Abstracts

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Biochemistry and nutrition

GLYCEROLIPID BIOSYNTHESIS IN RAT ADIPOSE TISSUE: EFFECT OF AGE, SITE OF ADIPOSE TISSUE AND CELL SIZE. Subhash C. Jamdar and Linda J. Osborne (Medical Research Institute, Florida Institute of Technology, 3325 W. New Haven Avenue, Melbourne, FL 32901) *Biochimica et Biophysica Acta 665*:145–153 (1981). Adipose glycerolipid formation was studied in relation to age, site of adipose tissue and cell size in developing rats. Glycerolipid formation was measured in vitro in the presence of [^{14}C] glycerol 3-phosphate, palmitate, ATP, CoA and Mg^{2+} by using adipocyte homogenates derived from various age groups of animals. In the epididymal fat pads, glycerolipid formation was very low in 15-day-old rats, increased 5–6 fold in 30-day-old rats and reached maximal at 60 days of age. This rise in glycerolipid formation with age was related to increase in the adipocyte size. At later ages, in spite of the increase in adipocyte size, glycerolipid formation declined. The decline in glycerolipid formation with age was also apparent in the perirenal and subcutaneous adipocytes. Adipocyte homogenates derived from various age groups of animals and from various fat depots formed phosphatidate, diacylglycerol and triacylglycerol as the major reaction products from [^{14}C]glycerol 3-phosphate. The proportions of the various lipids formed changed significantly as a function of age of the animal. In addition, changes in the adipocyte size with age were determined. Adipose tissues from 15-day-old rats contained smaller adipocytes with a mean cell diameter around 30 μm. As the rats grew older, the presence of more larger adipocytes was detected in their fat depots. These studies suggest that adipose glycerolipid synthesis changes with age and anatomical location of the adipose organ independently of the adipocyte size.

CHARACTERIZATION OF PHOSPHOLIPIDS AND LOCALIZATION OF SOME PHOSPHOLIPID SYNTHETIC AND SUBCELLULAR MARKER ENZYMES IN SUBCELLULAR FRACTIONS FROM RABBIT LUNG. Alan Jobe, Machiko Ikegami, Isabelle Sarton-Miller, Sally Jones and Grace Yu (Fetal-Maternal Research Laboratories, Department of Pediatrics, Harbor-UCLA Medical Center, Torrance, CA 90509) *Biochimica et Biophysica Acta 666*: 47–57 (1981). Following an alveolar wash, lung tissue from 3-day-old rabbits was gently homogenized and fractionated into organelle, cytosol, microsome and low density microsome fractions. The organelle fraction was further separated on isopycnic continuous sucrose gradients. All fractions were characterized by phosphatidylcholine and protein content, saturated-to-total phosphatidylcholine ratios, and phospholipid compositions. Rabbits were injected with radioactively labeled palmatic acid 10 min to 8 h before killing, and the specific activities (cpm/μmol) of phosphatidylcholine recovered from fractions from continuous gradients were measured. Each fraction was assayed for the presence of four subcellular marker enzymes: NADPH: cytochrome *c* reductase, succinate: cytochrome *c* reductase, 5′-nucleotidase and UDPgalactose galactosyltransferase, and four phospholipid biosynthetic enzymes: cholinephosphotransferase, glycerolphosphate phosphatidyltransferase, phosphatidic acid phosphatase and lysophosphatidylcholine acyltransferase. Results were as follows: 1. Lung microsomes can be fractionated into low (under 1 M sucrose) and high (over 1 M sucrose) density fractions. The low density fractions have more sphingomyelin, more 5′-nucleotidase and UDPgalactose galactosyltransferase activity and probably represent in part plasma membrane and Golgi fragments. 2. The organelle fraction contains a spectrum of particulate matter with phospholipids characteristic of endoplasmic reticulum fragments at high density (1.5 M sucrose) and lamellar bodies at low density (0.5 M sucrose). 3. NADPH: cytochrome *c* reductase was present in relatively low density fractions from the continuous gradient but not at densities characteristic of lamellar bodies, while residual 5′-nucleotidase activity remained in lamellar body fractions. Cholinephosphotransferase and glycerolphosphate phosphatidyltransferase were confined to high density fractions from the continuous gradients while phosphatidic acid phosphatase and lysophosphatidylcholine acyl transferase activities were detected across the gradients. The various microsomal enzymes had disparate specific activity profiles across the continuous gradients. 4. After injection of radioactively labeled palmitic acid, radioactively labeled phosphatidylcholine sequentially enters less dense fractions with time. The distribution of the radioactively label supports the hypothesis that surfactant phospholipids move sequentially through a series of high density subcellular particles toward the low density lamellar bodies.

STUDIES OF DIACYLGLYCEROL CHOLINEPHOSPHOTRANSFERASE AND DIACYLGLYCEROL ETHANOLAMINEPHOSPHOTRANSFERASE ACTIVITIES IN *TETRAHYMENA* MICROSOMES. Y. Kameyama, S. Yoshioka, I. Hasegawa, and Y. Nozawa (Dept. of Biochem. Gifu Univ., School of Medicine, Tsukasamachi-40, Gifu, Japan) *Biochim. Biophys. Acta 665*(2):195–204 (1981). Microsomes isolated from *Tetrahymena pyriformis* synthesized phosphatidylcholine and phosphatidylethanolamine by CDPcholine: 1,2-diacylglycerol cholinephosphotransferase (EC 2.7.8.2) and CDPethanolamine: 1,2-diacylglycerol ethanolaminephosphotransferase (EC 2.7.8.1), utilizing ethanol-dispersed dioleoylglycerol. Cholinephosphotransferase and ethanolaminephosphotransferase activities have similar dependences on $MgCl_2$ and $MnCl_2$, but the latter was more effective than the former for both enzyme activities. The *V* values for 1,2-dioleoylglycerol obtained at optimal conditions were 1.8 nmol/min per mg microsomal protein for cholinephosphotransferase and 0.6 nmol/min per mg microsomal protein for ethanolaminephosphotransferase. Both enzymes could not utilize 1,3-dioleoylglycerol or 1-oleoylglycerol as substrates. Cholinephosphotransferase had an apparent K_m for CDPcholine of 11.7 μM with 1,2-dioleoylglycerol and was inhibited by CDP-ethanolamine competitively. On the other hand, ethanolaminephosphotransferase has an apparent K_m for CDPenthanolamine of 8 μM and CDP-choline was a noncompetitive inhibitor of ethanolaminephosphotransferase activity. Furthermore, despite the marked alteration of phospholipid composition occurring during the temperature acclimation of *Tetrahymena* cells, both enzyme activities showed similar dependences on growth and incubation temperatures. This may imply that the final step of de novo synthesis of two major phospholipids does not participate in the thermally induced modification of the profile of phospholipid polar head group in membranes.

ADAPTIVE MODIFICATION OF MEMBRANE LIPIDS IN *TETRAHYMENA PYRIFORMIS* DURING STARVATION: ALTERATIONS IN PHOSPHOLIPID COMPOSITION AND POSITIONAL DISTRIBUTION OF FATTY ACYL CHAINS. Reiko Kasai, Takehito Watanabe, Hirofumi Fukushima, Hisaya Iida and Yoshinori Nozawa (Department of Biochemistry, Gifu University School of Medicine, Tsukasamachi-40, Gifu, Japan) *Biochimica et Biophysica Acta 666*:36–46 (1981). *Tetrahymena* cells decreased to half of their normal size during a 24 h starvation period, with a large reduction in total phospholipid content per cell. Upon transferring to the non-nutrient medium, there was a progressive increase in 2-aminoethylphosphonolipid (AEPL) concentration and a corresponding decrease in the phosphatidylethanolamine (PE), present solely in diacyl form, while the level of phosphatidylcholine (PC) fraction remained rather constant. The alkyl-acyl PC and alkyl-acyl and diacyl AEPL showed relatively strong resistance to catabolic attack in a nutrient-free medium, although diacyl types (PC and PE) were reduced drastically. γ-Linolenate, which is a principal fatty acid of starved cells, increased remarkably, with a compensatory decrease in palmitoleate. The hydrolysis with phospholipase A_2 revealed the characteristic segregation of saturated and unsaturated acids between the 1- and 2-position, respectively, of PE, diacyl PC and diacyl PC and diacyl AEPL. The increase of pentadecylate at the 1-position and the decrease of palmitoleate at the 2-position were observed in diacyl forms of PE, PC and AEPL. On the other hand, the 2-position of 1-*O*-alkyl-2-acyl-phosphatidylcholine and 1-*O*-alkyl-2-acyl-(2-aminoethyl)phosphonolipid was occupied mainly by γ-linolenate together with cilienate ($18{:}2^{\Delta 6,11}$) and linoleate. γ-Linolenate at the 2-position of 1-*O*-alkyl-2-acyl-phosphatidylcholine was increased, with a decrease of linoleate, whereas an increase in unusual fatty acid, cilienate and a decrease in linoleate

were observed with no alteration of γ-linolenate in 1-*O*-alkyl-2-acyl-(2-aminoethyl)phosphonolipid under starvation. These results indicate that hexadecyl/18:3-PC, saturated/18:1-AEPL and hexadecyl/18:2$^{\Delta 6,11}$ (or 18:3)-AEPL, which contain either an ether linkage or a C-P bond, would play a crucial role in adaptation to nutrient-free environment in *Tetrahymena.*

THE LIPIDS OF GROSSLY NORMAL HUMAN AORTIC INTIMA FROM BIRTH TO OLD AGE. Saul S. Katz (From McGill University Clinic, Royal Victoria Hospital, Montreal, Quebec H3A 1A1 Canada) *The Journal of Biological Chemistry 256*(23):12275-12280 (1981). Lipid content, composition, and lipid physical states of 86 grossly normal human aortic intimas were studied to determine changes occurring from birth to the eighth decade. Newborn intimas were 71.4% phospholipid, 23.8% cholesterol, 2.4% cholesterol ester, and 2.4% triglyceride. This composition was in the one-phase phospholipid region of the cholesterol-cholesterol esterphospholipid phase diagram, with the phospholipid bilayers saturated with cholesterol ester and 73% saturated with free cholesterol. As predicted by the phase diagram, microscopy of fresh tissue and of the pure extracted lipids showed the phospholipid phase and rare or no lipid droplets. Lipid compositions of the first four decades fell on a straight line in the phase diagram joining the compositions of newborn intimas and low density lipoprotein (LDL). This suggested a continued accumulation of LDL-derived lipids to age 40. Polarized light microscopy showed that at least some of the accumulated lipid existed as isotropic cholesterol ester droplets which were an order of magnitude larger than LDL particles. LDL-derived lipids could account for 16.8% of total intimal lipids in the first decade, and 35.3%, 53.9%, and 72.0% of total lipids in the second, third, and fourth decades, respectively. Cholesterol ester fatty acid composition of intimas also approached the fatty acid composition of LDL in the second and third decades and was almost the same as LDL after age 30. Since cholesterol saturation and the relative amount of cholesterol oleate did not increase during the first four decades, local cholesterol esterification by cellular mechanisms probably accounts for very little of the increased intimal cholesterol ester. After the fourth decade, cholesterol saturation increased markedly but intimal compositions never entered the three phase region of the phase diagram, and cholesterol monohydrate crystals were never seen in lesion-free intima. Lipid content relative to DNA (milligrams per mg) increased from 2.13 at birth to 22.35 by the eighth decade. Thus, lipid accumulation in normal intima up to age 40 is characterized by increasing LDL lipids, either as LDL particles, cholesterol ester-rich droplets, or even connective tissue-bound lipids. After age 40, another process supervenes resulting in an increasing saturation of intimal lipids with free cholesterol.

EFFECT OF VITAMIN D DEFICIENCY ON IN VITRO LABELING OF CHICK INTESTINAL PROTEINS: ANALYSIS BY TWO-DIMENSIONAL ELECTROPHORESIS. Nancy C. Kendrick, Charles R. Barr, Doreen Moriarity, and Hector F. DeLuca (From the Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin–Madison, Wisconsin 53706) *Biochemistry 20*:5288-5294 (1981). Duodenal tissue from vitamin D_3 replete chicks was labeled in vitro for 2.5 h with ^{3}H-labeled amino acids and then combined with tissue from severely vitamin D deficient chicks incubated in an identical fashion with ^{14}C-labeled amino acids. Four double-labeled samples thus obtained were separated into pellet and cytosol fractions by centrifugation, the acidic proteins in each fraction separated by two-dimensional electrophoresis, and the separated proteins analyzed for changes in ^{3}H/^{14}C ratio by the McConkey method of double-label autoradiography. The ^{3}H/^{14}C ratios for proteins with high isotope incorporation were then determined by direct measurement of radioactivity. Of the 100 proteins resolved in the pellet fraction, two were found with ^{3}H/^{14}C ratios greater than base line. Protein 1 (M_r2700) was identified by comigration as membrane-associated, vitamin D induced calcium binding protein. Protein 23 (M_r7600 and unknown identity) was increased in ^{3}H/^{14}C ratio by 2.7-, 2.0, 2.8-, and 1.4-fold over base line in samples I-IV, respectively; this protein stained very faintly with Coomassie blue. The ^{3}H/^{14}C ratio of β- and γ-actin was low in each sample, being 0.61, 0.54, 0.28, and 0.67 of the base-line ratio in samples I-IV, respectively. In the cytosolic fractions, the calcium binding protein was found to have a ^{3}H/^{14}C ratio approaching infinity in each sample. In addition, an unidentified cytosolic protein (M_r ~ 76000) was found to be enriched 1.5-, 2.2-, 4.9-, and 1.7-fold over the base-line ratio. This protein also stained faintly with Coomassie blue. The observed deviations from the ^{3}H/^{14}C base-line ratios probably reflect differences in rates of protein synthesis brought about either directly by 1,25-dihydroxyvitamin D_3 or indirectly via changes in serum calcium or other serum factors.

POLYOXYETHYLATED CHOLESTEROL DERIVATIVES: ORGANIC SYNTHESIS, CELLULAR UPTAKE AND EFFECT ON LIPID METABOLISM IN CULTURED SKIN FIBROBLASTS. Avedis K. Khachadurian, Chien H. Fung, Theodorus Van Es and Frank F. Davis (Departments of Medicine and Biochemistry, College of Medicine and Dentistry of New Jersey-Rutgers Medical School, Piscataway, NJ 08854, and Department of Biochemistry and the Bureau of Biological Research, Rutgers University, New Brunswick, NJ 08903) *Biochimica et Biophysica Acta 665*:434-441 (1981). Polyoxyethylated derivatives of cholesterol were synthesized by addu ction of ethylene oxide to the 3-β-hydroxy position of cholesterol. Derivatives with the methoxy terminal were prepared by condensing methoxypoly(oxyethylene)methane sulfonate with cholesterol. Derivatives with nine ethoxy groups were isolated by the combined use of high pressure liquid chromatography and thin-layer chromatography and identified by proton magnetic resonance. In cultured human skin fibroblasts, uptake of polyoxyethylated [^{3}H] cholesterol was linear up to a concentration of 60 μM. Uptake at 10 μM concentration was linear for 5 h and amounted to 2.5% of the total added label per h. All of the cellular radioactivity was recovered in polyoxyethylated cholesterol and no label appeared in other lipid fractions. Polyoxyethylated cholesterol (10 μM) inhibited the incorporation of acetate into cholesterol and the activity of 3-hydroxy-3-methylgultaryl-CoA reductase, 50% inhibition occurring at 1 and 3 h, respectively. After 18 h incubation, 50% inhibition of both activities occurred at 5 μM and near total inhibition at 25 μM. The incorporation of leucine into protein, uridine into RNA and thymidine into DNA were not affected significantly. Methoxypolyoxyethylated cholesterol gave similar results. There were no differences between normal and homozygous familial hypercholesterolemic fibroblasts which lack membrane receptors for low density lipoproteins. Incorporation of acetate into fatty acids was suppressed by polyoxyethylated cholesterol but not by 25-hydroxycholesterol. Polyoxyethylated cholesterol had no effect on the activity of fatty acid synthetase. Efflux of polyoxyethylated cholesterol from cells had a rapid phase amounting to 55% in the first 2 h, followed by a slow leakage of 15% in the next 20 h. Recovery of the activity of 3-hydroxy-3-methylglutaryl-CoA reductase was faster than that of incorporation of acetate to cholesterol and fatty acids but all these activities remained below the control levels, suggesting that the polyoxyethylated cholesterol retained within the cell continues to exert its inhibitory effect on lipid synthesis.

EFFECT OF GUAR GUM ON BLOOD LIPIDS. A.R. Khan, G.Y. Khan, A. Mitchel and M.A. Qadeer (Depts. of Dietetics and Nutrition, Florida International University, Miami, FL) *Am. J. Clin. Nutr. 34*(11):2446-2449 (1981). The effect of guar gum in capsule form on serum total cholesterol, triglycerides, and lipoprotein cholesterol (very low-density lipoproteins, low-density lipoproteins, and high-density lipoproteins) was studied in healthy volunteers in this double-blind study. Twenty-four subjects, equally divided into treatment and placebo groups, received 9.0 g/day of guar and glucose in 600 mg identical capsules, respectively, for 4 wk under conditions of constant body weight and dietary stability. Guar significantly lowered serum total cholesterol and low-density lipoprotein cholesterol, but had no significant effect on serum triglycerides, high-density lipoproteins and very low-density-lipoprotein cholesterol. No significant changes were seen in blood lipids in the placebo group.

STIMULATION OF CHOLESTEROL BINDING TO STEROID-FREE CYTOCHROME P-450$_{scc}$ BY POLY(L-LYSINE): THE IMPLICATION IN FUNCTIONS OF LABILE PROTEIN FACTOR FOR ADRENOCORTICAL STEROIDOGENESIS. T. Kido and T. Kimura (Department of Chemistry, Wayne State University, Detroit, MI 48202) *J. Biol. Chem. 256*(16):8561-8568. At the low concentration of 2 nM, poly9L-lysine stimulated the binding of dioleoylglycerophosphocholine liposomal cholesterol to steroid-free cytochrome P-450$_{scc}$, which was purified from bovine adrenocortical mitochondria. Ca^{2+} and K^+ ions displayed similar effects at much higher concentrations. These stimulatory effects were more significant at lower temperatures. Regardless of the presence of these activators, the maximal binding of cholesterol was observed between 20 and 30 mol % in dioleoylglycerophosphocholine liposomes. The examination of phospholipid fatty acyl groups on the cholesterol binding revealed that the initial binding rates decreased in the following order: (18_c:2)>(18_c:1)>(18_c:3)>(18_t:1)>(18_c:1, 18:0) in the presence and absence of the lysine polypeptide. When 7α-, 7β-, and 25-hydroxycholesterol were used as ligands, the stimulatory effect of the lysine polypeptide was not seen, indicating the specificity for cholesterol. When the cholesterol side chain cleavage activity was determined by the reconstituted system consisting of adrenodoxin reductase, adrenodoxin, and P-450$_{scc}$, the stimulatory effects by poly(L-lysine) were observed. Polyglycine, poly(L-glutamic acid), putrescine, spermidine, spermine, and L-lysine had no effect, while poly(D-lysine) and poly(L-arginine) had activities much less than the lysine polypeptide.

COMPARATIVE STUDIES OF ADAPTIVE RESPONSES OF

FATTY ACID SYNTHETASE ACTIVITIES IN RAT LIVER AND ADIPOSE TISSUE. Manok Kim and Charles E. Elson (Department of Nutritional Sciences, University of Wisconsin, Madison, WI 53706) *J. Nutr. 111*:1985–1995 (1981). The specific activities of the fatty acid synthetases in the cytosolic fractions of livers and epididymal fat pads from fed, fasted or refed rats were determined. Refed rats received diets which provided, as the primary energy sources, sucrose or starch (75%) or both (59%) with beef tallow or safflower oil (16%) or the monosaccharide components (75%). From these determinations of fatty acid synthetase activity, the relative contribution of each tissue to the rat's overall lipogenic capacity was estimated. In rats fed a cereal-based stock diet ad libitum the adipose tissue accounted for 58% of total activity. During a 2-day fast the hepatic activity decreased from 14 units/100 g body weight to 2.7 units whereas the adipose tissue activity fell from 19 to 13.4 units/100 g body weight. At this time, 82.8% of the activity was in the latter tissue. After refeeding the sucrose diet for 2 days, the hepatic activity had increased 45-fold to 122.3 units/100 g body weight while the adipose tissue activity increased only 2.2-fold to 29.1 units, 19% of the total activity. We estimate that these adaptive responses deteriorate slowly (12.3 days, adipose tissue; 15.4 days, liver) with continued refeeding to the levels present in rats fed the cereal-based stock diet. Activation of residual or constituative enzyme was the major factor in the adipose tissue response whereas activation and induction played roles in the hepatic response. Responses to the refeeding of the other diets were of lesser magnitude. Generally our estimates based on fatty acid synthetase activities are consistent with other estimates based on radiolabel incorporation, NADPH generation and rate-limiting enzyme activities.

