Abstracts

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Biochemistry and nutrition

FATTY ACID AND LIPID COMPOSITION OF THE MONKEY RETINA IN DIET-INDUCED HYPERCHOLESTEROLEMIA. B.T. Hyman, M.H. Haimann, M.L. Armstrong, and A.A. Spector (Departments of Biochemistry, Ophthalmology and Internal Medicine, Univ. of Iowa, Iowa City, IA 52242) *Atherosclerosis 40*(3,4):321-328 (1981). We determined the fatty acid composition of the lipids of cynomolgus monkey retina in animals fed commercial chow or a saturated fat, cholesterol-enriched atherogenic diet for 100 days. Doxosahexaenoic acid (22:6) accounted for 25.8% of the ethanolamine phosphoglyceride fatty acids, 17.6% of the serine plus inositol phosphoglyceride fatty acids, 8.4% of the choline phosphoglyceride fatty acids and 5.8% of the netural lipid fatty acids in the retinas of the chow-fed animals. Therefore, monkey retinas, like those of other mammalian species, ordinarily contain large amounts of 22:6. Retinas from the monkeys fed the atherogenic diet contained less 22:6 as well as other polyunsaturates in each of the phospholipid classes. The decrease in polyunsaturates was compensated for by increases in palmitic, stearic, and oleic acids. There was no difference in the amount of phospholipid, the distribution of phospholipid classes, or the amount of cholesterol in the retinas of the monkeys fed the atherogenic diet. These results indicate that the single type of lipid alteration produced in the retina by a diet enriched in saturated fat and cholesterol is a decrease in the polyunsaturated of the retinal phospholipids. The reduction in retinal 22:6 content might have significance for photoreceptor function.

SECRETION AND TURNOVER OF VERY LOW DENSITY LIPOPROTEIN TRIACYLGLYCEROLS IN RATS FED CHRONICALLY DIETS RICH IN GLUCOSE AND FRUCTOSE. R. Kannan, N. Baker, and K.R. Bruckdorfer (Tumor-Lipid Laboratory, Research Service, Veterans Administration, Wadsworth Med. Center, Los Angeles CA 90073) *J. Nutr. 111*(7):1216-1223 (1981). Very low density lipoproteins (VLDL) were isolated from serum after intravenous injection of rats with 1-^{14}C-palmitic acid. These lipoproteins were in turn injected into tail veins of rats which had been fed ad libitum for 21 days on fat-free diets in which the source of carbohydrates was glucose or fructose. Groups of rats were killed at intervals up to 10 minutes after injection and the rates of decline of serum triacylglycerol (TG) and of serum VLDL-TG turnover specific radioactivity were measured. The half-lives of VLDL-TG turnover were very short (approximately 1 minute in both groups) compared to those described previously for rats fed conventional diets or for fasted animals, but the higher plasma TG concentrations in fructose-fed rats were as reported elsewhere. From this information and the serum VLDL-TG concentrations in the two dietary groups, it was possible to estimate the rate of VLDL secretion from the liver which was found to be 75% greater in the fructose-fed rats. No differences were found in the total lipoprotein lipase activity in acetone powders of white adipose tissue from other rats fed fructose and glucose.

EFFECT OF DIETARY LEVEL OF ASCORBIC ACID ON THE GROWTH, HEPATIC LIPID PEROXIDATION, AND SERUM LIPIDS IN GUINEA PIGS FED POLYCHLORINATED BIPHENYLS. N. Kato, K. Kawai, and A. Yoshida. (Dept. of Agricultural Chem., Nagoya Univ., Nagoya 464, Japan) *J. Nuttr. 111*: 1727-1733 (1981). Rats exposed to polychlorinated biphenyls (PCB) or other xenobiotics exhibit an increase in tissue and urinary ascorbic acid, serum cholesterol and hepatic lipid peroxidation. To clarify the physiological role of ascorbic acid in exposure to PCB' we studied the influence of dietary levels of ascorbic acid (30-2,000 ppm) on the growth, serum lipids and hepatic lipid peroxidation in guinea pigs fed 50 ppm PCB-containing diets. The results showed that the growth depression due to PCB was ameliorated by increasing dietary ascorbic acid. The increases in serum cholesterol and phospholipid and in hepatic lipid peroxidation due to PCB were suppressed by larger amounts of ascorbic acid, which inversely correlated with the changes in growth. PCB intake also increased serum levels of cortisol and triglyceride, but these effects were not influenced by dietary level of ascorbic acid. The optimum requirement of ascorbic acid in the guinea pigs fed PCB was 800-2,000 ppm in the diet for the changes in the growth, serum cholesterol and hepatic lipid peroxidation.

SYNTHESIS OF $\Delta^{5,22}$-CHOLESTADIEN-3β-OL FROM $\Delta^{5,7,22}$-CHOLESTATRIEN-3β-OL BY A LIVER ENZYME. M.J. Koroly and M.E. Dempsey (Departments of Surgery and Anatomy, Harvard Medical School & Massachusetts General Hospital, Boston, MA 02114) *Lipids 16*(10):755-758 (1981). The rat liver enzyme system, which catalyzes reduction of $\Delta^{5,7,24}$-cholestatrien-3β-ol to cholesterol (Δ^{5}-cholesten-3β-ol), converted radiolabeled $\Delta^{5,7,22}$-cholestatrien-3β-ol to $\Delta^{5,22}$-cholestadien-3β-ol, but not to cholesterol. This enzyme system thus contains membrane-bound Δ^{7}- and Δ^{24}-reductases and no Δ^{22}-reductase. Kinetic and competition studies showed that the enzyme system contains a single $\Delta^{5,7}$-sterol Δ^{7}-reductase, which is not influenced by unsaturation at the Δ^{22}-position of the sterol side chain. The identity of $\Delta^{5,22}$-cholestadienol was established by chromatographic, spectral and chemical analyses. Use of the enzyme system and readily available $\Delta^{5,7,22}$-cholestatrienol provides a facile procedure for specific production of $\Delta^{5,22}$-cholestadien-3β-ol in quantity.

PLASMA LECITHIN:CHOLESTEROL ACYLTRANSFERASE IN COPPER-DEFICIENT RATS. B.W.C. Lau and L.M. Klevay (Dept. of Biochem., Univ. of North Dakota and U.S. Dept. of Agriculture, Science and Education Adm., Grand Forks Human Nutrition Research Center, Grand Forks, ND 58202) *J. Nutr. 111*:1698-1703 (1981). Some groups of people with high risk of ischemic heart disease have low lecithin:cholesterol acyltransferase activity in plasma and vice versa. Because we hypothesized a relationship between inadequate copper nutriture and the risk of ischemic heart disease, we meausred plasma lecithin:cholesterol acyltransferase in copper deficient male Sprague-Dawley rats. Deficiency was verified by the presence of anemia, hypercholesterolemia and low copper concentrations in kidney and skeletal muscle. Three experiments showed a significant decrease (22-32% reduction) in enzyme activity in deficiency. Copper may be required for the synthesis of the enzyme or as a constituent of the enzyme.

VITAMIN A TURNOVER IN RATS AS INFLUENCED BY VITAMIN A STATUS. K.C. Lewis, M.H. Green, and B.A. Underwood (Nutrition Program, The Pennsylvania State Univ., University Park, PA 16802) *J. Nutr. 111*(7):1135-1144 (1981). Vitamin A turnover was studied in rats fed vitamin A-sufficient (+A) or vitamin A-deficient (-A) diets for 24-25 days. Hepatic vitamin A stores of the +A group (543 μg) were significantly larger than those of -A group (11 μg) and similarly, the plasma vitamin A concentration of the +A group (56 μg/dl) was significantly higher than that of the -A group (26 μg/dl). Rats were injected intravenously with plasma containing tritium-labeled retinol (^{3}H-ROH) obtained from vitamin A-deficient donor rats previously fed ^{3}H-ROH. Plasma samples from injected recipients were collected over a 48-hour period. Kinetic analysis of plasma tracer concentration versus time curves indicated that the data fit a three-pool model. The plasma vitamin A turnover rate of the +A group was significantly more rapid than that of the -A group (5.19 versus 1.98 μg/hr). Plasma fractional turnover rates for the +A group (1.31 hour^{-1}) were not significantly different from those of the -A group (0.90 hour^{-1}). The data suggest that for both dietary groups, the metabolism of retinol associated with the prealbumin and retinol-binding protein complex involved extensive recycling among the liver, plasma, interstitial fluid and peripheral tissues.

INFLUENCE OF FAT AND CARBOHYDRATE SOURCE ON RATE OF FOOD PASSAGE OF SEMIPURIFIED DIETS FOR LAYING HENS. G.G. Mateos and J.L. Sell (Department of Animal Science, Iowa State University, Ames, Iowa 50011) *Poultry Sci. 60*(9):2114-2119 (1981). An experiment was conducted with While Leghorn hens to determine the influence of supplemental yellow grease and carbohydrate source on rate of food passage (ROP). Two levels of yellow grease (0 and 7%) and two carbohydrate sources (sucrose and starch) were tested in a complete 2 X 2 arrangement. The ROP was determined by utilizing either Cr_2O_3 or ^{144}Ce as nonabsorbable markers. First appearance of the markers in the excreta and percentages of the markers ingested that were

recovered in feces 9.5 to 11.5 hr after feeding were criteria used to determine ROP. The ROP varied with the composition of the diet. Diets containing starch had a slower ROP than diets containing sucrose (first appearance time was 156 vs 127 min, respectively). Also, yellow grease supplementation decreased ROP from 150 to 133 min. The ROP of sucrose-containing diets was decreased more by fat supplementation than the ROP of starch-containing diets (32 vs 3 min respectively). Similar trends were observed when ROP was measured as percentage of marker recovered in feces 9.5 to 11.5 hr after feeding. The results show that supplemental fat decreased ROP in chickens. This observation may help in understanding the nature of the extrametabolic or extracaloric effect of fat in poultry diets. With decreased ROP, the diet will be more thoroughly digested and absorbed and, thereby, more energy may be derived from a diet if fat is added than if not.

METABOLIZABLE ENERGY OF SUPPLEMENTAL FAT AS RELATED TO DIETARY FAT LEVEL AND METHODS OF ESTIMATION. G.G. Mateos and J.L. Sell (Department of Animal Science, Iowa State University, Ames, Iowa) *Poultry Sci. 60*(7): 1509-1515 (1981). White Leghorn laying hens were fed practical diets containing 0, 5, 10, 15, 20, 25, or 30% yellow grease. The nitrogen-corrected metabolizable energy (ME) of yellow grease was estimated from apparent digestibility data and from ME's of the diet determined experimentally. The ME's of yellow grease were consistently higher when dietary ME data were used in the calculations than when lipid digestibility data were used (8380, 8770, 8567, 9050, 8408, and 8473 kcal/kg vs 8360, 8344, 8278, 8456, 8212, and 8540 kcal/kg for diets containing 5, 10, 15, 20, 25, and 30% fat, respectively). A comparison also is presented of ME values for yellow grease obtained by conventional calculation or by use of regression analysis. Regardless of the method of calculation, the ME data indicated that supplemental yellow grease exerted an extrametabolic effect on dietary ME, although the magnitude of this effect varied with the mathematical approach used.

FATTY ACID AND CHOLESTEROL SYNTHESIS FROM SPECIFICALLY LABELED LEUCINE BY ISOLATED RAT HEPATOCYTES. M.M. Mathias, A.C. Sullivan, and J.G. Hamilton (Roche Research Center, Hoffmann-La Roche Inc., Nutley, NJ 07110) *Lipids 16(10):73*

Lipids 16(10):739-743 (1981). Hepatocytes isolated from female rats meal-fed a high-glucose diet were incubated in Krebs-Henseleit bicarbonate medium containing 16.5 mM glucose, ^3H_2O, and ^{14}C-labeled amino acids (-)-Hydroxycitrate depressed the incorporation of ^3H_2O and [^{14}C] alanine into fatty acids and cholesterol. Incorporation of [U-^{14}C] leucine into lipids was not affected but incorporation of ^3H_2O into lipids was decreased significantly by (-)-hydroxycitrate. (-)-Hydroxycitrate depressed the incorporation of radioactivity from [2-^{14}C] leucine into fatty acids and cholesterol by 61 and 38% respectively, and stimulated the incorporation of radioactivity from [4,5-^{3}H] leucine 35 and 28%. As [2-^{14}C] leucine labels the acetyl-CoA pool and [4,5-^{3}H] leucine labels the acetoacetate pool, it was concluded that mitochondrial 3-hydroxy-3-methylglutaryl-CoA is not incorporated intact into cholesterol, and that acetoacetate can be activated effectively in the liver cytosol for support of cholesterol and fatty acid synthesis.

EFFECT OF ESSENTIAL FATTY ACID DEFICIENCY ON MATERNAL, PLACENTAL, AND FETAL RAT TISSUES. N.K. Menon, C. Moore, and G.A. Dhopeshwarkar (Laboratory of Nuclear Medicine and Radiation Biology, University of California, 900 Veteran Ave., Los Angeles, CA 90024) *J. Nutr. 111*(9):1602-1610 (1981). Prolonged dietary deprivation is needed to produce essential fatty acid (ETA) deficiency. But lack of EFA also impairs reproductive function. Inclusion of small amounts of linoleic acid in the diet can overcome this difficulty; further, if large amounts of oleic acid are included in the diet, this competes with the utilization of 18:2 producing EFA deficiency. Using this approach, female rats were fed a fat-free diet containing 5% oleic acid w/w (with 2-3% 18:2) as the only source of fat for 4 months. They were mated and on the 21st day of gestation, the fatty acids of fetal tissues, placenta, maternal liver and plasma were analyzed and compared to controls on a stock diet. Fetuses from the experimental group were smaller and contained higher amounts of 18:1 and 20:3ω9 indicating EFA deficiency. The ω6 fatty acids in the polar lipids of placenta of the EFA-deficient group were not significantly lower than the controls, in spite of lower concentrations in the maternal plasma, suggesting a unique capacity of the placenta to concentrate ω6 fatty acids, which in turn may be utilized for prostaglandin synthesis needed for inducing labor and other vascular changes in the fetus.

ω- AND (ω-1)-HYDROXYLATION OF 1-DODECANOL BY FROG LIVER MICROSOMES. Y. Miura (Department of Biochemistry, Teikyo University School of Medicine, 2-11-1 Kaga, Itabashi-ku, Tokyo 173, Japan) *Lipids 16*(10):721-725 (1981). Frog liver microsomes catalyzed the hydroxylation of 1-dodecanol into the corresponding ω- and (ω-1)-hydroxy derivatives. The hydroxylation rate for 1-dodecanol was much lower than that for lauric acid. Both NADPH and O_2 were required for hydroxylation activity. NADH had no effect on the hydroxylation. The hydroxylating system was inhibited 49% by CO at a CO:O_2 ratio of 4.0. The formation of ω-hydroxydodecanol was more sharply inhibited by CO than was the formation of (ω-1)-hydroxyldodecanol, implying that more than one cytochrome P-450 was involved in the hydroxylation of 1-dodecanol and that CO has a higher affinity for the P-450 catalyzing the ω-hydroxylation. The formation of laurate during the incubation of 1-dodecanol with frog liver microsomes suggests that a fatty alcohol oxidation system is also present in the microsomes. NAD^+ was the most effective cofactor for the oxidation of 1-dodecanol and $NADP^+$ had a little effect. Pyrazole (an inhibitor of alcohol dehydrogenase) had a slight inhibitory effect on the oxidation and sodium azide (an inhibitor of catalase) had no effect.

METABOLIC DISCRIMINATION BETWEEN CHOLESTEROL AND β-AMYRIN BY *Phytophthora cactorum*. W.D. Nes, G.A. Saunders, and E. Heftmann (Plant Physiology and Chemistry Research Unit, Western Regional Research Center, Science and Education Administration, U.S. Department of Agriculture, Berkeley, CA 94710) *Lipids 16*(10):744-748 (1981). When cultures of *Phytophthora cactorum* were incubated on solid medium for 3 weeks in the dark at 20 C with [^{14}C] cholesterol or [^{14}C]-β-amyrin, the [^{14}C] cholesterol was assimilated into the advancing mycelium to a greater extent than the [^{14}C] β-amyrin. Examination of the mycelium and medium for radioactive metabolites showed significant differences in the metabolism of the 2 labeled compounds. Cholesterol was converted mainly to esters and to some extent to glycosides, whereas β-amyrin was only slightly converted to esters and not at all to glycosides.

PREDICTION OF THE THREE-DIMENSIONAL STRUCTURES OF COMPLEXES OF LYSOZYME WITH CELL WALL SUBSTRATES. M.R. Pincus and H.A. Scheraga (Baker Laboratory of Chemistry, Cornell University, Ithaca, New York 14853) *Biochemistry 20*(14):3960-3965 (1981). The conformational energies of complexes of alternating copolymers of *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc) with hen egg white lysozyme have been computed. This involved a complete search of the conformational space at the active site of the enzyme available to these substrates and minimization of the conformational energies of the noncovalent complexes. The hexasaccharide $(GlcNAcMurNAc)_2$-$(GlcNAc)_2$ binds preferentially on the "left" side of the active-site cleft. The alternating copolymer $(GlcNAcMurNAc)_3$, however, binds with its F-site residue preferentially on the "right" side of the active-site cleft. This result can explain the higher rate of catalysis for the cell wall substrate (the alternating copolymer). The relative affinities of the disaccharide GlcNAc-MurNAc for all sequential pairs of sites A-F (including E and F sites on *both* sides of the cleft) are determined. It is found that the highest affinity of this disaccharide is for sites C and D and "right-side" sites E and F. The energy of the recently determined X-ray crystallographic structure of MurNAc-GlcNAc-MurNAc bound to the B,C, and D sites of hen egg white lysozyme has been minimized. The D ring is undistorted and binds close to the surface of the active-site cleft. The structure can be extended into sites E and F by addition of two GlcNAc residues, but only on the left side of the active-site cleft. This indicates that polymers bound with their D-site residues near the surface of the cleft must bind to sites E and F on the left side of the cleft.

