Abstracts

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Biochemistry and nutrition

REDUCED TRIGLYCERIDEMIA AND INCREASED HIGH DENSITY LIPOPROTEIN CHOLESTEROL LEVELS AFTER TREATMENT WITH ACIPIMOX, A NEW INHIBITOR OF LIPOLYSIS. C.R. Sirtori, G. Gianfranceschi, M. Sirtori, F. Bernini, G. Descovich, U. Montaguti, L.M. Fuccella, and L. Musatti (Instit. of Pharmacology and Pharmacognosy, Univ. of Milan, Milan and II. Medical Clinic, Univ. of Bologna, Bologna, Italy) *Atherosclerosis 38*(3,4):267-71 (1981). Acipimox (5-methylpyrazine carboxylic acid 4-oxide) is a new inhibitor of lipolysis with long-lasting activity, whose plasma lipid lowering potential was demonstrated in early clinical trials. The hypolipidemic effect of acipimox was investigated in two double-blind cross-over trials versus placebo. The first trial, carried out in 12 type IV patients, showed a significant triglyceride lowering effect (-35%) following 4 weeks of drug administration at a 250 tid dose. The same regimen, maintained for 9 weeks in 18 type IIa patients, failed to induce a significant reduction of total cholesterolemia. However, in 10 subjects, in whom lipoprotein cholesterol fractionation was carried out, a significant reduction of low density and a highly significant increase in high density lipoprotein cholesterol levels (respectively -11% and +20%) were observed.

THE FATE OF CHOLESTERYL LINOLEYL ETHER AND CHOLESTERYL LINOLEATE IN THE INTACT RAT AFTER INJECTION OF BIOLOGICALLY LABELED HUMAN LOW DENSITY LIPOPROTEIN. Y. Stein, G. Halperin and O. Stein (Lipid Research Laboratory, Dept. of Medicine B, Hadassah University Hospital, Jerusalem, Israel) *Biochim. Biophys. Acta 663*(2):569-574 (1981). In vitro labeling of low density lipoproteins (LDL) with [7α(n)-^{3}H] cholesteryl linoleyl ether, and with [4-^{14}C] cholesteryl linoleate was achieved by a modification of the method developed for labeling of very low density lipoproteins. [^{3}H] Cholesteryl linoleyl ether and [^{14}C] cholesteryl linoleate were cosonicated with partially delipidated high density lipoprotein (HDL) and the HDL was purified by centrifugation at d = 1.063. LDL was labeled by incubation of the labeled HDL in the presence of the d > 1.25 fraction of human plasma and reisolated at d = 1.063. The ^{3}H/^{14}C ratio in the labeled LDL was the same as in the HDL. The labeled LDL had the same lipid composition and ultrastructural appearance as the non-incubated LDL. After injection into rats, both labels disappeared at similar rates and the $t_{1/2}$ between 1-24 h was 7.0 h. Up to 8 h after injection of labeled LDL, 94-97% of ^{3}H and ^{14}C radioactivity in the plasma was precipitable by heparin-manganese. 24 h after injection, 28% of the [^{3}H] cholesteryl linoleyl ether was recovered in the liver, 6% in small intestine and 34% in the carcass, and the rest was distributed among all other ogans; total recovery of ^{3}H label was 89 ± 3.0%. The present findings indicate that as in the rat there is no transfer of esterified cholesterol among plasma lipoproteins, LDL is catabolized by both the liver and extrahepatic tissues.

BILE LIPID ALTERATIONS IN TAURINE-DEPLETED MONKEYS. Z.F. Stephan, M.J. Armstrong, and K.C. Hates (Dept. of Nutrition, Harvard Schl. of Public Health, 665 Huntington Ave., Boston, MA 02115) *Am. J. Clin. Nutr. 34*(2):204-210 (1981). Newborn cebus and cynomolgus monkeys, differing in their ingerent taurine-glycine conjugation of bile acids, were fed taurine-free soy protein infant formula (Isomil) with or without added taurine. Taurine depletion in cebus monkey, an obligate taurine cojugator (97%), did not reduce bile acid conjugation with taurine, or did it alter bile acid pool size, biliary lipid composition, or theoretical maximal cholesterol solubility in bile. Conversely, taurine depletion in cynomolgus, a species normally conjugating with some glycine, significantly reduced conjugation of taurine with bile acids from 84 to 64%, essentially doubling that of glycine from 16 to 36%. Furthermore, theoretical maximal cholesterol solubility in cynomolgus bile improved significantly as a result of taurine depletion. This improvement was associated with increased percentage distribution of biliary phospholipid from 17 to 33%, in turn reflecting an increase in the taurochenodeoxycholate to taurocholate ratio from 0.7 to 2.9. Concomitant increase in biliary cholesterol concentration associated with increased glycine conjugation precluded any changes in the percent saturation of bile which remained constant at 130% for both dietary groups of cynomolgus. Taurochenodeoxycholate uniquely conserved taurine in the face of body taurine depletion. Taurine availability thus potentially has a substantial influence on bile acid characteristics and cholesterol solubility in a glycine conjugating primate.

AGE-STRAIN INTERRELATIONS IN LIPID METABOLISM OF RATS. J.A. Story, E. Gomolinski, S.K. Czarnecki, S.A. Tepper and D. Kritchevsky (Wistar Institute of Anatomy and Biology, Philadelphia, PA 19104) Various aspects of lipid metabolism were compared in Fisher 344 (F) and Sprague-Dawley (SD) rats aged 2, 6, 12, 18 and 24 months. The analyses included free and total cholesterol of serum and liver, LCAT, hepatic HMG-CoA reductase, cholesterol 7α-hydroxylase, fatty acid synthetase, acetyl CoA carboxylase and cholesterol synthesis from acetate or mevalonate. The body weight of SD rats increases with age whereas that of F rats plateaus at 9-12 months. Liver and aorta cholesterol levels were comparable for the 2 strains. Serum cholesterol varied but was usually lower in F rats. HMG-CoA reductase and cholesterol 7α-hydroxylase activities were not significantly different. Cholesterol synthesis from acetate was significantly higher only in 2-month-old F rats; synthesis from mevalonate was similar at each level. Acetyl CoA carboxylase and fatty acid synthetase activity were generally higher in F rats at every age level. The major difference between F and SD rats is in their pattern of weight gain with age. Differences in lipid metabolism are most marked between the young (2-month) rats.

INCORPORATION OF PHOSPHATIDYLCHOLINE INTO SPHERICAL AND DISCOIDAL LIPOPROTEINS DURING INCUBATION OF EGG PHOSPHATIDYLCHOLINE VESICLES WITH ISOLATED HIGH DENSITY LIPOPROTEINS OR WITH PLASMA. A.R. Tall and P.H.R. Green (Gastroenterology Division, Dept. of Medicine, Columbia University, College of Physicians and Surgeons, New York, NY 10032) *J. Biol. Chem. 256*(4): 2035-44 (1981). Incubation of phosphatidylcholine vesicles with isolated high density lipoproteins (HDL) or with whole plasma results in transfer of phospholipid into HDL. To investigate the mechanisms of this process, small unilamellar vesicles of egg phosphatidylcholine were incubated with human HDL_3 or with plasma and were subsequently analyzed by density gradient ultracentrifugation and negative stain electron microscopy. Incubation of vesicles with isolated HDL_3 resulted in formation of both discoidal lipoproteins and phospholipid-enriched spherical lipoproteins during incubation of vesicles with isolated HDL_3, there is insertion of phospholipid into HDL_3, producing spherical lipoproteins which have some properties in common with HDL_2. Discoidal lipoproteins are also formed, probably as a result of interaction of vesicles with small amounts of apoA-I and apoA-II released from HDL_3. The uptake of phosphatidylcholine by HDL in whole plasma occurs by similar mechanisms.

THE EFFECT OF DIFFERENT PROPORTIONS OF CASEIN IN SEMIPURIFIED DIETS ON THE CONCENTRATION OF SERUM CHOLESTEROL AND THE LIPOPROTEIN COMPOSITION IN RABBITS. A.H.M. Terpstra, L. Harkes and F.H. van der Veen (Dept. of Human Nutr., Agricultural Univ., De Dreijen 11, 5703 BC Wageningen, The Netherlands) *Lipids 16*(2):114-9 (1981). The effect of different proportions of casein in semipurified diets on the concentration of serum cholesterol and the lipoprotein composition was studied in rabbits. Low-casein diets (10% w/w) resulted in serum cholesterol levels and growth rates that were lower than high-casein diets (40% w/w). An intermediate proportion of casein (20%) produced intermediate concentrations of serum cholesterol, but only minor differences in food intake and weight gain, compared with the high-casein group. In the animals with the highest values of total serum cholesterol (the 40% casein group), most of the serum cholesterol was transported in the very low density lipoprotein, whereas with moderate hypercholesterolemia (the 20% casein group), the low density lipoproteins were the main carriers of cholesterol. Elevation in lipoprotein cholesterol was associated in all groups with an increased ratio of cholesterol to protein, suggesting the formation of particles relatively rich in cholesterol. When the rabbits on the diet containing 10% casein were subsequently transferred to the 40% casein diet, a steep increase in the level of serum cholesterol occurred. Conversely, switching the rabbits on the 40%

casein diet to the 10% casein diet resulted in a decrease in the level of serum cholesterol.

RELATIONSHIP OF RAISED ATHEROSCLEROTIC LESIONS TO FATTY STREAKS IN CIGARETTE SMOKERS. R.E. Tracy, V.T. Toca, J.P. Strong and M.L. Richards (Dept. of Path., Louisiana State Univ. Medical Center, New Orleans, LA 70112) *Atherosclerosis 38*(3,4):347-57 (1981). Intimal surfaces of the 3 main coronary arteries combined and of the abdominal aorta, sudan-stained, were described by visual inspection as divided into normal intima (N), fatty streaks (F) and raised lesions (R) expressed as percentages. Subjects were 1320 black and white men aged 25-64 years autopsied in New Orleans. Histories of cigarette smoking, elicited by interview of wives or other associates of each deceased subject, were summarized as average number of cigarettes used per day during the preceding 10 years. The results were examined in terms of the model $N \rightleftarrows F \rightarrow R$ in which Class A causes are viewed as promoting the process from beginning to end while Class B agents act at the first or the second step but not both. Of many possible ways to interpret the patterns which were found, one especially simple formulation is compatible with the model. This interpretation suggests that (1) cigarette smoking had a large Class B effect, (2) a racial trait attached greater Class B atherogenesis to whites than to blacks, and (3) Class A effects were alike in both racial groups and all smoking categories. The interpretation further leads to the suggestion that the target tissue of smoking is the fatty streak such that slowly progressing or regressing fatty streaks, formed alike in smokers and non-smokers, are caused by smoking to cease to regress and to progress more rapidly.

SERUM LIPIDS, LIPOPROTEIN LIPIDS AND CORONARY HEART DISEASE IN PATIENTS WITH XANTHELASMA PALPEBRARUM. A. Watanabe, A. Yoshimura, T. Wakasugi, R. Tatami, K. Ueda, T. Haba, T. Kametani, J. Koizumi, S. Ito, M. Ohta, S. Miyamoto, H. Mabuchi and R. Takeda (Second Dept. of Internal Medicine, School of Medicine, Kanazawa University, Kanazawa, Japan) *Atherosclerosis 38*(3,4): 283-90 (1981). Serum lipids and lipoprotein lipids were studied in 53 patients with xanthelasma palpebrarum and 40 age-matched normal controls (NC). Patients were subdivided into patients with normolipidemia, hyperlipidemia or familial hypercholesterolemia (FH). In both male and female patients with hyperlipidemia or FH, the serum cholesterol (Chol) levels were significantly higher (SH) than in NC. In both male and female patients with normolipidemia or hyperlipidemia, the VLDL-Chol levels were SH than in NC. Male patients with FH showed SH levels of VLDL-chol than NC. Both male and female patients with normolipidemia, hyperlipidemia or FH showed SH levels of VLDL-chol, lower HDL-Chol levels and lower HDL-Chol/LDL-Chol ratios than NC. In both male and female patients with hyperlipidemia and in male patients with FH, the serum triglyceride (TG) levels were SH than in NC. Both male and female hyperlipidemic patients showed SH levels of VLDL-TG than NC. In male patients with FH, the VLDL-TG levels were significantly above the control levels. In male patients with normolipidemia, the LDL-TG levels were SH than in NC. In both male and female patients with hyperlipidemia or FH, the LDL-TG levels were SH than in NC. The HDL-TG levels in patients with normolipidemia (males) or FH (females) were significantly lower than in NC. The prevalence of coronary heart disease in patients with normolipidemia, hyperlipidemia or FH was 29.4%, 24.0% and 45.4%, respectively.

EFFECT OF DIETARY FAT AND CHOLESTEROL ON MILK COMPOSITION, MILK INTAKE AND CHOLESTEROL METABOLISM IN THE RABBIT. B.J. Whatley, J.B. Green and M.H. Green (Nutrition Program, The Pennsylvania State University, University Park, PA 16802) *J. Nutr. 111*(3:432-41 (1981). Sixteen female rabbits were fed one of four diets during lactation: 1) a commercial stock diet; 2) the stock diet with 5% added lard; 3) the stock diet with 5% lard and 0.25% cholesterol; or 4) the stock diet with 15% lard and 1% cholesterol. By days 30-35 of lactation, maternal plasma cholesterol concentrations were increased approximately 10-fold in group 3 and 100-fold in group 4 does compared to does in group 1. Milk cholesterol concentration was similar over lactation for does in groups 1, 2 and 3, but was approximately 2 times higher in group 4 does. Milk triglyceride and protein concentrations and milk intake by the pups were not significantly influenced by maternal diet. Average cholesterol intake of pups nursed by group 4 does was significantly higher than that of other pups. Although plasma cholesterol concentration was significantly increased in group 2, 3 and 4 pups at weaning (age 5 weeks), there was no significant effect of maternal diet on plasma cholesterol at 6, 7 or 11 weeks of age after pups had been weaned to the stock diet. Similarly, liver cholesterol concentration was increased in pups from group 4 does at weaning, but these differences were no longer apparent at 11 weeks of age. These data suggest that severe maternal hypercholesterolemia induced by dietary fat and cholesterol in the rabbit can result in increased milk cholesterol concentration and consequent cholesterol intake by the pups. However, the associated elevations in pup plasma and liver cholesterol levels do not persist when pups were weaned to a low fat/low cholesterol diet.

VALIDATION OF A DIETARY RECORD SYSTEM FOR THE ESTIMATION OF DAILY CHOLESTEROL INTAKE IN INDIVIDUAL OUTPATIENTS. E.C. White, D.J. McNamara, and E.H. Ahrens (Rockefeller University, New York, NY 10021) *Am. J. Clin. Nutr. 34*(2):199-203 (1981). In order to develop a reliable system for measuring daily chnolesterol intake in individual outpatients, studies were undertaken to establish the shortest time period (in days) for which it is necessary to obtain daily food intake records. Three volunteers were trained in dietary record-keeping and portion-size assessment, and instructed to self-select a low-cholesterol diet for 20 days. During the study period they maintained daily dietary records and collected dummy diets. Comparisons of cholesterol intake calculated from the dietary records (mean 144 mg/day, SD ± 13, n=60) to the values from chemical analysis (118 ± 28 mg/day) demonstrated that the calculated values were higher (mean 19%). More importantly, it was found that a minimum of 9 days' records of dummy diet analyses were required in order to reach an estimate of daily cholesterol intake that varied by less than 10% from the mean of the 20-days' values. In 100 outpatients trained to adhere to a moderately low-cholesterol intake and who maintained sequential dietary records for 9 days, it was found that the mean daily intake was 251 mg/day but that individual patients exhibited substantial daily variations in cholesterol intake (average coefficient of variation = 54%, range = 8.5 to 121.2%). These results demonstrate that, under conditions of training in dietary record-keeping and portion-size assessment, adherence to a low-cholesterol diet, and with collection of at least 9 days of dietary records, a reliable quantitative estimate of daily dietary cholesterol intake can be obtained in free-living outpatient populations.

NADH INHIBITION AND NAD ACTIVATION OF *ESCHERICHIA COLI* LIPOAMIDE DEHYDROGENASE CATALYZING THE NADH-LIPOAMIDE REACTION. K.D. Wilkinson and C.H. Williams, Jr. (Veterans Administration Medical Center and Department of Biological Chemistry, The University of Michigan, Ann Arbor, Michigan 48105) *J. Biol. Chem. 256*(5):2307-2314 (1981). A unique form of inhibition by NADH and partial reversal by NAD^+ has been demonstrated with *Escherichia coli* lipoamide dehydrogenase. Substrate inhibition by NADH is consistent with its reduction of the active two-electron reduced enzyme intermediate to the inactive four-electron reduced form. NAD^+ partially overcomes this inhibition by mass action reversal of this reduction. NAD^+ activation is only partial since the presence of both NAD^+ and NADH forces the accumulation of two binary enzyme-pyridine nucleotide complexes. These are intermediates in the two-electron to four-electron reduction of the enzyme and thus are not on the catalytic pathway. NAD^+ is also shown to inhibit by binding to the oxidized enzyme to give a dead-end complex. From the steady state rate equations, it is apparent that the degree of inhibition will depend on the oxidation-reduction potential for two-to four-electron reduction of the enzyme. Thus, the wide variation in the severity of NADH ianhibition between the *E. coli* and pig heart enzymes is explained by quantitative differences in the basic lipoamide dehydrogenase mechanism. A possible phsyiological role for this type of inhibition as a mechanism of control in *E. coli* is discussed.