EFFECT OF VITAMIN A DEFICIENCY ON GLYCOSYLATION OF RAT SERUM α_1-MACROGLOBULIN. Timothy C. Kiorpes, Roger S. Anderson and George Wolf (Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, MA 02139) *J. Nutr. 111*:2059–2068 (1981). We previously reported that synthesis of the serum glycoprotein α_1-macroglobulin (α_1-M) was decreased in vitamin A-deficient rats. In a reexamination of this conclusion we have shown that the primary synthetic change, reflected by labeled glucosamine uptake, was an underglycosylation which initially has no effect on the glycoprotein's concentration in serum. The severity of this lesion developed progressively and was independent of the glycosylation of total serum glycoprotein, at least up to 11 days after onset of the deficiency. Less glucosamine was demonstrated chemically in a α_1-M purified from rats in the first week of deficiency; α_1-M from more severely deficient rats showed significant depressions of mannose, galactose, and glucosamine.

RELATIONSHIP BETWEEN THE NUTRITIONAL STATUS OF ZINC AND CHOLESTEROL CONCENTRATION OF SERUM LIPOPROTEINS IN ADULT MALE RATS. S.I. Koo and D.A. Williams (Depts. of Biochemistry and Natural Science, Oral Roberts University, Tulsa, OK 74171) *Amer. J. Clin. Nutr. 34*(11):2376–2381 (1981). The effect of zinc status on the distribution of serum cholesterol among the major serum lipoproteins was assessed in adult male rats fed a zinc-deficient diet as compared with those of pair-fed and ad libitum-fed zinc-supplemented controls. All data were collected within the first 4 wk of dietary treatment before the manifestatio of any external symptoms of zinc deficiency. The acute zinc depletion produced a significant reduction in total serum cholesterol; the reduction was primarily due to the selective decline in high-density lipoprotein-cholesterol. Zinc depletion per se produced no significant alterations in very low-density lipoprotein- or low-density lipoprotein-cholesterol level. In addition, linear regression analysis based on the 36 pairs of serum zinc and high-density lipoprotein-cholesterol values showed a significant positive correlation between these two parameters. These results present new evidence for the close association between the nutritional status of zinc and high-density lipoprotein concentration in adult male rats and draw attention to the potential significance of zinc nutriture in relation to cardiovascular health.

LIPID DEPENDENCE OF THE MEMBRANE-BOUND D-LACTATE DEHYDROGENASE OF *ESCHERICHIA COLI*. Stephan Kovatchev, Winchil L.C. Vaz, and Hansjorg Eibl (From the Max-Planck-Institut für biophysikalische Chamie, D-3400 Göttingen-Nikolausberg, Federal Republic of Germany) *The Journal of Biological Chemistry 256*(20):10369–10374 (1981). The lipid dependence of the D-lactate dehydrogenase of *Escherichia coli* plasma membranes was investigated. The delipidated purified enzyme was shown to possess a small activity in the absence of lipid or detergent. Activation, in some cases up to 7-fold the lipid-free activity, was achieved in the presence of added deoxylysophosphatidylcholines, phosphatidylcholines, some phosphatidylethanolamines, methylphosphatidic acid, phosphatidylglycerol, and phosphatidylserine. The enzyme activity was examined in the presence of a homologous series of deoxylysophosphatidylcholines with acyl chains from 6 to 22 carbon atoms long. Activation was only observed when the deoxylysophosphatidylcholine was in its micellar form. Myristoyl deoxylysophosphatidylcholine was observed to be the best activator in this series of micelle-forming compounds. Deoxylysophosphatidylethanolamines did not activate the enzyme, but increasing the degree of *N*-methylation increased the extent of activation. In the phosphatidylcholine series, dimyristoyl-, dipalmitoyl-, and distearoyl-, the distearoyl analog was observed to be the best activator of the enzyme and the dimyristoyl analog was the poorest. Conversely, in the corresponding phosphatidylethanolamine series, the dimyristoyl analog was the only one capable of weakly activating the enzyme. The unsaturated egg phosphatidylethanolamine and 1-palmitoyl-2-oleoylphosphatidylethanolamine were better activators than their corresponding saturated analogs, but they were, nevertheless, weaker activators than the saturated acyl chain phosphatidylcholines. The best activation was obtained by adding phosphatidylserine and phosphatidylglycerol.

LIPID ABSORPTION IN UNANESTHETIZED, UNRESTRAINED RATS: EFFECTS OF 4-AMINOPYRAZOLOPYRIMIDINE AND ETHINYL ESTRADIOL. Brian R. Krause, Charles H. Sloop and Paul S. Roheim (Department of Physiology, Louisiana State University, Medical Center, New Orleans, LA 70119) *Biochimica et Biophysica Acta 665*:165–169 (1981). Emulsified triacylglycerol containing [^{14}C]palmitate was infused intraduodenally in unanesthetized, unrestrained animals treated with 4-aminopyrazolopyrimidine or pharmacologic doses of ethinyl estradiol. 4-Aminopyrazolopyrimidine practically eliminated the appearance of radioactivity in plasma but in ethinyl estradiol-treated animals the peak of radioactivity and shape of the plasma curve were similar to control, although lower in amplitude. A delayed appearance of radioactivity was also observed in 48-h- compared to 15-h-fasted controls, suggesting a requirement for induction of lipoprotein production prior to fat absorption.

INTERACTION OF TUBULIN WITH PHOSPHOLIPID VESICLES: II. PHYSICAL CHANGES OF THE PROTEIN. N. Kumar, R.D. Klausner, J.N. Weinstein, R. Blumenthal and M. Flavin (Lab. of Cell. Biol., Nat. Heart, Lung and Blood Inst. and the Lab. of Theoretical Biol., National Cancer Inst., Nat. Inst. of Health, Bethesda, MD) *J. Biol. Chem. 256*(11):5886–5889 (1981). We have shown that soluble tubulin wil bind to small unilamellar vesicles of dipalmitoy phosphatidylcholine. This association uniquely occurs at the lipid soluble tubulin will bind to small unilamellar vesicles of dipalmitoy phosphatidylcholine. This association uniquely occurs at the lipid phase transition. The tubulin, when bound to the vesicles, displays an altered tryptophan fluorescence characterized by a 5-nm blue shift in the emission maximum and a 22% decrease in fluorescence intensity, when compared to soluble tubulin. Tryptophans in vesicle-bound tubulin are less accessible to the aqueous collisional quenchers, acrylamide and iodide, than in soluble tubulin. Circular dichroism studies reveal an increase in α-helical content of tubulin as a result of vesicle interaction. Proteolytic digestion by trypsin of vesicle bound tubulin is slower than of soluble tubulin. The β subunit of tubulin is preferentially protected from trypsin by vesicle interaction. Furthermore, the pattern of tryptic cleavage products is altered by this interaction.

GLYCOSPHINGOLIPID-HIGH DENSITY LIPOPROTEIN-3 INTERACTIONS: I. TRANSFER OF GLYCOSPHINGOLIPID FROM PHOSPHATIDYLCHOLINE VESICLES TO HIGH DENSITY LIPOPROTEIN-3. Bill C.P. Kwok, Betty W. Shen, and Glyn Dawson (From the Departments of Biochemistry, Pediatrics and Medicine, Joseph P. Kennedy, Jr. Mental Retardation Research Center of the Pritzker School of Medicine, University of Chicago, Chicago, Illinois 60637) *The Journal of Biological Chemistry 256*(18):9698–9704 (1981). Single bilayer vesicles (d<1.02 g/ml) of ^{3}H-glycosphingolipids and [^{14}C] phosphatidylcholine in the molar ratio of 1:7 were prepared by ethanolic injection of the lipid mixture into buffer, concentrated, and incubated with human serum high density lipoprotein-3 (HDL_3; d = 1.14 g/ml) at 37°C. Equilibrium ultracentrifugation of the incubation mixtures on a 0–22% NaBr gradient revealed the presence of three discrete lipid-protein complexes of density 1.03, 1.06, and 1.12 g/ml (Peaks I, II, and III, respectively). Each peak was homogeneous upon reultracentrifugation and the protein and radioactivity eluted as a single peak upon Sepharose CL-6B chromatography. Compositional analysis showed peak I to contain 2.6% protein (apo-A-I peptide) and 4.3% cholesterol, peak II to contain 17.6% protein (apo-A-I peptide) and 6.3% cholesterol, and peak III to have a composition similar to HDL_3. Electron microscopy of negatively stained samples confirmed the homogeneity of the peaks and the similarity between peak III and HDL_3. Peak II particles were larger than HDL_3; peak I particles resembled fused or aggregated vesicles which could be removed by ultracen-

trifugation; disc-shaped particles were not seen in any of the fractions. Direct incubation of HDL_3 or human serum with ^{3}H-glycosphingolipid dispersions did not yield a glycolipid·HDL_3 complex as judged by density gradient ultracentrifugation and Sepharose CL-6B chromatography. However, incubation of ^{3}H-glycolipid/phosphatidylcholine vesicles with serum did result in transfer of ^{3}H-glycolipid to the HDL fraction. It was concluded that glycolipids incorporated into a lipid membrane structure can interact with, and become incorporated into, high density lipoprotein.

NEUTRON SMALL ANGLE SCATTERING ON SELECTIVELY DEUTERATED HUMAN PLASMA LOW DENSITY LIPOPROTEINS: THE LOCATION OF POLAR PHOSPHOLIPID HEADGROUPS. Peter Laggner, Gerhard M. Kostner, Ulrike Rakusch, and David Worcester. (From the Institut fuer Roentgenfeinstrukturforschung der Oesterreichischen Akademie der Wissenschaften und des Forschungzenstrums Graz, Steyregasse 17, A-8010 Graz, Austria, and the European Molecular Biology Laboratory, Hamburg Outstation at Deutsches Elektronen-Synchroton, Hamburg, Germany; The Institut fuer Medizinische Biochemie, Universitaet Graz, Graz, Austria, and the Institute Max von Laue-Paul Langevin, Grenoble, France) *The Journal of Biological Chemistry 256*(22):11832–11839 (1981). Human plasma low density lipoproteins (LDL) were deuterated in the phospholipid headgroup region by exchange with phospholipid headgroup region by exchange with phosphatidylcholine-$N(CD_3)_3$-apolipoprotein A complexes. The exchange was associated with a net transfer of phosphatidylcholine to LDL leading to an increase in total phospholipid content by 27%. Practically all of the endogenous phosphatidylcholine including lysophosphatidylcholine, and about one-third of the sphingomyelin pool was found to be exchangeable. Immunochemically, deuterated LDL was identical with native LDL. The hydrodynamic and ultrastructural properties were closely similar for the two particle species apart from a slight increase in overall particle size by about 2%. Both native and deuterated LDL were investigated by neutron small angle scattering at several representative contrasts in H_2O/D_2O buffers. Subtraction of the scattering amplitudes of native from deuterated LDL resulted in a radius of gyration of 103 ± 5 Å for the $N(CD_3)_3$ groups, and in a structure factor resembling that of a thin, spherical shell.

β-OXIDATION OF THE GEOMETRIC AND POSITIONAL ISOMERS OF OCTADECENOIC ACID BY RAT HEART AND LIVER MITOCHONDRIA. Larry D. Lawson and Ralph T. Holman (The Hormal Institute, University of Minnesota, 801 16th Avenue, N.E., Austin, MN 55912) *Biochimica et Biophysica Acta 665*:60–65 (1981). The *cis* and *trans* isomers of Δ^4 through Δ^{16} octadecenoic acid, all present in partially hydrogenated soybean oil, were compared as substrates for β-oxidation by isolated rat heart and liver mitochondria. The fatty acids were converted to their coenzyme A esters and oxygen uptake rates measured polarographically in the presence of L-malate, L-carnitine, ADP, and optimum albumin. The *cis* isomers were catabolized in a similar pattern by heart and liver. The even-positioned *cis* isomers were oxidized significantly more slowly than adjacent odd-positioned isomers. Most odd-positioned *cis* isomers were oxidized as rapidly as oleoyl-CoA. The pattern of catabolism of the *trans* isomers, however, was different from the *cis* isomers. Liver mitochondria oxidized most even-positioned *trans* isomers significantly more rapidly than adjacent odd-positioned isomers. The same pattern was observed with heart mitochondria only for the *trans* isomers in which the double bond was located near the middle of the acyl chain. Heart mitochondria oxidized nearly all the *trans* isomers significantly more slowly than stearoyl-CoA; however, liver mitochondria oxidized the even-positioned *trans* isomers nearly as rapidly as stearoyl-CoA. Both heart and liver mitochondria oxidized the *cis* isomers, especially Δ^9 and Δ^{11}, significantly more rapidly than their respective *trans* isomers, with three notable exceptions: Δ^8, Δ^{10}, and Δ^{14}. 3-Hydroxyacyl-CoA epimerase and Δ^3-*cis*-Δ^2-*trans*-enoyl-CoA isomerase account for most of the observed β-oxidation patterns. An additional and more efficient pathway for the β-oxidation of *n*-6 fatty acids is suggested.

DECLINING MORTALITY IN CORONARY HEART DISEASE. Robert I. Levy (From the National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland) *Arteriosclerosis 1*:312–325 (1981). Since 1968, there has been a dramatic, unprecedented decline in mortality from cardiovascular disease in the United States, especially from coronary heart disease and stroke. The decline has now been confirmed as real and has been observed in all age, sex, and race groups. Possible causes of the decline in coronary heart disease mortality include the development of the concept of acute coronary care, new drugs, sophisticated surgical techniques such as coronary artery bypass, noninvasive diagnostic methods for earlier disease detection, and the identification of specific cardiovascular risk factors. The decline has been temporally related to risk factor awareness and modification (cigarette smoking cessation, hypertension control, diet change, and reduction in cholesterol). Thus, both primary prevention through lifestyle changes and improved treatment regimes have played a role in the decline.

FATTY ACID COMPOSITION OF LIPIDS IN THE MATERNAL DIET AND ESTABLISHMENT OF A LACTOBACILLUS SP. STRAIN IN THE DIGESTIVE TRACT OF SUCKLING GNOTOBIOTIC MICE AND RATS. C. Lhuillery, Y. Demarne, F. Dubos, J-V. Galpin, R. Ducluzeau, and P. Raibaud (Station de Recherches de Nutrition I.N.R.A.-C.N.R.Z. 78350 Jouy-en-Josas, France) *Am. J. Clin. Nutr. 34*(8):1513–1519 (1981). Adult gnotobiotic mice and rats, monoassociated with a homofermentative strain of Lactobacillus sp. of intestinal origin, were fed either a commercial rodent chow A or a semi-synthetic diet B. Similar numbers of lactobacilli were established in their gastrointestinal tract whatever diet they ate. The lactobacilli were established in the digestive tract of the newborn of A mothers at 2 days but were hardly established in mouse or rat pups of B mothers during the first 15 neonatal days. They became established more slowly in the digestive tracts of rat pups of A mothers than in their mouse homologues. Comparative analysis of milk lipids in the A and B series showed a linoleic acid content which was three times higher in the B than in the A series. Two diets S and H differing only by their lipid fractions which, respectively, presented the same fatty acid compositions as lipids from diets A and B were then given to two others lots of Lactobacillus monoassociated mice. The establishment kinetics of the strain were the same in the mouse pups of these two lost as precendently in the A series. However, the fatty acid composition of milk lipids in lot H was very similar to that precedently observed in the B series. The difference observed in the establishment kinetics of the Lactobacillus strain in the digestive trace of suckling gnotobiotic mice was thus attributed to other dietary factors than the fatty acid composition of the maternal diet.

RECONSTITUTION OF DELIPIDATED BACTERIORHODOPSIN WITH ENDOGENOUS POLAR LIPIDS. C. Lind, B. Hojeberg, and H.G. Khorana (Department of Biology and Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139) *J. Biol. Chem. 256*(16):8298–8305 (1981). Delipidated bacteriorhodopsin has been reconstituted with endogenous polar lipids from *Halobacterium halobium*. The vesicles (diameter, 250–500 Å) formed are very stable, relatively homogeneous in bacteriorhodopsin and lipid content, and almost optically clear; a minor turbid fraction can be separated by gel filtration. Bacteriorhodopsin in the reconstituted vesicles has an inside out orientation and, on illumination, translocates protons efficiently from the medium to the interior of the vesicles in the presence of the ionophore valinomycin. In the absence of the latter, both the rate and the extent of light-dependent proton uptake by the vesicles are decreased 3–6- and 5–15-fold, respectively, depending on the salt in the assay medium. Both the stimulation by valinomycin and the proton-translocating activity are higher in NaCl than in KCl. Bacteriorhodopsin in these vesicles, as in purple membrane, undergoes light adaptation in indicated by a red shift (7–8 nm) of the absorption maximum. At low pH, the absorption maximum of reconstituted protein shows a 50-nm red shift, possibly due to protonation of an ionizable group which interacts with the chromophore. The latter group appears to be accessible only from the external medium.

EFFECTS OF AN ATHEROGENIC DIET ON APOLIPOPROTEIN E BIOSYNTHESIS IN THE RAT. Yen-Chiu Lin-Lee, Yoshio Tanaka, Chin-Tarng Lin, and Lawrence Chan (From the Departments of Medicine and Cell Biology, Baylor College of Medicine, and the Methodist Hospital, Houston, Texas 77030) *Biochemistry 20*: 6474–6480 (1981). The effects of an atherogenic diet on apolipoprotein E biosynthesis in the rat liver were studied by immunocytochemical and biochemical techniques. Two groups of rats were fed either a normal laboratory chow or a special atherogenic diet containing 5% lard, 1% cholesterol, 0.35% taurocholic acid, and 0.1% propylthiouracil for 25 days. The atherogenic diet fed animals developed increased plasma cholesterol concentrations (134 ± 22 vs. 70 ± 15 mg/dL) and apolipoprotein E (apoE) concentrations (42.5 ± 10.2 vs. 15.6 ± 4.8 mg/dL). Plasma albumin levels were unchanged (7.3 ± 3.0 vs. 7.4 ± 2.0 g/dL). When liver sections from these animals were studied by indirect immunoperoxidase staining, by using a rabbit anti-rat apoE serum, the number of apoE-positive grains increased from 5.45 ± 0.49 to 6.79 ± 0.54 per hepatocyte, and the intensity of staining of individual grains also increased. Rat liver slices were incubated in vitro in culture medium containing [^{35}S]methionine for 90 min at 37°C. Radioactivity incorporated into immunoprecipitable apoE were 2.89 ± 0.2% of Cl_3AcOH-precipitable radioactivity in special diet fed animals compared to 1.32 ± 0.1% in normal controls. Again, radioactivity incorporated into immunoprecipitable albumin was unchanged (11.9% in special diet group vs. 11.5% in controls). Total RNA was isolated from the

liver of both groups of animals. Poly(A) RNA was purified by oligo-(dT)-cellulose chromatography. Translation of the poly(A) RNA in a wheat germ system in vitro indicates that the atherogenic diet fed rat liver contained significantly higher concentrations of apoE mRNA activity (3.17% of total Cl_3AcOH-precipitable cpm) when compared to that of the controls (1.5% of total activity). In contrast, albumin mRNA activity in these RNA samples stayed constant at 9.1% (atherogenic diet fed) and 8.9% (controls), respectively. Our observations have provided unequivocal evidence that feeding the rats the special atherogenic diet for 25 days leads to an increase in apoE synthesis in the liver. This increase was mediated at least in part by an accumulation of translatable apoE mRNA activity in this organ.