LYSOPHOSPHATIDYLCHOLINE ACYLTRANSFERASE ACTIVITY DURING EXPERIMENTAL CHOLELITHIASIS. F.A. Rutledge II, D.M. Hickman, J.J. Dunn, C.F. Frey, and R.S. Matson (Veterans Administration Medical Center, Martinez, CA 94553) *Lipids 16*(10):714-720 (1981). The accumulation of (1-palmitoyl)-lysophosphatidylcholine, lysolecithin, in gallbladder bile was observed during the first week of cholesterol-induced experimental cholelithiasis using the prairie dog model for cholesterol gallstone formation. Gallbladder fluid transport function decreased as bile lysolecithin concentration increased. These observations suggest that lysolecithin plays an important, early role in the etiology of gallstone disease. Furthermore, the relative activities of hepatic and gallbladder mucosa lysophosphatidylcholine acyltransferase and acylcoenzyme A hydrolases may be responsible for the turnover of gallbladder bile lysolecithin.

HEPATIC VITAMIN A DEPLETION AFTER CHRONIC ETHANOL CONSUMPTION IN BABOONS AND RATS. M. Sato and C.S. Lieber (Lab. of Liver Disease and Nutr. and Alcohol Research and Treatment Center, Bronx Veterans Administration Med. Center and Mount Sinai School of Med., New York, NY 10468) *J. Nutr.*

111(11):2015-2023 (1981). To evaluate possible effects of alcohol consumption on vitamin A and retinol-binding protein (RBP) status, baboons were pair-fed a nutritionally adequate liquid diet containing 50% of total calories either as ethanol or isocaloric carbohydrate. Fatty liver developed after 4 months of ethanol feeding with a 59% decrease ($P<0.001$) in hepatic vitamin A levels, and fibrosis or cirrhosis developed after 24-84 months with a 95% decrease ($P<0.001$). Similarly, hepatic vitamin A levels of rats fed ethanol (36% of total calories) were decreased after 3 weeks (42%, $P<0.001$) and continued to decrease up to 9 weeks. In contrast, vitamin A contents in the kidney and testis were increased 2-3 fold in ethanol-fed rats after 9 weeks. Serum vitamin A and RBP levels were not significantly changed in rats. When dietary vitamin A was increased 5-fold, hepatic vitamin A was again decreased in ethanol-fed rats. When dietary vitamin A was virtually eliminated, the depletion rate of vitamin A from endogenous hepatic storage was 2.5 times faster in ethanol-fed rats than in controls. It is concluded that chronic ethanol consumption decreases hepatic vitamin A, and that some mechanisms other than malnutrition and malabsorption may be involved in this process.

COMPARISONS OF BODY FAT ESTIMATED FROM TOTAL BODY WATER AND SKINFOLD THICKNESSES OF UNDERNOURISHED MEN. G.B. Spurr, M. Barac-Nieto, H. Lotero, and H.W. Dahners (Dept. of Physiological Sciences and the Metabolic Unit, Dept. of Medicine, Univ. Hospital, Universidad del Valle, Cali, Colombia) *Am. J. Clin. Nutr. 34*(9):1944-1953 (1981). In 49 chronically undernourished adult males, classified as having mild, intermediate, or severe nutritional compromise, comparisons were made of body fat calculated from total body water with values obtained from triceps and scapular skinfolds. These same comparisons were followed in 19 of the severely undernourished subjects during a 2½-month period of dietary repletion. Results indicate that the correlations between fat estimates obtained from body water and skinfolds are good ($r>0.8$) in mildly undernourished subjects, but that they are progressively reduced as the nutritional compromise becomes more severe until statistical significance disappears. Dietary repletion of 2½-month duration did not restore a statistically significant relationship between fat and triceps and scapular skinfolds. These data imply that the triceps and scapular skinfolds do not adequately represent body fat in chronically undernourished adult males and that new empirical equations are required which take into account nutritional status andpossible shifts in fat deposit sites in chronic undernutrition.

EFFECTS OF VITAMIN E DEFICIENCY ON THE RELATIVE INCORPORATION OF ^{14}C-ARACHIDONATE INTO PLATELET LIPIDS OF RABBITS. C.C. Tangney and J.A. Driskell (Dept. of Human Nutrition and Foods, Virginia Polytechnic Institute and State Univ., Blacksburg, VA 24061) *J. Nutr. 111*:1839-1847 (1981). The potential influence of vitamin E in vivo on the transformation of ^{14}C-arachidonate (^{14}C-ArA) in washed platelets from vitamin E-depleted, pair-fed control and ad-libitum-fed control rabbits was investigated. We considered rabbits vitamin E-depleted when their plasma total tocopherol levels were <50% of initial values. By week 7 deficient rabbits exhibited significantly lower ($P < 0.01$) plasma and platelet tocopherol levels and significantly higher ($P < 0.01$) urinary creatine:creatinine ratios than those of controls. Thrombin treatment of platelet suspensions that were previously incubated with ^{14}C-ArA resulted in significnatly greater ($P < 0.05$) losses of radiolabel from phospholipids (PL) and significantly greater ($P < 0.05$) ^{14}C incorporation into prostaglandin E_2 (PGE_2) fractions from deficient animals than those of either control group. The significantly greater ($P < 0.05$) losses in lipid phosphorous content of phosphatidylcholine (PC) fractions corroborated the significantly diminished ($P < 0.05$) radiolabel detected in the PC fraction of deficient animals with thrombin activation. Such findings suggest an enhancement of thrombin-induced phospholipase A_2 (PLPA) activity in platelets of vitamin E-deficient rabbits compared to that of controls.

EFFECTS OF HIGH GLUCOSE AND HIGH LARD DIETS ON THE ACTIVITIES OF RAT LIVER GLYCOSYLTRANSFERASES. H.M. Tepperman, R. Silver, J. DeWitt, and J. Tepperman (Dept. of Pharmacology, State Univ. of New York, Upstate Medical Center, Syracuse, NY 13210) *J. nutr. 111*:1734-1741 (1981). The activities of several enzymes involved in glycoprotein synthesis were measured in the livers of rats (L) fed diets with 67% of calories as lard and compared with those of rats (G) fed 67% glucose diets for 5-9 days. Glucosamine synthetase activity was not influenced by diet, but the greater in the livers of the rats fed the glucose diet than in L rat livers. The content of UDP-N-acetylglucosamine was also higher in G livers than in the L group. Activities of glycosidases and of sugar nucleotide pyrophosphatases and phosphatases were the same on both diets. Serum total hexosamine was higher in L than in G rats. These findings are discussed in relation to earlier reports that liver plasma membranes from G rats contained more glycoprotein carbohydrate than L membranes.

PLASMA LEVELS OF VITAMIN D METABOLITES IN DIPHOSPHONATE-TREATED RATS. U. Trechsel, C.M. Taylor, J.A. Eisman, J.-P. Bonjour and H. Fleisch (Dept. of Pathophysiol., Univ. of Berne, Mertenstrasse, Berne, Switzerland) *Clin. Sci. 61*(4):471-476 (1981). 1. Protein-binding assays have been used to measure plasma 1,25-dihydroxy-vitamin D [1,25-$(OH)_2$D] as well as 25-hydroxy-vitamin D [25-(OH)D] in rats given 10 mg of phosphorus (P) day^{-1} kg^{-1} as ethane-1-hydroxy-1,1-d phosphonate (EHDP). 2. In control animals given a normal laboratory chow plasma 25-(OH)D and 1,25-$(OH)_2$D were about 40 nmol/l and 300 pmol/l respectively. 3. EHDP produced a decrease of plasma 1,25-$(OH)_2$D to below 50 pmol/l in 2 days. 4. Both in control and in EHDP-treated rats plasma 1,25-$(OH)_2$D increased when dietary calcium (Ca) was restricted to 0-1%, or dietary P to 0-2%, indicating that the well-known stimulatory effect of Ca or P deprivation was at least partially effective in EHDP-treated rats. 5. In response to an increase of the oral supply of vitamin D_3 to 65 nmol/day the plasma level of 25-(OH)D rose in both control and EHDP groups. Plasma 1,25-$(OH_2$D was not increased above the normal value in control rats. In EHDP-treated rats, however, plasma 1,25-$(OH)_1$D rose to a level equal to that in controls, suggesting that the effect of EHDP on plasma 1,25$(OH)_2$D can be overcome at high precursor concentration.

CHOLESTEROL RESPONSE IN INBRED STRAINS OF RATS, *Rattus norvegicus*. L.F.M. Van Zutphen and M.G.C.W. Den Bieman (Vakgroep Zoötechniek, Veterinary Faculty, Yalelaan 17, Univ. of Utrecht, Utrecht, The Netherlands) *J. Nutr. 111*:1833-1838 (1981). A hypercholesterolemic diet fed to rats revealed significant interstrain differences in plasma cholesterol levels. Hyperresponding and hyporesponding strains could be distinguished from normoresponding strains within 3 weeks. The increase in plasma cholesterol level was more than 300 mg/100 ml in the hyperresponding strains BN/Cpb, SD/Cpb and WE/Z and less than 50 mg/100 ml in the hyporesponding strains S3/Cpb and SHR/Cpb. These differences were primarily genetically determined: the calculated coefficient of genetic determination (g^2) of the response was 0.84. The response is not correlated with variation in plasma esterase or alkaline phosphatase isozyme patterns.

CONTENT OF *TRANS*-OCTADECENOIC ACID IN VEGETARIAN AND NORMAL DIETS IN SWEDEN, ANALYZED BY THE DUPLICATE PORTION TECHNIQUE. B. Åkesson, B.-M. Johansson, M. Svensson, and P.-A. Öckerman (Dept. of Clinical Chemistry, University Hospital, S-221 85 Lund, Sweden) *Am. J. Clin. Nutr. 34*(11):2517-2520 (1981). The intake of *trans* fatty acids by subjects adhering to the normal Swedish diet or to different vegetarian regimes was studied, using chemical analysis of duplicate portions. *Trans*-octadecenoic acid was 5.0, 3.9, and 1.8% of dietary fatty acid in the normal, lactovegetarian, and vegan diets, respectively, corresponding to 2.0, 1.3 and 0.5% of energy intake. The results are related to the content of *trans*-octadecenoic acid in some edible fats.

MECHANISM OF LONG CHAIN FATTY ACID PERMEATION IN THE ISOLATED ADIPOCYTE. Nada A. Abumrad, Ray C. Perkins, Jane H. Park, and Charles R. Park (Department of Physiology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232) *The Journal of Biological Chemistry* Vol. 256, No. 17, Issue of September 10, pp. 9183-9191, (1981). The mechanism of permeation of [^{14}C]oleate into isolated rat adipocytes has been examined. The initial rates of uptake of the fatty acid were determined at 23°C as a function of the concentration of unbound fatty acid in the medium. Under the conditions employed, the following observations were made. 1) The rates were maximal and linear for at least 15 s and were the same in the presence or absence of glucose. 2) In the absence of glucose, all fatty acid taken up was recovered in the cell as unesterified fatty acid, whereas about 75% was esterified in the presence of the sugar. Thus uptake appeared to be independent of fatty acid metabolism. 3) Influx of fatty acid was strongly inhibited by phloretin, particularly at low concentrations of unbound fatty acid. Efflux was also blocked. (Phloretin in an albumin-free medium at 0°C was a very effective stop solution for abruptly terminating fatty acid fluxes and for washing cells without loss of unesterified fatty acid.) 4) The fatty acid taken up was not bound to the cell surface but probably was largely bound within the cell. 5) Uptake was not limited by dissociation of fatty acid from albumin in the medium nor by an interaction between albumin and the cell surface. From these considerations, we concluded that the uptake measurements were valid estimates of the influx of fatty acid. Partial saturation was observed as a function of external concentration of unbound fatty acid with a $k_{0.5}$ of 6×10^{-8} M.

LECITHIN: CHOLESTEROL ACYLTRANSFERASE (LCAT) MASS; ITS RELATIONSHIP TO LCAT ACTIVITY AND CHOLES-

TEROL ESTERIFICATION RATE. John J. Albers, Chin Hong Chen, and Janet L. Adolphson (Department of Medicine, Northwest Lipid Research Clinic, Harborview Medical Center, University of Washington School of Medicine, Seattle, WA) *J. Lipid Res. 22*: 1206–1213 (1981). The relationship between plasma lecithin: cholesterol acyltransferase mass and enzyme activity and between mass and plasma cholesterol esterification rate was determined in 25 adult volunteers without overt disease (14 normolipidemic and 11 hyperlipidemic). Furthermore, the relationship of lecithin: cholesterol acyltransferase mass and cholesterol esterification rate to lipids, apoproteins, age, and ideal body weight was assessed. Lecithin:cholesterol acyltransferase mass determined by radioimmunoassay was highly correlated with enzyme activity assayed using a heated plasma substrate ($r = 0.636$) and with the molar cholesterol esterification rate determined either by radioassay ($r = 0.621$). Lecithin:cholesterol acyltransferase mass was also positively correlated with total cholesterol ($r = 0.608$), unesterified cholesterol ($r = 0.562$), age ($r = 0.544$), and percent ideal body weight ($r = 0.619$), but was not significantly correlated with log triglyceride, high density lipoprotein cholesterol, or apolipoproteins A-I, A-II, or D.

IMMOBILIZATION OF A SPIN-LABELED FATTY ACID CHAIN COVALENTLY ATTACHED TO Ca^{2+}-ATPase FROM SARCOPLASMIC RETICULUM SUGGESTS AN OLIGOMERIC STRUCTURE. Jens P. Andersen, Pierre Fellmann, Jesper V. Møller, and Philippe F. Devaux. *Biochemistry 20*:4928–4936 (1981). A spin-labeled fatty acid chain (16-doxylstearic acid), linked by an ester bond to a maleimide residue, was covalently attached to the Ca^{2+}-ATPase of rabbit sarcoplasmic reticulum. Three different hydrophobic environments of Ca^{2+}-ATPase were investigated by electron spin resonance: (i) native sarcoplasmic reticulum, (ii) reconstituted vesicles obtained by exchange of the endogenous lipids with egg lecithin, and (iii) Ca^{2+}-ATPase solubilized by the nonionic detergent octaethylene glycol monododecyl ether ($C_{12}E_8$). All spectra were composite, with a major component corresponding to strongly immobilized probe (called "immobilized component"). The percentage of immobilized component seemed to decrease with increasing temperature or in the presence of a small amount of $C_{12}E_8$, under nonsolubilizing conditions. Quantitation of the percentage of immobilized component was performed by linear combination of pairs of appropriately selected spectra, for which it could be assumed that changes predominantly were caused by differences in the proportion of immobile and mobile components and not by changes in line shape. We propose that the immobilized component arises from lipid chains trapped in protein oligomers, which exist in native sarcoplasmic reticulum. Partial dissociation of the oligomers may be induced by a rise of temperature or by perturbation of membrane structure with a low concentration of detergent.

KINETIC PARAMETERS OF THE LIPOPROTEIN TRANSPORT SYSTEMS IN THE ADRENAL GLAND OF THE RAT DETERMINED *IN VIVO*, COMPARISON OF LOW AND HIGH DENSITY LIPOPROTEINS OF HUMAN AND RAT ORIGIN. John M. Andersen and John M. Dietschy (Departments of Pediatrics and Internal Medicine, The University of Texas Health Science Center at Dallas, Dallas, Texas 75235) *J. Biol. Chem. 256*(14):7362–7370 (1981). These studies were done to characterize the rate of uptake of high (HDL) and low (LDL) density lipoprotein cholesterol by the adrenal gland of the rat under *in vivo* conditions. Animals were pretreated for 4 days with 4-aminopyrazolo[3,4-*d*]pyrimidine to essentially eliminate endogenous plasma lipoproteins and with aminoglutethimide for 1 h to block conversion of cholesterol to adrenal hormones. Such animals were then infused with varying amounts of HDL and LDL from both human and rat plasma, and this led to rapid accumulation of cholesterol, both free and esterified, in the adrenal glands over the subsequent 4-h period of observation. This uptake process was linear with respect to time and manifested saturation kinetics with respect to the steady state level of plasma lipoprotein cholesterol. These data provide additional support for the concept that the adrenal gland (and ovary) of the rat can take up HDL and LDL cholesterol by separate mechanisms. However, from the quantitative data on the kinetics of these uptake processes, it is apparent that in this species HDL cholesterol must be the major substrate for the synthesis of adrenal hormones.

EFFECTS OF HYPOCHOLESTEROLEMIA AND CHRONIC HORMONAL STIMULATION ON STEROL AND STEROID METABOLISM IN A LEYDIG CELL TUMOR. Mario Ascoli (Division of Endocrinology, Department of Medicine and Department of Biochemistry, Vanderbilt Medical School, Nashville, TN 37232) *J. Lipid Res. 22*:1247–1253 (1981). The studies presented herein were done to investigate the effects of drug-induced hypocholesterolemia and chronic hormonal stimulation on cholesterol metabolism and steroid biosynthesis in a functional Leydig cell tumor. It was found that 4-aminopyrazolo(3,4-d)-pyrimidine (4-APP)–induced hypocholesterolemia had no effect on *a)* the amount of cholesterol present in the tumor, *b)* cholesterol biosynthesis, and *c)* steroid production. Chronic stimulation with choriogonadotropin also had no effect on the amount of cholesterol present in the tumor, but it increased steroid production and cholesterol biosynthesis. These results suggest that the Leydig tumor cells primarily use intracellular cholesterol for steroid biosynthesis. Other data show that 4-APP treatment reduces gonadotropin binding in the Leydig tumor cells.--Ascoli, M. Effects of hypocholesterolemia and chronic hormonal stimulation on sterol and steroid metabolism in a Leydig cell tumor.

METABOLISM OF LIPID LABELED VERY LOW DENSITY LIPOPROTEIN FROM LAYING TURKEY HENS IN LAYING TURKEY HENS AND IMMATURE TURKEYS. W.L. Bacon (Dept. of Poultry Science, Ohio Agricultural Research and Development Center, Wooster, Ohio 44691) *Poultry Sci. 60*(7):1525–1536 (1981). Labeled very low density lipoprotein of laying turkey hens (VLDL-L) was prepared by injecting 1-14C-palmitate and subsequently isolating the VLDL-L by ultracentrifugation at d=1.006. The isolated VLDL-L then was injected into recipient laying hens, immature males, or immature females. Size exclusion chromatography of recipient laying hen plasma showed no remnant particles of smaller size or greater density than the injected VLDL-L up to 400 min postinjection. In the immature birds of either sex, remnant particles of greater density and smaller size than the injected VLDL-L were present when blood samples were withdrawn at 5 (males) or 1 (females) min postinjection. In laying females, both VLDL-L-triglyceride (VLDL-L-TG) and phospholipids (VLDL-L-PL) had identical fractional clearance rates of .00253 min^{-1} and had parallel rates of disappearance. The irreversible loss of VLDL-L-TG was 12.8 g/day while it was 4.8 g/day for VLDL-L-PL. VLDL-L-TG decayed with a single exponential decay component in both immature males and females. The VLDL-L-PL decayed in a more complex pattern in the immature birds, showing more than a single exponential decay component.

