Abstracts

Biochemistry and nutrition

INTESTINAL SECRETION OF LIPIDS AND LIPOPROTEINS DURING CARBOHYDRATE ADSORPTION IN THE RAT. N.L. Keim and J.A. Marlatt (Dept. of Nutr. Sci., Univ. of Wisconsin, Madison, WI 53706) *J. Nutr. 110*(7), 1354-64 (1980). To assess the effects of carbohydrate ingestion on the characteristics and quantities of triglyceride-rich lipoproteins produced by the intestine mesenteric lymph was collected from rats receiving intraduodenal infusions of saline, sucrose or starch. Lymph total lipid (mg/hour) was greatest during saline infusion, decreased during sucrose and became significantly less ($P < 0.05$) during starch infusion. Lipoproteins were isolated from lymph by ultracentrifugation followed by gel filtration chromatography. During saline infusion total lipid in very low density lipoproteins (VLDL) was 2-fold higher than total lipid in chylomicrons; with both carbohydrate infusions this ratio increased to seven. Both carbohydrates significantly lowered the rate of secretion of total lipid as chylomicrons. The VLDL lipid secretion rates during sucrose infusion were equivalent to those during saline, whereas starch infusion tended to decrease VLDL lipid secretion rates. These results suggest that during the process of carbohydrate absorption, carbohydrate does not enhance the flow of intestinal VLDL into the circulation; digestion and absorption of complex carbohydrates in fact may retard the intestinal contribution of triglyceride-rich lipoproteins.

PURIFICATION AND PROPERTIES OF BOVINE AORTIC LIPOPROTEIN LIPASE. D.A. Wisner, Jr., K. Shirai and R.L. Jackson (Div. of Lipoprotein Res., Depts. of Pharm. and Cell Biophys., Biological Chem. and Med., Univ. of Cincinnati Med. Center, Cincinnati, OH 45267) *Artery 6*(6), 410-36 (1980). Lipoprotein lipase of bovine aortic intima has been purified to homogeneity by affinity chromatography on heparin-Sepharose. As determined by polyacrylamide gel electrophoresis in sodium dodecyl sulfate, the purified enzyme had a molecular weight of approximately 60,000, required apolipoprotein C-II for activity and was inhibited by 1.0 M NaCl. Optimum lipolytic activity was in the pH range of 8.0-8.5. Bovine skimmed milk lipoprotein lipase was also purified and its properties compared to those of the aortic enzyme. Based on these comparative studies, we conclude that bovine aortic and milk lipoprotein lipase have similar properties.

INTERCORRELATIONS AMONG PLASMA HIGH DENSITY LIPOPROTEIN, OBESITY AND TRIGLYCERIDES IN A NORMAL POPULATION. M.J. Albrink, R.M. Krauss, F.T. Lindgren and J. Von Der Groeben (Dept. of Med., West Virginia Univ. Schl. of Med., Morgantown, WV 26505) *Lipids 15*(9), 668-76 (1980). The interrelationships among fatness measures, plasma triglycerides and high density lipoproteins (HDL) were examined in 131 normal adult subjects. A high correlation was found among the various fatness measures. These measures were negatively correlated with total HDL, reflecting the negative correlation between fatness measures and HDL_2 (as the sum of HDL_{2a} and $_{2b}$). Fatness measures showed no relationship to HDL_3. There was also an inverse correlation between triglyceride concentration and HDL_2. No particular fatness measure was better than any other for demonstrating the inverse correlation with HDL but multiple correlations using all of the measures of obesity improved the correlations. Partial correlations controlling for fatness did not reduce any of the significant correlations between triglycerides and HDL_2 to insignificance. The weak correlation between fatness and triglycerides was reduced to insignificance when controlled for HDL_2.

LINOLEIC ACID HEMOLYSIS OF ERYTHROCYTES. N.J. Bai, T. George and S. Krishnamurthy (Dept. of Biochem., T.D. Medical College, Alleppey 688 006 India) *Indian J. Biochem. Biophys. 17*(2), 139-43 (1980). Erythrocytes of man and rat readily hemolysed on incubation with linoleic and oleic acids, but not with myristic, caproic, palmitic and stearic acids. Antioxidants and other inhibitors of lipid peroxidation did not inhibit lysis by fatty acids. Loss of cellular K^+ and reduced glutathione prior to hemolysis indicated membrane damage of erythrocytes. Sulphydryl agents enhanced hemolysis while reduced glutathione prevented it. Preincubation of cells with linoleic acid increased the fragility of cells. Linoleic acid hemolysis of red cells is not caused by lipid peroxidation but is probably due to changes in cell permeability, consequent on direct fatty acid interaction with stromal components.

RATE OF FAT COMPENSATION AND GROWTH EFFICIENCY OF LIPECTOMIZED SPRAGUE DAWLEY RATS. J.W. Bailey and D.B. Anderson (Dept. of Animal Sci., Univ. of Illinois, Urbana, IL 61801) *J. Nutr. 110*(9), 1785-92 (1980). The objective of this experiment was to measure changes in food efficiency and to determine the rate of fat compensation following the surgical removal of adipose tissue in Sprague Dawley rats. Dissected fat weight was significantly less in lipectomized animals at all body weights except 465 g. Gradual fat compensation up to 465 g was due to partial regeneration of the right inguinal fat pad as well as compensatory growth of other (intact) fat depots. Epididymal fat pads showed no signs of regeneration. Lipectomized animals had similar food intake but gained weight more rapidly than shams. Lipectomized rats had more food available for production when calculated maintenance energy requirements were subtracted from total food intake. Efficiency of utilization of production food did not differ significantly between treatments. Compensatory weight gain seen in lipectomized rats was therefore due to greater ad-libitum food consumption above maintenance requirements.

