology, Dept. of Poultry Science, and Division of Nutritional Sciences, Cornell University, Ithaca, New York 14853) *Proc. Soc. Exp. Biol. Med.* 166, 1-5 (1981). Three side chain analogs of all-*rac*- α -tocopherol (all-*rac*- α -T) were evaluated for vitamin E activity on the basis of their efficacies in preventing exudative diathesis (ED) and in supporting growth and survival of selenium-deficient chicks. Results showed that 2RS, 4'RS- α -T-C₁₁ (lacking one isopentyl unit) and 2RS- α -t-nC₁₃ (with an unbranched 13-carbon side chain) had the following activities relative to

those of all-*rac*- α -T: 69 and 50%, respectively, for supporting growth; 37 and 46%, respectively, for preventing exudative diathesis; 13 and 44%, respectively, for sustaining chick survival. 2*RS*- α -T-C₈ (lacking two isopentyl units) demonstrated no biological activity at the levels fed.

THE CONTRIBUTION OF CHYLOMICRON CHOLESTEROL TO MILK CHOLESTEROL IN THE RAT. P.M. Kris-Etherton and I.D. Frantz, Jr. (Depts. of Food Science and Nutrition, Medicine and Biochemistry, University of Minnesota, St. Paul, Minnesota 55108 and Minneapolis, Minnesota 55455) *Proc. Soc. Exp. Biol. Med.* 165, 502-7 (1980). The contribution of chylomicron cholesterol to total milk cholesterol was studied in the lactating rat. Pregnant rats were fed a high-fat, high-cholesterol diet or standard rat diet. On the 13th day of lactation, dams were intubated with 25 μ Ci [³H]cholesterol (25 μ Ci/ μ mole). Milk was collected at 5 and 10 hr following intubation. There was a lower specific activity of chylomicron cholesterol in dams fed the high-fat, high-cholesterol diet compared with control animals at 5, 8, and 10 hr after intubation ($P < 0.05$) with radiolabeled cholesterol. There was no difference in the specific activity of milk cholesterol between both groups at 5 hr. These data indicate that chylomicron cholesterol does not significantly contribute to milk cholesterol levels in the lactating rat. At 8 and 10 hr following intubation with radiolabeled cholesterol, the specific activity of plasma cholesterol from dams fed the high-fat, high-cholesterol diet was lower than that from control animals ($P < 0.05$). Taken together, these data suggest that plasma lipoproteins other than chylomicrons contribute significantly to milk cholesterol levels.

EFFECTS OF DIETARY TRANS ACIDS ON THE BIOSYNTHESIS OF ARACHIDONIC ACID IN RAT LIVER MICROSOMES. N. Kurata and O.S. Privett (The Hormel Institute, University of Minnesota, Austin, MN 55912) *Lipids* 15, 1029-36 (1980). Effects of dietary *trans* acids on the interconversion of linoleic acid was studied using the liver microsomal fraction of rats fed a semipurified diet containing fat supplements of safflower oil (SAFF), hydrogenated coconut oil (HCO) at 5 and 20% levels or a 5% level of a supplement containing 50.3% linoleic and 24.3% elaidic acids devoid of *cis,cis*-linoleic acid (TRANS). Growth rate was suppressed to a greater extent with the animals fed the 20% than the 5% level of the HCO-supplemented diets and still further by the TRANS diet compared to the groups fed the SAFF diets. Food intake was greater in the groups fed the HCO than the SAFF-supplemented diets, demonstrating the marked effect of an essential fatty acid (EFA) deficiency on feed efficiency. In contrast to an EFA deficiency produced by the HCO supplement, which stimulated the *in vitro* liver microsomal biosynthesis of arachidonic acid, diets containing the TRANS supplement exacerbated the EFA deficiency and depressed 6-desaturase activity of the liver microsomal fraction. It is suggested that dietary *trans* acids alter the physical properties of the 6-desaturase enzyme system, suppressing its activity, which increases the saturation of the tissue lipids and, in turn, the requirement for EFA or polyunsaturated fatty acids.

