abstracts
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• Fats and Oils

RESEARCH ON AUTOOXIDATION AND ANTIOXIDATION. M.H. El-Mallah, H. Abdul-Karim and H.M. El-Khalafy (Natl. Res. Center, Dokki, Cairo, Egypt). *Seifen-Öle-Fette-Wachse* 100(19), 469–74 (1974). Comparative antioxidant potencies of individual antioxidants as well as their combinations have been studied using the active oxygen method. Synergistic and antagonistic effects exhibited by the antioxidant combinations were also evaluated. The combination gossypol-Santoquin showed a synergistic effect, however α-tocopherol-Santoquin showed an antagonistic action. The other twenty combinations have been evaluated at two oxidation levels using mixed methyl esters of shark liver oil as fatty substrate. Mechanisms of both synergistic and antagonistic actions were postulated.

OZONOLYSIS OF LONG-CHAIN CYCLIC ACETALS:FORMATION OF MONOESTERS. W.J. Baumann and T.H. Madson (Univ. of Minnesota, Hormel Inst., Austin, Minn. 55912). *J. Lipid Res.* 15, 528–9 (1974). A procedure is described for the quantitative conversion of saturated long-chain cyclic acetals of diols to the corresponding 0-acyl diols. Acetal ozonolysis proceeds in ethyl acetate-methylene chloride solution at −16 to −18C without overoxidation.

A METHOD FOR DETERMINATION OF SATURATED PHOSPHATIDYLCHOLINE. D. Shimojo, M. Abe and M. Ohta (Dept. of Biochemistry, Sapporo Med. College, Sapporo, Japan). *J. Lipid Res.* 15, 525–7 (1974). Phosphatidylcholine preparations containing saturated and unsaturated molecular species were subjected to $KMnO_4$-$NaIO_4$ oxidation in aqueous acetic acid, which left only disaturated species intact. After the oxidation, the remaining intact phosphatidylcholine was separated by thin-layer chromatography. The procedure could be used as a simple and rapid method for microdetermination of the saturated species in phosphatidylcholine preparations containing more than 0.1 μmole of the saturated species. The contents of the saturated species in native phosphatidylcholines obtained from rat lung tissue and washings by this procedure were 35.7% and 58.3%, respectively.

RELATIONSHIPS BETWEEN VOLATILE COMPOUNDS, STORAGE CONDITIONS, DEODORIZATION AND FLAVOR SCORES OF MILK FATS AND DRY WHOLE MILKS. A. Tamsma, A. Kontson and F.E. Kurtz (Dairy Products Lab., Eastern Regional Res. Ctr., Philadelphia, PA 19118). *J. Dairy Sci.* 57, 1143–8 (1974). Butterfat, deodorized butterfat, dry whole milk and a dry whole milk-like product containing 26% deodorized butterfat were stored for 3 and 6 mo at 4 and 27C. Initially and after each storage period, samples were evaluated for flavor after reconstituting to whole milk with 3.3% fat, the butterfats by homogenizing with skim milk, and the powders by reconstituting with water. At the same periods the butterfats and fats extracted from powders were steam deodorized at 50C and 1 torr, and the volatile compounds were collected and gas chromatographed. The chief volatile compounds, quantitatively, were identified as caproic, caprylic, capric, and lauric acids, nonanone, undecanone, tridecanone, and pentadecanone methyl ketones, and octa, deca, dodeca, and tetradeca delta lactones. Their concentrations are reported, and relationships to flavor scores were examined. Lactone concentration, deodorization, storage time and temperature and deodorization-temperature interaction were significant factors. Flavor scores after storage were related, in a linear model, to numerical values for these significant factors.

THE DYNAMIC STRUCTURE OF FATTY ACYL CHAINS IN A PHOSPHOLIPID BILAYER MEASURED BY DEUTERIUM MAGNETIC RESONANCE. A. Seelig and J. Seelig (Dept. of Biophys. Chem., Biocntr. of the Univ. of Basel, CH-4056 Basel, Klingelbergstrasse 70, Switzerland). *Biochemistry* 13, 4839–45 (1974). Deuterium magnetic resonance of selectively deuterated lipids opens a new avenue for probing the structure of lipid membranes. The method provides quantitative information on the ordering and the motional anisotropy of the various parts of the lipid molecules without resorting to perturbing probes. This is demonstrated for nonsonicated bilayers of L-α-dipalmitoyllecithin. The lipid molecules are deuterated at nine different carbon atoms of the fatty acyl chains. The deuterium data differ from spin-label electron spin resonance experiments. This may be attributed to a perturbation of the bilayer by the spin-label group.

THE NONEQUIVALENCE OF THE PHOSPHORUS ATOMS IN CARDIOLIPIN. G.L. Powell and J. Jacobus (Depts. of Chem. and Biochem., Clemson Univ., Clemson, S.C. 29631). *Biochemistry* 13, 4024–6 (1974). Cardiolipin possesses two nonequivalent phosphorus atoms. This conclusion, based on symmetry considerations, is consistent with the available ^{31}P nuclear magnetic resonance and phospholipase D hydrolysis data both sets of data being difficult to rationalize on alternative grounds. Predictions concerning expected chemical reactions of cardiolipin, based on generally applicable symmetry arguments, are advanced.

STRUCTURE OF THE MAJOR COMPLEX FORMED BY INTERACTION OF PHOSPHATIDYLCHOLINE BILAMELLAR VESICLES AND APOLIPOPROTEIN-ALANINE (APO-C-III). J.D. Morrisett, J.G. Gallagher, K.C. Aune and A.M. Gotto, Jr. (Depts. of Med. (J.D.M. and A.M.G.) and Biochem (K.C.A. and A.M.G.), Baylor College of Med. and the Methodist Hosp., Houston, Tx. 77025). *Biochemistry* 13, 4765–71 (1974). Apolipoprotein-alanine (ApoLP-Ala) from human plasma very low density lipoproteins has been shown previously to interact with phosphatidylcholine (PC) as determined by ultracentrifugal flotation, circular dichroism, and intrinsic tryptophan fluorescence. This protein also causes spherical bilamellar vesicles of phosphatidylcholine to become aligned as linear stacks or rouleaux when negatively stained and viewed by electron microscopy. The complex formed by interaction of PC vesicles and ApoLP-Ala *in solution* has been studied further by analytical gel filtration, analytical ultracentrifugation, and quasi-elastic light scattering. The complex eluted from a Sepharose 6B column at approximately the same volume as PC vesicles alone, but well ahead of ApoLP-Ala. Upon titration of PC vesicles with apoprotein, the observed sedimentation coefficient increased from 1.19 S to a limiting value of 4.93 S, which was first reached when the protein-lipid ratio was 0.23 g/g.

STEROL METABOLISM. XXXII. RADIATION-INDUCED OXIDATION OF ISOMERIC CHOLESTEN-3β-OLS. M.J. Kulig and L.L. Smith (Div. of Biochem., Dept. of Human Biol. Chem. and Genetics, Univ. of Texas Med. Branch, Galveston, Tx. 77550). *J. Org. Chem.* 39, 3398–402 (1974). The air oxidation induced by ^{60}Co γ *radiation of* cholest-4-en-3β-ol, 5α-cholest-6-en-3β-ol, and 5α-cholest-7-en-3β-ol yielded allylic hydroperoxides and other oxidized derivatives. The Δ^4-sterol gave cholest-4-en-3-one, 6β-hydroperoxycholest-4-en-3-one, 3β-hydroxycholest-4-ene 6α-hydroperoxide, and cholest-4-ene-3β, 6α-diol. The Δ^6-sterol gave cholesterol 7α- and 7β-hydroperoxides, the epimeric cholest-5-ene-3β, 7-diols, 3β-hydroxycholest-5-en-7-one, and 5α-cholest-6-3β, 5-diol but no 3β-hydroxy-5α-cholest-6-ene 5-hydroperoxide. The Δ^7-*sterol gave the epimeric* 3β-hydroxy-5α-cholest-7-ene 6-hydroperoxides, the epimeric 5α-cholest-7-ene-3β,6-diols, 3β-hydroxy-5α-cholest-7-en-6-one, and cholesta-5,7-dien-3β-ol. Pyrolysis of either Δ-6-hydroperoxide gave the corresponding 5α-cholest-7-ene-3β,6-diol, 3β-hydroxy-5α-cholest-7-en-one, and cholesta-5,7-dien-3β-ol. Reaction pathways for oxidations by radiation-induced processes of the isomeric Δ^4-, Δ^5-, Δ^6- and Δ^7-sterols and for their photosensitized oxidations in which *singlet molecular oxygen is implicated were compared.*

TEMPERATURE CHARACTERIZATION OF THE CONDUCTANCE OF THE EXCITABILITY INDUCING MATERIAL CHANNEL IN OXIDIZED CHOLESTEROL MEMBRANES. R. Latorre, O. Alvarez and P. Verdugo (Dept. de Biologia, Facultad de Ciencias, Casilla 653 and Dept. de Fisiologia y Biofisica, Facultad de Med., Univ. de Chile, Santiago, Chile). *Biochim. Biophys. Acta* 367, 361–5 (1974). The temperature dependence of the voltage-dependent excitability inducing material channel has been studied in thin lipid membranes containing only one channel. It is shown that the conductance of the open configuration has a temperature dependence that is just that expected from the temperature dependence of the bathing electrolyte. The conductance of the closed channel decreases increasing temperature.

PROBLEMS IN THE STEREOCHEMICAL DESIGNATION OF TOCOPH-

EROLS AND RELATED COMPOUNDS. O. Hoffman-Ostenhof (Ludwig-Boltzmann-Forschungsstelle fur Biochemie, and Inst. fur Allgemeine Biochemie der Univ. Wien, Vienna, Austria). *Am. J. Clin. Nutr.* 27, 1105–9 (1974). Whereas there are no serious problems concerning the nomenclature and the stereochemical designation of the natural tocopherols and tocotrienols, there exist considerable difficulties in naming the synthetic products with vitamin E activity. These natural compounds and the synthetic materials seem to differ only quantitatively in their biological effects. In the tocopherols, there are three chiral centers, the position 2,4′ and 8′; it is known that in natural α-tocopherol the configuration is 2R, 4′R,8′R. Owing to a slight dextrorotatory effect exhibited by the natural compound on plane-polarized light in certain solvents, it has been called d-α-tocopherol, whereas the product obtained by condensation of trimethylquinol with natural phytol or phytylbromide, respectively, originally got the name dl-α-tocopherol. This material is not a racemate but a mixture of the two epimer α-tocopherols with the configurations 2R, 4′R,8′R and 2S,4′S,8′S. The acetate of this material is the present International Standard for Vitamin E Activity. In recent years a product obtained by condensation of trimethylquinol and synthetic phytol or isophytol, respectively, is extensively used wherever vitamin E activity is desired. This material also has usually been referred to as dl-α-tocopherol, although it is a mixture of all eight possible stereoisomers in the form of four racemates. We deal here with mixtures of stereoisomers. So far, no system for such mixtures has ever been devised, but the importance of the tocopherols makes the creation of such a system necessary.

ROLE OF FAT IN FLAVOR OF CHEDDAR CHEESE. E.A. Foda, E.G. Hammond, G.W. Reinbold and D.K. Hotchkiss (Dept. of Food Technol., Iowa State Univ., Ames 50010). *J. Dairy Sci.* 57, 1137–42 (1974). The flavor of Cheddar cheese made from various kinds of fats homogenized into skim milk was compared with cheese made from natural milk emulsion. Milk fat homogenized into skim milk gave better flavors than other fats but was inferior to the natural milk emulsion. Commercial fats with composition and physical properties similar to milk fat seemed to give off-flavors during cheese ripening and performed less well than an inert mineral oil. Deodorized milk fat was not significantly different from milk fat in flavor production. Adding dimethyl sulfide and milk-fat-globule membrane material did not significantly improve cheese flavor. The addition of gum acacia as an emulsifying agent made the flavor more Cheddar-like, indicating that the water-fat interface is important in development of Cheddar flavor.

INTERESTERIFICATION OF GLYCERIDE OILS. W.H. DeGroot and M.H. Hilder (Lever Bros. Co.). *U.S. 3,852,315.* In the process, a glyceride oil is maintained below its boiling point at a temperature of 100–275C and an alkali metal having a melting point substantially below the temperature of the interesterified mixture is extruded directly into it. The alkali metal is maintained in solid form in the immediate vicinity of its point of entry to provide a seal between its supply and the interesterification mixture. The molten alkali metal is then distributed throughout the interesterification mixture after which the undesired soap is separated from the interesterified product.

STABILIZATION OF FOODS WITH AN ANTIOXIDANT. S.J. Bishov and A.S. Henick (U.S. Secy. of the Army). *U.S. 3,852,502.* The antioxidant comprises a compound selected from the group consisting of BHA, BHT and α-tocopherol, and autolyzed yeast protein which acts as a synergist for the other antioxidant.

PROCESS FOR COMPLETE SEPARATION OF CONSTITUENTS OF RICE BRAN AND THE LIKE. S. Mihara, Y. Inaba, K. Tachibana, T. Endo and E. Yasui (Nakataki Pharm. Industry Co.). *U.S. 3,852,504.* The starting material consists of non-deoiled seeds or brans of cereal grains which contain the natural amounts of oil or bran together with protein, crude fiber, starch, phytin, water soluble carbohydrates and water. The process comprises (1) adjusting the pH of the starting material to 1–6 with aqueous acid and mechanically pulverizing the mixture. The resulting mixture consists of a solid phase containing large size crude fiber particles and small size starch particles and a liquid phase containing protein bonded with oil emulsified in an aqueous phase containing phytin and water soluble carbohydrates dissolved therein. The process further comprises (2) separating from the mixture and recovering the crude fiber and starch particles; (3) filtering the liquid phase to recover separately the emulsified oil and the water soluble constituents; (4) extracting the oil from the protein with a solvent to recover each separately; and (5) adjusting the pH of the aqueous phase to 8–10 to precipitate phytin following which it is separated from the water soluble carbohydrates.

INTERESTERIFICATION PROCESS. A.J. Haighton and H.R. Kattenberg (Lever Bros. Co.). *U.S. 3,855,254.* In the process for accelerating the directed interesterification of a mixture of glycerides containing fatty acid radicals with 2–26 carbon atoms, the reaction is carried out at −30 to 60C in the presence of 0.01–0.5% of alkali metals or their catalytically active derivatives under conditions at which the glyceride mixture is alternately subjected for 5–60 minutes to a temperature of 1–15C below the cloud point of a randomized mixture of the same glycerides and for 15–300 minutes to a temperature of at least the cloud point of the same mixture. The temperature is cycled at least three times until the cloud point of the mixture obtained is at least 5C above the cloud point of the randomized mixture.

OIL OF TEMPEH. P. Gyorgy. *U.S. 3,855,256.* Oil of tempeh, useful as an antioxidant or stabilizing agent for edible oils and fats, is prepared by extracting tempeh, a fermented soybean product, with a liquid solvent consisting of a low molecular weight aliphatic hydrocarbon and a low molecular weight oxygen containing polar aliphatic organic compound. The liquid solvent extract phase is separated from the remainder and the solvent separated from the oil of tempeh.

• Biochemistry and Nutrition

EFFECT OF ESSENTIAL FATTY ACID DEFICIENCY ON THE ARRHENIUS PLOT OF ACETYLCHOLINESTERASE FROM RAT ERYTHROCYTES. B. Bloj, R.D. Morero and R.N. Farias (Inst. de Quimica Biol., Facultad de Bioquimica, Quimica y Farmacia, Univ. Nacional de Tucuman, Chacabuco 461, San Miguel de Tucuman, Rep. Argentina). *J. Nutr.* 104, 1265–72 (1974). Arrhenius plot of erythrocyte acetylcholinesterase was studied at different pH values in four groups of rats. Two groups were fed EFA-sufficient diets with lard or corn oil as the dietary fat. The other two groups were fed EFA-deficient diets: a basic, fat-free diet, and the same supplemented with hydrogenated beef fat. The Arrhenius plot of membrane-bound acetylcholinesterase from EFA-sufficient animals was found to have a breakpoint about 20C at pH 8.0, with lower activation energy at higher temperatures. The enzyme from EFA-deficient animals exhibited a breakpoint about 28C, the activation energies being lower than that of the enzyme from EFA-sufficient animals above and below this point. Solubilization of the membrane with Triton X-100 led to a shift in the breakpoint and to an increase in the activation energies in the enzyme from EFA-deficient animals. No changes were detected with preparations from EFA-sufficient animals after the treatment. After reconstitution of membrane-like material from the soluble EFA-deficient preparation, the distinctive enzymatic behavior was restored. The results indicate that the Arrhenius plot of the acetylcholinesterase is changed when the enzyme is bound to an EFA-deficient membrane.