CHOLESTEROL AND CHOLESTERYL ESTERS OF EGGS FROM VARIOUS AVIAN SPECIES. J. Bitman and D.L. Wood (USDA, Beltsville Agr. Res. Center, Beltsville, MD 20705) *Poult. Sci. 59*(9), 2014-23 (1980). A method was developed to analyze the cholesterol and cholesteryl esters of egg yolk by gas liquid chromatography (GLC). After the cholesteryl ester fraction was isolated by chromatography on a silica gel (Hi-Flosil) column, individual esters were quantitatively separated and determined by GLC on glass columns packed with SP-2340. The cholesterol content and nature of the cholesteryl esters in eggs from various avian species were determined. Birds were classified according to feeding habits as follows: domestic fowl eating grain and plant materials-White Leghorn chicken, Silver-penciled Plymouth Rock chicken, turkey, Japanese quail; wild plant-eating birds; wild birds eating aquatic plants and animals; wild aquatic carnivorous birds; wild mammal-eating birds. Although egg size varied from 7 to 121 g, total cholesterol content ranged only from 12 to 25 mg/g yolk. Cholesterol present as ester ranged from 1 to 26% in the 14 birds studied. There was no consistent pattern in egg cholesterol content or in the percentage of cholesteryl esters in the species studied. Differences existed, however, in the composition of the cholesteryl esters and in the composition of egg yolk fatty acids that distinguished one bird species with particular feeding habits from another.

LIPOPROTEIN-X: CARBON-13 NUCLEAR MAGNETIC RESONANCE STUDIES ON NATIVE, RECONSTITUTED, AND MODEL SYSTEMS. J.R. Brainard, J.A. Hamilton, E.H. Cordes, J.R. Patsch, A.M. Gotto, Jr., and J.D. Morrisett. (Dept. of Chemistry, Indiana University, Bloomington, Indiana 47401) *Biochemistry 19*, 4266-73 (1980). Lipoprotein-X (LP-X), a lipoprotein isolated from human cholestatic plasma by ethanol-acetate precipitation and zonal ultracentrifugation, has been studied by ^{13}C NMR at 67.9 MHz. Spectra of LP-X and its three subfractions are markedly different from those of normal human high-density lipoprotein$_3$ (HDL_3) or low-density lipoprotein (LDL). The three LP-X subfractions isolated by zonal ultracentrifugation gave spectra which are identical, within experimental error, as judged qualitatively from their appearance and quantitatively from the line widths of selected resonances. In addition, ^{13}C NMR spectra of sonicated total LP-X lipids are similar to spectra of the intact native lipoprotein. This study suggests that motions of lipids in LP-X as probed by ^{13}C NMR are similar to the motions of lipids found in model vesicular systems, that the motions of the cholesterol rings and phospholipid fatty acyl chains are significantly more restricted in LP-X than in HDL_3 and LDL, and that the motions of the phosphoryl moieties in all three systems are similar.

STRUCTURAL REORGANIZATIONS IN LIPID BILAYER SYSTEMS: EFFECT OF HYDRATION AND STEROL ADDITION ON RAMAN SPECTRA OF DIPALMITOYLPHOSPHATIDYLCHOLINE MULTILAYERS. S.F. Bush, R.G. Adams, and I.W. Levin (Lab. of Chemical Physics, National Inst. of Arthritis, Metabolism and Digestive Diseases National Inst. of Health, Bethesda, MD 20205) *Biochemistry 19*(19), 4429-36 (1980). Vibrational Raman spectroscopy was used to investigate the conformational behavior of dipalmitoyl phosphatidylcholine (DPPC) bilayers perturbed by cholesterol and water, two membrane components whose lipid interactions involve different regions of the bilayer matrix. These data indicate that hydration confers a mobility to the head-group, glycerol, and carbonyl moieties. Shifts in the CN symmetric and PO_2 antisymmetric stretching modes, occuring on the addition of approximately four molecules of water, indicate a conformational rearrangement within the polar head group. After approximately

four molecules of water are added to the DPPC system, the spectral features of the gel system [70% (w/w) water] indicate that no further head-group changes nor increases in either acyl chain trans/gauche or lattice disorder arise on further hydration.

HEPATIC Δ^9 and Δ^6 DESATURASE ACTIVITIES DURING THE RECOVERY PERIOD FOLLOWING CARBON TETRACHLORIDE POISONING. J-P. Carreau, D. Frommel, T.T. Nguen and P. Mazliak (Laboratoire de Physiologie Cellulaire, CNRS, ERA 323, Universite Pierre et Marie Curie, 4 place Jussieu 75230 Paris Cedex 05, France) *Lipids 15*(9), 631-6 (1980). The liver microsomal Δ^9 and Δ^6 desaturase activities have been studied in rats with carbon tetrachloride-induced hepatitis. Immediately after poisoning, significant decreases were observed for both types of desaturase activity. However, recovery kinetics were slower for the Δ^6 desaturase than for the Δ^9 desaturase. The activities of NADH-ferricyanide and NADH-cytochrome C reductases, proteins involved in the electron transfers associated with microsomal desaturation, were also measured. There was a fall in both activities after poisoning, but this decrease was less than that of the desaturase activities.

INTERACTION OF LECITHIN: CHOLESTEROL ACYLTRANSFERASE AND CHOLESTERYL ESTER TRANSFER PROTEIN IN THE TRANSPORT OF CHOLESTERYL ESTER INTO SPHINGOMYELIN LIPOSOMES. T. Chajek, L. Aron, and C.J. Fielding (Cardiovascular Research Institute and Dept. of Physiology, University of California, San Francisco, CA 94143) *Biochemistry 19*(16), 3673-7 (1980). When isolated lecithin:cholesterol acyltransferase was incubated with cholesterol-lecithin liposomes in the presence of apolipoprotein A-1, cholesteryl ester accumulated until a maximal ester/lecithin weight ratio of 0.03 was reached. The inhibition of transferase associated with accumulation of cholesteryl ester was relieved by addition of additional lecithin-cholesterol liposomes but not by addition of sphingomyelin liposomes containing the same proportion of substrate unesterified cholesterol. These results indicate that it is the accumulation of cholesteryl ester product which directly inhibits transferase activity. Cholesteryl ester incorporated directly into the liposomes or synthesized from free cholesterol via the transferase reaction was equally transferred to sphingomyelin acceptor liposomes, indicating that the cholesteryl ester in these particles formed a single miscible pool for transfer.