HYPERLIPIDEMIA IN GUINEA-PIGS INDUCED BY ASCORBIC ACID DEFICIENCY—THE EFFECTS OF CHOLESTEROL, DL-ETHIONINE AND AFLATOXIN. F. Yokota, Y. Igarashi, and R. Suzue (The National Institute of Nutrition, 1, Toyama-cho, Shinjuku-ku, Tokyo 162, Japan) *Atherosclerosis 38* (3,4):249-54 (1981). A study was made of hyperlipidemia caused by ascorbic acid deficiency and of the effects of cholesterol, DL-ethionine and aflatoxin on plasma lipoprotein fractions of normal and scorbutic guinea pigs. The plasma lipoprotein fractions of scorbutic animals showed a significantly higher level of pre-β-lipoprotein and a lower level of α-lipoprotein. By adding DL-ethionine to the control group, the pre-β-lipoprotein fraction was remarkably elevated and by adding cholesterol, the α-lipoprotein level was greatly reduced and the β-lipoprotein level was increased. Addition of aflatoxin to the control diet resulted in a rather high concentration of α-lipoprotein and a low pre-β-lipoprotein level. High concentrations of triglyceride and phospholipid were seen in the plasma of scorbutic guinea pigs. The probable cause of hyperlipidemia induced by ascorbic acid deficiency is partly retarded degradation of cholesterol resulting from imparied 7α-hydroxylation, and partly that ascorbic acid deficiency may affect other enzyme systems that control triglyceride of phospholipid metabolism, such as lipoprotein lipase activity, or synthesis of breakdown of these enzymes.

ABDOMINAL AND CARCASS FAT IN FIVE BROILER STRAINS. W.A. Becker, J.V. Spencer, L.W. Mirosh, and J.A. Verstrate (Department of Animal Sciences, Department of Food Science and Tech-

nology, Washington State University, Pullman, Washington 99164) *Poultry Sci. 60*(4):693-7 (1981). Ten males and 10 females from each of five commercial broiler strains were weighed and slaughtered at 55 days of age. Over all, mean live body weight was 2112 g for males and 1702 g for females and abdominal fat was 2.9% (males) and 3.3% (females). Mean total fat in whole bird was 13.4% (males) and 15.5% (females). There were no statistically significant differences between strains at the 5% level. Correlation coefficients with percent abdominal fat were .29 for body weight (males) and .36 (females), for percent carcass fat .51 (males) and .77 (females), and for fat free carcass .26 (males) and .07 (females). Abdominal fat represented 22% of the total fat for males and females. The results obtained were similar to those found previously in this laboratory with one strain of broilers.

RELATIONSHIPS BETWEEN CHOLECALCIFEROL METABOLISM AND GROWTH IN CHICKS AS MODIFIED BY AGE, BREED AND DIET. A. Bar and S. Hurwitz (Institute of Animal Science, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel) *J. Nutr. 111*(3):399-404 (1981). Fast-growing heavy (White Rock) chicks, fed a vitamin D-deficient diet, exhibited a higher activity of kidney 25-hydroxycholecalciferol-1-hydroxylase (1-hydroxylase) than slow-growing light (White Leghorn × Rhode Island Red) chicks fed the same diet. 1-Hydroxylase and duodenal calcium-binding protein (CaBP) declined with age. Feeding of low energy diets with or without vitamin D resulted in a slower rate of growth and reduced 1-hydroxylase activity and CaBP concentration. Severe dietary restriction of either calcium or phosphorus resulted in a lower growth rate as well as a duodenal CaBP as compared to a moderate mineral restriction. The severe dietary calcium restriction also resulted in a lower 1-hydroxylase activity than that resulting from a moderate restriction. The results clearly indicate that high 1-hydroxylase activity and a high intestinal CaBP are associated with a high growth rate.

THE ROLE OF VITAMIN E IN THE NUTRITION OF PREMATURE INFANTS. E.F. Bell and L.J. Filer, Jr. (Dept. of Pediatrics, Univ. of Iowa College of Medicine, Iowa City, IA 52242) *Am. J. Clin. Nutr. 34*(3):414-22 (1981). Vitamin E (α-tocopherol) has been credited with a variety of beneficial effects in the premature newborn infant. It has been thought that deficiency of vitamin E is at least partly responsible for the anemia which often occurs 4 to 6 wk after premature birth, and routine dietary supplementation with vitamin E is frequently recommended. However, critical analysis reveals that published controlled studies of vitamin E supplementation do not agree on the magnitude or even the existence of this protective effect against anemia. Analysis of commonly used feeding practices suggests that the dietary ratio of α-tocopherol to polyunsaturated fatty acids is generally sufficient to prevent manifestations of vitamin E deficiency without supplementation. Large parenteral doses of vitamin E have been purported to protect premature infants exposed to oxygen-enriched environments and mechanical ventilation from the complications of retrolental fibroplasia and bronchopulmonary dysplasia. Subsequent studies, however, have not yet substantiated encouraging early reports of these protective effects. At present, there seems to be no clearly established need for supplementing the premature infant's usual dietary intake of vitamin E.

SELECTION OF A STRAIN OF RATS WITH SPONTANEOUS HIGH CHOLESTEROLEMIA. J.P. Boissel, B. Crouzet, M.C. Bourdillon and N. Blaes (INSERM, Unite 63, 22 Avenue du Doyen Lepine, 69500 Bron, France) *Atherosclerosis 39*(1):11-18 (1981). By means of an inbred selection procedure utilizing positive assortative mating, high (SHC: spontaneous high cholesterolemic) developed. This procedure was shown to be much more efficient in increasing than in lowering the blood cholesterol level. The diet used throughout selection was normal laboratory chow; therefore the high and low blood cholesterol levels occurred spontaneously. Since the 6th generation there has been no overlap between the blood cholesterol values of the animals of the two strains. The cholesterol increase with aging was found to be strongly related to sex, but weakly to the genes governing the differences between SHC and SLC rats. Cholesterol enhancement following a hyperlipidic diet did not differ between strains or sexes. It appears that the SHC rat strain could be an interesting model, particularly in pharmacological research.

TRIACYLGLYCEROL HYDROLYSIS BY CELLS ISOLATED FROM LACTATING RAT MAMMARY GLAND. R.A. Clegg (Dept. of Biochem., The Hannah Res. Inst., Ayr, KA 6 5HL, U.K.) *Biochim. Biophys. Acta 663*(3):598-612 (1981). The hydrolysis of exogenous trioleoylglycerol emulsions by suspensions of cells prepared from lactating rat mammary gland has been investigated. Cell integrity remains high throughout short incubations, during which extracellular hydrolysis of trioleoylglycerol proceeds at a mean rate of 1.9 nmol oleate released/min per mg protein. This hydrolysis shows partial dependence upon added serum and partial inhibition by protamine sulphate—both characteristic properties of lipoprotein lipase-catalyzed lipolysis. Evidence is presented consistent with the hypothesis that a surface-located lipoprotein lipase is responsible for the observed lipolysis. During trioleoylglycerol hydrolysis, non-esterified oleate does not accumulate in the cells or in the medium in quantities stoicheiometric with glycerol release. Analyses indicate that it passes into the cells without prior equilibration with the extracellular oleate pool(s). The possible relevance of these findings to the physiological mechanism of fatty acid uptake from triacylglycerol at the capillary endotheium is discussed.

THE ROLE OF PHOSPHOLIPID AND FACTOR $VIII_a$ IN THE ACTIVATION OF BOVINE FACTOR X. G. van Dieijen, G. Tans, J. Rosing and H.C. Hemker (Department of Biochemistry, Biomedical Centre, State University of Limburg, Maastricht, The Netherlands) *J. Biol. Chem. 256*(7):3433-42 (1981). The kinetic parameters of bovine factor X activation by bovine factor IX_a have been determined in the absence and presence of Ca^{2+}, thrombin-activated bovine factor VIII ($VIII_a$), and phospholipid. Factor IX_a in the absence of Ca^{2+}, factor $VIII_a$, and phospholipid is able to catalyze factor X activation. Addition of Ca^{2+} has little effect on the kinetic constants of factor X activation by factor IX_a. The presence of 10 μM phospholipid dramatically decreases the K_m for factor X. The V_{max} of factor X_a formation slightly increases when more phospholipid is present in our experiments, and there is a considerable increase of the K_m for factor X at higher phospholipid concentrations. Therefore, the K_m measured in the presence of phospholipid has to be regarded as an apparent K_m. The possible explanations for this phenomenon are discussed. In order to exert its stimulating effect on factor X activation factor VIII has to be activated with thrombin. Our results show that factor IX_a is an enzyme which can activate factor X at a very low rate. The stimulating effect of phospholipid in factor X activation is mainly due to an effect on the K_m for factor X, bringing it within the range of the plasma concentration. The stimulatory effect of factor $VIII_a$ is explained by its 200,000-fold increase of the V_{max} of factor X_a formation.

CHANGES IN FATTY ACYL CHAIN COMPOSITION OF RAT HEART PHOSPHOLIPIDS INDUCED BY NORADRENALINE. A. Emilsson and S. Gudbjarnason (Department of Chemistry, Science Institute, University of Iceland, Dunhaga 3, Reykjavik, Iceland) *Biochim. Biophys. Acta 664*(1):82-8 (1981). The effect of noradrenaline on fatty acyl chain composition of rat heart phospholipids was studied in vivo. The rats received increasing amounts of noradrenaline for 15 days. The noradrenaline stress caused significant alterations in the fatty acyl chain composition of the two major phospholipids in heart muscle, whereas the phospholipid content remained unchanged. In phosphoatidylcholine, there was 50% diminution in linoleic acid and a decrease in oleic acid with a concomitant increase in stearic, arachidonic and docosahexaenoic acids. In phosphatidylethanolamine, the docosahexaenoic acid increased by 25% accompanied by a decrease in oleic and arachidonic acids. The possible causes and consequences of these changes are discussed.

LIPOPROTEINS OF FETAL AND NEWBORN CALVES AND ADULT STEER: A STUDY OF DEVELOPMENTAL CHANGES. T.M. Forte, J.J. Bell-Quint and F. Cheng, Donner Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720) *Lipids 16*(4):240-5 (1981). Serum lipoproteins in fetal and newborn calves were characterized and compared with those of adult animals. Fetal calf serum contains only low density (LDL) and high density (HDL) lipoproteins; the LDL is the major lipoprotein class. Fetal LDL are ca. 36.0 nm diameter and are morphologically unusual in that particles form linear aggregates or "chains" in which LDL have flattened, parallel sides. These particles contain only apolipoprotein B and are high in polar lipids. Fetal HDL consist of 8.2-nm, round particles which contain large amounts of cholesteryl ester thus suggesting an active lecithin: cholesterol acyltransferase system in the fetal state. The major protein in fetal HDL is apolipoprotein A-I (80%); however, another component with a molecular weight (MW) of ca. 9,000 is also present. Newborn calves possess very low density (VLDL) lipoproteins which have a mean diameter of 61 nm and are similar in size and composition to those of adult animals; their apolipoprotein composition is principally apolipoprotein B, although C-apolipoproteins and apolipoprotein A-I are also present. The LDL of neonatal and adult animals are round particles ca. 19.0 nm diameter which contain less polar lipids than the fetal animal. Apolipoprotein B is the major protein in newborn LDL, but adult LDL additionally contains a protein of 27,000 MW which probably represents apolipoprotein A-I from overlapping α-migrating particles in this region. The altered morphology and composition of fetal LDL, together with the lack of VLDL, suggest that the LDL particles may be synthesized de novo.

DIFFERENTIAL EFFECTS OF DIETARY FATTY ACIDS ON THE ACCUMULATION OF ARACHIDONIC ACID AND ITS METABOLIC CONVERSION THROUGH THE CYCLOOXYGENASE AND LIPOXYGENASE IN PLATELETS AND VASCULAR TISSUE. C. Galli, E. Agradi, A. Petroni and E. Tremoli (Inst. of Pharmacology and Pharmacognosy, Univ. of Milan, Via A. Del Sarto 21 20129 Milan, Italy) *Lipids 16*(3):165-72 (1981). Semisynthetic diets containing either corn oil (CO) or butter (B) (11 and 2.2 in % as linoleic acid, respectively) were fed to male rabbits for periods of 3 weeks and 3 months. The CO diet, in respect to the B diet, induced higher levels of linoleic acid (LA) and lower levels of arachidonic acid (AA) in platelet phospholipids, lower levels of AA in aortic phosphatidylinositol (PI) and accumulation of both LA and AA in liver lipids. The threshholds for aggregation with AA, but not with collagen, were higher in the CO group and the formation of thromboxane B_2 (TXB_2) from [^{14}C] AA, but not from endogenous substrate after collagen stimulation, was lower in the same group. Formation of PGE_2-like material by incubated aortas was higher in the B group. In the CO group, platelet cyclooxygenase appeared to be selectively depressed. The correlations among diet-induced fatty acid changes in platelet and aortic lipids, platelet aggregation and thromboxane and prostacyclin formation are discussed.

STRUCTURE OF *ESCHERICHIA COLI* MEMBRANES. GLYCEROL AUXOTROPHS AS A TOOL FOR THE ANALYSIS OF THE PHOSPHOLIPID HEAD-GROUP REGION BY DEUTERIUM MAGNETIC RESONANCE. H.U. Gally, G. Pluschke, P. Overath, and J. Seelig (Department of Biophysical Chemistry, Biocenter, University of Basel, CH-4056 Basel, Switzerland) *Biochemistry 20*(7):1826-31 (1981). Glycerol selectively deuterated at various positions was synthesized and supplied to the growth medium of *Escherichia coli* strain T131 GP, which is defective in endogenous glycerol synthesis as well as glycerol degradation and lacks the ability to synthesize cardiolipid. The procedure enables the stereospecific labeling of the membrane phospholipids (∿80% phosphatidylethanolamine, ∿20% phosphatidylglycerol). Deuterium magnetic resonance spectra were obtained for cell membranes and lipid dispersions either from total lipid extracts or from purified phosphatidylglycerol or ethanolamine. When glycerol deuterated at various positions was used, all resonances of the phospholipid glycerol backbone and the terminal glycerol moiety in phosphatidylglycerol could be assigned. The results indicate that the molecular conformation of the glycerol backbone is independent of the phospholipid species investigated and is also not altered by the presence of high amounts of membrane proteins. For the quantitative interpretation of the deuterium magnetic resonance splittings, a model is proposed which assumes essentially free rotation around the glycerol C(2)-C(3) bond combined with an asymmetric and restricted jump process around the C(1)- C(2) bond. This model is compatible with known X-ray structures of phospholipid molecules. The two deuterons of both the glycerol backbone C(1) and c(3) segments were found to be magnetically inequivalent. Stereoselective monodeuteration eliminated one set of quadrupole splittings in both cases.

THE PARTICIPATION OF STEROL CARRIER PROTEIN$_2$ IN THE CONVERSION OF CHOLESTEROL TO CHOLESTEROL ESTER BY RAT LIVER MICROSOMES. K.L. Gavey, B.J. Noland and T.J. Scallen (Dept. of Biochem., School of Medicine, University of New Mexico, Albuquerque, New Mexico 87131) *J. Biol. Chem. 256*(6):2993-9 (1981). Since SCP_2 is required for the synthesis of cholesterol by microsomal membranes, it was decided to test the hypothesis that SCP_2 might also participate in enzymatic reactions which utilize cholesterol as a substrate. The reaction studied in the present investigation was the conversion of cholesterol to cholesterol esters (acyl-CoA cholesterol acyltransferase) by rat liver microsomes. The results show that when exogenously added [4-^{14}C] -cholesterol is the substrate, SCP_2 produces a striking increase in cholesterol ester biosynthesis by rat liver microsomes. Although the effect of SCP_2 was most clearly seen with exogenously added cholesterol, it was also demonstrated when [1-^{14}C] oleoyl-CoA was the labeled substrate and the incorporation of labeled oleate into cholesterol ester was determined. Although it was demonstrated that microsomes could bind large amounts of cholesterol in the absence of SCP_2, the bound cholesterol was ineffective as a substrate for microsomal acyl-CoA cholesterol acyltransferase. However, the microsomally bound cholesterol became an effective substrate for the enzyme upon the addition of SCP_2. The results demonstrate the SCP_2 participates in the utilization of cholesterol via the microsomal conversion of cholesterol to cholesterol ester. We also conclude that SCP_2 may participate in the intracellular transport of cholesterol, in particular, the delivery of either exogenous (dietary) cholesterol or endogenous cholesterol to acyl-CoA cholesterol acyltransferase in the endoplasmic reticulum.