THE UPTAKE AND METABOLISM OF CHYLOMICRON-REMNANT LIPIDS BY NONPARENCHYMAL CELLS IN PERFUSED LIVER AND BY KUPFFER CELLS IN CULTURE. Patrick M. Lippiello, Jan Dijkstra, Mieke van Galen, Gerrit Scherphof, and B. Moseley Waite (From the Department of Biochemistry, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina 27103 and the Laboratory of Physiological Chemistry, University of Groningen, Bloemsingel 10, Groningen, The Netherlands) *The Journal of Biological Chemistry 256*(14):7454-7460 (1981). The role of nonparenchymal liver cells in the metabolism of triacylglycerol-rich lipoproteins was examined by monitoring the uptake of radiolabeled chylomicron remnants by Kupffer and endothelial cells in perfused rat liver, and by incubation of labeled remnants with Kupffer cells maintained in culture. Remnants were radiolabeled in all of the major lipid classes as follows: [^{14}C] phosphatidylcholine; [^{14}C] cholesterol and [^{14}C] cholesterol ester; [^{3}H] oleoyl mono-, di-, and triacylglycerol, ^{3}H-phospholipid, ^{3}H-fatty acid, and [^{3}H] cholesterol ester. In perfused liver, 66% of the ^{3}H-lipids and 61% of the ^{14}C-lipids were taken up by the whole liver in 10 min. Following isolation of nonparenchymal cells by pronase digestion and centrifugal elutriation, 4% of the ^{3}H-lipids and 13% of the ^{14}C-lipids were associated with Kupffer cells, compared to 1% of the ^{3}H-lipids and 3% of the ^{14}C-lipids with endothelial cells. Relative to remnants, the overall ^{3}H/^{14}C isotopic ratio was decreased in both cell types. By difference from total liver uptake, parenchymal cells accounted for 61% and 45% of the ^{3}H- and ^{14}C-lipids, respectively, suggesting that relatively more [^{3}H] oleoyl lipids were taken up by these cells. The radiolabel distribution in total liver and both nonparenchymal cell types revealed a marked decrease in [^{3}H] oleate in the monoacylglycerol, triacylglycerol, and fatty acid fractions, and a significant incorporation of [^{3}H] oleate into phospholipid. There was no accumulation of [^{14}C] cholesterol ester in Kupffer or endothelial cells, despite appreciable uptake by the whole liver. In the absence of other cell types present *in situ*, Kupffer cells in culture demonstrated both efficient uptake of ^{3}H-lipids and incorporation of [^{3}H] oleate into phospholipid for up to 2 h, with an increased ^{3}H/^{14}C isotopic ratio relative to remnants. There was no accumulation of [^{14}C] cholesterol ester in cultured cells. Although both the uptake and metabolism of remnant lipids by nonparenchymal cells are quantitatively significant, it can be inferred from these results that the major contribution *in vivo* is by parenchymal cells.

DEMONSTRATION OF THE OCCURRENCE OF INACTIVE FATTY ACID SYNTHETASE IN RAT LIVER BY IMMUNOTITRATION AND ITS *IN VITRO* PARTIAL ACTIVATION. Frank A. Lornitzo, Sarvagya S. Katiyar, Rajinder N. Puri, and John W. Porter (From the Lipid Metabolism Laboratory, William S. Middleton Memorial Veterans Hospital and the Department of Physiological Chemistry, University of Wisconsin, Madison, Wisconsin 53706) *The Journal of Biological Chemistry 256*(16):8498-8505. (1981). Direct immunotitrations of rat liver fatty acid synthetase in crude tissue homogenates with monospecific rabbit anti-rat liver fatty acid synthetase antibody enabled us to make a comparison of fatty acid synthetase protein and activity (percentage of maximal activity) as a function of the nutritional state in normal, diabetic, and insulin- and glucagon-insulin treated animals. Previous results, in which large changes in fatty acid synthetase activity were related to protein synthesis and degradation rather than to enzyme activation, were confirmed. It was also shown that fatty acid synthetase activation does not occur immediately on synthesis but follows the synthesis of fatty acid synthetase protein. In order to characterize the enzymatically inactive protein found on immunotitration and to develop an *in vitro* system for fatty acid synthetase activation, conditions were sought to obtain large amounts of fatty acid synthetase protein free from, or low in, activity. It was found that treatment of hypophysectomized rats with triiodothyronine meets these requirements, yielding milligram quantities of inactive fatty acid synthetase protein with less than 2% of maximal activity. A part of the inactive fatty acid synthetase was found to be the apoenzyme as indicated by β-ketoreductase and thioesterase activities, by its ability to incorporate label from [G^{3}H] CoA, and by its partial *in vitro* activation, which led to an increase in overall synthetase activity in crude and partially purified cell-free systems. The components required for activation include magnesium ion and a transferase fraction prepared from livers of 48-h fasted, 12-h refed rats.

BILE SALT INDUCTION OF 7α- AND 7β-HYDROXYSTEROID DEHYDROGENASES IN *CLOSTRIDIUM ABSONUM*. Ian A. Macdonald and Paul D. Roach (Departments of Medicine and Biochemistry, Dalhousie University, Halifax, Nova Scotia B3H 4H7 Canada) *Biochimica et Biophysica Acta 665*:262-269 (1981). Eight strains of *Clostridium absonum* grown in the presence of $4 \cdot 10^{-4}$ M deoxycholate contained both NADP-dependent 7α- and 7β-hydroxysteroid dehydrogenase activities. In one strain studied in detail, significant amounts of NADP-dependent 7α- and 7β-hydroxysteroid dehydrogenase and NAD-dependent 7α-hydroxysteroid dehydrogenase activities were demonstrated only when cells were grown in the presence of deoxycholate or chenodeoxycholate, both optimal at $4 \cdot 10^{-4}$ M. When the bile salt was deleted from the medium, only a trace of 7α-hydroxysteroid dehydrogenase was present and 7β-hydroxysteroid dehydrogenase was absent. Other bile salts including cholate, ursodeoxycholate and keto bile salts were less effective as inducers. Addition of cholate to medium already containing deoxycholate at a suboptimal concentration enhanced the induction, while addition of ursodeoxycholate suppressed the induction. Further enhancement of 7α- and 7β-hydroxysteroid dehydrogenase could be obtained by additions of deoxycholate (up to a total of $6 \cdot 10^{-4}$ M) during the growth of the organisms (in log phase). As enzyme enhancement is blocked by addition of rifampicin to the medium, the authors conclude that the enzymes are bile salt-inducible. Growth curve studies revealed an optimal enzyme yield at a harvest time of approx. 6-9 h. We have preliminarily characterized several inducible enzyme components: an NADP-dependent 7β-hydroxysteroid dehydrogenase as well as both NAD- and NADP-dependent 7α-hydroxysteroid dehydrogenases.

REASSEMBLED MODEL LIPOPROTEINS: LIPID DYNAMICS IN RECOMBINANTS OF HUMAN APOLIPOPROTEIN A-II AND DIMYRISTOYLPYOSPHATIDYLCHOLINE. William W. Mantulin, John B. Massey, Antonio M. Gotto, Jr., and Henry J. Pownall (From the Department of Medicine, Baylor College of Medicine and The Methodist Hospital, Houston, Texas 77030) *The Journal of Biological Chemistry 256*(21):10815-10819 (1981). Dimyristoylphosphatidylcholine (DMPC) and apolipoprotein A-II (apoA-II) combine to form three isolatable complexes of molar stoichiometry of 240:1 75:1, and 45:1, respectively. Steady state fluorescence depolarization studies with diphenylhexatriene (DPH) and parinaric acid incorporated into the DMPC/apoA-II complexes reveal that increasing the protein content reduces fluidity and increases the apparent lipid phase transition (T_c). Time-resolved studies with DPH reveal hindered rotational motion and a loss of fluidity with increasing protein content, arising from an increase in the order of the lipid assay. The parinaric acid probes could detect no lateral phase separation of lipid ensembles in the DMPC/apoA-II complexes, suggesting that the DMPC adjacent to the protein is as mobile as bulk lipid. Measurements of the excimer-forming properties of a pyrene lecithin analog show that the increasing protein content in the DMPC/apoA-II complexes reduces lateral diffusion of the lipid.

HIGH DENSITY LIPOPROTEIN SUBFRACTIONS ISOLATED BY HEPARIN-SEPHAROSE AFFINITY CHROMATOGRAPHY AND THEIR ROLE IN CHOLESTERYL ESTER TRANSFER TO VERY LOW DENSITY LIPOPROTEINS. Yves L. Marcel, Camilla Vézina, Diane Emond, Roy B. Verdery, and Ross W. Milne (Laboratory of Lipoprotein Metabolism, Clinical Research Institute of Montreal, 110, Pine Avenue West, Montreal H2W 1R7, Quebec, Canada) *J. Lipid Res. 22*:1198-1205 (1981). Normal human plasma HDL was applied to a column of heparin-Sepharose in the presence of $MnCl_2$ and three fractions were obtained by stepwise elution with increasing NaCl concentrations: a non-retained fraction (NR, 78% of protein) and two retained fractions (R_1 and R_2, 18 and 2.5% of protein, respectively). Both unesterified and esterified cholesterol increased from NR to R_1 to R_2 but the increment was more pronounced for unesterified cholesterol. ApoA-II to apoA-I ratio was lower in R_1 compared to NR but R_1 contained more apoC than NR. ApoE increased from NR to R_1 to R_2 (0.07, 0.4, and 14% of protein in each fraction, respectively) while apoB was found only in R_2. Agarose gel electrophoresis and immunoadsorbers for apoB and apoE showed that R_2 consisted of two major lipoprotein populations, one containing apoB and some apoE and the other containing apoE and no apoB. Cholesteryl ester transfer between each HDL subfraction and VLDL in the presence of partially purified cholesterol ester transfer protein was studied. NR and R_1 gave the highest initial rates of transfer for labeled cholesteryl ester which were corroborated by significant mass transfer of cholesteryl esters. From these results, we concluded that there is no connection between cholesteryl ester transfer and apoE. On the other

hand, transfer from R_2 to VLDL followed different kinetics with a high zero hour transfer but with subsequently lower rates when compared to NR and R_1. The cholesteryl ester transfer activity in R_2 was mainly due to the presence of apoE-containing lipoproteins whereas those containing apoB had minimal transfer activity. However, because this transfer of label was not translated into significant mass transfer of cholesteryl ester to VLDL, the apoE-containing lipoproteins appear involved mainly in the equilibration of cholesteryl esters.

EFFECTS OF TEMPERATURE ACCLIMATION ON *NEUROSPORA* PHOSPHOLIPIDS: FATTY ACID DESATURATION APPEARS TO BE A KEY ELEMENT IN MODIFYING PHOSPHOLIPID FLUID PROPERTIES. C.E. Martin, D. Siegel, and L.R. Aaronson (Dept. of Biological Sciences, Rutgers Univ.-DC, New Brunswick, NJ 08903) *Biochim. Biophys. Acta 665*:399–407 (1981). Experiments were conducted on the effect of growth temperature on phospholipids of *Neurospora*. Strains grown at high (37°C) and low (15°C) temperatures show large differences in the proportions of phospholipid fatty acid α-linolenate (18:3) which can vary by 10-fold over this temperature range. Changes in the phospholipid base composition are less dramatic; the most significant is an increase in phosphatidylethanolamines at low temperatures accompanied by a concomitant decrease in phosphatidylcholine. It appears that phospholipid fatty acid desaturation is closely regulated with respect to growth temperature. Over the 37 to 15°C growth temperature range there appear to be at least two desaturase systems in *Neurospora* which are under different controls. Production of 18:1 and 18:2 species appears to occur at high levels over the entire temperature range, whereas the production of 18:3 seems to be inversely related to growth temperature. Shifting 37°C-acclimated cultures to 15°C produces a growth lag period of approximately 3 h, during which the level of 18:3 increases markedly. Differential scanning calorimetry of phospholipids from 37°C cells shows a phase transition at -22°C while lipids from 15°C cultures exhibit a phase transition with reduced enthalpy at about -41°C. The data are consistent with the idea that phospholipid composition in *Neurospora* is under strict control and suggest that membrane fluidity is regulated with respect to growth temperature through changes in membrane lipid composition.

RESPONSES OF SERUM LIPOPROTEINS TO DIETARY CHOLESTEROL AND TYPE OF FAT IN THE BABOON. Henry C. McGill, Jr., C. Alex McMahan, Arthur W. Kruski, Jim L. Kelley, and Glen E. Mott (From the Department of Pathology, The University of Texas Health Science Center, and the Southwest Foundation for Research and Education, San Antonio, Texas) *Arteriosclerosis 5*: 337–344 (1981). We examined the effects of dietary cholesterol (<0.01 and 1.7 mg/Kcal) and type of fat (saturated, coconut oil; polyunsaturated, corn oil) on very low density plus low density lipoprotein (VLDL + LDL) and high density lipoprotein (HDL) cholesterol in 24 young baboons (12 male, 12 female) (*Paplo* sp.) in a crossover design experiment. The oils contributed 40% of calories. Total serum cholesterol concentration on the low cholesterol-polyunsaturated fat diet averaged 120 mg/dl; on the high cholesterol-saturated fat diet, 245 mg/dl; and on the other two cholesterol-fat diet combinations, about 200 mg/dl. There was a significant interaction between cholesterol and type of fat in their effects on VLDL + LDL cholesterol, but not in their effects on HDL cholesterol. Dietary cholesterol elevated VLDL + LDL cholesterol when fed with both types of fat, but elevated it more when fed with polyunsaturated fat than with saturated fat. Saturated fat elevated VLDL + LDL cholesterol when dietary cholesterol was low, but not when dietary cholesterol was high. Saturated fat consistently elevated HDL cholesterol more than did dietary cholesterol. The response of apolipoprotein B concentrations to dietary components was similar to that of VLDL + LDL cholesterol. These results indicate that dietary cholesterol and type of fat have different effects on the distribution of cholestrerol among the major serum lipoproteins of the baboon.

THE EFFECT OF STEROL ON THE ENERGY PRODUCING CAPACITY OF YEAST MITOCHONDRIA. C.A. McLean-Bowen and L.W. Parks (Dept. of Microbiol., Oregon State Univ., Corvallis, OR) *Chem. Phys. Lipids 29*(2):137–145 (1981). The mitochondrial transmembrane potential ($\Delta\Psi$) of yeast mitochondria has been determined. Comparison of mutants which accumulate sterols other than ergosterol with isogenic wild-types shows no correlation between sterol structure and the ability to generate a trans-membrane potential.

STRUCTURE OF THE CHICKEN APO VERY LOW DENSITY LIPOPROTEIN II GENE. Frits C.P.W. Meijlink, Alfred D. van het Schip, Annika C. Arnberg, Bé Wieringa, Geert AB, and Max Gruber (From the Department of Biochemistry, University of Groningen, Nijenborgh 16, 9747 AG Groningen, The Netherlands) *The Journal of Biological Chemistry 256*(18):9668–9671 (1981). We describe two cloned genomic DNA fragments, both bearing the entire apo very low density lipoprotein II gene. Electron microscopy and restriction enzyme mapping showed that this gene is split into at least four coding sequences by three or more intervening sequences. A very short exon at the 5′-end of the gene is separated by a 1.5-kilobase intron from the second exon, which codes for the AUG initiation codon of the mRNA.

IDENTIFICATION OF PHOSPHOLIPID AS AN ESSENTIAL PART OF BOVINE VITAMIN K-DEPENDENT CARBOXYLASE. Menno de Metz, Cees Vermeer, Berry A.M. Soute, and H. Coenraad Hemker (From the Department of Biochemistry, Biomedical Centre, State University of Limburg, 6200 MD Maastricht, The Netherlands) *The Journal of Biological Chemistry 256*(21):10843–10846 (1981). Vitamin K-dependent carboxylase from bovine liver contains phospholipid (primarily phosphatidylcholine), which is essential for its *in vitro* activity. Sepharose-bound carboxylase can be depleted of phospholipids, either by washing the enzyme with detergents or by phospholipase treatment. The enzyme can be reconstituted by adding mixed micelles of phosphatidylcholine and cholate to the Sepharose-bound proteins.

INHIBITORS OF STEROL SYNTHESIS: DIFFERENTIAL EFFECTS OF 14α-HYDROXYMETHYL-5α-CHOLEST-7-ENE-3β,15α-DIOL AND 14α-HYDROXYMETHYL-5α-CHOLEST-6-ENE-3β,15α-DIOL ON STEROL SYNTHESIS IN CELL-FREE HOMOGENATES OF RAT LIVER. L.R. Miller, R.A. Pascal, Jr., and G.J. Schroepfer, Jr. (Depts. of Biochemistry and Chemistry, Rice University, Houston, TX 77001) *J. Biol. Chem. 256*(15):8085–8091 (1981). 14α-Hydroxymethyl-5α-cholest-7-ene-3β,15α-diol and 14α-hydroxymethyl-5α-cholest-6-ene-3β,15α-diol have been shown to be potent inhibitors of the synthesis of digitonin-precipitable sterols in mouse L-cells and in primary cultures of fetal mouse liver cells and to cause a reduction in the levels of activity of 3-hydroxy-3-methylglutaryl-CoA reductase in the same cells. In the present study, we have found that both sterols have a second, but distinct, site of action, distal to the formation of mevalonic acid. 14α-Hydroxymethyl-5α-cholest-7-ene-3β,15α-diol has been found to be a potent inhibitor of the synthesis of digitonin-precipitable sterols from labeled mavalonate in cell-free preparations of rat liver. In contrast, 14α-hydroxymethyl-5α-cholest-6-ene-3β,15α-diol had only a slight effect on the synthesis of digitonin-precipitable sterols from labeled mevalonate in cell-free rat liver preparations.

EFFECT OF ASCORBIC ACID ON COPPER AND CHOLESTEROL IN ADULT CYNOMOLGUS MONKEYS FED A DIET MARGINAL IN COPPER. D.B. Milne, S.T. Omaye and W.H. Amos, Jr. (Nutrition Technology Division, Letterman Army Institute of Research, Presidio of San Francisco, CA 94129) *Am. J. Clin. Nutr. 34*(11):2389–2393 (1981). The effects of prolonged consumption of high levels of dietary ascorbic acid on copper metabolism and cholesterol in adult monkeys fed a diet low or marginal in copper were investigated. Small reductions in serum copper and in serum ceruloplasmin levels were observed when high levels of ascorbic acid were fed. During the period of copper depletion there was a gradual but significant increase in serum cholesterol. The level of ascorbic acid supplementation had no effect during this phase. When copper was added back to the diet, serum cholesterol levels leveled off or declined in the monkeys receiving the low (1 mg/day/kg body weight) dose of ascorbic acid. Cholesterol levels continued to increase in the group receiving the higher ascorbic acid supplement (25 mg/day/kg body weight). These data suggest that high levels of ascorbic acid supplementation may make dietary copper relatively unavailable for regulating cholesterol metabolism.

PARAQUAT AND NADPH-DEPENDENT LIPID PEROXIDATION IN LUNG MICROSOMES. Hara P. Misra and Lee D. Gorsky (From the Laboratory for Energy-Related Health Research, University of California, Davis, California 95616) *The Journal of Biological Chemistry 256*(19):9994–9998 (1981). Since there exists some controversy in the literature as to whether paraquat augments microsomal lipid peroxidation via superoxide anion (O_2^-), the role of paraquat and active oxygen species in NADPH-dependent lung microsomal lipid peroxidation was investigated. Incubation of buffered aerobic mixture of bovine lung microsome and NADPH, in the presence or absence of exogenously added iron, resulted in a progressive formation of lipid peroxides whose accumulation could be followed at 535 nm as malondialdehyde. Paraquat strongly inhibited this lipid peroxidation. Thus, malondialydehyde formation was 50% inhibited by 4×10^{-5} M paraquat in the reaction mixture. The malondialdehyde color development by lipid peroxides was not affected by this concentration of paraquat. Lipid peroxidation was also strongly inhibited by singlet oxygen scavengers, *e.g.* dimethylfuran and diphenylfuran, and by catalase. Hydroxyl radical scavengers, *e.g.* mannitol, benzoate, and ethanol, had little effect

in malondialydehyde production. Superoxide dismutase, which removes O_2^- efficiently, did not inhibit malondialdehyde production by lung microsomes and rather enhanced its formation. A scheme in which paraquat and active O_2 species may be involved with microsomal lipid peroxidation is presented.