INTRACELLULAR MOVEMENT OF CHOLESTEROL IN RAT ADRENAL CELLS: KINETICS AND EFFECTS OF INHIBITORS. J.F. Crivello and C.R. Jefcoate (Dept. of Pharmacology, Univ. of Wisconsin Med. Schl., Madison, WI 53706) *J. Biol. Chem. 255*(17), 8144-51 (1980). Adrenocorticotropin (ACTH)-stimulated cholesterol transport to mitochondria has been quantitated in rat adrenals in vivo and in adrenal cell suspensions. It is concluded that microfilaments and microtubules are involved in the transport of cholesterol both to and from the mitochondria while coupling of protein synthesis and steroidogenesis is expressed ultimately entirely on the rate-limiting transference of cholesterol to cytochrome P-450 within the mitochondria. Rat adrenal cell suspensions did not show the marked stimulation of steroidogenesis by cytochalsin B in the presence of serum lipoproteins which has been reported for Y-1 adrenal tumor cells. The in vivo effects of cytochalsin on rat adrenals suggest that intracellular transfer of cholesterol rather than transfer from plasma lipoproteins is critical to the rate of corticosterone synthesis during short term stimulation of rat adrenals.

EFFECTS OF DIETARY ESSENTIAL FATTY ACID CONCENTRATION UPON PROSTANOID SYNTHESIS IN RATS. J. Dupont, M.M. Mathias and P.T. Connally (Dept. of Food Sci. and Nutr., Colorado St. Univ., Ft. Collins, CO 80523) *J. Nutr. 110*(8), 1695-702 (1980). The effects of dietary linoleate as zero to 27% of energy, fed to female rats for 6 months, in relation to ability of whole blood to synthesize PG during clotting at 37 C was studied. Synthesis of PGE_1, PGE_2, $PGF_{2\alpha}$ and TXB_2 after 10 or 40 minutes of incubation of whole blood was determined by assay of serum concentration by radioimmunoassay. Fatty acid composition of serum phospholipids, cholesteryl esters and acyl glycerides was determined. The concentration of TXB_2 was found to be about 20-40 times that of PGE_2 and $PGF_{2\alpha}$. The decline of PGE_1 synthesis with 2-7% linoleate calories was correlated with 20:3n-6 concentration and PGE_2 and $PGF_{2\alpha}$ production with 20:4. With dietary linoleate concentration greater than 7.4% PG synthesis had a linear increase up to 27% of kilocalories, not correlated with fatty acids.

EFFECTS OF VITAMIN A AND ASCORBIC ACID ON IN VITRO CHOLESTEROL BIOSYNTHESIS IN THE RAT. J.G. Elliott and P.A. Laschance (Dept. of Food Sci., Rutgers Univ., New Brunswick, NJ 08903) *J. Nutr. 110*(7), 1488-96 (1980). In order to determine the effect of various doses of vitamin A and the interaction between vitamin A and ascorbic acid on cholesterol synthesis, male weanling rats were fed four levels of vitamin A as retinyl acetate (0, 20, 436 and 6,666 IU/g diet) and two levels of ascorbic acid (0 and 1 mg/g diet) for 28 days except the highest level of retinyl acetate which was fed for only 3 days. The incorporation of [2-^{14}C] mevalonic acid into cholesterol intermediates, fatty acids and bile acids was determined in liver slices prepared from rats fed the above diets. The results may be summarized as follows: (a) ascorbic acid synthesis was reduced in both a deficiency and excess of vitamin A; (b) ascorbic acid in the diet prevented or blocked the decrease in liver ascorbic acid in vitamin A deficiency but not at the highest level of retinyl acetate (6,666 IU/g); (c) retinyl acetate inhibited the incorporation of [2-^{14}C] mevalonic acid into cholesterol, lanosterol, dimethylallyl alcohol, geranol and farnesol, but had no inhibitory effect on the incorporation into squalene, nerolidol or bile acids, and (d) ascorbic acid had no inhibitory effect on cholesterol synthesis and no interaction between retinyl acetate and ascorbic acid was observed.

PREDICTION OF THE PARAMETERS OF WHOLE BODY CHOLESTEROL METABOLISM IN HUMANS. D.S. Goodman, F.R. Smith, A.H. Seplowitz, R. Ramakrishnan and R.B. Dell (Depts. of Med. and Pediatrics and the Arteriosclerosis Res. Center, Columbia Univ. College of Physicians and Surgeons, New York, NY 10032) *J. Lipid Res. 21*(6), 699-713 (1980). Total body turnover of cholesterol was studied in 54 subjects by fitting a three-pool mathematical model to plasma decay curves of 32-49 weeks duration following [^{14}C] cholesterol injection. Fifteen subjects were normal, 10 hypercholesterolemic, 21 hypertriglyceridemic, and 8 had both hypercholesterolemia and hypertriglyceridemia; 21 had a familial form of hyperlipidemia. In every subject in this hetergeneous population, the three-pool model gave the best fit for the data. An extensive search was conducted for relationships between model parameters and physiological variables (body size, serum lipid levels, age, and sex). Both linear and nonlinear relationships, and those involving interactions between pairs of variables, were explored. Fifty different forms of the model parameters and 53 forms of the physiological variables were examined. To guard against declaring statistical significance when none was present, subjects were first randomly divided into two matched groups.