STIMULATION OF GLYCOLIPID SYNTHESIS AND EXCHANGE BY HUMAN SERUM HIGH DENSITY LIPOPROTEIN-3 IN HUMAN FIBROBLASTS AND LEUKOCYTES. B.C.P. Kwok, G. Dawson, and M.C. Ritter (Depts. of Biochemistry, Pediatrics, and Medicine, Pritzker School of Medicine, Univ. of Chicago, Chicago, IL 60637) *J. Biol. Chem.* 256, 92-8 (1981). Upon exposure to either human skin fibroblasts or human circulating leukocytes, the composition of human serum high density lipoprotein-3 (HDL₃) was modified by the apparent loss of apolipoprotein A-II and a 2- to 4-fold increase in glycosphingolipid content. Exposure of HDL₃ to leukocytes produced an increase in the content of lactosylceramide, which is the major glycolipid in leukocytes, whereas exposure of HDL₃ to human skin fibroblasts produced predominantly an increase in trihexosylceramide, which is the major glycolipid in fibroblasts. Other protein components of HDL₃ (such as apolipoprotein A-I) were unaffected and there were no major changes in either neutral lipid or phospholipid composition. The increase in glycosphingolipid content of both cells and reisolated HDL₃ particles was HDL₃ concentration-dependent up to a concentration of 1 mg/ml and appeared to be the result of a stimulation of cellular glycolipid synthesis by HDL₃ and subsequent transfer to HDL₃ in the medium. A similar stimulation could not be produced by either low density lipoprotein or lipoprotein-deficient human serum. The coaddition of HDL₃ and lipoprotein-deficient serum reduced both the loss of apolipoprotein A-II and the change in HDL₃ glycolipid content, suggesting

that modification of the apolipoprotein A-II peptide may enhance the ability of HDL₃ to acquire new glycolipid from cells.

ISOLATION AND IDENTIFICATION OF 25-HYDROXYVITAMIN D₂ 25-GLUCURONIDE: A BILIARY METABOLITE OF VITAMIN D₂ IN THE CHICK. L.W. LeVan, H.K. Schnoes and H.F. DeLuca (Dept. of Biochem., College of Agricultural and Life Sciences, Univ. of Wisconsin—Madison, Madison, WI 53706) *Biochemistry* 20, 222-6 (1981). The biliary metabolites of vitamin D₂ obtained from chickens dosed with ³H-labeled vitamin D₂ were investigated. Most of the biliary radioactivity migrated as charged compounds on diethylaminoethyl-Sephadex chromatography, and the charged fraction could be resolved into several components by reversed-phase high-pressure liquid chromatography. A major charged metabolite was further purified by reversed-phase high-pressure liquid chromatography. This compound was found to be β -glucuronidase sensitive and to yield 25-hydroxyvitamin D₂ upon milk acid hydrolysis. The metabolite was converted first to the methyl ester and then to silylated and acetylated derivatives, which were subjected to mass spectrometry. The structure of the original metabolite was established as 25-hydroxyvitamin D₂ 25- β -D-glucuronic acid. This 25-hydroxyvitamin D₂ 25- β -glucuronide is a major biliary metabolite of vitamin D₂ in the chick and may play a role in the chick's discrimination against vitamin D₂.

THE LONG TERM EFFECTS OF DIETARY CHOLESTEROL UPON THE PLASMA LIPIDS, LIPOPROTEINS, CHOLESTEROL ABSORPTION, AND THE STEROL BALANCE IN MAN: THE DEMONSTRATION OF FEEDBACK INHIBITION OF CHOLESTEROL BIOSYNTHESIS AND INCREASED BILE ACID EXCRETION. D.S. Lin and W.E. Connor (The Divisions of Metabolism and Nutrition and Cardiology, the Department of Medicine and Clinical Research Center, University of Oregon Health Sciences Center, Portland, OR 97201) *J. Lipid Res.* 21, 1042-52 (1980). In order to study the metabolic responses of humans consuming a diet moderately high in cholesterol content, we carried out a long-term sterol balance study, up to 25 weeks in duration. Two subjects, one normocholesterolemic and one hypercholesterolemic, were given, in sequence, a very low cholesterol diet and then a diet containing 1000 mg cholesterol per day. Of the possible compensatory mechanisms against cholesterol overloading from the diet, two mechanisms were partially effective: cholesterol biosynthesis decreased (feedback inhibition) and bile acid excretion increased. Cholesterol absorption remained unchanged after the high cholesterol diet and was not a compensatory mechanism despite earlier assumptions that it might be. In spite of these compensatory mechanisms, the cholesterol feeding led to a 44% increase in the plasma cholesterol levels of these subjects. The predominant component of the plasma cholesterol increase was in the cholesterol transported by LDL and with presumably greater atherogenicity as a result. In the hypercholesterolemic subject, the LDL/HDL ratio increased and there was a net storage of cholesterol in the body. Storage of cholesterol did not occur in the normal subject.