EFFECT OF LIPID MEMBRANE STRUCTURE ON THE ADENOSINE 5'-TRIPHOSPHATE HYDROLYZING ACTIVITY OF THE CALCIUM-STIMULATED ADENOSINETRIPHOSPHATASE OF SARCOPLASMIC RETICULUM. Bryant M. Moore, Barry R. Lentz, Mathias Hoechli, and Gerhard Meissner (From the Departments of Biochemistry and Nutrition (B.M.M., B.R.L., and G.M.) and Anatomy (M.H.), The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514) *Biochemistry 20*:6810–6817 (1981). An active Ca^{2+}-stimulated, Mg^{2+}-dependent adenosinetriphosphatase (Ca^{2+}-ATPase) isolated from rabbit skeletal muscle sarcoplasmic reticulum membranes has been incorporated into dilauroyl-, dimyristoyl-, dipentadecanoyl-, dipalmitoyl-, and palmitoyloleoylphosphatidylcholine bilayers by using a newly developed lipid-substitution procedure that replaces greater than 99% of the endogenous lipid. Freeze-fracture electron microscopy showed membranous vesicles of homogeneous size with symmetrically disposed fracture-face particles. Diphenylhexatriene fluorescence anisotropy was used to define the recombinant membrane phase behavior and revealed more than one transition in the membranes. Enzymatic analysis indicated that saturated phospholipid acyl chains inhibited both overall ATPase activity and Ca^{2+}-dependent phosphoenzyme formation below the main lipid phase transition temperature (T_m) of the lipid-replaced membranes. At temperatures above T_m, ATPase activity but not phosphoenzyme formation was critically dependent on acyl chain length and thus bilayer thickness. No ATPase activity was observed in dilauroylphosphatidylcholine bilayers. Use of the nonionic detergent dodecyloctaoxyethylene glycol monoether demonstrated that the absence of activity was not due to irreversible inactivation of the enzyme. Increased bilayer thickness resulted in increased levels of activity. An additional 2-fold rise in activity was observed when one of the saturated fatty acids in dipalmitoylphosphatidylcholine was replaced by oleic acid, whose acyl chain has a fully extended length comparable to that of palmitic acid. These results indicate that the Ca^{2+}-ATPase requires for optimal function a "fluid" membrane with a minimal bilayer thickness and containing unsaturated phospholipid acyl chains.

REDUCED LDL- AND INCREASED HDL-APOPROTEINS IN PATIENTS WITH HYPERCHOLESTEROLAEMIA UNDER TREATMENT WITH BEZAFIBRATE. R. Mordasini, W. Riesen, P. Oster, M. Keller, G. Middelhoff, and P.D. Lang (Medical Dept. and Institute for Clinical Protein Research, Tiefenauhospital, Univ. of Berne, Switzerland) *Atherosclerosis 40*:153–158 (1981). The effect of bezafibrate on serum lipids, lipoproteins and the apoproteins A-I, AII and B was studied in 18 patients with primary hypercholesterolaemia. Total cholesterol was lowered by 20% ($P<0.05$), LDL-cholesterol by 24% ($P<0.05$), and apo B by 14% ($P<0.05$), which is comparable to the effect obtained with anion exchange resins but with far fewer side-effects. HDL increased significantly during bezafibrate treatment both by measurement of HDL-cholesterol (+19%, $P<0.05$) and A-II (+23%, $P<0.05$). This increase of HDL and the decrease of triglycerides was maintained for 6 weeks of placebo treatment after cessation of bezafibrate, while serum total and LDL cholesterol as well as apo B returned to their baseline levels.

EFFECT OF DIHYDROXYLATED METABOLITES OF VITAMIN D_3 ON CALCIUM ABSORPTION IN URAEMIC MAN. N. Muirhead, G.R.D. Catto, S. Gvozdanovic, D. Gvozdanovic and N. Edward. (Department of Medicine and Department of Biomedical Physics and Bioengineering, University of Aberdeen, Foresterhill, Aberdeen, Scotland, U.K.) *Clinical Science 61*:723–727 (1981). 1. Peak ^{47}Ca absorption and 7 day ^{47}Ca retention were measured by a whole-body radioactivity counting technique in 10 haemodialysis patients before and after treatment with 1,25-dihydroxycholecalciferol [1,25-$(OH)_2D_3$] and 24,25-dihydroxycholecalciferol [24,25-$(OH)_2D_3$]. 2. Before treatment all patients had low peak ^{47}Ca absorption and 7 day ^{47}Ca retention. 3. After treatment with 1,25-$(OH)_2D_3$ (0·25–1 μg/day for 4–12 months) peak ^{47}Ca absorption and 7 day ^{47}Ca retention returned to normal. 4. After treatment with 24,25-$(OH)_2D_3$ (2 μg/day for 4–12 months) peak ^{47}Ca absorption and 7 day ^{47}Ca retention remained at pretreatment levels. 5. It is concluded that physiological doses of 24,25-$(OH)_2D_3$ have no effect on calcium absorption or retention in uraemic man.

PLASMA LIPIDS AND LIPOPROTEINS OF JAPANESE ADULTS AND UMBILICAL CORD BLOOD. Tsuguhiko Nakai, Toshitaka Tamai, Shiro Yamada, Takeshi Kobayashi, Takio Hayashi, Yasumori Kutsumi, Koji Oida and Ryoyu Takeda (The Second Department of Internal Medicine, School of Medicine, Kanazawa University 13-1, Takaramachi, Kanazawa City, Japan 920, Department of Internal Medicine, Cancer Research Institute, Kanazawa University) *Artery 9*(2):132–150 (1981). By means of sequential ultracentrifugation of plasma from 85 control healthy Japanese subjects and from 15 maternal infant pairs, very low density lipoprotein (VLDL: $d < 1.006$ g/ml), low density lipoprotein (LDL: $1.006 < d < 1.063$ g/ml) and high density lipoprotein (HDL: $d > 1.063$ g/ml) were obtained and analyzed for cholesterol (Ch), triglyceride (TG), phospholipid (PL) and apolipoprotein B (Apo B). These results were compared to values in Western countries reported in the literature. HDL-Ch/LDL-Ch of adult Japanese was much higher than that of subjects in Western countries, especially in males. Umbilical cord (U) plasma-Ch, TG, PL and Apo B were 73.3 ± 15.9, 51.0 ± 10.4, 110.0 ± 21.0 and 47.6 ± 15.7 (mean ± S.D.) mg/100 ml, respectively. In cord blood the concentrations of Ch, TG and PL in all lipoprotein density classes were less than one half of those in normal adults. Cord blood has higher levels of HDL-Ch and lower levels of LDL-Ch than normal adults. Therefore, HDL-Ch/LDL-Ch ratio in cord blood (1.58 ± 0.32) was far higher than the ratio in normal adults (0.50 ± 0.11). In cord blood, HDL was the predominant Ch, TG and PL carrying lipoprotein. HDL-Ch/LDL-Ch ratios were 1.58 in Japan, 1.14 in USA, 0.89 in Australia and 0.69 in GDR. HDL-Ch/LDL-Ch ratio in Japanese cord blood was the highest among the values reported in the literature. The relationships between maternal and infant lipoproteins were more significant in HDL than in VLDL or LDL. The positive correlation between maternal HDL and infant HDL may suggest the possibility of placental transfer of intact HDL particles.

ARACHIDONIC ACID 15-LIPOXYGENASE FROM RABBIT PERITONEAL POLYMORPHONUCLEAR LEUKOCYTES: PARTIAL PURIFICATION AND PROPERTIES. Shuh Narumiya, John A. Salmon, Frank H. Cottee, Barry C. Weatherley, and Roderick J. Flower (From the Department of Prostaglandin Research, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, United Kingdom) *The Journal of Biological Chemistry 256*(18): 9583–9592 (1981). Arachidonic acid 15-lipoxygenase was purified from rabbit peritoneal polymorphonuclear leukocytes. The enzyme was recovered in the cytosol fraction after sonication and purified about 250-fold by acetone precipitation, column chromatography on CM52, Sephadex G-150, and hydroxyapatite. The enzyme catalyzed the conversion of arachidonic acid to 15-hydroperoxy-5,8,11,13-eicosatetraenoic acid (15-HPETE), which then decomposed to a mixture of 15-hydroxy-5,8,11,13-eicosatetraenoic acid (15-HETE), 15-keto-5,8,11,13-eicosatetraenoic acid, 13-hydroxy-14,15-epoxy-5,8,11-eicosatrienoic acid, and 11,14,15-trihydroxy-5,8,12-eicosatrienoic acid. The enzyme was specific for oxygenation at carbon 15 of arachidonic acid. The apparent molecular weight of the enzyme was about 61,000 as measured by Sephadex G-150 gel filtration chromatography. The enzyme was sensitive to sulfhydryl-blocking reagents such as *p*-chloromercuribenzoic acid. The enzyme activity was inhibited by eicosatetraynoic acid (ETYA) or 3-amino-1-(*m*-(trifluoromethyl)-phenyl)-2-pyrazoline (BW755C), but not by indomethacin up to 200 μg/ml.

ACIDIC PHOSPHOLIPIDS, UNSATURATED FATTY ACIDS, AND LIMITED PROTEOLYSIS MIMIC THE EFFECT OF CALMODULIN ON THE PURIFIED ERYTHROCYTE Ca^{2+}-ATPase. V. Niggli, E.S. Adunyah, and E. Carafoli (Laboratory of Biochemistry, Swiss Federal Institute of Technology, CH-8092, Zürich, Switzerland) *J. Biol. Chem. 256*(16):8588–8592 (1981). The purified Ca^{2+}-pumping ATPase of human erythrocyte membranes (Niggli, V., Adunyah, E.S., Penniston, J.T., and Carafoli, E. (1981) *J. Biol. Chem. 256*:295–401) can be stimulated, in the absence of calmodulin, by other treatments. 1. A variety of acidic phospholipids (phosphatidylserine, cardiolipin, phosphatidylinositol, and phosphatidic acid) stimulate the V_{max} and decrease the $K_m(Ca^{2+})$ of the isolated enzyme to the same extent as calmodulin. Unsaturated fatty acids (oleic and linoleic acid) have the same effect as phospholipids but at lower concentrations. Neutral phospholipids (phosphatidylcholine, sphingomyelin, and phosphatidylethanolamine) have no effect on the enzyme. The minimal proportion of acidic phospholipids in the environment of the enzyme necessary for full stimulation is about 40%. 2. The isolated enzyme, after reconstitution in phosphatidylcholine liposomes in the absence of calmodulin, can be activated by limited proteolysis. The trypsinized enzyme has the same high V_{max} and high affinity for Ca^{2+} of the enzyme in the presence of calmodulin.

TRANSBILAYER DISTRIBUTION IN SMALL UNILAMELLAR PHOSPHATIDYLGLYCEROL-PHOSPHATIDYCHOLINE VESICLES. J.R. Nordlund, C.F. Schmidt, and T.E. Thompson (From the Department of Biochemistry, University of Virginia School of Medicine, Charlottesville, Virginia 22908) *Biochemistry 20*:6415–6420 (1981). The transbilayer distribution of the phospholipids in small unilamellar vesicles comprised of egg phosphatidylglycerol

(PG) and egg phosphatidylcholine (PC) was ascertained by ^{31}P NMR. These vesicles, containing 10–75 mol % PG, were formed by sonication (pH 7.6) and fractionated by centrifugation. Data from spectra accumulated in the presence and absence of a paramagnetic shift reagent, Mn^{2+}, indicated that the phospholipids are randomly arranged across the bilayer. The absence of compositional asymmetry, which contradicts earlier reports, is also exhibited by small unilamellar vesicles (50 mol % PG) prepared by the rapid ethanol injection method. Control experiments showed that Mn^{2+} did not induce fusion, permeate the vesicles, or cause the phospholipids to migrate across the bilayer. It has been proposed that the transbilayer distribution of charged phospholipids in membranes is a consequence of the different surface charge densities on the opposing sides of the membrane. Our results suggest that it is the difference in the effective polar headgroup volumes of the components rather than the net charge of one component that determines the packing constraints for mixtures of phospholipids with the same acyl chains, at least in highly curved bilayers.

SIDE CHAIN HYDROXYLATION OF C_{27}-STEROIDS AND VITAMIN D_3 BY A CYTOCHROME P-450 ENZYME SYSTEM ISOLATED FROM HUMAN LIVER MITOCHONDRIA. Helge Oftebro, Kristin Saarem, Ingemar Björkhem, and Jan I. Pedersen (Institute for Nutrition Research, School of Medicine, University of Oslo, Blindern, Oslo 3, Norway and Department of Clinical Chemistry and the Research Center at Huddinge Hospital, Karolinska Institute, Stockholm, Sweden) *J. Lipid Res. 22*:1254–1264 (1981). The present study was undertaken to obtain information on the involvement of cytochrome P-450 in the 26-hydroxylation of bile acid intermediates and in the 25-hydroxylation of vitamin D_3 in human liver mitochondria. Cytochrome P-450 was solubilized from human liver mitochondria and purified two times to a specific content of 0.125 nmol per mg protein. Furthermore, a ferredoxin was isolated from the mitochondria and partly purified. This iron-sulfur protein had properties similar to bovine adrenal ferredoxin. A mitochondrial NADPH-ferredoxin reductase was also isolated and purified to homogeneity. This enzyme was a flavoprotein with properties very similar to the bovine adrenal NADPH-ferredoxin reductase. The cytochrome P-450 preparation catalyzed 26-hydroxylation of C_{27}-steroids and 25-hydroxylation of vitamin D_3 when reconstructed with NADPH, the ferredoxin and the ferredoxin reductase. With different substrates the following turnover numbers (nmol product × nmol P-450^{-1} × min^{-1}) were found: cholesterol, 8; 5-cholestene-3β,7α-diol, 10; 7α-hydroxy-4-cholesten-3-one, 23; 7α,12α-dihydroxy-r-cholesten-3-one, 27; 5β-cholestane-3α,7α-diol, 28; 5β-cholestane-3α,7α, 12α-triol, 41; and vitamin D_3, 0.16. The hydroxylation reactions were inhibited by CO and metryapone. The human liver mitochondrial ferredoxin and ferredoxin reductase could be replaced by adrenal ferredoxin and adrenal ferredoxin reductase without reduction of activity, but they could not be replaced by microsomal NADPH-cytochrome P-450 reductase. It is concluded that human liver mitochondria contain cytochrome P-450 involved in the oxidation of the side chain of C_{27}-steroids and vitamin D_3.

ARACHIDONIC ACID METABOLISM IN RABBIT RENAL CORTEX: FORMATION OF TWO NOVEL DIHYDROXYEICOSATRIENOIC ACIDS. Ernst H. Oliw, John A. Lawson, Alan R. Brash, and John A. Oates (From the Department of Parmacology, Vanderbilt University, School of Medicine, Nashville, Tennessee 37232 and the Department of Pharmacology, Karolinska Institutet, S-10401 Stockholm, Sweden) *The Journal of Biological Chemistry 256*(19): 9924–9931 (1981). [1-^{14}C] Eicosatetraenoic (arachidonic) acid was incubated with a low speed (17,000 × *g*) rabbit renal cortical subernatant or with a cortical microsomal suspension fortified with NADPH for 15 min at 37°C. The products which were less polar than prostaglandins on reversed phase high performance liquid chromatography were identified by gas chromatography-mass spectrometry. Both the fortified microsomes and the low speed supernatant formed significant amounts of two novel metabolites, 11,12-dihydroxy-5,8,14-eicosatrienoic acid and 14,15-dihydroxy-5,8,11-eicosatrienoic acid. Other identified products were 19- and 20-hydroxyeicosatetraenoic acid, 19-oxoeicosatetraenoic acid, and in the low speed supernatant, eicosatetraen-1,20-dioic acid. The metabolites were not formed in significant amounts by high speed cortical supernatant or by nonfortified cortical microsomes. Carbon monoxide inhibited formation of these compounds, indicating that they may be formed by the cytochrome P-450-linked renal monooxygenase systems.

ROLE OF THE ENOYL REDUCTASE DOMAIN IN THE REGULATION OF FATTY ACID SYNTHASE ACTIVITY BY INTERDOMAIN INTERACTION. A.J. Poulose and P.E. Kolattukudy (Institute of Biological Chemistry and Biochemistry/Biophysics Program, Washington State University, Pullman, WA 99164) *J. Biol. Chem. 256*(16):8379–8383. Fatty acid synthase from the uropygial gland of goose was inhibited by increasing the [NADP]:[NADPH] ratio. NADP inhibition of the overall activity of fatty acid synthase, the ketoreductase, and the enoyl reductase were competitive with respect to NADPH with K_i values of 6 μM, 40 μM, and 260 μM, respectively. The other component activities of fatty acid synthase were not affected by NADP with one exception; the condensing activity was severely inhibited. This inhibition was noncompetitive with respect to malonyl-CoA and hexanoyl-CoA. The inhibition of condensing activity by NADP was abolished by selective modification of the NADPH binding site of the enoyl reductase domain with pyridoxal phosphate. That the binding of NADPH, which triggers dimerization of fatty acid synthase peptides, occurs at the enoyl reductase domain was shown by the inability of NADPH to dimerize the pyridoxal phosphate-modified enzyme although this enzyme could be dimerized by high ionic strength. Proteolysis of the native enzyme was inhibited by NADPH but the proteolysis of the modified enzyme was not. These results strongly suggest that enoyl reductase domain of fatty acid synthase plays a key role in the interdomain interactions which regulates the activity of the enzyme via the [NADP]:[NADPH] ratio.

CHOLESTEROL, COCONUTS, AND DIET ON POLYNESIAN ATOLLS: A NATURAL EXPERIMENT: THE PUKAPUKA AND TOKELAU ISLAND STUDIES. I.A. Prior, F.D. Davidson, C.E. Salamond, Z. Czochanska (Epidemiology Unit, Wellington Hospital, Health Dept., and the Chemistry Division, Dept. of Scientific and Industrial Research, Wellington New Zealand) *Am. J. Clin. Nutr. 34*(11):1552–1561 (1981). Two populations of Polynesians living on atolls near the equator provide an opportunity to investigate the relative effects of saturated fat and dietary cholesterol in determining serum cholesterol levels. The habitual diets of the atoll dwellers from both Pukapuka and Todelau are high in saturated fat but low in dietary cholesterol and sucrose. Coconut is the chief source of energy for both groups. Tokelauans obtain a much higher percentage of energy from coconut than the Pukapukans, 63% compared with 34%, so their intake of saturated fat is higher. The serum cholesterol levels are 35 to 40 mg higher in Tokelauans than in Pukapukans. These major differences in serum cholesterol levels are considered to be due to the higher saturated fat intake of the Tokelauans. Analysis of a variety of food samples, and human fat biopsies show a high lauric (12:0) and myristic (14:0) content. Vascular disease is uncommon in both populations and there is no evidence of the high saturated fat intake having a harmful effect in these populations.

INTERACTION OF FREE CHOLESTEROL AND APOPROTEINS OF LOW AND HIGH DENSITY LIPOPROTEINS WITH ISOLATED RABBIT HEPATOCYTES. Jean Pruss O'Malley, Patricia A. Soltys, and Oscar W. Portman (Department of Nutrition and Metabolic Diseases, Oregon Regional Primate Research Center, Beaverton, OR 97006 and Department of Biochemistry, Oregon Health Sciences University, Portland, OR 97201) *J. Lipid Res. 22*: 1214–1224 (1981). Primary cultures of rabbit heptocytes were incubated with rabbit high density (HDL) and low density (LDL) lipoproteins in order to compare the surface transfer of free cholesterol with the uptake of apoproteins. Hepatocytes were maintained for various intervals with either LDL or HDL which contained both ^{125}I-labeled protein and free [4-^{14}C] cholesterol. After a 3-hr incubation with an LDL concentration equivalent to 25% of the normal rabbit serum level, the percentage of media ^{14}C in hepatocytes was 2.3 times greater than the percentage of ^{125}I; cells that had been incubated with HDL showed an eight-fold selectivity for ^{14}C. Although the influx of free cholesterol from HDL was greater than that from LDL, there was no difference between the uptake of LDL protein and of HDL protein. The degradation of lipoproteins labeled with [^{3}H]-leucine or ^{125}I was compared. Hepatocytes incubated with lipoproteins labeled with [4-^{14}C] cholesterol showed a greater influx of cholesterol from HDL_2 than from LDL. The efflux of labeled cellular cholesterol was also greater to HDL_2 than to LDL, whether the cellular cholesterol was labeled by prior exchange with labeled HDL_2 or by endogenous synthesis of cholesterol from [2-^{3}H] mevalonic acid lactone.