SMOOTH MICROSOMES: A TRAP FOR CHOLESTERYL ESTER FORMED IN HEPATIC MICROSOMES. S. Hashimoto and A.M. Fogelman (Res. Service, Wadsworth Veterans Admin. Hosp. Center, Los Angeles, CA 90073) *J. Biol. Chem. 255*(18), 8678-84 (1980). Acyl-CoA:cholesterol acyltransferase was found predominantly (85%) in RNA-rich microsomes, the rest being in RNA-poor and smooth microsomes. However, the esterified cholesterol concentration of smooth microsomes was 2-fold greater than that of RNA-rich microsomes, suggesting the possibility of an interaction between RNA-rich and smooth microsomes. The distribution of cholesteryl ester between microsome subfractions was examined after incubation of a mixture of RNA-rich and smooth microsomes with [1-^{14}C] palmitoyl-CoA. The entrapment of radioactive cholesteryl ester in the smooth microsomes could not be accounted for by passive transfer of cholesteryl ester from RNA-rich microsomes to smooth microsomes. It is proposed that cholesterol in the smooth microsomal membranes may have been esterified by acyl-CoA: cholesterol acyltransferase located on the surface of RNA-rich microsomes with the resulting cholesteryl ester retained in the smooth microsomes. This hypothesis was strengthened by the observation that acyl-CoA:cholesterol acyltransferase was located on the cytoplasmic surface of the RNA-rich microsomal vesicle.

PHOTOSYNTHESIS OF PREVITAMIN D_3 IN HUMAN SKIN AND THE PHYSIOLOGIC CONSEQUENCES. M.F. Holick et al., (Endocrine Unit, Massachusetts General Hosp., Boston, MA 02114) *Science 210*(10), 203-5 (1980). Photosynthesis of previtamin D_3 can occur throughout the epidermis and in the dermis when hypopigmented Caucasian skin is exposed to solar ultraviolet radiation. Once previtamin D_3 is formed in the skin, it undergoes a temperature-dependent thermal isomerization that takes at least 3 days to complete. The vitamin D-binding protein preferentially translocates the thermal product, vitamin D_3, into the circulation. These processes suggest a unique mechanism for the synthesis, storage, and slow, steady release of vitamin D_3 from the skin into the circulation.

SYNTHESIS OF [3β-^{3}H]-3-EPIVITAMIN D_3 AND ITS METABOLISM IN THE RAT. S.A. Holick, M.F. Holick, J.E. Frommer, J.W. Henley, and J.A. Lenz ((Endocrine Unit, Massachusetts General Hosp., and Harvard Med. Schl., Boston, MA 02114) *Biochemistry 19*(17), 3933-7 (1980). 3-Epivitamin D_3, the 3α epimer of vitamin D_3, was synthesized, and its biological activity in the rat was evaluated. It was found to be $\sim$4 times less active on a weight basis than vitamin D_3 with respect to intestinal calcium transport, bone calcium mobilization, and calcification score as determined by the line-test assay. Tritiated 3-epivitamin D_3 was prepared, and its metabolism in the rat was compared with that of vitamin D_3 to

investigate the reasons for this diminished activity. 3-Epivitamin D_3 was converted to two polar metabolites, for which the chromatographic properties and the origin of biosynthesis (in the liver and kidney, respectively) correspond to 25-hydroxy-3-epivitamin D_3 and 1α,25-dihydroxy-3-epivitamin D_3. The fact that the concentration of 1α,25-dihydroxy-3-epivitamin D_3 in the intestine is half that of 1α,25-dihydroxyvitamin D_3 may be one explanation for the reduced biological activity of this epimer.

EFFECT OF FEEDING PROTECTED LIPIDS ON FATTY ACID SYNTHESIS IN OVINE TISSUES. R.L. Hood, L.J. Cook, S.C. Mills and T.W. Scott (CSIRO Div. of Food Res., PO Box 52, North Ryde, NSW 2113, Australia) *Lipids 15*(9), 644-50 (1980). The effects of including protected lipid supplemented in the sheep diet have been studied by measuring the incorporation of [1-^{14}C] acetate into tissue fatty acids in vivo and in vitro. Supplementing the diet with protected lipid significantly ($P<0.05$) depressed lipogenesis in adipose tissue both in vivo and in vitro. However, when protected lipids of different fatty acid composition were given to lambs, the protected safflower oil supplement containing high levels of linoleic acid was the only treatment to cause a significant ($P<0.05$) depression in fatty acid synthesis in adipose tissue, the major site of lipogenesis in the sheep. Larger adipose cells in the lipid-supplemented sheep indicate that these sheep were fatter than those receiving the basal diet. Therefore, supplemented wethers deposited more fat than sheep receiving the basal diet and this fat was derived from the supplement rather than from de novo synthesis.

RECEPTOR BINDING OF CHOLESTEROL-INDUCED HIGH-DENSITY LIPOPROTEINS CONTAINING PREDOMINANTLY APOPROTEIN E TO CULTURED FIBROBLASTS WITH MUTATIONS AT THE LOW-DENSITY LIPOPROTEIN RECEPTOR LOCUS. T.L. Innerarity, R.E. Pitas, and R.W. Mahley (Gladstone Foundation Lab. for Cardiovascular Disease, Univ. of California, San Francisco, CA 94140) *Biochemistry 19*(18), 4359-65 (1980). Previous equilibrium and kinetic studies have shown that cholesterol-induced high-density lipoproteins (HDL_C), which contain only the arginine-rich (E) apoprotein (apo-E), have an affinity for normal human fibroblasts that was 20 times that of human low-density lipoprotein (LDL). Also, 4 times as many LDL particles as HDL_C particles were required to saturate the normal human fibroblast surface receptors. The most feasible explanation was that each HDL_C particle bound to approximately four receptors. Here we considered two other possibilities: (a) that HDL_C bind to one receptor, suppressing three other receptors and (b) that HDL_C and LCL bind to different but adjacent receptors, such that binding of either blocks the binding of the other. These studies further support the concept that apo-E HDL_C bind to multiple cell surface receptors.

