Plans for Spring Meeting Ladies' Program announced

Chairwoman Barbara Allen has announced various activities planned for the Ladies Program of the Spring Meeting in Dallas, April 27-30.

Sunday afternoon registration will be followed with a mixer Sunday night. After a continental breakfast Monday morning, a tour of Olla Podrida, Dallas's enclosed market place for artisans and craftsmen, is planned. After lunch, participants will have a short trip to North Park to view the "Miracle of Pentecost," America's largest original religious painting presented with sound and light. On the return trip to the hotel, a tour will be conducted through Dallas residential areas.

Tuesday, following a brunch at Neiman-Marcus's Zodak Room, a tour of the Dallas Garden Center will be conducted. An informal lecture on indoor plants and terrariums also will be held at the Garden Center.

Wednesday morning will be open with several events offered. Small group walking tours will be organized, as well as tours of the Dallas Theatre Center and the Quadrangle, another shopping area. A sewing demonstration and bridge parties will be held at the hotel. ■

abstracts

• Fats and Oils

PARTITION OF FATTY ACIDS. R.B. Simpson, J.D. Ashbrook, E.C. Santos and A.A. Spector (Lab. of Biophysical Chem., Natl. Inst. of Arthritis, Metabolism, and Digestive Diseases, and Lab. of Applied Studies, Div. of Computer Res. and Tech., Natl. Inst. of Health, Bethesda, Md. 20014). *J. Lipid Res.* 15, 415–22 (1974). The partition ratios of radioactive fatty acids between *n*-heptane and a physiological buffer at 37C were measured. The fatty acids included the saturated acids with an even number of carbons from 10 to 18 and the unsaturated acids oleic, linoleic, and linolenic. In addition, the partition ratios of decanoate, myristate, and palmitate were determined over a wide pH range. Any *single* plot of partition ratio vs. aqueous concentration of an acid gave a nearly straight line, a finding consistent with very little association in the aqueous phase. In the case of the acids with 16 and 18 carbon atoms, however, comparison of the constants calculated from these plots with the assumption of no aqueous phase association revealed several inconsistencies. These inconsistencies cannot be resolved completely by assuming the existence of fatty acid association in the aqueous solution. We believe that at least some of the deviations are due to the presence of trace quantities of radioactive impurities in the labeled fatty acids. For example, purification of a sample of supposedly pure [1-^{14}C]myristate by a series of solvent extractions increased the partition ratio by a factor of 1.5.

LIPIDS IN PLANT TISSUE CULTURES. III. VERY LONG-CHAIN FATTY ACIDS IN THE LIPIDS OF CALLUS CULTURES AND SUSPENSION CULTURES. S.S. Radwan, H.K. Mangold and F. Spener (Bundesanstalt für Fettforschung, 44 Munster (Westf.), Germany). *Chem. Phys. Lipids* 13, 103–7 (1974). The lipids in plant tissue cultures contain in addition to the common saturated and unsaturated fatty acids even- and odd-numbered fatty acids having chain-lengths up to 26 carbon atoms.

SKIN LIPIDS: THEIR BIOCHEMICAL UNIQUENESS. N. Nicolaides (Dept. of Med. (Dermatology), Univ. of Southern Calif., Los Angeles, Calif. 90033). *Science* 186, 19–26 (1974). Two key words characterize the uniqueness of skin lipids: complexity and perversity. Each suggests a function. Complexity manifests itself in the large number and variety of both saturated and unsaturated fatty chains synthesized by human skin. Functionally, this allows each individual to have a distinct odor or chemical fingerprint. Perversity manifests itself when one compared the lipids synthesized by skin with those synthesized by internal tissues. For example, skin makes odd instead of only even chains, branched instead of only straight chains, free instead of only esterified acids, places double bonds in unusual positions in the fatty chains, extends chains to extreme lengths, and accumulates intermediates in the synthesis of a biologically valuable compound such as cholesterol. Functionally, these products may pose metabolic problems to potential pathogens and thus contribute to the survival of only compatible microorganisms.

QUANTITATIVE COMPOSITION OF COLD-PRESSED ORANGE OILS. P.E. Shaw and R.L. Coleman (U.S. Citrus and Subtropical Products Lab., Southern Region, U.S. Dept. of Agr., Agr. Res. Service, Winter Haven, Fla. 33880). *J. Agr. Food Chem.* 22, 785–7 (1974). One early-season, two midseason and four late-season cold-pressed orange oils were analyzed quantitatively by gas chromatography (glc) on a nonpolar column where glc response factors and percent nonvolatile material were determined to afford weight percent for each of the 17 main oil components. Late- and midseason oils were similar in composition by this analysis, although late-season oil is considered a better flavoring ingredient for orange products. The early oil had smaller quantities of linalool and some aldehydes than the others. A synthetic mixture of 15 components prepared for response factor determinations had a citrus-like aroma, but it was not like that of cold-pressed orange oil. The octanal to decanal ratio (measured on a polar column) and the citral to saturated aldehyde ratio in orange oil were comparable to earlier reported results.

PROCESS FOR PREPARING STEROLS FROM TALL OIL PITCH. D.V. Julian (Procter & Gamble). *U.S. 3,840,570.* The process comprises the steps of (1) dissolving a plant-derived sterol source in a solvent mixture comprising a lower alcohol, a liquid hydrocarbon, and 0.5–10% water and allowing the phases to separate; (2) saponifying the sterol esters obtained from the hydrocarbon phase with an alkali metal base in a lower alcohol solvent; and (3) dissolving the sterols in a polar, aprotic organic solvent in which soaps are insoluble, discarding the soaps, and recovering the sterols from the solvent.

PREPARATION OF HEAT-RESISTANT AND LIGHTFAST FATTY ACIDS. S.S. Naskar and G. Reneckhoff (Dynamit Nobel Ag.). *U.S. 3,840,574.* The process comprises heating the crude fatty acids of C_8–C_{18} chain length with a polycarboxylic acid selected from the group consisting of an aliphatic poly- or hydroxypolycarboxylic acid containing 2–10 carbon atoms, and a cycloaliphatic polycarboxylic acid containing 5–10 carbon atoms or a lower alkyl ester of the polycarboxylic acid to temperatures of 180–280C for a period sufficient to improve the heat-resistance and light fastness of the fatty acids. The treated fatty acids are subsequently distilled off under vacuum.

EGG PRODUCT. D.R. Strong and S. Redfern (Standard Brands, Inc.). *U.S. 3,840,683.* A cholesterol-free egg product comprises egg white, nonfat milk solids, vegetable oil and a coloring agent comprising a mixture of beta-carotene and an extract of plant xanthophylls present in amounts sufficient to impart the color of whole eggs to the product.

CHEESE CRUMBLES. S.F. Ziccarelli (Beatrice Foods Co.). *U.S. 3,843,808.* A cheese flavored, shelf stable and self preserving composition comprises 10–70% dry cheese solids and 30–50% of finely divided fat particles having an average size not greater than 0.5 mm. The fat has a melting point in the

range 90–125F. The fat particles are coated with 1–20% of an enrobing agent which prevents coalescence of the particles.

LOW-FAT EGG PRODUCT. R.D. Seeley (Anheuser-Busch, Inc.). *U.S. 3,843,811.* A low-fat, low-cholesterol frozen egg mix comprises 4–6% liquid whole egg, 92.5–88.5% liquid egg white, 2–2.6% potato flour, 0.1–0.2% carboxymethyl cellulose, 1.4–1.8% nonfat dry milk, and sufficient citric acid to provide a pH in the mix of 6.8–7.9. The mix contains 0–1.1% fat, 8–18% protein and less than 0.05% cholesterol.

DEEP FAT FRYING WITH EDTA ESTERS TO REDUCE DARKENING. E.R. Lowrey and V.E. Weis (Procter & Gamble). *U.S. 3,846,457.* A process for reducing the rates of darkening and/or foaming of fats and oils during frying consists of adding to the fats 1–1000 parts per million of alkyl esters of ethylenediamine tetraacetic acid.

REDUCING OIL CONTENT OF FRIED POTATOES. M. Nonaka, E. Hautala and M.L. Weaver (U.S. Secy. of Agriculture). *U.S. 3,846,572.* A process for preparing fried potato products of decreased oil content consists of (a) cutting peeled potatoes into strips, (b) imparting a surface freeze to the strips by immersing them for 11 seconds in difluorodichloromethane at −21.6F, (c) leaching the strips in water at 125F for 20 minutes, (d) par-frying the strips in oil at 323F for 1 minute, and (e) immersing the strips with agitation in oil-free difluorodichloromethane at −21.6F for 1–2 minutes.

FOOD FRYER WITH CONTINUOUSLY FILTERED COOKING OIL. W.A. Morris. *U.S. 3,849,309.* The filtering arrangement comprises a pair of concentric open frame support cylinders having an independent cylinder of filter paper positioned between them. The filter paper is made of mercerized wood pulp fiber saturated with a melamine-formaldehyde resin.