ESSENTIAL FATTY ACID STATUS IN CYSTIC FIBROSIS AND THE EFFECTS OF SAFFLOWER OIL SUPPLEMENTATION. J.D. Lloyd-Still, S.B. Johnson and R.T. Holman (The Hormel Inst., Univ. of Minnesota, 801 16th Ave. N.E., Austin, MN 55912) *Am. J. Clin. Nutr.* 34, 1-7 (1981). The fatty acid compositions of serum phospholipids, cholesteryl esters, triglycerides, and free fatty acids were determined on a group of cystic fibrosis patients. These were compared with similar data from random hospitalized patients of the same age groups of both sexes. Fatty acid patterns in all lipid classes were skewed in the direction of essential fatty acid deficiency, but the differences were most dramatic in phospholipids. Many calculated parameters useful as indices of essential fatty acid status indicated that essential fatty acid deficiency exists in cystic fibrosis. Treatment of 11 cystic fibrosis patients with safflower oil (1 g/kg/day) failed to correct the aberrations in fatty acid pattern. The biochemical data suggest that there may be an impairment in conversion of linoleate to arachidonate as well as an impairment of absorption.

THE INFLUENCE OF DIETARY ENERGY AND AMINO ACID LEVELS ON ABDOMINAL FAT PAD DEVELOPMENT OF THE BROILER CHICKEN. C.J. Mabray and P.W. Waldroup (Dept. of Animal Sci., Univ. of Arkansas, Fayetteville, AR 72701) *Poultry Sci.* 60, 151-9 (1981). A study was conducted to determine the effects of dietary energy and amino acid content on the weight of the abdominal fat pad of broiler chicks. Diets containing 2970, 3190, and 3410 ME kcal/kg were formulated to contain 70 to 120% of the 1977 NRC amino acid requirements. These diets

were fed to broiler chicks for 57 days. After slaughter in a commercial processing plant carcasses were evaluated. Through the dietary manipulations it was possible to produce carcasses of widely varying abdominal fat pad size. Although total fat pad weight was directly influenced by the dietary energy level of the diets, covariance adjustments for differences in body weight markedly reduced the influence of dietary energy. The degree of fatness could be significantly reduced by increasing the dietary amino acid levels within a given energy level.

EFFECTS OF INGESTING SOY OR EGG LECITHINS ON SERUM CHOLINE, BRAIN CHOLINE AND BRAIN ACETYLCHOLINE. S.G. Magil, S.H. Zeisel, and R.J. Wurtman (Lab. of Neuroendocrine Regulation, Dept. of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, MA 02139) *J. Nutr.* 111, 166-70 (1981). Rats were fed lecithins, derived from eggs or soybeans, to determine whether the fatty acid composition of the phosphatidylethanolamine altered choline availability. Rats were fed either a single meal containing 5 g phosphatidylethanolamine or a lecithin-containing diet for 3 weeks, including approximately 5 g phosphatidylethanolamine per day. Each form of dietary lecithin elevated blood choline, brain choline and brain acetylcholine significantly ($P < 0.05$). There was no difference in response to egg- or soy-derived lecithin.