EFFECTS OF 1,3-BUTANEDIOL-1,3-DIOCTANOATE AND CORN OIL ON LIPIDS OF CHICK PLASMA, LIVER, AND SKIN. R.K. Johnston, J.W. Frankenfeld, and R.L. Squibb (Labs. of Disease and Environmental Stress, Rutgers Univ., New Brunswick, NJ 08903) *Poult. Sci. 59*(9), 2098-104 (1980). A synthetic energy source, 1,3-butanediol-1,3-dioctanoate (BDDO), and corn oil were fed at the 10% level in diets for chicks recovering from Newcastle disease virus at two levels of severity. There were little differences in plasma lipid concentrations between corn oil and BDDO groups. Liver lipid analyses showed significantly higher ($P\leq.01$) triglyceride concentration for the BDDO group as compared with corn oil. In both trials, total liver lipid content was higher in all components for the BDDO group. Triglycerides and total lipids were notable lower ($P\leq.01$) in skin of the BDDO group as compared with corn oil. Liver and skin lipid variations were attributed to different metabolic routes for BDDO and corn oil. The data suggest that BDDO tends to allow liver synthesis of triglycerides while suppressing lipid storage in the skin.

EFFECT OF CHOLECALCIFEROL AND 1,25-DIHYDROXY-CHOLECALCIFEROL ON THE INTESTINAL ABSORPTION OF ZINC IN THE CHICK. S.I. Koo, C.S. Fullmer and R.H. Wasserman (Dept. of Physical Biology, New York St. College of Veterinary Medicine, Cornell Univ., Ithaca, NY 14853) *J. Nutr. 110*(9), 1813-8 (1980). The effect of cholecalciferol on the intestinal absorption of ^{65}Zn was assessed in zinc-deficient and zinc-replete rachitic chicks, using the in situ ligated loop techniques. Cholecalciferol did not significantly affect ^{65}Zn absorption in either group, although the synthesis of the intestinal calcium-binding protein (CaBP) in both groups was similar. In an analogous study, 1,25-dihydroxy-cholecalciferol increased ^{47}Ca absorption and induced the synthesis of CaBP but exerted no effect on ^{65}Zn absorption in zinc-deficient rachitic chicks. When fed a diet adequate in cholecalciferol, more CaBP was present in the intestine of the zinc-adequate group than in the zinc-deficient group, possibly due to the greater rate of growth and therefore the greater need for calcium by the former group. These results suggest that cholecalciferol and its most active metabolite do not directly affect zinc absorption and, by inference, that the vitamin D-dependent transport mechanism is not involved in zinc homeostasis, or in the interaction between calcium and zinc.

COMPARATIVE STUDIES ON COMPOSITION OF CARDIAC PHOSPHOLIPIDS IN RATS FED DIFFERENT VEGETABLE OILS. J.K.G. Kramer (Animal Res. Inst., Res. Branch, Agriculture, Canada, Ottawa, ON Canada K1A 0C6) *Lipids 15*(9), 651-60 (1980). Male Sprague-Dawley rats were fed diets for 1 or 16 weeks, containing 20% by weight vegetable oils differing widely in their oleic, linoeic and linolenic acid content. No significant changes were observed in the level of the cardiac lipid classes. The results suggest that dietary linolenic acid of less than 15% does not inhibit the conversion of linoleic to arachidonic acid but the subsequent conversion of arachidonic acid to the C_{22} polyunsaturates was greatly reduced. It is quite evident from the results of this study that the incorporation of oleic acid and the substitution of linolenic for linoleic acid-derived C_{22} polyunsaturated fatty acids into cardiac phospholipids was related to the dietary concentration of these fatty acids and was not peculiar to any specific oil. Even though it is impossible to estimate the effect of such changes in cardiac phospholipids on membrane structure and function, results are discussed which suggest that the resultant membrane in the Sprague-Dawley male rat is more fragile, leading to greater cellular breakdown and focal necrosis.

A SPONTANEOUSLY SEASONAL HYPERCHOLESTEROLEMIC ANIMAL: PLASMA LIPIDS AND LIPOPROTEINS IN THE EUROPEAN BADGER (*MELES MELES* L.). P.M. Laplaud, L. Beaubatie, and D. Maurel (Laboratoire de Biochimie medicale, Faculte de medecine et de pharmacie, 2 rue du Dr. Marcland, 87032 Limoges Cedex, France) *J. Lipid Res. 21* (6), 724-38 (1980). The European badger has previously been shown to exhibit yearly cycles of locomotor activity, endocrine secretions, and body weight, as well as seasonal variations in plasma cholesterol. Over a period of 2 years, we have followed the plasma levels of free and esterified cholesterol, triglycerides and phospholipids, and of plasma lipoproteins (by means of polyacrylamide gel electrophoresis, agarose column chromatography and preparative and analytical ultracentrifugation). Some preliminary observations on the qualitative characteristics of the plasma apoproteins, obtained by application of electrophoretic techniques, are also described. Our results provide evidence for considerable synchronous and spontaneous variations of each of the plasma lipid components studied, all of them reaching a maximum in late autumn/early winter, then decreasing to a minimum in early spring. Our findings thus suggest that the badger may provide a useful model for future experiments regarding the hormonal regulation of plasma lipid transport as well as the metabolism and physiopathological implications of some cholesterol-rich lipoproteins.