EFFECTS OF FEEDING CHENODEOXYCHOLIC ACID ON METABOLISM OF CHOLESTEROL AND BILE ACIDS IN GERM-FREE RATS. B.E. Gustafsson, B. Angelin, I. Bjorkhem, K. Einarsson, and J-A Gustafsson (Department of Clinical Chemistry, Huddinge Sjukhus, Stockholm, Sweden) *Lipids 16*(4):228-33 (1981). The aim of this investigation was to study the influence of chenodeoxycholic acid administration on cholesterol and bile acid synthesis in germ-free rats. Seven rats were fed a basal diet and 2 groups of 4 rats received the same diet supplemented with 0.4 and 1% chenodeoxycholic acid, respectively. After 6 weeks, feces were collected in one 3- and one 4-day pool for analysis of cholesterol and bile acids. When the sampling period was finished, the rats were killed and theliver microsomal fractions isolated. The activities of HMG CoA reductase and cholesterol 7α-hydroxylase were determined, the 7α-hydroxylase by a mass fragmentographic method. The 2 dominating bile acids in the untrated rats were cholic acid and β-muricholic acid. During treatment with chenodeoxycholic acid, 60-70% of this bile acid was converted into α- and β-muricholic acid, indicating a high activity of the 6β-hydroxylase. The excretion of cholic acid was almost completely inhibited and the 7α-hydroxylase activity was decreased ca. 75% in the rats fed 1% chenodeoxycholic acid. The activity of the hepatic HMG CoA reductase activity was unchanged. The fecal excretion of cholesterol increased 2-3 times. An accumulation of cholesterol was seen in the rats treated with 1% chenodeoxycholic acid, which was probably a result of the decreased catabolism of cholesterol to bile acids.

REGULATION OF TRIACYLGLYCEROL SYNTHESIS IN THE LIVER: A DECREASE IN DIACYLGLYCEROL ACYLTRANSFERASE ACTIVITY AFTER TREATMENT OF ISOLATED RAT HEPATOCYTES WITH GLUCAGON. H.P. Haagsman, C.G.M. DeHaas, M.J.H. Geelen and L.M.G. Van Golde (Laboratory of Veterinary Biochemistry, State University of Utrecht, Biltstraat 172, 3572 BP Utrecht, The Netherlands) *Biochim. Biophys. Acta 664*(1):74-81 (1981). Isolated rat hepatocytes were used to investigate the possibility of a short-term effect of glucagon on the synthesis of triacylglycerols in the liver. Incubation of hepatocytes in the presence of glucagon, followed by homogenization in a buffer containing F^- (50 mM) and EDTA (2.5 mM), resulted in a 53% decrease in activity of microsomal diacylglycerol acyltransferase (EC 2.3.1.20). the only enzyme that is exclusively involved in the synthesis of triacylglycerols. The activity of cholinephosphotransferase (EC 2.7.8.2), which also uses diacylglycerols as substrate, was not decreased after exposure of the hepatocytes to glucagon. This may imply that triacylglycerol synthesis can be regulated independently of phosphatidylcholine synthesis. The activity of diacylglycerol acyltransferase in microsomes isolated from a homogenate of whole liver could be reduced by preincubating the microsomes with Mg^{2+} (5 mM), ATP (1 mM) and 105000X G supernatant. The enzyme G supernatant in the presence of dithiothreitol (5 mM). Fluoride (50 mM) inhibited this reactivation. It is concluded that the activity of diacylglycerol acyltransferase is subject to hormonal short-term control, possibly via a phosphorylation-dephosphorylation mechanism.

PLASMA LIPID CONCENTRATION AND LIVER OUTPUT OF LIPOPROTEINS IN RATS FED COCONUT FAT OR SUNFLOWER OIL. A.T. Høstmark, Ø. Spydevold and E. Eilertsen (Institute of Hygiene, University of Oslo, Gydas vei 8, Oslo 3, Norway) *Artery 7*(5):367-83 (1980). Male rats were fed a purified diet for 8 months containing either 10% coconut fat or sunflower oil. The plasma triglyceride (TG) concentration in rats fed coconut fat increased 3-4 fold while no major changes were observed in the TG level of rats fed the sunflower oil diet. Although plasma total cholesterol (Chol) increased with age in both groups there were no significant differences as a function of dietary fat. In rats fed the sunflower oil diet the percentage of plasma alpha lipoproteins was lower and that of pre-beta lipoprotein higher than in coconut fat fed rats. After 8 months the isolated livers of animals from both diet groups were perfused in vitro. The output of very low density lipoprotein (VLDL) protein, Chol and TG from perfused livers was similar in both groups. On the other hand, VLD from livers of the sunflower oil group had lower TG to Chol ratios than those of the other diet group. The liver output of protein and Chol in lipoproteins of d=1.006-1.210 g/mL was higher in the sunflower oil group than in the coconut fat group. The TG to Chol ratio in these lipoproteins was similar for both groups, but the protein to TG, and protein to Chol ratio was highest in lipoproteins of the sunflower oil group. A positive correlation between protein and cholesterol was observed for both VLDL and the heavier lipoproteins. The results suggest that diets rich in polyunsaturated fatty acids can persistently maintain low plasma TG levels and appreciably modify the hepatic output and composition of lipoproteins.

THE CONTENT OF DIACYLGLYCEROL, TRIACYLGLYCEROL AND MONOACYLGLYCEROL AND A COMPARISON OF THE STRUCTURAL AND METABOLIC HETEROGENEITY OF DIACYLGLYCEROLS AND PHOSPHATIDYLCHOLINE DURING RAT LUNG DEVELOPMENT. K. Ishidate and P.A. Weinhold

(Veterans Administration Medical Center and Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48105) *Biochim. Biophys. Acta 664*(1):133-47 (1981). The content of diacylglycerol in fetal rat lung is approx. 36% of the adult and rapidly increases to adult levels by 1 day after birth. Triacylglycerol content is also low (23%) and increases to adult levels between 1 and 2 days following birth. Monoacylglycerol content is relatively low at all stages of development. The overall pattern of molecular species of phosphatidylcholine was similar to the pattern for diacylglycerol. The relative incorporation of radioactivity into disaturated, monoene and diene species of phosphatidylcholine in fetal lung was very similar to that for the corresponding diacylglycerol species. The rate of the reaction from the disaturated species of diacylglycerol to the disaturated species of phosphatidylcholine, calculated from the in vivo data, was one of the higher rates and inciated considerable potential for the synthesis of disaturated phosphatidylcholine via this route. The overall results suggests that de novo synthesis of disaturated phosphatidylcholine from the disaturated species of diacylglycerol can be a major route for the synthesis of dipalmitoylphosphatidylcholine in fetal lung.

PHOSPHOLIPID IS REQUIRED FOR THE PROCESSING OF PRESECRETORY PROTEINS BY DETERGENT-SOLUBILIZED CANINE PANCREATIC SIGNAL PEPTIDASE. R.C. Jackson and W.R. White (Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03755) *J. Biol. Chem. 256*(5):2545-50 (1981). The ability of canine pancreatic signal peptidase to remove the signal peptide portion of presecretory proteins in a translocation-independent assay is shown to require phospholipid. Sodium deoxycholate extracts of canine pancreatic rough microsomes containing both signal peptidase and phospholipid were delipidated by gel filtration chraomtography on Sepharose CL-6B equilibrated with 0.2% deoxycholate. Column fractions were assayed for signal peptidase activity both with and without the addition of ethanol-extracted soybean phospholipid at a final concentration of 1.0 mg/mL. A peak of signal peptidase activity was detected only when the fractions were assayed with added phospholipid. Phospholipid assays demonstrated that the peak of signal peptidase activity was cleanly separated from phospholipid. The ratio of protein to phospholipid in the deoxycholate extract of rough microsomes was 1.76 while that of the most active signal peptidase fractions ranged from 46.1 to 138. The peak of signal peptidase activity exhibited an apparent Stokes radius of 55 Å. Highly purified preparations of phosphotidylcholine were most effective in restoring activity to delipidated signal peptidase. Phosphatidylinositol was much less effective. Phosphatidylserine, phosphatidylethanolamine, sphingomyelin, and lysophosphatidylcholine were all ineffective.

EFFECT OF ASCORBIC ACID ON PLASMA LIPIDS AND LIPOPROTEINS IN HEALTHY YOUNG WOMEN. A.R. Khan and F.A. Seedarnee (Dept. of Dietetics and Nutr., Floride International Univ., Miami, FL 33199) *Atherosclerosis 39*(1):89-95 (1981). Plasma total cholesterol, triglycerides, and lipoprotein cholesterol (VLDL, LDL, HDL) were studied in 13 normal young female volunteers (21-28 yr) given 1 g/day ascorbic acid for 4 weeks under conditions of constant body weight and dietary stability. The results are negative and indicate that ascorbate treatment had no effect on plasma lipids or lipoprotein cholesterol.

BREED DIFFERENCES IN NUTRITIONALLY INDUCED HYPERLIPOPROTEINEMIA IN THE RABBIT. C. Lacombe and M. Nibbelink (Universite Paul Sabatier, Institut de Physiologie Era 412, Cnrs, Rue F, Magendie F-31400 Toulouse, France) *Artery* 7(5):419-427 (1980). The influence of a high-fat, cholesterol-free diet on the lipoprotein pattern is compared in New Zealand hyperresponder and Fauve de Bourgogne hyporesponder rabbits. Quantitative as well as qualitative differences were observed. The high-fat diet induces an increase in both LDL and HDL in the Fauve de Bourgogne, the ratio VLDL + LDL/HDL cholesterol being unchanged. By contrast, New Zealand rabbits present a greater increase in plasma cholesterol which is mainly transported by the VLDL and the LDL. Consequently the proportion of HDL cholesterol is markedly decreased. The breed difference in response to the diet is also noted in lipoproteins composition since only New Zealand rabbits show abnormal VLDL. These results are discussed in terms of differences in susceptibility to experimental atherosclerosis reported between the two strains.

DYNAMIC PROPERTIES OF THE LIPID-WATER INTERFACE OF MODEL MEMBRANES AS REVEALED BY LIFETIME-RESOLVED FLUORESCENCE EMISSION SPECTRA. J.R. Lakowicz and D. Hogen (University of Maryland, Department of Biological Chemistry, Baltimore, MD 21201) *Biochemistry 20*(5):1366-73 (1981). We examined the dynamic properties of the lipid-water interface region of model membranes, on the nano-second time scale, by using the fluorescent probe 2-*p*-toluidinylnaphthalene-6-sulfonic acid (TNS). In particular, we examined the steady state emission spectra of TNS as its average lifetime was decreased by oxygen quenching. Under these quenching conditions the centers of gravity (ν_{cg}) of the emission spectra shift to shorter wavelengths. The lifetime dependence of these shifts reveals the time dependence of membrane relaxation around the excited-state dipole moment of TNs. For these lipids, the spectral relaxation times and the temperature dependence of the relaxations are similar in magnitude. Most relaxation times fall in the range of 0.6-6 ns, and except for the ether analogue, the activation energies for spectral relaxation are 10 ± 2 kcal/mol. The average energy loss during spectral relaxation was 1000 cm^{-1}. However, for the saturated phosphatidylcholines at temperatures below their transition temperatures, smaller relaxation losses were observed ($\sim$600 cm^{-1}). We attribute these smaller losses to ordering of the polar head groups around the ground-state dipole moment of TNS. Overall, these results indicate that the dynamic properties of the lipid-water interface region are similar among the phosphatidylcholines and depend only slightly on the chemical composition and phase state of the acyl side chains.

REGULATION OF CHOLESTEROL BIOSYNTHESIS AND 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE ACTIVITY BY CHYLOMICRON REMNANTS IN ISOLATED HEPATOCYTES AND PERFUSED LIVER. M.R. Lakshmanan, R.A. Muesing and J.C. LaRosa (Lipid Research Laboratory, Veterans Administration Medical Center, Washington, D.C. 20422) *J. Biol. Chem. 256*(6):3037-43 (1981). Newly synthesized and native chylomicrons from cholesterol-fed rats and native chylomicrons from three individuals with type V lipoproteinemia failed to inhibit the overall rate of cholesterol synthesis in isolated hepatocytes from meal-fed rats when incubated for 60 min at 37 C. In contrast, the corresponding chylomicron remnants prepared *in vitro* from the respective native chylomicron preparations by treatment with post-heparin plasma or bovine milk lipoprotein lipase inhibited hepatocyte cholesterol synthesis markedly (47-95%) under identical conditions. The chylomicron remnants markedly inhibited the incorporation of labeled acetate but not that of mevalonate into hepatocyte cholesterol indicating thereby that the remnants must inhibit cholesterol biosynthesis at a step prior to the formation of mevalonate. Since the conversion of 3-hydroxy-3-methylglutaryl coenzyme A to mevalonate is the only step prior to mevalonate formation from acetylcoenzyme A that involves the incorporation of tritium from ^3H_2O via NADPH, it is obvious that the remnants inhibit at the 3-hydroxy-3-methylglutaryl-CoA reductase step.

PROTEOLYTIC DEGRADATION OF LOW DENSITY LIPOPROTEINS BY ARTERIAL SMOOTH MUSCLE CELLS: THE ROLE OF INDIVIDUAL CATHEPSINS. D.S. Leake and T.J. Peters (Division of Clinical Cell Biology, MRC Clinical Research Centre, Watford Road, Harrow, Middlesex, HA1 3UJ, United Kingdom) *Biochim. Biophys. Acta 664*(1):108-16 (1981). Low density lipoproteins have been implicated in the pathogenesis of atherosclerosis. A study has therefore been made of their proteolytic degradation by homogenates of cultured smooth muscle cells from the pig aorta. The pH otimum of proteolysis of ^{125}I-labeled low density lipoproteins was 4.25, thus suggesting the involvement of lysosomal cathepsins. Proteolysis at acid pH started to become saturated at low density lipoprotein concentrations of approx. 20 μg of protein/mL, but did not obey Michaelis-Menten kinetics. After a lag period of approx. 10 min, proteolytic degradation was linear with time up to at least 4 h incubation, but showed a sigmoidal relationship with homogenate concentration. When cathepsin D was inhibited by pepstatin, the proteolysis of ^{125}I-labeled low density lipoproteins was inhibited by more than 90%, whereas when cathepsin B was inhibited by leupeptin, the rate of proteolysis decreased by approx. 50%. Antipain, which inhibits both cathepsins A and B, did not inhibit proteolysis any more than leupeptin, thus suggesting a minor role, if any, for cathepsin A. A combination of pepstatin and either leupeptin or antipain inhibited proteolysis completely. Cathepsins B and D acted synergistically in the degradation of ^{125}I-labeled low density lipoproteins.

CHOLESTEROL KINETIC ANALYSES IN COPPER-DEFICIENT RATS. I.M. Lin and K.Y. Lei (Nutrition Program, College of Agriculture and Home Economics, Mississippi Agriculture and Forestry Experiment Station, Mississippi State University, Mississippi State, MS 39762) *J. Nutr. 111*(3):450-7 (1981). Ninety weanling male Sprague-Dawley rats were randomly allotted into two treatment groups (copper-deficient and adequate; less than 2 mg and 8 mg Cu/kg of diet. Feed and distilled water were provided ad libitum for 16 weeks. After 5 weeks of treatment, the rats were injected intraperitoneally with 6.35 μCi [4-^{14}C] cholesterol/kg body weight. A disappearance curve of the serum cholesterol specific activity (SA) was obtained for each treatment group by killing one animal at 1, 2, 7, 14, 28, 42, 56, 70 and 77 days after the tracer injection. Disappearance curves of serum cholesterol SA were constructed for the rats fed the copper-adequate and copper-deficient diets. Each

disappearance curve of cholesterol SA in the serum was subjected to a kinetic two-pool analysis. The size of the fast turning over cholesterol pool (pool A) and the half-life of pool A were significanly greater in rats fed the copper-deficient diet than the controls. In addition, copper deficiency decreased the rate of removal of cholesterol from pool A (K_{AA}), decreased the irreversible removal of cholesterol from pool A (K_A) and reduced the rate of transport of cholesterol (K_{AB}) from pool A to the slowly exchangeable pool (pool B). However, copper deficiency did not alter the production rate of cholesterol (PR_A) in pool A. Thus, the reduction in the rate of removal of cholesterol from pool A might have been responsible for the hypercholesterolemia observed previously in rats fed copper-deficient diets.

ADSORPTION OF DIVALENT CATIONS TO A VARIETY OF PHOSPHATIDYLCHOLINE BILAYERS. L.J. Lis, W.T. Lis, V.A. Parsegian and R.P. Rand (Department of Biological Sciences, Brock University, St. Catharines, Ontario, Canada) *Biochemistry 20*(7): 1771-7 (1981). We have determined the degree of binding of divalent cations to several kinds of phosphatidylcholine (PC) bilayers. This has been done by measuring the electrostatic interbilayer repulsive force that results when multilamellar lattices are exposed to $Mn^{2+}Cl_2$ solutions. Divalent cations bind to dipalmitoylphosphatidylcholine in the sequence $Ca^{2+} \cong Cd^{2+} \cong Mn^{2+} > Ca^{2+} \cong Mg^{2+} > Ba^{2+}$. Among the different synthetic lipids, preference for Ca^{2+} is in the sequence DOPC < DPLC < DMPC ≅ DPPC ≅ DSPC. The density of bound charge is proportional to the density of polar groups on the bilayer surface. Phosphatidylcholines with mixed hydrocarbon chains, such as egg PC or 1:1 mixtures of synthetic PC's, form two distinct lamellar phases in $CaCl_2$ solutions. In all cases the electrostatic force between bilayers decays exponentially with their separation but more slowly than expected from ionic double-layer theory. We suggest that the electric fields from opposing surfaces perturb the zwitterionic charge-binding polar groups and continuously modify their ion binding affinities as the bilayers approach.