METABOLISM OF IODINATED VERY LOW DENSITY LIPOPROTEIN IN THE RAT. AUTORADIOGRAPHIC LOCALIZATION IN THE LIVER. O. Stein, D. Rachmilewitz, L. Sanger, S. Eisenberg and Y. Stein (Dept. of Exp. Med. and Cancer Res. Hebrew Univ.-Hadassah Med. Schl. and Lipid Res. Lab., Dept. of Med. B, Hadassah Univ. Hosp., Jerusalem, Israel). *Biochim. Biophys. Acta* 360, 205–16 (1974). Very low density lipoprotein, labeled with ^{125}I, was injected into rats and its uptake by the liver was studied with the help of radioautography. Five minutes after injection saturation of uptake by the liver was observed when the injected dose exceeded 1.0 mg protein; at 30 min the uptake increased with the injected dose up to 3.5 mg protein. At these two time intervals, the radioautographic reaction in the liver (which represents mostly protein radioactivity) was concentrated mainly at the sinusoidal cell boundary, while only 40% of the grains were seen over the hepatocyte cytoplasm. At 120 min after injection 80% of the label was intracellular and many grains were localized to secondary lysosomes. The present findings indicated that the parenchymal liver cells are responsible for the uptake of a portion of the protein of injected very low density lipoprotein and that interiorization of the labeled product is preceeded by its accumulation at the cell boundary.

INTERACTION OF HUMAN SERUM HIGH DENSITY LIPOPROTEIN APOPROTEIN WITH PHOSPHOLIPIDS. A.W. Kruski and A.M.

Scanu (Depts. of Med. and Biochem. of the Pritzker Schl. of Med., Univ. of Chgo., and Franklin McLean Memorial Res. Inst., Chicago, Il. 60637). *Chem. Phys. Lipids* **13**, 27–48 (1974). The interaction of the apoprotein of human serum high density lipoprotein-3 (apo HDL_3) with aqueous dispersion of natural and synthetic phospholipids (PL) was investigated at a temperature above the transitions of the PL hydrocarbon chains and also above their critical micellar concentration. This protein is known to contain two major polypeptides: apo A-I and apo A-II. The protein: PL mixtures (weight ratio, 2, 1 or 0.5) were subjected to sonic irradiation and then fractionated by either CsCl or D_2O-sucrose density gradient ultracentrifugation. Usually three bands were obtained, the relative mass distribution of which depended upon the nature of the PL and the ratio of the interacting components: one band contained the PL-poor protein (d 1.28/g/ml), another, the uncombined PL ($d \leqslant 1.08$ g/ml), and the third band, both protein and PL.

INCREASED POLYUNSATURATED FATTY ACID YIELDS IN MILK OF COWS FED PROTECTED FAT. W. Mattos and D.L. Palmquist (Dept. of Dairy Sci., Ohio Agr. Res. and Develop. Ctr., Wooster 44691). *J. Dairy Sci.* **57**, 1050–4 (1974). Three Jersey cows were fed: (1) Control diet (corn silage, alfalfa hay, grain concentrate); (2) Control plus 3.6 kg/day full-fat soyflour; and (3) Control plus 3.6 kg/day formaldehyde-protected full-fat soyflour. Changes in rumen fatty acids, diet digestibility, and total yield of milk fatty acids were measured. Ether extract digestibility increased when the experimental diets were fed where as nitrogen digestibility was reduced by the protected supplement. Proportion of rumen acetate was lower when the unprotected soyflour diet was fed. Milk and fat yields were increased by the experimental diets. The weight percent as well as absolute yields of milk fatty acids from 6 to 16 carbons were reduced whereas butyrate and all 18 carbon fatty acids were increased by the experimental diets. Linoleic acid weight percent and absolute yield more than doubled in milk fat from animals receiving the protected supplement. Increased mammary gland uptake of 18 carbon fatty acid inhibited de novo synthesis of fatty acids by mammary tissue.

EFFECTS OF FREE AND PROTECTED FORMS OF COD LIVER OIL ON MILK FAT SECRETION IN THE DAIRY COW. J.E. Storry, P.E. Brumby, A.J. Hall and B. Tuckley (Natl. Inst. for Res. in Dairying Shinfield, Reading RG2 9AT, England). *J. Dairy Sci.* **57**, 1046–9 (1974). Diets of six lactating cows were supplemented with cod liver oil, either free or protected by encapsulation in formaldehyde-treated casein. Protected oil increased yields in milk of 20 to 22 carbon fatty acids but not yield of total milk fat. An inhibition of mammary uptake of plasma fatty acids is proposed to account for this. Free oil similarly increased yields of 20 to 22 carbon fatty acids in milk and additionally reduced the ratio of acetate to propionate in rumen digesta, yields of 4 to 18 carbon fatty acids in milk, and yield of total milk fat. Reduced syntheses of fatty acids in the rumen and mammary gland and reduced mammary uptake of plasma fatty acids are proposed to account for effects of free cod liver oil.

EFFECT OF AMOUNT AND FREQUENCY OF FEEDING SAFFLOWER OIL ON RELATED MILK, BLOOD, AND RUMEN COMPONENTS. R.B. Rindsig and L.H. Schultz (Dept. of Dairy Sci., Univ. of Wisc., Madison, Wisc. 53706). *J. Dairy Sci.* **57**, 1037–45 (1974). Two trials with 12 Holstein cows in early to mid-lactation were similar in experimental design. In Trial 1, four cows were assigned to each of the following treatments: (1) Control (conventional ration); (2) Control + 250 ml safflower oil fed once daily; and (3) Control + 500 ml safflower oil fed once every other day. In Trial 2, four cows were assigned to each of the following treatments for the experimental period: (1) Control; (2) Control + 500 ml safflower oil fed once daily; and (3) Control + 500 ml safflower oil fed once every 4th day. Milk fat percent and milk fat production decreased in all oil-fed groups versus their respective controls, with no significant differences between oil treatments within each trial. Plasma cholesterol was elevated with oil feeding, and a tendency for a negative arteriovenous difference for free fatty acids was observed. Uptake of triglyceride 18-carbon fatty acids by the mammary gland was selective in Trial 2. There were no significant differences in milk yield, protein, solids-not-fat, rumen volatile fatty acids, pH, arterial amounts, arteriovenous differences for blood glucose and acetate, and plasma triglycerides in either trial.

REGULATION OF 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE IN HEPATOMA TISSUE CULTURE CELLS BY SERUM LIPOPROTEINS. E.S. Kirsten and J.A. Watson (Dept. of Biochem. and Biophys., Univ. of Calif. San Francisco, San Francisco, Calif. 94143). *J. Biol. Chem.* **249**, 6104–9 (1974). The rate of 3β-hydroxysterol synthesis in hepatoma tissue culture (HTC) cells can be modified by the quality and quantity of serum lipoprotein in the culture medium. Cells maintained in medium which contained lipoprotein-poor serum have a steady state rate of 3β-hydroxysterol synthesis which is 3- to 4-fold greater than cells grown in medium containing unfractionated dialyzed serum. This increase is also reflected by a similar change in the catalytic activity of the rate-limiting enzyme for sterol biosynthesis (3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (EC 1.1.1.34). The addition of serum lipoproteins to medium containing lipoprotein-poor serum led to a rapid decrease in catalytic activity of HMG-CoA reductase; a $t_{1/2}$ for decay of 1.5 to 2 hours was calculated from steady state kinetics analysis. These results suggest that the regulatory site of action of serum lipoproteins is at a posttranscriptional level and that their net effect is to decrease the rate of synthesis of HMG-CoA reductase.

STRUCTURAL FEATURES OF STEROIDS WHICH INITIATE PROLIFERATION OF DENSITY-INHIBITED 3T3 MOUSE FIBROBLASTS. C.R. Thrash, Tsung-Shang Ho and D.D. Cunningham (Dept. of Med. Microbiol., Calif. College of Med., Univ. of Calif., Irvine, Irvine, Calif. 92664). *J. Biol. Chem.* **249**, 6099–6103 (1974). The ability of cortisol to initiate DNA synthesis and division of density-inhibited 3T3 mouse fibroblasts appears to correlate with its glucocorticoid activity. Steroids which possess high glucocorticoid activity in vivo such as triamcinolone acetonide, dexamethasone, cortisol, corticosterone, and aldosterone stimulated both DNA synthesis and cell division. The relative potency of these active steroids was related to their relative glucocorticoid potency. Steroids which are devoid of glucocorticoid activity such as testosterone, β-estradiol, tetrahydrocortisol, and progesterone did not initiate DNA synthesis or cell division. The only exception to the correlation between stimulatory and glucocorticoid activity was cortisone. Cortisone was inactive; however, its in vivo glucocorticoid activity apparently depends upon its conversion to cortisol.

CHOLESTEROL PRECURSOR POOLS FOR THE SYNTHESIS OF CHOLIC AND CHENODEOXYCHOLIC ACIDS IN RATS. K.A. Mitropoulos, N.B. Myant, G.F. Gibbons, S. Balasubramaniam and B.E.A. Reeves (Med. Res. Council Lipid Metab. Unit, Hammersmith Hosp. London, W12 OHS, United Kingdom). *J. Biol. Chem.* **249**, 6052–6 (1974). The rates of excretion and specific activities of total bile acids, cholic acid, chenodeoxycholic acid and cholesterol excreted in the bile were measured during a diurnal cycle in bile fistula rats fed [^{3}H]cholesterol for 4 to 5 weeks. One [^{3}H]cholesterol-fed rat received a constant infusion of [2-^{14}C]mevalonic acid and another received a constant infusion of 7α-hydroxy[4-^{14}C]cholesterol during collection of the bile. The rates of excretion of the four biliary constituents measured exhibited diurnal rhythm, with a maximum during the night and a minimum at noon. The specific activities of [^{3}H] chenodeoxycholate and [^{3}H]cholesterol did not change appreciably during the diurnal cycle. The specific activity of [^{14}C]chenodeoxycholate fell markedly during the night and was higher than that of [^{14}C]cholate throughout the diurnal cycle. These observations indicate that newly synthesized hepatic cholesterol is the preferred substrate for the formation of cholic acid, but they raise the possibility that some chenodeoxycholic acid is formed from a pool of cholesterol other than that from which cholic acid is formed.

STIMULATION OF THE SYNTHESIS OF VERY LOW DENSITY LIPOPROTEINS IN ROOSTER LIVER BY ESTRADIOL. K.L. Luskey, M.S. Brown and J.L. Goldstein (Divs. of Med. Genetics and Gastroenterology-Liver, Dept. of Internal Med., Univ. of Texas Southwestern Med. Schl., Dallas, Tx. 75235). *J. Biol. Chem.* **249**, 5939–47 (1974). A sensitive, specific, and rapid immunochemical method for the measurement of the synthesis of lipoproteins in rooster liver is described. Incubation of liver slices with [^{3}H]leucine resulted in the incorporation of radioactivity into protein material that could be precipitated from crude extracts by a monospecific antibody directed against the antigen common to plasma very low density and low density lipoproteins (very low density lipoprotein antigen). Use of this assay permitted the demonstration of a 4-fold increase in the rate of hepatic synthesis of the very low density lipoprotein antigen occurring 16 hours after the administration of estrogen to roosters. Since under these conditions as much as 18% of the total protein synthesized by the rooster liver represented very low density lipoprotein antigen, this system may provide

a model for studying not only the effect of a steroid hormone on specific gene expression but also the mechanism of regulation of very low density lipoprotein synthesis in higher animals.

THE INDUCTION OF HEPATIC CHOLESTEROL SYNTHESIS IN THE RAT BY LECITHIN MESOPHASE INFUSIONS. L. Jakoi and S.H. Quarfordt (Vet. Admin. Hosp., and Dept. of Med., Duke Univ. Med. Ctr., Durham, N.C. 27705). *J. Biol. Chem.* 249, 5840–4 (1974). A 4-hour intravenous infusion of egg lecithin in the rat produced a significant increase in hepatic cholesterol synthesis from acetate in both liver slices and homogenates. Direct in vitro additions of lecithin to the homogenates produced no change in the conversion of acetate to cholesterol. Mevalonate conversion to cholesterol in both homogenates and slices was unchanged by lecithin infusions. An increase of similar magnitude in the activity of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase was seen after the lecithin infusion. This increase in enzyme activity was associated with a significantly lower microsomal cholesterol content. The increase in cholesterol synthesis was prevented when the infused lecithin was made to molar ratios of cholesterol to lecithin greater than 0.5:1. Cholesterol-lecithin infusions with molar ratios of 1:1 appeared to inhibit conversion of acetate to cholesterol. The cholesterol content of the microsomes following these infusions was greater than control. Pretreatment of the animals with cycloheximide abolished the activation of cholesterol synthesis by lecithin, but actinomycin D had no effect.

INHIBITION OF GLUCOSE 6-PHOSPHATE DEHYDROGENASE BY PALMITOYL COENZYME A. A. Kawaguchi and K. Bloch (Conant Chem. Labs., Harvard Univ., Cambridge, Mass. 02138). *J. Biol. Chem.* 249, 5793–5800 (1974). Low concentrations of palmitoyl coenzyme A inhibit yeast glucose-6-P dehydrogenase. This inhibition is prevented or reversed by bovine serum albumin, mycobacterial polysaccharides, or alkylated cyclodextrins. Sephadex chromatography and sucrose density gradient centrifugation show that palmitoyl-CoA inhibits by dissociating the tetrameric dehydrogenase to dimeric, enzymatically inactive subunits. These structural changes are accompanied by firm binding of palmitoyl-CoA to the dimeric subunit and by release of NADP. After removal of the (nondialyzable) inhibitor from the palmitoyl-CoA-dimer complex by alkylated cyclodextrin the subunits reaggregate to enzymatically active tetramer. Reaggregation does not require NADP. In contrast to palmitoyl-CoA, sodium dodecyl sulfate dissociates yeast dehydrogenase to monomers. It is concluded that palmitoyl-CoA, unlike the synthetic anionic detergent, perturbs the dehydrogenase subunit structure in a controlled manner which may be important for regulating the activity of lipogenic enzymes. The dimeric glucose-6-P dehydrogenase from *Leuconostoc mesenteroides* is inhibited only by high concentrations of palmitoyl-CoA. In this instance palmitoyl-CoA neither binds to the enzyme nor dissociates it. *Torulopsis utilis* glucose-6-P dehydrogenase, also dimeric, is irreversibly converted to inactive monomers by low concentrations of palmitoyl-CoA.

CHARACTERIZATION OF THE RAT APOLIPOPROTEINS. I. THE LOW MOLECULAR WEIGHT PROTEINS OF RAT PLASMA HIGH DENSITY LIPOPROTEINS. P.N. Herbert, H.G. Windmueller, T.P. Bersot and R.S. Shulman (Molecular Disease Branch, Natl. Heart and Lung Inst., and the Lab. of Nutr. and Endocrinology, Natl. Inst. of Arthritis, Metabolism, and Digestive Diseases, Natl. Insts. of Health, Bethesda, Md. 20014). *J. Biol. Chem.* 249, 5718–24 (1974). The apolipoproteins from rat plasma high density lipoproteins were separated into three fractions by Sephadex gel chromatography. Five low molecular weight proteins were purified from the last Sephadex fraction by ion exchange chromatography. All five proteins have apparent homologues among the human apolipoproteins. The A-II apoprotein has COOH-terminal alanine, a blocked NH_2 terminus and contains no histidine, tryptophan or cysteine. Unlike human A-II, it appears to occur in monomeric form. The C-I apoprotein has COOH-terminal alanine, NH_2-terminal aspartic acid, contains 16% lysine, and lacks valine, tyrosine, and cysteine. The C-II apoprotein has NH_2-terminal threonine, COOH-terminal glutamic acid and contains no cysteine. Two forms of the C-III apoprotein were found with COOH-terminal proline and NH_2-terminal aspartic acid. One form, C-III-0, contains no carbohydrate, while the other, C-III-3, contains 3 moles of sialic acid and 1 mole of galactosamine per mole of protein.

SEQUENTIAL RENAL LIPID CHANGES IN WEANLING RATS FED A CHOLINE-DEFICIENT DIET. A.J. Monserrat, E.A. Porta, A.K. Ghoshal and S.B. Hartman (Centro de Patologia Exp., Dept. de Patologia, Facultad de Med., Univ. de Buenos Aires, Buenos Aires, Argentina). *J. Nutr.* 104, 1496–1502 (1974). To clarify conflictive aspects related to the possible pathogenic role of renal lipid changes in the usually fatal renal necrosis occurring in choline-deficient rats, a severely hypolipotropic basal diet that induces renal necrosis was fed ad libitum to male rats for 5 days. Control rats were pair-fed the same basal diet supplemented with choline. Renal lipid changes were sequentially studied at days 2, 4 and 5, and the values of these determinations were expressed using different base parameters to facilitate adequate interpretation. Since at day 5 almost 50% of the choline-deficient rats had renal necrosis, the data obtained at this time were separated into those preceding and those accompanying necrosis. The analyses of the results obtained under these conditions suggested that the most significant prenecrotic lipid change is a decrease in the renal content of phospholipids occurring shortly before necrosis (day 5). At this time the levels of sphingomyelin in the nonnecrotic kidneys of choline-deficient rats were significantly higher than those of the control rats while the levels of phosphatidylinositol were significantly lower. It is concluded that contrary to recent proposals, the possibility still exists that a renal phospholipid deficit and/or other more subtle changes in the individual renal phospholipids may play a role in the pathogenesis of this condition.