CHOLESTERYL ESTER SYNTHESIS IN MACROPHAGES: STIMULATION BY β -VERY LOW DENSITY LIPOPROTEINS FROM CHOLESTEROL-FED ANIMALS OF SEVERAL SPECIES. R.W. Mahley, T.L. Innerarity, M.S. Brown, Y.K. Ho and J.L. Goldstein (Gladstone Foundation Laboratories for Cardiovascular Disease, University of California, San Francisco, San Francisco, CA 94140) *J. Lipid Res.* 21, 970-80 (1980). Animals fed cholesterol accumulate several types of cholesterol-rich lipoproteins in their plasma and ultimately develop cholesteryl ester deposition in tissue macrophages. We demonstrate that β -VLDL obtained from cholesterol-fed animals of several other species, including monkeys, rabbits, and rats, also causes cholesteryl ester accumulation in monolayers of mouse peritoneal macrophages, as monitored by an increase in the rate at which the cells incorporate exogenous [14 C]oleate into cholesteryl [14 C]oleate. The β -VLDL from these three other species were effective at low concentrations and exhibited saturation kinetics, suggesting that they entered macrophages by receptor-mediated endocytosis. The current findings suggest that β -VLDL from cholesterol-fed animals has the general property of stimulating cholesteryl ester synthesis and accumulation in macrophages.

EFFECTS OF BIOTIN ON LIPIDS AND OTHER CONSTITUENTS OF PLASMA OF HEALTHY MEN AND WOMEN. M.W. Marshall, P.G. Kliman, V.A. Washington, J.F. Mackin and B.T. Weinland (USDA, SEA, Human Nutrition Center, Nutrition Institute Lipid Nutrition Laboratory, Beltsville, MD 20705) *Artery* 7, 330-51 (1980). A double-blind study of the effects of biotin supplementation (0.9 mg/day) of self-selected diets on plasma lipids and other plasma constituents was carried out in 40 men and women, age 30 to 60 years, for 71 days. Comparison of percent change from control levels showed significant treatment effects on more plasma constituents than did comparison of the means of the actual levels. In an analysis of period by period changes, the largest differences were observed generally during the first two weeks after supplementation when positive changes for biotin-treated men and women differed significantly from changes for the placebo-treated in total lipid, total phospholipid, and alpha + beta lipoprotein cholesterol. At the end of the study, these levels were at or below initial levels. Plasma biotin levels were elevated by biotin supplementation. There was a negative correlation between biotin levels and total plasma lipids. Responses of some lipid constituents of plasma were greater in volunteers who initially had normal levels of lipids. It is concluded that human requirements for biotin should be studied by new approaches and with modern techniques. Questions raised regarding the role of biotin in the lipid metabolism of normal men and women should be investigated further.

PLATELET FUNCTIONS AND FATTY ACID COMPOSITION OF PLATELET PHOSPHOLIPIDS IN SPONTANEOUSLY HYPERTENSIVE RATS FED SATURATED OR POLYUNSATURATED FATS. L. McGregor and S.R. Renaud (INSERM, Unit 63, 22 Ave Doyen Lepine, 69500 Lyon-Bron, France) *Atherosclerosis* 38, 129-36 (1981). Spontaneously hypertensive rats as compared to their normotensive controls presented a markedly higher platelet activity both in coagulation and aggregation, as triggered by thrombin. By comparing animals fed saturated or polyunsaturated fat, it could be observed that the saturated fat diet induced similar

results on platelet functions to those observed in hypertension. In addition, the saturated fat diet further increased the platelet response of the hypertensive animals. The most significant change induced by the saturated fat diet in the fatty acid composition of the platelet phospholipids was an increase in 20:3 ω 9, further enhanced in the hypertensive animals. In the polyunsaturated diet-fed rats, it was mostly 20:4 which was more elevated in the platelet phospholipids of the hypertensive. As a result, it was the sum of 20:3 ω 9 + 20:4 in the platelet phospholipids, which appeared to be the most significantly related, in the 4 groups of animals, to the response of platelets to thrombin induced aggregation.

CHOLESTEROL ABSORPTION IN MAN: EFFECT OF ADMINISTRATION OF CLOFIBRATE AND/OR CHOLESTYRAMINE. D.J. McNamara, N.O. Davidson, P. Samuel, and E.H. Ahrens, Jr. (Rockefeller University, New York, NY 10021) *J. Lipid Res.* 21, 1058-64 (1980). Cholesterol absorption measurements were carried out in a free-living out-patient population by a plasma isotope-ratio method. The method was applied in 150 hyperlipidemic male out-patients, ingesting a standardized diet containing 250mg cholesterol per day, who had been randomized into four different drug-treatment groups: 1) no medication, 2) clofibrate, 3) cholestyramine, or 4) both clofibrate and cholestyramine. Cholesterol absorption (as percent of the oral dose) was increased in patients receiving cholestyramine and decreased in those receiving clofibrate; the group on the combined medication had the same percent absorption as the control group. Pre-test administration of cholestyramine caused a 38% decrease in cholesterol absorption. These results demonstrate that the isotope-ratio method of measuring cholesterol absorption is a reproducible procedure applicable to a free-living out-patient population, and that the hypolipidemic drugs, clofibrate and cholestyramine, significantly affect cholesterol absorption in man. The data also show that the results of measurements of cholesterol absorption can be profoundly altered by the type and timing of medication in relationship to the test meal of labeled cholesterol.