POLYUNSATURATED PROTECTED LIPID: EFFECT ON MILK PHOSPHOLIPIDS. D.M. Barbano and J.W. Sherbon (Dept. of Food Science, Cornell University, Ithaca, NY 14853) *J. Dairy Sci. 64*(11): 2170–2174 (1981). Five lactating Holsteins were fed a complete mixed control ration for 20 days. Total milk phospholipid and milk fat produced per day increased during protected lipid feeding, whereas milk produced per day remained the same throughout the study. Diglycerides from fresh milk had larger amounts of polyunsaturated fatty acids during protected lipid feeding. In contrast to milk diglycerides, there was little change in fatty acid composition of milk phospholipids in response to the protected lipid diet. Independence of bovine milk phospholipid fatty acid composition from influence of dietary fatty acid composition is important to the understanding of mammary fat synthesis. Stability of fatty acid composition in bovine milk phospholipids sharply contrasts with changes in milk diglycerides and triglycerides.

MONOCLONAL ANTIBODIES TO THE LOW DENSITY LIPOPROTEIN RECEPTOR AS PROBES FOR STUDY OF RECEPTOR-MEDIATED ENDOCYTOSIS AND THE GENETICS OF FAMILIAL HYPERCHOLESTEROLEMIA. U. Beisiegel, W.J. Schneider, J.L. Goldstein, R.G.W. Anderson and M.S. Brown (Depts. of Molecular Genetics, Internal Medicine, and Cell Biology, University of Texas Health Science Center, Dallas, TX 75235) *J. Biol. Chem. 265* (11): 11923–11931 (1981). Monoclonal antibodies directed against the low density lipoprotein (LDL) receptor have been prepared by immunization of mice with a partially purified receptor from bovine adrenal cortex. Spleen cells from the mice were fused with the Sp2/0-Ag14 line of mouse myeloma cells. The most extensively studied monoclonal antibody, designated immunoglobulin-C7, reacts with the human and bovine LDL receptor, but not with receptors from the mouse, rat, Chinese hamster, rabbit or dog. In normal fibroblasts, the receptor-bound monoclonal antibody was taken up and degraded at 37°C at a rapid rate similar to that for LDL. The current data demonstrate the usefulness of monoclonal antibodies as probes for the study of the cellular and genetic factors involved in receptor-mediated endocytosis.

MONOCLONAL ANTIBODIES TO THE LOW DENSITY LIPOPROTEIN RECEPTOR AS PROBES FOR STUDY OF RECEPTOR-MEDIATED ENDOCYTOSIS AND THE GENETICS OF FAMILIAL HYPERCHOLESTEROLEMIA. Ulrike Beisiegel, Wolfgang J. Schneider, Joseph L. Goldstein, Richard G.W. Anderson, and Michael S. Brown (Departments of Molecular Genetics, Internal Medicine, and Cell Biology, University of Texas Health Science Center at Dallas, Dallas, Texas 75235) *J. Biol. Chem. 256*(22):11923–11931 (1981). Monoclonal antibodies directed against the low density lipoprotein (LDL) receptor have been prepared by immunization of mice with a partially purified receptor from bovine adrenal cortex. Spleen

cells from the mice were fused with the Sp2/0-Ag14 line of mouse myeloma cells. The most extensively studied monoclonal antibody, designated immunoglobulin-C7, reacts with the human and bovine LDL receptor, but not with receptors from the mouse, rat, Chinese hamster, rabbit, or dog. ^{125}I-labeled monoclonal antibody binds to human fibroblasts in amounts that are equimolar to ^{125}I-LDL. In fibroblasts from 6 of 8 patients with the receptor-negative form of homozygous familial hypercholesterolemia, which have less than 5% of normal LDL binding, the amount of monoclonal antibody binding was also less than 5% of normal. The current data demonstrate the usefulness of monoclonal antibodies as probes for the study of the cellular and genetic factors involved in receptor-mediated endocytosis.

CHOLESTEROL ESTERIFICATION BY RAT ADRENAL GLAND. INHIBITION BY LOCAL ANESTHETICS IN VITRO. F.P. Bell (Diabetes-Atherosclerosis Research, The Upjohn Company, Kalamazoo, MI 49001, USA) *Biochim. Biophys. Acta* 666:58-62 (1981). Cholesterol esterification was studied in rat adrenal gland, adrenal homogenates and isolated adrenal microsomes. In whole gland and homogenates, the local anesthetic, lidocaine, inhibited the incorporation of [1-^{14}C]oleate and [1-^{14}C]oleoyl-CoA, respectively, into labeled cholesteryl esters in a dose-dependent manner. Inhibition of sterol esterification in the preparations reached 50% at about 2 mM. Various other local anesthetics (tetracine, dibucaine and benzocaine) also inhibited sterol esterification in adrenal homogenates but were more potent than lidocaine; in each case, 90% inhibition occurred at anesthetic levels of 1 mM. Since sterol esterification in the adrenal gland is a function of microsomal acyl-CoA: cholesterol acyltransferase (EC 2.3.1.26), the enzyme was assayed in isolated adrenal microsomes in the presence of lidocaine while using [^{14}C]oleoyl-CoA as a substrate for labeled cholesteryl ester formation. Inhibition of the enzyme by lidocaine was confirmed, with 50% inhibition occurring between 0.5 and 0.75 mM lidocaine. Lidocaine may be useful as a tool in studies on the regulation of adrenal sterol esterification.

VARIATIONS IN THE ACTIVITY OF MICROSOMAL PALMITOYL-CoA HYDROLASE IN MIXED MICELLE SOLUTIONS OF PALMITOYL-CoA AND NON-IONIC DETERGENTS OF THE TRITON X SERIES. R.K. Berge, E. Slinde, and M. Farstad (Laboratory of Clinical Biochemistry, Univ. of Bergen, Haukeland Sykehus, N-5016, Bergen, Norway) *Biochim. Biophys. Acta* 666:25-35 (1981). The kinetics of palmitoyl-CoA hydrolase were influenced by both the availability of the substrate and formation of micelles. At palmitoyl-CoA concentrations below the critical micelle concentration, addition of non-ionic detergent increased the activity until the critical micelle concentration of the mixed micelles was reached. At palmitoyl-CoA concentrations above the critical micelle concentration, inhibition of the activity was observed, but addition of detergents of the Triton X series reversed the inhibition. Maximum palmitoyl-CoA hydrolase activity was found when the ratios (w/v) of palmitoyl-CoA:Triton X-100 and palmitoyl-CoA:Triton X-405 were approximately 0.35 and 0.05, respectively. At these ratios no inhibition was observed even at concentrations of palmitoyl-CoA and Triton X-100 10-15 times above the mixed critical micelle concentrations. The results indicate that monomer palmitoyl Co-A is the substrate and that monomer forms of the nonionic detergents of the Triton X series activate the enzyme. Isolated microsomal lipids activated the microsomal palmitoyl-CoA hydrolase, suggesting that a hydrophobic environment is advantageous for interaction between enzyme and substrate in vivo. The maximum activity in the presence of mixed micelles is discussed in relation to a model where mixed micelles are regarded as artificial membranes to which the enzyme may adhere in an equilibrium with the monomer substrate and detergent in the monomer form. It is suggested that intracellular membranes may resemble mixed micelles in equilibrium with detergent-active substrates.

METABOLISM OF LEUKOTRIENE D BY PORCINE KIDNEY. Kerstin Bernström and Sven Hammarström (Department of Chemistry, Karolinska Institutet, S-10401 Stockholm, Sweden) *Journal of Biological Chemistry 256*:(18):9579-9582 (1981). An enzyme from porcine kidney converted leukotriene D_4 into a less polar metabolite. The structure of this compound was 5-hydroxy-6-*S*-cysteinyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid (leukotriene E_4). Analogous products, *viz.* 5-hydroxy-6-*S*-cysteinyl-7,9,11-eicosatrienoic acid (leukotriene E_3), 5-hydroxy-6-*S*-cysteinyl-7,9,11-*trans*-14-*cis*-eicosatetraenoic acid (11-*trans*-leukotriene E_4), and 5-hydroxy-6-*S*-cysteinyl-7,9,11,14,17-eicosapentaenoic acid (leukotriene E_5) were formed from leukotrienes D_3, 11-*trans*-D_4, and D_5, respectively. Leukotriene E_4 induced slow reacting substance-like contractions of guinea pig ileum but was less potent (8-12 times) than leukotriene C_4. The biological potency of 11-*trans*-leukotriene E_4 was similar to that of leukotriene E_4.

THE EFFECTS OF INSULIN AND GLUCAGON ON THE RELEASE OF TRIACYLGLYCEROLS BY ISOLATED RAT HEPATOCYTES ARE MERE REFLECTIONS OF THE HORMONAL EFFECTS ON THE RATE OF TRIACYLGLYCEROL SYNTHESIS. A.C. Beynen, H.P. Haagsman, L.M.G. Van Golde and M.J.H. Geelen (Laboratory of Veterinary Biochemistry, State University of Utrecht, Biltstraat 172, 3572 BP Utrecht, The Netherlands) *Biochimica et Biophysica Acta, 665*:1-7 (1981). Elsevier/North-Holland Biomedical Press. 1. Isolated hepatocytes from meal-fed donor rats secrete newly synthesized very-low-density lipoproteins (VLDL) when incubated in a simple bicarbonate buffer. When incubated with ^3H_2O for 2 h, 72-81% of the ^{3}H-labelled triacylglycerols secreted by the hepatocytes were recovered in VLDL. The secretion of newly synthesized triacylglycerols shows a lag phase of about 30 min. 2. Insulin stimulates the secretion of newly synthesized VLDL triacylglycerols, whereas glucagon has an inhibitory effect on this process. 3. When hepatocyte triacylglycerols were labelled by preincubating the cells with ^3H_2O or [1-^{14}C] oleate and the cells were subsequently washed and further incubated in radioisotope-free buffer containing hormones, it was observed that the release of the prelabelled triacylglycerols is not hormone-sensitive. This suggests that insulin and glucagon do not affect the release of triacylglycerols per se. 4. It is concluded that the effects of insulin and glucagon on the overall process of triacylglycerol secretion are reflections of the hormone-determined rate of triacylglycerol synthesis.

SATURABLE HIGH AFFINITY BINDING, UPTAKE AND DEGRADATION OF RAT PLASMA LIPOPROTEINS BY ISOLATED PARENCHYMAL AND NON-PARENCHYMAL CELLS FROM RAT LIVER. Theo J.C. Van Berkel, Johan K. Kruijt, Teus Van Gent and Arie Van Tol (Department of Biochemistry I, Medical Faculty, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands) *Biochimica et Biophysca Acta, 665*: 22-23 (1981). Elsevier/North-Holland Biomedical Press. 1. Freshly isolated rat parenchymal and non-parenchymal liver cells bind iodinated rat very low density lipoprotein (VLDL) remnants, low density lipoproteins (LDL) and high density lipoproteins (HDL) in a saturable way. The apparent K_m values for the binding of VLDL remnants are 6-20-fold lower than for LDL or HDL. The binding per mg cell protein to non-parenchymal cells is 5-8-fold higher than to parenchymal cells. Competition experiments indicate that rat VLDL-remnants, LDL and HDL, but not human LDL compete for the same surface receptor. It is concluded that the source of common recognition could be apolipoprotein E and that the interaction with the receptor is also influenced by the apolipoproteins A and C. The high apparent affinity of the receptor for VLDL remnants might be the result of multiple receptor occupancy of this lipoprotein. The presence of a 5-8-fold higher concentration of the described lipoprotein receptor in non-parenchymal cells as compared to parenchymal cells explains the relatively high uptake of VLDL remnants (as compared to LDL and HDL) as well as the relative contribution of parenchymal and non-parenchymal cells to the total hepatic uptake of lipoproteins in vivo. 2. The greater part (70-80%) of the parenchymal and non-parenchymal cell-associated apolipoproteins, LDL or HDL, remains bound to the external surface of the cells, during in vitro incubation at 37°C. High-affinity degradation of apolipoprotein(s) by isolated liver cells is dependent on the specific lipoprotein. During a 3 h incubation at 37°C, 37-49% of the total cell-associated ^{125}I-labeled VLDL remnants. Degradation of the different lipoproteins by non-parenchymal liver cells occurs at a 3-6 times higher rate per mg cell protein than by parenchymal cells. It is suggested that the rate-limiting step in the degradation of apolipoprotein by isolated liver cells is their transport to intracellular degradation sites.

CARNITINE DEFICIENCY INDUCED DURING HEMODIALYSIS AND HYPERLIPIDEMIA: EFFECT OF REPLACEMENT THERAPY. M. Bertoli, P.A. Battistella, L. Vergani, A. Naso, M.L. Gasparotto, G.F. Romagnoli, and C. Angelini (Haemodialysis Service and the Neurological Department, Univ. Hospital of Padua, Padua, Italy) *Amer. J. Clin. Nutr. 34*(8):1496-1500 (1981). Plasma carnitine levels were studied in 14 uremic patients before, during, and after hemodialysis. The predialysis plasma carnitine levels were normal but fell during dialysis (half-life 3.6 h). Plasma carnitine levels rose quickly in the first 6 h after dialysis, after which time the rise was more gradual. Muscle carnitine was significantly reduced in the dialyzed patients ($p < 0.005$) compared with controls. In four patients lipid droplets were observed in muscle. Ten patients on maintenance hemodialysis exhibited plasma hyperlipidemia and low muscle carnitine. These individuals were given DL-carnitine (50 mg/kg body weight) intravenously after each dialysis. At the end of a 2-month carnitine treatment, plasma triglyceride levels were found to be reduced ($p < 0.001$) and muscle carnitine content significantly increased ($p < 0.005$). These findings suggest that carnitine may be useful in treatment of hypertriglyceridemia and muscle carnitine

deficiency states induced during maintenance hemodialysis.

CHEMICAL AND BIOLOGICAL STUDIES ON 5,6-EPOXYRETINOL, RETINOL, AND THEIR PHOSPHORYL ESTERS. Pangala V. Bhat, Peter P. Roller, and Luigi M. De Luca (National Cancer Institute, National Institutes of Health, Bethesda, MD 20205) *J. Lipid Res. 22*:1069–1078 (1981). Studies are reported on chemical synthesis, ultraviolet absorption spectral characteristics, and mass spectral fragmentation of 5,6-epoxyretinol and 5,6-epoxyretinyl-phosphate. These compounds were separated from each other and from other retinoids by a reverse phase high pressure liquid chromatographic system. A comparative study on the lability to acid of 5,6-epoxyretinylphosphate and retinylphosphate was conducted. The retroretinoid anhydroretinol is formed chemically from retinylphosphate by acid hydrolysis and biologically from retinol in cultured, spontaneously-transformed mouse fibroblasts, 3T12 cells. Similarly, acid hydrolysis of 5,6-epoxyretinylphosphate (absorption maxima 324,310,296 nm) in methanol yielded a low polarity retinoid with absorption maxima at 364,346, and 330 nm, similar to the absorption spectra of retrovitamin A_1 and retrovitamin A_2. Mass spectral analysis was found to be in agreement with a retrostructure and permitted identification of the compound as a methoxyretrovitamin A_1 methyl ether. A similar retroretinoid was formed biologically from 5,6-epoxyretinol in spontaneously-transformed mouse 3T12 cells. Thus, it appeared that these cells have the ability to convert the primary alcohols into retroretinoids, which are also formed by acid treatment of the phosphate esters. The adhesive properties of 3T12 cells were highly enhanced by culturing in the presence of 10^{-6} to 10^{-5} M 5,6-epoxyretinol or -retinoic acid, in analogy with the response of these cells to the parent retinoids. Moreover, in another test of biological activity, 5,6-epoxyretinylphosphate functioned as a highly active acceptor of [^{14}C]D-mannose from GDP-[^{14}C]mannose in a reaction catalyzed by rat liver membranes. Thus, 5,6-epoxyretinoids appear to be as active as the parent retinoids in these in vitro tests of biological activity, even though they do not replace vitamin A in its growth function in vivo.

SEX PHEROMONE BIOSYNTHESIS FROM RADIOLABELED FATTY ACIDS IN THE REDBANDED LEAFROLLER MOTH. L.B. Bjostad and W.L. Roelofs (Dept. of Entomology, New York State Agricultural Experiment Station, Geneva, NY 14456) *J. Biol. Chem. 256*(15):7936–7940 (1981). Radiolabeled fatty acids in dimethyl sulfoxide were applied topically to sex pheromone glands of young adult female *Argyrotaenia velutinana*. Glands treated with sodium [1-^{14}C]acetate incorporated radiolabel into the sex pheromone components and into dodecanoic, tetradecanoic, hexadecanoic, octadecanoic, (Z)-11-tetradecenoic, and (E)-11-tetradecenoic fatty acyl moieties. In contrast, very little radiolabel was incorporated into unsaturated 16-carbon fatty acyl moieties and almost none into unsaturated 18-carbon fatty acyl moieties, although these moieties were abundant in the gland. A similar pattern of incorporation was observed for glands treated with [U-^{14}C]hexadecanoic acid. In glands treated with [1-^{14}C]hexadecanoic acid, a smaller proportion of the label was incorporated into the 14-carbon fatty acyl moieties than in glands treated with [U-^{14}C]hexadecanoic acid, indicating that 14-carbon fatty acyl moieties arose from chain-shortening of hexadecanoyl moieties. In glands treated with (E)-11-[1-^{14}C]tetradecenoic acid, much more label was incorporated into (E)-11-tetradecenyl acetate than with the other substrates, showing that most of the labeled acetate arose from reduction and acetylation of the labeled fatty acyl moiety.

AC CALORIMETRY OF DIMYRISTOYLPHOSPHATIDYLCHOLINE MULTILAYERS: HYSTERESIS AND ANNEALING NEAR THE GEL TO LIQUID-CRYSTAL TRANSITION. S.G. Black and G.S. Dixon (Department of Physics, Oklahoma State University, Stillwater, Oklahoma 74078) *Biochemistry 20*(23):6740–6744 (1981). The gel to liquid-crystal phase transition in dimyristoylphosphatidylcholine liposomes was studied with 0.4-Hz ac calorimetry. The ac heat capacity on heating scans exhibited a peak in the vicinity of 23.9°C with a full width at half-maximum of 0.15–0.20°C. The enthalpy change was 4.8 kcal/mol, in good agreement with conventional differential scanning temperature by 0.1–0.5°C, the width increased to 0.25–0.40°C, and the apparent enthalpy change was only 40% of that observed on heating. Both the heating and cooling heat capacities were stable for at least 20 min in quasi-isothermal conditions. Following a 1 h anneal at 10°C, the heating scans were quite reproducible. The results have been interpreted in terms of the nucleation and subsequent annealing of small ordered domains in the bilayer on freezing the acyl chains. No peak associated with the pretransition was observed, as expected since the relaxation time for the degrees of freedom that produce the pretransition is much longer than the period of the 0.4-Hz temperature wave.