FILTER BED ASSEMBLY FOR CLEANSING COOKING OIL. W.A. Wecker, Sr. (Wacker-Dearborn Corp.). *U.S. 3,849,312.* The assembly comprises a base plate, a perforate filter plate, a filter element and means for clamping the package together. The package can be disassembled for cleaning and replacement.

AEROSOL DISPENSING SYSTEM FOR ANHYDROUS EDIBLE FAT COMPOSITIONS. V.D. Seijpal, W.J. Lueschen and B. Weinstein (American Home Products Corp.). *U.S. 3,849,580.* A dispensing system for comestible spreads not requiring refrigeration or included preservatives or corrosion inhibitors for prolonged shelf life comprises (a) a pressure tight container, (b) a dispensing nozzle, (c) 2–20% of food grade propellent, and (d) 80–98% of an anhydrous edible fat composition having a solid fat index of 5–30% at 70 F and selected from the class consisting of anhydrous butter, anhydrous solid and liquid vegetable oils.

METHOD OF PRODUCING A FONDANT PRODUCT. A.M. Aartsen (Cooperatieve Vereniging Suiker Unie U.A.). *U.S. 3,849,583.* The method of producing a dry fondant product capable of admixture with liquid to form a sugar glaze which will not flow away incidental to thawing after having been frozen comprises the steps of (a) forming a mixture of hard fat, liquid oil, and emulsifier having a melting point of 20–40C; (b) melting the fat component; (c) blending the fat component with a dry, granular sugar component at a level of 5–10%. The dry sugar component is obtained from a sugar solution cooked to 90–95% solids by mixing into it 30–70% of sugar particles having a size of less than 40 microns, and then mixing, breaking and cooling the mixture to a moisture content of 2%.

• Fatty Acid Derivatives

WHIPPABLE TOPPING COMPOSITION. H. Kubota, S. Nakayama and T. Tateishi (Fuji Oil Co.). *U.S. 3,840,682.* The composition comprises an edible oil or fat having a melting point of not less than 10C, 0.08–3% of an unmodified phospholipid, and 0.1–3% of an edible hydrophilic surface active agent. The surface active agent is nonionic and is selected from the group consisting of higher fatty acid esters of polyglycerols, higher fatty acid esters of polyoxyethylene sorbitan, higher fatty acid esters of saccharose and monoglycerides of malic and citric acid.

ALUMINUM COMPLEX SOAP GREASE. W.W. Bailey and P.R. McCarthy (Gulf Res. & Dev. Co.). *U.S. 3,843,528.* A lubricating grease composition comprises a major proportion of a mineral oil lubricating base having an aniline point below 230F thickened to the consistency of a grease with an aluminum complex soap of benzoic acid and a saturated fatty acid having 20–22 carbon atoms. A small amount of precipitated calcium carbonate is added to improve the antiwear and extreme pressure properties of the grease.

ISOMERIZATION OF 1,2-DIGLYCERIDES TO 1,3-DIGLYCERIDES. W.T.M. de Groot (Lever Bros. Co.). *U.S. 3,845,087.* The 1,2-diglyceride is maintained in the solid state within 20C of the initial melting point for a period from 2 hours to 2 months until the weight ratio of the 1,3-diglyceride to the 1,2-diglyceride is greater than 6:1. The fatty acids are the straight chain ones containing 4–24 carbon atoms.

N-SUBSTITUTED FATTY ACID AMIDE LUBRICANTS. F.C. Magne, R.R. Mod, G. Sumrell and W.E. Parker (U.S. Secy. of Agriculture) *U.S. 3,846,449.* The compound claimed is N,N-dibutyl-9,10-epithiostearamide.

ESTERS OF LACTIC ACID AND FATTY ALCOHOLS. J.D. Zech (ICI America Inc.). *U.S. 3,846,479.* One example of the composition is an ester obtained by subjecting a mixture of succinic acid or anhydride or adipic acid, lactic acid, and a fatty alcohol to esterification reaction conditions.

N-SUBSTITUTED FATTY ACID AMIDE LUBRICANTS. F.C. Magne, R.R. Mod, G. Sumrell and W.E. Parker. *U.S. 3,849,321.* The composition comprises paraffin oil and 5–10% of N,N-dibutyl-(9(10)-hydroxy-(9)10-dibutylphosphato)stearamide. It is useful as a lubricant because of its antiwear and extreme pressure lubricating properties. See also *U.S. 3,849,454.*

SULFURIZED FATTY OILS. P.C. Vienna and M.J. Den Herder (Standard Oil Co.). *U.S. 3,850,825.* The compositions consist of a blend of 80–95% prime burning lard oil and 5–20% alkyl oleate sulfurized to contain 8–12% bound sulfur and not more than 0.3% free sulfur. The alkyl oleate is a mixture of alkyl esters of saturated and unsaturated fatty acids having 14–18 carbons. Not more than 50% of the fatty acids are unsaturated and not more than 10% contain conjugated double bonds. The alkyl group has 1–3 carbon atoms.

SPERM OIL SUBSTITUTE. R.L. Zipf (Werner G. Smith, Inc.). *U.S. 3,850,827.* A lubricant comprises a blend of fatty acids having 10–24 carbon atoms and mono- and diesters of the fatty acids with glycols selected from the group consisting of simple glycols, each having 2–6 carbon atoms, polymers of the glycols having molecular weights up to 282, and mixtures of the two. At least 10% of the mono- and diesters are monoesters. The blend has a hydroxyl number in the range 15–70, an acid number less than 15, a pour point below 30F, an S.U.S. viscosity at 100F of 90–120, and an iodine number of 0–190.

• Biochemistry and Nutrition

FLUX OF FREE FATTY ACIDS AMONG HOST TISSUES, ASCITES FLUID AND EHRLICH ASCITES CARCINOMA CELLS. P. Mermier and N. Baker (Radioisotope Res., Veterans Admin. Wadsworth Hosp. Center, Los Angeles, Calif. 90073). *J. Lipid Res.* 15, 339–51 (1974). The role of plasma free fatty acids (FFA) in the transport of fatty acids from host tissues to Ehrlich ascites carcinoma in mice was studied. [9,10^{3}H]Palmitate complexed to mouse serum (albumin) was injected either intraperitoneally or intravenously into unanesthetized tumor-bearing mice. The incorporation of radioactivity into tumor extracellular fluid FFA, tumor cell FFA, neutral lipid, phospholipid, water-soluble material in cells and fluid, plasma FFA, host carcass total lipid fatty acids, and water-soluble (i.e., nonlipid) material was measured. In addition, the quantity of fatty acid in each of the above lipid fractions was determined. The data were analyzed by multicompartmental analysis (SAAM) using a digital computer, and fractional rate constants of FA movement within and out of the host-tumor system were calculated. These rate constants and pool size measurements were used to estimate the corresponding fluxes. Although FFA in the tumor's extracellular fluid were replaced rapidly, almost none of the newly formed fluid FFA was derived from plasma FFA. Moreover, the transfer of FFA from the tumor extracellular fluid FFA to plasma FFA was virtually negligible.

METABOLIC PATTERNS AND INSULIN RESPONSIVENESS OF ENLARGING FAT CELLS. M. DiGirolamo, M.D. Howe, J. Esposito, L. Thurman and J.L. Owens (Div. of Endocrinology, Dept. of Med., Emory Univ. Schl. of Med., Atlanta, Ga. 30303). *J. Lipid Res.* 15, 332–8 (1974). The rate and pattern of glucose metabolism, basal lipolysis, and intracellular concen-

tration of free fatty acids were determined in isolated epididymal fat cell preparations (mean volume 30–800 pl) from rats on the basis of fat cell number and in relation to the cell volume. The effects of increasing glucose concentrations in the medium and of insulin on the cellular metabolic activities were compared. Expanding fat cell volume correlated positively and significantly ($P < 0.001$) with the synthesis of glyceride glycerol from glucose (correlation coefficient, $r = 0.919$), with rates of basal lipolysis ($r = 0.663$), and with intracellular free fatty acid accumulation ($r = 0.796$); it correlated negatively and significantly with glucose conversion to glyceride fatty acids ($r = 0.814$, $P < 0.01$). The differences in patterns of glucose metabolism and basal lipolysis between small (<100 pl) and large (>400 pl) fat cells were not modified by insulin or by increments in glucose concentration. The results indicate that the reduced capacity of the large fat cells to respond to insulin cannot be attributed solely to a limited capacity of the cells to take up and metabolize increasing amounts of glucose.