BIOSYNTHETIC CONTROL OF THE NATURAL ABUNDANCE OF CARBON 13 AT SPECIFIC POSITIONS WITHIN FATTY ACIDS IN ESCHERICHIA COLI. EVIDENCE REGARDING THE COUPLING OF FATTY ACID AND PHOSPHOLIPID SYNTHESIS. K.D. Monson and J.M. Hayes (Biogeochemical Labs. and Depts. of Chem. and Geol., Indiana Univ., Bloomington, IN 47405) *J. Biol. Chem.* 255, 11435-41 (1980). Stable carbon isotope ratios (13 C/ 12 C) at natural abundance levels have been determined for individual carbon atoms in each of the major phospholipid fatty acids of *Escherichia coli* grown on glucose as the sole carbon source. Two models were constructed for the isotope effects and carbon flow pathways which must be responsible for the observed isotopic fractionations. Depletion of carbon 13 in the carboxyl groups of myristic and palmitoleic acids (relative to carbonyl groups in precursor acyl-ACP's) was observed to occur at this branching site. Only one of the models was consistent both with this observation and with the observation that exogenous fatty acids are incorporated into phospholipids but are not elongated. The successful model has free fatty acid as the intermediate product coupling fatty acid biosynthesis to phospholipid synthesis. Essential to this pathway are those reactions catalyzed by thioesterases I and II as well as acyl-ACP synthetase, enzymes whose roles have previously been unknown *in vivo*.

PLACENTAL TRANSPORT OF *trans* FATTY ACIDS IN THE RAT. C.E. Moore and G.A. Dhopheshwarkar (Lab. of Nuclear Medicine and Radiation Biology, University of California, 900 Veteran Ave., Los Angeles, CA 90024) *Lipids* 15, 1023-8 (1980). Placental transport of 9-*trans* [1- 14 C] octadecenoic (elaidic) and 9-*trans*,1,2-*trans* [1- 14 C] octadecadienoic (linoelaidic) acids was demonstrated in rats. Differences in specific activities of plasma, placental and fetal total lipids indicated a decreasing concentration gradient for both *cis* and *trans* isomers of octadecenoic and octadecadienoic acids. Distribution of radioactivity in various lipid components was determined by thin layer chromatography. Irrespective of the label, the highest percentage of total radioactivity was carried by triglycerides (TG) in maternal plasma and was incorporated mainly in phospholipids (PL) of fetal tissues. A nearly equal distribution of the label was found between PL and TG of placental lipids. Radioactivity of fatty acid methyl esters (FAME) determined by radio-gas liquid chromatography indicated that after injection of linoelaidate, radioactivity of maternal plasma, placental and fetal tissue FAME was associated only with t,t-18:2. Following injection of elaidate, all the radioactivity

in placental FAME was associated with t-18:1; however, in fetal tissues, the label was distributed between 16:0 and t-18:1. These findings suggest that, in contrast to linoleic acid, rat fetal tissues can metabolize elaidic acid via β oxidation to form acetyl CoA and palmitic acid.