ROLE OF PHOSPHATIDYLINOSITOL IN ATTACHMENT OF ALKALINE PHOSPHATASE TO MEMBRANES. M.G. Low and D.B. Zilversmit (Dept. of Biophysics, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298) *Biochemistry 19*(17), 3913-8 (1980). The mechanism of release of alkaline phosphatase from membranes by phosphatidylinositol-specific phospholipase C from *Staphylococcus aureus* was studied. Alkaline phosphatase was readily released from pig kidney microsomes by phospholipase C but not by a variety of other treatments, e.g high ionic strength, extremes of pH, divalent cations, chelating agents, or analogues of the polar head group of phosphatidylinositol. Alkaline phosphatase released from microsomes by phospholipase C did not bind to phospholipid vesicle containing phosphatidylinositol. The ability of butanol-extracted alkaline phosphatase to bind to phospholipid vesicles was destroyed by added phosphatidylinositol-specific phospholipase C. Hydrolysis of added phosphatidylinositol by endogenous phospholipase activity in butanol extracts was also accompanied by loss of binding ability. Loss of binding ability was paralleled by a decrease in the apparent molecular weight of alkaline phosphatase. These results indicate that alkaline phosphatase is attached to membranes by a strong interaction with phosphatidylinositol.

INFLUENCE OF GRADED LEVELS OF FAT ON UTILIZATION OF PURE CARBOHYDRATE BY THE LAYING HEN. G.G. Mateos and J.L. Sell (Dept. of Animal Science, Iowa St. Univ., Ames, IA 50011) *J. Nutr. 110*(9), 1894-903 (1980). An experiment involving 140 SCWL laying hens was conducted to investigate the influence of graded levels of supplemental fat on the nitrogen-corrected metabolizable energy (ME) of diets containing different carbohydrates. The ME of each carbohydrate increased with each increment of supplemental fat. When yellow grease constituted 0 or 9% of the diet, the respective ME values of the carbohydrates (kcal/kg), expressed in a relative index form with the ME of sucrose in diets without added fat set equal to 100, were 100 and 110 for sucrose, 96 and 103 for starch, 93 and 100 for maltose, 92 and 101

for glucose, 90 and 98 for fructose and 89 and 98 for the glucose + fructose mixture. The data indicate that supplemental fat enhanced the utilization of energy from nonlipid dietary constituents. The mechanism by which fat exerts this influence on utilization of dietary energy is not known, but the possible relationship between decreased rate of food passage resulting from supplemental fat and energy utilization is discussed.

CHARACTERISTICS OF PHOSPHOLIPIDS IN HUMAN LUNG CARCINOMA. M. Nakamura, T. Onodera, T. Akino (Third Dept. of Internal Medicine, Sapporo Medical College, Sapporo 060, Japan) *Lipids 15*(9), 616-23 (1980). Human lung carcinoma tissues with histological types of adenocarcinoma, squamous cell and small cell carcinoma were investigated for phospholipids. There were marked differences in the phospholipids between these lung carcinoma and normal lung tissue. A marked decrease in saturated phosphatidylcholine (PC), predominantly the dipalmitoyl species, was noted in the carcinoma, although they still contained 17-20% of the saturated classes. The lung carcinoma contained less phosphatidylglycerol (PG) and lyso-*bis*-phosphatidic acid and more cardiolipin and phosphatidylinositol (PI) than the normal lung tissue. These alterations observed in the lung carcinoma appeared to show that they lose the characteristic feature of phospholipids in the lung tissue. The differences in the lipid composition among different cell types of lung carcinoma were also noted. The squamous cell and small cell carcinoma contained more triacylglycerol and relatively higher dienes I (monoenoic-monoenoic) and lower dienes II (saturated-dienoic) of PG, respectively, as compared to adenocarcinoma.

METABOLITES OF 1α,25-DIHYDROXYVITAMIN D_3 IN RAT BILE. B.L. Onisko, R.P. Esvelt, H.K. Schnoes, and H.F. DeLuca (Dept. of Biochem., College of Agr. and Life Sci., Univ. of Wisconsin-Madison, Madison, WI 53706) *Biochemistry 19*(17), 4124-30 (1980). Metabolites of 1α,25-dihydroxy[3α-^{3}H]vitamin D_3 in rat bile were studied. The water-soluble metabolites (77-91% of the total bile radioactivity) are predominantly acids (retained by DEAE-Sephadex chromatography) that become chloroform soluble after methylation with diazomethane. Calcitroic acid was isolated from this fraction (after conversion to the methyl ester) and unambiguously identified by chromatographic and mass spectrometric studies. The chloroform phase of extracted bile contains small amounts (1.1-8.1% of the metabolites in bile, dependent on dose given) of unchanged 1α,25-dihydroxyvitamin D_3 and no 1α,24(R),25-trihydroxyvitamin D_3. In addition, the chloroform phase contained a mixture of 24-dehydro- and 24-dehydro-1α-hydroxyvitamin D_3 and calcitroic acid methyl ester. The latter compound was shown to be an artifact resulting from the use of methanol as part of the solvent mixture for the initial bile extractions.

THE EFFECT OF VITAMIN B_{12} ON THE TOLERANCE OF CHICKS FOR HIGH LEVELS OF DIETARY FAT AND CARBOHYDRATE. M.B. Patel and J. McGinnis (Dept. of Animal Sciences, Washington St. Univ., Pullman, WA 99164) *Poult. Sci. 59*(10), 2279-86 (1980). Three experiments were conducted with White Leghorn chicks hatched from hens fed diets varying in levels of protein, fat, and vitamin B_{12}. Adding animal fat at a level of 10% in the chick diet caused growth depression of vitamin B_{12} deficient chicks, regardless of protein or energy level of hen or chick diet. Increasing the level of fat to 20% in the chick diet caused further growth depression and increased mortality. Feed efficiency of vitamin B_{12} deficient chicks was severely depressed by each additional increment in the fat level. Increasing protein content from 20 to 30% in the chick diet resulted in severe growth depression and poor feed efficiency. Although the added fat in the 30% protein chick diet depressed growth of chicks hatched from hens fed the 16 and 32% protein with added fat, it improved growth of those hatched from hens fed the similar diets with no added fat. Added fat in the 30% protein chick diet also improved feed efficiency of all chicks regardless of breeder diet treatments.