EFFECT OF 1,3-BUTANEDIOL ON HEPATIC FATTY ACID SYNTHESIS AND METABOLITE LEVELS IN THE RAT. D.R. Romsos, P.S. Belo and G.A. Leveille (Dept. of Food Sci. and Human Nutr., Mich. State Univ., East Lansing, Mich. 48824). *J. Nutr.* 104, 1438–45 (1974). The effect of 1,3-butanediol on hepatic fatty acid synthesis and metabolite levels in the rat was examined. Hepatic fatty acid synthesis was depressed in rats fed 1,3-butanediol. An intraperitoneal injection of 1,3-butanediol increased blood glucose levels but did not affect hepatic fatty acid synthesis in the rat. Addition of 1,3-butanediol to the incubation buffer depressed glucose, but not acetate, conversion to fatty acids by rat liver slices. Methylene blue inhibition of hepatic fatty acid synthesis was partially reversed by the addition of 1,3-butanediol to the incubation buffer. Hepatic β-hydroxybutyrate levels and the β-hydroxybutyrate:acetoacetate ratio were increased when, 1,3-butanediol was fed. Lactate and pyruvate levels were lower in freeze-clamped liver preparations from rats fed 1,3-butanediol than those observed in control animals. Further, the lactate:pyruvate ratio was increased, suggesting that the hepatic cytoplasmic NADH/NAD^+ ratio was increased. Hepatic long-chain acyl CoA levels were also increased when 1,3-butanediol was fed. It is suggested that the shift in the cytoplasmic redox state and the increase in hepatic long-chain acyl CoA levels are involved in the decrease in hepatic fatty acid synthesis observed when rats are fed 1,3-butanediol.

RESONANCE RAMAN SPECTROSCOPY OF RHODOPSIN IN RETINAL DISK MEMBRANES. A.R. Oseroff and R.H. Callender (Dept. of Mol. Biophys. and Biochem., Yale Univ., New Haven, Conn. 06520). *Biochemistry* 13, 4243–8 (1974). Low-temperature resonance Raman spectroscopy has been used to study the conformation and interactions of retinal within its opsin binding site in disk membrane vesicles formed from bovine retinal rod outer segments. At 80K, laser irradiation within the visible absorption band produces well-defined photostationary states containing only rhodopsin, isorhodopsin, and bathorhodopsin. A double wavelength, pump-probe technique has been devised to distinguish scattering from these three components. In addition to the conventional Raman "probe" laser beam, a "pump" beam at a different wavelength is used to modify the composition of the photostationary state. The fixed wavelength probe holds the resonant enhancement factors constant so that changes in the spectra that are induced by the pump beam are directly related to the composition of the photostationary state. The Raman data demonstrate that in situ, retinal and opsin are joined by a protonated Schiff base.

1α-HYDROXYVITAMIN D_3. AN ANALOG OF VITAMIN D WHICH APPARENTLY ACTS BY METABOLISM TO 1α,25-DIHYDROXYVITAMIN D_3. J.E. Zerwekh, P.F. Brumbaugh, D.H. Haussler, D.J. Cork and M. R. Haussler (Dept. of Biochem., College of Med., Univ. of Ariz., Tucson, Ariz. 85724). *Biochemistry* 13, 4097–4102 (1974). 1α-Hydroxyvitamin D_3 (1α-OH-D_3) is a synthetic sterol with biological characteristics similar to those of 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2$-D_3), the apparent hormonal form of vitamin D. The synthetic sterol is virtually equipotent to the natural hormone, in vivo, and has been utilized recently to treat patients with defects in vitamin D metabolism. However, no information is presently available on its biochemical mode of action. In order to determine if 1α-OH-D_3 functions

by binding directly to target tissue receptors for 1α,25-$(OH)_2$-D_3 or is first metabolized to 1α,25-$(OH)_2$-D_3, we have carried out a detailed examination of the comparative biologic effects as well as the receptor binding properties of the two sterols. In chronic administration studies, the synthetic sterol was slightly more antirachitic than 1α,25-$(OH)_2$-D_3, with both sterols being 2–6 times more active than native vitamin D_3. The conversion of 1α-OH-D_3 to 1α,25-$(OH)_2$-D_3 was also observed in intestinal mucosa homogenates, in vitro, further verifying the occurrence of this enzymatic reaction.

TRITON X-100 IN 4 M UREA AS AN EXTRACTION MEDIUM FOR MEMBRANE PROTEINS. II. MOLECULAR PROPERTIES OF PURE CYTOCHROME b_{559}: A LIPOPROTEIN CONTAINING SMALL POLYPEPTIDE CHAINS AND A LIMITED LIPID COMPOSITION. H.S. Garewal and A.R. Wasserman (Dept. of Biochem., McGill Univ., Montreal, Quebec, Canada). *Biochemistry* **13**, 4072–9 (1974). The molecular composition of electrophoretically homogeneous preparations of chloroplast cytochrome b_{559} was examined. The partial specific volume was 0.91; the hydrodynamic molecular weight, likely as a complex with Triton X-100, was 117,000. The equivalent dry weight per mole of heme, using Triton-depleted samples, was 111,000. The protein content (41%) was equivalent to 45,900 g/mol of heme. Since *only one size of polypeptide chain* was found [5600(±1000)] about eight small chains were calculated to be present per one heme and thus per molecule. N-terminal analysis revealed at least three kinds of polypeptide chains (Glu, Asp, and Thr as N-terminals). The Triton X-100 content was less than 5–6% and migrated as a single spot different from those of the two unknown polar lipids. Neither polar lipid was a glycolipid. The major polar lipid was not a phospholipid, but the minor component might have been a phospholipid. Each of the unknown migrated chromatographically like unknown polar lipids of chloroplast-grana membranes. Cytochrome b_{559} contained no non-heme iron and no detectable hexose.

BIOSYNTHESIS OF LIPIDS IN GOLGI COMPLEX AND OTHER SUBCELLULAR FRACTIONS FROM RAT LIVER. L.M.G. Van Golde, J. Raben, J.J. Batenburg, B. Fleischer, F. Zambrano and S. Fleischer (Lab. of Vet. Biochem., State Univ. of Utrecht, Utrecht, The Netherlands). *Biochim. Biophys. Acta* **360**, 179–92 (1974). Golgi complex, rough and smooth microsomes, plasma membranes, mitochondria and nuclei from rat liver were isolated and their purity assessed using specific marker enzymes. The various subcellular fractions were assayed for the following processes: biosynthesis of sphingomyelin, CDP-diglycerides, phosphatidylinositol, phosphatidylserine, the conversion of phosphatidylserine into phosphatidylethanolamine, the formation of lecithin via N-methylation, and the activation of palmitic and octanoic acids. None of these processes were found to be present in Golgi complex. The endoplasmic reticulum appears to be the principal site in the cell for the synthesis of sphingomyelin, CDPdiglycerides, phosphatidylinositol, phosphatidylserine and the formation of lecithin. Mitochondria are, however, the only site in the cell where phosphatidylserine is decarboxylated. This activity appears to be localized in the inner membrane. The activation of palmitate is localized predominantly in endoplasmic reticulum and mitochondria, though some activity was detected in plasma membranes as well. All other cell organelles, including Golgi and probably nuclei, did not contain significant palmitoyl-CoA synthetase activity.

REDUCTION OF BLOOD SERUM CHOLESTEROL. M. Winitz (NASA). *U.S. 3,849,554.* A method for lowering blood serum cholesterol in humans consists of administering as the sole source of sustenance a defined diet composition consisting of vitamins, minerals, a source of amino acids, a source of essential fatty acid and a carbohydrate component consisting of glucose, maltose, polysaccharides of glucose and mixtures of these.

TRITIATED BILE ACIDS: PROBLEMS AND RECOMMENDATIONS. D.K. Panveliwalla, D. Pertsemlidis and E.H. Ahrens, Jr. (Rockefeller Univ., N.Y. 10021). *J. Lipid Res.* **15**, 530–2 (1974). Kinetic studies of cholic and chenodeoxycholic acids were carried out in three patients by simultaneous intravenous administration of appropriate pairs of ^3H- and ^{14}C-labeled compounds. The results obtained indicated two sources of error: chemical impurity and loss tritium by biological exchange. Precautions are listed for use of tritiated bile acids in studies of pool sizes and turnover rates.

LIQUID-GEL PARTITION CHROMATOGRAPHY OF VITAMIN A COMPOUNDS; FORMATION OF RETINOIC ACID FROM RETINYL ACETATE IN VIVO. Y.L. Ito, M. Zile, H. Ahrens and H.F. DeLuca (Dept. of Biochem., College of Agr. and Life Sci., Univ. of Wisconsin-Madison, Madison, Wisc. 53706). *J. Lipid Res.* **15**, 517–24 (1974). A clear separation of retinol, retinal and retinoic acid has been achieved by liquid-gel partition chromatography on Sephadex LH-20 with solvent mixtures of chloroform, Skellysolve B and methanol. A mixture of retinyl esters, retinol, retinal and retinoic acid has been resolved on hydroxyalkoxypropyl Sephadex using Skellysolve B and acetone. There is no decomposition of any of the vitamin A compounds during chromatography, and recovery is complete. The combination of mildness and potential for resolution makes liquid-gel partition chromatography a superior tool for the separation of vitamin A compounds. This method has been applied to the study of vitamin A metabolism at physiological levels in the vitamin A-deficient rat. Retinyl palmitate, an ester of retinoic acid, retinal, retinol, retinoic acid and a polar metabolite have been demonstrated in various tissues of the rat 12 hr after a dose of 2 μg of [11-^{14}C]retinyl acetate.

EFFECT OF ALTERATIONS OF THE SPECIFIC ACTIVITY OF THE INTRACELLULAR ACETYL CoA POOL ON APPARENT RATES OF HEPATIC CHOLESTEROGENESIS. J.M. Dietschy and M.S. Brown (Gastrointestinal-Liver Univ, Dept. of Internal Med., Univ. of Texas Southwestern Med. Schl. at Dallas, Dallas, Tx. 75235). *J. Lipid Res.* **15**, 508–16 (1974). We have previously shown significant dilution of the specific activity of the intracellular acetyl CoA pool when radio-labeled acetate is used as the precursor in liver slice experiments. In the present study, using liver from animals subjected to various manipulations known to alter the rate of cholesterogenesis, the specific activity of the intramitochondrial acetyl CoA pool was 27–49% of the theoretical specific activity expected if no endogeneous dilution occurred. Because of the cytosolic acetyl CoA pool that gives rise to cholesterol is not in equilibrium with the intramitochondrial pool, these values cannot be used to correct the flux of labeled carbon from [^{14}C]acetate into cholesterol. However, because [^{14}C]octanoate is rapidly oxidized intramitochondrially to acetyl CoA, which feeds both the intra- and extramitochondrial metabolic pathways, [^{14}C]octanoate can be utilized to determine true flux rates of C_2 units into cholesterol and other products. Data also are presented that show very good agreement between the corrected C_2 flux rate from octanoate into cholesterol and microsomal HMG CoA reductase activity in the same liver under conditions in which the synthetic rates were varied over a 100-fold range.

PHOSPHOLIPIDS OF CLOSTRIDIUM BUTYRICUM. V. EFFECTS OF GROWTH TEMPERATURE ON FATTY ACID, ALK-1-ENYL ETHER GROUP AND PHOSPHOLIPID COMPOSITION. G.K. Khuller and H. Goldfine (Dept. of Microbiol., Schl. of Med., Univ. of Penn., Philadelphia, Pa. 19174). *J. Lipid Res.* **15**, 500–7 (1974). Many anaerobic bacteria have a high proportion of 1-alk-1′-enyl ethers (plasmalogens) among their phospholipids. We have examined the effects of growth temperature on the phospholipid, fatty acid and alk-1-enyl group compositions of *Clostridium butyricum*. When the growth temperature was decreased from 37C to 25C, the proportion of glycerol phosphoglycerides (the sum of phosphatidylglycerol and the corresponding plasmalogen) increased at the expense of the ethanolamine and N-methylethanolamine phosphoglycerides. When the temperature was lowered from 37C to 25C, the fatty acids had progressively more unsaturated and cyclopropane chains and fewer saturated chains. The alk-1-enyl groups, in particular those from the ethanolamine and N-methylethanolamine plasmalogens, were more saturated at 30C than at 37C. When the growth temperature was lowered to 25C, there was little further change in the degree of unsaturation of the alk-1-enyl groups.

REGULATION OF CHOLESTEROL STORAGE IN ADIPOSE TISSUE. A. Angel and J. Farkas (Dept. of Med. and Inst. of Med. Sci., Univ. of Toronto, Toronto, Canada). *J. Lipid Res.* **15**, 491–9 (1974). Adipose tissue is a major site of cholesterol storage. In an attempt to define mechanisms controlling this process, a variety of nutritional and metabolic alterations were employed and their effects on adipose tissue cholesterol levels were determined by direct chemical analysis. When rats were raised on Purina chow, a linear increase in the cholesterol/DNA ratio in relation to animal weight (from 120 g [5–6 wk] to 700 g [2 yr]) occurred. The rate of cholesterol accumulation was related to the dietary cholesterol load. Cholesterol accumulation by adipose tissue also occurred in rats on a cholesterol-free diet and reached levels exceeding those observed in animals fed on a diet containing 0.05 or 0.1% (w/w) cholesterol. Comparison of obese mice with nonobese littermate controls showed that the size of the adipose cholesterol pool was proportional to the degree of adipocity because the

amount of cholesterol stored per unit glyceride mass was identical. Adipose tissue cholesterol was not affected by animal sex. Thus, adipose tissue cholesterol levels were dependent on animal age, dietary cholesterol load, early nutritional deprivations, and the size of the adipose organ itself.

ISOLATION AND CHARACTERIZATION OF GLUCOSYLSPHINGOSINE FROM GAUCHER'S SPLEEN. S.S. Raghavan, R.A. Mumford and J.N. Kanfer (E.K. Shriver Ctr. for Mental Retardation, W.E. Fernald State Schl., Waltham, Mass. 02154). *J. Lipid Res.* **15**, 484–90 (1974). Glucosylsphingosine has been isolated for the first time as a natural constituent from Gaucher's spleen. On thin-layer chromatography, it migrates with authentic glucosylsphingosine, yielding a positive color reaction with ninhydrin for the amino group and with α-naphthol-sulfuric acid for the carbohydrate residue. N-Acylation with palmitic acid gave rise to glucosylceramide, which was cleaved by purified glucosylceramide: β-glucosidase to ceramide. Gas-liquid chromatography of the trimethylsilyl derivative showed a retention time similar to authentic glucosylsphingosine. Gas-liquid chromatographic analysis of the trimethylsilyl derivatives after methanolysis revealed the presence of only glucose and C_{18}-sphingosine. Mass spectral data further supported the structural identity with glucosylsphingosine.

POSTNATAL DEVELOPMENT OF ADIPOCYTE CELLULARITY IN THE NORMAL RAT. M.R.C. Greenwood and J. Hirsch (Rockefeller Univ., N.Y. 10021). *J. Lipid Res.* **15**, 474–83 (1974). It has been shown by several investigators that adipocyte number is stable in mature human beings and several species of rodents. Although the number of new cells appearing in the adipose depot can be measured histometrically and by Coulter counting of osmium-fixed cells, such methods do not distinguish between "lipid filling" of preexistent adipocytes and synthesis of new adipocytes. The experiments reported here using in vivo injection of [^{3}H]thymidine show that synthesis of new adipocytes in the Sprague-Dawley rat continues after birth and ceases before sexual maturity. Furthermore, during the second and third postnatal weeks, a "bed" of preadipocytes is synthesized. Preadipocytes may take as long as 30 days to appear as mature adipocytes.

VALIDATION OF A DUAL-ISOTOPE PLASMA RATIO METHOD FOR MEASUREMENT OF CHOLESTEROL ABSORPTION IN RATS. D.B. Zilversmit and L.B. Hughes (Graduate Schl. of Nutr. and Section of Biochem., Mol. and Cell Biol., Div. of Biol. Sci., Cornell Univ., Ithaca, N.Y. 14850). *J. Lipid Res.* **15**, 465–73 (1974). Several methods for measuring cholesterol absorption in the rat have been compared. After administration of an oral dose of labeled cholesterol (^{14}C or ^{3}H) and an intravenous dose of colloidal labeled cholesterol (^{3}H or ^{14}C) the ratio of the two labels in plasma or whole blood 48 hr or more after dosing compared closely to the ratio of areas under the respective specific activity-time curves. The area ratio method is independent of a time lag between the appearance of oral and intravenous label in the bloodstream. Both measures of cholesterol absorption agree fairly well with a method based on measuring the unabsorbed dietary cholesterol in a pooled fecal sample. The plasma isotope ratio method for cholesterol absorption gave the same results in rats practicing coprophagy as in those in which this practice was prevented. The addition of sulfaguanidine to the diet lowered cholesterol absorption as measured by the plasma isotope ratio to the same degree as that measured by the fecal collection method.

CHARACTERIZATION OF THE PLASMA LIPOPROTEINS OF THE GENETICALLY OBESE HYPERLIPOPROTEINEMIC ZUCKER FATTY RAT. G. Schonfeld, C. Felski and M.A. Howald (Lipid Res. Ctr., Depts. of Preventive Med. and Med., Washington Univ. Schl. of Med., St. Louis, Mo. 63110). *J. Lipid Res.* **15**, 457–64 (1974). The plasma lipoproteins of the Zucker fatty rat were characterized with respect to lipid and apoprotein composition, and results were compared with those obtained from lean controls. Information on apoproteins was obtained from gel filtration experiments and electrophoresis on polyacrylamide gels. Very low density lipoproteins (VLDL) were increased severalfold in fatties, and 78% of their mass was triglycerides compared with 60% in the controls. Low density (LDL) and high density (HDL) lipoproteins were increased by a factor of 2, although their compositions were similar to those of the controls. Levels of apoVLDL, apoLDL, and apoHDL were five, two and two times higher, respectively, in the fatties, and the two most rapidly moving subunit peptides on polyacrylamide gels were disproportionately elevated in the apoproteins. The slower of these two bands was present in relatively greater amounts than the faster one in fatties. The increased capacity for catabolism may be a response to the altered secretory rates.