THE EFFECTS OF SUBFRACTIONS OF HIGH DENSITY LIPOPROTEIN ON CHOLESTEROL EFFLUX FROM CULTURED FIBROBLASTS: REGULATION OF LOW DENSITY LIPOPROTEIN RECEPTOR ACTIVITY. John F. Oram, John J. Albers, Marian C. Cheung, and Edwin L. Bierman (From the Division of Metabolism and Endocrinology and the Northwest Lipid Research Clinic, Department of Medicine, University of Washington School of Medicine, Seattle, Washington 98195) *The Journal of Biological Chemistry 256*(16):8348–8356 (1981). When cultured human fibroblasts were incubated with medium of varying composition, the activity of the low density lipoprotein (LDL) receptor was strongly correlated with the amount of cholesterol that egressed from the cell. Particles isolated from lipoprotein-deficient serum ($d > 1.25$)

by anti-apoprotein (apo) A-I affinity chromatography were as effective as the $d > 1.25$ serum in activating the LDL receptor, suggesting that the acceptors for cellular cholesterol in human lipoprotein-deficient serum are apo A-I-containing particles. Incubations with high density lipoprotein (HDL) subfractions designated as "very high" density lipoprotein (VHDL, $d = 1.21 - 1.25$) and HDL_3 ($d = 1.100 - 1.21$) enhanced LDL receptor activity while incubations with HDL_2 ($d = 1.063 - 1.100$) had a slight inhibitory effect. Based on apo A-I content, VHDL was a more potent activator than HDL_3. Incubations with HDL_3 also enhanced the rate of sterol synthesis, inhibited cholesterol esterification, and decreased cell cholesterol, while incubations with HDL_2 had the opposite effect. When apo E was removed from HDL by heparin-Sepharose affinity chromatography, HDL_3 retained its ability to activate the LDL receptor while HDL_2 was still inhibitory. Addition of chloroquine and compactin to the medium, to block supply of cholesterol to the cell, resulted in cell death between 12 and 24 h of incubation with VHDL and HDL_3, but not with HDL_2. With blocking agents present, incubations with VHDL, but not HDL_2, decreased the cellular free cholesterol content within 12 h. These results suggest that net efflux occurs only in the presence of HDL particles from $d > 1.100$ serum fractions. The greater the density of the apo A-I-containing particles, the greater the ability to remove cholesterol from the cells.

THERMODYNAMICS OF LIPID-PROTEIN ASSOCIATION: THE FREE ENERGY OF ASSOCIATION OF LECITHIN WITH REDUCED AND CARBOXYMETHYLATED APOLIPOPROTEIN A-II FROM HUMAN PLASMA HIGH DENSITY LIPOPROTEIN. Henry J. Pownall, Diane Hickson, and Antonio M. Gotto, Jr. (From the Department of Medicine, Baylor College of Medicine, and The Methodist Hospital, Houston, Texas 77030) *The Journal of Biological Chemistry 256*(19):9849–9854 (1981). Apolipoprotein A-II is an apoprotein which in human plasma high density lipoproteins exists as a disulfide-linked dimer of 17,400 molecular weight. It is known to spontaneously bind phospholipids with an affinity that has so far eluded measurement. We have prepared and isolated ^{3}H-reduced and carboxymethylated apoA-II ([^{3}H] RCM-A-II, M_r = 8700) and measured its free energy of association, ΔG_a, by equilibrium methods. The ΔG_a values were measured for dimyristoyl phosphatidylcholine multilayers (-7.1 ± 0.4 kcal) and single bilayer vesicles (-8.0 ± 0.2 kcal), bovine brain sphingomyelin vesicles (-7.2 ± 0.4 kcal), brominated egg lecithin in the absence (-8.7 ± 0.4 kcal) and presence of 33 mol % cholesterol (-7.9 ± 0.4 kcal), and human plasma high density lipoproteins (-7.3 ± 0.5 kcal). With dimyristoyl phosphatidylcholine in 0.3 M guanidine-HCl, ΔG_a decreased to -6.1 ± 0.3 kcal. The free energy of association was insensitive to temperature even close to T_a where a large enthalpy of association has been observed by microcalorimetry. These results represent the first quantitative measurement of the affinity of an apolipoprotein for a lipid or lipoprotein; the measured values are much less than those based upon the sum of the free energies of transfer of the individual amino acid side chains of RCM-A-II. These results suggest that the free energy of association of RCM-A-II with lipids and lipoproteins is entropy-driven, largely due to the hydrophobic effect; that the enthalpy of association is compensated by an additional entropy term; and that the hydrophobic amino acid residues of RCM-A-II associate with a region of the lipid or lipoprotein that is much more polar than the interior of a lipid bilayer. This region must include the bilayer surface and the first 4 or 5 methylene units. Our results show that RCM-A-II transfers from the phospholipid phase to the aqueous phase, and suggests that native apoA-II probably does not, or does so at a much lower rate.

EFFECTS OF CASEIN VERSUS SOY PROTEIN DIETS ON SERUM CHOLESTEROL AND LIPOPROTEINS IN YOUNG HEALTHY VOLUNTEERS. Joop M.A. van Raaij, M.Sc., Martijn B. Katan, Ph.D., Joseph G.A.J. Hautvast, M.D.D.N. (Cantab), and Ruud J.J. Hermus, Ph.D. (From the Department of Human Nutrition, Agricultural University, 6703 BC Wageningen, The Netherlands) *The American Journal of Clinical Nutrition 34*:1261–1271 (1981). The effects of casein and soy protein on serum cholesterol levels and lipoprotein composition were studied in 69 healthy volunteers (18 to 28 yr of age) under strict dietary control. Subjects were fed for 6 wk on diets containing 13% of energy as protein, 38% as fat (P/S ratio = 0.6) and about 380 mg cholesterol per day. Of the protein in the diets 65% consisted of casein or soy protein or a 2:1 mixture of casein and soy protein. After a control period of 10 days during which all the subjects received the casein-soy diet, 20 subjects continued on this diet for the next 4 wk as a base-line control, 25 subjects switched to the casein diet, and the remaining 24 subjects switched to the soy diet. Both food records and chemical analysis of double portions revealed that the diets were completely identical except for the type of protein. Average serum cholesterol levels at the end of the control period were 152 ± 27 mg/dl (3.93 ± 0.69 mmol/l) and 153 ± 23 mg/dl (3.95 ± 0.60 mmol/l) (mean ± SD) for the casein and soy group, respectively. At the end of the test period the levels were 149 ± 24 and 150 ± 23 mg/dl, respectively; thus there was no significant change on either diet. On the casein diet there was no change in the low-density lipoprotein cholesterol concentration, and only a slight, nonsignificant increase in the high-density lipoprotein cholesterol concentration. On the soy diet, however, there was a significant decline in low-density lipoprotein-cholesterol (-6.6 mg/dl; -0.17 mmol/l) and a significant increase in high-density lipoprotein-cholesterol (+5.8 mg/dl; +0.15 mmol/l). The decline in low-density lipoprotein cholesterol in the soy group was significantly different from the small change in the casein group, but the difference in increase in high-density lipoprotein cholesterol in the soy and the casein group was only weakly significant. This suggests that soy protein could have a slight beneficial effect on the distribution of cholesterol over the various lipoprotein fractions, even at constant total cholesterol concentration.

RELATIONSHIP OF WEIGHT LOSS AND CIGARETTE SMOKING TO CHANGES IN HIGH-DENSITY LIPOPROTEIN CHOLESTEROL. S.W. Rabkin, E. Boyko, and D.A. Streja (Depts. of Medicine and Social and Preventive Medicine, Univ. of Manitoba, Winnipeg, Manitoba, Canada R3E OZ3) *Am. J. Clin. Nutr. 34*(9):1764–1768 (1981). To determine the effect of weight loss on serum high-density lipoprotein cholesterol (HDL-C), we measured serum HDL-C as well as total cholesterol and triglycerides in 65 subjects (56 women and 9 men, mean age 41.1 ± 1.5 (±SEM yr) before and after a weight reduction program. At entry into the program there was a significant correlation between HDL-C and several indices of overweight—relative weight, body mass index and sum of skinfold thickness. For all subjects, despite a significant weight loss of 4.5 kg or 5.8% of initial body weight and significant decrease in sum of skinfold thickness. For women, but not men, a weak negative correlation between change in HDL-C and change in weight or percentage change in weight was observed. However, in the subset of women who were current cigarette smokers a significant ($p<0.01$) correlation was observed between change in HDL-C and change in weight ($r= -0.876$) and percentage change in weight ($r= =0.881$). Thus a modest weight loss is not usually associated with a significant increase in serum HDL-C concentration except in cigarette smoking women.

ON DETERMINING THE EXTENT OF SIDE-POOL SYNTHESIS IN A THREE-POOL MODEL FOR WHOLE BODY CHOLESTEROL KINETICS. Rajasekhar Ramakrishnan, Ralph B. Dell, and DeWitt S. Goodman (Departments of Pediatrics and Medicine and the Arteriosclerosis Research Center, College of Physicians and Surgeons, Columbia University, New York, NY) *J. Lipid Res. 22*: 1174–1180 (1981). Whole body cholesterol turnover is well described by a three-pool model. This model has eight unknown parameters: three masses, three synthesis rates, and two intercompartmental exchange rates. Only six parameters can be estimated by fitting the model to the plasma specific radioactivity-time curve which results from the intravenous injection of labeled cholesterol. Additional information is obtained if a precursor of cholesterol, labeled with a different isotope, is also injected. Equations are derived to enable the calculation of all eight model parameters from the two sum-of-exponentials equations that are fitted to the two tracer curves. The characteristics of a satisfactory precursor are discussed.

OVARIAN AND UTERINE LIPIDS OF RATS ADMINISTERED EXCESS VITAMIN A. D.N. Rao and U.K. Misra (Dept. of Biochemistry, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantinagar, U.P. India) *Acta Vitaminol. Enzymol. 3*(1):3–7 (1981). The effect of administering 33 mg retinol either alone or with 10 mg ascorbic acid for two days on neutral lipids and phospholipids of ovary and uterus and on the incorporation of acetate-1 ^{14}C and $NaH_2{}^{32}PO_4$ into the lipids has been studied. Vitamin A reduced ovary and uterus weight. Ascorbic acid prevented the weight decrease only in the ovary. Vitamin A increased total cholesterol, esterified cholesterol, triglycerides, phosphatidyl choline and phospatidyl ethanolamine in ovary but in uterus all the neutral lipids were increased and phospholipids decreased. Vitamin C administration did not affect vitamin A induced changes in phospholipids. Both the cholesterogenesis and lipogenesis from acetate-1 ^{14}C was increased in ovary and uterus of rats given vitamin A. Incorporation of $NaH_2{}^{32}PO_4$ was increased in phosphatidyl choline of ovary and uterus rats given vitamin A.

KETONE BODIES, GLUCOSE AND GLUTAMINE AS LIPOGENIC PRECURSORS IN HUMAN DIPLOID FIBROBLASTS. W. Douglas Reed, H. Ronald Zielke, Peter J. Baab and Pinar T. Ozand (Department of Pediatrics, University of Maryland School of Medicine, Baltimore, MD; and the Walter P. Carter Center Research Unit, Baltimore, MD 21201) *Lipids 16*(9):677–683 (1981). Incorporation of [^{14}C] from acetoacetate, D(-)- and L(+)-3-hydrox-

ybutyrate, glucose, glutamine, acetate and palmitate in cellular lipids were studied in cultures in human diploid fibroblasts (HDF). The results showed that acetoacetate was 2–10 times more effective as a lipogenic precursor than was either D- or L-3-hydroxybutyrate. Its extent of incorporation into lipids was 2- to 8-fold more than the other precursors examined under conditions when the overall rates of nonsaponifiable and saponifiable lipogenesis as measured by ^3H_2O incorporation were essentially unchanged. Acetoacetate supported both saponifiable and nonsaponifiable lipid syntheses with half-saturation values (K_m app.) of 185 μM and 30 μM, respectively. Glucose stimulated acetoacetate incorporation into lipids whereas, conversely, acetoacetate inhibited [^{14}C]glucose incorporation into lipids. The presence of low density lipoproteins (LDL) cholesterol (@40 μg cholesterol/mL) inhibited the incorporation of [^{14}C] from acetoacetate 56% into nonsaponifiable lipids; the inhibition was consistently higher (75%) when [^{14}C]glucose or glutamine were the precursors. The loss of 3-hydroxy-3-methyl-glutaryl CoA (HMG CoA) reductase activity upon addition of LDL-cholesterol was greater than the suppression of [^{14}C] incorporation from acetoacetate or glucose into nonsaponifiable lipids. In the presence of glucose, [^{14}C]acetoacetate was incorporated into 3-βOH sterols (digitonin precipitable) 7.7 ± 1.1 times more effectively than was [^{14}C]glucose. The results suggest that HDF would be a suitable model to investigate the effects of various precursors of HMG CoA on the rate of cholesterol biosynthesis.

INCORPORATION INTO LIPID CLASSES OF PRODUCTS FROM MICROSOMAL DESATURATION OF ISOMERIC *TRANS*-OCTADECENOIC ACIDS. T. Riisom and R.T. Holman (The Hormel Institute, University of Minnesota, Austin, MN 55912) *Lipids 16*(9): 647–654 (1981). The microsomal desaturation of positional isomers of *trans*-octadecenoic acids is effected by the Δ9-desaturase and, with concomitant geometric isomerization, *cis,trans*- and *cis,cis*-octadecadienoic acids of unusual structure are formed. Incorporation of the substrates and their products into lipids varied from 50.5% for incubations with 14–18:1 to 81.0% for 6–18:1. A detailed study of the composition of each of the major lipid classes, i.e., phospholipids, triacylglycerol and cholesteryl esters, as well as the composition of the free fatty acid fraction, revealed a complex picture. Generally, the *c,c*-18:2 products were enriched in the phospholipid fraction, whereas the *c,t*-18:2 appeared preferentially in cholesteryl esters. The 18:1 substrates themselves did not show marked preferences for any of the lipid classes. Phospholipase A_2 action on phosphatidylcholine and phosphatidylethanolamine demonstrated enrichment of the *c,c*- and the *c,t*-18:2 formed by desaturation of *t*11–18:1 varied from this pattern, probably due to their conjugated double bond structures. Linoleic acid, *c*9,*c*12–18:2, formed during desaturation of *t*12–18:1, surprisingly showed enrichment in the 1-position of phosphatidylcholine. Incubation experiments with *t*5- and *t*6-isomers using liver microsomes from rats fed a corn-oil-supplemented diet showed conversion and incorporation rates similar to the rates obtained with microsomes from EFA-deficient rats. The fatty acid composition of lipid classes and the distributions of products and substrate between the 1- and 2-positions of phosphatidylcholine also agreed with results obtained using microsomes from EFA-deficient rats.

IN VIVO SYNTHESIS OF LIPID-LINKED OLIGOSACCHARIDES IN THE LIVERS OF NORMAL AND VITAMIN A-DEFICIENT RATS. Gloria Chi Rosso, Charles J. Bendrick, and George Wolf (From the Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139) *The Journal of Biological Chemistry 256*(16):8341–8347 (1981). [1-^{14}C]Glucosamine, [2-^{3}H]mannose, or [^{14}C]galactose were injected into vitamin A-deficient and pair-fed control rats at an early stage of deficiency. The livers were homogenized, centrifuged, and the oligosaccharide-lipids extracted into chloroform:methanol:water (10:10:3). This fraction, when labeled with glucosamine, reached a maximum at 35 min after injection, and remained constant to 110 min. The fraction was chromatographed on a DEAE-cellulose-acetate column and was eluted at an ammonium acetate concentration of 20 mM, corresponding to oligosaccharide bound to dolichylpyrophosphate. Fractionation on Bio-Gel P-4 of the oligosaccharide produced by mild acid hydrolysis of the oligosaccharide-lipid, gave a major peak (I) followed by a broad minor peak (II) of smaller molecular weight. Peak II from deficient liver was always greater than from normal liver (25.91 ± 8.89 *versus* 6.28 ± 5.46% of total oligosaccharide). In a recovery experiment, vitamin A was given intragastrically to deficient rats. It caused virtual disappearance of peak II between 4 and 8 h after administration. Rechromatography of peak II on a longer column yielded several distinct peaks of smaller molecular weight than peak I, with the principal peak (peak F) having a K_d of 0.486, corresponding to that of a standard oligosaccharide (mannose)$_5$(*N*-acetylglucosamine)$_2$. Peak I had a K_d of 0.285, corresponding to that of a standard oligosaccharide (glucose)$_3$(mannose)$_9$(*N*-acetylglucosamine)$_2$. We conclude that normal rat liver accumulates an oligosaccharide linked to dolichylpyrophosphate consistent with the structure (glucose)$_3$(mannose)$_9$(*N*-acetylglucosamine)$_2$, whereas vitamin A deficiency causes an increased pool of smaller molecular weight oligosaccharide-lipids, the principal one being consistent with (mannose)$_5$(*N*-acetylglucosamine)$_2$-dolichylpyrophosphate.

ON THE DIFFERENTIAL SUPPRESSION OF CHOLESTEROL SYNTHESIS BY LOW DENSITY LIPOPROTEIN IN B AND T LYMPHOCYTES. Ajit Sanghvi, Carl Wight and Vijay Warty (Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261) *Biochimica et Biophysica Acta 665*:48–52 (1981). Cholesterol synthesis and its suppression by low density lipoprotein cholesterol were measured in purified B and T peripheral blood lymphocytes. After preincubation for 53 h in lipoprotein-deficient serum, both B and T cells exhibited increased cholesterol synthesis as compared with synthesis measured in cells immediately after their isolation from blood and without preincubation with lipoprotein-deficient serum. The magnitude of this increase was far greater in T cells in comparison with that in B cells in all subjects studied. But, whereas there was an immediate and progressive suppression of cholesterol synthesis in lipoprotein-deficient serum-incubated T cells as the concentration of low density lipoprotein cholesterol in the medium was increased, synthesis in lipoprotein-deficient serum-incubated B cells remained insensitive to the presence of low density lipoprotein in the medium. Hydroxymethyl-glutaryl-CoA reductase activity was observed also to follow a similar pattern in both cell types. These observations may imply that one or more events, including binding of low density lipoprotein to its receptor, internalization and degradation of low density lipoprotein receptor complex finally leading to suppression of hydroxymethyl-glutaryl-CoA reductase activity and cholesterol synthesis, fail to take place in B cells.

MOUSE α_1-FETOPROTEIN AND ALBUMIN: A COMPARISON OF THEIR BINDING PROPERTIES WITH ESTROGEN AND FATTY ACID LIGANDS. Lia Savu, Claudine Benassayag, Geneviève Vallette, Névéna Christeff, and Emmanuel Nunez (From the Unité d'Enseignement et de Recherche Biomédicale des Saints-Pères, Paris 75006, France) *The Journal of Biological Chemistry 256*(18):9414–9418 (1981). The binding of estradiol-17β (E_2), diethylstilbestrol (DES), and polyene fatty acids, in particular arachidonate (C20:4), to α_1-fetoprotein (α-FP) and albumin purified from mouse embryo sera was studied using equilibrium dialysis and electrophoretic techniques. E_2, arachidonate, and DES all bind to α-FP, but with decreasing strength. E_2 is a high affinity, low capacity ligand (K_a ~ 0.8 × 10^8 M^{-1} and ~ 0.3 sites/mol of α-FP at 25°C); arachidonate is a weaker ligand disposing of more sites (K_a ~ 0.3 × 10^7 M^{-1} and 4–5 sites/mol of α-FP); the binding of DES is of comparatively low affinity and capacity (K_a ~ 0.2 × 10^7 M^{-1} and n ~ 0.7/mol of α-FP). In spite of different structures and equilibrium parameters, E_2, DES, and arachidonate are able to compete with each other for binding to the fetoprotein. The C22:4 and C22:6 fatty acids are also efficient concentration-dependent inhibitors of E_2 or DES binding. Albumin binds the fatty acids and DES, but equilibrium parameters are different from those of α-FP. In particular, arachidonate is a better ligand for albumin, where it interacts with at least two classes of apparent sites (K_{a_1} ~ 0.3 × 10^8 M^{-1} and n_1 ~ 1; K_{a_2} ~ 0.2 × 10^7 M^{-1} and n_2 ~ 30). In contrast to α-FP, albumin virtually does not bind E_2. Also no competition could be demonstrated between DES and fatty acid ligands for binding to albumin. None of the studied interactions, with either albumin or α-FP, was modified even by high doses of bilirubin. The possible functions of the various binding activities present in fetal sera in the process of growth are discussed.

THE EFFECTS OF LOW CHOLESTEROL, HIGH POLYUNSATURATED FAT, AND LOW FAT DIETS ON PLASMA LIPID AND LIPOPROTEIN CHOLESTEROL LEVELS IN NORMAL AND HYPERCHOLESTEROLEMIC SUBJECTS. E.J. Schaefer, R.I. Levy, N.D. Ernst, F.D. Van Sant, and H.B. Brewer, Jr. (Building 10, Room 7N-117, National Institutes of Health, Bethesda, MD 20205) *Am. J. Clin. Nutr. 34*(9):1758–1763 (1981). The effects of various cholesterol-lowering diets on plasma lipid and lipoprotein cholesterol levels were assessed in normal and hypercholesterolemic subjects. The base-line diet was an ad libitum hospital diet of normal composition. Diet A was a 20% protein, 40% carbohydrate, 40% fat, polyunsaturated:saturated fat ratio 0.1 to 0.3, 250 to 300 mg cholesterol diet, diet B was identical to diet A except that the polyunsaturated/saturated fat ratio was 1.8 to 2.2, and diet C was a 20% protein, 80% carbohydrate, very low fat (5 to 10 g), polyunsaturated/saturated fat ratio 0.1 to 0.3, 150 to 200 mg cholesterol diet. Diet A (low cholesterol) caused mean reductions in plasma, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol of 5.9, 5.6, and 6.3%, respectively, in 11 normal subjects. Diet B (low cholesterol, high polyunsaturated

fat) caused significant decreases in plasma cholesterol, LDL cholesterol and HDL cholesterol of 17.0, 16.2, and 17.4%, respectively, in 12 normal subjects; and reductions of 11.0, 10.8, and 17.1%, respectively, in 19 hypercholesterolemic subjects. Diet C (low cholesterol, very low fat) produced significant mean decreases in plasma, LDL, and HDL cholesterol of 26.7, 29.9, and 27.9%, respectively, in 11 normal subjects, and in 9 hypercholesterolemic patients of 22.6, 27.2, and 28.6%, respectively. The reductions in plasma cholesterol caused by these diets were therefore due to decreases in both LDL and HDL cholesterol with no significant changes in the LDL cholesterol:HDL cholesterol ratio.