OXIDATIVE DEMETHYLATION IN STEROL METABOLISM. Inhibition by NADH, a required cofactor. D.R. Brady (Ctr. for Nuclear Studies, Memphis State Univ., Memphis, Tenn. 38152) *J. Biol. Chem. 256*(20):10442–19448 (1981). Oxidative demethylation of 4-methyl sterols by rat liver microsomes has been shown to be inhibited by low levels of NADH. Using snake venom-treated microsomes to remove endogenous NADH-utilizing activities, we observed inhibition of overall demethylation at concentrations of NADH as low as 2.5 μM. At 20 μM NADH, overall demethylation had been reduced to 60% of optimal activity. In the presence of NADH, oxidative metabolism of [30,31-^{14}C]4,4-dimethyl-5α-cholest-7-en-3β-ol resulted in formation of an intermediate which was metabolized anaerobically to $^{14}CO_2$ in the presence of NAD^+. This intermediate and its acetylated derivative had coincident mobility on silicic acid thin layer chromatograms with 3β-hydroxyl-[^{14}C]4β-methyl-5α-cholest-7-en-[^{14}C]4α-carboxylic acid sterol and its acetylated derivative. The inhibition by NADH is transitory. Normal rates return after~5 min if $NADH/NAD^+ = 0.2$, and after ~15 min if $NADH/NAD^+ = 2$. At an $NADH/NAD^+$ ratio of 10, the return to normal rates is even slower, exceeding 30 min. Further, the inhibition, though transitory, occurs again upon introduction of additional NADH to the assay mix.

INTERACTION OF MYELIN BASIC PROTEIN WITH DIPALMITOYLPHOSPHATIDYLGLYCEROL: DEPENDENCE ON THE LIPID PHASE AND INVESTIGATION OF A METASTABLE STATE. J.M. Boggs, D. Stamp, and M.A. Moscarello (From the Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada M5G 1X8) *Biochemistry 20*:6066–6072 (1981). The basic protein of myelin binds electrostatically to acidic lipids but has several hydrophobic segments which are believed to intercalate into the lipid bilayer. Differential scanning calorimetry (DSC) and fatty acid spin-labels were used to investigate the dependence of this intercalation on the phase state of dipalmitoylphosphatidylglycerol. After interaction with the lipid in the liquid-crystalline phase, basic protein decreased the phase transition temperature but had a much greater effect on cooling scans than on heating scans, if heating was performed from a low temperature. If the sample had been supercooled, an exothermic transition also occurred in heating scans, suggesting that the phase formed on supercooling is metastable. Incubation at the temperature of the exothermic transition for a short time resulted in conversion of the sample to a state which melted with a temperature and enthalpy only slightly less than those of the pure lipid. This could also be achieved by prolonged storage of the sample at a low temperature. Below the phase transition, the protein had a pronounced immobilizing effect on a spin-labeled fatty acid with the nitroxide moiety located near the terminal methyl. Supercooling, prolonged cooling, and incubation at the temperature of the exothermic transition for a few minutes all increased the degree of this immobilization, which was greater than that produced by polylysine or divalent cations.

ESTERIFICATION OF AN ENDOGENOUSLY SYNTHESIZED LIPOXYGENASE PRODUCT INTO GRANULOCYTE CELLULAR LIPIDS. Robert W. Bonser, Marvin I. Siegel, Sophia M. Chung, Randy T. McConnell, and Pedro Cuatrecasas (From the Molecular Biology Department, Wellcome Research Laboratories, Research Triangle Park, North Carolina 27709) *Biochemistry 20*:5297–5301 (1981). The human promyelocytic leukemia cell line HL60 can be induced to differentiate into mature granulocytes by exposure to Me_2SO. [1-^{14}C]Arachidonic acid incubated overnight with these cells was incorporated mainly into membrane phospholipids. Stimulation of these cells with the calcium ionophore, A_{23187}, resulted in a rapid release of esterified arachidonic acid from phosphatidylethanolamine and phosphatidylcholine. The released arachidonic acid was metabolized via both the cyclooxygenase and lipoxygenase pathways into three major hydroxylated products, 12-L-hydroxy-5,8,10-heptadecatrienoic acid (HHT), 5(S)-hydroxy-6,8,11,14-icosatetraenoic acid (5-HETE), and 5-(S),12(R)-dihydroxy-6,8,10,14-icosatetraenoic acid (leukotriene B). Arachidonic acid was also incorporated into triacylglycerols and phosphatidylinositol. The lipoxygenase product, 5-HETE, was rapidly esterified into cellular lipids. Thirty minutes after ionophore stimulation, 55% of the total 5-HETE synthesized was esterified into phopholipids and 35% incorporated into acylglycerols. In contrast, the other hydroxylated derivatives of arachidonic acid (HHT and leukotriene B) were not incorporated into acylglycerols or phospholipids. Esterification of hydroxylated metabolites of arachidonic acid into membrane phospholipids may serve to regulate a number of granulocyte functions.

PORTAL BLOOD CONCENTRATIONS OF CONJUGATED CHOLIC AND CHENODEOXYCHOLIC ACIDS RELATIONSHIP TO BILE SALT SYNTHESIS IN LIVER CELLS. Kathleen M. Botham, Margaret E. Lawson, Geoffrey J. Beckett, Iain W. Percy-Robb and George S. Boyd (Department of Biochemistry, University of Edinburgh, Hugh Robson Building, George Square, Edinburgh, EH8 9XD and Department of Clinical Chemistry, University of Edin-

burgh, The Royal Infirmary, Edinburgh, EH3 9YW (U.K.) *Biochimica et Biophysica Acta 665*:81-87 (1981). 1. Rats were maintained in a strictly controlled environment of 12 h illumination and 12 h darkness. At regular intervals during the light/dark cycle the portal blood conjugated cholic acid and conjugated chenodeoxycholic acid concentrations were measured. The bile salt concentrations exhibited similar diurnal rhythms, the highest concentrations occurring in the middle of the dark phase. 2. The concentrations of conjugated cholic and chenodeoxycholic acids in the portal blood of rats fed a diet containing the bile sat sequestrant, cholestyramine, were significantly lower than those found in rats given a control diet. 3. During total biliary drainage the portal blood concentrations of conjugated cholic and chenodeoxycholic acids fell to a minimum 6-8 h after the start of the experiment, whereas bile salt synthesis in hepatocytes isolated from the rats was not increased until at least 13 h after the commencement of total biliary drainage. 4. These results suggest that the concentrations of bile salts in the portal blood do not affect directly the diurnal fluctuation in rates of bile salt synthesis, as the response of synthesis to a change in portal blood bile salt concentrations is too slow. 5. When the rats were given a small supplement of cholesterol in the diet to suppress hepatic cholesterologenesis prior to being subjected to total biliary drainage, the changes in the portal blood concentrations of conjugated cholic and chenodeoxycholic acids and the synthesis of the two bile salts by isolated hepatocytes were similar to those found in rats given the control diet. 6. The rise in bile salt production seen during biliary drainage may not be dependent exclusively on a preceding increase in cholesterol synthesis.

THE EFFECT OF VARIOUS INTAKES OF ω3 FATTY ACIDS ON THE BLOOD LIPID COMPOSITION IN HEALTHY HUMAN SUBJECTS. H.B. Bronsgeest-Schoute, C.M. van Gent, J.B. Luten, and A. Ruiter (Institute for Fishery Products TNO, P.O. Box 183, 1970 AD Ijmuiden, The Netherlands) *Am. J. Clin. Nutr. 34*(9):1752-1757 (1981). In a study with 52 healthy volunteers, the effect of different amounts of ω3 fatty acids on the blood lipid composition was investigated. Doses of 1.4, 2.3, 4.1, and 8.2 g of ω3 fatty acids were administered to these subjects daily over a period of 4 wk. Total cholesterol, high-density lipoprotein cholesterol, total triglycerides, and glucose were determined in blood serum and hemoglobin in whole blood in all individuals before, during, and after the intake of ω3 fatty acids. In pooled serum samples, the lipoprotein composition and the fatty acid composition of blood lipids were determined. All dosages caused a shift in the fatty acid composition of blood serum lipids in favor of ω3 fatty acids and at the expense of ω6 and/or ω9 acids. In the sterol esters, only the percentage of C20:5ω3 increased. Maximum shifts depended on the amount of ω3 acids ingested and were evident within 1 to 2 wk. Two wk after the last ingestion of ω3 acids, the fatty acid composition of blood serum lipids had returned to the original state. In the groups receiving 8.2 of ω3 fatty acids, there was a significant decrease in serum triglyceride and very low-density lipoprotein levels, which is in accord with earlier observations. In the other parameters, including cholesterol and high-density lipoprotein cholesterol, no decrease or increase was observed.

DE NOVO FATTY ACID SYNTHESIS IN THE PERFUSED RAT LUNG: INCORPORATION OF PALMITATE INTO PHOSPHOLIPIDS. K.F. Buechler and R.A. Thoades (Depts. of Physiology and Biochemistry, Indiana Univ. School of Medicine, 1100 West Michigan St. Indianapolis, IN 46223 USA) *Biochim. Biophys. Acta 665*: 393-398 (1981). 1. The incorporation of exogenously derived [^{14}C]-palmitate and endogenously synthesized [^{3}H] palmitate (from $^{3}H_2O$) was measured in the isolated perfused lung. 2. Over 40% of the fatty acid esterified into lung desaturated phosphatidylcholine was derived from de novo synthesis. 3. A major portion of the palmitate synthesized de novo was incorporated in the 2 position of disaturated phosphatidylcholine. 4. Streptozotocin-induced diabetes and the compound 5-(tetradecyloxy)-2-furoic acid markedly inhibited de novo fatty acid synthesis while the incorporation of exogenously supplied palmitate increased into disaturated phosphatidylcholine, primarily in the 2 position. 5. Treatment with insulin resulted in an increase in [^{14}C] glucose incorporation into lung phospholipid, with the largest increase appearing in the glyceride-glycerol fraction of the phosphatidylcholine species. 6. Insulin neither stimulated de novo fatty acid synthesis nor increased exogenous palmitate incorporation. 7. These data show: (1) that de novo fatty acid synthesis in the perfused rat lung is involved in the remodeling reactions in the synthesis of phosphatidylcholine, and (2) that diabetes affects the relative contribution of de novo synthesized and exogenously supplied palmitate available for the esterification of lung phospholipid.

SECRETION OF LIPOPROTEINS, APOLIPOPROTEIN A-I AND APOLIPOPROTEIN E BY ISOLATED AND PERFUSED LIVER OF RAT WITH EXPERIMENTAL NEPHROTIC SYNDROME. S. Calandra, E. Gherardi, M. Fainaru, A. Guaitani and I. Bartošek (Istituto di Patologia Generale, Università di Modena, Via Campi 287, 41100 Modena (Italy), Department of Medicine, Haddassah Medical School, Jerusalem (Israel) and Istituto di Ricerche Farmacologiche 'Mario Negri', Milano (Italy)) *Biochimica et Biophysica Acta 665*:331-338 (1981). Nephrotic syndrome induced in the rat by the administration of puromycin aminonucleoside is accompanied by a hyperlipoproteinemia characterized by an elevation of all plasma lipoproteins, particularly of VLDL (1.006 g/ml) and HDL_1 (1.050-1.090 g/ml). The increase of HDL_1 is due to the accumulation of a lipoprotein species floating mainly in the density interval 1.050-1.090 g/ml, in which apolipoprotein A-I replaces apolipoprotein E as the major constituent peptide. This lipoprotein has been designated nephrotic HDL. The present study was conducted to establish whether nephrotic liver secreted more lipoproteins than the control liver and, in addition, produced a lipoprotein similar to nephrotic HDL found in plasma. Isolated livers from control and nephrotic rats were perfused with a lipoprotein-free medium for 3 h in a recirculating system. Lipoproteins were isolated by ultracentrifugation; apolipoprotein A-I and apolipoprotein E were measured in the whole perfusate at various time intervals. Nephrotic liver secreted twice as much VLDL and HDL_2 and 30% more LDL and HDL_1 than the control liver. This was accompanied by an increased secretion of both apolipoprotein A-I and apolipoprotein E, the levels of which were 6.5- and 2-fold, respectively, of those found in the control perfusates at the end of the perfusion. In view of the increased secretion of apolipoprotein A-I, the apolipoprotein A-I to apolipoprotein E ratio was much higher in the perfusates of nephrotic livers than in those of the controls. The concentration of apolipoproteins A-I and E in plasma of nephrotic rats was 7- and 2-fold, respectively, of that found in the plasma of the controls. In the perfusates of the nephrotic livers, we could not find a HDL_1 (1.050-1.090 g/ml) rich in apolipoprotein A-I similar to that isolated from plasma (nephrotic HDL). We suggest that the latter is formed in the circulation from the intravascular modification of HDL_2 secreted in excess by the liver.

PULMONARY PHOSPHATIDIC ACID PHOSPHOHYDROLASE: DEVELOPMENTAL PATTERNS IN RAT LUNG. P.G. Casola and F. Possmayer (Dept. of Biochem. and Dept. of Obstetrics and Gynaecology, Univ. of Western Ontario, London, Ontario N6A 5A5, Canada) *Biochim. Biophy. Acta 665*(2):177-185 (1981). The development profiles of the phosphatidic acid phosphohydrolase activities in developing rat lung were determined using aqueously dispersed phosphatidic acid, membrane-bound phosphatidic acid and lipid vesicles prepared from extracts of PA_{mb} as the substrates. The specific activities of the PA_{aq}-dependent phosphohydrolase activities in the homogenates, microsomes or cytosol did not change appreciably prior to birth but the microsomal and to a lesser extent the homogenate activities increased after birth. The PA_{mb}- and $PA_{1\,v}$-dependent activities increased between 18-21 days gestation (term 22) and thereafter declined. Fractionation of adult rat lung cytosol on Bio-Gel A5m columns produced PA_{aq}-dependent phosphohydrolase activities in the void volume and a major PA_{aq}-dependent activity with an apparent molecular mass (M_r) of 130 kdaltons which was followed by a broad shoulder. The 130 kdalton activity peak was not present in fetal and +1 day neonatal cytosols but the shoulder was still present. Thermal denaturation curves for the phosphatidic acid-dependent activities in fetal, neonatal and adult cytosols were biphasic with a rapidly inactivated component and a more slowly inactivated component. Little developmental change was noted in the thermal denaturation patterns of the PA_{mb}-dependent activities. However, the thermal denaturation patterns of the PA_{aq}-dependent activities revealed an increase in the heat stability of the more rapidly inactivated component with the +3 day neonatal and the adult cytosols.

PULMONARY PHOSPHATIDIC ACID PHOSPHOHYDROLASE: DEVELOPMENTAL PATTERNS IN RABBIT LUNG. P.G. Casola and F. Possmayer (Dept. of Biochem. and Dept. of Obstetrics and Gynaecology, Univ. of Western Ontario, London, Ontario N6A 5A5, Canada) *Biochim. Biophys. Acta 665*(2):186-194 (1981). The developmental patterns of the phosphatidic acid phosphohydrolase activities in developing rabbit lung were determined using both aqueously dispersed phosphatidic acid (PA_{aq}) and membrane-bound phosphatidic acid (PA_{mb}) as the substrates. The specific activities and the total activities of the PA_{mb}-dependent phosphohydrolase activities in the microsomes and to a lesser extent in the homogenates increased between 26 and 30 days gestation (term 31), but decreased in the adult. Fractionation of adult cytosol on Bio-Gel A5m revealed PA_{aq}-dependent activities in the void volume (V_O) (50% total), a peak with an apparent molecular mass (M_r)=150 kdaltons (25% total) and a peak with M_r=110 kdaltons (25% total). Thermal denaturation studies on the PA_{mb}-dependent activities in the cytosols produced biphasic curves with a rapidly inactivated component and a relatively heat-stable component. The thermal

denaturation profiles for the PA_{mb}-dependent activities remained relatively unaltered throughout fetal development.

DECREASED PROSTACYCLIN SYNTHESIS IN VITAMIN E-DEFICIENT RABBIT AORTA. A.C. Chan and M.K. Leith (Dept. of Foods and Nutrition, The Univ. of Manitoba, Winnipeg, Manitoba, Canada, R3T 2N2) *Am. J. Clin. Nutr. 34*(11):2341–2347 (1981). Rabbit aorta predominantly synthesizes prostacyclin (PGI_2), a potent inhibitor of platelet aggregation and a strong vasodilator. We report here the effect of vitamin E depletion and repletion on endogenous release of PGI_2 by rabbit aorta. Serum pyruvate kinase was monitored for myopathy. The endogenous release of PGI_2 by the aorta, detected as its stable metabolite 6-keto-$PGF_1\alpha$, was inhibited by indomethacin and was inversely related to the size of aorta sections. Deficient animals for 48 h completely restored the PGI_2 release to a level comparable to the control values. The data showed that PGI_2 synthesis by aorta can be influenced by dietary vitamin E.

URINARY STEROID METABOLITES AND THE OVERGROWTH OF LEAN AND FAT TISSUES IN OBESE GIRLS. D.B. Cheek, J.E. Graystone, R.F. Seamark, J.E.A. McIntosh, G. Phillipou, and J.M. Court (Royal Childrens Hospital Research Foundation, Univ. of Melbourne) *Am. J. Clin. Nutr. 34*(9):1804–1810 (1981). Studies were made of steroid metabolites excreted in the urine of 17 obese girls 11.4 to 16.8 yr and 17 normal girls 11 to 17 yr. Creatinine excretion (muscle mass), total body water (or deuterium space), lean body mass and body fat were determined in the obese girls. Extracellular volume (corrected bromide space) was also measured and by difference with body water, intracellular water or soft tissue cell mass was calculated. In normal girls 24-h creatinine excretion was determined, but body water was predicted from height and weight. It was found, as in previous studies, that the obese girls had excess muscle mass and soft tissue cell mass for height. The excess growth of muscle, lean tissue, and body length in obese girls correlated with increments in oxosteroid (17 ketosteroid) excretion. The overall weight increase correlated with increased excretion of corticosteroid metabolites—a finding of interest since a physiological Cushing's syndrome was postulated for fat girls many years ago. When the normal and obese girls were divided by age at 14 yr and the subgroups compared (normal obese) the younger girls showed differences with respect to height, weight, total body water, fat and percentage fat. Differences in steroid metabolites were not found. In older girls the same findings were made again, but here it was clear that the increments in body size, particularly muscle mass, correlated with augmented oxosteroid excretion. Evidence is cited that these findings are not just related to a larger steroid pool in obese girls.