METABOLISM OF FREE AND ESTERIFIED CHOLESTEROL AND APOLIPOPROTEINS OF PLASMA LOW AND HIGH DENSITY LIPOPROTEINS. O.W. Portman, M. Alexander and J.P. O'Malley (Dept. of Nutr. and Metabolic Diseases, Oregon Regional Primate Res. Center, Beaverton, OR 97006) *Biochim. Biophys. Acta 619*(3), 545-58 (1980). We studied the patterns of equilibration of free and esterified cholesterol between lipoprotein fractions of plasma separated by heparin-Mn^{2+} and of their disappearance from plasma and appearance in liver and bile. Within 10 min after the injection of lipoproteins that had labeled free cholesterol, the bile contained labeled free cholesterol and within 20 min labeled bile acids. Both biliary cholesterol and bile acids initially were enriched 5-10-fold with the isotope that was originally contained in plasma HDL. Hepatic cholesterol was less enriched than bile cholesterol with the isotope of HDL. Although cholesteryl esters were equilibrated slowly between lipoprotein classes, their overall rate of removal from plasma was identical to that for the apolipoproteins of ^{125}I-labeled LDL and ^{125}I-labeled HDL during the first 2 h after injection. Labeled free cholesterol initially disappeared from the plasma compartment several times more rapidly than the esterified form or the lipoprotein apolipoprotein. Thus, cholesteryl esters probably interact with cells as part of intact lipoproteins, since they are not exchanged with cellular cholesterol like plasma free cholesterol.

EFFECT OF SATURATED AND POLYUNSATURATED FAT DIETS ON THE COMPOSITION AND STRUCTURE OF HUMAN LOW DENSITY LIPOPROTEINS. H.J. Pownall, J. Shephard, W.W. Mantulin, L.A. Sklar and A.M. Gotto, Jr. (Dept. of Med., Baylor College of Med. and the Methodist Hosp., Houston, TX 77030) *Atherosclerosis 36*, 229-314 (1980). We have studied the effect of diets of saturated and polyunsaturated fat on the composition, structure and thermal behavior of human plasma low density lipoproteins (LDL). We find that relative to a saturated fat diet one of polyunsaturated fat produces: (1) An increase in the unsaturated acyl content of all lipid classes of LDL. (2) An increase in the triglyceride content of LDL. (3) A decrease in the temperature of the thermotropic liquid crystal to isotropic liquid phase transition of LDL. (4) No effect on the temperature of LDL denaturation. In spite of major changes in the fatty acid composition of the major lipid of LDL, cholesteryl esters, polyunsaturated fat diet raised LDL triglyceride levels, our calorimetric and spectroscopic results suggest that the thermal properties and the structure of LDL at 37 C are sensitive to the composition of dietary fat.

EFFECT OF HIGH-FAT, HIGH-BEEF DIET AND OF MODE OF COOKING OF BEEF IN THE DIET ON FECAL BACTERIAL ENZYMES AND FECAL BILE ACIDS AND NEUTRAL STEROLS. B.S. Reddy et al. (Naylor Dana Inst. for Disease prevention, American Health Foundation, Dana Road, Valhalla, NY 10595) *J. Nutr. 110*(9), 1880-7 (1980). The effect of a high-fat, high-beef diet and of method of preparation of beef in the diet on the fecal bile acids and neutral sterols and on the activities of fecal bacterial β-glucoronidase, cholesterol dehydrogenase and 7α-dehydroxylase was studied in healthy men and women, 24-41 years old, who were consuming a customary mixed-western diet. The mode of cooking beef in the high-fat, high-beef experimental diets had no influence on the fat and protein content of the diets but the fat content of experimental diets was high compared to customary mixed-western diet. Fecal bacterial β-glucornidase activity and fecal secondary bile acid and cholesterol metabolite levels were significantly higher during the experimental diet periods but the fecal bacterial activities of 7α-dehydroxylase and cholesterol dehydrogenase were unaffected. The mode of cooking beef in experimental diets had no influence on the fecal bacterial enzymes and on the excretion of fecal bile acids and cholesterol metabolites.

INVESTIGATION OF THE ESSENTIAL BOUNDARY LAYER PHOSPHOLIPIDS OF CYTOCHROME *c* OXIDASE USING TRITON X-100 DELIPIDATION. N.C. Robinson, F. Strey, and L. Talbert. (Dept. of Biochemistry, University of Texas Health Science Center at San Antonio, San Antonio, TX 78284) *Biochemistry 19*(16), 3645-61 (1980). Beef heart cytochrome *c* oxidase was initially delipidated by incubation of the complex in 5% Triton X-100 followed by separation of the resulting detergent-protein complex from the detergent-lipid mixed micelles by sedimentation through a glycerol gradient containing 1% Triton X-100. These results are interpreted in terms of three classes of boundary layer phospholipids that have different affinities for the enzyme. The one with the lowest affinity is nonessential for activity and can be replaced by a variety of exogenous phospholipids and detergents. The second class is more tightly bound to the enzyme, requiring high concentrations of Triton X-100 for its removal, and is also nonessential for activity. The third class has the highest affinity for cytochrome c oxidase and is comprised of two to three molecules of DPG that are either tightly bound at the enzyme surface or buried in the cytochrome *c* oxidase complex. These DPG molecules are essential for the maximal activity of the complex and cannot be replaced by exogenous phospholipids and detergents, other than DPG, without loss of activity.