KETONE BODIES, GLUCOSE AND GLUTAMINE AS LIPOGENIC PRECURSORS IN HUMAN DIPLOID FIBROBLASTS. W. Douglas Reed, H. Ronald Zielke, Peter J. Baab and Pinar T. Ozand (Department of Pediatrics, University of Maryland School of Medicine, Baltimore, MD and the Walter P. Carter Center Research Unit, Baltimore, MD 21201) *Lipids 16*(9):677–683 (1981). Incorporation of [^{14}C] from acetoacetate, D(-)- and L(+)-3-hydroxybutyrate, glucose, glutamine, acetate and palmitate in cellular lipids were studied in cultures in human diploid fibroblasts (HDF). The results showed that acetoacetate was 2–10 times more effective as a lipogenic precursor than was either D- or L-3-hydroxybutyrate. Its extent of incorporation into lipids was 2- to 8-fold more than the other precursors examined under conditions when the overall rates of nonsaponifiable and saponifiable lipogenesis as measured by $^{3}H_2O$ incorporation were essentially unchanged. Acetoacetate supported both saponifiable and nonsaponifiable lipid syntheses with half-saturation values (K_m app.) of 185 μM and 30 μM, respectively. Glucose stimulated acetoacetate incorporation into lipids whereas, conversely, acetoacetate inhibited [^{14}C] glucose incorporation into lipids. The presence of low density lipoproteins (LDL) cholesterol (@40 μg cholesterol/mL) inhibited the incorporation of [^{14}C] from acetoacetate 56% into nonsaponifiable lipids; the inhibition was consistently higher (75%) when [^{14}C] glucose or glutamine were the precrusors. The loss of 3-hydroxy-3-methyl-glutaryl CoA (HMG CoA) reductase activity upon addition of LDL-cholesterol was greater than the suppression of [^{14}C] incorporation from acetoacetate or glucose into nonsaponifiable lipids. In the presence of glucose, [^{14}C] acetoacetate was incorporated into 3-βOH sterols (digitonin precipitable) 7.7 ± 1.1 times more effectively than was [^{14}C] glucose. The results suggest that HDF would be a suitable model to investigate the effects of various precrusors of HMG CoA on the rate of cholesterol biosynthesis.

TIME COURSE OF CHANGES IN PORCINE MYOCARDIAL PHOSPHOLIPID LEVELS DURING ISCHEMIA: A REASSESSMENT OF THE LYSOLIPID HYPOTHESIS. Nisar A. Shaikh and Eugene Downar (From the Department of Medicine, Univeristy of Toronto, Toronto, Ontario, Canada) *Circ Res 49*:316–325 (1981). This study was performed to determine the early and delayed metabolic effects of myocardial ischemia on the major membrane phospholipids and to reassess the potential role of lysophospholipids in the genesis of malignant dysrhythmias induced by ischemia. Samples taken from in situ hearts before and at various intervals up to 40 minutes after abrupt ligation of LAD were extracted by the classical Folch technique with modifications to avoid artifactual lysophospholipid production and losses. Following thin layer chromatography of lipid extracts, phospholipid fractions were quantified by phosphorus estimation and lysophospholipids by a more sensitive method employing gas liquid chromatography. The total phospholipid content with the exception of lysophospholipids remained essentially constant throughout the early phases of acute ischemia, but fell by 6 and 14% after 8 and 24 hours, respectively. At 8 minutes, lysophospholipid levels in ischemic myocardium were significantly increased by 60% compared to pre-occlusion controls in the ischemic zone and by 25% in post-occlusion controls. They changed little thereafter. The molecular species of lysophospholipids remained unchanged throughout the period of ischemia studied. The mole fraction of other phospholipids as well as their fatty acyl and aldehyde profiles also were unchanged. Despite significant elevations in lysophospholipids levels, their absolute quantities were very small (0.6% of total phospholipid P) and 15-fold smaller than that reported in vitro to simulate electrophysiological manifestation of ischemia. However, such small amounts in vivo, if produced in the microenvironment of certain membrane-bound enzymes along with acidosis, hypoxia, and fatty acids, could be potentially deleterious to cell functions.

GLYCOSPHINGOLIPID-HIGH DENSITY LIPOPROTEIN 3 INTERACTIONS: II. CHARACTERIZATION OF THE GLYCOSPHINGOLIPID COMPONENT OF MODIFIED HIGH DENSITY LIPOPROTEIN. Betty W. Shen, Bill C.P. Kwok, and Glyn Dawson (From the Departments of Biochemistry, Pediatrics and Medicine, Joseph P. Kennedy Jr. Mental Retardation Research Center of the Pritzker School of Medicine, University of Chicago, Chicago, Illinois 60637) *The Journal of Biological Chemistry 256*(18):9705–9710 (1981). Incubation of [^{3}H] glycosphingolipid/egg phosphatidylcholine ([^{3}H] GSL/PC) mixed vesicles with human high density lipoprotein-3 (HDL_3) resulted in the formation of a GSL·HDL_3 complex which peaked at a density similar to native HDL[3] after density gradient ultracentrifugation. The transfer of either neutral glycolipid (*e.g.* galactosylceramide) or negatively charged glycolipid (*e.g.* monosialoganglioside G_{M1}) resulted in a lipid (GSL and PC)-enriched particle, deficient in apo-A-I and free cholesterol, but otherwise of very similar composition and properties to native HDL_3. The yield and density of the final product was influenced by the initial ratio of lipid to lipoprotein and the time of incubation. Quantitative analysis of the amount of GSL transfer as a function of lipid and lipoprotein concentrations revealed that only 69% of the glycolipid in the mixed vesicles was available for transfer. This is consistent with the concept of random distribution of lipids during the formation of mixed vesicles in which only the lipids in the outer layer of th vesicles are available for exchange. The kinetics of GSL and PC transfer was first order with respect to both lipid and lipoprotein concentrations, with a half-life in the range of 2–3 h. The external location of G_{M1} inserted into the HDL_3-like particle was evaluated by the ability of the complex to bind [^{14}C] acetylated cholera toxin. Neither native HDL_3 (in which the presence of surface glycolipids can be demonstrated by galactose oxidase-Na$B^{3}H_4$ labeling) nor a [^{3}H] galactosylceramide·HDL_3 complex could bind cholera toxin under the same conditions. Thus the oligosaccharide unit of G_{M1} was located on the surface of HDL_3 particles in a manner similar to that of cell membranes. [^{3}H] GSL/[^{14}C] PC·HDL_3 complexes also served as donors of [^{3}H] GSL and [^{14}C] PC to unlabeled GSL/PC mixed vesicles, indicating that the transferring process was reversible.

INTERACTION OF LIPOPROTEIN LIPASE WITH PHOSPHOLIPID VESICLES: ROLE OF APOLIPOPROTEIN C-II AND HEPARIN. K. Shirai, N. Matsuoka, and R.L. Jackson (Div. of Lipoprotein Research, Depts. of Pharmacology and Cell Biophysics, Biological Chemistry and Medicine, Univ. of Cincinnati Medical Center, Cincinnati, OH 45267) *Biochim. Biophys. Acta 665*:504–510 (1981). Lipoprotein lipase is bound to heparin-like molecules at the surface of capillary endothelial cells. For maximal activity, the enzyme requires apolipoprotein C-II, a protein constituent of triacylglycerol-rich lipoproteins. In this report, the interactions of apolipoprotein C-II, heparin and sonicated vesicles of dipalmitoylphosphatidylcholine with purified bovine milk lipoprotein lipase were studied by gel filtration on Bio-Gel A5m. In the presence of vesicles of dipalmitoylphosphatidylcholine (1 mg), lipoprotein lipase (25 μg) associated with phospholipids even in the absence of apolipoprotein C-ii. With limited phospholipid (40 μg), the amount of enzyme which associated with lipid decreased in the presence of apolipoprotein C-II (20 μg). Human plasma apolipoprotein C-III, another protein constituent of triacylglycerol-rich lipoproteins, also caused a decrease in the amount of enzyme associated with phospholipid. These results suggest that apolipoprotein C-II does not increase the activity of the enzyme by facilitating its interaction with a lipid interface. In the absence of lipid, lipoprotein lipase and apolipoprotein C-II (molar ration, 1:1) eluted from Bio-Gel A5m as two separate components. The interaction of heparin with lipoprotein lipase was studied using a specific [^{3}H] heparin, which was isolated by affinity chromatography on immobilized lipoprotein lipase; the [^{3}H] heparin eluted with 0.6 M NaCl. Specific [^{3}H] heparin coeluted with lipoprotein lipase when the enzyme was associated with phospholipid; the [^{3}H] heparin was released from the enzyme by 0.75 M NaCl.

DETERMINANTS OF HYPOCHOLESTEROLEMIC RESPONSE TO SOY AND ANIMAL PROTEIN-BASED DIETS. R.L. Shorey, B. Bazan, G.S. Lo, and F.H. Steinke (Graduate Nutrition Division, GEA 115, Univ. of Texas at Austin, Austin, TX 78712) *Am. J. Clin. Nutr. 34*(9):1769–1778 (1981). The effect of substituting soy for animal protein in mixed diets was determined in young men with mildly elevated plasma cholesterol, 218 to 307 mg/dl. The diets were low in cholesterol, 200 mg/day, with 13 to 16% of energy as protein, 30 to 35% as fat, and a polyunsaturated to saturated fat ratio of 0.5. Of protein 65% was from either mixed animal proteins or isolated soy protein products made comparable by the addition of extracted animal fats. Fresh egg yolk was added to balance the cholesterol content of the diets. Proteins from grains and vegetables were identical to both menus and contributed about 35% of dietary protein. Twenty of 24 subjects decreased plasma cholesterol at the end of the protocol. Subjects were classified as responders or nonresponders as a function of greater or lesser than mean reduction in cholesterol for the groups. Mean decreases in plasma cholesterol, 16 and 13%, for responders in the animal and soy groups were significant, $p < 0.01$ and 0.05, respectively. Responders in both groups had higher initial plasma cholesterol values than nonresponders. Although plasma high-density lipoprotein cholesterol decreased

slightly, the high-density lipoprotein cholesterol to cholesterol ratio (high-density lipoprotein cholesterol/total cholesterol) remained constant for most individuals. The hypocholesterolemic effects were similar for both animal and soy protein diets. Compared to previous dietary intake, responders significantly decreased dietary protein ($p<0.05$) and fat ($p<0.05$) while on the experimental diet. All groups significantly decreased dietary cholesterol.

RADIOIMMUNE PRECIPITATION OF 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE FROM CHINESE HAMSTER FIBROBLASTS. M. Sinensky and R. Torget (Eleanor Roosevelt Institute, University of Colorado Health Sciences Center, Denver, CO 80262) *J. Biol. Chem. 256*(22):11774–11779 (1981). Antibody prepared against 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase of rat liver can be shown to inhibit this enzyme in extracts prepared from cultured Chinese hamster ovary (CHO-K1) cells. The molecular weight (53,000) of the HMG-CoA reductase subunits of rat liver and Chinese hamster liver is identical with a (^{35}S)methionine-labeled polypeptide that can be precipitated from CHO-Kl lysates by this antibody used on conjunction with protein A Sepharose. It is shown that 25-hydroxycholesterol which lowers HMG-CoA reductase activity in cultured fibroblasts blocks the incorporation of labeled methionine into this polypeptide. Furthermore, the antibody immune precipitates two other polypeptides with molecular weights of 127,000 and 60,000. The latter polypeptide responds to 25-hydroxycholesterol in the same fashion as the 53,000-dalton polypeptide. In a dominant 25-hydroxycholesterol-resistant mutant of the CHO-Kl cell, 25-hydroxycholesterol did not inhibit incorporation of labeled methionine into either the 53,000- or 60,000-dalton polypeptides.

GLYCEROGLUCOLIPIDS OF THE MUCOUS BARRIER OF DOG STOMACH. Amalia Slomiany, Nina I. Galicki, Koichi Kojima, Zofia Banas-Gruszka and Bronislaw L. Slomiany (Gastroenterology Research Laboratory, Department of Medicine, New York Medical College, Research Center, Metropolitan Hospital, New York, NY 10029) *Biochimica et Biophysica Acta 665*:88–91 (1981). Distribution of glyceroglucolipids in the mucous barrier of dog stomach fundus, body and antrum was investigated. Surface mucus lining and preformed intracellular mucus were obtained by instillation of the ligated stomach compartments with 2 M NaCl. Lipids were extracted from the dialyzed and lyophilized samples, and the glycolipids were separated into neutral and acidic fractions. The glyceroglucolipids contained in each fraction were purified into individual components by thin-layer chromatography and were quantified. The content of glyceroglucolipids (μmol glucose/g protein) in the antral portion of the stomach was 2.1 times greater than that of the fundus and 3.4 times greater than that of the body. All three areas of the stomach contained neutral and sulfated glyceroglucolipids. However, the level of sulfated glycerolglucolipids (μmol glucose/g protein) was three times higher in the antrum as compared to the fundus and four times higher as compared to the body. The neutral and sulfated glyceroglucolipids were present in a molar ratio of 1.0:0.7 in the fundus, 1.0:1.1 in the body, and 1.0:1.5 in the antrum.

EFFECT OF LEAD INGESTION ON FUNCTIONS OF VITAMIN D AND ITS METABOLITES. C.M. Smith, H.F. DeLuca, Y. Tanaka, and K.R. Mahaffey (Dept. of Biochem., College of Agricultural and Life Sciences, Univ. of Wisconsin-Madison, Madison, WI 53706) *J. Nutr. 111*(8):1321–1329 (1981). A study of the effect of ingestion of lead on the metabolism and function of vitamin D was carried out in rats fed diets varying in calcium and phosphorus content. The ingestion of 0.82% lead as lead acetate suppressed plasma levels of 1,25-dihydroxycholecalciferol in rats fed either a low phosphorus or a low calcium diet while it had no effect on this parameter in rats fed either a high calcium diet or a normal phosphorus diet. Most important, the ingestion of lead totally blocked the intestinal calcium transport response to cholecalciferol, 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol. On the other hand, the ingestion of lead acetate had no influence on the mobilization of calcium from bone, the elevation of serum inorganic phosphorus and in the mineralization of rachitic bone in the same animals. Thus by the feeding of 0.82% lead as acetate, the physiologic responses to vitamin D and its metabolites in intestine and bone could be separated. Although the effect of 0.82% lead on the intestinal responses to vitamin D and its metabolites was greatest in animals fed a low calcium or a low phosphorus diet, it was present with all diets tested.

CATABOLISM OF THE APOLIPOPROTEINS OF HDL IN CONTROL AND NEPHROTIC RATS. Charles E. Sparks, Steven D. Tennenberg and Julian B. Marsh (Department of Physiology and Biochemistry, The Medical College of Pennsylvania, Philadelphia, PA 19129) *Biochimica et Biophysica Acta 665*:8–12 (1981). ^{125}I-labeled high density lipoprotein (HDL) from control rats, or from rats made nephrotic by puromycin aminonucleoside, was injected into control or nephrotic rats. At 5 and 20 h, the amount and distribution of label remaining in apolipoproteins HDL, A-I, E, A-IV and the C apolipoproteins was measured after ultracentrifugal isolation of HDL and SDS-polyacrylamide gel electrophoretic separation of each apolipoprotein. There were no significant differences in the removal rates of apolipoprotein HDL or of the individual apolipoproteins when the removal of HDL of controls was compared to HDL of nephrotics. HDL from nephrotic rats contains less than 10% of either the apolipoprotein A-IV or apolipoprotein E content of control HDL, indicating that neither apolipoprotein A-IV nor apolipoprotein E play a significant role in determining the catabolic fate of rat HDL. In severely nephrotic animals the apolipoprotein C content of HDL was reduced to 50% of control values and the apolipoprotein A-I content of HDL rose to 87% of the total apolipoprotein. The individual apolipoproteins of HDL from either nephrotics or controls were catabolized at the same rates irrespective of the degree of nephrosis or altered HDL apolipoprotein composition. The apparent fractional catabolic rates for apolipoprotein HDL and for each of the apolipoproteins, determined after 20 h, did not differ from one another, and all were reduced by half in the nephrotic rats compared to the normal controls. These results support the concept that HDL is catabolized as a particle mediated by apolipoprotein A-I recognition, and they reinforce earlier work indicating that increased synthesis is the dominant factor responsible for increased plasma HDL concentrations in experimental nephrosis.

INTERACTION BETWEEN MACROPHAGES AND AORTIC SMOOTH MUSCLE CELLS: ENHANCEMENT OF CHOLESTEROL ESTERIFICATION IN SMOOTH MUSCLE CELLS BY MEDIA OF MACROPHAGES INCUBATED WITH ACETYLATED LDL. O. Stein, G. Halperin and Y. Stein (Department of Experimental Medicine and Cancer Research, Hebrew University-Hadassah Medical School, and Lipid Research Laboratory, Department of Medicine B, Hadassah University Hospital, Jerusalem, Israel) *Biochimica et Biophysica Acta 665*:477–490 (1981). Mouse peritoneal macrophages were cultured for 24 h in Dulbecco-Vogt medium containing 10% calf serum. This medium was replaced with Dulbecco-Vogt medium containing 1% bovine serum albumin to which all subsequent additions were made. Medium changes, accompanied by appropriate additions, were made every 48 or 72 h and the media were used for incubation of aortic smooth muscle cells, prelabeled with [^{3}H] cholesterol. The amount of labeled cholesteryl ester in the smooth muscle cells incubated for 48 h with macrophage media which had been collected 48–144 h after addition of acetylated LDL was increased 3–4 times above that present prior to postincubation. A marked increment in cholesteryl ester mass occurred also after incubation of smooth muscle cells with macrophage media conditioned with acetylated LDL and this effect was shared by maleylated LDL, but not by other negatively charged compounds. The present results offer another view of the possible interactions between macrophages and smooth muscle cells. A modified lipoprotein, not recognized by smooth muscle cells, is ingested by macrophages, which leads to accumulation of esterified cholesterol. Part of the esterified cholesterol undergoes hydrolysis and is excreted back into the medium, leading to enrichment of the lipoproteins in the medium with free cholesterol. This enrichment with free cholesterol promotes cholesterol esterification in smooth muscle cells.

MODULATION OF ENZYME ACTIVITIES IN ISOLATED LYMPHOCYTE PLASMA MEMBRANES BY ENZYMATIC MODIFICATION OF PHOSPHOLIPID FATTY ACIDS. Marta Szamel and Klaus Resch (From the Institute for Virus Research, German Cancer Research Center, D-6900 Heidelberg, Federal Republic of Germany and Division of Molecular Pharmacology, Department of Pharmacology and Toxicology, Medical School of Hannover, D-3000 Hannover, Federal Republic of Germany) *The Journal of Biological Chemistry 256*(22)11618–11623 (1981). Plasma membranes were purified from calf thymus lymphocytes after disrupting the cells by nitrogen cavitation. The phospholipids were modified by incubation of the plasma membranes in the presence of lysophosphatidylcholine with increasing concentrations of the coenzyme A derivatives of long chain fatty acids. Control experiments documented that with the conditions used, all added acylcoenzyme As were converted nearly quantitatively into membrane-associated phosphatidylcholine. Lysophosphatidylcholine alone, or lysolecithin acyltransferase-mediated incorporation of palmitic acid (16:0) had no effects on any of the enzyme activities tested. Incorporation of oleic acid (18:1) had a small but consistent effect, similar to that obtained with incorporation of polyunsaturated fatty acids. Incorporation of linoleic acid (18:2) or arachidonic acid (20:4) markedly modulated plasma membrane-associated enzymes with a peak effect at the same degree of fatty acid substitution. The activity of (Na^+ + K^+)-ATPase was increased, which was reversed

to ground levels at higher incorporation rates. Concomitantly, the specific activity of Mg^{2+} ATPase, γ-glutamyltransferase, and alkaline *p*-nitrophenyl phosphatase were decreased. The data show that enzyme-driven phospholipid modification offers a physiological tool for the study of the interrelation of membrane proteins with the structure of phospholipids. Moreover, they suggest a crucial role of lysolecithin acyltransferase in the initiation of lymphocyte activation.