RESTRAINT OF CHOLESTEROL ACCUMULATION IN TISSUE POOLS ASSOCIATED WITH DRASTIC SHORT-TERM LOWERING OF SERUM CHOLESTEROL LEVELS BY CLOFIBRATE OR CHOLESTYRAMINE IN HYPERCHOLESTEROLEMIC SWINE. D.N. Kim, K.T. Lee, J.M. Reiner and W.A. Thomas (Dept. of Pathol., and Specialized Center of Res. in Atherosclerosis, Albany Med. Coll., Albany, N.Y. 12208). *J. Lipid Res.* **15**; 326–331 (1974). In this study, young growing swine were made hypercholesterolemic (~300 mg/dl) by feeding milk and eggs for 7 wk. They were then divided into three groups (untreated, clofibrate-treated and cholestyramine-treated), and the diet was continued for an additional 3–4 wk. A cholesterol balance study was carried out in the terminal week. When the swine were killed, the total carcass content of cholesterol was determined, as well as contents of individual tissues. Both drugs caused a 50% reduction in serum cholesterol levels. The total carcass cholesterol contents were significantly lower in both treatment groups than in the untreated group. The difference was due largely to lower concentrations in the plasma and in bulk tissues. [4-^{14}C]Cholesterol was fed 7 days before the animals were killed, and specific activities of cholesterol in individual tissues were determined terminally. These gave a broad spectrum of values in tissues (excluding central nervous system) ranging rather evenly from 33% of plasma specific activity in the aorta to 100% in some tissues. The balance data suggest that cholestyramine reduces the enterohepatic bile acid pool and cholesterol absorption but increases fecal output of bile acids and total body cholesterol synthesis.

ENDOGENOUS PHOSPHOLIPIDS OF SWINE LIVER: EFFECT OF FAT DEPRIVATION ON MOLECULAR SPECIES OF PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLETHANOLAMINE. J.M. Shaw and N.R. Bottino (Dept. of Biochem. and Biophys., Texas A&M Univ., College Station, Texas 77843). *J. Lipid Res.* **15**, 317–25 (1974). Three 1-yr-old swine and two 2.5-wk-old swine were fed a fat-free diet for 1 month and 5 months, respectively. The hepatic phosphatidylcholine and phosphatidylethanolamine were fractionated by silver ion thin-layer chromatography. A distinctive feature of the chromatographic procedure was the development of the chromatograms at low temperatures: −10C for phosphatidylcholine and 4C for phosphatidylethanolamine. The chromatographic fractions were hydrolyzed with phospholipase A_2, and the fatty acids were characterized. Significant concentrations of odd-chain saturated and unsaturated fatty acids were found in the swine deprived of fat for 5 months. The major molecular species of phosphatidylcholine in both groups contained monoenoic fatty acids: 16:0/18:0 (n–9), 18:0–18:1(n–9), and 18:1(n–9)/18:1(n–9). Their concentrations changed only slightly with the diet. The molecular species of phosphatidylethanolamine were more sensitive to dietary changes. In the swine deprived of fat for 1 month, about 50% of the molecular species of phosphatidylethanolamine contained tetraenoic fatty acids: 16:0/20:4(n–6), 18:0/20:4(n–6), and 18:0(n–9)/20:4(n–6).

UTILIZATION OF ASCITES PLASMA VERY LOW DENSITY LIPOPROTEIN TRIGLYCERIDES BY EHRLICH CELLS. D.E. Brenneman and A.A. Spector (Depts. of Biochem. and Internal Med., Univ. of Iowa, Iowa City, Iowa 52242). *J. Lipid Res.* **15**, 309–16 (1974). Much of the lipid present in the ascites plasma in which Ehrlich cells grow is contained in very low density lipoproteins (VLDL). Chemical measurements indicated that triglycerides were taken up by the cells during in vitro incubation with ascites VLDL. When tracer amounts of radioactive triolein were incorporated into the ascites VLDL, the percentage uptakes of glyceryl tri[1-^{14}C]oleate and triglycerides measured chemically were similar. The cells also took up [2-^{3}H]glyceryl trioleate that was added to VLDL, but the percentage of available ^{3}H recovered in the cell lipids was 30–40% less than that of ^{14}C from glyceryl tri[1-^{14}C]oleate. This difference was accounted for by water-soluble ^{3}H that accumulated in the incubation medium, suggesting that extensive hydrolysis accompanied the uptake of VLDL triglycerides. Radioactive fatty acids derived from the VLDL triglycerides were incorporated into cell phospholipids, glycerides, and free fatty acids, and they also were oxidized to CO_2. Triglyceride utilization increased as the VLDL concentration was raised. These results suggest that one function of the ascites plasma VLDL may be to supply fatty acid to the Ehrlich cells and that the availability of fatty acid to this tumor is determined in part by the ascites plasma VLDL concentration.

SECRETION OF APOLIPOPROTEINS IN VERY LOW DENSITY AND HIGH DENSITY LIPOPROTEINS BY PERFUSED RAT LIVER. S.P. Noel and D. Rubinstein (Dept. of Biochem., McGill Univ., Montreal, Canada). *J. Lipid Res.* **15**, 301–8 (1974). The incorporation of labeled amino acids into the peptides of very low density lipoproteins (VLDL) and high density lipoproteins (HDL) secreted by perfused rat liver was studied using a Ringer-albumin solution in the perfusate in place of serum to diminish exchange of peptides between VLDL and HDL. Among the lipoproteins, the greatest release of protein, greatest incorporation of amino acid, and highest specific activity were found in VLDL. After separation of the delipidated peptides by electrophoresis on polyacrylamide gel, the incorporation into VLDL peptides was found to be 5–10 times as great as into HDL peptides. There was virtually no incorporation into the peptides of low density lipoproteins (LDL). Approximately 25% of the radioactivity incorporated into perfusate VLDL failed to enter the 13% polyacrylamide gel. The remaining radioactivity was distributed primarily among three peptide bands; one, found in the upper portion of the gel, contained 45% of the total, most of the remainder being found in two rapidly migrating bands. These three peptides appear to approximate those of human apo-C in relative electrophoretic mobility. Most of the HDL peptide radioactivity entering the running gel was found in a band that migrates slightly faster than the main VLDL band. A portion of the radioactivity of this major HDL band did not enter the running gel unless β-mercaptoethanol was present.

BIOSYNTHESIS OF RETINAL IN BOVINE CORPUS LUTEUM. A.M. Gawienowski, M. Stacewicz-Sapuncakis and R. Longley (Dept. of Biochem., Univ. of Mass., Amherst, Mass. 01002). *J. Lipid Res.* **15**, 375–9 (1974). Bovine corpus luteum tissue was sliced and incubated with β-[15,15′-^{3}H]carotene. The conversion of radioactive β-carotene into radioactive retinal was substantiated utilizing column chromatography, thin-layer chromatography, high-speed liquid chromatography, and a derivative formation. Lowering of the incubation temperature to 20C or boiling the tissue eliminated the conversion of β-carotene to retinal. In addition, other carotenoids and possible oxidation products of β-carotene in the corpus luteum were investigated. Our results indicate that the bovine corpus luteum possesses the ability to synthesize retinal in situ, which may play a role in reproductive functions.

PHOSPHOLIPID METABOLISM BY PHAGOCYTIC CELLS. PHOSPHOLIPASES A_2 ASSOCIATED WITH RABBIT POLYMORPHONUCLEAR LEUKOCYTE GRANULES. R. Franson, P. Patriarca and P. Elsbach (Dept. of Med., New York Univ. Schl. of Med., New York 10016). *J. Lipid Res.* **15**, 380–8 (1974). Polymorphonuclear leukocytes obtained from sterile peritoneal exudates in rabbits contain two phospholipid-splitting activities (phosphatidylacylhydrolases EC 3.1.1.4), one most active at pH 5.5 and the other between pH 7.2 and 9.0 Hydrolysis of phospholipid was demonstrated using *Escherichia coli* labeled during growth with [1-^{14}C]oleate and then autoclaved to inactivate *E. coli* phospholipases and to increase the accessibility of the microbial phospholipid substrates. The acid and alkaline phospholipase activities are both membrane bound, calcium dependent, and heat stable, and they appear to be specific for the 2-acyl position of phospholipids. Evidence was also obtained suggesting that the *E. coli* envelope phospholipids with oleate in position 2 are more readily degraded than those with palmitate. The two activities are associated with azurophilic as well as specific granules (obtained by zonal centrifugation) and with phagosomes (isolated after ingestion of paraffin particles by the granulocytes). Phospholipase A activities at pH 5.5 and pH 7.5 degrade the two major phospholipids of *E. coli*, phosphatidylethanolamine and phosphatidylglycerol, to the same extent, but the phospholipase activity at acid pH does not hydrolyze micellar dispersions of phosphatidylethanolamine.