INFLUENCE OF DIETARY CHOLESTEROL AND FAT ON SERUM LIPIDS IN MEN. Wanda Chenoweth, Margaret Ullmann, Ronald Simpson and Gilbert Leveille (Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI 48824) *J. Nutr. 111*:2069–2080 (1981). The effect of changes in dietary cholesterol and fat on serum lipids was studied in 32 healthy men (mean age = 24.8 years). Subjects were fed a controlled diet for 10 days providing 42 to 45% of the total calories from fat, a P/S ratio of 0.3 to 0.5 and two eggs per day. During the next eight weeks, 16 subjects received each of the following diets for four weeks in a crossover design: 1) a control diet with two eggs per day or 2) the control diet with eggs replaced by a cholesterol-free egg substitute. The remaining 16 subjects received each of the following diets in a similar crossover design: 1) a modified-fat diet containing 35% of the total calories from fat, a P/S ratio $\geq$ 1.0 and two eggs per day or 2) the same modified-fat diet with the egg substitute replacing the eggs. The two-week cycle of menus repeated throughout the study included a wide variety of foods commonly consumed in this country. Although the response of individual subjects varied, analysis of variance showed a significant decrease in serum total cholesterol related to replacement of eggs with the egg substitute and to modification in the type and amount of dietary fat. A significant diet-treatment interaction or sequencing effect was not found. Change in cholesterol intake related to addition or deletion of two eggs in the daily diet had no significant effect on serum triglycerides, high density lipoprotein cholesterol, or relative lipoprotein concentrations.

DIACYLGLYCEROL-CARRYING LIPOPROTEIN OF HEMOLYMPH OF THE LOCUST AND SOME INSECTS. Haruo Chino and Kyoko Kitazawa (Biochemical Laboratory, Institute of Low Temperature Science, Hokkaido University, Sapporo, Japan) *Lipid Res. 22*:1042–1052 (1981). The diacylglycerol-carrying lipoprotein (DGLP) was purified from hemolymph of the locust, *Locusta migratoria*, by a rapid method which included a specific precipitation at low ionic concentration and DEAE-cellulose column chromatography. The final preparation was highly homogeneous as judged by gel electrophoresis, electron microscopy, and immunodiffusion. The locust DGLP molecule was almost spherical in shape with a diameter of about 130 Å. The molecular weight, determined by a sedimentation equilibrium method, was approximately 580,000. The total lipid content amounted to about 40%. The lipids comprised diacylglycerol (33% of total lipid), hydrocarbon (21%), cholesterol (8%), and phospholipids (36%). The hydrocarbon fraction contained a number of *n*-alkanes and methylalkanes ranging from C_{25} to C_{38} in chain length. Mannose (3%) and glucosamine (0.5%) were associated with the apoprotein of DGLP. Apoprotein of locust DGLP consisted of two subunits, heavy chain (mol wt 250,000) and light chain (mol wt 85,000); carbohydrate (mannose) was associated only with the heavy chain. Tests of physiological function of DGLPs from locust, cockroach, and silkworm suggest that the insect DGLP serves multiple roles as a true carrier molecule in transporting diacylglycerol, cholesterol, and hydrocarbon from sites of storage, absorption, and synthesis to sites where these lipids are utilized as metabolic fuel, precursors for triacylglycerol and phospholipid synthesis, or structural components of cell membrane and cuticle. In addition, the insect DGLPs displayed no species-specificity in terms of the functions, whereas they were immunologically distinguishable.

ADIPOCYTES AND ADIPOSITY IN ADULTS. W.C. Chumlea, Ph.D., A.F. Roche, M.D., Ph.D., D.Sc., R.M. Siervogel, Ph.D., J.L. Knittle, M.D., and P. Webb, M.D. (Fels Research Institute and Dept. of Pediatrics, Wright State Univ. School of Med., Yellow Springs, OH 45387) *Am. J. Clin. Nutr. 34*(9):1798–1803 (1981). Measures of adipocyte size and body density were collected from 217 nonobese adults 20–50 yr of age, and estimates of total body fat, percentage body fat, and adipocyte number were calculated. Women had a greater percentage body fat than men in every age group except the oldest. Women had significantly greater amounts of total body fat and larger adipocytes than men in the 20- to 24-yr group, but men had significantly greater amounts of total body fat than the women in the 45- to 50-yr age group. Adipocyte number, total body fat, and percentage body fat are each positively correlated with age in both sexes. Adipocyte size is not correlated with age but is positively correlated with total and percent body fat in men and women irrespective of age. These cross-sectional data suggest that adipocyte number, rather than being stable during adulthood, increases with age and is associated with corresponding increases in total and percentage body fat.

NUTRIENT INTAKE, ADIPOSITY, PLASMA TOTAL CHOLESTEROL, AND BLOOD PRESSURE OF RURAL PARTICIPANTS IN THE (VERMONT) NUTRITION PROGRAM FOR OLDER AMERICANS (TITLE III). R.P. Clarke, M.S., E.D. Schlenker, Ph.D., and S.B. Merrow, M.Ed. (Dept. of Human Nutrition and Foods, College of Agriculture, University of Vermont, Terrill Hall, Burlington, VT 05405) *Am. J. Clin. Nutr. 34*(9):1743–1751 (1981). The interrelationships of obesity, hypertension, elevated plasma cholesterol (risk factors), and intakes of selected nutrients were examined among elderly subjects attending a congregate meal program in Vermont. Mean nutrient intakes were significantly higher for 22 males compared to 69 females. Mean plasma cholesterol levels were higher in females. Age, systolic and diastolic blood pressure, and indices of adiposity showed no sex differences. Intakes of total fat and animal protein increased in males but plasma cholesterol decreased with age. Systolic blood pressure in females increased while body mass index decreased with age. A higher proportion of females had plasma cholesterol levels $\geq$ 260 mg/100 ml and a higher proportion of females than males $>$ 73 yr of age had blood pressures at risk level. There was a greater proportion of females than males with both elevated plasma cholesterol levels and adiposity. Similarly the females had greater incidence of the combination of any two risks. No males, compared to 9% of females, were in all three risk categories.

VITAMIN D STATUS AND BONE HISTOMORPHOMETRY IN GROSS OBESITY. J.E. Compston, S. Vedi, J.E. Ledger, A. Webb, J-C. Gazet, and T.R.E. Pilkington (Gastrointestinal Research Unit, Rayne Institute and Dept. of Surgical Pathology, St. Thomas' Hospital, London, England) *Am. J. Clin. Nutr. 34*(11):2359–2363 (1981). Plasma 25-hydroxyvitamin D concentrations and bone histomorphometry were investigated in 24 grossly obese subjects. The mean plasma 25OHD concentration was significantly lower in the obese group than in age-matched, healthy controls. Subnormal values were found in four obese subjects and in a further two subjects, who were investigated at the end of the summer, plasma 25-hydroxyvitamin D levels were at the lower end of the normal winter range. Bone histology was abnormal in two patients. In one, mild osteomalacia and secondary hyperparathyroidism were present while in the other patient the appearances suggested increased bone turnover, possibly as a result of healing osteomalacia. We conclude that gross obesity is associated with an increased risk of vitamin D

deficiency, probably because of reduced exposure to uv radiation. Histological evidence of metabolic bone disease may also occur. Preoperative vitamin D deficiency may contribute in some patients to the development of metabolic bone disease after intestinal bypass.

EFFECT OF VITAMIN E ON PROTHROMBIN LEVELS IN WARFARIN-INDUCED VITAMIN K DEFICIENCY. J.J. Corrigan, Jr., M.D. and L.L. Ulfers, R.N. (Dept. of Pediatrics, University of Arizona Health Sciences Center, Tucson, AZ 85724) *Am. J. Clin. Nutr. 34*(9):1701-1705 (1981). Rats rendered lightly vitamin K deficient with warfarin (0.01 mg/100 g, IP) and given the equivalent of 1000 units of vitamin E/kg IM for 7 days, showed a marked reduction in functional factor II activity, but normal factor II levels using Echis venom on coagulation analysis. In 12 humans receiving warfarin, vitamin E was administered in doses of 100 or 400 units/day orally for 4 wk. The results in these patients showed no significant change in the prothrombin time, factor II coagulant activity, or factor II antigen (by electroimmunoassay). However, by using a ratio of factor II coagulant activity to immunoreactive protein, significant reduction was observed when compared to pretreatment ratios. These data suggest that vitamin E acts at the step mediated by vitamin K and not in the synthesis of the factor II precursor. Although the administration of high doses of vitamin E in animals, and possibly humans, with vitamin K deficiency potentiates the vitamin K deficiency, this effect is not clinically obvious with 400 IU/day or less.

LIPID DEPENDENCE OF GLUCOSE-6-PHOSPHATE PHOSPHOHYDROLASE: A STUDY WITH PURIFIED PHOSPHOLIPID TRANSFER PROTEINS AND PHOSPHATIDYLINOSITOL-SPECIFIC PHOSPHOLIPASE C. Richard C. Crain and Donald B. Zilversmit (From the Division of Nutritional Sciences and Section of Biochemistry, Molecular and Cell Biology, Division of Biological Sciences, Cornell University, Ithaca, New York 14853) *Biochemistry 20*:5320-5326 (1981). The nonspecific and phosphatidylcholine-specific transfer proteins from beef liver and the phosphatidylinositol-specific phospholipase C from *Staphylococcus aureus* were used to modify the phospholipid composition of microsomal membranes in order to study the dependence of glucose-6-phosphate phosphohydrolase (EC 3.1.3.9) activity on membrane phospholipids. Incubation of microsomes with dipalmitoylphosphatidylcholine-containing unilamellar vesicles and either transfer protein produced a membrane in which this disaturated phospholipid contributed up to 43% of the total phosphatidylcholine. Incubation of microsomes and phosphatidylcholine unilamellar vesicles with nonspecific transfer protein caused a net increase in the membrane phosphatidylcholine content and a net decrease in the phosphatidylethanolamine and phosphatidylinositol levels, whereas in incubations with the phosphatidylcholine transfer protein, no change in phospholipid class composition occurred. Incubations of microsomes and phosphatidylcholine/phosphatidylethanolamine (3:1 mol/mol) unilamellar vesicles with the nonspecific transfer protein also caused an increased phosphatidylcholine content and a decreased phosphatidylinositol content but no change in the phosphatidylethanolamine level. Similar incubations in the presence of phosphatidylcholine-specific transfer protein had no effect on the phospholipid composition.

TRANSFER AND EXCHANGE OF PHOSPHOLIPID BETWEEN SMALL UNILAMELLAR LIPOSOMES AND RAT PLASMA HIGH DENSITY LIPOPROTEINS: DEPENDENCE ON CHOLESTEROL CONTENT AND PHOSPHOLIPID COMPOSITION. Jan Damen, Joke Regts and Gerrit Scherphof (Laboratory of Physiological Chemistry, University of Groningen, Bloemsingel 10,9712 KZ Groningen, The Netherlands) *Biochimica et Biophysica Acta 665*: 538-545 (1981). We investigated the ability of small unilamellar liposomes of various lipid compositions to maintain their integrity in the presence of rat plasma or plasma fractions. Liposomal damage was determined in terms of release of an entrapped water-soluble marker, carboxyfluorescein, and, simultaneously, loss of liposomal ^{14}C-labeled phospholipid. Complete retentions of carboxyfluorescein during a 30-min incubation with plasma could be attained with liposomes containing at least 40 mol% cholesterol. By substituting sphingomyelin for phosphatidylcholine a considerable prolongation of solute retention was achieved. Sphingomyelin/cholesterol (molar ratio 3:2) liposomes retained nearly all of the entrapped dye during a 20-h incubation with plasma, while phosphatidylcholine/cholesterol liposomes lost as much as 40%. Incorporation of cholesterol also reduces the transfer of ^{14}C-labeled phospholipid from liposomes to plasma HDL. While transfer of phosphatidylcholine was partially reduced, transfer of sphingomyelin was completely inhibited under the same conditions. Isolated HDL was unable to bring about the extent of carboxyfluorescein release and phospholipid transfer which was observed with whole plasma but required the addition of lipoprotein-free plasma. The subfraction of HDL that is rich in apolipoprotein E (HDL_1) plays at most a minor role in liposome-plasma interactions. Native HDL phospholipids were biosynthetically labeled with ^{32}P and isolated HDL was incubated with ^{14}C-labeled liposomes in presence of lipoprotein-free plasma. In this way we demonstrated that, whereas phosphatidylcholine transfer from cholesterol-poor liposomes is mainly an unidirectional process leading to excessive liposome degradation, phosphatidylcholine transfer from cholesterol-rich liposomes involves an exchange process and is attended by almost complete retention of entrapped solute.

PHOSPHOLIPID SYNTHESIS IN HUMAN EMBRYO FIBROBLASTS INFECTED WITH HERPES SIMPLEX VIRUS TYPE 2. Larry W. Daniel, Moseley Waite, Louis S. Kucera, Lynn King and Iris Edwards (Departments of Biochemistry and Microbiology and Immunology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103) *Lipids 16*(9):655-661 (1981). The effect of herpes simplex virus type 2 infection on the synthesis of phospholipids in human embryo fibroblasts was determined at temperatures permissive (35 C) or nonpermissive (42C) for virus replication. Incorporation of [^{32}P]i was decreased by herpes simplex virus type 2 infection after 6 hr, which corresponds to the time of initiation of progeny virus production. No differences were observed in the relative incorporation of [^{32}P]i into phospholipid classes. In another series of experiments, cells were labeled with [^{3}H]ethanolamine before infection and with [^{14}C]ethanolamine after infection. The incorporation of [^{14}C]ethanolamine was also decreased after 6 hr of infection. When choline was substituted for ethanolamine, a similar, although less pronounced, decrease in incorporation was seen in infected cells compared to mock-infected cells. During abortive infection at 42 C, incorporation of [^{3}H]thymidine into cellular DNA was stimulated, but the incorporation of phospholipid precursors was decreased. Total phospholipid composition and phospholipid acyl group composition were not changed appreciably during abortive or productive infection, regardless of whether the cells were labeled before or after infection. In conclusion, these data indicated that, during herpes simplex virus type 2 infection, the incorporation of lipid precursors into phospholipid was decreased. The stimulation of cellular DNA synthesis previously observed during abortive infection at 42 C was not paralleled by a detectable stimulation of total phospholipid synthesis. Neither productive nor abortive infection resulted in significant phospholipid compositional changes in the host cell; however both resulted in a marked inhibition of phospholipid synthesis.

METABOLIC FATE OF VLDL APOLIPOPROTEINS B AND E IN HEPATECTOMIZED RATS. Roger A. Davis, Arthur D. Hartman, Ladislav Dory, Brian J. Van Lenten and Paul S. Roheim (Department of Physiology, Louisiana State University Medical School, New Orleans, LA 70119) *Biochimica et Biophysica Acta, 665*: 154-164 (1981). The metabolic fate of VLDL apolipoproteins B and E was examined in functionally hepatectomized rats. 1 h after hepatectomy, there was almost complete absence of ultracentrifugally isolated VLDL lipid and protein including apolipoproteins B and E. Analysis of apolipoprotein concentrations by electroimmunoassay showed hepatectomy did not affect the total serum concentrations of apolipoproteins B and E; thus, hepatectomy caused a redistribution of these apolipoproteins from VLDL to higher density lipoproteins. In the LDL (d = 1.03 - 1.063 g/ml) fraction, hepatectomy increased the concentrations of free cholesterol (40%), esterified cholesterol (57%) and protein (18-67%), due to an increase in apolipoproteins B (22-48%) and E (250-300%). After hepatectomy, the HDL fraction accumulated the greatest total amount of apolipoprotein E. Since the majority of apolipoprotein E was isolated in the d > 1.21 g/ml fraction after sequential ultracentrifugation, the redistribution of apolipoproteins B and E was further defined by fractionation of serum on 5 M agarose columns. Electroimmunoassay of the column fractions showed that the apolipoprotein B peak eluted before the apolipoprotein E peak. Although a considerable portion of apolipoprotein E eluted with A-I, the peak of apolipoprotein E eluted before the A-I peak in both groups. These data suggest that a portion of apolipoprotein E is associated with particles which are smaller than LDL but are larger than A-I-rich HDL. Hepatectomy caused an accumulation of apolipoprotein B in LDL, and apolipoprotein E and cholesterol in particles which were smaller than LDL and may represent HDL_1. It is likely that under normal physiological conditions the liver plays a role in the removal of these apolipoprotein E-rich particles which are derived, at least in part, from the metabolism of VLDL.

MECHANISM OF THE AGE-RELATED DECREASE OF EPINEPHRINE-STIMULATED LIPOLYSIS IN ISOLATED RAT ADIPOCYTES: β-ADRENERGIC RECEPTOR BINDING, ADENYLATE CYCLASE ACTIVITY, AND CYCLIC AMP ACCUMULATION. Elizabeth M. Dax, John S. Partilla, and Robert I. Gregerman (Geron-

tology Research Center, National Institute on Aging, National Institutes of Health at Baltimore City Hospitals and the Departments of Medicine, Baltimore City Hospitals and Johns Hopkins University School of Medicine, Baltimore, MD) *J. Lipid Res. 22*:934–943 (1981). β-adrenergic binding ([^{3}H] dihydroalprenolol), adenylate cyclase activity, and cAMP accumulation were measured in adipocytes to investigate whether the mechanism of decreased hormone-sensitive lipolytic response with age was mediated through membrane-associated events. The dose of epinephrine required for half maximal stimulation of glycerol release (ED_{50}) was significantly lower in 2-month-old rats (0.8 ± 0.2 μM) than in mature (6- and 12-month-old) rats (5.2 ± 1.5 and 6.2 ± 1.5 μM, respectively). In 24-month-old rats the ED_{50} (0.7 ± 0.2 μM) was less than in mature rats. Maximum rates of hormone-stimulated glycerol release (per 10^6 cells) was highest in the two mature groups and decreased by 50% in the old rats ($P < 0.01$). Lipolytic changes were independent of cell size. β-adrenergic receptor number (50–90 thousand sites/cell) and affinity (K_D 4–5 nM) were the same in each age group. ED_{50} and maximum level of hormone-stimulated adenylate cyclase activity did not change with age. The ED_{50} of cAMP accumulation of young rats was 3 ± 4 and 25 ± 5 μM in 6- and 12-month-old rats, respectively. The results suggest that age-related decrease of epinephrine-sensitive lipolysis in old rats may be due to alterations of the lipolytic pathway distal to the receptor-adenylate cyclase complex and the generation of cyclic AMP.