L FIBROBLAST PHOSPHOLIPID ACYL GROUP COMPOSITION AND TRIACYLGLYCEROL LEVELS: RESPONSE TO CONTINUOUS FATTY ACID INFUSION. R.D. Lynch and S.M. Liffmann (Department of Biological Sciences, University of Lowell, One University Avenue, Lowell, Massachusetts 01854) *Proc. Soc. Exp. Biol. Med. 166*(3):462-8 (1981). Two methods of administering fatty acids to cells in culture were compared for their effects on growth, phospholipid acyl group composition, and triacylglycerol formation. Solutions of fatty acid salts were delivered to suspension cultures equal to that infused was added as a single dose complexed to albumin at the start of the 48-hr incubation period. With an initial cell density of 2.5×10^5/mL, 5-18 μmol of Na oleate could be delivered by either method with no adverse effects on the cells. Linoleate (18:2) at the high end of this range, however, decreased culture growth by as much as 50% when the infusion method was employed and 15% when the fatty acid was added as a single dose. At low doses, 5 μmol of 18:2 caused the dienoic acyl group content of phospholipid to increase 22-fold, while the single-dose method increased it by a factor fo 18. Time course studies showed that the triacylglycerol content in the latter case increased to max levels from the 6th to the 24th hr at least 60 times greater than that of control cells not supplemented with fatty acid. The max increase in triacylglycerol above control levels during infusion of 18:2 was only 8-fold and occurred at 24 hr. The infusion method at low levels of 18:2 is at least as effective as the single-dose method in modifying phospholipid acyl group composition, but had the advantage of maintaining lower levels of triacylglycerol.

CHANGES IN SYNTHESIS OF STEROLS AND FATTY ACIDS ASSOCIATED WITH INHIBITION OF GROWTH OF L-M CELLS AT HIGH CELL DENSITY. W.A. Maltese, B.A. Reitz and J.J. Volpe (Dept. of Pediatrics, Washington Univ. Schl. of Med., P.O. Box 14871, 500 S. Kingshighway, St. Louis, MO 63178) *Biochim. Biophys. Acta 663*(3):645-652 (1981). The relationship between cell density and de novo synthesis of sterols and fatty acids has been studied in monolayer cultures of L-M cells grown in serum-free medium. Incorporation of radioactivity from [^{14}C] acetate or ^3H_2O into sterols and fatty acids declined sharply as cultures approached stationary phase. The activities of 3-hydroxy-3-methylglutaryl-CoA reductase and 3-hydroxy-3-methylglutaryl-CoA synthase declined in conjunction with the decrease in sterol synthesis; however, the activity of acetoacetyl-CoA thilase did not decrease until after sterol synthesis had begun to decline. The data suggest that lipogenesis is regulated in coordination with the changes in the rate of cell proliferation that occur when L-M cells attain a high density in monolayer culture. Moreover, these studies establish the feasibility of using the L-M cell culture system to investigate the relationship between cell density and the enzymatic regulation of lipogenesis.

EFFECTS OF ISOLEUCINE DEPRIVATION ON SYNTHESIS OF STEROLS AND FATTY ACIDS IN LM-CELLS. W.A. Matlese, B.A. Reitz and J.J. Volpe (Depts. of Pediatrics, Neurology and Biological Chemistry, Washington University School of Medicine, St. Louis, Missouri 63110) *J. Biol. Chem. 256*(5): 2185-2193 (1981). The effects of isoleucine deprivation on *de novo* synthesis of sterols and fatty acids have been studied in mouse LM-cells cultured in a chemically defined, lipid-free medium. Removal of isoleucine from the medium resulted in cessation of cell growth within 24 h. The cessation of cell division was accompanied by coordinate decreases in incorporation of radioactivity from [^{14}C]-acetate or ^3H_2O into 3-β-hydroxysterols and fatty acids. The decline in sterol synthesis in isoleucine-starved cells was accompanied by suppression of the activities of acetoacetyl-CoA thiolase, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase, and HMG-CoA reductase. The fact that addition of low density lipoprotein-cholesterol, bovine serum albumin palmitate, or mevalonate to isoleucine-starved cultures did not prevent arrest of growth, suggests that the declines in sterol synthesis and fatty acid synthesis are consequences of, rather than causes of, the cessation of cell division.

MECHANISM OF LIPID-PROTEIN INTERACTION IN THE PLASMA LIPOPROTEINS: IDENTIFICATION OF A LIPID-BINDING SITE IN APOLIPOPROTEIN A-II. S.J.T. Mao, R.L. Jackson, A.M. Gotto, Jr., and Houston, TX 77030) *Biochemistry 20*(6):1676-80 (1981). Apolipoprotein A-II (apoA-II) is a dimeric 77-residue apoprotein of human high-density lipoproteins. Previous studies indicate that residues 56-77 in the apoprotein do not bind phospholipid whereas residues 47-77 form a complex with dimyristoylphosphatidylcholine (DMPC). To further delineate the lipid-binding region between residues 47 and 77, we have prepared synthetic fragments of apoA-II corresponding to residues 54-77, 52-77, and 50-77 and have tested each fragment for its ability to interact with vesicles of DMPC. The interaction of the fragments was determined by changes in secondary structure as measured by circular dichroism and by isolation of peptide-DMPC complexes by ultracentrifugation in density gradients of KBr. By these criteria, only fragment 50-77 binds DMPC. Since the 56-77 fragment does not associate with phospholipid, we propose that the addition of residues Thr-Pro-Leu-Ile-Lys-Lys (corresponding to residues 50-55) to the 56-77 fragment gives the peptide the necessary sequence information for lipid binding. We conclude that residues 50-55 are important in lipid binding and that the hydrophobic center formed by Leu-Ile plays an important role.

THE FORMATION OF SPHINGOMYELIN FROM POHSPHA–TIDYLCHOLINE IN PLASMA MEMBRANE PREPARATIONS FROM MOUSE FIBROBLASTS. W-D. Marggraf, F.A. Anderer and J.N. Kanfer (Department of Biochemistry, University of Manitoba, Winnipeg, Manitoba R3E 0W3, Canada) *Biochim. Biophys. Acta 664*(1):61-73 (1981). The enzymatic formation of radioactive sphingomyelin from (^{14}C) choline-labeled phosphatidylcholine was demonstrated to reside exclusively in the plasma membrane fraction of mouse fibroblasts. This activity has several properties in common with the phosphoatidylcholine ceramide phosphocholine transferase of mouse liver microsomes. The enzyme has little if any phospholipase C activity and isotope dilution experiments suggest that phosphatidylcholine is the substrate rather than it is converted to CDPcholine, phosphocholine, free choline or glycerophosphocholine prior to the transfer reaction. The activity is stimulated by the addition of bovine serum albumin and $MnCl_2$ to the incubation mixtures. The plasma membrane localization of the enzyme suggests that it may have a central role in the biosynthetic pathways for sphingomyelin in mouse fibroblasts.

PLACENTAL DEVELOPMENT AND FATTY ACID METABOLISM IN PIGS FED *AD LIBITUM* OR RESTRICTED DURING GESTATION. R.J. Martin and G.J. Hausman (Department of Foods and Nutrition, College of Home Economics, University of Georgia, Athens, GA 30602) *Proc. Soc. Exp. Biol. Med. 166*(4):472-8 (1981). Placental metabolism and histochemistry were studied during three periods of gestation in pigs fed either *ad lib* or restricted. Placental tissue fatty acid synthesis and esterification were depressed in those pigs which were restricted. Palmitate oxidation to carbon dioxide was not influenced by dietary manipulation. Between the gestational age of 45 and 112 days, placental villi increased in length and maternal and chorionic epithelial cells decreased in height. A comparison of maternal and fetal placental cells revealed differences in lipid content and fatty acid synthesis. It is proposed that both maternal and fetal placentas are capable of responding to maternal nutritional state and may be important in altering metabolites available to the fetus.

PHYSICAL PROPERTIES OF LIPID-PROTEIN COMPLEXES FORMED BY THE INTERACTION OF DIMYRISTOYLPHOSPHATIDYLCHOLINE AND HUMAN HIGH-DENSITY APOLIPOPROTEIN A-II. J.B. Massey, M.F. Rohde, W.B. Van Winkle, A.M. Gotto, Jr., and H.J. Pownall (Dept. of Medicine, Baylor College of

Medicine, and The Methodist Hospital, Houston, TX 77030) *Biochemistry 20*(6):1569-74 (1981). Apolipoprotein A-II (apoA-II) from human plasma high-density lipoproteins associates with dimyristoylphosphatidylcholine (DMPC) to give complexes whose structure is determined by the temperature crystalline transition

PHYSICAL PROPERTIES OF LIPID-PROTEIN COMPLEXES FORMED BY THE INTERACTION OF DIMYRISTOYLPHOSPHATIDYLCHOLINE AND HUMAN HIGH-DENSITY APOLIPOPROTEIN A-II. J.B. Massey, M.F. Rohde, W.B. Van Winkle, A.M. Gotto, Jr., and H.J. Pownall (Dept. of Medicine, Baylor College of Medicine, and The Methodist Hospital, Houston, TX 77030) *Biochemistry 20*(6):1569-74 (1981). Apolipoprotein A-II (apoA-II) from human plasma high-density lipoproteins associates with dimyristoylphosphatidylcholine (DMPC) to give complexes whose structure is determined by the temperature at which the reaction is conducted. The temperature dependence is related to the gel → liquid crystalline transition temperature, T_c, of DMPC which occurs at 23.9 C. At $T < T_c$ (20 C), $T = T_c$, and $T > T_c$ (30 C), three different complexes can be isolated. At 20 C, a 75:1 (molar ratio of lipid to protein) complex is formed. At 24 C, two different complexes may be formed. One is similar to the one formed at 20 C and the other is a complex with a DMPC:apoA-II ratio of 240:1. At 30 C, a 45:1 complex was formed. Electron microscopy reveals that the negatively stained complexes are arranged in rouleaux having subunits with average dimensions of 175 × 60, 250 × 62, and 500 × 55 Å for the 45:1, 75:1, and 240:1 complexes, respectively. The multiple lipid-protein species formed by apoA-II and DMPC suggest the possible existence of more than one macromolecular species of lipid and apoA-II in the plasma.

THERMODYNAMICS OF LIPID-PROTEIN INTERACTIONS: INTERACTION OF APOLIPOPROTEIN A-II FROM HUMAN PLASMA HIGH-DENSITY LIPOPROTEINS WITH DIMYRISTOYLPHOSPHATIDYLCHOLINE. J.B. Massey, A.M. Gotto, Jr., and H.J. Pownall (Dept. of Medicine, Baylor College of Medicine, and The Methodist Hospital, Houston, TX 77030) *Biochemistry 20*(6):1575-84 (1981). Apolipoprotein A-II (apoA-II), the second most abundant protein of the human high-density lipoproteins, spontaneously associates with dimyristoylphosphatidylcholine (DMPC) to give multiple products whose composition, structure, and properties are a sensitive function of the temperatures and of the initial lipid to protein ratio at which they are formed. We have studied the thermodynamics of this association by calorimetric and spectroscopic methods. Complexes having a DMPC/apoA-II molar ratio of 75:1 are formed at 20 and 24 C; a 240:1 complex is also formed at 24 C, and a 45:1 complex is formed at 30 C. Additionally, in the presence of a large excess of lipid at 24 C, the 75:1 complex can be converted to a 240:1 complex. These temperature regions are respectively below, at, and above the transition temperature, T_c, of DMPC (23.9 C). The enthalpy of association of *lyso*myristoylphosphatidylcholine (LMPC) with apoA-II at 30 C was identical with that of DMPC. Correlation of circular dichroic and calorimetric data shows that the enthalpy of α-helix formation of apoA-II which accompanies its association with DMPC is exothermic. These results demonstrate that the energetics of apolipoprotein association with phospholipids are a function of accompanying structural changes in both the lipid and the protein.

PHOSPHOLIPID ASYMMETRY IN SEMLIKI FOREST VIRUS GROWN ON BABY HAMSTER KIDNEY (BHK-21) CELLS. G. van Meer, K. Simons, J.A.F. Op den Kamp and L.L.M. van Deenen (Laboratory of Biochemistry, University of Utrecht, Transitorium 3, De Uithof, 3508 TB Utrecht, The Netherlands) *Biochemistry 20*(7):1974-81 (1981). The distribution of the different phsopholipid classes over the two leaflets of the membrane of Semliki Forest virus, grown on baby hamster kidney cells (BHK-21), was studied. To localize the phospholipids we have used a phosphatidylcholine-specific exchange protein, a nonspecific exchange protein, phospholipases A_2 and C, sphingomyelinase, and the amino group labeling reagent trinitrobenzenesulfonate. When low concentrations of exchange proteins and phospholipases were used, only those phospholipids present in lysed or otherwise defective virions were detected. Up to 10% of the phospholipids are present in such particles, and this amount correlated well with the amount of ribonuclease-degradable RNA present in each virus preparation. In the actual localization experiments, carried out with higher concentrations of exchange proteins and phosholipases as well as with trinitrobenzenesulfonate, the various independent techniques yielded identical results. Phosphatidylserine could not be localized. Calculations, based on the size of the Semliki Forest virus and the number of phospholipid molecules per virion, indicate it to be unlikely that the phospholipids which could not be localized are present in the inner bilayer leaflet. The present data on phospholipid composition and distribution have been compared with previous results on the lipid distribution in plasma membrane derived structures. It is concluded that an asymmetric distribution of phospholipids inthe plasma membrane of various mammalian cells is apparent.

IN VIVO EXCHANGE OF CHOLESTERYL ESTERS FROM LOW DENSITY LIPOPROTEINS TO HIGH DENSITY LIPOPROTEINS. P.J. Nestel and T. Billington (Cardiovascular Metabolism and Nutrition Research Unit, Baker Medical Research Institute, Melbourne, Australia, 3181) *Artery* 7(5):395-403 (1980). Labeled cholesteryl esters were injected within low density lipoproteins (LDL) in four subjects. In all experiments, cholesteryl esters transferred to HDL initially, at the rate of about 3% per hour. Very low density lipoprotein cholesteryl esters became labeled later, possibly by transfer from HDL. The simultaneous labeling of HDL free cholesterol by a second isotope (injected within mevalonic acid) showed that the rapid labeling of HDL cholesterol esters from LDL was substantially faster than that from the esterification of HDL free cholesterol. While this process does not show net transfer of cholesteryl esters from LDL to HDL it indicates a pathway for equilibration of cholesteryl esters amosng plasma lipoproteins.

EFFECTS OF CALCIUM CHANNEL BLOCKERS ON ARACHIDONATE-INDUCED SUDDEN DEATH IN RABBITS. S. Okamatsu, R.C. Peck, and A.M. Lefer (Department of Physiology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvanie 19107) *Proc. Soc. Exp. Biol. Med. 166*(4): 551-5 (1981). The effects of the calcium channel blockers, nisoldipine and verapamil, on arachidonate-induced sudden death were investigated in rabbits. Sodium arachidonate (2 mg/kg) was injected into the vena cava producing death within 3 min (2.5 ± 0.3 min) in all untreated rabbits. Seventy-five percent of the rabbits pretreated by nisoldipine (0.2 mg/kg) were protected from arachidonate-induced sudden death, showed inhibition of thromboxane synthesis, and the absence of intravascular thrombosis in pulmonary vessels. Verapamil also protected rabbits from sudden death but to a lesser extent than that of nisoldipine. Inhibition of thromboxane synthesis and prevention of platelet-induced pulmonary thrombosis appear to be related to the effects of the calcium channel blockers in arachidonate-induced sudden death.

SEQUENTIAL CYCLES OF CHOLESTEROL AND DOLICHOL SYNTHESIS IN MOUSE SPLEENS DURING PHENYLHYDRAZINE-INDUCED ERYTHROPOIESIS. J.E.R. Potter, M.J. James, and A.A. Kandutsch (Jackson Laboratory, Bar Harbor, ME 04609). *J. Biol. Chem. 256*(5):2371-6 (1981). The results presented demonstrate that erythropoiesis in the mouse spleen was associated with cyclic changes in cholesterol and dolichol synthesis which were independent of each other. Changes in the rate of cholesterol synthesis were correlated with the level of 3-hydroxy-3-methylglutaryl-CoA reductase activity and with the rate of cell division (thymidine incorporation into DNA), but a cycle of dolichol synthesis appeared to be independent of both these parameters. Consequently the incorporation of [^{14}C] acetate into dolichol increased 20- to 30-fold compared to the rate of cholesterol synthesis 5 to 6 days following phenylhydrazine treatment. The pathway for dolichol synthesis branches from that leading to cholesterol at the level of the intermediate farnesyl pyrophosphate, subsequent to the reaction catalyzed by the regulatory enzyme 3-hydroxy-3-methylglutaryl-CoA reductase. Previous studies showed that in several cell culture lines, and in liver, dolichol synthesis and cholesterol synthesis are regulated in a coordinated way by reductase activity. However, during the process of spermatogenesis and in developing mouse brain the regulation of dolichol synthesis appeared to be independent of cholesterol synthesis. Independent regulation of dolichol synthesis is now demonstrated in a third differentiating tissue, the erythropoietic spleen, and the results suggest that a cycle of dolichol synthesis is specifically associated with one or more stages of erythroid differentiation.