TRANSPORT OF LIPIDS IN INSECTS. L.I. Gilbert and H. Chino (Dept. of Biol. Sci., Northwestern Univ., Evanston, Il. 60201). *J. Lipid Res.* **15**, 439–56 (1974). Many insect species are almost completely dependent on lipids for their metabolic needs, although this is usually a function of developmental stage. The primary storage organ is the fat body, which can constitute 50% of the fresh weight of the insect and also acts as the major metabolic center (analogous to the vertebrate adipose tissue and liver). Bathing the fat body (and all other tissues and organs) is the hemolymph, the main functions of which are to transport nutrient substrates to utilization sites and to deliver metabolic wastes to the excretory system. Although neutral lipids are stored as triglycerides, in times of need they appear to be endergonically released into the hemolymph as diglycerides in the majority of insects thus far studied (particularly silkmoths and locusts). Indeed, diglycerides constitute the largest neutral lipid fraction in the hemolymph of silkmoths, locusts, cockroaches, bugs, etc. In the hemolymph the diglyceride is found as a constituent of specific lipoproteins, and specific lipoprotein class (lipoprotein I; high density lipoprotein) appears to be necessary for the transport diglyceride from the fat body cell into the hemolymph. The other major insect growth hormone, juvenile hormone, is transported by hemolymph lipoproteins in silkmoths and locusts and by a lower molecular weight hemolymph protein in the tobacco hornworm.

PHYSICAL-CHEMICAL STUDIES OF PHOSPHOLIPIDS AND POLY (AMINO ACIDS) INTERACTIONS. Kam-Yee Yu, J.J. Baldassare and C. Ho (Dept. of Biophys. and Microbiol. and Dept. of Biochem., Faculty of Arts and Sci., Univ. of Pittsburgh, Pittsburgh, Pa. 15260). *Biochemistry* **13**, 4375–81 (1974). The interactions of phospholipid vesicles with poly(L-glutamic acid) and poly(L-tyrosine) were investigated as a model for the molecular interactions between proteins and phospholipids in biological membranes. We have used the spin-label and glucose permeability techniques to study the interactions between poly(amino acids) and phosphatidylcholine. The spin-labels that we used are the spin-labeled stearic acids and the spin-labeled phosphatidylcholines. The spin-label results suggest that these two poly(amino acids) interact on the surface of the phosphatidylcholine vesicles and that this interaction might cause a lateral tightening up of the polar region of the phospholipid molecule, but the flexibility gradient in the methylene chain is still preserved in the model membranes. In addition, the slower rate of glucose permeability in the complexes provides another piece of evidence that there is a tightening up of the bilayer structure of the phosphatidylcholine vesicles upon complex formation with negatively charged poly(amino acids) in aqueous solution.

NUCLEAR MAGNETIC RESONANCE STUDIES OF THE INTERACTIONS OF SONICATED LECITHIN BILAYERS WITH POLY(L-GLUTAMIC ACID). Chin-An Chang and S.I. Chan (Lawrence Berkeley Lab., Univ. of Calif., Berkeley, Calif. 94720). *Biochemistry* **13**, 4381–5 (1974). The interactions of sonicated lecithin vesicles with poly(L-glutamic acid) have been studied by high-resolution proton magnetic resonance (pmr) spectroscopy. The choline methyl protons of the lecithin vesicles showed a large decrease in pmr intensities upon mixing with the poly(L-glutamic acid). The observed change in pmr intensities is shown to be mainly due to the interactions between the lecithin vesicles and poly (L-glutamic acid). Electron microscopy study showed isolated vesicles for the pure lecihtin solution, but clusters of vesicles when the poly(L-glutamic acid) is present in the solution. The pmr spectra of poly(L-glutamic acid) further indicated that the polypeptide remained in the random coil form upon mixing with the lecithin vesicles.

STUDIES ON THE SUBSTRATE SPECIFICITY OF THE PHOSPHOLIPASE A_1 OF THE PLASMA MEMBRANE OF RAT LIVER. M. Waite and P. Sisson (Dept. of Biochem., Bowman Gray Schl. of Med. of Wake Forest Univ., Winston-Salem, N.C. 27103). *J. Biol. Chem.* **249**, 6401–5 (1974). The phospholipase A_1 from the plasma membranes of the rat liver catalyzes a transacylation between acylglycerols in addition to hydrolysis. Only the acyl group from position 1 can be donated; that in position 2 is not utilized. Compounds which contain either primary or secondary hydroxyls can be utilized as acyl acceptor molecules. The acceptor molecule may not be bulky, however, as shown by the lack of utilization of diglycerides, monoacylglycerophosphorylethanolamine and cholesterol. The addition of mono- and diacylglycerols with acyl chains of different length and unsaturation caused varying degrees of inhibition. Dialkyl-

glycerols did not inhibit the enzyme as well as diacylglycerols which demonstrates that the nature of the bond on the glycerol affects the binding of the lipid to the enzyme. These results substantiate our earlier postulate that the enzyme has separate binding sites for acyl donor and acyl acceptor molecules.

VITAMIN A DEFICIENCY AND REPRODUCTION IN RHESUS MONKEYS. B.A. O'Toole, R. Fradkin, J. Warkany, J.G. Wilson and G.V. Mann (Children's Hosp. Res. Found., Mental Retardation Res. Ctr., Univ. of Cincinnati Med. College, Cincinnati, Ohio 45229). *J. Nutr.* **104**, 1513–24 (1974). Maternal vitamin A deficiency results in a variety of congenital malformations in several mammalian species, but there are only a few reports of malformations in children attributable to this deficiency. It seemed of interest to test experimentally the teratogenicity of this deficiency in a subhuman primate. Ten mature female and one male *Macaca mulatta* were fed a vitamin A-free diet for a period of 24–36 months. Plasma vitamin A levels were followed; when levels dropped to 10 μg/100 ml or lower, supplements of 400 IU of vitamin A were given twice weekly. Assays were also performed on liver biopsy tissue to confirm the state of vitamin A depletion. Eight pregnancies occurred in females fed deficient diets; four of these were not in a state of vitamin A depletion at conception. Three pregnancies resulted in abortion, four in viable offspring, and one was interrupted at day 72 by hysterotomy. No congenital malformations were observed, but two young were born with xerophthalmia, and one developed the condition after a 2-year period of vitamin A deficiency. Thus, *Macaca mulatta* may be maintained for 2–3 years on a vitamin A-free diet and may reproduce if the diet is supplemented with very low doses of vitamin A.

CALORIGENIC ACTIONS OF OLEIC ACID AND GLUCAGON INFUSIONS IN ANESTHETIZED DOGS. P.C. Weiser and F. Grande (Jay Phillips Res. Lab., Mount Sinai Hosp., Minneapolis, Minn. 55404). *Proc. Soc. Exp. Biol. Med.* **147**, 80–4 (1974). Infusion of oleic acid at the rate of 1.0 nmole/kg/hr for 1 hr produced significant elevations of oxygen uptake in anesthetized dogs. The elevations of oxygen uptake (ml O_2/kg/min) after 30 min of infusion showed a high correlation with the corresponding elevations of plasma FFA ($r = 0.91$, $N = 17$, $P < 0.01$). Glucagon infusion produced elevation of oxygen uptake, as previously described. The increase in oxygen uptake produced by glucagon was the same when the hormone was infused alone and when it was infused during the infusion of oleic acid. Statistical analysis showed no interaction between the calorigenic effects of oleic acid and glucagon infusions. It is concluded that the calorigenic actions produced by infusing oleic acid and glucagon are additive and mediated by independent mechanisms.

BODY COMPOSITION AND PROTEIN UTILIZATION OF CHICKS FED GRADED LEVELS OF FAT. J.G. Velu and D.H. Baker (Dept. of Animal Sci., Univ. of Ill., Urbana, IL. 61801). *Poultry Sci.* **53**, 1831–8 (1974). Two experiments involving chicks fed a complete crystalline amino acid diet from day 8 to day 21 posthatching were employed to evaluate body composition changes and protein utilization as a function of dietary caloric density. In experiment 1 graded levels of corn oil from 5 to 25% of the diet were fed. Chicks gained as rapidly and utilized the dietary nitrogen as efficiently when 5% corn oil was fed as when higher levels were fed. As caloric density of the diet increased, chicks ate to meet their energy need and therefore cut back on feed (hence, protein) intake. Nonetheless, each increment of protein intake was used at the same efficiency. Body fat increased and body protein decreased with each incremental increase in dietary corn oil. In experiment 2 it was established that protein utilization remained constant over a wide range of protein intake (below the requirement for maximal protein retention), regardless of whether protein intake differences resulted from a change in caloric density of the diet or from a change in the dietary concentration of the complete amino acid mixture. Energy retention appeared to reach a maxima at a dietary concentration of 15% corn oil or 4.19 kcal. M.E./g.

VITAMIN E-POLYUNSATURATED LIPID RELATIONSHIP IN DIET AND TISSUES. L.A. Witting (Texas Woman's Univ., Box 23975, TWU Station, Denton, Tx. 76201). *Am. J. Clin. Nutr.* **27**, 952–9 (1974). It is firmly established that the requirement for vitamin E is related to the fatty acid composition of the tissue lipids. Unfortunately, the complex relationships between dietary lipid composition and tissue lipid composition are not understood or appreciated by many investigators. Frequently one sees reports of drastic changes made in diet lipids and attempts to measure effects attributable to the change long before the establishment of new tissue equilibria. Data from animal studies are not directly applicable to adult man unless careful attention is paid to the extremely slow approach of certain tissues to equilibrium noted in the experiments of Dayton et al. and others. The kinetics of lipid autoxidation are relatively complex and it is not surprising that attempts to make predictions regarding events in biological systems have not always been successful. When the analogies are correctly stated, excellent correlation is noted between lipid autoxidation in vitro and lipid peroxidation in vivo in various studies including those of Green and co-workers.

VITAMIN A AND LYSOSOMAL STABILITY IN RAT LIVER. P.R. Sudhakaran and P.A. Kurup (Dept. of Biochem., Univ. of Kerala, Trivandrum-695 001, India). *J. Nutr.* **104**, 1466–75 (1974). The stability of rat liver lysosomes has been studied in animals of varying vitamin A status. The activities of various hydrolytic enzymes-β-glucuronidase, β-hexosaminidase, hyaluronidase, cathepsins and arylsulfatase-in the lysosome-rich 15,000 *g* sediment and the 15,000 *g* supernatant and in the serum have been taken as criteria of lysosomal stability. Maximum lysosomal stability has been found in rats when the vitamin A intake ranged from 100 to 2,000 IU/day. Within this range the variation in lysosomal stability was not appreciable, but above and below this range of vitamin A intake, the stability was progressively decreased. There was no appreciable alteration in the total enzyme activity (sum of the nuclear, bound, and free activities) with variations in vitamin A status. Retinol in vitro had no effect on the enzyme activity released from the nuclear or lysosomal fraction by the action of Brij-35 or on the nonsedimentable activity. The action of vitamin A is therefore mainly a membrane effect. Proteoglycan fraction from normal rat liver had no effect on the lysosomal stability of the vitamin A-deficient rat in vitro, but lipid extract from normal rat liver increased the stability. This effect has been found to be due to the phospholipids present in the lipid extract.

ATHEROSCLEROSIS IN THE RHESUS MONKEY FED THREE FOOD FATS. D. Vesselinovitch, G.S. Getz, R.H. Hughes and R.W. Wissler (Dept. of Pathol. Univ. of Chicago, Chicago, Il. 60637). *Atherosclerosis* **20**, 303–21 (1974). Three groups of 6 male Rhesus monkeys were fed one of three diets, each containing 2% cholesterol and 25% lipid, either corn oil, butterfat or peanut oil, over a period of 50 weeks. These diets produced prompt elevation of serum lipids. The highest serum cholesterol concentrations were observed in animals fed butterfat and the levels in animals fed peanut oil and corn oil were similar and much lower. Gross examination of the arteries revealed a definite difference in the degree and the characteristics of aortic atherosclerosis among the three dietary groups. The butterfat diet produced severe aortic lesions, characterized by abundant lipid deposition and relatively little cell proliferation or collagen deposition. The most widespread and advanced atherosclerosis was observed in monkeys fed peanut oil. The aortic lesions in these animals were characterized by thick, fibrous plaques which were elevated to the point of apparent narrowing of the ostia of various vessels. In addition, the peanut oil ration produced not only the highest incidence of coronary artery involvement but also the most severe coronary narrowing. This study adds to the growing body of experimental evidence in several animal species that the tissue components and that the severity of atherosclerotic lesions can be greatly influenced by the food fat fed and that peanut oil is an unusually atherogenic fat, mainly because of the severe intimal proliferation and fibrosis that occur.

ETHANOL INHIBITION OF VITAMIN A METABOLISM IN THE TESTES: POSSIBLE MECHANISM FOR STERILITY IN ALCOHOLICS. D.H. Van Thiel, J. Gavaler and R. Lester (Div. of Gastroenterology, Dept. of Med., Univ. of Pittsburgh, Schl. of Med., Pittsburgh, Pa. 15261). *Science* **186**, 941–2 (1974). Vitamin A (retinol) is essential for spermatogenesis. Alcohol dehydrogenase, the enzyme responsible for ethanol metabolism, is also required for the conversion of retinol to bioactive retinal at the end organ site. Ethanol inhibits the oxidation of retinol by testicular homogenates containing alcohol dehydrogenase. Thus, a possible biochemical mechanism for the sterility of chronic alcoholics is identified.

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 Sec. Vet. Administration 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.

ASSAY, KINETICS AND LYSOSOMAL LOCALIZATION OF AN ACID CHOLESTERYL ESTERASE IN RABBIT AORTIC SMOOTH MUSCLE CELLS. T. Takano, W.J. Black, T.J. Peters and C. De Duve (Rockefeller Univ., New York, N.Y. 10021). *J. Biol. Chem.* **249**, 6732–7 (1974). A sensitive radioisotope microassay has been developed for the estimation of cholesteryl ester hydrolase (EC 3.1.1.13) in preparations of isolated rabbit aortic smooth muscle cells. Kinetic studies served to establish optimal assay conditions, which involve incubation in a total volume of 0.2 ml containing 5μM cholesteryl oleate tritiated in the cholesterol moiety, 0.4 mM egg yolk lecithin, 0.3 mM taurocholate, 25 μg of bovine serum albumin (defatted) per ml, and 0.05 M sodium acetate buffer, pH 4.25. The labeled cholesterol was separated from the ester by thin layer chromatography with double development. Hydrolysis of as little as 1 pmole of substrate could be detected by this method. Under these conditions no measurable activity was found at neutral or alkaline pH in either phosphate or Tris buffer. The enzyme is several times more active on cholesteryl esters of unsaturated fatty acids than on those of saturated fatty acids in the C_{18} and C_{20} series. Among esters of even numbered saturated fatty acids (C_{12} to C_{20}), cholesteryl myristate (C_{14}) was hydrolyzed fastest. Fractionation studies indicate that cholesteryl esterase is associated with lysosomes in aortic smooth muscle cells.

BLOOD LEVELS OF α-TOCOPHEROL IN A DISORDER OF LIPID PEROXIDATION: BATTEN'S DISEASE. A.N. Siakotos, N. Koppang, S. Youmans and C. Bucana (Dept. of Pathol., Indiana Univ. Schl. of Med., Indianapolis, Ind. 46202). *Am. J. Clin. Nutr.* **27**, 1152–7 (1974). Batten's disease is characterized by the onset of progressive mental regression and seizures. The brain cells of patients with this disease show the accumulation of large masses of a lipopigment, ceroid. Similar accumulations have been observed in the brain of a genetic strain of English setters. Previous studies by our laboratory have established that the accumulated lipopigment, ceroid, is clearly different from "age" pigment, or lipofusein, in a number of parameters, although some similarities exist between the two lipopigments. Since the proposed biological role of α-tocopherol is that of an antioxidant, this compound has been utilized in experimental therapeutic approaches with patients affected with Batten's disease. Our approach to this problem was to investigate the blood and tissue levels of α-tocopherol in this disorder. Early studies suggested that blood α-tocopherol levels were below normal values. However, a study on a large number of samples from both human patients and dogs with a similar disease clearly established that blood and tissue levels of α-tocopherol are significantly higher than normal age-matched controls. Obviously this "peroxidative disorder" progresses in spite of an ample supply of the biological antioxidant, α-tocopherol.

EFFECT OF DIFFERENT FATTY ACIDS ON GLYCEROLIPID SYNTHESIS IN ISOLATED RAT HEPATOCYTES. R. Sundler, B. Akesson and A. Nilsson (Dept. of Physiol. Chem., Univ. of Lund, S-220 07 Lund 7, Sweden). *J. Biol. Chem.* **249**, 5102–7 (1974). Glycerolipid synthesis from [^{3}H]glycerol and [^{14}C]dihydroxyacetone operated in enzymically isolated rat hepatocytes at a rate similar to that in the intact organ. Addition of albumin-bound fatty acids with more than 12 carbon atoms to the incubation medium markedly stimulated triacylglycerol synthesis from [^{3}H]glycerol whereas the effects on phospholipid synthesis were smaller and more dependent on fatty acid structure. Capric acid, lauric acid, and erucic acid inhibited both phosphatidylethanolamine and phosphatidylcholine synthesis whereas fatty acids with 16 to 18 carbon atoms were stimulatory or without effect. High proportions of labeled diacylglycerols containing 2 molecules of the added fatty acid were formed for all fatty acids. Unsaturated diacylglycerols were well utilized for phospholipid synthesis, while saturated ones were utilized to a lesser degree, especially for phosphatidylethanolamine synthesis. The low utilization of saturated diacylglycerols may represent one mechanism whereby the formation of phospholipid molecules with unsuitably high transition temperatures is avoided.