VITAMIN D NUTRITION IN RELATION TO SEASON AND OCCUPATION. M.S. Devgun, C.R. Paterson, B.E. Johnson, and C. Cohen (Depts. of Biochemical Medicine, Dermatology, and Geriatrics, Univ. of Dundee, Dundee, Scotland) *Am. J. Clin. Nutr. 34* (8):1501–1504 (1981). Seasonal variations in vitamin D nutrition were assessed by measurements of serum 25-hydroxycholecalciferol levels in outdoor workers, indoor workers and long-term hospital inpatients. All three groups showed seasonal changes and the outdoor workers had, as might be expected, the highest levels at all seasons. However, the highest levels of 25-hydroxycholecalciferol were found in October in the indoor workers and in November for the outdoor workers whereas the peak in ultraviolet exposure was in July. The possible reasons for this long lag are discussed; the most likely explanation is that vitamin D continues to be formed and stored during the autumn especially in outdoor workers.

SERUM AND HEPATIC NASCENT LIPOPROTEINS IN NORMAL AND HYPERCHOLESTEROLEMIC RATS. Peter J. Dolphin (Department of Biochemistry, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7) *J. Lipid Res. 22*:971–989 (1981). The lipids and apoproteins of serum, hepatic Golgi cisternae, and secretory vesicle lipoproteins from hypothyroid, hypercholesterolemic rats were analyzed and compared to homolgous lipoprotein fractions from euthyroid rats fed a low fat chow diet in order to determine the nature of the nascent lipoprotein particles and indicate post-secretory modifications. Normal rat hepatic Golgi and secretory vesicles contained predominantly triglyceride-rich very low density lipoprotein (VLDL) which had little associated apoC-II or C-III and was deficient in apoE when compared to serum VLDL. Small quantities of cholesteryl ester-enriched low density lipoprotein (LDL) containing apoB and apoE were also present. Hepatic fractions from hypercholesterolemic rats contained cholesteryl ester- and apoE-rich, triglyceride-depleted VLDL of similar size, immunodiffusion characteristics, ratio of immunoassayable apoB to apoE, and lipid composition, to hypercholesterolemic serum VLDL. Hepatic levels of LDL in hypercholesterolemic rats were markedly elevated and enriched in cholesteryl esters and apoE when compared to normal hepatic LDL. Cholesteryl ester-rich hepatic VLDL and LDL increased in size and in cholesteryl ester and apoE content during transit from the Golgi cisternae into the secretory vesicles. Triglyceride-rich VLDL only acquired apoE which was further supplemented upon secretion. Nascent VLDL and LDL represented LpB-LpE association complexes and no deficiency in any apoE isoprotein within the cholesteryl ester-rich serum lipoproteins was observed. Thus, dietary-induced hypercholesterolemia in hypothyroid rats results in a fatty liver whose lipoprotein secretory products contribute to the plasma pool of abnormal cholesteryl ester- and apoE-enriched lipoproteins.

EFFECTS OF FERROUS CHLORIDE AND IRON-DEXTRAN ON LIPID PEROXIDATION IN VIVO IN VITAMIN E AND SELENIUM ADEQUATE AND DEFICIENT RATS. J.J. Dougherty, W.A. Croft, and W.G. Hoekstra (Depts. of Biochemistry and Human Oncology, Univ. of Wisconsin-Madison, Madison, WI 53706) *J. Nutr. 111*(10):1784–1796 (1981). The effects of intraperitoneally injected ferrous chloride and iron-dextran on lipid peroxidation in vivo were assessed. Peroxidation was estimated by measuring ethane, a volatile autoxidation product of omega-3-unsaturated fatty acids. Rats supplemented with 0.1 ppm dietary selenium and rats supplemented with 0.1 ppm selenium and 200 IU vitamin E/kg were injected with ferrous chloride at 30 mg iron/kg, or with sodium chloride, or left uninjected. In both dietary groups ferrous chloride increased ethane production while sodium chloride did not, but iron-caused ethane increased was 8 times greater in the low E group. Iron-dextran injected at 500 mg iron/kg was fatal to rats fed a basal diet deficient in selenium and vitamin E or a diet supplemented with 0.5 ppm selenium; supplemental vitamin E at 200 IU/kg diet prevented this mortality. Iron-dextran quadrupled ethane production in rats fed the basal diet and tripled ethane production in rats fed the selenium-supplemented diet. Vitamin E supplementation prevented the iron-dextran-caused rise in ethane production. A histological examination of rats killed by iron-dextran showed severe generalized necrosis of the diaphragm and severe focal necrosis of thigh muscle. Vitamin E protected more effectively than selenium against iron-dextran-caused peroxidation as well as against acute iron-dextran-caused mortality.

UPTAKE AND 25-HYDROXYLATION OF VITAMIN D_3 BY ISOLATED RAT LIVER CELLS. S. Dueland, I. Holmberg, T. Berg, and J.I. Pedersen (Institute for Nutrition Research, Sch. of Med., Univ. of Oslo, Oslo 3, Norway) *J. Biol. Chem. 256*(20):10430–10434 (1981). The physiological roles played by hepatocytes and nonparenchymal cells of rat liver in the metabolism of vitamin D_3 have been investigated. Tritium-labeled vitamin D_3 dissolved in ethanol was administered intravenously to two rats. Isolation of the liver cells 30 and 70 min after the injection showed that vitamin D_3 had been taken up both by the hepatocytes and by the nonparenchymal liver cells. The relative proportion of vitamin D_3 that accumulated in the nonparenchymal cells increased with time. Perfusion of the isolated rat liver with [^{3}H] vitamin D_3 added to the perfusate confirmed the ability of both cell types to efficiently take up vitamin D_3 from the circulation. By a method based on high pressure liquid chromatography and isotope dilution-mass fragmentography it was found that isolated liver cells in suspension had a considerable capacity to take up vitamin D_3 from the medium. About 2.5 pmol of vitamin D_3 were found to be associated with each hepatocyte or nonparenchymal cell after 1 h of incubation. 25-Hydroxylation *in vitro* was found to be carried out only by the hepatocytes. The rate of hydroxylation was about the same whether the cells were isolated from normal or rachitic rats (3.5 and 4 pmol of 25-hydroxyvitamin D_3 formed per h per 10^6 cells, respectively). The possibility that the nonparenchymal cells might serve as a storage site for vitamin D_3 in the liver is discussed.

EFFECTS OF TWO ALBUMINS AND TWO DETERGENTS ON THE ACTIVITY OF BOVINE MILK LIPOPROTEIN LIPASE AGAINST VERY LOW DENSITY AND HIGH DENSITY LIPOPROTEIN LIPIDS. S. Eisenberg, D. Feldman, and T. Olivecrona (Lipid Research Laboratory, Dept. of Medicine B, Hadassah Univ. Hospital, Jerusalem, Israel and Dept. of Physiological Chemistry, Univ. of Umea, Umea, Sweden) *Biochim. Biophys. Acta 665*:454–462 (1981). In this study we have determined the effects of two commercial albumin preparations (Sigma and Pentex albumins) and two detergents (sodium deoxycholate and Triton X-100) on the activity of lipoprotein lipase purified from bovine milk against biosynthetically labeled triacylglycerol in very low density lipoprotein and biosynthetically labeled phosphatidylcholine in very low density and high density lipoproteins. Pentex albumin decreased the activity of lipoprotein lipase in all assays to about one-fourth to one-third of that observed with Sigma albumin. Quantitative differences were observed in the distribution of labeled surface constituents (^{32}P-labeled phospholipids, [^{3}H] cholesterol and ^{125}I-labeled apolipoprotein C) among density fractions during lipolysis of very low density lipoprotein carried out in the presence of Pentex or Sigma albumins. With Pentex albumin, more phospholipids and apolipoprotein C distributed to the density fraction of *d* 1.04–1.21 g/ml than with Sigma albumin. Sodium deoxycholate at a concentration of up to 2 mM had little effect in all assays. Triton X-100 decreased the activity of lipoprotein lipase against very low density lipoprotein lipids but increased the activity of the enzyme against high density lipoprotein lipids. The study has thus demonstrated marked quantitative differences of lipoprotein lipase activities when determined under slightly differing incubation conditions.

THE INFLUENCE OF DIETARY UNSATURATED *CIS* AND *TRANS* AND SATURATED FATTY ACIDS ON TISSUE LIPIDS OF SWINE. Charles E. Elson, Norlin J. Benevenga, David J. Canty, Robert H. Grummer, Joseph J. Lalich, John W. Porter and Arlow E. Johnston (Department of Nutritional Sciences, Department of Meat and Animal Sciences, Department of Pathology and Department of Physiological Chemistry, University of Wisconsin, Madison, WI 53706; and Oilseed Crops Laboratory, A.R., W.E.A., U.S.D.A., Northern Regional Research Center, Peoria, IL 61604) *Atherosclerosis 40*:115–137 (1981). A feeding trial was conducted to evaluate the effects of dietary *trans* unsaturated fatty acids (*trans* fat) and of the interplay of dietary saturated fatty acids (saturated

fat), *cis* unsaturated fatty acids, (*cis* fat) and *trans* fat on tissue lipids, particularly those effects suggestive of angiotoxicity. Swine were fed for 10 months a diet containing 17% added fat. Seven blends of varying proportions of the 3 fat components provided sufficient sample points to permit an examination of the interplay. Parameters under study included weight gain, serum cholesterol and triglyceride concentrations, lipoprotein lipid profile, total lipid and cholesterol concentrations of liver, heart and aorta, fatty acid composition of liver and aorta lipids and hepatic fatty acid synthesis and high levels of saturated or *cis* fat generally elicited responses consistent with results reported by others. The notable exception was the serum cholesterol concentration. Throughout the study, the swine were hypercholesterolemic. Swine fed the high saturated fat blend had serum cholesterol levels equal to those swine fed the high *cis* fat blend. Serum cholesterol levels in the swine fed the other fat blends were more elevated. Another apparent anomaly was the lower concentration of lipid in the aortas of swine fed the high-saturated fat diet. The impact of the *trans* fat was modulated by the relative proportions of saturated and *cis* fat in the diet. The impact of *trans* fat was of greater magnitude for most parameters when the fat blend was low in saturated fat. The sole parameter suggestive of *trans* fat-mediated angiotoxicity was the distribution of lipids in lipoprotein fractions. Swine fed diets containing *trans* fat had lower relative proportions of the α-lipoprotein lipids. Although hypercholesterolemic, the high fat diets were not overtly angiotoxic except when fed to swine that carried a specific immune-genetically defined low density lipoprotein.

PREPARATION, CHARACTERIZATION, AND INSULIN SENSITIVITY OF ISOLATED SWINE ADIPOCYTES: COMPARISON WITH ADIPOSE TISSUE SLICES. T.D. Etherton and C.S. Chung (Dept. of Dairy and Animal Science, The Pennsylvania State University, University Park, PA 16802) *J. Lipid Research 22*:1053–1059 (1981). The technique of Rodbell (*J. Biol. Chem.* 239:375) was modified considerably in order to isolate swine adipocytes without rupturing large cells. Cell size and diameter distributions were the same for adipocytes fixed with OsO_4 following isolation with collagenase and adipocytes liberated from OsO_4-fixed adipose tissue slices. Lipogenic rates were greater for isolated adipocytes compared with thin adipose tissue slices at low (0.5 mM) and high (10 mM) glucose concentrations (cells = 307 and 1100; slices = 139 and 744 nmoles glucose → lipid/10^6 cells/hr for 0.5 and 10 nM glucose, respectively, $P < 0.001$). Similar differences were found for glucose oxidation. Sensitivity to insulin was determined by measuring the stimulation of lipogenesis and glucose oxidation in the presence of 0, 1, 5, 25, and 100 ng/ml of purified porcine insulin at low (0.5 mM) and high (10 mM) glucose concentrations. Relative to basal incubations, the addition of insulin caused similar increases in glucose oxidation and lipogenesis for isolated adipocytes and adipose tissue slices when glucose concentration was 10 mM. These results indicate 1) that isolated swine adipocytes can be prepared without alterations in cell size of diameter distribution, and 2) that isolated adipocytes have higher rates of glucose oxidation and lipogenesis from glucose even though they retain a similar in vitro sensitivity to insulin.

THE EFFECT OF DECREASED PLASMA CHOLESTEROL CONCENTRATION ON CIRCULATING MEVALONATE METABOLISM IN RATS. Kenneth R. Feingold, Millie Hughes Wiley, Gordon MacRae, and Marvin D. Siperstein (Metabolism Section, Medical Service, Veterans Administration Medical Center and Cardiovascular Research Institute and Department of Medicine, University of California, San Francisco, CA) *J. Lipid Res. 22*:990–997 (1981). Circulating mevalonate is metabolized by two mechanisms: the sterol pathway leading to cholesterol and the shunt pathway resulting in CO_2 production. The kidney is the chief site of circulating mevalonate metabolism by both pathways. The present study investigated the effect of plasma cholesterol concentration on circulating mevalonate metabolism. 4-Aminopyrazolo(3,4-*d*)pyrimidine and Triton WR 1339 were utilized to induce "functional hypocholesterolemia". An enhancement of both renal total nonsaponifiable lipid synthesis (36–43%) and cholesterol synthesis (42%) from circulating mevalonate was observed when "functional hypocholesterolemia" was induced by either compound. Hepatic total nonsaponifiable lipid synthesis from circulating mevalonate was not enhanced in the Triton-treated animals, but 4-aminopyrazolo(3,4-*d*)-pyrimidine treatment increased accumulation of total labeled nonsaponifiable lipids and cholesterol. No increase in labeled total nonsaponifiable lipids or cholesterol in the carcass was observed after treatment with either compound. "Functional hypocholesterolemia" reduced the shunt pathway of circulating mevalonate metabolism by approximately 30%. This reduction occurred in both the renal and extrarenal shunt pathways. These data indicate that plasma cholesterol concentration regulates the in vivo metabolism of circulating mevalonate in that hypocholesterolemia reduces the shunt pathway and stimulates sterologenesis, an effect chiefly localized to the kidneys.

ACTIVATION OF CTP: PHOSPHOCHOLINE CYTIDYLYLTRANSFERASE IN RAT LUNG BY FATTY ACIDS. Douglas A. Feldman, Pamela G. Brubaker and Paul A. Weinhold (Veterans Administration Medical Center and Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48105) *Biochimica et Biophysica Acta 665*:53–59 (1981). CTP: phosphocholine cytidylyltransferase activity exists in both the microsome and cytosol fractions of adult lung, 36 and 59%, respectively. Although these enzyme activities are stimulated in vitro by added lipid activators (i.e. phosphatidylglycerol), there are significant levels of activity in the absence of added lipid. We have removed endogenous lipid material from microsome and cytosol preparations of rat lung by rapid extraction with isopropyl ether. The extraction procedure did not cause any loss of cytidylyltransferase activity in the cytosol. After the extraction the enzyme was almost completely dependent upon added lipid activator. Isopropyl ether extraction of microsome preparations produced a loss of 40% of the cytidylyltransferase activity, when measured in the presence of added phosphatidylglycerol. Lipid material extracted into isopropyl ether restored the cytidylyltransferase activity in cytosol. The predominant species of enzyme activator in the isopropyl ether extracts was fatty acid. A variety of naturally occurring unsaturated fatty acids stimulated the cytidylyltransferase to the same extent as phosphatidylglycerol. Saturated fatty acids were inactive.

ALBUMIN-LIPID INTERACTIONS: PROSTAGLANDIN STABILITY AS A PROBE FOR CHARACTERIZING BINDING SITES ON VERTEBRATE ALBUMINS. F.A. Fitzpatrick and M.A. Wynalda (From Drug Metabolism Research, The Upjohn Company, Unit 7256, Kalamazoo, Michigan 49008) *Biochemistry 20*:6129–6134 (1981). We determined the effect of vertebrate albumins on the stability of several physiologically relevant prostaglandins. All naturally occurring prostaglandins with a β-hydroxy ketone group decomposed by first-order kinetics, dependent on the albumin concentration in 0.1 M, pH 7.4, buffer at 37°C. Even subphysiological levels of albumin (1–20 mg/mL) significantly reduced the stability of these compounds in vitro. The prostaglandins with a β-hydroxy ketone responded to albumin in the order of their intrinsic stability; namely, less stable compounds were more susceptible. The destructive effect of albumin was nearly maximal at a 1:1 mole ratio of albumin (20 mg/mL):prostaglandin (100 μg/mL). Albumin had no destructive effect on prostaglandins without a β-hydroxy ketone. Albumins from different vertebrates varied in destructive severity, but all were effective. Near neutrality, in the absence of albumin, decomposition of E-type prostaglandins was practically suspended at the dehydration stage. In the presence of albumin, dehydration was accompanied by rapid isomerization reactions (e.g., $PGA_1 \rightarrow PGB_1$) that occur only at an elevated pH. The results suggest that albumin sequesters prostaglandins to one principal binding site and exposes them to its associated highly alkaline microenvironment. This results in a uniform and predictable influence on prostaglandin stability. Our proposed model system successfully reconciles apparently anomalous or contradictory reports regarding the effect of albumin on prostaglandin stability.

SERUM LIPIDS IN HUMANS FED DIETS CONTAINING BEEF OR FISH AND POULTRY. Margaret A. Flynn, Ph.D., Beth Heine, M.S., Georgia B. Nolph, M.D., H. Donald Naumann, Ph.D., Elaine Parisi, M.S., Deborah Ball, M.S., Gary Krause, Ph.D., Mark Ellersieck, Ph.D., and Susan S. Ward, M.S. (From the Departments of Family and Community Medicine, School of Medicine; Department of Human Nutrition, Foods and Food Systems Management, College of Home Economics; Department of Food Science and Nutrition, College of Agriculture; Agriculture Experiment Station; Clinical Research Center, School of Medicine, University of Missouri, Columbia, MO.) *The American Journal of Clinical Nutrition 34*: 2734–2741 (1981). One half of a group of 129 men and women (74 men and 55 women), in a crossover design ate, within a self-selected diet, one egg and at least 5 oz of beef daily for 3 months while the other half ate one egg and at least 5 oz of poultry and fish daily. Then they reversed their diets for 3 months. Blood samples were drawn by venipuncture before the study started and at the end of 3 and 6 months, for analyses of serum total cholesterol, triglycerides, and high density lipoprotein cholesterol. No statistically significant changes were found in serum lipids in men. In women serum triglycerides but not other serum lipids were significantly higher when poultry and fish had been ingested.