ACCUMULATION OF NEUTRAL LIPIDS BY HUMAN SKIN FIBROBLASTS: DIFFERENTIAL EFFECTS OF SATURATED AND UNSATURATED FATTY ACIDS. M.D. Rosenthal (Dept. of Biochem., Eastern Virginia Med. Schl., Norfolk, VA 23501) *Lipids 16*(3):173-82 (1981). The accumulation of neutral lipids by human skin fibroblasts grown in medium supplemented with fatty acids has been investigated. GM-10 cells incorporated exogenous fatty acids into both phospholipids and neutral lipids. More [^{14}C] oleate, linoleate, or linolenate was incorporated into triacylglycerol than was [^{14}C] palmitate or stearate. Supplementation of medium containing delipidized serum with unsaturated fatty acids resulted in fat more stimulation of [^{14}C] glycerol incorporation into triacylglycerol than did supplementation with saturated fatty acids. Palmitate- and stearate-fed cells incorporated sizable amounts of [^{14}C] fatty acids and [^{14}C] glycerol into diacylglycerol as well as triacylglycerol, especially at higher fatty acid concentrations. Increased oleate supplementation from lipid 10-300 μM resulted in increased triacylglycerol synthesis and accumulation of discrete cytoplasmic lipid droplets; palmitate concentrations above 70 μm

were toxic. Micrographs of the palmitate-fed cells showed electron translucent slits, suggesting solid depositions of saturated fat, rather than the discrete osmiophilic droplets found in oleate-fed cells. Although GM-10 cells can synthesize fully saturated triacylglycerols, these data suggest that in cells fed saturated fatty acids, solid depositions of neutral lipids may sequester diacylglycerols and thus limit triacylglycerol synthesis.

THE EFFECT OF SKIM MILK, YOGHURT, AND FULL CREAM MILK ON HUMAN SERUM LIPIDS. J.E. Rossouw, E.-M. Burger, P. Van Der Vyver and J.J. Ferreira (Medical Res. Council National Res. Inst. for Nutritional Diseases, Tygerberg, Republic of South Africa) *Am. J. Clin. Nutr. 34*(3):351-356 (1981). The hypothesis that certain milk products contain a cholesterol-lowering "milk factor" was tested in adolescent schoolboys whose diets were complemented with 2 L of skim milk, yoghurt, or full cream milk daily for 3 wk. After a fall in all serum lipids during the precomplementation wk, serum total cholesterol and low-density lipoprotein cholesterol continued to fall on skim milk; in contrast, it rose for the first 2 wk on yoghurt or full cream milk. These changes correlated with dietary fat and cholesterol intakes. Total cholesterol returned to base-line values during the 3rd wk on yoghurt or full cream milk. High-density lipoprotein cholesterol and percentage high-density lipoprotein/total cholesterol rose transiently in all three groups, with the highest levels being recorded on full cream milk. Serum triglycerides tended to decrease in all groups. No convincing evidence of a milk factor could be found, but skim milk appeared to have a cholesterol-lowering effect at least partly due to its low lipid content.

LIPOPROTEIN UPTAKE IN PERFUSED ARTERIES: INHIBITION BY 7-KETOCHOLESTEROL. G.G. Santillan, J.R. Schuh, S.I. Chan and R.J. Bing (Huntington Memorial Hospital and Huntington Institute of Applied Medical Research, 100 Congress Street, Pasadena, CA 91109) *Artery 7*(5):384-94 (1980). The effect of 7-ketocholesterol (7-KC), an oxygenated sterol (OS) on the uptake of low density lipoproteins (LDL) by artificially perfused porcine carotid arteries was studied. The perfusate consisted of Dulbecco's modified Eagle medium, supplemented with albumin pure ^{125}i-LDL. Prior exposure to 7-KC significantly inhibited LDL uptake (from 2.47 to 1.92 μg LDL p/gr artery). The degree of inhibition of LDL uptake was less than that previously observed for arterial influx of cholesterol.

SEMISYNTHESIS OF PHOSPHOLIPASE A_2. PREPARATION AND PROPERTIES OF ARGININE-6 BOVINE PANCREATIC PHOSPHOLIPASE A_1. G.J.M. van Scharrenburg, W.C. Puijk, M.R. Egmond, G.H. de Haas, and A.J. Slotboom (Laboratory of Biochemistry, State University of Utrecht, Transitorium 3, University Center "De Uithof", Padualaan 8, 3508 TB Utrecht, The Netherlands) *Biochemistry 20*(6): 1584-91 (1981). The major differences between porcine and bovine pancreatic phospholipases A_2 are the low affinity of the bovine enzyme for lipid-water interfaces and its low capacity to penetrate more densely packed monolayers of lecithins. In the proposed binding site for lipid-water interfaces the porcine enzyme has an Arg residue at position 6 which is Asn in the bovine enzyme. In order to study whether this difference affects the above-mentioned properties, a hybrid bovine phospholipase A_2 that has Arg at position 6 was prepared. Both the affinity of bovine [Arg^6] AMPA for lipid-water interfaces and its ability to penetrate bovine AMPA. In these respects bovine [Arg^6] AMPA was found to be almost identical with the porcine AMPA. Moreover, bovine-[Arg^6] AMPA possesses enhanced enzymatic activity as compared to bovine and porcine AMPA. It can be concluded that substitution of Asn^6 by Arg in bovine phospholipase A_2 improves the binding for lipid-water interfaces. The concomitant increase in enzymatic activity strongly suggests an effect of the lipid binding site on the active site.

GLUCONEOGENESIS IN ISOLATED CHICKEN HEPATOCYTES: EFFECT OF FATTY ACIDS, β-HYDROXY-BUTYRATE, ETHANOL, AND VARIOUS PYRUVATE/LACTATE RATIOS. P. Schultz and S.P. Mistry (Lab. of Nutritional Biochem., Dept. of Animal Sci., Univ. of Illinois, Urbana, IL 61801) *Poultry Sci. 60*(3):653-8 (1981). The effect of fatty acids, β-hydroxy-butyrate, ethanol, and different pyruvate/lactate ratios on gluconeogenesis in isolated chicken hepatocytes was investigated. Gluconeogenesis was significantly affected by a change in the oxidation-reduction (pyruvate/lactate) ratio, and this effect was greater than could be accounted for by the additive effects of these substrates. Substituting lactate with nongluconeogenic substrates, such as β-hydroxybutyrate or ethanol, increased the formation of glucose by 80 and 200%, respectively, demonstrating the beneficial effect of the increased reducing equivalents in the hepatocytes. Oleic acid *per se* had no effect but when added, complexed with albumin, it had a negative effect on gluconeogenesis.

REGULATION OF 3-HYDROXY-3-METHYLGLUTARYL-CoA REDUCTASE BY ENDOGENOUS STEROL SYNTHESIS IN CULTURED INTESTINAL MUCOSA. E.F. Stange, G. Preclik, A. Schneider, M. Alavi and H. Ditschuneit (Div. of Metabolism, Nutr. and Gastroent., Dept. of Internal Medicine, Univ. of Ulm, Ulm, F.R.G.) *Biochim. Biophys. Acta 663*(3):613-20 (1981). In vitro regulation of the key enzyme of cholesterol synthesis, 3-hydroxy-3-methylglutaryl-CoA reductase (EC 1.1.1.34) by compactin, a competitive inhibitor of the enzyme, and mevalonate was studied in rabbit ileum organ culture. Addition of compactin suppressed ileum homogenate reductase activity by over 80% at concentrations up to 0.5 μg/ml. In contrast, compactin at the same concentrations added to the culture medium induced reductase activity up to 240% of controls. When endogenous cholesterol synthesis was blocked by compactin, mucosal alkaline phosphatase activity decreased progressively, whereas medium activity from desquamated cells did not change. This distribution of the villous cell marker enzyme is characteristic of a decrease in crypt cell renewal and/or villous cell differentiation. This effect of compactin was also reversible with mevalonolactone. The reductase enzyme induced by compactin was probably latent intracellularly, since tissue cholesterol contents dropped sharply after blockade of endogenous sterol synthesis.

ARTERIAL INFLUX OF ESTERIFIED CHOLESTEROL FROM TWO PLASMA LIPOPROTEIN FRACTIONS AND ITS HYDROLYSIS IN VIVO IN HYPERCHOLESTEROLEMIC RABBITS. S. Steender and D.B. Zilversmit (Div. of Nutritional Sciences, Cornell Univ., Ithaca, NY 14853) *Atherosclerosis 39*(1):97-109 (1981). Arterial influx of esterified cholesterol from 2 plasma lipoprotein fractions, d<1.019 and d>1.019, and influx of plasma free cholesterol were determined in each of 15 hypercholesterolemic rabbits with approximately the same plasma cholesterol concentrations but with different extents of arterial lesions. The arterial influx of cholesteryl ester derived from d<1.019 lipoproteins was about equal to that derived from the d>1.019 fraction. The amount of cholesteryl ester in plasma d<1.019 was approximately 3 times that in d>1.019. Thus, per unit of cholesteryl ester concentration the d< 1.019 lipoproteins delivered about 1/3 as much cholesteryl ester to the artery as the lipoproteins in the higher density fractions. Some 5-40% of plasma esterified cholesterol which had entered the artery was hydrolyzed in the artery during the experimental period. The influx of free cholesterol that could not be accounted for by the influx of intact plasma lipoproteins was 5-80% of the free cholesterol influx. This excess probably represents free cholesterol influx by an exchange between the plasma lipoproteins and the intimal surface of the artery.

EFFECTS OF PREGNANCY AND STREPTOZOTOCIN DIABETES ON HEPATIC FATTY ACID METABOLISM. I. Wasfi, I. Weinstein and M. Heimberg (Department of Pharmacology, University of Missouri School of Medicine, Columbia, MO 65212) *Proc. Soc. Exp. Biol. Med. 166*(3):330-4 (1981). Nonpregnant female rats and pregnant rats were fed *ad libitum*. Diabetes was induced by a single intravenous injection of streptozotocin. Pregnant rats received streptozotocin on Day 17 of gestation. Diabetes in nonpregnant and pregnant animals decreased the output of triacylglycerol and increased ketogenesis by the isolated perfused liver in comparison to the corresponding nondiabetic group. In agreement with previous data, pregnancy increased output of triacylglycerol and decreased ketogenesis. Livers from pregnant diabetic rats secreted more triacylglycerol and fewer ketones than did livers from nonpregnant diabetic rats. Pretreatment of diabetic rats with insulin reduced ketogenesis toward normal values and increased output of triacylglycerol to values exceeding corresponding control values. Total net synthesis of triacylglycerol was increased by pregnancy and was decreased in livers from pregnant diabetic rats, when compared to their corresponding controls. The data suggest that certain hormonal changes induced by pregnancy act antagonistically to experimental insulin deficiency.

IN VIVO INFLUX, TISSUE ESTERIFICATION AND HYDROLYSIS OF FREE AND ESTERIFIED PLASMA CHOLESTEROL IN THE CHOLESTEROL-FED RABBIT. S. Stender and D.B. Zilversmit (Div. of Nutritional Sciences, Savage Hall, Cornell Univ., Ithaca, NY 14853) *Biochim. Biophys. Acta 663*(3):674-86 (1981). The influx of free and esterified cholesterol into various tissues of cholesterol-fed rabbits is calculated from the tissue [^{3}H] cholesterol and [^{14}C] cholesterol content—corrected for radioactivity in contaminating plasma—after a 3-6 h exposure to in vivo-labeled plasma. The plasma free cholesterol was labeled primarily with ^{3}H and the esterified cholesterol with ^{14}C or vice versa. The influx calculation is based on total ^{3}H and ^{14}C in tissues and two linear equations that take into account esterification and hydrolysis of sterol fractions by the tissues. The influx of free cholesterol was considerably higher than expected if free and esterified cholesterol had entered the tissues together as part of plasma lipoproteins. This excess of free

cholesterol influx can be ascribed to cholesterol exchange between plasma lipoproteins and tissues, which in several tissues amounted to more than 80% of the total free cholesterol influx. From tissue free and esterified cholesterol radioactivity, one can calculate that 20-70% of the newly entered esterified cholesterol was hydrolyzed by various tissues and that most tissues esterified less than 10% of newly entered cholesterol during the experimental period. However, esterification of plasma cholesterol by adrenals averaged 50% of that taken up during a 3-6 h period.

α-TOCOPHEROL AND SERUM LIPOPROTEINS. G.S. Sundaram, R. London, S. Manimekalai, P.P. Nair and P. Goldstein (Dept. of Obstetrics and Gynecology, Sinai Hosp. of Blatimore, MD 21215) *Lipids 16*(4):223-7 (1981). Twenty-six patients with clinically confirmed mammary dysplasia and five age-matched controls were treated with α-tocopherol, 600 mg/day. Serum samples collected on the 21st day of the menstrual cycle were analyed for cholesterol in lipoprotein fractions, isolated by a combination of precipitation and ultracentrifugation techniques. Eighty-five percent of patients showed objective and subjective remission from disease following therapy. In mammary dysplasia patients, the ratio of serum cholesterol/high density lipoprotein cholesterol was higher than those in age-matched controls, an abnormality which was corrected by α-tocopherol therapy. Furthermore, as a result of therapy, high density lipoproteins increased and ester cholesterol associated with low density lipoproteins decreased. The results suggest that α-tocopherol may serve as an effective agent in treating patients with benign breast disease, as well as correct the inherent abnormality in serum cholesterol distribution in mammary dysplasia patients.

EFFECT OF FIBER ON CHOLESTEROL METABOLISM IN THE *COTURNIX* QUAIL. C.D. Sutton, W.M. Muir, and J.J. Begin (Department of Animal Sciences, University of Kentucky, Lexington, Kentucky 40546) *Poultry* Sci. 60(4):812-7 (1981). This experiment involving 288 *Coturnix* quail was conducted to determine the effects of various fiber sources (alfalfa, wheat bran, dried brewer's grain, cellulose, and pectin) on serum, liver and egg yolk cholesterol, and egg production. The fiber sources were added at a rate to provide 6.25% fiber to a corn-soybean meal diet and fed for a 28-day experimental period. Serum, liver, and egg cholesterol levels were measured as pen composite samples at the end of the experimental period. No difference was exhibited in egg yolk cholesterol among any of the groups ($P>.10$). Liver and serum cholesterol levels were elevated in the birds fed the pectin and wheat bran diets as compared with that in birds fed the other fiber source; also, metabolizable energy intakes and eggs per hen day (EHD) were decreased for both groups. When data were adjusted for EHD by covariance analysis, the treatment differences no longer appeared ($P>.05$). These results indicate that 1) there is a basal quantity of cholesterol deposited in the egg on which fiber intake, energy consumed, or egg production have very little effect and that 2) there is an inverse relationship between serum and tissue cholesterol levels and the total quantity of cholesterol excreted *via* the egg.

ISOLATION, IDENTIFICATION, AND BIOLOGICAL ACTIVITY OF 25-HYDROXY-24-OXOVITAMIN D_3: A NEW METABOLITE OF VITAMIN D_3 GENERATED BY IN VITRO INCUBATIONS WITH KIDNEY HOMOGENATES. Y. Takasaki, T. Suda, S. Yamada, H. Takayama, and Y. Nishii (Department of Biochemistry, School of Dentistry, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142) *Biochemistry 20*(6):1681-86 (1981). A metabolite of 25-hydroxyvitamin D_3 has been isolated in pure form from incubation mixtures containing kidney homogenates of chicks given large doses of vitamin D_3. The isolation involved methanol-chloroform extraction and six steps of column chromatography. The metabolite was identified as 25-hydroxy-24-oxovitamin D_3 by means of ultraviolet absorption spectrometry, mass spectrometry, infrared spectrometry, nuclear magnetic resonance spectrometry, and specific chemical reactions. Use of a sensitive in situ technique revealed that 25-hydroxy-24-oxovitamin D_3 enhances intestinal calcium transport in rats approximately as effectively as 24,25-dihydroxyvitamin D_3 does. In contrast, 25-hydroxy-24-oxovitamin D_3 appeared to be less active than 24,25-dihydroxyvitamin D_3 in chicks 24 h after intravenous injection.

SOMATIC, ENDOCRINE, AND SERUM LIPID CHANGES DURING DETRAINING IN ADULT HAMSTERS. A.C. Tsai, J. Bach and K.T. Borer (Human Nutr.Program, Schl. of Public Health, Univ. of Michigan, Ann Arbor, MI 48109) *Am. J. Clin. Nutr. 34*(3):373-6 (1981). Effects of abrupt discontinuation of chronic exercise on body composition and serum lipid, insulin, and glucagon concentrations were examined in adult female hamsters. Thirty-six hamsters (100 to 120 g) were randomly allotted to two groups of 18 each for an 84-day study. One group served as controls and were sedentary throughout the experimental period; another group had access to voluntary running on horizontal discs during the first 42 days of the experimental period. Six hamsters from each group were killed at the end of the exercise period and 12 and 42 days after retirement. Results showed that hamsters engaged in high levels of voluntary activity increased food intake by about 10-20% and this effect persisted for about 10 days after retirement. Voluntary running resulted in a 60% reduction in body fat content and a 30% decrease in serum triglyceride levels. Exercise was also associated with an increase in serum insulin level. Increased food consumption and changes in serum insulin and glucagon may reflect compensatory adjustments to increased energy expenditure of exercise. Discontinuation of exercise resulted in a reversal of exercise effects on body fat, body cholesterol, and serum triglyceride levels.