KETOGENESIS FROM OLEATE AND OCTANOATE IN ISOLATED RAT HEPATOCYTES. J.A. O'Donnell III and R.A. Freedland (Dept. of Physiological Sciences, School of Veterinary Med., Univ. of Calif., Davis, CA 95616) *The J. of Nutr.* 110, 2365-73 (1980). Isolated hepatocytes were prepared from 48-hour starved male rats and incubated for 45 minutes with either 2.0 mM oleate or 2.0 mM octanoate. In an attempt to clarify the mechanism of antiketogenesis seen with 10.0 mM lactate, pyruvate, fructose, glycerol, ethanol and acetaldehyde, the metabolic inhibitors of α -2-amino-4-methoxy-trans-3-butenoic acid, n-butyl malonate and 3-mercaptopicolinic acid were added separately to the incubations. Experimental design eliminated increased esterification of fatty acids with α -glycerolphosphate or carnitine transferase as potential mechanisms of antiketogenesis. Thus, availability of mitochondrial oxaloacetate and competitive oxidation were two potential mechanisms studied in these experiments. The data indicate that under the experimental condi-

tions of this study the control of ketogenesis is related to both the availability of mitochondrial oxaloacetate and competitive oxidation with the relative importance depending upon the specific antiketogenic agent present. Results obtained with combinations of antiketogenic agents plus metabolic inhibitors showed that: a) fructose and glycerol antiketogenicity depends largely on increasing mitochondrial oxaloacetate; b) lactate and pyruvate depend upon both mechanisms, and c) acetaldehyde and ethanol depend primarily on competitive oxidation.

HUMAN PLASMA LIPID RESPONSES TO RED MEAT, POULTRY, FISH AND EGGS. B.C. O'Brien and R. Reiser (Texas Agricultural Experiment Station, Texas A&M University System, College Station, Texas 77843) *Amer. J. Clin. Nutr.* 33, 2573-80 (1980). The effect on total plasma cholesterol and high-density lipoprotein (HDL) cholesterol concentrations of middle-aged men pursuing their normal activities while reducing cholesterol intake to 300 mg or less per day and substituting fish and poultry for red meat in the diet was assessed. Twenty-nine men, ages 39 to 61 years with plasma cholesterol concentrations below 240 mg/dl consumed for 6 weeks each of four diets: 1) red meat, no fish or poultry and 3 eggs daily; 2) red meat, no fish or poultry, and no eggs; 3) fish and poultry, no red meat, and no eggs; 4) fish and poultry, no red meat, and 3 eggs a day. Group I consumed the diets in the order given and group II followed the reverse order. Mean plasma cholesterol and HDL cholesterol concentrations in group I were not significantly altered by any of the test diets. On the other hand, average plasma cholesterol concentrations for group II at the end of each diet period were significantly higher when eggs were added to either flesh diet. As in group I, HDL: cholesterol levels in group II were not significantly affected by these diets. Neither group I nor group II showed a significantly different response to the ingestion of fish and poultry versus red meat. The results of this study suggest that conscientious adherence to a low cholesterol fish and poultry diet failed to change plasma cholesterol concentrations beyond the normal range of variability for the majority of these subjects.

THE EFFECT OF BILE ACIDS AND LIPASE ON ABSORPTION OF TALLOW IN YOUNG CHICKS. D. Polin, T.L. Wing, P. Ki and K.E. Pell (Dept. of Poultry Sci., Michigan St. Univ., East Lansing, MI 48824) *Poultry Sci.* 59, 2738-43 (1980). White Leghorn chicks were fed diets with 4% tallow supplemented with one of the following bile acids at .04%: cholic acid, chenodeoxycholic acid, dehydrocholic acid, deoxycholic acid, or sodium taurocholate. Cholic acid improved the absorption of tallow but not significantly; chenodeoxycholic acid significantly improved tallow absorption during days 0 to 7 but decreased it during days 14 to 21. The bile acids, dehydrocholic acid, deoxycholic acid, and sodium taurocholate had no significant effect on absorption of tallow. In a 2 x 3 factorial design involving cholic acid and lipase, .04% cholic acid and/or .10% lipase significantly improved the absorption of tallow by 8 and 4% in chicks 1 and 3 weeks of age, respectively. Dry matter digestibility and efficiency appeared to be improved with the improvement of lipid absorption.

PUBLICATIONS ABSTRACTED

- American Journal of Clinical Nutrition, 9650 Rockville Pike, Bethesda, MD 20014.
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- Biochemica et Biophysica Acta, P.O. Box 1345, 1000 B.H. Amsterdam, The Netherlands.
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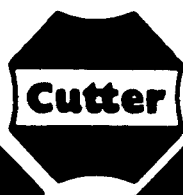
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