ROLE OF FATTY ACID SYNTHESIS IN THE CONTROL OF INSULIN-STIMULATED GLUCOSE UTILIZATION BY RAT ADIPOCYTES. Susan K. Fried, Marcelle Lavau, and F. Xavier Pi-Sunyer (Obesity Research Center and Institute of Human Nutrition, St. Luke's-Roosevelt Hospital Center, College of Physicians and Surgeons, Columbia University, New York, NY 10025 and Groupe

de Recherche sur la Physiopathologie de la Nutrition, INSERM U. 177, Institut Biomedical des Cordeliers, 15-21 Rue de l'Ecole de Medicine 75270, Paris Cedex 06) *J. Lipid Res. 22*:753-762 (1981). A decreased capacity for fatty acid synthesis is associated with a decreased insulin effect on glucose metabolism in large fat cells and fat cells from rats fed a high-fat diet. We have investigated the relationship between these processes by specifically inhibiting fatty acid synthesis with (−)-hydroxycitrate (2.5 mM), an inhibitor of citrate cleavage enzyme, and cerulenin (0.05 mM), an inhibitor of fatty acid synthetase. (−)-Hydroxycitrate and cerulenin decreased maximally insulin-stimulated fatty acid synthesis from (6-^{14}C] glucose to 10% and 25% of controls, respectively, while only (−)-hydroxycitrate decreased basal values. Oxidation of [1-^{14}C] glucose in the presence of insulin was markedly depressed by each inhibitor. Thus, the percent increase over basal value was decreased from 540% in controls to 151% and 154% by (−)-hydroxycitrate and cerulenin, respectively. In contrast, oxidation of [6-^{14}C] glucose was slightly enhanced by both inhibitors. Thus, oxidation of glucose via the pentose shunt was reduced, while Krebs cycle oxidation was unaffected. Basal and insulin-stimulated incorporation of [1-^{14}C] glucose and [6-^{14}C] glucose into glyceride-glycerol and basal lactate production was unchanged by the inhibition of fatty acid synthesis. Insulin-stimulated lactate production was halved by the inhibition of fatty acid synthesis. The enzymatic capacity of the fat cell for fatty acid synthesis is therefore an important determinant of insulin-stimulated glucose utilization.

ACYLATION OF 1-PALMITOYL-LYSOPHOSPHATIDYLGLYCEROL IN ALVEOLAR TYPE II CELLS FROM RAT LUNG. J.D. Funkhouser, J.J. Batenburg, and L.M.G. Van Golde (Laboratory of Veterinary Biochem., State Univ. of Utrecht, Biltstraat 172, 3572 BP Utrecht, The Netherlands) *Biochim. Biophys. Acta 666*:1-6 (1981). 1. Alveolar type II cells from adult rat lung have the enzymic capability to acylate 1-palmitoyl-lysophosphatidylglycerol, using palmitoyl-CoA to form dipalmitoylphosphatidylglycerol. 2. On a protein basis the acylation of 1-palmitoyl-lysophosphatidylglycerol is at least 2-fold more active in sonicated type II cells than in whole lung homogenates. 3. Both type II cells and whole lung homogenates show higher activity towards palmitoyl-CoA than oleoyl-CoA for acylation of 1-palmitoyl-lysophosphatidylglycerol. 4. Both in type II cells and in whole lung homogenates the rates of acylation of 1-palmitoyl-lysophosphatidylglycerol and 1-palmitoyl-lysophosphatidylcholine with palmitate are of the same order of magnitude, while the rate of acylation of 1-palmitoyl-lysophosphatidylethanolamine is much lower.

ALTERATION, BY EARLY UNDERFEEDING, OF CELLULAR MULTIPLICATION AND DIFFERENTIATION IN THE INGUINAL FAT PADS OF RATS. Anne-Marie Gaben-Cogneville, Thérèse Jahchan and Elisabeth Swierczewski (INSERM U 29, Hôpital Port-Royal, Paris Cedex 14, France) *J. Nutr. 111*:2098-2105 (1981). The effect of litter size on the incorporation of labeled thymidine (TdR) into DNA was studied in the stromal and the adipocyte fractions of the rat inguinal tissue. In experiment 1 the animals were kept in litters of 18 (UF) or 6 (control) from birth till 10 days. They were injected with [2-^{14}C] TdR at day 3 and killed at 60 minutes, 1, 3 and 7 days post-injection. In experiment 2, the pups were raised in litters of 18 during 3 (RF3), 6 (RF6) or 10 (RF10) days, and distributed again in litters of six. They were injected with [2-^{14}C] TdR or [$^{14}CH_3$]TdR at the beginning of the refeeding and killed as described previously. In all experiments the weight of the inguinal tissue was more reduced than the total body weight. In the UF, the proliferation was markedly reduced in cellular fractions as was the differentiation of stromal cells into adipocytes from six days of underfeeding. In the RF3 and the RF6 there was an attempt to recover the cell number as suggested by the recycling of the degradation products of TdR for DNA synthesis. In the RF10, cell multiplication and differentiation were strongly affected by the length of the deprivation period.

DIFFERENTIAL FATNESS GAIN OF LOW INCOME BOYS AND GIRLS. S.M. Garn, P.J. Hopkins, and A.S. Ryan (Center for Human Growth and Development, University of Michigan, Ann Arbor, MI 48109) *Am. J. Clin. Nutr. 34*(8):1465-1468 (1981). As shown in 564 girls and 553 boys followed for a period of 18 yr, long-term gain in both subscapular and triceps skin-fold thickness was higher in children of lower family income level than those of higher family incomes. This differential fatness gain accounts for the socioeconomic "reversal" of fatness in the female shown in cross-sectional studies and newly extends the phenomenon to both sexes. The finding that low-income children show a greater long-term increase in fatness bears on the prevention and control of obesity.

TYPE III HYPERLIPOPROTEINEMIA ASSOCIATED WITH APOLIPOPROTEIN E DEFICIENCY. Giancarlo Ghiselli, Ernst J. Schaefer, Pedro Gascon, H. Bryan Brewer, Jr. (Molecular Disease Branch, National Heart, Lung, and Blood Institute, Bethesda, Maryland 20205) *Science 214*:1239-1241 (1981). Subjects with type III hyperlipoproteinemia develop premature atherosclerosis and have hyperlipidemia due to an increase in cholesterol-rich very low density lipoproteins (VLDL) of abnormal electrophoretic mobility. Apolipoprotein E is a major protein constituent of VLDL and appears to be important for the hepatic uptake of triglyceride-rich lipoproteins. A new kindred of patients with type III hyperlipoproteinemia is described in which no plasma apolipoprotein E could be detected, consistent with the concept that type III hyperlipoproteinemia may be due to an absence or striking deficiency of apolipoprotein E.

ISOLATION AND CHARACTERIZATION OF LAMELLAR BODY MATERIAL FROM RAT LUNG HOMOGENATES BY CONTINUOUS LINEAR SUCROSE GRADIENTS. Helena Gilder, Rudy H. Haschemeyer, Gordon F. Fairclough, Jr., and Dennis C. Mynarcik (Departments of Surgery and Biochemistry, Cornell University Medical College, 1300 York Avenue, New York, NY 10021) *J. Lipid Res. 22*:1277-1285 (1981). A technique is described for isolating lamellar body material from rat lung. Membranes with relative densities ranging between 1.050 and 1.074 g/ml were isolated by centrifugation of crude lung homogenates upward through continuous linear sucrose gradients at 40,000 rpm (199,000 *g*) for 3 hr. Their protein and lipid content was characteristic of that of lamellar bodies. They were free of contaminating microsomal and mitochondrial marker enzymes but contained enzyme activities associated with lysosomes and Golgi complex. Longer or repeated centrifugation resulted in a reduced yield and an apparent transformation of some of the material to lower densities. Electron microscopy revealed that most of the images represent disrupted rather than intact lamellar bodies. Other methods for preparation of lamellar bodies entail either sedimentation or pelleting at interfaces between sucrose solutions. Such preparations are often contaminated with endoplasmic reticulum membranes and have apparently lost the more fragile bodies. The present technique reveals the heterogeneous nature of lamellar body material and should be useful in a search for lamellar body precursors and in the investigation of the mechanisms by which surfactant is synthesized or assembled.

INTESTINAL FATTY ACID ESTERIFICATION ACTIVITY IN JEJUNOILEAL BYPASS PATIENTS. Paul B. Goldberg, M.D., Yih-Fu Shiau, M.D., Gary M. Levine, M.D., and Ernest F. Rosato, M.D. (From the Gastroenterology Section of the Department of Medicine and Department of Surgery, Philadelphia Veterans Administration Medical Center and the University of Pennsylvania School of Medicine, Philadelphia, PA 19104) *The American Journal of Clinical Nutrition 34*:2742-2747 (1981). In four patients undergoing reversal of jejunoileal bypass we compared functioning (in continuity) with bypassed intestine in order to determine the effects of luminal contents. Total mucosal thickness, villus height, and crypt depth, as well as in vitro fatty acid esterification activity were determined. Morphological studies in segments exposed to luminal contents revealed that the ileum had a greater mucosal thickness than the jejunum ($p < 0.001$) and that the difference was reflected in both fillus height and crypt depth ($p < 0.001$). The functioning segments of both jejunum and ileum had greater mucosal thickness than corresponding bypassed segments consequent to a difference in villus height ($p < 0.001$) but not crypt depth. Despite similar exposure to luminal contents, total fatty acid esterification was significantly higher ($p < 0.001$) in the functioning jejunum than in the ileum. Jejunum incontinuity possessed higher esterification activity than bypassed jejunum. These results indicate that *1)* luminal contents are the most important modulator of intestinal fatty acid esterification activity and the absence of luminal contents in bypassed intestine leads to a significant reduction in esterification activity, *2)* the jejunum and ileum possess intrinsic differences in esterification activity even when both are exposed to an identical luminal environment.

EVIDENCE FOR THE EXISTENCE OF ONLY ONE TRIACYLGLYCEROL LIPASE OF RAT LIVER ACTIVE AT ALKALINE pH. Johanna E.M. Groener and Thomas E. Knauer (Department of Medicine and Department of Biophysics, Medical College of Virginia, Richmond, VA 23298) *Biochimica et Biophysica Acta 665*:306-316 (1981). There have been numerous reports suggesting the existence of two or more lipases in liver capable of hydrolyzing triacylglycerols at neutral to alkaline pH. We set out to determine if rat liver contains an alkaline triacylglycerol lipase, in addition to heparin-releasable lipase, which has an intracellular localization. We report here the results of studies concerning the pH dependence, subcellular localization and kinetic analysis of the alkaline lipase(s) of rat liver. Homogenates and cytosolic, microsomal and plasma membrane-enriched subfractions all exhibited an optimum of lipase activity at approx. pH 8.0. In no case was there evidence of multi-

ple pH optima in the alkaline ranges of conformity to Michaelis-Menten kinetics were calculated for the microsomal (0.91 ± 0.12 mM), cytosolic (1.55 ± 0.38 mM) and plasma membrane-enriched (1.02 ± 0.04 mM) subfractions. To determine if the com- and subfractions prepared from control livers with those prepared from livers perfused with collagenase. The loss (93%) of lipase activity from both the cytosolic and microsomal subfractions after collagenase perfusion was identical to the loss (93%) of activity from the homogenates, suggesting a common origin with the collagenase-sensitive alkaline lipase on plasma membrane. The characteristics of hydrolysis in vitro of triacylglycerol contained in artificial and natural substrate preparations by the alkaline lipase of rat liver were examined. The artificial substrate preparation was emulsified tri[1-^{14}C] oleoylglycerol prepared by sonication and the natural substrate preparation was a triacylglycerol-rich lipid fraction ('liver fat') prepared from rat liver homogenates. Although the curves were complex, apparent K_m values (mean ± S.E., n = 3 – 6) over the limited concentration ranges of conformity to Michaelis-Menten kinetics were calculated for the microsomal (0.91 ± 0.12 mM), cytosolic (1.55 ± 0.38 mM) and plasma membrane-enriched (1.02 ± 0.04 mM) subfractions.

EARLY CHANGES IN PHOSPHATIDYLINOSITOL AND ARACHIDONIC ACID METABOLISM IN QUIESCENT SWISS 3T3 CELLS STIMULATED TO DIVIDE BY PLATELET-DERIVED GROWTH FACTOR. Andreas J.R. Habenicht, John A. Glomset, Weiling C. King, Cynthia Nist, Carolyn D. Mitchell, and Russell Ross (From the Howard Hughes Medical Institute Laboratory, Departments of Medicine, Biochemistry and Pathology, and the Regional Primate Research Center, University of Washington, Seattle, Washington 98195) *The Journal of Biological Chemistry 256*(23):12329–12335 (1981). We added platelet-derived growth factor to cultures of quiescent Swiss 3T3 cells to investigate early changes in lipid metabolism related to initiation of cell cycle traverse. In a series of experiments that focused on lipid degradation we added the growth factor to cells that had been prelabeled with myoinositol, glycerol, or arachidonic acid. We observed the following mitogen-dependent effects: a decline of radioactivity in cell phosphatidylinositol within 2 to 5 min that progressed to 25 to 50% during the 1st h, a transient rise of radioactivity in cell diacylglycerol that peaked at 10 min, a gradual increase of radioactivity in monoacylglycerol in the medium, and a concomitant increase of radioactivity in medium-free fatty acid. In experiments that focused on lipid biosynthesis, we added the growth factor to cells and pulse-labeled them with radioactive precursors. We observed increased incorporation within 60 min of myoinositol into phosphatidylinositol, arachidonic acid into phosphatidylinositol, diacylglycerol, and phosphatidylethanolamine, and choline into phosphatidylcholine. These results support the possibility that action of platelet-derived growth factor on Swiss 3T3 cells leads to release of diacylglycerol from phosphatidylinositol, that some of the released diacylglycerol is hydrolyzed to monoacylglycerol and arachidonic acid, and that these lipid products are in part reconverted to phosphatidylinositol and other lipids.

CHOLESTERYL ESTER SYNTHESIS IN CANINE VEIN AND ARTERY. P.O. Hagen (Atherosclerosis Research Laboratory, Dept. of Surgery and Biochemistry, Duke University Medical Center, Durham, NC 27710) *Artery 9*(4):275–284 (1981). Accumulation of cholesteryl esters in arterial tissue is a prominent feature of human and experimental atherosclerosis. This accumulation does not occur in undisturbed venous tissue, but has been reported in veins which have been surgically placed into the arterial system as bypass grafts. The formation of cholesteryl esters and some properties of the fatty acyl-CoA:cholesterol acyltransferase system have been studied in microsomal preparations from canine arterial and venous tissue. The rate of synthesis of cholesteryl palmitate was five-fold faster in venous than in arterial preparations. There was no difference, however, in the apparent K_m values. Our results indicate that venous tissue possesses active fatty acyl-CoA:cholesterol acyltransferase activity which may be partly responsible for the accumulation of cholesteryl esters in veins grafted into the arterial system.

THE RELATIONSHIP OF PLASMA ESTRADIOL AND PROGESTERONE LEVELS TO THE FATTY LIVER HEMORRHAGIC SYNDROME IN LAYING HENS. F. Haghighi-Rad and D. Polin (Poultry Science Department, Michigan State University, East Lansing, Michigan 48824) *Poultry Sci. 60*(10):2278–2283 (1981). The relationship of plasma estradiol and progesterone levels to fatty liver-hemorrhagic syndrome (FLHS) was studied with three groups of 9 White Leghorn hens per group. One group was fed *ad libitum*; the other two groups were force-fed at 120% and 135% of their own pre-experimental daily feed intake. Force-feeding for 3 weeks produced FLHS. The average FLHS score was 1.6 for control, 3.7 for 120% force-fed, and 4.5 for 135% force-fed. The average liver fat contents were 31.3%, 75.1%, and 76.8% (dry matter basis), respectively. Plasma estradiol averaged 165 pg/ml in the control group and 194 and 247 pg/ml in groups force-fed at 120% and 135%, respectively. The correlation coefficient between plasma estradiol and FLHS was .72 ($P < .01$). No significant differences in plasma progesterone were obtained among the control and force-fed groups. The data indicate that high endogenous estrogen levels are associated with FLHS.

INFLUENCE OF DIABETES ON THE MYOCARDIUM AND CORONARY ARTERIES OF RHESUS MONKEY FED AN ATHEROGENIC DIET. Bunyad Haider, Chien K. Yeh, George Thomas, Henry A. Oldewurtel, Michael M. Lyons, and Timothy J. Regan *Circ Res 49*:1278–1288 (1981). To examine the influence of diabetes on the progression of coronary atherosclerosis and primary myocardial alterations in the rhesus monkey, a Purina or atherogenic diet was fed to nondiabetic animals of groups 1 and 2, respectively, and also to groups 3 and 4 with alloxan diabetes. After 18 months, cardiac studies were performed, by indicator dilution in the intact anesthetized state, at similar levels of heart rate and aortic pressure. Despite comparable basal hemodynamics, preload increments with saline evoked a stroke work response that was significantly less in both diabetic groups. Left ventricular end-diastolic pressure rose from 10.1 ± 1.4 mm Hg to 20.5 ± 2.7 in group 3, and from 11.1 ± 2.1 to 24.0 ± 3.3 in group 4, which were significantly higher elevations than occurred in the controls. End-diastolic volume rose much less in diabetics. Indices of contractility as well as left heart weight were normal. Hydroxyproline concentrations were 4.98 ± 0.33 g/mg dry weight in group 1, 5.16 ± 0.24 in group 2, 8.4 ± 0.35 in group 3, and 7.1 ± 0.37 in group 4. Soluble collagen was significantly diminished and the insoluble fraction enhanced in diabetics and was the apparent basis for enhanced wall stiffness. The collagen increment was most evident between myofibers. Cardiac cell organelles by electron microscopy, tissue cation concentrations, as well as the myocardial lactate response to pacing, were within normal limits. Cholesterol content of the coronary arteries, as a measure of the early atherosclerotic process observed as lipid streaks, was similarly increased in the nondiabetic and diabetic monkeys on lipid diets, with respective plasma cholesterols of 367 ± 55 and 409 ± 62 mg/100 ml. The diabetic-Purina group had a lower but significantly elevated coronary artery cholesterol, associated with higher plasma glucose and nonsterol lipid levels. Thus, in this primate model, diabetes did not intensify either the early coronary lesions induced by moderate hypercholesterolemia, nor were the myocardial changes associated with diabetes altered by the presence of moderate hypercholesterolemia.