THE CONTRIBUTION OF NEWLY SYNTHESIZED CHOLESTEROL TO BILIARY CHOLLESTEROL IN THE RAT. S.D. Turley, J.M. Dietschy (Dept. of Internal Medicine, University of Texas Health Science Center at Dallas, TX 75235) *J. Biol. Chem. 256*(5):2438-46 (1981). The current studies were undertaken to quantitate the proportion of biliary cholesterol that is newly synthesized in the rat and to determine whether this proportion is influenced by the rate of hepatic cholesterol synthesis. Female rats were subjected to total biliary diversion either immediately preceding, or 6 h following, the intravenous administration of [^{3}H] water, the amount of newly synthesized sterol secreted in the bile in relation to the total cholesterol secretion rate increased during the first few hours of diversion but by the 7th and 8th hours the proportion had become essentially constant at about 18%. When a continuous infusion of bile acid was given, the output of both total and labeled cholesterol increased several-fold, but the proportion of the total that was newly synthesized was unchanged. Under conditions, where biliary diversion was not commenced until 6 h following the administration of the [^{3}H] water, newly synthesized sterol gain contributed only 16% of the total biliary cholesterol. When the rate of hepatic cholesterol synthesis was increased 2.5-fold by feeding cholesteyramine, 34% of total biliary cholesterol was newly synthesized, whereas when synthesis was suppressed by feeding cholesterol or by fasting for 48 h this proportion decreased to 3.5% and 1.7%, respectively. However, there was no significant change in total biliary cholesterol output under these conditions. In other experiments, it was shown that the specific activity of the unesterified cholesterol in bile was significantly higher than that of any other tissue, including the liver, plasma, and several extrahepatic tissues.

EVIDENCE FOR A ROLE OF ARACHIDONIC ACID IN GLUCOCORTICOID-INDUCED CLEFT PALATE IN RATS. G.G. Tzortzatou, A.S. Goldman and W.C. Boutwell (Division of Human Genetics and Teratology, The Children's Hospital of Philadelphia and Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104) *Proc. Soc. Exp. Biol. Med. 166*(3):321-4 (1981). We have shown that arachidonic acid significantly reduces the production of cleft palate in rats by dexamethasone and that this corrective effect of arachidonic acid is blocked by indomethacin, an inhibitor of cyclooxygenase. Moreover, by using [^{3}H]-arachidonic acid as a tracer we have shown that dexamethasone treatment depresses significantly the free [^{3}H] arachidonic acid available to the microsomal cyclooxygenase in the fetal upper and lower jaws including th palate at the critical period of development. These observations suggest that glucocorticoids produce their palatal teratogenicity by limiting the release and consequently the availability of arachidonic acid at the critical period of development.

GLUTAMATE FORMED FROM LIGNOCERIC ACID BY RAT BRAIN PREPARATION IN THE PRESENCE OF PYRIDINE NUCLEOTIDE AND CYTOSOLIC FACTORS: A BRAIN-SPECIFIC OXIDATION OF VERY LONG CHAIN FATTY ACIDS. M. Uda, I. Singh, and Y. Kishimoto (John F. Kennedy Institute and the Department of Neurology, The Johns Hopkins University, School of Medicine, Baltimore, MD 21205) *Biochemistry 20*(5): 1295-300 (1981). When [1-^{14}C] lignoceric acid was incubated with a rat brain particulate fraction in the presence of heat-stable and heat-labile factors and NADPH, considerable radioactivity was found in the water-soluble fraction. The water-soluble product was purified to apparent homogeneity by combining gel filtration, steric exclusion high-performance liquid chromatography, and CM-Sepharose chromatography. NADPH could be replaced by NADP, NADH, or NAD with nearly equal results. α-Cyclodextrin increased the glutamate formation 3-5-fold, but it could not replace the heat-stable factor. CoA had an inhibitory effect. The enzymatic reaction was inhibited by EDTA, respiratory chain inhibitors, and an uncoupler. This glutamate formation was not detected in liver, kidney, and spleen. The radioactivity from [1-^{14}C] palmitic acid was also converted to a water-soluble material. The conversion required the two cytosolic factors but was not stimulated by α-cyclodextrin. Although the extraneural tissues, especially liver, could produce a water-soluble material, the cytosolic factors were not required for this. These observations suggest that in brain there is a unique

type of oxidation which has a high specificity for longer chain fatty acids.

LYMPHATIC ABSORPTION OF SHELLFISH STEROLS AND THEIR EFFECTS ON CHOLESTEROL ABSORPTION. G.V. Vahouny, W.E. Connor, T. Roy, D.S. Lin and L.L. Gallo (Department of Biochemistry, George Washington University, School of Medicine and Health Sciences, Washington DC 20037) *Am. J. Clin. Nutr. 34*(4):507-13 (1981). Studies have been conducted on the absorbability of individual sterols from a mixture of oyster sterols when administered intragastrically to rats with indwelling catheters in the left thoracic duct. In addition, the effect of oyster sterols on cholesterol absorption has been assessed using [4-^{14}C] cholesterol in the mixture, and compariosn against absorption of cholesterol alone. The order of absorbability (percentage absorption) of individual sterols from the mixture of oyster sterols was: cholesterol ≥ 26-carbon sterols ≥ dehydrocholesterol > 24-methylene cholesterol > brassicasterol > plant sterols. The absorption of noncholesterol sterols was 8.2 ± 0.8% of the fed dose, or less than half of that for an equivalent level of cholesterol alone. The presence of these sterols in mixtures containing cholesterol reduced lymphatic absorption of cholesterol by 25 to 40% compared to absorption of the same amount of cholesterol administered alone, or to an amount of cholesterol equal to the total ester sterols, respectively. These studies suggest that shellfish sterols are poorly absorbed, and, like plant sterols, effectively reduce dietary and/or endogenous cholesterol absorption from the intestine.

SELECTIVE UTILIZATION OF PALMITOYL LYSOPHOSPHATIDYLCHOLINE IN THE SYNTHESIS OF DISATURATED PHOSPHATIDYLCHOLINE IN RAT LUNG. A COMBINED IN VITRO AND IN VIVO APPROACH. G.P.H. Van Heusden, H.P.J.M. Noteborn and H. Van Den Bosch (Biochemistry Laboratory, The State University, Padualaan 8, NL-3584 CH Utrecht, The Netherlands) *Biochim. Biophys. Acta 664*(1):49-60 (1981). The acyl-CoA:lysophosphatidylcholine acyltransferase system in rat lung microsomes was found to utilize selectively 1-[1-^{14}C] palmitoyl-*sn*-glycero-3-phosphocholine when compared with 1-[9,10-$^{3}H_2$]-stearoyl-*sn*-glycero-3-phosphocholine. This result was found with either palmitoyl-CoA, linoleoyl-CoA or an equimolar mixture of these acyl donors and confirms recent data reported by Holub, Piekarski and Possmayer. The selective utilization of palmitoyl lysophosphatidylcholine from a mixture of lysophosphatidylcholine species may cause an increased isotopic ratio in phosphatidylcholine when compared with that of total lysophosphatidylcholine. This suggested a direct acylation by lung acyl-CoA:lysophosphatidylcholine acyltransferases. By contrast, when a mixture of 1-[9,10-$^{3}H_2$] palmitoyl-*sn*-glycero-3-phospho[*methyl*-^{14}C] choline and 1-stearoyl-*sn*-glycero-3-phospho[*methyl*-^{14}C] choline was injected, the $^{3}H/^{14}C$ ratio in disaturated lung phosphatidylcholine increased to about 1.4-fold that of the injected substrate. These data indicate that increased isotopic ratios in disaturated phosphatidylcholine of lung tissue, after intravenous injection of lysophosphatidylcholine, do not necessarily point to the involvement of lysophosphatidylcholine:lysophosphatidylcholine transacylase in disaturated phosphatidylcholine formation.

IN VITRO INTERACTION OF HUMAN HDL WITH HUMAN APOLIPOPROTEIN A-II. SYNTHESIS OF APOLIPOPROTEIN A-II-RICH HDL. P. Van Tornout, H. Caster, M.-J. Lievens, M. Rosseneu and G. Assmann (Dienst Wetenschappelijk Onderzoek, Algemeen Ziekenhuis Sint-Jan, Ruddershove, B-8000 Brugge, Belgium) *Biochim. Biophys. Acta 663*(3):630-6 (1981). The aim of this study was to define the specific affinity of human apolipoproteins A-I and A-II for HDL lipids and to investigate the possible transfer of apolipoproteins from the HDL molecule. For this purpose we incubated human HDL with increasing amounts of isolated apolipoprotein A-II. After incubation the reaction products were separated by gel chromatography and apolipoproteins A-I and A-II were quantified separately by immunoephelometry and HDL lipids by thin-layer chromatography. According to our results, apolipoprotein A-II progressively displaces apolipoprotein A-I from human HDL, and 2 mol apolipoprotein A-II substitute 1 mol apolipoprotein A-I to generate an HDL-like particle with identical lipid composition, hydrodynamic properties and lipid fluidity. These data indicate that apolipoprotein A-II is able to displace quantitatively apolipoprotein A-I from HDL in vitro, and that such a mechanism might contribute to the regulation of the $HDL_2 \longleftrightarrow HDL_3$ distribution in plasma.

DISTRIBUTION OF LIPOPROTEIN TRIGLYCERIDE AND LIPOPROTEIN CHOLESTEROL IN AN ADULT POPULATION BY AGE, SEX, AND HORMONE USE. THE PACIFIC NORTHWEST BELL TELEPHONE COMPANY HEALTH SURVEY. P.W. Wahl, G.R. Warnick, J.J. Albers, J.J. Hoover, C.E. Walden, R.O. Bergelin, J.T. Ogilvie, W.R. Hazzard and R.H. Knopp (Northwest Lipid Res. Clinic, Univ. of Washington, Seattle, WA 98104) *Atherosclerosis 39*(1):111-124 (1981). This report describes the distribution of lipoprotein triglyceride and lipoprotein cholesterol in employees of the Pacific Northwest Bell Telephone Company. Means, medians, and selected percentiles are presented for very low, low, and high density lipoproteins (VLDL, DLD, and HDL, respectively) in 606 randomly selected white subjects aged 20-59. Women who use sex hormones have significantly higher concentrations of triglycerides in all of the fractions across all age decades from 20 to 59 than do women not taking hormones. Men have the highest average VLDL triglyceride value but their average triglyceride concentrations in the LDL and HDL fractions approximate those of women not taking hormones. This study in a well-defined population provides references standards for lipoprotein triglyceride concentrations. These results can be used to evaluate the effect of sex hormone treatment on the lipoprotein triglyceride content in VLDL, LDL and HDL, and to assess triglyceride content as a potential risk factor in men and older women.

PURIFICATION OF THE LYSOSOMAL ACID LIPASE FROM HUMAN LIVER AND ITS ROLE IN LYSOSOMAL LIPID HYDROLYSIS. T.G. Warner, L.M. Dambach, J.H. Shin and J.S. O'Brien (University of California, San Diego, Department of Neurosciences, La Jolla, California 92093) *J. Biol. Chem. 256*(6):2952-7 (1981). The lysosomal acid lipase has been purified 2,500-fold to near homogeneity from human liver. The enzyme was converted to a soluble form by extraction of frozen tissue with Triton X-100. The enzyme, which required Triton X-100 buffers at all purification steps for optimal yields, was stabilized by the inclusion of 33% ethylene glycol during purification. Lectin chromatography on concanavalin A-Sepharose followed by chromatography on carboxymethylcellulose and Sephadex G-150 provided the highly purified enzyme in 17% yield. Sodium dodecyl sulfate-acrylamide gel electrophoresis indicated that the minimum molecular weight was about 29,000 ± 1,000. Minor protein contaminants at M_r = 58,500, 14,700, and 13,900 were present in the final preparation. A single protein band, with enzyme activity, was observed in nondenaturing acrylamide gels containing Triton X-100. Gel filtration on Sephadex G-150 in the presence of Triton X-100 gave an apparent molecular weight of about 125,000 ± 13,000. Trioleoylglycerol, cholesterol oleate, and 1,2- and 1,3-dioleoylglycerols were substrates for the purified enzyme giving apparent V_{max} values of 5,400, 1,400, 19,400, and 22,100 nmol min^{-1} mg of $protein^{-1}$, respectively, and K_m values of 0.8, 0.8, 0.9, and 1.2 mM, respectively. The recoveries of both trioleoylglycerol and cholesterol oleate hydrolytic activities were nearly identical at each purification step, suggesting that the acid lipase as single enzyme is responsible for lysosomal hydrolysis of the neutral lipids.

THE HEPATIC MICROSOMAL BIOTRANSFORMATION OF Δ^5-STEROIDS TO 5α,6β-GLYCOLS VIA α- AND β-EPOXIDES. T. Watabee, M. Kanai, M. Isobe and N. Ozawa (Laboratory of Drug Metabolism and Toxicology, Department of Hygienic Chemistry, Tokyo College of Pharmacy, 1432-1 Horinouchi, Hachioji-shi, Tokyo 192-93, Japan) *J. Biol. Chem. 256*(5):2900-7 (1981). Bovine liver microsomes converted four Δ^5-steroids, cholesterol, 5-cholestene, 20-methyl-6-pregnen-3β-ol, and pregnenolone, to the corresponding 5,6α-epoxides, 5,6β-epoxides, and 5,6β-glycols in the presence of an NADPH-generating system, ferrous ion, and ADP. All of the metabolites were separated and isolated by high performance liquid chromatography and identified by gas chromatography-mass spectroscopy. The microsomes catalyzed hydrolysis of the α- and β-epoxides of all the examined Δ^5-steroids to the corresponding 5α,6β-glycols. The Δ^5-steroid double bond oxidation reaction was mediated by microsomal lipid peroxidation but not by P-450. The reaction required ferrous ion and was inhibited with the metal-chelating agent, EDTA. The microsomal epoxidation took place at higher rates from the β-side of the Δ^5-steroid molecules than from the α-side to yield the α- and β-epoxides in the ratio 1:4. The well known potent inhibitor, 3,3,3-trichloro-1-propene oxide (TCPO), of hepatic microsomal epoxide hydrolase did not inhibit but remarkably stimulated the microsomal hydrolysis of xenobiotic epoxides, styrene oxide and safrole oxide.

MOLECULAR SPECIES OF BILIARY PHOSPHATIDYLCHOLINES IN GALLSTONE PATIENTS: THE INFLUENCE OF TREATMENT WITH CHOLIC ACID AND CHENODEOXYCHOLIC ACID. J. Ahlberg, T. Curstedt, K. Einarsson, and J. Sjövall (Department of Medicine and Surgery, Serafimerlasarettet, Department of Medicine, Huddinge University Hospital, and Department of Chemistry, Karolinska Institutet, Stockholm, Sweden) *J. Lipid Res.* 22(3): 404-9 (1981). Molecular species of phosphatidylcholines were analyzed in hepatic and gallbladder bile obtained from six subjects with adenomyoma of the gallbladder (gallstone-free controls) and 27 gallstone patients undergoing cholecystectomy. Seven of the gallstone patients had been treated with cholic acid and seven with chenodeoxycholic acid for at least 8 weeks before operation.

The two predominant species were 1-palmitoyl-2-oleoyl- and 1-palmitoyl-2-linoleoyl-*sn*-glycerophosphocholines which together accounted for 75-80% of the total amount of phosphatidylcholines. Minor species were 1-palmitoyl-2-palmitoleoyl-1-stearoyl-2-linoleoylleoyl-, and 1-palmitoyl-2-arachidonoyl-*sn*-glycerophosphocholines. Gallstone patients has a higher proportion of the 1-palmitoyl-2-oleoyl species and a concomitant lower proportion of the 1-palmitoyl-2-linoleoyl species than gallstone-free subjects. The ratio between the two species was about 0.7 and 0.4, respectively, in the hepatic bile of the two groups of patients. Treatment with bile acids was associated with a normalization of the pattern of phosphatidylcholines.

EFFECT OF SOME DIETARY ADDITIONS TO EITHER AN ARGININE-DEVOID DIET OR A DIET SUPPLEMENTED WITH OROTIC ACID REFED AFTER STARVATION ON LIVER LIPID CONTENT DURING ESSENTIAL FATTY ACID DEFICIENCY IN RATS. Y. Aoyama, A. Yoshida and K. Ashida (Lab. of Nutr. Biochem., Dept. of Ag. Chem., Nagoya Univ., Furo-cho, Chikusa, Nagoya, Japan) *J. Nutr. 111*(5):895-906 (1981). Refeeding either an arginine-devoied diet or a 14% casein diet supplemented with 1% orotic acid for 7 days to starved rats caused an increase in liver lipid content which was prevented by the addition of adenine, allopurinol and safflower oil, but not guanine, cytosine, thymine and uracil. When rats were refed the arginine-devoid diet unsupplemented or supplemented with guanine, cytosine, thymine and uracil, their serum triglyceride and cholesterol decreased or tended to decrease as compared with those of rats refed the arginine-devoid diet supplemented with either adenine or allopurinol or rats refed the arginine-devoid diet supplemented with either adenine or allopurinol or rats refed the arginine-supplemented diet. Thus, when the arginine-devoid diet unsupplemented or supplemented with arginine, adenine and allopurinol was refed, liver lipid content was inversely related to the serum triglyceride level.

IODINATION OF DOCOSAHEXAENOIC ACID BY LACTOPEROXIDASE AND THYROID GLAND IN VITRO: FORMATION OF AN IODOLACTONE. J.M. Bocynames, J.T. Watson, J.A. Oates and W.C. Hubbard (Department of Pharmacology, Vanderbilt University, Nashville, TN 37232) *Lipids 16*(5):323-8 (1981). In the presence of iodide, hydrogen peroxide and lactoperoxidase, docosahexaenoic acid (22:6ω3) was converted into iodinated compounds. The major product was identified as 5-iodo-4hydroxy-7,10,13,16,19-docosapentaenoic acid, ±lactone, on the basis of ^{125}I incorporation, mass spectrometry, chemical modifications and proton nuclear magnetic resonance spectroscopy. Iodolactonization of docosahexaenoic acid occurred in the rat thyroid in vitro and was inhibited by the peroxidase inhibitor methimazole. These data indicate that formation of an idolactone constitutes one pathway of docosehexaenoic acid metabolism which could be expressed in tissues containing an iodide peroxidase.