SEPARATION OF THE MAIN LIPOPROTEIN DENSITY CLASSES FROM HUMAN PLASMA BY RATEZONAL ULTRACENTRIFUGATION. J.R. Patsch, S. Sailer, G. Kostner, F. Sandhofer, A. Holasek and H. Braunsteiner (Med. Dept., Univ. of Innsbruck, and Dept. of Med. Biochem., Univ. of Graz, Austria). *J. Lipid Res.* 15, 356–66 (1974). The major lipoprotein density classes (chylomicrons-VLDL, LDL, HDL_2 and HDL_3) were isolated from human plasma in a two-step ultracentrifugal procedure using the Ti-14 zonal rotor. The isolation of the two major high density lipoprotein subclasses (HDL_2 and HDL_3) was achieved in a 24-hr run using a nonlinear NaBr gradient in the density range of 1:00–1.40. The lipoproteins with a density <1.063 found in the rotor's center were isolated in a second run of 140 min duration using a continuous linear NaBr gradient in the density range of 1.00–1.30. The isolated lipoproteins were analyzed for chemical composition and for electrophoretic mobility; purity of isolated fractions was checked by immunochemistry. The lipoproteins exhibited flotation rates, chemical compositions, and molecular weights similar to those found with the common sequential procedures in angle-head rotors. The amount of lipoprotein lipids in the bottom fraction of the zonal rotor was comparable to that of the angle-head rotor. The described method yields the main lipoprotein density classes free from albumin in a very short running time; compared with the rate-zonal techniques already in use; this method allows the quantitative separation of an additional lipoprotein density class (HDL_2) without increasing the running time.

DETERMINATION OF CLOFIBRATE IN BIOLOGICAL FLUIDS BY THIN-LAYER AND GAS-LIQUID CHROMATOGRAPHY. A. Sedaghat, H. Nakamura and E.H. Ahrens, Jr. (Rockefeller Univ., N.Y. 10021). *J. Lipid Res.* 15, 352–5 (1974). A specific and sensitive method is described for the detection of clofibrate in biological fluids. The drug is separated from associated fatty acids by thin-layer chromatography and the methyl ester is quantified by gas-liquid chromatography. Recovery is excellent, and any small losses are corrected with an internal recovery standard. Although more time-consuming than other available techniques, the method offers advantages for accurate studies of clofibrate metabolism.

STUDIES ON TRIGLYCERIDE SYNTHESIS IN LIPID MICELLES FROM ADIPOSE TISSUE. N. Matsuoka, Y. Saito, H. Okuda and S. Fujii (Dept. of Enzyme Phys., Inst. for Enzyme Res., Schl. of Med., Tokushima Univ., Tokushima 770). *J. Biochem.* 76, 359–64 (1974). Lipid micelles were prepared from isolated fat cells by treatment under hypotonic conditions as reported previously. The micelles were found to have triglyceride synthesizing activity as well as lipolytic activity. Adrenaline and dibutyryl cyclic AMP (DBcAMP) had no effect on triglyceride synthesis, but greatly stimulated lipolysis in the micelles. Calcium ions inhibited triglyceride synthesis and activated lipolysis. Triglyceride synthesizing activity was found in the pellet fraction obtained by centrifuging the micelles at 105,000xg for 60 min. This fraction also contained lipase [EC 3.1.1.3] and esterase [EC 3.1.1.1] activities. Calcium ions inhibited the triglyceride synthesizing activity and activated the lipase activity of the pellet fraction. Based on these results, it was suggested that calcium ions may be important in the lipid metabolism of the micelles.

INHIBITION OF LIPID BIOSYNTHESIS BY P-CHLOROPHENOXYISOBUTYRATE (CPIB) IN TETRAHYMENA PYRIFORMIS. Yoshinori Nozawa (Dept. of Biochem., Gifu Univ. Schl. of Med., Gifu). *J. Biochem.* 74, 1157–63 (1973). The effects of a potent hypocholesteremic agent, *p*-chlorophenoxyisobutyrate (CPIB or clofibrate), on the growth, lipid content and incorporation of ^{14}C-labeled precursors (sodium [1-^{14}C] acetate and [1-^{14}C] palmitate) into various lipid fractions of *Tetrahymena pyriformis*, strain E, were investigated. Addition of CPIB induced a striking decrease in incorporation of ^{14}C-labeled precursors into phospholipids. Synthesis from ^{14}C-acetate of a sterol-like lipid, tetrahymanol, which is the most abundant neutral lipid and exclusively rich in the surface membranes, was also markedly inhibited by CPIB. Experiments with ^{14}C-acetate showed increased incorporation of ^{14}C-radioactivity into triglyceride associated with a corresponding decrease in phospholipid biosynthesis.

HUMAN MILK LIPASES. 1. SERUM-STIMULATED LIPASE. O. Hernell and T. Olivecrona (Dept. of Chem., Section on Physiological Chem., Univ. of Umea, S-901 87 Umea, Sweden). *J. Lipid Res.* 15, 367–74 (1974). Lipase activity has previously been demonstrated in human milk. This study shows that there are two separate triglyceride lipases in human milk. One is mainly in the skim milk and is stimulated by bile salts; the other is mainly in the cream and is inhibited by bile salts but stimulated by serum. The serum-stimulated lipase was purified by affinity chromatography on heparin-substituted Sepharose 4B. This gave a 9500-fold purification over whole milk. Although polyacrylamide gel electrophoresis showed that the enzyme was not purified to homogeneity, it had the highest specific activity so far reported for a human serum-stimulated lipase. The purified enzyme was free from bile salt-stimulated lipase activity and had the characteristics of other serum-stimulated or so-called lipoprotein lipases. Thus, it was almost completely inhibited by 1 M NaCl. The purified enzyme was active against tributyrylglycerol also in the absence of exogenous serum factors.

PHOSPHOLIPIDS AND ACYL GROUPS OF SUBCELLULAR MEMBRANE FRACTIONS FROM HUMAN INTRACRANIAL TUMORS. G.Y. Sun and B.S. Leung (Lab. of Neurochem., Ohio Mental Retardation Res. Center, Cleveland, Ohio 44109). *J. Lipid Res.* 15, 423–31 (1974). The phospholipids from subcellular fractions of human intracranial tumors were examined. For comparison, microsomes were isolated from a fetal human brain and from the gray matter of adult human brains. The subcellular membranes of tumors had a higher protein-to-phospholipid ratio than the normal brain membranes. The microsomes from tumors had a lower proportion of diacylglycerophosphorylethanolamine and higher proportions of alkenylacylglycerophosphorylcholine and sphingomyelin (plus diacylglycerophosphorylinositol) than microsomes from the gray matter. Also, the ratios of alkenylacylglycerophosphorylethanolamine to diacylglycerophosphorylethanolamine were higher in the tumors than in the normal controls. The acyl groups of ethanolamine-phosphoglycerides in tumor microsomes had relatively more 18:1, 18:2, and 20:4(n–6) and less 18:0, 22:4(n–6) and 22:6 (n–3) than the adult brain gray matter. Except for the increase in 18:2, acyl group changes in choline phosphoglycerides between tumors and controls were not as extensive as in the ethanolamine phosphoglycerides. The characteristic features of phospholipids and their constituent acyl groups of tumors were often present in all the subcellular fractions.

TRITIATED GLYCEROL TRIETHER AS AN OIL PHASE MARKER IN MAN. V.P. Gerskowitch and R.I. Russell (Dept. of Gastroenterology and Univ. Dept. of Med., Royal Infirmary, Glasgow, G4 OSF, Scotland). *J. Lipid Res.* 15, 432–5 (1974). ^3H-labeled glycerol triether has been suggested as a marker of the oil phase during digestion and absorption of a lipid test meal. This study examines the behavior of this isotope in the human alimentary tract. The results suggest that it is completely recovered from the gastrointestinal tract, and thus it remains solely with the oil phase of emulsions in vivo and with the oil phase of intestinal aspirates. ^3H-labeled glycerol triether may thus be an appropriate marker of the oil phase for use in human studies of lipid absorption.

COMPARISON OF THE EFFECTS OF VITAMIN A AND ITS ANALOGS UPON RABBIT EAR CARTILAGE IN ORGAN CULTURE AND UPON GROWTH OF THE VITAMIN A-DEFICIENT RAT. D.S. Goodman, J.E. Smith, R.M. Hembry and J.T. Dingle (Dept. of Med., Columbia Univ. Coll. of Phys. and Surgeons, N.Y. 10032). *J. Lipid Res.* 15, 406–414 (1974). A study was conducted to explore the relationship between the effects of vitamin A upon cartilage and the biological role of vitamin A in maintaining growth and life. Retinol, retinoic acid, α-retinoic acid and R08–7699 (a cyclopentyl analog of retinoic acid) were highly effective in promoting the lysis of the extracellular matrix of cartilage grown in organ culture in vitro. Retinoic acid and its two analogs were quantitatively more active than was retinol in bringing about lysis of matrix and release of proteoglycan into the culture medium. A bioassay was then conducted to determine the ability of each compound to promote growth of vitamin A-deficient rats. In contrast to their effects upon cartilage, retinoic acid and its two analogs were considerably less active quantitatively than retinol in promoting growth of vitamin A-deficient rats. Moreover, the three acids tested showed graded biological activity in the growth bioassay, with α-retinoic acid showing reduced bioactivity (approx. one-fourth that of retinoic acid) and R08–7699 being virtually inactive. The lysis of cartilage produced by these compounds was presumably caused by release of lysosomal enzymes as a result of the membrane-labilizing effects of the compounds.