APPEARANCE OF THE INTESTINAL CYTOSOLIC RECEPTOR FOR 1,25-DIHYDROXYVITAMIN D_3 DURING NEONATAL DEVELOPMENT IN THE RAT. B.P. Halloran and H.F. Deluca (Dept. of Biochemistry, College of Agriculture and Life Sciences, University of Wisconsin-Madison, Madison WI 53706) *J. Biol. Chem. 256*(14):7338–7342 (1981). During the early suckling period in the rat, active calcium uptake by the intestine is refractory to stimulation by 1,25-dihydroxyvitamin D_3. To determine the role that the specific cytosolic receptor for 1,25-dihydroxyvitamin D_3 plays in this refractory state, the concentration of the receptor and its binding characteristics were measured during the neonatal period. From Scatchard analysis, the concentration of receptor in the adult intestine was 563 ± 64 fmol/mg of protein. At 28 and 21 days postpartum, receptor concentrations were 711 ± 129 and 251 ± 36 fmol/mg of protein, respectively. Estimates from sucrose density gradient profiles and Scatchard analyses indicated that, at 14 and 7 days postpartum, receptor concentrations were less than 50 fmol/mg of protein. Equilibrium dissociation constants were similar at all times measured and ranged from 0.38 to 0.52 nM. These results suggest that the lack of response observed in the early neonatal intestine to 1,25-dihydroxyvitamin D_3 may stem, at least in part, from the relative absence of receptors for 1,25-dihydroxyvitamin D_3. Furthermore, the appearance of the receptor may be the determining factor in the initiation of active calcium absorption in the intestine and its regulation.

SYNTHESIS AND RELEASE OF LIPIDS AND LIPOPROTEINS BY ISOLATED RAT JEJUNAL ENTEROCYTES IN THE PRESENCE OF SODIUM TAUROCHOLATE. Alan G.D. Hoffman, Peter Child and Arnis Kuksis (Banting and Best Department of Medical Research, 112 College Street, University of Toronto, Toronto M5G 1L6, Canada) *Biochimica et Biophysica Acta 665*:283–298 (1981). Isolated rat jejunal villus and crypt cells prepared by differential scraping and hyaluronidase dispersion were used in the presence of 8 mM sodium taurocholate to study the incorporation of *sn*-[^{3}H] glycerol-2-monoleate, [1-^{14}C] palmitate, [1-^{14}C] acetate, L-[4,5(n)-^{3}H] leucine and D-[1-^{14}C] glucosamine into cellular and medium lipids and proteins, respectively. The villus cells were capable of an apparently normal biosynthesis of triacylglycerols and phospholipids, as well as of proteins and glycoproteins despite an altered dye permeability and increased release of cytosolic and membrane

enzymes. About 20–30% of the newly formed triacylglycerols and about 35% of the newly formed phospholipids were secreted into the medium and were recovered as triacylglycerol-rich particles. Labelled proteins and glycoproteins were also recovered from this fraction. The crypt cells synthesized about one-half as much triacylglycerol and phospholipid as did the villus cells, but secreted little or no labelled lipid into the postincubation medium. The release into the medium of triacylglycerols synthesized by the villus cells was blocked by a pretreatment of the isolated cells with the microtubule disruptors, nocodazole, colchicine and colcemid; by the amino sugar, D-galactosamine; by the inhibitors of protein synthesis, puromycin and cycloheximide, and by the inhibitor of the biosynthesis of phosphatidylcholine, chlorocholine. These results indicate that the secretion of labelled lipids, proteins and glycoproteins by the upper villus enterocytes in the presence of sodium taurocholate is not entirely due to cell breakage and spillage of contents. It is concluded that incubations of isolated villus cells of rat jejunum with mixed micellar solutions containing 8 mM taurocolate are compatible with an apparently normal biosynthesis and secretion of triacylglycerol-rich particles.

EFFECT OF CIGARETTE SMOKE AND DIETARY CHOLESTEROL ON PLASMA LIPOPROTEIN COMPOSITION. J.L. Hojnacki, J. J. Mulligan, J.E. Cluette, R.R. Kew, D.J. Stack and G.L. Huber (Dept. of Biological Sciences, University of Lowell, Lowell, MA 01854) *Artery 9*(4):285–304 (1981). The effect of acute inhalation of cigarette smoke and consumption of dietary cholesterol on plasma lipoprotein composition in atherosclerosis-susceptible White Carneau pigeons was examined. Pigeons were assigned to four treatment groups: 1) Controls fed a chow diet ad libitum and retained in their cages throughout the study; 2) Sham pigeons fed a cholesterol-saturated fat diet and exposed to fresh air by the Lorillard smoking machine; 3) Low nicotine-low carbon monoxide (Lo-Lo) animals also fed the cholesterol diet and exposed to low concentrations of these cigarette smoke products; and 4) *High nicotine-high* carbon monoxide (HiHi) birds fed the cholesterol diet and subjected to high concentrations of these inhalants. Smoke-related differences appeared in HiHi high density lipoproteins (HDL) which contained relatively more free and esterified cholesterol and total lipid, but relatively and absolutely less total protein than HDL from Sham-smoked pigeons. Diet also altered the type of cholesteryl ester present in HDL with cholesteryl linoleate representing the predominant form in Control pigeons and cholesteryl oleate in cholesterol-fed birds. These results demonstrate that cigarette smoking can mediate alterations in lipoprotein composition independent of changes induced by dietary cholesterol and saturated fat.

ESSENTIAL FATTY ACID DEFICIENCY IN MALNOURISHED CHILDREN. R.T. Holman, S.B. Johnson, O.M. Mercuri, H.J. Itarte, M.A. Rodrigo, and M.W. De Tomas (Hormel Institute, University of Minnesota, Austin MN 55912) *Am. J. Clin. Nutr. 34*(11):1534–1539 (1981). Fatty acid patterns of major classes of lipids of serum were measured in forty Argentine children ages 2 to 24 months admitted to the hospital with chronic malnutrition. A normal control group of 48 children from the same population was also examined. Serum lipids were extracted and separated into phospholipids, cholesteryl esters, triglycerides, and free fatty acids. These were converted to methyl esters which were analyzed by gas chromatography. In chronic malnutrition, the fatty acid patterns of phospholipids and cholesteryl esters indicated changes characteristic of essential fatty acid deficiency of moderate degree. The total $\omega 6$ acids were found to be highly significantly diminished from normal, and the ratio $20{:}3\omega 9/20{:}4\omega 6$ was highly significantly increased. Decreased proportions of $\omega 6$ metabolites suggested impaired desaturase activity, and elevated ratios of $22{:}4\omega 6/20{:}4\omega 6$ and $20{:}2\omega 6/18{:}2\omega 6$ suggested increased chain elongation in chronic malnutrition.

METABOLISM OF C-APOLIPOPROTEINS: KINETICS OF C-II, C-III$_1$ AND C-III$_2$, AND VLDL-APOLIPOPROTEIN B IN NORMAL AND HYPERLIPOPROTEINEMIC SUBJECTS. Murray W. Huff, Noel H. Fidge, Paul J. Nestel, Timothy Billington, and Bruce Watson (Baker Medical Research Institute, Melbourne, Victoria 3181, Australia) *J. Lipid Res. 22*:1235–1246 (1981). The turnover and metabolism of the individual C apolipoproteins (C-II, C-III$_1$, and C-III$_2$) were studied following the injection of ^{125}I-labeled VLDL into 15 normal and hyperlipoproteinemic subjects. The C apolipoproteins from very low density lipoprotein (VLDL) and high density lipoprotein (HDL) were separated by analytical isoelectric focusing, and subsequent densitometric scanning and radioassay of the stained bands yielded values for specific activity. In 13 of 15 subjects, kinetics of C-II, C-III$_1$, and C-III$_2$ were best described by a one-pool model, whereas two subjects showed biexponential kinetics. The specific activity-time curves for VLDL and HDL were superimposable, indicating rapid exchange of all C apolipoproteins in hyperlipidemic as well as in normal subjects. In each subject the half-life was similar for C-II, C-III$_1$, and C-III$_2$, which suggests similar synthesis and catabolic mechanisms for each C apolipoprotein. The mass of exchangeable C-II (range 1.0–5.8 mg/kg), C-III$_1$ (2.6–20 mg/kg), and C-III$_2$ (2.0–13 mg/kg) increased with plasma triglyceride concentrations.

ALTERATIONS OF PLASMA LIPID AND LIPOPROTEIN LEVELS ASSOCIATED WITH BENSODIAZEPINE USE. The LRC Program Prevalence Study. Medication and Hormones Working Group: D. Hunninghake, R.B. Wallace, S. Reiland, E. Barrett-Connor, P. Wahl, J. Hoover, and G. Heiss (Depts. of Medicine and Pharmacology, Univ. of Minnesota Health Science Center, Minneapolis, MN 55455) *Atherosclerosis 40*:159–165 (1981). In a study of white adults from 10 North American Lipid Research Clinics populations, plasma lipid and lipoprotein levels in benzodiazepine (diazepam, chlordiazepoxide, flurazepam) users were compared to both the entire population of non-users and matched control non-users. Significantly higher plasma triglyceride and very low density lipoprotein cholesterol levels and by on method of analysis, lower high density lipoprotein cholesterol levels were noted in male benzodiazepine users. No significant differences were noted in total plasma cholesterol or low density lipoprotein cholesterol levels.

IN VIVO EFFECT OF CHOLESTEROL FEEDING ON THE SHORT TERM REGULATION OF HEPATIC HYDROXYMETHYLGLUTARYL COENZYME A REDUCTASE DURING THE DIURNAL CYCLE. Hans-Stephan Jenke, Marianne Lõwel, and Jürgen Berndt (From the Gesellschaft für Strahlen- und Umweltforschung, München, Institut für Toxikologie und Biochemie, Abteilung Zellchemie, 8042 Neuherberg, West Germany) *The Journal of Biological Chemistry 256*:9622–9625 (1981). Light-dark-cycled rats were fed a 3% cholesterol-supplemented diet at the beginning of the dark phase. Cholesterol-fed and control animals were taken at intervals throughout the following 12 h and the microsomal and solubilized hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase was isolated. Immunotitrations of this microsomal and solubilized enzyme were performed with a monospecific antibody to 3-hydroxy-3-methylglutaryl coenzyme A reductase. In contrast to the specific activity of the enzyme, which differs extremely during the diurnal cycle, the immunotitrations obtained from cholesterol-fed and control animals, yielded in identical antisera equivalence points. On the other hand, when the enzyme was phosphorylated *in vitro*, the antisera equivalence points corresponded to the alterations of the specific activity. In contrast to the results published by Higgins and Rudney (1973 *Nature New Biol.* 246, 60–61), our data prove that even the *in vivo* short term changes in enzyme activity are due to changes in the quantity of enzyme rather than to a modulation of the catalytic activity.

Fats and oils

CERAMIDE STRUCTURE OF SPHINGOMYELIN FROM HUMAN MILK FAT GLOBULE MEMBRANE. J-F. Bouhours and D. Bouhours (Laboratoire de Biochimie des Membranes, LBTM-CNRS, Universite Claude Bernard, 43 Boulevard du 11 Novembre 1918, 69622 Villeurbanne cedex, France) *Lipids 16* (10):726-731 (1981). Sphingomyelin was purified from human milk fat globule membrane and submitted to phospholipase C to yield ceramide. The structure of this ceramide was investigated by gas liquid chromatographic analyses of its components, fatty acids, and sphingoid bases. The structure of the native ceramide was confirmed by direct-inlet mass spectrometry. It was shown to contain a major base C_{18}-sphingosine associated with a high proportion (60%) of C_{20}, C_{22}, C_{24}, and $C_{24:1}$ nonhydroxylated fatty acids. As these very long-chain fatty acids might be of nutritive importance, the concentration of sphingomyelin in human milk and its distribution in cream and skim milk were established.

X-LINKED ICHTHYOSIS: INCREASED BLOOD CHOLESTEROL SULFATE AND ELECTROPHORETIC MOBILITY OF LOW-DENSITY LIPOPROTEIN. E.H. Epstein, Jr., R.M. Krauss, and C.H.L. Shackleton (Department of Dermatology, San Francisco General Hospital, Medical Center, San Francisco, CA 94110) *Science 214*(6):659-660 (1981). Plasma cholesterol sulfate concentration is increased in paitents with recessive X-linked ichthyosis, a disease in which steroid sulfatase activity is absent. In these patients, cholesterol sulfate is found primarily in the low-density lipoprotein fraction of plasma, and the electrophoretic mobility of these lipoproteins is greatly increased.

A NONDESTRUCTIVE SPRAY REAGENT FOR THE DETECTION OF PROSTAGLANDINS AND OTHER LIPIDS ON THIN LAYER CHROMATOGRAMS. S.K. Goswami and J.E. Kinsella

(Institute of Food Science, Cornell University, Ithaca, NY 14853) *Lipids 16*(10):759-760 (1981). The spray reagent 8-hydroxy-1,3,6-pyrenetrisulfonic acid trisodium salt (10 mg/100 ml methanol) is extremely sensitive for locating prostaglandins on thin layer chromatograms. This reagent does not alter the PG, nor interfere with liquid scintillation counting.

HIGH PRESSURE LIQUID CHROMATOGRAPHIC SEPARATION OF MOLECULAR SPECIES OF PHOSPHATIDIC ACID DIMETHYL ESTERS DERIVED FROM PHOSPHATIDYLCHOLINE. J.Y-K. Hsieh, D.K. Welch, and J.G. Trucotte (Dept. of Medicinal Chemistry, College of Pharmacy, Univ. of Rhode Island, Kingston, RI 02881) *Lipids 16* (10):761-763 (1981). A majority of the individual molecular species of phosphatidic acid dimethyl esters derived from multispecies egg yolk and soybean phosphatidylcholines have been separated by reverse-phase high pressure liquid chromatography. Two Partisil-10 ODS columns connected in tandem and the eluents acetonitrile or methanol/water (95:5) were used for molecular species resolution, based on total fatty acyl carbon number and degree of unsaturation.

THERMODYNAMICS OF DIHEXANOYLPHOSPHATIDYLCHOLINE AGGREGATION. R.E. Johnson, M.A. Wells, and J.A. Rupley (University Department of Biochemistry, University of Arizona, Tucson, Arizona 85721) *Biochemistry 20*(14):4239-4242 (1981). Heats of dilution of aqueous solutions of dihexanoylphosphatidylcholine were determined by use of a flow microcalorimeter to monitor an exponential dilution gradient. Three different models of micelle formation were tested: monomer in equilibrium with micelles of varied size, or with small aggegates and micelles. The heat of dilution data for low solute concentration could be fit only by assuming the existence of premicellar aggregates. The critical micelle concentration determined calorimetrically is 0.016 ± 0.002 M and is independent of the model. The enthalpy change for transfer of monomer into the micelle is 1.6 ± 0.2 kcal/mol; about one-third of this heat effect is produced in formation of the premicellar aggregation. Comparison of the calorimetric measurements with results obtained by using other methods indicates the complexity of the micellization process.

MINOR AND TRACE STEROLS IN MARINE INVERTEBRATES. 27.[1] ISOLATION, STRUCTURE ELUCIDATION, AND PARTIAL SYNTHESIS OF 25-METHYLXESTOSTEROL, A NEW STEROL ARISING FROM QUADRUPLE BIOMETHYLATION IN THE SIDE CHAIN. L. Niang Li, U. Sjöstrand, and C. Djerassi (Department of Chemistry, Standford University, Stanford, California 94305) *J. Org. Chem. 46*(10):3867-3870 (1981). A novel C_{31} sterol, 25-methylxestosterol, resulting from quadruple biomethylation in the side chain has been isolated as a trace constituent of the sterol fraction from a Carribbean sponge (*Xestospongia* sp.). Its structure (1,24-methylene-25,26,27-trimethyl-cholesterol) has been elucidated by spectroscopic methods and confirmed by partial synthesis. A biosynthetic route leading to 1 is proposed that is consistent with the hypothesis of stepwise biomethylations and with earlier discoveries of "extended" side chains among marine sterols.

AQUEOUS LIPID PHASES OF RELEVANCE TO INTESTINAL FAT DIGESTION AND ABSORPTION. M. Linström, H. Ljusberg-Wahren, K. Larsson, and B. Borgström (Department of Physiological Chemistry, University of Lund, P.O. Box 750, S-220 07 Lund 7, Sweden) #ιριλ˜ ο•(10):749-754 (1981). The phase behavior of monoglyceride/water systems, with oleic and linoleic acid as the dominating fatty acid residues, was investigated. Increased solubilization of triglycerides (oil) or oleic acid in the cubic liquid-crystalline phase formed by monoglyceride and water resulted in the formation of a reversed hexagonal liquid-crystalline phase followed by an L2-phase. The liquid-crystalline phases have different dispersion properties compared to each other in dilute micellar bile salt solutions. The cubic phase is found to be easily dispersed. The relevance of aqueous lipid phases other than micellar is discussed in relation to intestinal lipid digestion and absorption.

IMMUNOSPECIFIC TARGETING OF LIPOSOMES TO CELLS: A NOVEL AND EFFICIENT METHOD FOR COVALENT ATTACHMENT OF FAB' FRAGMENTS VIA DISULFIDE BONDS. F.J. Martin, W.L. Hubbell, and D. Papahadjopoulos (Cancer Research Institute and Dept. of Pharmacology, Univ. of CA, San Francisco, CA 94143) *Biochemistry 20*(14):4229-4238 (1981). An efficient method for covalently cross-linking 50K Fab' antibody fragments to the surface of lipid vesicles is reported. Coupling up to 600 μg of Fab'/=mol of phospholipid (about 6000 Fab' molecules per 0.2-μm vesicle) is achieved via a disulfide interchange reaction between the thiol group exposed on each Fab' fragment and a pyridyldithio derivative of phosphatidylethanolamine present in low concentration in the membranes of preformed large unilamellar vesicles. The coupling reaction is efficient, proceeds rapidly

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