EFFECTS OF ACUTE ADMINISTRATION OF CHLORINATED WATER ON LIVER LIPIDS. J.H.S. Chang and C.R. Vogt; G.Y. Sun and A.Y. Sun (Sinclair Comparative Med. Res. Farm and Dept. of Biochemistry, Univ. of Missouri, Columbia, MO 65201) *Lipids 16*(5):336-40 (1981). An acute administration of chlorinated water to rats caused "fatty liver" and indicated a more than 2-fold increase in liver triacylglycerols at 2 days after administration. The acyl group composition of triacylglycerols and phospholipids in both liver mitochondria and liver whole homogenate were also altered by the chlorine treatment. Among the phospholipid acyl groups, there was an increase in the proportion of 20:4 but a decrease in most other polyunsaturated acyl groups. The acyl group changes were more obvious with phosphatidylcholines than with phosphatidylethanolamines. Other phospholipids, including cardiolipin in the mitochondrial membranes, were not greatly altered. Both morphological and biochemical changes were maximum at 2 days after the treatment and were fully recovered after 10 days. The disturbance of a number of enzymatic processes in the liver membranes may account for a large part of the changes observed.

TEMPERATURE ACCLIMATION IN THE CRAYFISH: EFFECTS ON PHOSPHOLIPID FATTY ACIDS. T. Farkas and J.C. Nevenzel (Institute of Biochemistry, Biological Res. Center, Hungarian Academy of Sciences, H-6701 Szeged, Hungary) *Lipids 16*(5):341-6 (1981). Acclimation to different temperatures by a poikilothermous animal must include modification of its membrane lipids to maintain the proper physical properties. The simplest way to achieve this acclimation would seem to be by modification of the phospholipid fatty acids. In a freshwater crayfish, Procambarus clarkii, rapid changes in the degree of unsaturation of newly synthesized phospholipid fatty acids were correlated with changes in environmental temperature, both in whole animals and in slices of hepatopancreas tissue. At 5 C, the rate of fatty acid synthesis was about half that occurring at 23 C. Hepatopancreas tissue from animals acclimated to either 5 C or 23 C, when incubated for 2 hr at 5 C, incorporated a higher percentage of exogenous [1-^{14}C] acetate into polyunsaturated acids (27-38% of the radioactivity in total fatty acids) than when incubated at 23 C (12-14%); conversely, more saturated fatty acids were synthesized at 23 C (73-80% vs 51-73%). The higher average unsaturation of the fatty acids biosynthesized at 5 C constitutes an effective response to the animal's need for modification of lipids to maintain adequate membrane function at the lower environmental temperature.

THYROID CONTROL OVER BIOMEMBRANES: VI. LIPIDS IN LIVER MITOCHONDRIA AND MICROSOMES OF HYPOTHYROID RATS. F.L. Hoch, C. Subramanian, G.A. Dhopeshwarkar, and J.F. Mead (Depts. of Internal Medicine and Biological Chemistry, University of Michigan Medical School, 7696 Kresge Building, Ann Arbor, MI 48109) *Lipids 16*(5):328-35 (1981). The lipids of liver mitochondria prepared from normal rats and from rats made hypothyroid by thyroidectomy and injection with ^{131}INa contained similar amounts, per mg protein, of total lipids, phospholipids, neutral lipids and lipid phosphorus. Hypothyroidism caused a doubling of the relative amounts of mitochondrial cardiolipins (CL; to 20.5% of the phospholipid P) and an accompanying trend (although statistically not significant) toward decreased amounts of both phosphatidylcholines (PC) and phosphatidylserines (PS), with phosphatidylethanolamines (PE) remaining unchanged. The pattern of elevated 18:2 fatty acyl content and depleted 20:4 acyl groups of the mitochondrial phospholipids of hypothyroid preparations was reflected to varying degrees in the resolved phospholipids, with PC showing greater degrees of abnormality than PE, and CL showing none. Hypothyroidism produced the same abnormal pattern of fatty acyl distributions in liver microsomal total lipids as was found in the mitochondria. Hypothyroid rats, when killed 6 hr after injection of [1-^{14}C] labeled linoleate, showed the following abnormalities: the liver incorporated less label into lipids, and converted 18:2 not exclusively to 20:4 (as normals do) but instead incorporated the label mainly into saturated fatty acids. These data, together with the known decrease in β-oxidation, suggest that hypothyroidism involves possible defective step(s) in the conversion of 18:2 to 20:4.

COMPOSITION AND VARIABILITY OF THE BRANCHED-CHAIN FATTY ACID FRACTION IN THE MILK OF GOATS AND COWS. A.M. Massart-Leën, H. DePooter, M. Decloedt and N. Schamp (Dept. of Physiology, Veterinary Faculty, State University of Gent, Casinoplein 24, B-9000 Gent, Belgium) *Lipids 16*(5): 286-92 (1981). Branched-chain fatty acids of the milk fat of goats were analyzed by high resolution gas chromatography-mass spectrometry. Iso- and anteiso-acids predominated, but a range of other monomethyl-branched components, mostly with methyl-substitution on carbons 4 and 6, was present. Analysis of the milk fat of cows revealed the presence of iso- and anteiso-fatty acid; other monomethyl-substituted fatty acids, as found in the milk fat of the goat, were virtually absent. Only a trace amount of 6-methylhexadecanoate was detected. The difference between goats and cows in the effectiveness with which these animals metabolize propionyl-CoA and methylmalonyl-CoA is discussed.

EFFECT OF 1,25-DIHYDROXYVITAMIN D_3 ON PHOSPHOLIPID METABOLISM IN CHICK DUODENAL MUCOSAL CELL. RELATIONSHIP TO ITS MECHANISM OF ACTION. T. Matsumoto, O. Fontaine and H. Rasmussen (Departments of Cell Biology and Internal Medicine, Yale University School of Medicine, New Haven, Connecticut 06510) *J. Biol. Chem. 256*(7):3354-60 (1981). Pretreatment of the D-deficient chick with 1,25-dihydroxyvitamin D_3 increases *de novo* synthesis of phosphatidylcholine by a stimulation of CDP-choline:*sn*-1,2-diacylglycerol choline-phosphotransferase reaction. The time course of change in the incorporation of [^{3}H] choline and [^{14}C] ethanolamine into the brush border lipid fraction after 1,25-dihydroxyvitamin D_3 treatment correlates closely with the time course of change in calcium uptake into the brush border membrane vesicles. Prior treatment with cycloheximide does not block this increase in phosphatidylcholine synthesis. In addition, 1,25-dihydroxyvitamin D_3 administration increases the incorporation of [^{3}H] arachidonic acid into the phosphatidylcholine fraction of the brush border to a great extent but does not increase the incorporation of [^{3}H] palmitic acid into the phosphatidylcholine fraction. The incorporation of these ^{3}H labeled fatty acids into diacylglycerol is not changed by 1,25-dihydroxyvitamin D_3. These data indicate that 1,25-dihydroxyvitamin D_3 enhances the synthesis of phosphatidylcholine independent of new protein synthesis, and also increases the incorporation of unsaturated fatty acids into phosphasynthesis, and also increases the incorporation of unsaturated fatty acids into phosphatidylcholine. From these results we suggest that changes in phospholipid metabolism in the enterocyte are the mechanisms by which 1,25-dihydroxyvitamin D_3 acts to enhance calcium entry across the brush border membrane.

PHOSPHOLIPID ACYL GROUP COMPOSITION IN NORMAL AND TUMORAL NERVE CELLS IN CULTURE. D. Montaudon, J.C. Louis and J. Robert (Laboratoire de Biochimie Médicale A, Université de Bordeaux II, 146, rue Léo-Saignat, 33076 Bordeaux-Cédex, France) *Lipids 16*(5):293-7 (1981). We have studied the fatty acid composition of total phosphoglycerides from various types of nerve cells in culture. Primary cell cultures were compared with tumoral cell strains. Glial cells exhibited no chracteristic pattern when compared to neurons. Tumoral cell phosphoglycerides contained much higher levels of octadecenoic acid and lower levels of C-20 and C-22 polyunsaturated fatty acids than normal cell phosphoglycerides. This observation seems to be a general feature in tumoral cell membranes. It could be of interest in respect to the membrane fluidity of cancer cells.

COMPARISON OF THE EFFECTS OF COLESTIPOL HYDROCHLORIDE AND CLOFIBRATE ON PLASMA LIPIDS AND LIPOPROTEINS IN THE TREATMENT OF HYPERCHOLESTEROLEMIA. A.H. Seplowitz, F.R. Smith, L. Berns, H.A. Eder and D.S. Goodman (Arteriosclerosis Res. Center, Dept. of Med., Columbia-Presbyterian Med. Center, New York, NY) *Atherosclerosis 39*(1): 35-43 (1981). The effects of colestipol HCl resin and clofibrate on plasma lipid and lipoprotein levels were compared in 65 patients with primary hypercholesterolemia. Patients were randomly assigned to treatment with colestipol (in progressive doses of 15, 20, and 30 g/day), clofibrate (2 g/day), or placebo resin; lipoprotein levels were determined at months 0, 2, 4, 6, and 9. The colestipol group received both colestipol and clofibrate during months 7 through 9 of the study. After 6 months of treatment, mean plasma total cholesterol fell from 333 to 266 ($P<0.01$) on colestipol, and from 329 to 270 ($P<0.05$) on clofibrate. More patients responded, however, to colestipol than to clofibrate. Both drugs also produced significant reduction in LDL cholesterol levels, and clofibrate lowered plasma triglycerides as well. HDL cholesterol levels did not change significantly on either medication. The placebo group showed no change in any of the parameters studies. A significant difference was not observed between the effects of 15 g/day of colestipol and those of the higher doses studied. Addition of clofibrate to colestipol did not enhance the latter's hypocholesterolemic action.

KINETIC ANALYSIS OF THE MALONYL COENZYME A DECARBOXYLATION AND THE CONDENSATION REACTION OF FATTY ACID SYNTHESIS. APPLICATION TO THE STUDY OF MALONYL COENZYME A INACTIVATED CHICKEN LIVER FATTY ACID SYNTHETASE. K.R. Srinivasan and S. Kumar (Dept. of Biochem., College of Med. and Dentistry of NJ, NJ Med. Sch., Newark, NJ) *Biochem. 20*:3400-4 (1981). A kinetic analysis of the decarboxylation of malonyl-CoA and the condensation-CO_2 exchange reaction of fatty acid synthesis has been carried out. The analysis supported by experimental evidence defines conditions under which the decarboxylation of malonyl-CoA quantitatively reflects the activity for the condensation reaction between enzyme-bound acyl and malonyl groups. $NADP^+$ decreases the release of $^{14}CO_2$ from radiolabeled malonyl-CoA by lowering the rates of the processes leading to the formation of triacetic acid lactone. These analyses have been used to explain the mechanism of malonyl-CoA mediated inactivation of chicken liver fatty acid synthetase and are appropriate for determining the functional condensing site of the polyfunctional polypeptide chains comprising the dimeric enzyme.

ARTERIAL INFLUX OF ESTERIFIED CHOLESTEROL FROM TWO PLASMA LIPOPROTEIN FRACTIONS AND ITS HYDROLYSIS IN VIVO IN HYPERCHOLESTEROLEMIC RABBITS. S. Steender and D.B. Zilversmit (Div. of Nutr. Sci. and Sec. of Biochem., Mol. and Cell Biol., Div. of Biol. Sci., Cornell Univ., Ithaca, NY) *Atherosclerosis 39*:97-109 (1981). Arterial influx of esterified cholesterol from 2 plasma lipoprotein fractions, $d<1.019$ and $d>1.019$, and influx of plasma free cholesterol were determined in each of 15 hypercholesterolemic rabbits with aprpoximately the same plasma cholesterol concentrations but with different extents of arterial lesions. The arterial influx of cholesteryl ester derived from $d<1.019$ lipoproteins was about equal to that derived from the $d>1.019$ fraction. The amount of cholesteryl ester in plasma $d<1.019$ was approximately 3 times that in $d>1.019$. Thus, per unit of cholesteryl ester concentration the $d<1.019$ lipoprotein delivered about 1/3 as much cholesteryl ester to the artery as the lipoproteins in the higher density fractions. Some 5-40% of plasma esterified cholesterol which had entered the artery was hydrolyzed in the artery during the experimental period. The influx of free cholesterol that could not be accounted for by the influx of intact plasma lipoproteins was 5-80% of the free cholesterol influx. This excess probably represents free cholesterol influx by an exchange between the plasma lipoproteins and the intimal surface of the artery.

EFFECT OF 2-HEXADECYNOIC ACID ON CULTURED 7288C HEPATOMA CELLS. G.C. Upreti, M. Matocha and R. Wood (Dept. of Biochemistry and Biophysics, Texas Agricultural Experiment Station, Texas A&M University System, College Station, TX 77843) *Lipids 16*(5):315-22 (1981). The effects of 2-hexadecynoic acid on the growth and lipid metabolism of cultured 7288 (HTC) cells have been evaluated. Growth was inhibited by the acetylenic acid: the LD_{50} was 35-85 μM as determined by two methods at low and high cell densities. Reduced growth did not result from damaged plasma membranes as determined by α-amino isobutyrate leakage. DNA synthesis was unaffected by the acetylenic acid and the effect on RNA and protein synthesis appeared to be secondary to the effects on lipid metabolism. The 2-hexadecynoic acid inhibited lipid metabolism of the HTC cells at least at two levels. Data from both mass studies and radioactive acetate distributions in cellular and media lipids indicated that fatty acid elongation and acylation, especially triglyceride synthesis, were inhibited.

ISOLATION AND IDENTIFICATION OF 24(R)-HYDROXYVITAMIN D_3 FROM CHICKS GIVEN LARGE DOSES OF VITAMIN D_3. J. Wichmann, H.K. Schnoes, and H.F. DeLuca (Dept. of Biochem., College of Ag. and Life Sci., Univ. of Wisconsin-Madison, Madison, Wisconsin) *Biochemistry 20*:2350-53 (1981). A new metabolite of vitamin D was isolated from the blood plasma of chicks given large doses of vitamin D_3. The isolation involved methanol-chloroform extraction and four column chromatographic steps. The metabolite was identified by high- and low-resolution mass spectroscopy, chemical derivatization, and comigration with authentic standard as 3β,24(R)-dihydroxy-9,10-seco-5,7,10(19)-cholestatriene (24(R)-hydroxyvitamin D_3). No detectable 24-(R)-hydroxyvitamin D_3 was recovered from 16 L of plasma from chicks receiving physiologic levels of vitamin D_3.

DNA LABELING OF RAT EPITHELIAL TISSUES IN VITAMIN A DEFICIENCY. M.H. Zile, E.C. Bunge and H.R. Deluca (Dept. of Biochem., Col. of Ag. and Life Sci., Univ. of Wis.-Madison, Madison, Wis.) *J. of Nutr. 111*(5):777-88 (1981). We examined the effect of vitamin A nutritional status on cell division in various epithelial tissues of the rat. Tissues were examined by histological methods, and DNA labeling was assessed by autoradiography. Mild vitamin A deficiency decreased the DNA labeling index in the trachea and the epidermis, while not altering the histological appearance and the DNA labeling index of the cornea, jejunum and colon were not altered by mild vitamin A deficiency. We concluded that a diminished proliferation of epithelial cells is a manifestation of suboptimal vitamin A availability in that tissue.

Fats and oils

KINETICS OF HYDROGEN ION DIFFUSION ACROSS PHOSPHOLIPID VESICLE MEMBRANES. C.M. Biegel and J.M. Gould (Program in Biochem. and Biophys., Dept. of Chem., Univ. of Notre Dame, Notre Dame, Indiana) *Biochem. 20*:3474-3479. The membrane-impermeant, pH sensitive fluorescence probe 8-hydroxy-1,3,6-pyrenetrisulfonate can be entrapped with the internal aqueous compartment of unilamellar phospholipid vesicles, where it serves as a reliable indicator of internal aqueous hydrogen ion concentration. These findings indicate that the intrinsic permeability of unilamellar vesicle membranes to hydrogen ions is surprisingly high and much greater than the observed permeabilities of other small ions.

EFFECT OF STEROL STRUCTURE AND EXOGENOUS LIPIDS ON THE TRANSBILAYER DISTRIBUTION OF STEROLS IN THE MEMBRANE OF *MYCOPLASMA CAPRICOLUM*. S. Clejan, R. Bittman and S. Rottem (Dept. of Chem., Queens College of the City Univ. of New York, Flushing, NY) *Biochemistry 20*:2200-4 (1981). Stopped-flow kinetic measurements of the association of filipin with sterols in intact cells and isolated membranes of *Mycoplasma capricolum* were used to study the effects of varying the phospholipid composition and the sterol structure on sterol distribution in the membrane. The phospholipid composition and content of the membrane were varied by growing cells in an albumin-containing medium with cholesterol, palmitic and oleic acids, and various concentration of exogenous phospholipids. Thus, the behavior of the alkyl-substituted sterols differs from that of cholesterol. The extent to which a sterol is distributed asymmetrically between the two halves of the bilayer is not related to the extent to which maximum growth is produced. These results suggest that growth supporting sterols need not be translocated extensively.