FEEDING STUDIES DESIGNED TO DETERMINE WHETHER COMPETITIVE REACTIONS BETWEEN ACIDS OF THE OLEATE AND LINOLEATE FAMILIES FOR DESATURATION CHAIN ELONGATION OR INCORPORATION REGULATE THE FATTY ACID COMPOSITION OF RAT LIVER LIPIDS. H. Sprecher (Dept. of Physiol. Chem., 333 West Tenth Ave., Ohio State Univ., Columbus, Ohio 43210). *Biochim. Biophys. Acta* **369**, 34–44 (1974). Several groups of rats were raised on a fat-free diet and all fed a constant amount of octadeca-6,9,12-trienoate, but various amounts of octadeca-6,9-dienoate. Analysis of the total liver lipids showed that as the dietary level of octadeca-6,9-dienoate increased, the level of arachidonate remained at a level between 80-99% of that found in those rats receiving only its precursor octadeca-6,9,12-trienoate. Conversely, when both acids were fed, the level of eicosa-5,8,11-trienoate was never greater than 62% of that found in the fat free controls. Thus, competitive reactions for the chain elongation of the two dietary acids do not represent a major in vivo metabolic control point. Competitive feeding experiments using eicosa-5,8,11-trienoate and arachidonate show that arachidonate is preferentially incorporated into liver lipids. Competitive feeding experiments between linoleate and eicosa-8,11-dienoate support the hypothesis that different desaturases introduce double bonds at the 6- and 5-positions in the biosynthesis of polyunsaturated fatty acids.

PHOSPHOLIPID METABOLISM IN THE EGGS AND EMBRYOS OF THE SEA URCHIN ARBACIA PUNCTULATA. E. Schmell and W.J. Lennarz (Dept. of Physiol. Chem., Johns Hopkins Univ. Schl. of Med., Baltimore, Md. 21205). *Biochemistry* **13**, 4114–21 (1974). The incorporation of labeled phospholipid precursors into the phospholipids of *Arbacia punctulata* eggs and of embryos prior to the first cell cleavage has been investigated. Incorporation of [^{3}H]choline into phosphatidylcholine was not detected in either eggs or embryos although fertilization resulted in a fourfold stimulation of [^{3}H]choline uptake into the cells. Both eggs and embryos, when incubated with [^{3}H]ethanolamine, incorporated radioactivity into phosphatidylethanolamine. The incorporation by unfertilized eggs was three to four times greater than that observed in embryos. However, when eggs were preincubated with [^{3}H]ethanolamine and subsequently fertilized, the resultant preloaded eggs and embryos behaved identically in the incorporation of ethanolamine into phosphatidylethanolamine. Although in both cases the major inositol-labeled lipid was phosphatidylinositol, when eggs were preloaded with [^{3}H]inositol and then fertilized, an increase in the proportion of label in the polyphosphoinositides was detected in the resultant embryos, when compared to the unfertilized eggs.

REGULATION OF METABOLITE TRANSPORT IN RAT AND GUINEA PIG LIVER MITOCHONDRIA BY LONG CHAIN FATTY ACYL COENZYME A ESTERS. E. Shrago, A. Shug, C. Elson, T. Spennetta and C. Crosby (Depts. of Med. and Nutr. Sci., Univ. of Wisc. and the Vet. Administration Hosp., Madison, Wisc. 53706). *J. Biol. Chem.* **249**, 5269–74 (1974). Long chain fatty acyl coenzyme A esters were found to be potent inhibitors of adenine nucleotide translocation in both rat and guinea pig liver mitochondria. There was a positive correlation of inhibition with the carbon chain length and carnitine-dependent oxidation of the fatty acyl-CoA esters. Octanoyl-CoA was completely ineffective at concentrations up to 50 μM. Esters containing a greater number of carbon atoms produced significant inhibition at concentrations as low as 5 μM. Both saturated and unsaturated acyl-CoA esters were effective inhibitors, although there was some difference in effectiveness depending upon the concentration. Palmitoyl-CoA was shown to inhibit binding competitively with ADP in both nucleotide depleted mitochondria and Lubrol membrane fragments. Phosphoenolpyruvate, known to be transported on the tricarboxylate carrier, also was found to be transported by the adenine nucleotide translocase in both rat and guinea pig liver mitochondria. In addition the tricarboxylate as well as adenine nucleotide translocator was inhibited by atractylate as well as long chain fatty acyl-CoA esters. These results suggest an important function of long chain fatty acyl-CoA esters in the regulation of mitochondrial metabolite transport.

SYNTHESIS OF FATTY ACIDS IN THE PERFUSED MOUSE LIVER. D.M.W. Salmon, N.L. Bowen and D.A. Hems (Dept. of Biochem., Imperial College, London S.W.7, U.K.). *Biochem. J.* **142**, 611–8 (1974). Fatty acid synthesis de novo was measured in the perfused liver of fed mice. The total rate, measured by the incorporation into fatty acid of ^{3}H from ^3H_2O (1–7

μmol of fatty acid/h per g of fresh liver), resembled the rate found in the liver of intact mice. Perfusions with L-[U-^{14}C]lactic acid and [U-^{14}C]glucose showed that circulating glucose at concentrations less than about 17 mM was not a major carbon source for newly synthesized fatty acid, whereas lactate (10mM) markedly stimulated fatty acid synthesis, and contributed extensive carbon to lipogenesis. The identification of 50% of the carbon converted into newly synthesized fatty acid lends further credibility to the use of ^3H_2O to measure hepatic fatty acid synthesis. The total rate of fatty acid synthesis, and the contribution of glucose carbon to lipogenesis, were directly proportional to the initial hepatic glycogen concentration. The proportion of total newly synthesized lipid that was released into the perfusion medium was 12–16%. The major products of lipogenesis were saturated fatty acids in triglyceride and phospholipid. The rate of cholesterol synthesis, also measured with ^3H_2O, expressed as acetyl residues consumed, was about one-fourth of the basal rate of fatty acid synthesis. These results are discussed in terms of the carbon sources of hepatic newly synthesized fatty acids, and the effect of glucose, glycogen and lactate in stimulating lipogenesis, independently of their role as precursors.

LIPOGENESIS IN RABBIT ISOLATED FAT-CELLS. E.D. Saggerson (Dept. of Biochem., Univ. College London, Gower St., London WC1E 6BT, U.K.). *Biochem. J.* 142, 477–82 (1974). Fat-cells isolated from rabbit perirenal adipose tissue were incubated with the following U-^{14}C-labelled substrates: 5 mM-glucose (+insulin), 5mM-pyruvate, 5mM-lactate, 5mM-glucose+5mM-acetate (+insulin), and the relative rates of incorporation of these substrates into glyceride fatty acids determined. In general total rates of fatty acid synthesis were similar whatever substrate was supplied to the cells. Rabbit fat-cells incorporated [U-^{14}C]acetate into fatty acids and CO_2 as well in the absence of glucose as in the presence of this substrate. The disposition of the utilization of glucose-derived carbon through various metabolic pathways was determined. Extra-mitochondrial and mitochondrial activities were determined for 11 enzymes. The cells contained a very low activity of pyruvate carboxylase, undetectable NADP-malate dehydrogenase activity and a high mitochondrial phosphoenolpyruvate carboxylase activity. Various rabbit fat-cell metabolic parameters based on the measurement of ^{14}C incorporation and enzyme activity were compared with the same parameters previously measured in rat and guinea-pig fat-cells. In general guinea pig occupied a position between rat and rabbit with respect to these parameters. The profiles of substrate incorporation into fatty acids and of relative enzyme activities in rabbit fat-cells indicated that the operation of a 'citrate-cleavage' pathway may not be obligatory for the supply of lipogenic acetyl units.

PARTIAL PURIFICATION AND PROPERTIES OF PHOSPHATIDYLSERINE SYNTHETASE FROM ESCHERICHIA COLI. C.R.H. Raetz and E.P. Kennedy (Dept. of Biol. Chem., Harvard Med. Schl., Boston, Mass. 02115). *J. Biol. Chem.* 249, 5038–45 (1974). CDP-diglyceride: L-serine phosphatidyltransferase (phosphatidylserine synthetase) of *Escherichia coli* is tightly associated with ribosomes in crude cell-free extracts. The synthetase has now been separated from ribosomes by extraction with solutions containing 5 M NaCl and has been purified 100-fold. The partially purified enzyme is devoid of contaminating hydrolytic activities and nearly free of RNA. The enzyme catalyzes exchange reactions of CMP with CDP-diglyceride, and of serine with phosphatidylserine. Furthermore, the enzyme catalyzes the formation of CDP-diglyceride from phosphatidylserine and CMP, although the equilibrium strongly favors synthesis of phosphatidylserine. A phosphatidyl-enzyme intermediate may thus be involved in the action of this enzyme. Under the conditions employed in the assay system, however, this intermediate seems to be unstable, since the synthetase also hydrolyzes phosphatidylserine and CDP-diglyceride at a slow rate.

CREATINE KINASE AND MYOFIBRILLAR PROTEINS IN HEREDITARY MUSCULAR DYSTROPHY AND VITAMIN E DEFICIENCY. R.E. Olson (Dept. of Biochem., St. Louis Univ. Schl. of Med., St. Louis, Mo. 63104). *Am. J. Clin. Nutr.* 27, 1117–29 (1974). Although the myopathy of vitamin E deficiency imitates many features of hereditary muscular dystrophy, the molecular basis of these similarities remains unexplained. To test the hypothesis that vitamin E does regulate the synthesis of specific proteins required for normal muscle function, a series of studies has been carried out in my laboratory to examine general protein synthesis and the synthesis of creatine kinase and selected myofibrillar proteins in vitamin E deficiency and hereditary muscular dystrophy in animals. Creatine kinase was selected for a number of studies because it has served as a marker for the onset of dystrophy in a variety of animals and man. It was shown by isotope-labeling studies of the purified enzyme from muscle in vitamin E-deficient rabbits, that the turnover rate was twice as great as that for the normal enzyme. It was found that the yield of polysomes per gram of vitamin E-deficient muscle was 4 times higher than that from corresponding controls. Total muscle RNA levels were 2 to 3 times higher with increased muscle RNase. Because of reports of abnormalities in isoenzyme distribution of creatine kinase in human dystrophy and in hereditary dystrophy in the mouse, it was decided to investigate the nature of the creatine kinase occurring in vitamin E deficiency and in hereditary muscular dystrophy in the New Hampshire Red chick. Enzymes from normal, vitamin E-deficient, and dystrophic animals were similar in size, shape, charge, number of essential SH groups and kinetic properties.

PURIFICATION AND PROPERTIES OF A MEMBRANE-BOUND PHOSPHOLIPASE A_1 FROM MYCOBACTERIUM PHLEI. M. Nishijima, Y. Akamatsu and S. Nojima (Dept. of Chem., Natl. Inst. of Health, Shinagawa-ku, Tokyo 141, Japan). *J. Biol. Chem.* 249, 5658–67 (1974). A phospholipase A_1 bound tightly to the membranes of *Mycobacterium phlei* cells was purified approximately 500-fold to near homogeneity by extraction with Triton X-100, delipidation with organic solvents, solubilization with sodium dodecyl sulfate, column chromatographies on Sephadex G-200 in the presence of sodium dodecyl sulfate and on DEAE-cellulose in the presence of BRIJ 58, and sodium dodecyl sulfate polyacrylamide gel electrophoresis. Two active fractions with molecular weights of 27,000 and 45,000 were obtained. Appropriate concentrations of Triton X-100 caused a 7-fold increase in activity for hydrolysis of phosphatidylethanolamine and a slight increase in activity for lysophosphatidylethanolamine, but a higher concentrations this detergent inhibited both activities. Other ionic detergents suppressed enzyme activity at all concentrations tested. The effects on this phospholipase A_1 of various metal ions and enzyme inhibitors closely resembled their effects on the lysophospholipases so far reported.

INTERACTIONS OF DIETARY α-TOCOPHEROL, OXIDIZED MENHADEN OIL AND ETHOXYQUIN ON CHANNEL CATFISH (ICTALURUS PUNCTATUS). T. Murai and J.W. Andrews (Univ. of Ga. Agr. Exptl. Station and Skidaway Inst. of Oceanography, P.O. Box 13687, Savannah, Ga. 31406). *J. Nutr.* 104, 1416–31 (1974). A 3 × 3 × 2 factorial feeding study with channel catfish *(Ictalurus punctatus)* included the following dietary treatments: 0,25, and 100 mg/kg *dl*-α-tocopherol; 0, 10, and 100 g/kg oxidized menhaden oil; and 0 and 125 mg/kg ethoxyquin. Fish fed diets containing oxidized menhaden oil without supplemental α-tocopherol or ethoxyquin exhibited poor growth, food conversion and survival rates; high incidence of three distinct gross syndromes—exudative diathesis, muscular dystrophy and depigmentation; fatty livers; anemia; and pronounced histological changes in muscle fibers, kidney, and pancreatic tissue. Data from this study suggest that 25 mg/kg α-tocopherol and 125 mg/kg ethoxyquin, or 100 mg/kg α-tocopherol should provide adequate protection in most practical catfish diets.

CANINE LIPOPROTEINS AND ATHEROSCLEROSIS. I. ISOLATION AND CHARACTERIZATION OF PLASMA LIPOPROTEINS FROM CONTROL DOGS. R.W. Mahley and K.H. Weisgraber (Section on Exp. Atherosclerosis, Natl. Heart and Lung Inst., Natl. Insts. Health, Bethesda, Md. 20014). *Circulation Res.* 35, 713–21 (1974). Canine plasma lipoproteins were fractionated into four distinct classes by ultracentrifugation combined with Geon-Pevikon block electrophoresis and characterized with respect to physical and chemical properties. The distribution of plasma lipids and lipoproteins was quite unlike that in man, the dog having approximately five to six times as much high density as lower density lipoproteins. Despite the marked difference in distribution, human lipoprotein equivalents were present. Very low density lipoproteins (VLDL) isolated at density less than 1.006 g/ml were triglyceride-rich particles ranging in size from 260 to 900 Å in diameter. The HDL_1 particles ranged in size from 100 to 350 Å in diameter and appeared to be unlike any of the commonly described human lipoproteins. High density lipoproteins called HDL_2 isolated in the density range from 1.087 to 1.21 g/ml were protein-rich particles ranging in size from 55 to 85 Å. The apolipoprotein patterns of VLDL, LDL, and HDL_2 on polyacrylamide gel electrophoresis were similar to those of the corresponding lipoproteins of man.

II. CHARACTERIZATION OF THE PLASMA LIPOPROTEINS ASSOCIATED WITH ATHEROGENIC AND NONATHEROGENIC HYPERLIPIDEMIA. R.W. Mahley, K.H. Weisgraber and T. Innerarity. *Ibid.*, 722–33. Characterization of the hyperlipoproteinemia induced by feeding high-cholesterol diets to hypothyroid dogs was undertaken in an attempt to identify a lipoprotein pattern or a specific lipoprotein responsible for the atherosclerosis associated with such hyperlipoproteinemia. Various degrees of hyperlipidemia and atherosclerosis were produced during the diet, which was imposed for 3 months to more than a year. Dogs referred to as hyporesponders did not develop significant atherosclerosis despite plasma cholesterol levels ranging from two to five times normal or up to 750 mg/100 ml. This nonatherogenic hyperlipidemia was characterized by an increase in the LDL (low density lipoprotein) and HDL_c classes (HDL_c refers to a broad spectrum of cholesterol-enriched particles which resemble high density lipoproteins). Dogs referred to as hyperresponders developed significant and often complicated atherosclerosis. Their plasma cholesterol levels were in excess of 750 mg/100 ml, and most of the increased cholesterol was present in lipoproteins with density less than 1.006 g/ml. Several classes lipoproteins were isolated by ultracentrifugation and purified by Geon-Pevikon block electrophoresis. We suggest that the spectrum of lipoproteins with density less than 1.006 g/ml represents "remnants" that accumulate because of defective catabolism of lipoproteins synthesized to carry excess dietary cholesterol.

THE INTERACTION OF GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE WITH HUMAN ERYTHROCYTE MEMBRANES. C.F. McDaniel and M.E. Kirtley (Dept. of Biol. Chem., Univ. of Maryland Schl. of Med., Baltimore, Md. 21201). *J. Biol. Chem.* 249, 6478–85 (1974). Human erythrocyte ghosts retain most of the enzyme glyceraldehyde 3-phosphate dehydrogenase bound to the membrane. The bound dehydrogenase, 3×10^5 molecules per ghost, can be eluted with increasing concentrations of salt, with 50% elution occurring at 0.16 M NaCl, pH 7.5. Exogenous glyceraldehyde phosphate dehydrogenase can bind to the membrane. Two types of binding sites have been detected. The high affinity sites bind a maximum of 3×10^5 molecules of dehydrogenase per ghost with a K_a less than 10^{-8} M for the enzyme obtained from either erythrocytes or rabbit muscle. The low affinity sites bind up to 63×10^5 molecules per ghost with K_a greater than 10^{-7} M. Enzyme bound to the membrane in this way can be re-eluted with NaCl, indicating freely reversible interaction between the enzyme and membrane. Ghosts prepared and sealed in the presence of $MgSO_4$ bind very little glyceraldehyde phosphate dehydrogenase and no binding occurs with high affinity. Ghosts incubated at 37C in NaCl become partially sealed. Such ghosts also bind the dehydrogenase both at the high and low affinity sites. However, at each type of site part of the bound enzyme is cryptic, i.e. its activity is masked unless a detergent, Triton X-100, is present. Because both the enzyme and membrane are well characterized they form a valuable model system for examining enzyme-membrane interactions in vitro.