EFFECTS OF TOCOPHEROL DEFICIENCY ON LIPID METABOLISM IN THE ARTERIAL WALL OF RATS ON NORMAL AND HIGH CHOLESTEROL DIETS. K. Shirai et al. (The Second Dept. of Internal Medicine, School of Medicine, Chiba Univ., Chiba, Japan) *Artery 6*(6), 484-506 (1980). The effects of dietary tocopherol deficiency on arterial wall enzymes involved in lipid synthesis and hydrolysis were studied in rats receiving normal diets and diets supplemented with 1% cholesterol. When tocopherol was depleted from either the normal or high cholesterol diets, the following changes occurred in the arterial wall: increase in thiobarbituric acid reactive substances; decrease in lysosomal acid lipase and acid cholesteryl esterase; decrease in the microsomal enzymes, acyl CoA synthetase, triglyceride synthesizing activity, cholesteryl ester

synthesizing activity, neutral lipase and neutral cholesteryl esterase; increase in microsomal CPT. The results of these studies suggest that dietary tocopherol plays and important role in both lipid synthesis and degradation in the arterial wall, and the results may account for the accumulation of lipids and lipoperoxides in atherosclerotic lesions.

LIPID DEPOSITION IN THE MEDIA OF HUMAN CORONARY ARTERIES. D. Sinapius (Dept. of Path., Univ. of Goettingen, Goettingen, F.R.G) *Atherosclerosis 37*(1), 87-96 (1980). The coronary arteries of 52 unselected hearts obtained at autopsy and showing various types and degrees of intimal hyperplasia and atherosclerosis were investigated by staining for lipids and by histochemistry with regard to the lipid deposits in the media. Lipids, as demonstrated by histochemical staining are similar to those of the intimal lesions. The extracellular lipid contains largely cholesterol and a small amount of phospholipids; intracellular lipid is composed of cholesterol esters (as indicated by the spherical crystals in the droplets) and of a small amount of phospholipids, probably sphingomyelins. Medial lipids have apparently infiltrated from the intima. The presence of lipids in the media seems to reflect a stage in the transmural removal of lipids from the intima to the adventitia, and indicates an efflux of lipids from the vascular wall. Atherosclerotic plaques with large atheromas are very often associated with intracellular lipid deposition in the media (73% of sections). Athermas of younger persons are more frequently involved than those of older people.

THE INTERACTION OF VITAMIN A AND ZINC: IMPLICATIONS FOR HUMAN NUTRITION. N.W. Solomons and R.M. Russel (Dept. of Nutr. and Food Sci., Massachusetts Inst. of Tech., Cambridge, MA) *Am. J. Clin. Nutr. 33*(9), 2031-40 (1980). In recent years, evidence in experimental animals and man, regarding a multifaceted interaction between vitamin A and zinc has been accumulating. These studies have clarified older clinical observations in various human diseases while, at the same time, raising important diagnostic and therapeutic implications in several secondary (conditioned) deficiency states. Since the laboratory and experimental animal aspects of the vitamin A, zinc interaction have last been reviewed a number of important implications for human nutrition have emerged. In this paper, we attempt to link these experimental finding with classical and modern clinical observations, and to present a contemporary overview of nutritional interrelationships of zinc and vitamin A.

EFFECT OF ACUTE VIGOROUS EXERCISE ON LIPOPROTEIN LIPASE ACTIVITY OF ADIPOSE TISSUE AND SKELETAL MUSCLE IN PHYSICALLY ACTIVE MEN. M-R. Taskinen, E.A. Nikkila, S. Rehynen and A. Gordin (Third Dept. of Med., Univ. of Helsinki, and Res. Inst., Minerva, Helsinki, Finland) *Artery 6*(6), 471-83 (1980). Ten well-trained men ran a distance of 20 km in the morning after overnight fasting. Lipoprotein lipase (LPL) activity was determined from heparin eluates of adipose tissue and skeletal muscle ($P<0.01$) and 20% in adipose tissue ($P<0.05$) during the running. No significant change occurred in serum lipid or lipoprotein concentrations. The plasma insulin decreased and plasma glucagon increased during the exercise. The muscle LPL increment was significantly related to the fall of insulin/glucagon ratio. The results show that during exercise the skeletal muscle is adapted for increased uptake of circulating triglycerides which are either utilized immediately or used for restoration of muscle lipid stores after the end of exercise.

UNIDIRECTIONAL FLUX RATE OF CHOLESTEROL AND FATTY ACIDS INTO THE INTESTINE OF RATS WITH DRUG-INDUCED DIABETES MELLITUS: EFFECT OF VARIATIONS IN THE EFFECTIVE RESISTANCE OF THE UNSTIRRED WATER LAYER AND THE BILE ACID MICELLE. A.B.R. Thomson (Div. of Gastroenterology, Dept. of Med., Univ. of Alberta, Edmonton, Canada) *J. Lipid Res. 21*(6), 687-98 (1980). A previously validated

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in vitro technique was used to study the unidirectional flux rate (J_d) of cholesterol and a homologous series of saturated fatty acids (FA) into the jejunum and ileum of rats with alloxan or streptozotocin-induced diabetes mellitus (DM), under conditions of variable resistance of the intestinal unstirred water layer (UWL), and under conditions of varying concentrations of taurodeoxycholic acid (TDC). The results suggest that a) the passive permeability of the diabetic intestine to fatty acids is increased in DM but this difference is lost as the animals age; b) the J_d of cholesterol is greater in the jejunum and ileum in DM under conditions of variable concentrations of both cholesterol and bile acids; and c) the bile salt micelle functions in both DM and in controls to solubilize cholesterol and provide the source from which the cholesterol partitions prior to its uptake by the intestinal mucosal membrane.

PUBLICATIONS ABSTRACTED

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