GEL TO LIQUID-CRYSTALLINE TRANSITION TEMPERATURES OF WATER DISPERSIONS OF TWO PAIRS OF POSITIONAL ISOMERS OF UNSATURATED MIXED-ACID PHOSPHATIDYLCHOLINES. P.J. Davis, B.D. Fleming, K.P. Coolbear,

and K.M.W. Keough (Dept. of Biochem., Memorial Univ. of Newfoundland, St. John's, Newfoundland, Canada) *Biochem. 20*:3633-6 (1981). The gel to liquid-crystalline phase transition temperatures of dispersions of mixed-acid *sn*-1,2-lecithins which contain one unsaturated and one saturated fatty acid have been studied by differential scanning calorimetry. The differences in transition temperatures for the isomers of a pair containing the same two acids were consistent with those observed for positional isomers of saturated mixed-acid lecithins in that the isomer of the pair which had the longer fatty acid in the *sn*-1 position had the lower temperature. Differences in the chain lengths of the fatty acids at the two positions of the glycerol appear to predominate over differences in the depths of the double bonds in the bilayer in determining the transition temperatures.

ANALYSIS OF AUTOXIDIZED FATS BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY: VII. VOLATILE THERMAL DECOMPOSITION PRODUCTS OF PURE HYDROPEROXIDES FROM AUTOXIDIZED AND PHOTOSENSITIZED OZIDIZED METHYL OLEATE, LINOLEATE AND LINOLENATE. E.N. Frankel, W.E. Neff and E. Selke (Northern Regional Res. Center, Agricultural Res., Science and Education Administration, U.S. Dept. of Agriculture, Peoria, IL 61604) *Lipids 16*(5):279-85 (1981). To clarify the sources of undesirable flavors, pure hydroperoxides from autoxidized and photosensitized oxidized fatty esters were thermally decomposed in the injector port of a gas chromatograph-mass spectrometer system. Major volatile products were identified from the hydroperoxides of methyl oleate, linoleate and linolenate. Although the hydroperoxides from autoxidized esters are isomerically different in position and concentration than those from photosensitized oxidized esters, the same major volatile products were formed but in different relative amounts. Distinguishing volatiles were, however, produced from each type of hydroperoxide. The 9- and 10-hydroperoxides of photosensitized oxidized methyl oleate were thermally isomerized in the injector port into a mixture of 8-, 9-, 10- and 11-hydroperoxides similar to that of autoxidized methyl oleate. Under the same conditions, the hydroperoxides from autoxidized linoleate and linolenate did not undergo significant interconversion with those from the corresponding photosensitized oxidized esters. The compositions of the major volatile decomposition products are explained by the classical scheme involving carbon-carbon scission on either side of alkoxy radical intermediates. Secondary reactions of hydroperoxides are also postulated, and the hydroperoxy cyclic peroxides from methyl linoleate (photosensitized oxidized) and methyl linolenate (both autoxidized and photosensitized oxidized) are suggested as important precursors of volatiles.

FREE RADICAL POLYMERIZATION AND LIPID BINDING OF LYSOZYME REACTED WITH PEROXIDIZING LINOLEIC ACID. J. Funes and M. Karel (Dept. of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, MA 02139) *Lipids 16*(5):347-53 (1981). Insolubilization and polymerization of proteins exposed to peroxidizing lipids may be due either to cross-linking with incorporation of fragments of the lipid oxidation products, or to free radical transfer from lipid to protein and subsequent free radical polymerization of protein. The second mechanism which has been proposed was inferred from measurements of electron spin resonance signals in proteins. In this study, uniformly labeled linoleic acid, [^{14}C(U)]LA, was reacted with lysozyme. Volatile oxidation products of LA were also used in some experiments. Incubation was done in the absence of water. Oligomers of lysozyme, as well as the monomer, were isolated after incubation, and the [^{14}C] label incorporated into each fraction was determined. The results show that the dominant mechanism of protein polymerization after exposure to peroxidizing linoleic acid is the transfer of free radical from lipid to protein, and subsequent free radical polymerization.

INTRA- AND INTERMOLECULAR INTERACTIONS OF PHOSPHOLIPID HEADGROUPS WITHIN A TWO-DIMENSIONAL HEXAGONAL LATTICE. H. Frischleder, R. Krahl and E. Lehmann (Karl-marx Univ., Sektion Physik, 7010 Leipzig, G.C.R.) *Chem. and Phys. of Lipids 28*(3):291-304 (1981). Using different types of atom-atom potential functions the energetically most stable conformations of glycero-phosphatidylethanolamine (GPE) in a two-dimensional hexagonal lattice were calculated. The results show that the conformational behaviour of the phospholipid headgroups is determined mainly by the intramolecular electrostatic repulsion between the phosphate groups ester oxygen lone pairs. The best agreement with X-ray torsion angles was obtained reducing the CNDO-APSG net atomic charges by a factor of $3^{-1/2}$. The energetically preferred regions of headgroup torsion angles give a molecular model of the headgroup reorientational process in agreement with NMR results.

MEMBRANE FUSION THROUGH POINT DEFECTS IN BILAYERS. S.W. Hui, T.P.Stewart, and L.T. Boni (Biophysics Dept., Roswell Park Memorial Institute, Buffalo, NY 14263); and P.L. Yeagle (Biochem. Dept., State Univ. of New York, Buffalo 14214) *Science 212*(4497):921-2 (1981). Fusion between bilayers of mixed egg phosphatidylcholine and soybean phosphatidylethanolamine was induced by freezing and thawing. Contact points between bilayers were observed by freeze fracture electron microscopy, and isotropic molecular motional averaging was detected by phosphorus-31 nuclear magnetic resonance under fusion conditions. A molecular model of point defect structure is proposed as an intermediate stage of fusion.

4-DEMETHYL-, 4-MONOMETHYL- AND 4,4-DIMETHYLSTEROLS IN SOME VEGETABLE OILS. A. Kornfeldt and L.B. Croon (Food Technology Division, Alfa-Laval AB, Box 500, S-147 00 Tumba, Sweden) *Lipids 16*(5):306-14 (1981). The content of 4-demethyl- 4-monomethyl- and 4,4-dimethylsterols in 13 vegetable oils was found to vary between 0.10-1.5%, 0.01-0.08% and 0.02-0.29%, respectively. The largest amount of demethylsterols was found in maize and wheat germ oils, whereas the largest amounts of the dimethylsterols were found in olive and linseed oils. The predominating demethylsterols were sitosterol, campesterol, stigmasterol and Δ^5-avanasterol. Among the 4-monomethylsterols, obtusifoliol, gramisterol, cycloeucalenol and citrostadienol predominated, but usually more than 10 components were found in this fraction. The composition of the 4,4-dimethylsterol fraction was also rather complex, with the 9,19-cyclopropanesterols together with α- and β-amyrin predominating. In most of the oils, characteristically high or low percentages of some sterols were found, and a few specific sterols were also noted. A scheme useful for characterization is presented.

LIPID ANALYSIS OF CELLS AND CHROMATOPHORES OF *RHODOPSEUDOMONAS SPHAEROIDES*. G.V. Marinetti and K. Cattieu (Univ. of Rochester Med. Center, 601 Elmwood Ave., Rochester, NY) *Chem. and Phys. of Lipids 28*(3):241-51 (1981). The phospholipids and fatty acid analysis of four strains of *Rodopseduomonas sphaeroides* and of chromatophores from two strains show some differences and also show the presence of an unusual polar neutral lipid which is ninhydrin positive and which on acid hydrolysis yields ornithine and an unidentified amino compound. This lipid is called aminolipid-X and has a fatty acid composition very different from the phospholipids. Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) contain a very small amount of plasmolgen froms as determined by combined mild alkaline hydrolysis, acetic acid hydrolysis and phospholipase A_2 hydrolysis. The reaction of intact cells and chromatophores with trinitobenzenesulfonate (TNBS), fluorodinitrobenzene (FDNB) and isethionylacetimidate (IA) show that 78% of the total PE in chromatophores is localized on the outer membrane surface. In intact cells about 15-35% of the total PE is localized on the outer surface of plasma membrane.

EFFECTS OF MOLECULAR STRUCTURE ON POSITIVE AND NEGATIVE AZEOTROPIES IN MIXED MONOLAYERS OF LIPIDS. H. Matuo, K. Motomura and R. Matuura (Dept. of Chem., Faculty of Sci., Kyushu Univ., Fukuoka, Japan) *Chem. and Phys. of Lipids 28*(3):281-9 (1981). In order to clarify the effect of molecular structure on the nature of azeotropic transformations in mixed monolayers, many systems of the positive and negative azeotropic types are examined in this study. Two-dimensional phase diagrams and apparent molar energy changes which are associated with the phase transition from the liquid-expanded to the liquid-condensed state are evaluated using a previously developed thermodynamic treatment. There is a maximum in the phase diagram of the positive azeotropic type and the excess apparent molar -energy change is positive over the entire compositional range. Steric hindrance of the hydrophilic groups seems to be the important factor in the behavior of the positive azeotropic type. For the negative azeotropic type there is a minimum in the phase diagram and the excess apparent molar energy change in negative over the entire compositional range. The two long acyl chains of dipalmitoyl lecithin leads to a stronge interaction between the two components.

EFFECT OF FATTY ACIDS AND MONOGLYCERIDES ON PERMEABILITY OF LIPID BILAYER. N. Muranushi, N. Takagi, S. Muranishi and H. Sezaki (Faculty of Pharm. Sci., Kyoto Univ., Kyoto, Japan) *Chem. and Phys. of Lipids 28*(3):269-79 (1981). The effect of fatty acids and monoglycerides on barrier properties of liposomal membranes prepared from egg phosphatidylcholine was investigated. The incorporation of these lipids as liposomal membrane components induced the alteration of the permeability to less permeable liposomally entrapped drugs, sulfanilic acid and procainamide ethobromide (PAEB). These results indicated that the increase in the membrane permeability caused by fatty acids and monoglycerides associated with the disorder in the membranes'

interior and the interaction of the incorporated lipid with the polar head group of phospholipid.

PERMEABILITY OF PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLETHANOLAMINE BILAYERS. M. Singer (Dept. of Med., Queen's Univ., Kingston, Ontario) *Chem. and Phys. of Lipids* 28(3):253-67 (1981). Sodium and glucose effluxes were measured in liposomes formed from a series of saturated phosphatidylcholines (PC) and phosphatidylethanolamines (PE). Vesicles composed of a saturated PC display a local permeability maximum in the region of the lipid transition temperature. The height of this maximum is predominantly a function of the thickness of the hydrocarbon chain region. Liposomes formed from a saturated PE do not display such a permeability maximum and in these vesicles the permeability process appears to be controlled by the head group region. It is postulated that the control exerted by the ethanolamine group is due to the reorganization of water structure it induces at the bilayer surface.

ACYL EXCHANGE BETWEEN OLEOYL-CoA AND PHOSPHATIDYLCHOLINE IN MICROSOMES OF DEVELOPING SOYA BEAN COTYLEDONS AND ITS ROLE IN FATTY ACID DESATURATION. S. Stymne and G. Glad (Dept. of Food Hygiene, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden) *Lipids* 16(5):298-305 (1981). Microsomes of developing soya bean cotyledons transfer oleate from oleoyl-CoA to phosphatidylcholine (PC) by two different mechanisms: one in which oleate transfer is accompanied by the release of free CoA and another which results in the exchange of oleate from oleoyl-CoA for unsaturated 18-carbon fatty acids of PC. The acyl exchange can be demonstrated only when bovine serum albumin is present in the incubation medium. ATP-dependent acyl-CoA synthetase is not involved in the exchange process, which apparently does not require any cofactors. In light of this exchange process, the oleate desaturase system was reinvestigated in order to determine what the actual substrate for this system is. Upon incubation of microsomes with high concentrations of [^{14}C] oleoyl-CoA, bovine serum albumin and NADH, it could be conclusively demonstrated that most oleic acid is desaturated while part of the PC molecule. The amounts of [^{14}C] linoleoyl-CoA formed could be explained entirely by the acyl exchange. The physiological significance of the acyl exchange system is discussed. A new method for separation of acyl-CoA from other lipids and free CoA using reversed phase column chromatography also is described.

^{31}P NMR STUDY OF HEAD GROUP BEHAVIOR IN SONICATED PHOSPHATIDYLCHOLINE LIPOSOMES IN THE GEL AND IN THE LIQUID STATE. V. Viti and M. Minetti (Lab. di Bio. Cellulare e Immuno., Istituto Superiore de Sanita, Viale Regina Elena, Rome) *Chem. Phys. of Lipids* 28(3):215-25 (1981). We measured the ^{31}P{^{1}H} Nuclear Over Effect (NOE) as a function of temperature and of ^{1}H irradiation frequency, the linewidth $\Delta\nu_{1/2}$ as a function of temperature and the relaxation time T_1 above and below the thermal transition temperature, of the ^{31}P-NMR signal in sonicated liposomes of 1,2-dimiristoyl-3-*sn*-phosphatidylcholine (DMPC), 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine (DPPC) and 1,2-distearoyl-3-*sn*-phosphatidylcholine (DSPC). The same measurements were repeated in the presence of high molecular weight dextrans. They strongly reduce the NOE and produce longer relaxation times T_1. According to the current models, we were able to evaluate in the different situations, the correlation time of the internal motion τ_G and the distance r between interacting groups in the region of the polar head groups. While the first parameter changes abruptly through the phase transitions and under the effect of dextrans, the latter does not appear modified in any case. These results are discussed in terms of a conformational change of the phosphocholine head groups.

EICOSA-5,11,14-TRIENOIC AND OCTADEC-5-ENOIC ACIDS OF THE REPRODUCTIVE TRACT OF THE MALE HOUSE CRICKET (*ACHETA DOMESTICUS*) and field cricket (*Cryllus* spp.). R.E. Worthington, U.E. Brady, and H.L. Hitchcock (Dept. of Food Science, University of Georgia Experiment Station, Experiment, GA 30212) *Lipids* 16(5):351-4 (1981). The reproductive tracts (testes, seminal vesicles, accessory glands, ejaculatory duct) of male house crickets (*Acheta domesticus*) and field crickets (*Cryllus* spp.) contain ca. 6% of eicosa-5,11,14-trienoate in total tissue fatty acids. This fatty acid is concentrated primarily in the phosphatidylcholine fraction of phospholipids (12.6%) and occurs in no more than trace amounts (<0.1%) in neutral lipids. The 18:1 fatty acid fraction of total tissue lipid fatty acids consisted of 2 isomers with unsaturation in the Δ5 and Δ9 positions in proportions of ca. 33 and 67 mol%, respectively.

CHARACTERISTICS AND COMPOSITION OF INDIAN CASSAVA SEED AND OIL. R. Prasada Rao, G. Azeemoddin, D. Atchyuta Ramayya and S.D. Thirumala Rao, Oil Technological Research Institute, Anantapur, India. J. Food Sci. Tech. (India) Vol. 17; 266, 1980. A local variety of cassava (*Manihot esculenta* Crantz, *M. Utilissima* Phol) gave seed yield of 400 kg/ha. Seed contained 26% orange-yellow coloured oil and 18.5% protein. The oil has low unsaponifiable matter and the following fatty acid composition (weight %) as determined by gas-liquid chromatography: 8:0, 0.5; 10:0, 0.8; 12:0, 4.6; 14:0, 1.5; 16:0, 11.4; 18:0, 4.6; 18:1, 25.1 and 18:2, 51.4.

Drying oils and paints

OFF-LINE SYSTEM FOR HANDLING GAS CHROMATOGRAPHIC FATTY ACID DATA. E. Lanza, B.M. Golden, J. Zyren and H.T. Slover. *J. Chromat. Sci. 18* 126-32 (1980). A data gathering system for handling GLC fatty acid analyses is described. (World Surface Coatings Abs. No. 462.)

SURVEY OF THE USES OF FATTY SUBSTANCES IN PAINTS AND RELATED PRODUCTS. I. A. Poluzzi. *Riv. Ital. delle Sostanze Grasse 56*, 187-200 (1979). This survey describes the main paint oils (including tall oil); chemical products derived from fats, e.g., epoxidized oils and polyamides; synthetic fatty acids; fatty alcohols; vinyl monomers from fatty acids; and metallic soaps and additives based on fatty acids. (World Surface Coatings Abs. No. 462.)

PHYSIOCHEMICAL INVESTIGATION OF VEGETABLE OILS MODIFIED WITH PENTADIENE-1,3. A.D. Gladun et al. *Lakokras. Mat.* **1980**(3), 41-3. A method for solving the vegetable oil shortage by modification with oligomeric dienes is proposed. The physiochemical properties of sunflower seed oil modified with pentadiene-1,3 (piperylene) were determined. (World Surface Coatings Abs. No. 466.)

MICROSCOPIC DISTRIBUTION OF LINSEED OIL AFTER APPLICATION TO WOOD SURFACE. M.H. Schneider, *J. Coatings Tech.* 52 No. 665, 64-7 (1980). Scanning electron microscopic studies demonstrated the non-uniform distribution of linseed oil in wood, suggesting multiple penetration mechanisms. (World Surface Coatings Abs. No. 462)

OBTAINING A PAINT BINDER FROM RAPESEED OIL. W. Orzechowska and Z. Wolniewicz. *Przem. Chem.* 59 No. 2, 95-7 (1980). Conditions for preparing a varnish with good drying properties from dicyclopentadiene and rapeseed oil, viz. rate of addition of dicyclopentadiene, and temperature and time of reaction, have been established. Viscosity and drying properties of the products have been examined. (World Surface Coatings, Abs. No. 462)

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