A SELENIUM AND VITAMIN E RESPONSIVE CONDITION IN THE LAYING HEN. J.D. Latshaw and M. Osman (Dept. of Poultry Sci. and Ohio Agr. Res. and Development Ctr., Ohio State Univ., Columbus, Ohio 43210). *Poultry Sci.* 53, 1704–8 (1974). Diets composed mostly of low selenium corn and torula yeast were fed to laying hens to produce a selenium and/or vitamin E deficiency. The basal diet was also supplemented with 101.U. of vitamin E per kg., or 0.10 mg/kg. selenite selenium or a combination of vitamin E and selenium. Hens fed the basal diet decreased in egg production during the third month and for the remainder of the experiment, a decrease which was mostly corrected by vitamin E, and completely corrected by selenium. Fertility and hatchability of eggs were also low on the basal diet, and were also partly corrected by vitamin E, and completely corrected by selenium. The selenium content of lyophilized egg white and yolk is reported along with the selenium content of several tissues. Gross deficiency signs of the laying hen are also described.

INHIBITION OF MEMBRANE ADENOSINE TRIPHOSPHATASE BY ALPHA-TOCOPHEROL AND ITS DERIVATIVES. K. Kawai, M. Nakao, T. Nakao and G. Katsui (Dept. of Biochem., Tokyo Med. and Dental Univ., Schl of Med., Yushima, Bunkyo, Tokyo). *Am. J. Clin. Nutr.* 27, 987–94 (1974). Although it has been reported that α-tocopherol acts as a lipid antioxidant, that it inhibits various enzymes involved in biological oxidation, and that α-tocopherol stabilizes the cell membrane, the exact function of the vitamin in the cell membrane is still unknown. Using partially and highly purified Na,K-ATPase preparations, the effects of tocopherols and their derivatives were tested as sonicated suspensions in water. α-Tocopherol and α-tocopherylquinone inhibited the Na,K-ATPase with one-half maximal inhibition of 5.7×10^{-5}M and 3.1×10^{-5}M, respectively (near the physiological vitamin E concentration in blood, 3×10^{-5}M). Other tocopherol derivatives and fat-soluble vitamins did not show such strong inhibition. For example, the K_i value of α-tocopheryl acetate was 50 times greater than that of free α-tocopherol. BHT, cysteine and DTT did not affect the inhibition. Ca ATPase from muscle was similarly inhibited. On the other hand, ouabain-insensitive ATPase from rabbit brain and liver was slightly affected. There seemed to be no effect on acetylcholinesterase from human erythrocytes, and little or no effect on 5-nucleotidase and G6Pase from rabbit brain. Some kinetical examination of α-tocopherol and α-tocopherylquinone was performed.

MALONATE METABOLISM IN RAT BRAIN MITOCHONDRIA. A.H. Koeppen, E.J. Mitzen and A.A. Ammoumi (Res. Service (Neurology), Vet. Admin. Hosp., and Depts. of Neurol. and Biochem., Albany Med. College of Union Univ., Albany, N.Y. 12208). *Biochemistry* 13, 3589–95 (1974). Rat brain mitochondria were found to have malonyl-CoA decarboxylase activity and a malonate-activating enzyme (malonyl-CoA synthetase). The decarboxylating enzyme had an apparent k_m of 0.5 mM and a pH optimum of 8.3. Methylmalonyl-CoA was an effective competitive inhibitor. Malonyl-CoA decarboxylase was tightly bound to mitochondria and could not be released by ultrasonication or by treatment with Triton X-100 (1%). The reaction product of malonyl-CoA decarboxylation, acetyl-CoA, was released into the incubation medium and not further oxidized by the Krebs cycle. Acetyl-CoA was hydrolyzed by acetyl-CoA hydrolase (EC 3.1.2.1) which was also associated with rat brain mitochondria. Malonyl-CoA decarboxylase from rat brain mitochondria differs from the decarboxylase in liver mitochondria which is localized in the matrix and which is readily released by ultrasonication. The liver enzyme has a pH optimum of 7.0. Rat brain mitochondria activated malonate to malonyl-CoA in the presence of ATP, CoA-SH, and Mg^{2+}. The enzyme had an apparent k_m of 0.08 mM and a pH optimum of 7.3. Malonyl-CoA synthetase was tightly bound to mitochondria or mitochondrial particles. Transfer of the CoA-SH moiety to malonate occurred also from succinyl-CoA and acetoacetyl-CoA. The reaction of acetoacetyl-CoA with malonate was probably catalyzed by succinyl-CoA: 3-oxo-acid CoA-transferase (EC 2.8.3.5).

PHOSPHOLIPID EXCHANGE BETWEEN MEMBRANES. PURIFICATION OF BOVINE BRAIN PROTEINS THAT PREFERENTIALLY CATALYZE THE TRANSFER OF PHOSPHATIDYLINOSITOL. G.M. Helmkamp, Jr., M.S. Harvey, Karel W.A. Wirtz and L.L.M. Van Deenen (Lab. of Biochem., State Univ. of Utrecht, The Netherlands). *J. Biol. Chem.* 249, 6382–9 (1974). Using an assay that measures the transfer of phosphatidyl [^{3}H]inositol from rat liver microsomes to liposomes consisting of phosphatidylcholine (98 mole %) and phosphatidylinositol (2 mole %), two proteins have been isolated from homogenates of bovine brain cortical tissue which specifically stimulate the above phospholipid exchange. Protein I, purified 508-fold, has a molecular weight of approximately 29,000 and an isoelectric point of 5.2, and protein II, purified 426-fold, has a molecular weight of approximately 30,000 and an isoelectric point of 5.5. Among the phospholipids examined in various transfer systems, both proteins exhibited a marked preference for phosphatidylinositol transfer; phosphatidylcholine, but not phosphatidylethanolamine, could also be transferred between membranes. The capacities to transfer phosphatidylinotisol and phosphatidylcholine were inhibited simultaneously by tryptic degradation of each protein; however, protein II was significantly more sensitive to this treatment than protein I. The effects of membrane concentration (microsomal protein or liposomal phospholipid) on relative rates of phosphatidylinositol transfer have been determined. Estimations of K_m and V with respect to each membrane have been made. These properties of the brain phospholipid transfer proteins are discussed in relationship to those other proteins which function in the redistribution of phospholipid between membranes.

ROTATIONAL AND SEGMENTAL MOTIONS IN THE LIPIDS OF HUMAN PLASMA LIPOPROTEINS. J.A. Hamilton, C. Talkowski, R.F. Childers, E. Williams, A. Allerhand and E.H. Cordes (Dept. of Chem., Indiana Univ., Bloomington, Ind. 47401). *J. Biol. Chem.* 249, 4872–8 (1974). Natural abundance ^{13}C NMR spectra of high density, low density, and very low density lipoproteins isolated from human plasma have been recorded and assigned, and the spin lattice relaxation times (T_1) of some well resolved resonances have been determined. The ^{13}C

NMR spectra reflect differences in protein-lipid ratios and in lipid composition between the three classes of lipoproteins. All three lipoproteins yield numerous narrow ^{13}C resonances assignable to the lipid component. The high density lipoprotein also yields several narrow peaks that arise from the protein moiety. All of the above narrow resonances must originate from liquid-like lipid and protein components. T_1 values of lipid carbon resonances of lipoproteins and some model systems yielded semiquantitative information about rotational and segmental mobilities of the lipid components of lipoproteins. The T_1 values of C_6 of the cholesteryl moiety of high and low density lipoproteins revealed that the fused ring systems occupy regions of much higher microviscosity than that of the aqueous environment. T_1 values of carbons of fatty acyl chains yielded information about segmental motions of these chains in the environment of the lipoprotein complex. Segmental mobilities of fatty acyl chains are identical, within experimental error, in high density and low density lipoproteins, despite marked compositional (and probably structural) differences.

SUCROSE AND VARIOUS CARBOHYDRATE-CONTAINING FOODS AND SERUM LIPIDS IN MAN. F. Grande, J.T. Anderson and A. Keys (Lab. of Physiol. Hygiene, Univ. of Minn., Minneapolis, Minn. 55455). *Am. J. Clin. Nutr.* **27**, 1043–51 (1974). The effects of several high carbohydrate foods on fasting serum cholesterol, phospholipids and triglyceride levels were tested in 12 young men. The men were distributed into four groups, each of which was given a diet containing a different supplement. The four supplements (500 kcal of carbohydrate each) contained, respectively, sucrose, wheat flour, mixed fruits, and mixed vegetables. Egg white was added as needed to equalize protein in all the supplements. In consecutive 2-week periods, the supplements were exchanged so that in four periods each group received every supplement. These results indicate that in 2-week dietary periods sucrose does not cause higher fasting serum cholesterol, phospholipids or triglycerides than either wheat flour or dry leguminous seeds in amounts containing isocaloric quantities of starch.

THE EFFECT OF PHOSPHOLIPASE ON THE BINDING OF ASIALOGLYCOPROTEINS BY RAT LIVER PLASMA MEMBRANES. J. Lunney and G. Ashwell (Lab. of Biochem. and Metab., Natl. Inst. of Arthritis, Metab., and Digestive Diseases, Natl. Insts. of Health, Bethesda, Md. 20014). *Biochim. Biophys. Acta* **367**, 304–15 (1974). The ability of hepatic plasma membranes to bind desialylated glycoproteins has been shown to be markedly diminished by prior treatment of the membranes with phospholipase A or phospholipase C. In the latter case, the decreased binding capacity was correlated with the loss of membrane-bound phosphate over a wide range of enzyme concentration. However, upon solubilization of the membrane associated binding protein, the sensitivity to phospholipase-induced inhibition of binding was eliminated. Additional evidence is presented to support the concept that the observed inhibition is a consequence of non-specific changes in the membrane phospholipids and that phospholipid, per se, does not participate directly in the mechanism of binding.

PATHOPHYSIOLOGY OF VITAMIN E DEFICIENCY IN MONKEYS. K.C. Hayes (Dept. of Nutr., Harvard Schl. of Public Health, Boston, Mass. 02115). *Am. J. Clin. Nutr.* **27**, 1130–40 (1974). In a comparative study of vitamin E deficiency in Old and New World monkeys, 14 cynomolgus and 12 cebus monkeys were selected at 1 year of age from animals raised in our primate nursery and were fed either a diet containing 8% coconut oil or stripped safflower oil with or without added tocopherol. During the first year 0.2% cholesterol was added to all diets as an atherogenic stress. Plasma lipids, complete hematology, and plasma tocopherol values were monitored sequentially and complete histopathology, including electron microscopy, was performed terminally. Sudden death of undetermined cause occurred in 3 monkeys fed the safflower oil without vitamin E diet. After approximately 1 year, deficient cebus monkeys consuming safflower oil developed a progressive, macrocytic, hemolytic anemia. Anemia did not occur with the coconut oil diet. Despite the effect on vascular cells, tocopherol did not influence the extent or severity of atherogenesis induced by cholesterol feeding. Safflower oil was associated with more severe deficiency disease than coconut oil, and cebus monkeys were more adversely affected than cynomolgus even though the deficiency syndrome was similar in the two species.

VITAMIN E DEFICIENCY ANEMIA IN OLD AND NEW WORLD MONKEYS. L.M. Ausman and K.C. Hayes. *Ibid.*, 1141–51. In a study of vitamin E deficiency in two species of laboratory raised juvenile monkeys (12 cebus, 14 cynomolgus), diets containing 22% of the calories as coconut or stripped safflower oil with or without vitamin E were fed for 32 months. Signs of deficiency were recognized in the unsupplemented safflower oil-fed cebus monkeys after 12–13 months when severe anemia (hematocrit = 12–15), depressed appetite, weight loss and plasma tocopherol concentrations less than 100 μg/dl were observed. Comparably treated cynomolgus monkeys became clinically ill after 2 years and developed moderate anemia (hematocrit = 25–30), anorexia, muscular weakness and severe weight loss. Anemia in both species was macrocytic and was accompanied by reticulocytosis as high as 35% in some of the cebus monkeys. No monkeys fed coconut oil developed anemia. In vitro hemolysis tests paralleled the clinical findings in that red cells from the cebus hemolyzed earlier and to a greater degree than those from the cynomolgus. Vitamin E protected against the anemia and in vitro hemolysis. A higher percentage of polyunsaturated fatty acid in red blood cells from the cebus was thought to predispose them to hemolysis.

THE PREMATURE INFANT; VITAMIN E DEFICIENCY AND RETROLENTAL FIBROPLASIA. L. Johnson, D. Schaffer and T.R. Boggs, Jr. (Dept. of Pediatrics, Univ. of Pa. Med. Schl., Sect. on Newborn Pediatrics, Pa. Hosp., and Div. of Pediatric Ophthalmol., Childrens Hosp. of Philadelphia, Philadelphia, Pa.). *Am. J. Clin. Nutr.* **27**, 1158–73 (1974). Immature human infants develop retrolental fibroplasia in the absence of oxygen abuse, though fortunately much less frequently and to a less severe degree than in its presence. The incidence of acute stage retrolental fibroplasia among 173 infants weighing less than 2,500 g who were cared for at the Pennsylvania Hospital from 1968 to 1972 was 19% with an incidence of 32% in the 1,000- to 1,500-g weight group and of 75% among the six survivors in the 1,000-g weight category. The severity of the disease did not usually exceed grade 2 active retrolental fibroplasia but six instances of grade 3 disease and one of grade 4 were noted. Human retinal vessels normally develop entirely in the intrauterine environment. They are peculiarly sensitive to changes in O_2 tension. Therefore, in the prematurely born, these vessels are exposed to abnormally high O_2 tensions even in the absence of O_2 therapy. Premature infants are, to a greater or lesser degree, deficient in vitamin E, the natural antioxidant of biological membranes. Retinas are examined weekly during the period of vessel immaturity or proliferative retrolental fibroplasia, and biweekly or monthly during the stage of stabilization and regression of the disease process. The greatest effect is found in the more premature infants (under 1,500 g birth weight).

COMPARISON OF ALPHA-TOCOPHERYL NICOTINATE AND ACETATE ON SKIN MICROCIRCULATION. M. Kamimura (Sapporo Med. Schl., Sapparo, Japan). *Am. J. Clin. Nutr.* **27**, 1110–6 (1974). Clinical observations were made on persons with microcirculatory disturbances after administration of α-tocopheryl nicotinate. It was observed that the clinical symptoms of severe acrocyanosis and "feeling cold" were cured. All subjects were given the cooling-rewarming test. The experimental results indicated the tocopheryl nicotinate had strong effects on the microcirculation of the hand. The data show that α-tocopheryl nicotinate was more effective than the α-tocopheryl acetate and a mixture of α-tocopheryl acetate and nicotinic acid in reducing the mean rewarming time and that the effectiveness of α-tocopheryl nicotinate was not due to the synergic action of the tocopherol and nicotinic acid, but to the independent action of α-tocopheryl nicotinate.

CONTRIBUTION OF THE FATTY ACIDS OF THREE LOW DENSITY SERUM LIPOPROTEINS TO BOVINE MILK FAT. R.F. Glascock and V.A. Welch (Biochem. Dept., Natl. Inst. for Res. in Dairying, Shinfield, Reading, RG2 9AT, England). *J. Dairy Sci.* **57**, 1364–70 (1974). Tritium-labeled palmitic acid was put into the rumen of a lactating cow and the precipitable lipoproteins, consisting of a mixture of one lipoprotein of very low density and two others of low density, were separated from serial samples of arterial jugular, and mammary venous serum. Specific radioactivities of lipids in arterial and jugular venous serum were equivalent. There were arteriovenous differences in the fatty acid composition of the triglycerides of the lipoproteins. Specific radioactivity-time curves showed that the triglyceride fatty acids of two of the lipoproteins, which together accounted for 90% of the triglycerides carried by all three, behaved as a single pool to which those of the third did not belong. From mean concentrations and specific radioactivities, 45% and 47% of milk glyceride palmitic and mixed fatty acids, respectively, had been derived from precipitable lipoproteins. The relative contribution of each subfraction was also calculated. A net transfer of radioactivity

from triglycerides to nonesterified fatty acids, in which there was no arteriovenous difference in concentration, showed that fatty acids had exchanged between the two lipids during passage through the udder. The specific radioactivity-time curve of the triglyceride palmitic acid of the two lipoproteins which formed a single pool could be described by the sum of four exponential functions. This palmitic acid must have been in equilibrium with a larger pool containing at least 9 g.