INTERMEDIATE-DENSITY LIPOPROTEIN AND CHOLESTEROL-RICH VERY LOW DENSITY LIPOPROTEIN IN ANGIOGRAPHICALLY DETERMINED CORONARY ARTERY DISEASE. R. Tatami, H. Mabuchy, K. Ueda, R. Ueda, T. Haba, T. Kametani, S. Ito, J. Koizumi, M. Ohta, S. Miyamoto, A. Nakayama, H. Kanaya, H. Oiwake, A. Genda, and R. Takeda (Second Dept. of Internal Medicine, Kanazawa Univ. School of Med., Kanazawa, Ishikawa, Japan) *Circulation 64*(6):1174–1184 (1981). The relationship between the concentrations of intermediate-density lipoprotein and other lipoproteins and the extent of coronary artery disease (CAD) was studied in 182 consecutive patients evaluated by selective coronary cineangiography. On univariate analysis, the extent of CAD correlated significantly and positively with very low density lipoprotein (VLDL) cholesterol, IDL cholesterol and low-density lipoprotein (LDL) cholesterol, and negatively with high-density lipoprotein (HDL) cholesterol. Analysis of four subgroups divided by IDL cholesterol and LDL cholesterol levels indicated that moderately increased levels of IDL cholesterol were closely associated with a high frequency of CAD. Moreover, multivariate regression analysis demonstrated that IDL cholesterol for men, LDL cholesterol for men and women and HDL cholesterol for men were significant variables of use in the final weighting procedure. IDL cholesterol was closely associated with cholesterol-rich VLDL. This study shows that IDL and cholesterol-rich VLDL combine to contribute to the development of CAD.

MEASUREMENT OF PHOSPHATIDYLCHOLINE TRANSFER PROTEIN IN RAT LIVER AND HEPATOMAS BY RADIOIMMUNOASSAY. Tom Teerlink, Ben J.H.M. Poorthuis, Theo P. Van der Krift and Karel W.A. Wirtz (Laboratory of Biochemistry, State University of Utrecht, Transitorium 3, Padualaan 8, 3508 TB Utrecht, The Netherlands) *Biochimica et Biophysica Acta 665*:74–80 (1981). An antiserum was raised against the phosphatidylcholine transfer protein from rat liver by immunization of rabbits. The antiserum was shown to be specific for this protein. A double-antibody radioimmunoassay from the phosphatidylcholine transfer protein was developed. In order to economize the use of second antibody (immunobeads), the specific anti-phosphatidylcholine transfer protein-IgG fraction isolated by affinity chromatography was used. Phosphatidylcholine transfer protein was labelled with ^{125}I by the glucose oxidase-lactoperoxidase method and purified from the reaction mixture by affinity chromatography. Approx. 80% of the tracer was immunoprecipitable. The operating range of the assay was from 4 to 50 ng of transfer protein. This assay was used to determine the levels of phosphatidylcholine transfer protein in the 105 000 × *g* supernatant fractions of rat liver and Morris hepatomas 7777,7787 and 9633. The values obtained for the tumors were in good agreement with results previously obtained by immunotitration of the phosphatidylcholine transfer activity (Poorthuis, B.J.H.M., Van der Krift, T.P., Teerlink, T., Akeroyd, R., Hostetler, K.Y. and Wirtz, K.W.A., Biochim. Biophys. Acta 600 (1980) 376–386). For normal and host liver, the values determined by the radioimmunoassay were 2–4-fold higher.

EFFECT OF PROBUCOL ON SERUM LIPOPROTEIN LEVELS IN NORMAL AND DYSLIPOPROTEINEMIC MICE. M. Tomikawa, T. Nakayasu, K. Tawara, and U. Abiko (Laboratory of Biochemistry, Research Institute, Daiichi Seiyaku Co., Ltd., Edogawa-ku, Tokyo 132, Japan) *Atherosclerosis 40*:101–113 (1981). The effect of probucol was studied on serum lipoprotein levels in normal and cholesterol-fed, hypercholesterolemic mice. In normal mice, probucol caused a significant reduction in LDL + VLDL cholesterol at daily doses about 25–50 mg/kg and also in HDL cholesterol at higher doses. In cholesterol-fed mice, probucol treatment decreased LDL + VLDL cholesterol at daily doses exceeding 200 mg/kg and also HDL cholesterol at a daily dose of 800 mg/kg. The ratio of LDL + VLDL cholesterol to HDL cholesterol was significantly reduced by treatment at 25–100 mg/kg in normal mice and at 200 mg/kg in hypercholesterolemic mice. The ratio was not reduced at doses above these ranges. These dose-effect relationships were not modified by duration of probucol treatment. These findings suggest that there is an optimum dosage of probucol to lower LDL + VLDL cholesterol and the atherogenic index, and that the actual optimum dosage for the beneficial effect depends on blood lipid levels or types of hyperlipidemia. This may be important in the clinical application of this drug, because a negative correlation has been demonstrated between HDL cholesterol levels and ischemic heart disease. Clofibrate treatment did not affect serum lipid levels significantly in either normal or cholesterol-fed mice. Probucol was again effective in lowering LDL cholesterol values in cholesterol-fed mice which had previously been treated with clofibrate for 2 weeks without any beneficial effect.

ACTIVATION OF THE METABOLISM OF THE FATTY ACYL GROUP IN GRANULOCYTE PHOSPHOLIPIDS BY PHORBOL MYRSITATE ACETATE. J-S. Tou (Dept. of Biochemistry, Tulane Univ. School of Medicine, New Orleans, LA 70112) *Biochim. Biophys. Acta 665*:491–497 (1981). Phorbol myristate acetate is known to reproduce the stimulated oxidative activities characteristic of phagocytosis and its initial action is on the cell membrane. In the present study the effect of phorbol myristate acetate on the metabolism of the fatty acyl groups of granulocyte phospholipids was examined and compared with that of phagocytic stimuli. Phorbol myristate acetate stimulated the labeling of phosphatidylethanolamine, phosphatidylcholine and phosphatidylinositol by [1-^{14}C] palmitic acid but not by [U-^{14}C] glycerol, whereas starch granules selectively increased the labeling of phosphatidylinositol by both radioactive tracers. Labeled palmitic acid was found at both *sn*-1 and *sn*-2 positions of phospholipids and more radioactivity was recovered from the 2-position. The radioactivity at both positions was enhanced in stimulated cells. These data suggest that phorbol myristate acetate increased palmitic acid incorporation into glycerophospholipids by increasing the acylation of the lyso derivatives and that starch granules enhanced the formation of phosphatidylinositol via de novo synthesis and acylation of the lyso derivative as well. Both phorbol myristate acetate and starch granules selectively augmented the incorporation of [1-^{14}C] arachidonic acid into phosphatidylinositol which exhibited the highest specific radioactivity among the phospholipids in control and in stimulated cells. The possible significance of the increased incorporation of arachidonic acid into phosphatidylinositol is discussed.

PHOSPHORUS-31 NUCLEAR MAGNETIC RESONANCE SPECTRA CHARACTERISTIC OF HEXAGONAL AND ISOTROPIC PHOSPHOLIPID PHASES GENERATED FROM PHOSPHATIDYLETHANOLAMINE IN THE BILAYER PHASE. Ann M. Thayer and Susan J. Kohler (From the Department of Chemistry, Carr Laboratory, Mount Holyoke College, South Hadley, Massachusetts 01075) *Biochemistry 20*:6831–6834 (1981). ^{31}P nuclear magnetic resonance (NMR) spectroscopy is recognized as a technique which yields information concerning both the dynamics and organization of phospholipid molecules in biological membranes and phospholipid dispersions. In this theoretical paper, we examine the relationship between the conformation of the phospholipid molecule and the shape of the predicted ^{31}P NMR spectrum. Using a simple model of rotation of the phospholipid molecule about its long axis, we show that it is possible to generate spectra previously considered typical of the bilayer ($\sigma\| < \sigma\perp$), isotropic ($\sigma\| \cong \sigma\perp$), and hexagonal II ($\sigma\| > \sigma\perp$) packing arrangements by simply changing the phospholipid head-group conformation while *retaining the molecules in the bilayer phase.*

THE EFFECTS OF ENERGETIC STEADY STATE, PYRUVATE CONCENTRATION, AND OCTANOYL-(−)-CARNITINE ON THE RELATIVE RATES OF CARBOXYLATION AND DECARBOXYLATION OF PYRUVATE BY RAT LIVER MITOCHONDRIA. Will Davis-van Thienen and E. Jack Davis (From the Indiana University School of Medicine, Department of Biochemistry, Indianapolis, Indiana 46223) *The Journal of Biological Chemistry 256*(16): 8371–8378 (1981). Rat liver mitochondria were incubated with controlled concentrations of pyruvate over a range of energetic and respiratory steady states, in the presence and absence of octanoyl-(−)-carnitine in order to evaluate conditions and effectors which perturb the absolute and relative rates of flux through pyruvate carboxylase and pyruvate dehydrogenase. Control experiments using saturating concentrations of pyruvate are also reported. With high [pyruvate] (10 mM) as sole substrate, carboxylation rate was very rapid in the resting state, and was diminished in a stepwise manner on stimulation of respiration with increasing amounts of ATPase. Carboxylase flux rates did not correlate with [acetyl-CoA] or acetyl-CoA/CoASH ratio, or with changes in the mitochondrial ATP/ADP until ATPase in excess of maximal respiratory stimulation was added. Octanoyl carnitine stimulated carboxylation further, and this rate was better sustained in stimulated respiratory states. This stimulation is correlated qualitatively with elevation of the acetyl-CoA/CoA ratio. With pyruvate alone, pyruvate dehydrogenase flux was maximally stimulated, independent of the energetic state. The latter was suppressed by octanoyl carnitine in all respiratory states, but only marginally. It is concluded that the energetic state of the liver cell, and especially the availability of fatty acids, can trigger a very effective coordinated switch in gating pyruvate carbon to oxaloacetate or acetyl-CoA. Endocrine signals may initiate this gating by altering these parameters.

LIPID INSERTION OF CHOLERA TOXIN AFTER BINDING TO G_{M1}-CONTAINING LIPOSOMES. Maurizio Tomasi and Cesare Montecucco, with the technical assistance of Mario Santato (From the Laboratorio di Biologia Cellulare ed Immunologia, Istituto Superiore di Sanità, Roma, Italy and the Centro Consiglio Nazionale delle Richerche per la Fisiologia dei Mitocondri e Laboratorio di Biofisica e Biologia Molecolare, Istituto di Patologia Generale, Università di Padova, Padova, Italy) *The Journal of Biological Chemistry 256* (21):11177–11181 (1981). The technique of hydrophobic photolabeling with photoreactive lipids was used to study the topology of interaction of cholera toxin with liposomes containing galactosyl-*N*-acetylgalactosaminyl-[*N*-acetyl neuraminyl]-galactosyl glucosyl ceramide (G_{M_1}). The toxin appears to locate itself superficially on the lipid bilayer. The interaction is mediated only by the $\gamma\beta_5$ part. On reduction of the disulfide bridge joining the α and γ subunits, the α subunit penetrates deeply into the lipid bilayer. The mere binding of cholera toxin to G_{M_1} is not sufficient to allow the insertion of the enzymatically active α subunit in the membrane. Some processing, which may involve a modification of covalent bonds of the toxin molecule (such as that caused by reduction) appears to be necessary. The specific reduction of the α-γ disulfide bond on the external surface of the membrane as a prerequisite for the membrane penetration of the α subunit is discussed.

EFFECTS OF CATECHOLAMINES ON FREE FATTY ACID RELEASE FROM BONE MARROW ADIPOSE TISSUE. Marie-Antoinette Tran, Dang Tran Lac, and Michel Berlan (Laboratoire de Physiologie Appliquée et Pharmacologie Médicale (Pr. Montastruc), Faculté de Médecine, 37 allées Jules Guesde, 31000—Toulouse, and Département de Pathologie Ostéo-Articulaire (Pr. Arlet), Faculté de Médecine, Chemin du Vallon, Toulouse Rangueil, France) *J. Lipid Res. 22*:1271–1276 (1981). We have studied the effect of epinephrine and isoproterenol on free fatty acid (FFA) mobilization from bone marrow adipose tissue in dog tibia after constant-flow autoperfusion of the nutrient artery by ipsilaterial femoral arterial blood. The perfusions of epinephrine (0.025 μg/min) or isoproterenol (0.005 μg/min) significantly increased the FFA level in the nutrient vein of the tibia. Moreover, our data demonstrate that in vitro the bone marrow adipose tissue was less responsive to catecholamines than omental adipose tissue. It can be concluded that bone marrow adipose tissue is able to release FFA after administration of catecholamines but to a lesser extent than in other adipose tissue (omental adipose tissue). These results support the hypothesis that the bone marrow adipose tissue is involved in local nutrition rather than in the total energy supply of the animal.

DIETHYLSTILBESTROL TREATMENT MODULATES THE ENZYMATIC ACTIVITIES OF PHOSPHATIDYLCHOLINE BIOSYNTHESIS IN ROOSTER LIVER. C. Vigo, H.B. Paddon, F.C. Millard, P.H. Pritchard, and D.E. Vance (Dept. of Biochemistry, Univ. of British Columbia, Vancouver, B.C. V6T 1W5 Canada) *Biochim. Biophys. Acta 665*:546–550 (1981). The effect of diethylstilbestrol injection on the activities of phosphatidylcholine biosynthetic enzymes in rooster liver has been determined. Choline kinase activity was stimulated within 4 h after the first hormone injection. By the third day enzyme activity reached 5.47 $nmol \cdot min^{-1} \cdot mg^{-1}$ protein compared to control values (1.83 $nmol \cdot min^{-1} \cdot mg^{-1}$ protein) which were unchanged during the experiment. CTP:phosphocholine cytidylyltransferase activity was unaffected until Day 3 when its activity was 50% that of control values. When assayed in the presence of exogenous phospholipid, no significant change was noted in cytidylyltransferase activity. The activity of CDPcholine: 1,2-diacylglycerol phosphocholinetransferase was not altered by the hormone injections. The activity of phosphatidylethanolamine-*N*-methyltransferase gradually increased so that by Day 3, the enzyme activity was elevated 2-fold (0.12 to 0.24 nmol methyl group transferred per mg microsomal protein). These results are consistent with earlier in vivo studies.

INTERACTION OF SERUM LIPOPROTEINS AND A PROTEOGLYCAN FROM BOVINE AORTA. P. Vijayagopal, S.R. Srinivasan, B. Radhakrishnamurthy and G.S. Berenson (Depts. of Med. and Biochemistry, Louisiana State University School of Medicine, New Orleans, LA 70112) *J. Biol. Chem. 256*(15):8234–8241 (1981). The interactions of a chondroitin sulfate-dermatan sulfate proteoglycan with serum very low (VLDL), low (LDL), and high density (HDL_3) lipoproteins were studied with special reference to the nature of the interaction of LDL and the proteoglycan. The proteoglycan formed insoluble complexes with VLDL and LDL, but no complex was formed with HDL_3. The proteoglycan (40 μg/ml) converted 98% of added LDL (150 μg of cholesterol/ml) into insoluble complex at a Ca^{2+} concentration of 30 mM. Physiologic concentrations of albumin inhibited insoluble complex formation with VLDL and LDL by 61.5 and 40.7%, respectively. The proteoglycan formed soluble complexes with LDL even in the absence of Ca^{2+}. Optimum soluble complex formation occurred when the medium contained 166.6 μg of LDL cholesterol/ml and 66.6 μg of proteoglycan. Specific modifications of the lysine and/or arginine residues of LDL prevented complex formation with the proteoglycan, thus indicating the requirement of the positive charges of the protein moiety of LDL in the interaction. Removal of the protein core or the glycosaminoglycan chains of the proteoglycan prevented interaction with LDL. Desulfation of the proteoglycan molecule also inhibited complex formation with LDL. Thus, the native state of the arterial proteoglycan molecule confers optimum charge density for specific interaction with serum apoprotein B-containing lipoproteins.

EVIDENCE FOR A REGULATORY ROLE OF CTP: CHOLINE PHOSPHATE CYTIDYLYLTRANSFERASE IN THE SYNTHESIS OF PHOSPHATIDYLCHOLINE IN FETAL LUNG FOLLOWING PREMATURE BIRTH. Paul A. Weinhold, Douglas A. Feldman, Mary M. Quade, Joseph C. Miller and Robert L. Brooks (Veterans Administration Medical Center and Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48105) *Biochimica et Biophysica Acta 665*:134–144 (1981). The sequence of reactions which function to incorporate choline into phosphatidylcholine was investigated in lung from fetuses following premature delivery. The rate of [*methyl*-^{14}C] choline incorporation by rat lung slices into phosphatidylcholine increases following premature delivery at both 20 and 21 days gestation. The increase in choline incorporation is primarily due to an increased specific activity of phosphorylcholine resulting from a decreased pool size of phosphorylcholine. The decrease in the concentration of phosphorylcholine following premature delivery is apparently caused by an increased activity of cytidylyltransferase which leads to an increase in the conversion of phosphorylcholine to phosphatidylcholine. The total activity of choline kinase, cytidylyltransferase, cholinephosphotransferase and phosphatidate phosphohydrolase did not change significantly. However, the cytidylyltransferase activity in the microsome fraction increased following premature delivery at 20 and 21 days gestation. The amount of cytidylyltransferase in the H form in the cytosol fraction increased following premature delivery at 21 days gestation but not at 20 days gestation. The results are interpreted to indicate that the active form of cytidylyltransferase in lung cells is the membrane-bound enzyme and this fraction increased following premature delivery at 21 days gestation but not at 20 days gestation. The results are interpreted to indicate that the active form of cytidylyltransferase in lung cells is the membrane-bound enzyme and this form increases following birth resulting in an increased synthesis of phosphatidylcholine.

COFACTOR REQUIREMENTS FOR 7α-DEHYDROXYLATION OF CHOLIC AND CHENODEOXYCHOLIC ACID IN CELL EXTRACTS OF THE INTESTINAL ANAEROBIC BACTERIUM, *EUBACTERIUM* SPECIES V.P.I. 12708. (B.A. White, A.F. Cacciapuoti, R.J. Fricke, T.R. Whitehead, E.H. Mosbach, and P.B. Hylemon (Department of Microbiology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298 and Lipid Research Laboratory, Department of Surgery, Beth Israel Medical Center, New York, NY 10003) *J. Lipid Res. 22*:891–989 (1981). The characteristics of 7α-dehydroxylase, a bile acid-biotransforming enzyme, were determined using dialyzed cell extracts of *Eubacterium* sp. V.P.I. 12708. 7α-Dehydroxylase was induced by cholic acid in this organism. Induction by cholic acid resulted in the differential synthesis of at least five new polypeptides with molecular weights of 77,000, two at 56,000, 27,000 and 23,500, as determined by both one and two-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis. The relative molecular weight of 7α-dehydroxylase activity was estimated by anaerobic Bio-Gel A 1.5 M gel filtration chromatography to be 114,000. NAD^+ was the only cofactor found to consistently stimulate 7α-dehydroxylase activity in dialyzed cell extracts. The specific activity increased 4- to 6-fold with either cholic or chenodeoxycholic acid as a substrate in the presence of NAD^+. NAD^+ was also required for the reduction of the Δ^6-intermediate to deoxycholic acid. Other pyridine or flavin nucleotides were ineffective cofactors when added alone. These studies show that 7α-dehydroxylase is an inducible enzyme and requires NAD^+ as a cofactor in this bacterium.

INHIBITION OF THE LIPOLYTIC ACTION OF β-ADRENERGIC AGONISTS IN HUMAN ADIPOCYTES BY α-ADRENERGIC AGONISTS. Elizabeth E. Wright and Evan R. Simpson (Cecil H. and Ida Green Center for Reproductive Biology Sciences and the Departments of Obstetrics and Gynecology and Biochemistry, University of Texas Southwestern Medical School, 5323 Harry Hines Boulevard, Dallas, TX 75235) *J. Lipid Res. 22*:1265–1270 (1981). The aim of this study was to define the role of the α-adrenergic receptor in the regulation of lipolysis by human adipocytes. Glycerol production by isolated human adipocytes was stimulated by the pure β-adrenergic agonist isoproterenol in a dose-dependent fashion. This stimulation of lipolysis was inhibited by the

α-adrenergic agonists methoxamine, phenylephrine, and clonidine. Epinephrine-stimulated lipolysis was potentiated by the α-adrenergic antagonists, dihydroergocryptine, phentolamine, phenoxybenzamine, and yohimbine. Whereas the attenuation of β-adrenergic agonist-stimulated lipolysis by α-adrenergic agonists was reversed completely by the α_2-adrenergic antagonist yohimbine, the α_1-antagonist prazosin did not reverse such attenuation. It is concluded that α-adrenergic agonists act as antilipolytic agents in human adipocytes and that this action may result from the interaction of these compounds with a population of α_2-adrenergic receptors.