CUTICULAR LIPIDS OF INSECTS. VI. CUTICULAR LIPIDS OF THE GRASSHOPPERS MELANOPLUS SANGUINIPES AND MELANOPLUS PACKARDII. C.L. Soliday, G.J. Blomquist and L.L. Jackson

(Dept. of Chem., Montana St. Univ., Bozeman, Montana 59715). *J. Lipid Res.* **15**, 399–405 (1974). The cuticular lipids of the grasshoppers *Melanoplus sanguinipes* and *Melanoplus packardii* contain 60 and 68% alkanes and 28 and 18% secondary alcohol wax esters, respectively, with lesser amounts of normal and sterol wax esters, triglycerides, alcohols, sterols and free fatty acids. All the hydrocarbons are saturated, and four types of alkanes are present: *n*-alkanes, 3-methylalkanes, internally branched monomethylalkanes, and internally branched dimethylalkanes. The principal *n*-alkanes in both insects are C_{29} and C_{27}, with a range from C_{21} to C_{33}. Trace amounts of 3-methylalkanes of 28, 30, and 32 total carbons are present. The principal internally branched monomethylalkanes are C_{32} and C_{34}, whereas the main dimethylalkane contains 35 carbons. The *n*-alkanes do not correspond in chain length to the secondary alcohols. The primary alcohols range from C_{22} to C_{32} in both insects, with C_{24} and C_{26} predominating. The fatty acids in the triglyceride and free fatty acid fractions range from C_{12} to C_{24} in *M. sanguinipes* and from C_{12} to C_{18} in *M. packardii*.

FORMATION AND METABOLISM IN VITRO OF 5,6-EPOXIDES OF CHOLESTEROL AND β-SITOSTEROL. L. Aringer and P. Eneroth (Dept. of Chem., Karolinska Inst., and Hormone Lab., Dept. of Obstetrics and Gynecology, Karolinska Sjukhuset, S-104 01 Stockholm 60, Sweden). *J. Lipid Res.* **15**, 389–98 (1974). The formation of 5α,6α- and 5β,6β-epoxides of cholesterol and β-sitosterol in rat liver subcellular fractions has been studied. The results show that the epoxidation seems to occur only in connection with the nonspecific tissue oxidation of the sterols. The β-epoxides were formed in three- to fourfold excess over the α-epoxides. Both cholesterol epoxides were efficiently converted by a microsomal hydrolase into the 3β,5α,6β-triol. The conversion was less extensive with β-sitosterol epoxides, especially the β-epoxide. The possible biological significance in the formation of the sterol epoxides and the triols was evaluated by their ability to inhibit the microsomal cholesterol 7α-hydroxylase. Only the cholesterol epoxides and especially the β-epoxide were active in this respect.

A STUDY OF METHODS OF IDENTIFICATION AND ESTIMATION OF LP(A) LIPOPROTEIN AND OF ITS SIGNIFICANCE IN HEALTH, HYPERLIPIDAEMIA AND ATHEROSCLEROSIS. K.W. Walton, J. Hitchens, H.N. Magnani and M. Khan (Dept. of Experimental Pathol., Univ. of Birmingham, Great Britain). *Atherosclerosis* **20**, 323–46 (1974). The Lp(a) lipoprotein was originally suggested to be a qualitative autosomal dominant genetic marker confined to about 35% of Caucasian populations, when these were screened by a gel-diffusion technique. Using a more sensitive and quantitative immunochemical technique, the Lp(a) lipoprotein is found to be detectable in 75% of a random British population and to show quantitative variation in concentration between individuals. In hyperlipidaemic subjects (with the exception of subjects with the Type V abnormality) there is an overall increase in Lp(a) reactivity with a tendency for higher individual values to occur, regardless of the type of hyperlipidaemia. In individuals with coronary heart disease, hypertriglyceridaemia and the presence of a pre-beta-lipoprotein band on electrophoresis, no evidence was obtained to suggest that the electrophoretic abnormality was directly due to the increased frequency of Lp(a) reactivity found in such subjects. Using the immunofluorescent technique, the Lp(a) antigen has been demonstrated in the arterial atherosclerotic lesions of Lp(a) reactors in a topographic distribution closely similar to that of LDL.

HEPATIC AND SERUM LIPID PATTERNS DURING DEVELOPMENT OF PHENOBARBITAL INDUCED FATTY LIVERS IN RATS. D.J. Tuma, M.F. Sorrell and A.J. Barak (Liver Study Unit, Gastroenterology Section V.A. Hosp. and the Depts. of Med. and Biochem., Univ. of Neb. Med. Ctr., Omaha, Neb. 68105). *Proc. Soc. Exp. Biol. Med.* **146**, 953–6 (1974). Lipids were measured in the livers and sera of rats that had received phenobarbital injections for 3 days and 5 days. The same lipid patterns including free cholesterol, cholesterol esters, phospholipids, triglyceride and fatty acids were determined in the livers and sera of rats 3 days and 5 days following withdrawal of the drug. Liver lipids increased with time while the rats were injected with phenobarbital and decreased toward normal levels once the drug was withdrawn. During phenobarbital administration as well as during withdrawal the serum lipid levels did not change. These results suggest that decreased lipid transport and enhanced lipid mobilization may not play major roles in the induction of the phenobarbital fatty liver.

THE INFLUENCE OF DIETARY ALTERATIONS, FASTING AND COMPETITIVE INTERACTIONS ON THE MICROSOMAL CHAIN ELONGATION OF FATTY ACIDS. H. Sprecher (Dept. of Physiol. Chem., Ohio State Univ., Columbus, Ohio 43210). *Biochim. Biophys. Acta* **360**, 113–23 (1974). When rats were raised on a fat-free diet and fasted for 24, 48 and 72 h the rates for the chain elongation of palmitic acid, octadeca-6,9-dienoic acid and octadeca-6,9,12-trienoic acid were all depressed to about the same extent. When rats raised on a balanced diet were fasted for 72 h and then refed either a balanced diet or a fat-free diet the rates for the chain elongation of octadeca-6,9-dienoic acid and octadeca-6,9,12-trienoic acid returned only to the level of non-fasted controls. When fasted rats were refed the balanced diet the chain elongation of palmitic acid was four times greater than the non-fasted controls. When rats were refed the fat-free diet the chain elongation of palmitic acid was 12 times greater than for the non-fasted controls. These findings suggest that rat liver microsomes may contain more than one chain elongating system. Competitive substrate experiments also suggest that hepatic microsomes contain more than one fatty acid chain elongation system.

STEROL BIOSYNTHESIS BY THE SEA URCHIN ECHINUS ESCULENTUS. A.G. Smith and L.J. Goad (Dept. of Biochem., Univ. of Liverpool, P.O. Box 147, Liverpool L69 3BX, U.K.). *Biochem. J.* **142**, 421–7 (1974). The 4-demethyl sterols of *Echinus esculentus* consisted of cholesterol as the major component, with lower concentrations of nine other C_{26}, C_{27}, C_{28} and $C_{29}\Delta^5$ sterols. [2-^{14}C]Mevalonic acid was readily incorporated by the urchin into squalene, lanosterol and desmosterol but only to a small extent into cholesterol. [26-^{14}C]Demosterol did not appear to be reduced to give cholesterol, but conversion of 5α-[2-$^3H_2$]lanost-8-en-3β-ol into cholesterol was observed. No C-24 dealkylation of [4-^{14}C]sitosterol or metabolism of [4-^{14}C]cholesterol could be detected.

STEROL TO PHOSPHOLIPID MOLAR RATIOS OF L CELLS WITH QUALITATIVE AND QUANTITATIVE VARIATIONS OF CELLULAR STEROL. L. Sokoloff and G.H. Rothblat (Wistar Inst. of Anatomy and Biol., Philadelphia, Pa. 19104). *Proc. Soc. Exp. Biol. Med.* **146**, 1166–72 (1974). In this study paired values of cellular sterol and phospholipid were determined after L cells were grown in medium known to induce changes in the level and kinds of cellular sterol. The sterol to phospholipid molar ratios computed from the paired values showed differences because, while the level of cellular sterol changed in response to exogenous sterol, no statistically significant differences in the levels of cellular phospholipid were apparent. When L cells were grown in the presence of concentrations of exogenous cholesterol ranging from 0 to 40 μg/ml, the sterol to phospholipid molar ratio ranged from 0.21 ± 0.01 to 0.33 ± 0.04, and, although the cellular sterol ranged from a low level almost exclusively of desmosterol (97%) to a 60% higher level almost exclusively of cholesterol (91%), no statistically significant differences were observed in the amount of cellular phospholipid. In another instance of growth in lipid-free medium a sterol to phospholipid molar ratio as low as 0.16 ± 0.03 was observed. In cell fractions enriched for surface membrane material, sterol to phospholipid molar ratios of 0.27 ± 0.02 and 0.47 ± 0.04 were observed for cells grown respectively in lipid-free and 7.5% serum supplemented medium. Additional analysis by thin-layer chromatography of the total lipid extract showed no apparent differences in the relative amounts of phospholipid classes.