BINDING AND DEGRADATION OF LOW DENSITY LIPOPROTEINS BY CULTURED HUMAN FIBROBLASTS. COMPARISON OF CELLS FROM A NORMAL SUBJECT AND FROM A PATIENT WITH HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLEMIA. J.L. Goldstein and M.S. Brown (Divs. of Med. Genetics and Gastroenterology-Liver, Dept. of Internal Med., Univ. of Tex. Southwestern Med. Schl., Dallas, Tex. 75235). *J. Biol. Chem.* **249**, 5153–62 (1974). ^{125}I-Labeled low density lipoproteins were found to associate with monolayers of cultured normal fibroblasts by two processes—one of high affinity and one of low affinity. The high affinity association appeared to represent binding of the low density lipoprotein to specific receptor sites on the cell surface. This binding process exhibited saturation kinetics at low concentrations of the lipoprotein and competition by related molecules such as very low density lipoproteins. In addition, this process was stimulated by the presence of calcium in the culture medium and could be destroyed by limited treatment of the cells with pronase. The other process, designated low affinity uptake, may represent nonspecific endocytosis since the uptake was proportional to the lipoprotein concentration in the medium with no apparent saturation and because it showed no competition by very low density lipoproteins, no stimulation by calcium, and no destruction by pronase treatment. The ^{125}I-labeled low density lipoproteins associated with normal cells by either the high or low affinity process were degraded by proteolysis to trichloroacetic acid-soluble material, most of which was ^{125}I-tyrosine.

THE EFFECT OF THE HYPOCHOLESTEROLEMIC DRUG CLOFIBRATE ON LIVER MITOCHONDRIAL BIOGENESIS. A ROLE FOR NEUTRAL MITOCHONDRIAL PROTEASES. A.R.L. Gear, A.D. Albert and J.M. Bednarek (Dept. of Biochem., Univ. of Va., Charlottesville, Va. 22901). *J. Biol. Chem.* **249**, 6495–504 (1974). The hypocholesterolemic drug, clofibrate, raises hepatic levels of mitochondria by over 100%, with maximum increase at 2 to 4 days after drug administration, and a slow rise continuing at 10 days. This time response was similar to that seen after partial hepatectomy. However, several clear differences exist. During the doubling in mitochondrial content, no change in particle size or specific content of DNA or RNA was observed, whereas during liver regeneration the former decreases by 50% and the latter increases by 300%. The half-life of 5.8 days for normal mitochondria was not altered significantly by clofibrate, using [*guanidinio*-^{14}C]arginine. However, the initial rate of short term incorporation of [^{35}S]methionine into control mitochondria in vivo was 60% of that for drug-tested animals, while the in vitro efficiency of [^{14}C]leucine incorporation was reversed; mitochondria from drug-treated animals were about 61% as active as those from controls. Enzyme activity of drug-treated animals was not significantly altered for inner membrane or matrix enzymes. However, a 30 to 40% fall in specific activity of three outer membrane enzymes was noted from 2 to 15 days. This resembles changes seen during early liver regeneration.

RELATIONSHIPS BETWEEN COENZYME Q AND VITAMIN E. K. Folkers (Inst. for Med. Res., Univ. of Texas, Austin, Tx. 78712). *Am. J. Clin. Nutr.* **27**, 1026–34 (1974). Coenzyme Q and vitamin E have limited organic structural features in common, but their biochemical roles in life processes can be distinctly different. Vitamin E is a chromanol; the quinone analog of vitamin E related to coenzyme Q is a plastoquinone which has no role in nutrition, but functions in photosynthesis. Coenzyme Q is a quinone and its chromanol, similar to vitamin E, has no role in life processes. The organic chemical and biochemical differentiation between coenzyme Q and vitamin E were less clear in the early 1960's, but fortunately led to a study of coenzyme Q in experimental animal diseases caused by diets low in vitamin E. These studies demonstrated the lifesaving and vitamin activity of coenzyme Q in rabbits, monkeys, rats, hamsters, chickens and turkeys. Coenzyme Q has indispensable, electron transfer functions in three mitochondrial oxidases, and functions in the Golgi apparatus, and is a component of a cytoplasmic aldehyde oxidase. Vitamin E does not coenzymatically substitute for coenzyme Q in any CoQ_{10}-enzyme which is in agreement with their organic structural differences. The biosynthesis of coenzyme Q_{10} in human tissue requires many of the known vitamins and essential minerals and underscores the importance of coenzyme Q in nutritional sciences.

EFFECT OF VITAMIN B_{12} DEPRIVATION ON THE IN VIVO LEVELS OF COENZYME A INTERMEDIATES ASSOCIATED WITH PROPIONATE METABOLISM. E.P. Frenkel, R.L. Kitchens, L.B. Hersh and R. Frenkel (E.L. Overton Hematol.-Oncol. Res. Lab., Depts. of Internal Med. and Biochem., Univ. of Tex. Southwestern Med. Schl., Dallas, Tx. 75235). *J. Biol. Chem.* **249**, 6984–91 (1974). The in vivo coenzyme A intermediates involved in the metabolic pathway in which vitamin B_{12} serves as a coenzyme (conversion of methylmalonyl-CoA to succinyl-CoA) were measured in the livers of control and B_{12}-deprived animals. Succinyl-CoA was assayed by the succinate thiokinase arsenolysis of succinyl-CoA coupled with 5′,5′-dithiobis (2-nitrobenzoic acid) measurement of the liberated coenzyme A. Methylmalonyl-CoA was measured by coupling the succinyl-CoA assay with methylmalonyl-CoA mutase. The assays were shown to be specific and reproducible and provided excellent recovery of added exogenous coenzyme A derivatives. Unexpectedly, succinyl-CoA levels were found to be on an average 4-fold greater in the livers of the B_{12}-deprived animals than in the controls. In individual liver samples the propionyl-CoA to methylmalonyl-CoA ratio was approximately 2:1. Acetyl-CoA levels were not reduced by the presence of increase endogenous propionyl-CoA, but were actually increased in livers from the B_{12}-deprived group. Thus, the present study provides a method of measurement of the CoA intermediates in the B_{12}-dependent pathway in the intact liver and demonstrates that B_{12} deficiency results in an increase in propionyl-CoA and methylmalonyl-CoA as well as in succinyl-CoA, an intermediate beyond the site of action of coenzyme B_{12}.

SUBSTRATE SPECIFICITY AND MECHANISM OF ACTION OF ACETOACETATE COENZYME A TRANSFERASE FROM RAT HEART. A. Fenselau and K. Wallis (Dept. of Physiol. Chem., Johns Hopkins Univ. Schl. of Med., Baltimore, Md. 21205). *Biochemistry* **13**, 3884–8 (1974). The specificity of succinyl-CoA: acetoacetate CoA transferase (as a partially purified preparation from rat heart) was examined by employing various analogs of succinate. Diacids with a connecting chain length of $\geqslant 3$ methylene groups are inactive; oxalate and malonate are competitive inhibitors (K_i = 15 and 21 mM, respectively). Analogs with substitution into the ethylene group of succinate are generally inactive, except for inhibition by 2,2-difluorosuccinate and perfluorosuccinate (K_i = 6.4 and 18 mM, respectively). 3-Sulfopropanoate and compounds with substitution into one of the carboxyl groups (monomethyl succinate, succinamate, maleamate, and *N*-ethylmaleamate) also inhibit competitively (with K_i values from 11 to 33 mM), but do not serve as substrates. Only maleate proved to be a substrate (K_m = 35 mM *vs.* a K_m value for succinate of 28 mM) with a V_{max} one-ninth of that for succinate. Fumarate is ineffective; acetylenedicarboxylate weakly inhibits. Finally, usage of acetoacetate is inhibited competitively succinate and maleate; the other inhibitors to succinate display mixed-type inhibitions for acetoacetate, indicating their potential usefulness for studies on the metabolism of ketone bodies.

LIPOPROTEIN LIPASE: PROPERTIES OF THE ENZYME ISOLATED FROM POST-HEPARIN PLASMA. P.E. Fielding, V.G. Shore and C.J. Fielding (Cardiovascular Res. Inst., Univ. of Calif., San Francisco, Calif. 94143). *Biochemistry* **13**, 4318–23 (1974). Lipoprotein lipase purified from rat post-heparin plasma was characterized in terms of its amino acid, carbohydrate, and lipid composition. Molecular weight determinations by several procedures indicate a monomeric molecular weight of about 37,000; apparent dimer and tetramer forms were also identified. The purified lipase is a glycoprotein, but it does not contain heparin. It did not bind heparin in solution or covalently attached to agarose beads. The purified lipase retained approximately 1 mol of phospholipid/mol of protein.

COMPARATIVE PROPERTIES OF THE MEMBRANE-SUPPORTED AND SOLUBILIZED ENZYME SPECIES. *Ibid.*, 4324–30. The catalytic rate of lipoprotein lipase has been determined before and after solubilization from the perfused rat heart. Both the apparent K_m and k_c values were closely similar for the membrane-bound and soluble lipase. This result was confirmed for the major plasma very low density lipoprotein fraction (S_f 100–400) and for two subfractions of rat lymph chylomicrons (S_f 100–400 and $S_f > 400$). These findings suggest a superficial binding site for lipoprotein lipase at the capillary wall, and the absence of major conformational change during solubilization. Both membrane-bound and soluble lipase showed catalytic

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● **Abstracts** . . . . . (Continued from page 182A)

rates about twofold greater with chylomicron than with very low density lipoprotein triglyceride. These findings are discussed in the light of recent ideas on the mechanism of lipoprotein lipase activity.

THE INTERACTION OF APOLIPOPROTEIN-SERINE WITH PHOSPHATIDYLCHOLINE. R.L. Jackson, J.D. Morrisett, J.T. Sparrow, J.P. Segrest, H.J. Pownall, L.C. Smith, H.F. Hoff and A.M. Gotto, Jr. (Depts. of Med. and Biochem., Baylor College of Med. and the The Methodist Hosp., Houston, Tex. 77025). *J. Biol. Chem.* **249**, 5314–20 (1974). Apolipoprotein-serine (apoLP-Ser) is one of the protein constituents of human plasma very low density lipoproteins (VLDL). The protein has 57 amino acids with 1 residue each of methionine and tryptophan. Cleavage of apoLP-Ser with cyanogen bromide yields the NH_2-terminal fragment (CNBr I) and COOH-terminal fragment (CNBr II) having 38 and 19 residues, respectively. CNBr II contains the single residue of tryptophan. We have studied the interaction of egg phosphatidylcholine with apoLP-Ser and its cyanogen bromide fragments. ApoLP-Ser strongly inhibited the reactivation of defatted β-hydroxybutyrate dehydrogenase; 10 μg of the apoprotein inhibited reactivation by 85%. CNBr I and CNBr II inhibited reactivation to a lesser extent than did the intact apoprotein. The α helical content of apoLP-Ser was about 56%, as determined by circular dichroism. These findings are interpreted in light of a theory of phospholipid-binding involving amphipathic helical regions that contain positively and negatively charged amino acids and are consistent with the localization of such regions in the primary chemical structure of the molecule.

EFFECTS OF METHYLATION ON THE STABILITY OF NUCLEIC ACID CONFORMATIONS: STUDIES AT THE MONOMER LEVEL. J.D. Engel and P.H. von Hippel (Inst. of Mol. Biol. and Dept. of Chem., Univ. of Oregon, Eugene, Ore. 97403). *Biochemistry* **13**, 4143–58 (1974). High-resolution proton magnetic resonance measurements are reported on mono- and dimethylated analogs of adenine, cytosine, and guanine. These measurements demonstrate (for the adenine derivatives) and confirm (for the analogs of cytosine) that rotation of the aminoethyl group is restricted about the nitrogen-ring carbon bond, and that this rotation is characterized by activation enthalpies of +11 to +18 kcal/mol. The two possible rotamers of m^6A and m^4C lie in the plane of the ring, and show an ~20:1 preference for syn positioning of the methyl group (relative to N_1 of m^6A and N_3 of m^4C), resulting in both cases in preferential interference of the methyl group with Watson-Crick interbase hydrogen bonding. Rotation about the C_2-N_{10} bond in m^2G and m_2^2G does not appear to be restricted. These results indicate that the replacement of A or C by m^6A or m^4C in a base-paired polynucleotide structure should destabilize the Watson-Crick double-helical structure by ~1.0–1.8 kcal/mol of methyl substituent. This prediction is consistent with polymer data in the literature, based on melting point depression measurements of ordered structures.

ASCORBIC ACID AND CHOLESTEROL LEVELS IN PASTORAL PEOPLES IN KENYA. J.D.G. Davies and J. Newson (Nairobi Labs., Res. Div., P.O. Box 45789, Nairobi, Kenya). *Am. J. Clin. Nutr.* **27**, 1039–42 (1974). Pastoral tribes of Kenya with a high cholesterol intake were sampled. There was a positive correlation between levels of serum cholesterol and both plasma and leukocyte ascorbate. The possible relevance of these findings to atherogenesis is discussed.

THE EFFECTS OF DIET CHOLESTEROL ON THE SYNTHESIS OF RAT SERUM APOLIPOPROTEINS. J. Frinka and R. Reiser (Dept. of Biochem. and Biophys., Texas A and M Univ., College Station, Tx. 77843). *Biochim. Biophys. Acta* **360**, 322–38 (1974). The major objectives of this study were to determine the effects of diet cholesterol on serum lipoprotein apoprotein synthesis and to test the hypothesis that the synthesis of serum high density lipoprotein apoproteins may be interrelated with cholesterogenesis. The relative rates of synthesis of serum apoproteins were determined by assay apoprotein radioactivity at 30-min intervals after intraperitoneal injection of [4,5-^{3}H] leucine. The rate of incorporation of labeled leucine into apoproteins of high density lipoproteins was significantly less in male rats ingesting 1% cholesterol for 14 days than in those animals receiving no cholesterol. Reduction of high density lipoprotein apoprotein synthesis was accompanied by an increase in the synthesis of very low density lipoprotein apoprotein. Rapid changes in both apoprotein and cholesterol synthesis occurred during the first 3½ days after 0.5% cholesterol was included in the diet. Reduction of high density lipoprotein apoproteins and cholesterol synthesis, determined by simultaneously injecting [4,5-^{3}H]leucine and [1-^{14}C]acetate, was evident as early as 1½ days after animals had access to cholesterol diets.

A SOLID-PHASE RADIOIMMUNOASSAY FOR PLASMA PROGESTERONE. K.K. Dighe and W.M. Hunter (Med. Res. Council Radioimmunoassay Team, 2 Forrest Rd., Edinburgh EH1 2QW, U.K.). *Biochem. J.* **143**, 219–31 (1974). A detailed procedure is presented for the assay of plasma progesterone. The routine assay is based on the use of antiserum which is covalently linked to microcrystalline cellulose, the double-antibody method being used as a reference separation system. This procedure gives high precision accompanied by small and acceptable losses of antiserum titre but without loss of sensitivity when compared with the double-antibody method. Ethanol is first added to the plasma (10 vol. of plasma + 1 vol. of ethanol) after which a single extraction with light petroleum yields a constant recovery [92.4 ± 1.2 (S.D.) % of added [^{3}H] progesterone] and obviates the need for tracer recoveries on each sample being assayed. Distortions of the response curve owing to solvent residues have been almost eliminated. The assay can measure progesterone at all stages of the menstrual cycle when volumes of 200 μl of plasma are used and this permits the detection of the periovulatory rise at its inception. Detailed specificity studies are presented for the assay end point itself and these are related to the responses to be expected in extracts of plasma. Progesterone-like activity was found in urine and a fourfold increase in excretion rates was observed between the follicular and luteal phase of the normal menstrual cycle.

INFLUENCE OF REPEATED OXYTOCIC TREATMENTS ON COMPOSITION OF BOVINE MILK FAT. C.W. Dill, G.T. Lane and S.N. Hartsfield (Animal Sci. Dept., Texas A&M Univ., College Station, Tx. 77843). *J. Dairy Sci.* **57**, 1164–9 (1974). Fatty acid composition of bovine milk fat was not affected significantly by prolonged forced milk release. Monoglyceride content increased markedly with this treatment while phospholipid content was depressed slightly. Mastitis, as measured by the California Mastitis Test, produced little effect on fatty acid composition of milk fat.

HEXOSE TRANSPORT IN ISOLATED BROWN FAT CELLS. A MODEL SYSTEM FOR INVESTIGATING INSULIN ACTION ON MEMBRANE TRANSPORT. M.P. Czech, J.C. Lawrence, Jr. and W.S. Lynn (Depts. of Biochem. and Med., Duke Univ. Med. Ctr., Durham, N.C. 27710). *J. Biol. Chem.* **249**, 5421–7 (1974). Direct measurement of 3-0-[^{3}H]methyl-D-glucose uptake by brown fat cells with the use of a rapid filtration procedure was found to provide a suitable index of the D-glucose transport system activity. The rate of net 3-0-methylglucose influx across the fat cell membrane was 10 to 20 times greater than the uptake rate of L-[^{14}C]glucose which enters the cells by simple diffusion. After equilibration the concentration of 3-0-methylglucose in the intracellular water space was the same as that in the medium, indicating that 3-0-methylglucose uptake occurs by facilitated diffusion. Detectable phosphorylation of this D-glucose analogue by brown fat cells under the conditions of these studies did not occur. These results indicate that glucose uptake in brown fat cells is determined by both the activity of the membrane transport system and the rate of intracellular utilization and that alteration of either process can modulate glucose entry rates.