LECITHIN:CHOLESTEROL ACYLTRANSFERASE AND SERUM FATTY ACIDS. J.K. Yao, P.J. Palumbo and P.J. Dyck (Peripheral Nerve Research Laboratory and Dept. of Internal Medicine, Mayo Clinic and Mayo Foundation, Rochester, MN 55901) *Artery 9*(4): 262–274 (1981). The rate of cholesterol esterification in vitro was significantly lower (~20%) in non-obese diabetics than in non-obese controls. More specifically, this decrease was found to be in cholesteryl linoleate synthesis. The concentration of serum free fatty acid, particularly of oleic acid, was 2–3 times higher in diabetic patients than in controls. The decreased rate of serum cholesterol esterification was associated with an increase in serum free fatty acid concentration and with a decrease of linoleic acid in serum phospholipids.

CHOLESTERYL ESTER TRANSFER FROM PHOSPHOLIPID VESICLES TO HUMAN HIGH DENSITY LIPOPROTEINS. Patricia M. Young and Peter Brecher (Departments of Biochemistry and Medicine, Boston University School of Medicine, Boston, MA 02118) *J. Lipid Res. 22*:944–954 (1981). The exchange of cholesteryl esters between different lipoproteins was reported to be mediated by a protein present in human plasma. In this study we have examined the movement of cholesteryl ester from unilamellar phospholipid vesicles to high density lipoprotein (HDL). Experimental conditions were established so that vesicles containing egg yolk lecithin and cholesteryl oleate (molar ratio of 86:1) could be incubated with human HDL so that neither disruption of particles nor transfer of lipid occurred. Addition of human lipoprotein-deficient plasma to the system promoted the transfer of cholesteryl oleate, but not phospholipid, from vesicles to HDL. Cholesteryl oleate transfer was dependent upon amount of HDL or lipoprotein-deficient plasma added and occurred when either HDL_2 or HDL_3 were present. Addition of unesterified cholesterol to the vesicles did not influence cholesteryl ester transfer to HDL. When phospholipid vesicles containing both cholesteryl oleate and triolein (molar ratio 86:1:1) were incubated with HDL and lipoprotein-deficient plasma, only cholesteryl oleate was transferred from the vesicles to HDL. The results indicated that a protein present in rabbit and human plasma is effective in the selective, unidirectional transport of cholesteryl esters from a phospholipid bilayer to a plasma lipoprotein.

SELECTIVE ELEVATION OF PLASMA FREE CHOLESTEROL CONCENTRATION BY ADMINISTRATION OF ESTROGEN IN THE PRESENCE OF TOTAL BILIARY OBSTRUCTION. B. Zumoff, R.S. Rosenfeld, and L. Hellman (Clinical Research Center and the Institute for Steroid Research, Montefiore Hospital, and the Medical Center, The Bronx, NY) *Atherosclerosis 40*(2):139–144 (1981). On the basis of clinical observations suggesting interactive effects of biliary obstruction and estrogen therapy on plasma cholesterol levels, a prospective study of the effect of ethinyl estradiol on plasma lipid levels was carried out in a patient with total biliary obstruction. A daily dose of 50 mg of ethinyl estradiol raised the plasma free cholesterol concentration from 265 mg/dl to 550 mg/dl over a period of 3 weeks; there was no change in plasma ester cholesterol concentration. Withdrawal of the estrogen was followed by a fall to baseline of the free cholesterol concentration over a 45-day period; once again there was no change in ester cholesterol. Plasma phospholipid concentration rose and fell in direct proportion to the changes in free cholesterol; plasma triglyceride concentration was unaffected by the estrogen. To account for the results of this study, it is suggested that the already elevated plasma levels of lipoprotein-X in biliary obstruction are further elevated by estrogen administration.

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

Fats and oils

SIDE-CHAIN OXIDATION OF MONOOXYGENATED C_{27}- AND C_{29}-STEROIDS IN RAT LIVER MITOCHONDRIA AND 18000 $\times g$ SUPERNATANT. Leif Aringer and Lennart Nordström (Hormone Laboratory, Department of Obstetrics and Gynecology and the Department of Clinical Chemistry, Karolinska Sjukhuset, S-104 01 Stockholm 60, Sweden) *Biochimica et Biophysica Acta 665*: 13–21 (1981). The 24-, 25- and 26-hydroxylation of 4-cholesten-3α-ol, 4-cholesten-3β-ol, 5-cholesten-3α-ol, 5-cholesten-3β-ol, 5α-cholestan-3α-ol, 5α-cholestan-3β-ol, 5β-cholestan-3α-ol, 5β-cholestan-3β-ol, 4-cholesten-3-one, 5α-cholestan-3-one, 5β-cholestan-3-one and the 24α-ethyl derivatives of 5α-cholestan-3β-ol, 5β-cholestan-3α-ol, 5β-cholestan-3β-ol and 4-cholesten-3-one were studied in rat liver mitochondria (8500$\times g$ sediment fractions fortified wih isocitrate) and in rat liver microsomes (18000$\times g$ supernatants supplemented with NADPH). In the mitochondria, all C_{27}-substrates and probably all C_{29}-substrates were found to be ω-hydroxylated. From 24α-ethyl-5α-cholestan-3β-ol and 24α-ethyl-4-cholesten-3-one two ω-hydroxylated products were identified. All C_{27}- but no C_{29}-steroids were found to be 24- and 25-hydroxylated. The yields of ω-hydroxylated metabolites were much higher than those of the 24- and 25-hydroxylated products. The ω-hydroxylation of the C_{29}-steroids amounted to 3–50% of that found for the corresponding C_{27}-steroids. In the 18000$\times g$ supernatant only one substrate, 5β-cholestan-3α-ol, was found to be 25- and 26-hydroxylated and no 24-hydroxylation of any steroid could be detected.

AUTOXIDATION OF BIOLOGICAL MOLECULES. 2. THE AUTOXIDATION OF A MODEL MEMBRANE. A COMPARISON OF THE AUTOXIDATION OF EGG LECITHIN PHOSPHATIDYLCHOLINE IN WATER AND IN CHLOROBENZENE. L.R.C. Barclay and K.U. Ingold (Contribution from the Division of Chemistry, National Research Council of Canada, Ottawa, Canada K1A OR6) *J. Am. Chem. Soc. 103*:6478–6485 (1981). The kinetics of the autoxidation of egg lecithin phosphatidylcholine in homogeneous solution in chlorobenzene and as a bilayer dispersion in 0.1 M aqueous NaCl has been studied at 30°C under 760 torr of O_2. The autoxidations were initiated by the thermal decomposition of di-*tert*-butyl hyponitrite. The efficiency of chain initiation, e, was determined by the induction period method using α-tocopherol as the chain-breaking antioxidant. In chlorobenzene e was ca. 0.66 but in the aqueous dispersion e was only ca. 0.091. The reduced efficiency of initiation in the bilayer is attributed to a reduction in the fraction of *tert*-butoxyls which escape from the solvent cage, and this in turn is due to the fact that the bilayer has a high microviscosity. The rate of autoxidation of the egg lecithin in chlorobenzene is proportional to the lecithin concentration and to the square root of the rate of chain initiation, and is virtually independent of the oxygen pressure, which means that this autoxidation follows the usual kinetic rate law. In the aqueous dispersion the concentration of egg lecithin in the bilayer cannot be altered, but since the rate of autoxidation is proportional to the square root of the rate of chain initiation and is virtually independent of the oxygen pressure, the usual kinetic rate law would also appear to be followed. The oxidizability of egg lecithin in chlorobenzene is 0.61 $M^{-1/2}s^{-1/2}$, and in the aqueous dispension it is 0.0165 $M^{-1/2}s^{-1/2}$. The reduction in oxidizability in the bilayer is attributed to the diffusion of the peroxyl radical center, which is a polar moiety, out of the autoxidizable, nonpolar, interior region of the bilayer and into the non-autoxidizable, polar surface region. As a consequence, chain progogation will be retarded and chain termination will be accelerated.

STEROL RELEASE IN MAMMALIAN CELLS: THE ROLE OF A LIPOSOMAL-FREE ALBUMIN-PHOSPHOLIPID COMPLEX. Lester C. Bartholow and Robert P. Geyer (Department of Nutrition, Harvard School of Public Health, 665 Huntington Avenue, Boston,

MA 02115) *Biochimica et Biophysica Acta 665*:40–47 (1981). Human serum albumin, the purity of which was confirmed by two-dimensional gel electrophoresis and high-pressure liquid chromatography, is able to bind dipalmitoyl phosphatidylcholine and the resulting albumin-phospholipid complex can cause marked sterol release from animal and human cells in tissue culture. The complex-induced sterol release was synergistic, exceeding by 4–5-fold the sum of sterol release in the presence of albumin and phospholipid individually. Isolation of the albumin-phospholipid complex by gel permeation chromatography yielded a liposomal-free fraction which caused full sterol release. No synergistic release of sterol occurred when cells were incubated with human serum albumin and dilinoleoyl phosphatidylcholine.

PHASE EQUILIBRIA IN BINARY MIXTURES OF DIMYRISTOYLPHOSPHATIDYLCHOLINE AND CARDIOLIPIN. T. Berclaz and H.M. McConnell (Stauffer Laboratory for Physical Chemistry, Stanford University, Stanford, CA 94305) *Biochemistry 20*(23): 6635–6640 (1981). Paramagnetic resonance spectra of the spin-label 2,2,6,6-tetramethylpiperidinyl-1-oxy have been used to study phase separations in binary mixtures of dimyristoylphosphatidylcholine and cardiolipin. Two different samples of cardiolipin were used: (i) One sample contained calcium ions at a mole ratio of calcium: cardiolipin = 1:2; the experimental data support the view that cardiolipin is present in the bilayer membrane as calcium ion linked dimers, $(CL)_2Ca^{2+}$. (ii) A calcium-free sodium cardiolipin sample yielded remarkable spin-label partition data that were quite different from those obtained in the presence of Ca^{2+}. In both cases the spin-label data provide evidence for compound formation and for fluid-fluid immiscibility in the bilayer membrane.

CHARACTERIZATION OF HUMAN HIGH-DENSITY LIPOPROTEINS BY GRADIENT GEL ELECTROPHORESIS. Patricia J. Blanche, Elaine L. Gong, Trudy M. Forte and Alex V. Nichols (Donner Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720) *Biochimica et Biophysica Acta 665*: 408–419 (1981). Gradient gel electrophoresis in conjunction with automated densitometry was applied to the identification and estimation of subpopulations of high-density lipoproteins (HDL) in the ultracentrifugal $d \leq 1.200$ fraction from human plasma. The frequency distribution of relative migration distances (R_F values) of subpopulation peaks in HDL patterns of a group (n=194) of human subjects showed five apparent maxima: two in the R_F range associated with the HDL_2 subclass, and three in the R_F range of the HDL_3 subclass. HDL within R_F intervals bounding these maxima were designated $(HDL_2b)_{gge}$, $(HDL_2a)_{gge}$, $(HDL_3a)_{gge}$, $(HDL_3b)_{gge}$ and $(HDL_3c)_{gge}$ and were shown to correspond approximately to material determined by analytic ultracentrifugation within the HDL_{2b}, HDL_{2a} and HDL_3 components. Material represented by the HDL_{2a} component, as resolved by three-component analysis of the ultracentrifugal Schlieren pattern, was found by gradient gel electrophoresis to be polydisperse in particle size. Mean hydrated densities and particle sizes of HDL corresponding to those with R_F values of the frequency maxima were: 1.085 g/ml and 10.57 nm in the $(HDL_2b)_{gge}$; 1.115 g/ml and 9.16 nm in the $(HDL_2a)_{gge}$; 1.136 g/ml and 8.44 nm in the $(HDL_3a)_{gge}$; 1.154 g/ml and 7.97 nm in the $(HDL_3b)_{gge}$; and 1.171 g/ml and 7.62 nm in the $(HDL_3c)_{gge}$. The mean hydrated density values of the subpopulations within the $(HDL_3a)_{gge}$ and $(HDL_3b)_{gge}$ were comparable to those of the HDL_{3L} and HDL_{3D} components recently characterized by zonal ultracentrifugation. High order and statistically significant correlations between densitometric scans of the $(HDL_2b)_{gge}$, $(HDL_2a)_{gge}$ and $(HDL_3)_{gge}$ material, as obtained from gradient gels, and plasma concentrations of the HDL_{2b}, HDL_{2a} and HDL_3 components, as obtained from analytic ultracentrifugation, were demonstrated.

CARBON-13 NUCLEAR MAGNETIC RESONANCE STUDIES OF CHOLESTEROL–EGG YOLK PHOSPHATIDYLCHOLINE VESICLES. James R. Brainard and Eugene H. Cordes (From the Department of Chemistry, Indiana University, Bloomington, Indiana 47401) *Biochemistry 20*:4607–4617 (1981). Proton-decoupled natural abundance ^{13}C nuclear magnetic resonance spectra at 63 kG were obtained for isolated single-bilayer egg yolk phosphatidylcholine-cholesterol vesicles containing a variable phospholipid/cholesterol ratio. Numerous well-resolved single carbon resonances of phospholipid and cholesterol carbons were observed. Carbon resonances from different parts of the phospholipids show markedly different behavior as a function of cholesterol content of the vesicles. The line widths of resonances for carbon atoms in the headgroup region and the *sn*-3 carbon of the phospholipid glycerol backbone are relatively independent of cholesterol content. In contrast, resonances from the *sn*-1 and *sn*-2 carbon atoms of the glycerol backbone and the envelopes containing the olefinic and aliphatic carbon resonances of the fatty acyl chains of the phospholipids broaden markedly with increasing content of cholesterol. The most prominent cholesterol ring resonance is that for C6. This is, in part, due to its location in a clear window of the spectrum, where it is unobscured by interfering phospholipid resonances. These data suggest that in vesicles the rotation of cholesterol is more anisotropic than that of epicholesterol and that the stereochemistry of the C3 hydroxyl group of cholesterol is at least partly responsible for the highly anisotropic rotation of the steroid ring within the bilayer.

THEORY FOR NUCLEAR MAGNETIC RELAXATION OF PROBES IN ANISOTROPIC SYSTEMS: APPLICATION TO CHOLESTEROL IN PHOSPHOLIPID VESICLES. James R. Brainard and Attila Szabo (From the Department of Medicine, Baylor College of Medicine, Houston, Texas 77030 (J.R.B.), and the Department of Chemistry, Indiana University, Bloomington, Indiana 47405 (A.S.)) *Biochemistry 20*:4618–4628 (1981). The nuclear magnetic relaxation of a nucleus in a cylindrical probe embedded in a bilayer vesicle is considered. The probe is assumed to diffuse freely about its unique (C_∞) symmetry axis with an effective correlation time $\tau\parallel$, and the C_∞ axis moves in a potential which is azimuthally symmetric about a director, with an effective correlation time $\tau\perp$. The overall isotropic rotational correlation time of the membrane is τ_M. Dipolar relaxation and, in the special case that the relevant tensors are axially symmetric, quadrupolar and chemical shift anisotropy relaxation are treated. An expression for the appropriate correlation function is derived which depends on the above effective correlation times, on the order parameter of the C_∞ axis of the probe, and on the angle (β) which in the case of dipolar relaxation of a protonated ^{13}C nucleus is between the ^{13}C-H vector and the C_∞ axis of the probe. A significant feature of this formulation of the dynamics is that no assumptions need be made concerning the relative order of magnitudes of the effective correlation times and the Larmor frequencies (e.g., such as the extreme narrowing limit).

THERMAL INTERACTION OF LYSINE AND TRIGLYCERIDES. Dennis Breitbart and Wassef Nawar (Department of Food Science & Nutrition, University of Massachusetts, Amherst, Massachusetts 01003) *J. Agric. Food Chem. 29*:1194–1196 (1981). Mixtures of lysine and either tributyrin or tricaproin were heated under vacuum for 1 h at 250°C, and the volatile decomposition products were analyzed by gas chromatography and mass spectrometry. Interaction products included the fatty acid amide, secondary amides, a tertiary amide, and a series of pyridine compounds not previously isolated. Most of the identified products can be accounted for by well-known reactions.

LIPID-PROTEIN MULTIPLE BINDING EQUILIBRIA IN MEMBRANES. Jaakko R. Brotherus, O. Hayes Griffith, Martti O. Brotherus, Patricia C. Jost, John R. Silvius, and Lowell E. Hokin (From the Department of Medical Chemistry, University of Helsinki, Siltavuorengpenger 10, 00170 Helsinki 17, Finland (J.R.B. and M.O.B.), the Institute of Molecular Biology, University of Oregon, Eugene, Oregon 94703 (O.H.G., P.C.J., and J.R.S.), and the Department of Pharmacology, University of Wisconsin, Madison, Wisconsin 53706 (L.E.H.)) *Biochemistry 20*:5261–5267 (1981). Phospholipids at the lipid-protein interface of membrane proteins are in dynamic equilibrium with fluid bilayer. In order to express the number of binding sites (N) and the relative binding constants (K) in terms of measurable quantities, the equilibrium is formulated as an exchange reaction between lipid molecules competing for hydrophobic sites on the protein surface. Experimental data are reported on two integral membrane proteins, cytochrome oxidase and (Na,-K)-ATPase, reconstituted into defined phospholipids. Electron spin resonance measurements on reconstituted preparations of beef heart cytochrome oxidase in 1,2-dioleoyl-*sn*-3-phosphatidylcholine containing small quantities of the spin-labeled phospholipid 1-palmitoyl-2-(14-proxylstearoyl)-*sn*-3-phosphatidylcholine (PC*) gave a linear plot of bilayer/bound PC* vs. the lipid/protein ratio as predicted by the theory, with $K \simeq 1$ and $N = 40$ (normalized to heme aa_3). This demonstrates that the spin-label moiety attached to the hydrocarbon chain does not significantly perturb the binding equilibria. In the second experimental system, (Na,K)-ATPase purified from rectal glands of *Squalus acanthias* was reconstituted with defined phosphatidylcholines as the lipid solvent and spin-labeled phospholipids with choline or serine head groups (PC*, PS*) as the solute. The (Na,K)-ATPase has a larger number of lipid binding or contact sites (N = 60–65 per $\alpha_2\beta_2$ dimer) and exhibits a detectably larger average binding constant for the negatively charged phosphatidylserine than for the corresponding phosphatidylcholine.

CHARACTERIZATION OF SHORT-CHAIN ALKYL ETHER LECITHIN ANALOGUES: ^{13}C NMR AND PHOSPHOLIPASE STUDIES. R.A. Burns, Jr., J. M. Friedman, and M.F. Roberts (From the Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139) *Biochemistry 20*:5945–

5950 (1981). Several short-chain ether-linked lecithin analogues (*rac*-1,2-dihexyl-, *rac*-1,2-diheptyl-, and *rac*-1,2-dioctyl-*sn*-glycero-3-phosphocholine and L-1,2-diheptyl-*sn*-glycero-3-phosphocholine) have been synthesized and characterized. When dispersed in aqueous solution, these synthetic phospholipids form micelles (not bilayers) and can be used to investigate phospholipase action. Critical micellar concentrations are 1.5- to 2-fold lower than those of the comparable chain length diacyllecithins. This critical micelle concentration difference corresponds to the methylene ether being approximately 200 cal/mol more "hydrophobic" than an ester moiety. This value is compatible with the solvent free energy transfer potentials for ester/ether substitution in model compounds. ^{13}C NMR has been used to characterize the conformation and mobility of short-chain lecithins as monomers and micelles [Burns, R.A., Jr., & Roberts, M.F. (1980) *Biochemistry 19*, 3100]. ^{13}C T_1 relaxation times, chemical shift differences generated in the monomer/micelle transition, and interchain magnetic shift nonequivalence generated by micellization are similar at corresponding carbon positions in ester and ether lecithins. However, T_1 relaxation times do indicate greater fluidity near the terminal methyl end of ether lecithin chains. These data suggest that the carbonyl groups make little contribution to overall lipid conformation and mobility.

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