VARIABLE REGION SEQUENCE OF THE HEAVY CHAIN FROM A PHOSPHORYLCHOLINE BINDING MYELOMA PROTEIN. S. Rudikoff and M. Potter (Lab. of Cell Biol., Natl. Cancer Inst., Natl. Insts. of Health, Bethesda, Md. 20014). *Biochemistry* **13**, 4033–8 (1974). The variable region sequence of the heavy chain from McPC 603, a phosphorylcholine binding myeloma protein, has been determined primarily by use of the automated sequencer. The variable region of this protein contains methionine residues at positions 34 and 83. Three cyanogen bromide fragments were isolated from cleaved heavy chains and pepsin Fab's which accounted for this entire sequence. The sequence of this protein outside the hypervariable regions shows considerable homology to the variable regions of other mouse as well as human proteins suggesting a conservation of genes coding for heavy chains.

INDEPENDENCE OF CHOLESTEROL AND FATTY ACID BIOSYNTHESIS FROM CYCLIC ADENOSINE MONOPHOSPHATE CONCENTRATION IN THE PERFUSED RAT LIVER. P. Raskin, J.D. McGarry and D.W. Foster (Depts. of Internal Med. and Biochem., Univ. of Texas Southwestern Med. Schl. and V.A. Hosp., Dallas, Tx. 75235). *J. Biol. Chem.* **249**, 6029–32 (1974). Cyclic adenosine 3′:5′-monophosphate (cyclic AMP), when added in a concentration of 5 mM to incubations of rat liver slices with [1-^{14}C]acetate

or [1-^{14}C]octanoate, was found to depress markedly both cholesterol and fatty acid biosynthesis but not CO_2 or ketone body production. The inhibitory effects of the nucleotide were lost when its concentration was reduced to 0.5 mM. These findings, therefore, confirm and extend those made by others. However, it also was observed that perfusion of rat livers with the above mentioned substrates in the presence of sufficient glucagon to raise the tissue cyclic AMP level by at least 50-fold was totally without effect on rates of cholesterol or fatty acid synthesis. In addition, such treatment of the livers failed to reduce the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase in subsequently isolated microsomes. It is concluded that in the intact liver cholesterol and fatty acid synthesis are independent of acutely induced changes in the intracellular cyclic AMP concentration over a very wide physiological range.

CHANGES IN OPTICAL PARAMETERS OF MYELOMA PROTEINS WITH PHOSPHORYLCHOLINE BINDING. R. Pollet, H. Edelhoch, S. Rudikoff and M. Potter (Clinical Endocrinology Branch, Natl. Inst. of Arthritis, Metabolism and Digestive Diseases, Natl. Insts. of Health, Bethesda, Md. 20014). *J. Biol. Chem.* **249**, 5188–94 (1974). The effects of binding phosphorylcholine to the myeloma proteins TEPC 15 and MePC 603 and their monovalent fragments, Fab′, have been evaluated by fluorescence, polarization of fluorescence, difference absorption and circular dichroism. Changes in all of these parameters are observed with TEPC 15 Fab′ and in several with MePC 603 Fab′. These parameters measure various properties of the aromatic chromophores belonging to the amino acid residues tryptophan and tyrosine. Since these residues can also be located in the antibody binding site, perturbation experiments have been made. In addition, a decrease in relaxation time of TEPC 15 Fab′ has been observed with ligand binding. Fluorescent data also suggest that changes in energy transfer between aromatic residues occur with phosphorylcholine binding. Taken together, the data indicate that a small structural change is produced in the monovalent antibody molecules.

PERTURBATION OF LIPOSOMAL AND PLANAR LIPID BILAYER MEMBRANES BY BACITRACIN-CATION COMPLEX. R.I. MacDonald, R.C. MacDonald and N.W. Cornell (Dept. of Biol. Sci., Northwestern Univ., Evanston, Ill. 60201). *Biochemistry* **13**, 4018–24 (1974). The antibiotic bacitracin at concentrations between 10^{-3} and 10^{-2} M causes the release of trapped, low molecular weight marker from artificial lipid vesicles or liposomes. This antiliposome effect is a paradigm of the antibacterial effect on these grounds. Both actions are specifically enhanced by cadmium or zinc. The spectrophotometrically detected formation of the presumably biologically active divalent cation-bacitracin complex exhibits a pH dependence which is also characteristic of complex-induced liposome lysis. Microbiologically active concentrations of bacitracin and $CdCl_2$-i.e., ca. 10^{-5} M – lower the conductance of and induce instability in planar lipid bilayer membranes. As determined by microelectrophoresis, exposure to bacitracin alone does not materially change the negative surface charge density of lipid vesicles. In the presence of the antibiotic and cadmium-but not calcium-however, the liposomal potential is significantly more positive. The function of cadmium or zinc with respect to the antimicrobial effect of bacitracin, therefore, appears to be the promotion of the cell-antibiotic interaction. Cadmium apparently does not enhance the surfactant property of the antibiotic, insofar as it has little influence on the critical micellar concentration of bacitracin.

THE EFFECT OF MYO-INOSITOL DEFICIENCY ON LIPID METABOLISM IN RATS. I. THE ALTERATION OF LIPID METABOLISM IN MYO-INOSITOL DEFICIENT RATS. E. Hayashi, T. Maeda and T. Tomita (Shizuoka College of Pharmaceutical Sci., 2-2-1, Oshika, Shizuoka, Japan). *Biochim. Biophys. Acta* **360**, 134–45 (1974). A purified *myo*-inositol-deficient, balanced diet containing phthalysulfathiazole and hydrogenated cotton seed oil produced *myo*-inositol responsive lipodystrophy in rats in a short period of time without interfering with their growth. The deficient rats, compared to the control rats, had increased levels of triacylglycerols in the liver (2.6- and 5.3-fold higher for one and two weeks of treatment), a concomitant increase in the nonesterified fatty acid levels in the serum and raised levels of cholesterol in the liver. The alteration of lipid levels developed by the deficient diet was rectified completely by an administration of *myo*-inositol and partially by an administration of essential fatty acids. Gas-chromatographic analysis of the liver lipids showed that in the *myo*-inositol-deficient rats, the palmitic, palmitoleic and oleic acids increased significantly, while a significant decrease was noted in the levels of stearic, linoleic and arachidonic acids both in the triacylglycerol fraction and in the total liver lipids.

II. THE MECHANISM OF TRIACYLGLYCEROL ACCUMULATION IN THE LIVER OF MYO-INOSITOL-DEFICIENT RATS. *Ibid.*, 146–55. The mechanism of the triacylglycerol accumulation in liver which had been observed in the *myo*-inositol-deficient rats has been investigated. Fatty acid distribution in the deposited triacylglycerols was quite similar to that of epididymal fat pads and to that of the dietary fat. Reserpine treatment caused a reduction in serum non-esterified fatty acids and dramatically reduced the accumulation of triacylglycerols and cholesterol in the liver. Incorporation of [1-^{14}C]palmitate from the specifically labelled epididymal fat pads to liver lipids in the deficient rats was 2.7 times that of the control rats. Disappearance of intravenously injected [1-^{14}C]palmitate from liver was somewhat accelarated in the deficient rats. Incorporation of [1-^{14}C] acetate into liver fatty acids and that of [1-^{14}C]palmitate into liver triacylglycerols was unaltered. The L-glycerol 3-phosphate level in the liver was decreased by 25% and 38% after one and two weeks, respectively, of *myo*-inositol-deficiency treatment. The above results indicate that an accumulation of triacylglycerols in the liver of the *myo*-inositol-deficient rats is primarily due to an increased rate of fatty acid mobilization from adipose depots to the liver.

ALTERATIONS IN CHOLESTEROL AND FATTY ACID BIOSYNTHESIS IN RAT LIVER HOMOGENATES BY ARYLOXY ACIDS. R.J. Olson, T.E. Trumble and W. Gamble (Dept. of Biochem. and Biophys., Oregon State Univ., Corvallis, Ore. 97331). *Biochem. J.* **142**, 445–8 (1974). 2,4-Dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid inhibited the incorporation of [2-^{14}C]mevalonate into cholesterol and non-saponifiable lipids. Both compounds inhibited the conversion of [1-^{14}C]isopentenyl pyrophosphate into cholesterol and the synthesis of cholesterol and fatty acids from [2-^{14}C]acetate. There was no inhibition of the conversion of [1-^{14}C]mevalonate into CO_2. At low concentrations (0.5 mM) of the compounds there was a stimulation of acetate incorporation into fatty acids.

EFFECTS OF MANNOHEPTULOSE ON LIPID METABOLISM OF RATS. E. Koh and C.D. Berdanier (Dept. of Foods, Nutr. and Inst. Administration, College of Human Ecology, Univ. of Md., College Park, Md. 20740). *J. Nutr.* **104**, 1227–33 (1974). The effect of mannoheptulose on the hepatic synthesis of fatty acids and cholesterol was studied in young male BHE rats after 3 weeks of feeding a 45% carbohydrate-40% protein diet, a 65% sucrose diet, or a 65% protein diet. Half of the animals fed each diet received daily subcutaneous injections of 20 mg of mannoheptulose (MH) while the remaining animals were given daily injections of isotonic saline. MH treatment enhanced the incorporation of acetate into cholesterol in the animals fed the high protein diet, inhibited the incorporation of acetate into cholesterol in the animals fed the 45% carbohydrate-40% protein diet, and did not affect the incorporation of acetate into cholesterol in the sucrose-fed animals. The activity of fatty acid synthetase was not significantly affected by MH treatment. Sucrose-fed animals had the greatest fatty acid synthetase activity whereas the high protein-fed animals had the least enzyme activity. Serum triglyceride levels reflected, in part, the activity of the fatty acid synthetase. The results of these studies show that MH may have a direct effect on lipid metabolism in addition to the short-term effect on insulin release reported by other investigators.

INHIBITION OF STEROL SYNTHESIS IN CULTURED MOUSE CELLS BY CHOLESTEROL DERIVATIVES OXYGENATED IN THE SIDE CHAIN. A.A. Kandutsch and H.W. Chen (Jackson Lab. Bar Harbor, Maine 04609). *J. Biol. Chem.* **249**, 6057–61 (1974). Sterols derived from cholesterol by hydroxylation of the side chain in the 20α, 22α, 22β, or 25 position inhibited sterol synthesis from acetate and depressed the level of 3-hydroxy-3-methylglutaryl-CoA reductase (EC 1.1.1.34) activity in primary cultures of mouse fetal liver cells and in L cell cultures. Rates of acetate metabolism to fatty acids and CO_2, and rates of RNA and protein synthesis were not affected. Following the addition of the most potent inhibitors of the group, 25-hydroxycholesterol and 20α-hydroxycholesterol, to L cell cultures the enzyme activity diminished to one-half of the original amount within a period of 1 to 1.3 hours. Inhibitory potency was influenced by the location of the hydroxyl function on the side chain, by the completeness of the side chain, and by the introduction of a third functional group into the molecule.

THE PRIMARY STRUCTURE OF APOLIPOPROTEIN-SERINE. R.L. Jack-

son, J.T. Sparrow, H.N. Baker, J.D. Morrisett, O.D. Taunton and A.M. Gotto, Jr. (Dept. of Med., Baylor College of Med., and Methodist Hosp., Houston, Tex. 77025). *J. Biol. Chem.* 249, 5308–13 (1974). Apolipoprotein-serine (apoLP-Ser or apoC-I) is one of the apoprotein constituents of human plasma very low density lipoprotein. The protein has 57 amino acid residues, including one residue of methionine and is lacking histidine, cysteine, cystine, and tyrosine. The NH_2 terminus of apoLP-Ser is threonine and the COOH terminus is serine. Cleavage of apoLP-Ser with cyanogen bromide, followed by chromatography of the digest on Bio-Gel P-30 in 25% formic acid, yielded two fragments corresponding to the NH_2-terminal (CNBr I) and the COOH-terminal (CNBr II) fragments and accounting for the 57 residues of the intact protein. The amino acid sequences of the tryptic peptides from CNBr I and chymotryptic peptides from CNBr II were determined by conventional methods. The amino acid sequence of apoLP-Ser is as follows: Thr-Pro-Asp-Val-Ser-Ser-Ala-Leu-Asp-Lys-Leu-Lys-Glu-Phe-Gly-Asn-Thr-Leu-Glu-Asp-Lys-Ala-Arg-Glu-Leu-Ile-Ser-Arg-Ile-Lys-Gln-Ser-Glu-Leu-Ser-Ala-Lys-Met-Arg-Glu-Trp-Phe-Ser-Glu-Thr-Phe-Gln-Lys-Val-Lys-Glu-Lys-Leu-Lys-Ile-Asp-Ser.

EFFECTS OF DIETARY ROUGHAGE ON CHOLESTEROL ABSORPTION, CHOLESTEROL TURNOVER AND STEROID EXCRETION IN THE RAT. J. Balmer and D.B. Zilversmit (Div. of Nutr. Sci. and Section of Biochem., Molecular and Cell Biol., Div. of Biol. Sci., Cornell Univ., Ithaca, NY 14850). *J. Nutr.* 104, 1319–28 (1974). We have observed that commercial rat diet and its grain components desolubilize both cholesterol and sodium taurocholate from a micellar solution. We have also studied cholesterol absorption, plasma cholesterol concentration and turnover, and excretion of fecal steroids in rats fed either a commercial stock diet or a low or high residue semisynthetic diet. The percentage of an oral dose of radioactive cholesterol absorbed was shown to be approximately 60% in rats fed either the stock diet or the semisynthetic diet by two independent isotope ratio methods. Plasma cholesterol concentration was significantly lower in rats fed stock diet, while removal of radioactive cholesterol from plasma and excretion of radioactive steroids in feces were greater in rats fed stock diet than in those fed the low residue semisynthetic diet. Plasma cholesterol concentrations were lower and steroid excretion was increased in rats fed the semisynthetic diet plus a relatively nondigestible soybran supplement as compared to those receiving unsupplemented semisynthetic diet. Our data suggest that nondigestible components of the stock diet have a major influence on plasma cholesterol concentration and turnover and on fecal excretion of steroids, but that they do not inhibit absorption of dietary cholesterol.

HYPERLIPIDEMIA IN OFFSPRING OF IRON-DEFICIENT RATS. H.A. Guthrie, M. Froozani, A.R. Sherman and G.P. Barron (Nutr. Program, Penn. State Univ., University Park, Pa. 16802). *J. Nutr.* 104, 1273–8 (1974). The effects of maternal dietary iron restriction during gestation and/or lactation on serum lipids in offspring were studied in rats fed diets containing 307 ppm or 5 ppm of iron. At parturition, dams fed the iron-deficient diet and their newborn had tissue reserves of iron and hematocrit values significantly lower than groups fed the control diet. However, serum iron and total iron-binding capacity were similar in both groups. Storage iron was also lower at the end of lactation in both dams and their litters when the dam was fed an iron-deficient diet during gestation and lactation than when a control diet was fed in either or both periods. Pups of dams fed the deficient diet during both gestation and lactation had lipemic sera characterized by elevated triglycerides, cholesterol and phospholipids. In pups of dams fed a deficient diet during one period and control during the other or control during both periods, lipid values were significantly lower. No differences in serum lipids were found among groups of dams.

CHOLESTEROL-PHOSPHATIDYLCHOLINE INTERACTIONS IN VESICLE SYSTEMS. IMPLICATION OF VESICLE SIZE AND PROTON MAGNETIC RESONANCE LINE-WIDTH CHANGES. M.P.N. Gent and J.H. Prestegard (Dept. of Chem., Yale Univ., New Haven, Conn. 06520). *Biochemistry* 13, 4027–33 (1974). Vesicular structures composed of phosphatidylcholine (PC) and varying amounts of cholesterol or phosphatidylethanolamine (PE) have been prepared and examined with respect to their inherent vesicle size and resultant proton magnetic resonance spectra. The PC-PE system, which should have little variation in the nature of hydrocarbon interactions as a function of PE content, shows a simple monotonic increase of hydrocarbon chain proton line width and a concomitant increase in vesicle size on increasing the mole per cent PE. The PC-cholesterol system shows a more complex line width and size behavior. At low cholesterol content both size and line width increase in a manner similar to that observed in the PC-PE system but beyond 30 mol % cholesterol induced effects become much more pronounced. The results suggest that although overall chain motion is slowed in vesicles of low cholesterol content, chain conformations are not restricted much more than in PC-PE vesicles of comparable size. At higher cholesterol concentrations, the nature of the cholesterol-PC interaction must change to a more restrictive one suggesting a change in the mode of phospholipid-cholesterol interaction well below the 1:1 stoichiometry suggested for multilayer systems. The observed changes in vesicle size at low cholesterol content are interpreted on the basis of a thermodynamic model which ascribes major perturbations to a variation in the dependence of enthalpy on vesicle radius.

EFFECT OF LITHIUM UPON LIPID METABOLISM IN RATS. A.I. Fleischman, P.H. Lenz and M.L. Bierenbaum (Atherosclerosis Res. Group, St. Vincent's Hosp., Montclair, N.J. 07042). *J. Nutr.* 104, 1242–5 (1974). Based upon preliminary reports indicating a negative correlation between the lithium content of drinking water and the incidence of coronary heart disease, a study was undertaken to examine the possible mode of action of exogenous lithium. One hundred and twenty-five Holtzman albino rats, mean weight 500 g, were divided into five groups. Four were fed a 22% fat-2% cholesterol diet containing 0, 0.008, 0.02 and 0.08% lithium, respectively, for 60 days. The fifth group was a control fed a stock diet. Serum phospholipids, cholesterol, sodium, potassium, magnesium, and urea nitrogen were unaffected by exogenous lithium. Serum free fatty acids increased with increasing lithium as did the serum calcium. Inorganic phosphorus decreased with increasing lithium. Blood sugar decreased significantly at lithium concentrations above 0.008%. Liver weight, phospholipids and cholesterol were unaffected by exogenous lithium, but liver total lipids decreased with increasing lithium concentration. This decrease was due to a decrease in liver triglycerides. At the 0.08% concentration, liver free fatty acids decreased. The data suggest that exogenous lithium stimulates triglyceride metabolism since liver triglycerides decreased concomitant with an increase in serum free fatty acid.

LINOLEATE OXIDATION PRODUCTS AND CARDIOVASCULAR LESIONS. M.G. Cutler and R. Schneider (Dept. of Clinical Pharmacol., Med. Schl., Birmingham (Great Britain). *Atherosclerosis* 20, 383–94 (1974). Linoleate hydroperoxide administered to rats in a total dose of 353 mg by repeated subcutaneous injections increased the incidence of myocardial fibrosis. When administered subcutaneously to rabbits in doses of 700-1000 mg, it produced myocardial fibrosis and aortic lesions. Neither linoleate nor the degradation products of linoleate hydroperoxide produced these cardiovascular lesions in rabbits when given by repeated subcutaneous injections in a total dose of 750 mg. The aortic lesions in rabbits given injections of linoleate hydroperoxide were associated with an increased peroxide value in the vessel wall but were not attributable to raised levels of cholesterol of β-lipoprotein in the serum. Lipid peroxides did not accumulate in the heart of either species after administration of linoleate hydroperoxide, although the lipid peroxide content of the liver was raised. The feeding of cholesterol to rabbits was followed by the typical cardiovascular lesions and by an elevation of serum cholesterol and β-lipoprotein levels. There was no increase in the peroxide value of the aorta or heart in these rabbits.

DIETARY REQUIREMENTS FOR VITAMIN E AND SELENIUM MEASURED AT THE CELLULAR LEVEL IN THE CHICK. G.F. Combs, Jr., and M.L. Scott (Dept. of Poultry Sci. and Grad. Schl. of Nutr., Cornell Univ., Ithaca, N.Y. 14850). *J. Nutr.* 104, 1292–6 (1974). Dietary requirements for vitamin E in the presence of adequate selenium, and for selenium in the presence of adequate vitamin E, were determined in vitamin E-depleted chicks fed a semipurified basal diet low in selenium and vitamin E. Inhibition of in vitro ascorbic acid-stimulated peroxidation in hepatic microsomes was used as a parameter. Results showed that both nutrients were required for complete protection of these membranes. Vitamin E was required at 30 to 50 IU per kilogram diet. This level also was required for optimal growth and utilization of dietary vitamin E as plasma tocopherols in selenium-adequate chicks. Selenium was required at 0.06 ppm (as Na_2SeO_3) for inhibition of peroxidation. This level also was required for optimal growth in vitamin E-adequate chicks.

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• Abstracts . . . . . (Continued from page 110A)

ANTIOXIDANT EFFECTS ON SELENIUM AND VITAMIN E FUNCTION IN THE CHICK. *Ibid.*, 1297–1303. Experiments were conducted to determine the effects of high-level antioxidant feeding on selenium and vitamin E function in vitamin E-depleted chicks fed a semipurified basal diet low in selenium and tocopherols. Three different antioxidants were fed: ethoxyquin (6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline), ascorbic acid and vitamin A. Each antioxidant significantly reduced selenium requirements for growth and prevention of exudative diathesis and mortality in vitamin E-deficient chicks. Also, plasma activities of glutathione peroxidase of chicks receiving marginal selenium levels were significantly increased. Ethoxyquin and ascorbic acid treatments reduced the dietary vitamin E requirement for prevention of in vitro ascorbic acid-stimulated peroxidation in hepatic microsomes in selenium-adequate chicks. However, vitamin A treatment severely depressed the plasma concentration of tocopherols and increased microsomal peroxidation without response to dietary vitamin E. The results indicated that antioxidants increased the utilization of dietary selenium without severely affecting vitamin E antioxidant function, except for vitamin A which appeared to antagonize vitamin E absorption directly.

ISOLATION AND CHARACTERIZATION OF THE CYANOGEN BROMIDE PEPTIDES FROM THE α1 (III) CHAIN OF HUMAN COLLAGEN. E. Chung, E.M. Keele and E.J. Miller (Depts. of Pathol. and Biochem., and Inst. of Dental Res., Univ. of Alabama Med. Ctr., Birmingham, Ala. 35294). *Biochemistry* 13, 3459–64 (1974). The nine peptides derived from the human α1(III) chain by cyanogen bromide cleavage have been isolated and characterized with respect to amino acid composition and molecular weight. The peptides are recovered in equimolar amounts and together account for all the amino acids and molecular weight of the chain as obtained following pepsin digestion and solubilization of the native collagen. Characterization of the α1(III) peptides has also verified the unique compositional features of the latter chain. Thus, seven of the nine peptides contain more hydroxyproline than proline, and three of the peptides exhibit a glycine content somewhat higher than would be anticipated on the basis of one glycyl residue for every three amino acids. In addition, the two cysteinyl residues of α1(III) which provide sites for interchain disulfide bonding have been shown to occur in a typical collagen-like sequence near the COOH-terminal portion of the chain.

GLUCAGON AND PLASMA LIPOPROTEIN LIPASE. R. Caren and L. Corbo (Cedars-Sinai Med. Res. Inst. and the Div. of Med., Cedars-Sinai Med. Ctr., Los Angeles, Calif. 90048). *Proc. Soc. Exp. Biol. Med.* 146, 1106–10 (1974). Subcutaneous administration of 1.0 mg glucagon to 15 fasted normal people caused significant increase of plasma lipoprotein lipase-like activity in 1 hr. The activity is termed lipase-like since coconut oil emulsion rather than isolated chylomicrons was used as substrate. A linear relationship between lipolysis and time of incubation obtained indicating zero order kinetics. pH optimum of enzyme activity was 8.4. There was no correlation between depression of plasma triglycerides and increase of plasma lipoprotein lipase-like activity following glucagon administration. This was thought to be due to other effects of glucagon administration which alter plasma lipid levels. Incubation of whole blood with glucagon caused an increase of plasma lipoprotein lipase-like activity and a depression of plasma triglycerides that were significantly correlated. The in vitro action of glucagon when incubated with whole blood suggests that blood cell(s) may be a source of glucagon-induced plasma lipoprotein lipase and that they may play a role in the regulation of plasma triglycerides. Increase of plasma lipoprotein lipase-like activity by glucagon when added to whole blood suggests that the hormone acted by releasing preformed enzyme from blood cell(s) or by enzyme induction in the nucleated blood cells.

EFFECT OF VITAMIN A ALCOHOL ON THE SURFACE COAT AND CHARGES OF L1210 LEUKEMIC CELLS. D. Brandes, T. Sato, H. Ueda and J.O. Rundell (Dept. of Pathol., Baltimore City Hosp., Baltimore, Md. 21224). *Cancer Res.* 34, 2151–8 (1974). L1210 leukemic cells were treated with either vitamin A alcohol or neuraminidase. Ascites cells in vivo and cultures of an L1210 cell line in vitro were used. In untreated cells stained with ruthenium red, the glycoprotein surface coat appears as a thick, uninterrupted electron-dense band. Positive colloidal iron staining results in deposition of particles in similar continuous fashion. No gaps were detected with both stains. After negative colloidal staining, no particle deposition was seen on the cell surface. Treatment with vitamin A resulted in the following changes. Ruthenium red staining showed a thinner, discontinuous surface coat. Positive colloidal iron binding was markedly decreased, and particle deposition occurred in sparse clusters separated by extensive gaps. Negative colloidal particle binding was enhanced, with the appearance of patchy clusters separated by gaps. These results indicate a loss of surface coat material, reduction in negative cell surface charge, and exposure of positive changes induced by vitamin A. The striking similarities between the effects of the vitamin and neuraminidase suggest that release of lysosomal neuraminidase by vitamin A may play a role in the surface changes. A direct effect of the vitamin on the cell coat cannot be disregarded.

SULFATED MUCOPOLYSACCHARIDES AND BASOPHILIC LEUCOCYTES IN RABBITS ON A HIGH CHOLESTEROL/OIL DIET. T.K. Sue and L.B. Jaques (Hemostasis-Thrombosis Res. Unit, Dept. of Physiol., Univ. of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0). *Proc. Soc. Exp. Biol. Med.* 146, 1006–13 (1974). Rabbits were fed a diet containing 1% cholesterol-3% peanut oil for 13 weeks. It was found that blood basophil count, serum triglyceride and cholesterol increased during the development of atherosclerosis. The changes of blood basophil number showed a significant correlation with the changes of serum triglyceride level but less correlation with serum cholesterol. The major component in the mucopolysaccharide extracts of the rabbit blood basophils was identified as chondroitin sulphate A/C. The sulphated mucopolysaccharide content per basophil decreased in the course of hyperlipemia and hypercholesterolemia. These findings give evidence of the physiological role of basophils in metabolism of triglycerides.

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