LONG-TERM CHANGES OF SERUM CHOLESTEROL WITH CHOLESTEROL-ALTERING DRUGS IN PATIENTS WITH CORONARY HEART DISEASE. VETERANS ADMINISTRATION DRUG-LIPID COOPERATIVE STUDY. K.M. Detre and L. Shaw (West Haven VA Cooperative Studies Program Support Ctr., West Haven VA Hospital, West Haven, Conn.). *Circulation* **50**, 998–1005 (1974). In a controlled secondary prevention trial of estrogen and cholesterol-lowering drugs on 570 veterans, all of whom had had one or more episodes of acute myocardial infarction, changes in serum cholesterol were followed for at least five years. The findings included the following: Estrogen, 1.25 mg daily, had no appreciable effect on cholesterol level. Aluminum nicotinate, 4 g/day, resulted in a 20% reduction in cholesterol level for about 2½ years after which levels slowly rose to a level 12% below the baseline level in good adherers; poor adherers had smaller ultimate changes. Dextrothyroxine, 4 mg/day, had a sustained cholesterol-lowering effect of approximately 7% throughout the study. The above values were obtained after adjusting for an underlying upward trend in all cholesterol values during the five-year observation period. Discontinuation of both aluminum nicotinate and dextrothyroxine resulted in a significant rise in cholesterol within three months. The 6% rise in the cholesterol level found in the

control group over five years of follow-up could be attributed to aging and laboratory drift. Regression toward the mean rather than pharmacological effect accounted for the greater response to treatment of patients with high initial cholesterol.

CYCLIC ADENOSINE MONOPHOSPHATE-DEPENDENT PHOSPHORYLATION OF SPECIFIC FAT CELL MEMBRANE PROTEINS BY AN ENDOGENOUS MEMBRANE-BOUND PROTEIN KINASE. POSSIBLE INVOLVEMENT IN THE REGULATION OF INSULIN-STIMULATED GLUCOSE TRANSPORT. K-J. Chang, N.A. Marcus and P. Cuatrecasas (Dept. of Pharmacol. and Exptl. Therapeutics, and Dept. of Med., Johns Hopkins Univ. Schl. of Med., Baltimore, Md. 21205). *J. Biol. Chem.* 249, 6854–5 (1974). The phosphorylation of specific membrane proteins by an endogenous protein kinase has been studied in purified membrane fractions from rat adipocytes using trichloroacetic acid precipitation and sodium dodecyl sulfate polyacrylamide disc gel electrophoresis. The endogenous phosphorylation of two specific membrane proteins is completely dependent on the presence of cyclic adenosine 3′:5′-monophosphate (cyclic AMP) and magnesium ions. This phosphorylation occurs very rapidly at 24C, reaching maximal levels at 1 min. The number of sites specifically phosphorylated in the presence of cyclic AMP probably does not exceed 50,000 per fat cell, requiring the use of very high specific activity (10 to 50 Ci per mmole) [γ-^{32}P]ATP for these studies. The minimal molecular weights of the two specifically phosphorylated proteins are about 22,000 and 16,000 as determined by gel electrophoresis in the presence of sodium dodecyl sulfate. The same two proteins are phosphorylated when intact fat cells are exposed briefly to low concentrations of exogenous ATP, a process which results in the suppression of the insulin-stimulated rates of D-glucose transport.

REDUCTION OF SERUM TRIGLYCERIDE LEVELS BY POLYUNSATURATED FAT. STUDIES ON THE MODE OF ACTION AND ON VERY LOW DENSITY LIPOPROTEIN COMPOSITION. A. Chait, A. Onitiri, A. Nicoll, E. Rabaya, J. Davies and B. Lewis (Depts. of Chem. Pathol. and Med. and the Lipid Clinic, Royal Postgraduate Med. Schl., London, Great Britain). *Atherosclerosis* 20, 347–64 (1974). When isocaloric diets high in saturated fat or polyunsaturated fat were alternately fed to hyperlipidaemic and normal men, serum triglyceride was 35% lower ($P < 0.001$) and serum cholesterol 16% lower ($P < 0.001$) during the unsaturated fat diet than during saturated fat feeding. A similar response occurred in normal subjects and in patients with primary endogenous hyperglyceridaemia. Postprandial serum triglyceride levels were higher during saturated fat feeding than when unsaturated fat was fed. The difference was due to higher fasting levels, the increment due to alimentary lipaemia being similar during the two diets. There was no change in post-heparin lipolytic activity (PHLA) nor in fractional removal rate of triglyceride as measured by the intravenous fat tolerance test (IVFTT). Albumin-bound [9,10-^{3}H]palmitic acid and [1-^{14}C]linoleic acid were simultaneously infused and their relative rates of incorporation into very low density lipoprotein triglyceride (VLDL-TG) was measured in six normolipidaemic men. The results suggested that the saturated fatty acid was preferentially incorporated, and may imply that dietary polyunsaturated fat decreases the rate of secretion of VLDL-TG into plasma.

CHOLESTEROL METABOLISM IN THE EHRLICH ASCITES TUMOR. D.E. Brenneman, R. McGee and A.A. Spector (Depts. of Biochem. and Internal Med., Univ. of Iowa, Iowa City, Iowa 52242). *Cancer Res.* 34, 2605–11 (1974). Analyses of the Ehrlich ascites tumor during Days 11- to 14 of growth in the mouse peritoneal cavity revealed that the cells accumulate cholesterol at a rate of 5.7 nmoles/10^8 cells per hr. Incubations with either $^{3}H_2O$ or uniformly labeled glucose-^{14}C in vitro indicated that de novo cholesterol synthesis can account for only about 3% of the cholesterol accumulation in the Ehrlich cells. Since the peritoneal fluid in which the tumor cells grow is rich in very-low-density lipoproteins (VLDL), we wished to determine whether the cholesterol contained in these VLDL might be available for utilization by the cells. During incubation in vitro at 37C, the Ehrlich cells took up large quantities of cholesterol-4-^{14}C from VLDL. These findings demonstrate that the cholesterol contained in the ascites plasma VLDL potentially is available for utilization by the Ehrlich cells. Moreover, they suggest that VLDL may function as vehicles for transport of cholesterol from the tissues of the mouse to the growing tumor cells.

CHOLESTERYL ESTER SYNTHESIS IN NORMAL AND ATHEROSCLEROTIC AORTAS OF RABBITS AND RHESUS MONKEYS. P.I. Brecher and A.V. Chobanian (Cardiovascular Inst. and the Dept. of Med., Boston Univ. Schl. of Med., Boston, Mass. 02118). *Circulation Res.* 35, 692–701 (1974). The formation of cholesteryl ester in aortic tissue was studied using subcellular fractions from normal and atherosclerotic rabbit and rhesus monkey aortas. The properties of two enzyme systems capable of esterifying 1-^{14}C-oleic acid into cholesteryl ester in vitro were investigated, and increased activity was demonstrated for both systems as a result of cholesterol feeding. Microsomal preparations were used to study the ATP, CoA-dependent esterification which involves two enzymes, fatty acyl CoA:cholesterol acyltransferase. The properties of both enzymes were investigated, and an increase of about fourfold in activity of the acrltransferase was demonstrated in aortic microsomes as a result of cholesterol feeding for 3–6 months. Esterification of oleic acid into cholesteryl ester by aortic high-speed supernatant fractions at an acidic pH was also observed; the enzyme system involved did not require cofactors, and its activity greatly increased as a result of cholesterol feeding. These studies suggest that increased intracellular synthesis of cholesteryl ester by aortic tissue may contribute to its accumulation in atherosclerosis.

HYDROXYLATIONS OF BILE ACIDS BY RECONSTITUTED SYSTEMS FROM RAT LIVER MICROSOMES. I. Björkhem, H. Danielsson and K. Wikvall (Dept. of Chem., Karolinska Inst., Stockholm, Sweden). *J. Biol. Chem.* 249, 6439–45 (1974). The 7α-hydroxylation of taurodeoxycholic acid and the 6β-hydroxylation of taurochenodeoxycholic acid and lithocholic acid were studied with a reconstituted system from rat liver microsomes consisting of partially purified cytochrome P-450, NADPH-cytochrome P-450 reductase, a synthetic phosphatidylcholine and NADPH or an NADPH-generating system. 7α-Hydroxylase activity was observed with cytochrome P-450 from either male or female rats whereas 6β-hydroxylase activity was observed only with cytochrome P-450 from male rats. With male rats, the ratio between 7α-hydroxylase activity and 6β-hydroxylase activity was considerably higher in the reconstituted system than in the original microsomal fraction. The 6β-hydroxylase activity of the reconstituted system was more stable than the 7α-hydroxylase activity during prolonged storage at −20°. The rate of the hydroxylations in the reconstituted system was linear with the concentration of cytochrome P-450 and increased with the concentration of NADPH-cytochrome P-450 reductase up to a certain level and then remained constant. The catalytic activity of NADPH-cytochrome P-450 reductase per unit of NADPH-cytochrome *c* reductase activity was about the same regardless of the source of the preparation.

GAMMA TOCOPHEROL: METABOLISM, BIOLOGICAL ACTIVITY AND SIGNIFICANCE IN HUMAN VITAMIN E NUTRITION. J.G. Bieri and R.P. Evarts (Sect. on Nutr. Biochem., Lab. of Nutr. and Endocrinol., Natl. Inst. of Arthritis, Metabolism and Digestive Diseases, Natl. Insts. of Health, Bethesda, Md. 20014). *Am. J. Clin. Nutr.* 27, 980–6 (1974). Studies with labeled compounds showed that γ-tocopherol was absorbed from the intestine of rats about as efficiently as α-tocopherol but that γ-tocopherol disappeared faster from tissues after 24 hr. When these tocopherols were fed continuously in the diet as they occur naturally in corn and soybean oils, γ-tocopherol accumulated significantly in tissues to varying degrees. Adipose tissue had the highest concentration, heart, kidney and muscle were intermediate and plasma and liver were lowest. Reevaluation of the relative biological activity of γ-tocopherol in rats, chicks and hamsters gave an overall value of 10% that of α-tocopherol. Changing dietary fat patterns in the United States have resulted in soybean oil becoming the predominant dietary fat (70% of all vegetable fat), with the result that average diets have twice as much γ-tocopherol as α-tocopherol. These factors indicate that γ-tocopherol may contribute as much as 20% of the total vitamin E activity of United States diets, and that calculations based only on α-tocopherol significantly underestimate dietary vitamin E.

REGULATION OF LIPID SYNTHESIS IN CULTURED ANIMAL CELLS. A.W. Alberts, K. Ferguson, S. Hennessy and P.R. Vagelos (Dept. of Biochem., Div. of Biol. and Biomed. Sci., Washington Univ., St. Louis, Mo. 63110). *J. Biol. Chem.* 249, 5241–49 (1974). Fatty acid and sterol biosynthesis were studied in several cell lines in culture. Two of these cell lines, L-M and NCTC 1469, demonstrated an 8.6- and 7.2-fold increase, respectively, in acetate incorporation into fatty acids and a 5.2- and 2.4-fold increase, respectively, in acetate incorporation into sterols within 24 hours after serum-containing medium was replaced with serum-free medium. Three other cell lines, Chang liver, HeLa and NCTC 2544, demonstrated little change

in fatty acid or sterol synthesis 48 hours after serum removal from the medium, but they demonstrated up to an 8-fold increase in fatty acid synthesis and a 3-fold increase in sterol synthesis 48 hours after serum removal when insulin was present in the serum-free medium. In Chang liver cells fatty acid synthetase activity did not change during the first 24 hours after replacement of serum-containing medium with serum-free medium. In Chang liver cells low levels of actinomycin D and camptothecin, inhibitors of RNA synthesis, prevented the induction of fatty acid synthetase by serum removal or insulin addition. A combination of theophylline and cyclic adenosine 3′:5′-monophosphate also prevented induction.

RHODOPSIN. PURIFICATION AND RECOMBINATION WITH PHOSPHOLIPIDS ASSAYED BY THE METARHODOPSIN I → METARHODOPSIN II TRANSITION. M.L. Applebury, D.M. Zuckerman, A.A. Lamola and T.M. Jovin (Bell Labs., Murray Hill, N.J.). *Biochemistry* 13, 3448–58 (1974). Studies of the nature of interaction between the visual protein rhodopsin and the rod outer segment (ROS) membrane phospholipid components have been initiated. To assay this interaction, a flash photolysis instrument has been built with microsecond resolution allowing the kinetic observation of the spectroscopic intermediates metarhodopsin I_{380nm} → metarhodopsin II_{380nm} in the bleaching process of rhodopsin. A single first-order rate has been established for the kinetic appearance of metarhodopsin II_{380nm} in preparations of rhodopsin in its native disc membrane environment (ROS membranes) and for dodecyldimethylamine oxide (DDAO) detergent solubilized rhodopsin. A purification procedure has been developed for the preparation of rhodopsin free of phospholipid and detergent and the isolated protein can be recombined with phospholipids to obtain a "reconstituted" lipid-protein species of defined composition. The spectroscopic assay is a sensitive indication of the protein-lipid interaction. The transition is blocked for rhodopsin free of detergent and lipid but can be restored by addition of detergent.

A DOUBLE-BLIND TRIAL OF VITAMIN E IN ANGINA PECTORIS. T.W. Anderson and D.B.W. Reid (Dept. of Epidemiology and Biometries, Schl. of Hygiene, Univ. of Toronto, Toronto, Ontario, Canada). *Am. J. Clin. Nutr.* 27, 1174–8 (1974). The claim that large doses of vitamin E can relieve the symptoms of angina pectoris has been strenuously debated for over 20 years. The few clinical trials that were carried out (around 1950) gave negative results, but these trials have been criticized on the grounds of small numbers, poor design or inadequate dosage. The present trial was therefore carried out on 50 patients, using a randomized double-blind design, and a daily dosage of 3,200 IU of vitamin E or placebo. The results were not conclusive.

• Edible Proteins

BLAND SOY PROTEIN. W. Williams (Beatrice Foods Co.). *U.S. 3,852,480.* A method for producing a bland soy protein consists of suspending soy protein in an aqueous medium, adjusting the pH to between 1.5 and 4.0, adding an acid fungal protease, and allowing the protease to act at 40–55C until a bland protein is produced. The suspension is then neutralized to pH 6–8.

PRODUCTION OF VEGETABLE PROTEIN SEAFOOD SUBSTITUTES. J.M. Cabot (Central Soya Co.). *U.S. 3,852,484.* The method comprises (a) taking an aqueous solution of a vegetable protein essentially free of fibrous residue and other nonproteinaceous materials and adjusting the temperature to less than 150F and the pH to 4.5–4.8 whereby the major globulin fractions precipitate as a curd. The solids content of the curd is adjusted to 15–25% by removing some of the solution. (b) dispersing large particles or agglomerates in the curd, (c) rasing the pH of the curd to 5.5–7.0, (d) heating the curd in an extruder at 200–300F and extruding the heated curd at a pressure of 75–200 psi whereby the curd is modified to produce an extrudate having textural properties resembling those of seafood.

METHOD OF MAKING PUDDINGS CONTAINING SOY PROTEIN. P.J. Magnino, R.A. Hoer and R.E. Hahn (Ralston Purina Co.). *U.S. 3,852,503.* A process for forming a pudding with a pH of less than 4.2 and containing isolated soy protein comprises the steps of (a) preparing the isolate by isoelectric precipitation; (b) forming an aqueous slurry of the protein at pH 2.9–4.2 and solids content of 3–20%, practically instantly heating successive portions of the slurry to 250–320F for a period of time sufficient to inactivate the trypsin inhibitor, and then suddenly releasing the pressure on the slurry to flash off some of the water and cool the remaining slurry; and (c) blending 1.0–4.5% on a dry weight basis of the isolated soy protein with a mixture of pudding ingredients. The blended mixture has a moisture content of 65–70%, a pH less than 4.2, and a starch content of 3.5–7% on a dry weight basis. The blended mixture is heated at 240–320F for 45 seconds to 3 minutes and thereafter cooled to form a pudding.

METHOD OF FORMING PROTEIN FOOD PRODUCT. P.J. Magnino, R.A. Hoer and R.E. Hahn (Ralston Purina Co.). *U.S. 3,853,839.* The process described here is essentially the same as described in *U.S. 3,852,503.*

METHOD OF TREATING GLUTEN. R.A. Reiners, J.C. Pressick and L. Morris (CPC International). *U.S. 3,840,515.* A method for preparing a proteinaceous composition suitable for use as a food material comprises preparing a mixture of gluten and methanol, separating the gluten by means of countercurrent extraction, filtration, or centrifugation; mixing the gluten with aqueous methanol; heating the mixture at 110–140C; separating an aqueous methanol extract from the solid residue at 60–140C; and isolating a prolamine from the aqueous methanol extract.

PROCESS FOR PRODUCING FIBROUS PROTEIN PRODUCT. K.Y. Kim and N. Yagi (Minaminihon Rabuno Kyodo Kabishiki Kaisha). *U.S. 3,840,671.* The process comprises stirring at a temperature of 10–80C an aqueous solution of 5–30% of a nonfibrous protein adjusted to a pH of 6–9 with a gelating agent selected from the group consisting of calcium chloride and magnesium chloride and a protease for a time sufficient to convert the protein to a fibrous protein. The resulting product is stretched to impart an orientation to it, and the protease is subsequently inactivated.

METHOD OF BINDING FOODSTUFFS. A. Yamamoto, T. Ikemoto and R. Shimizu (Kyowa Hakko Kogyo Co.). *U.S. 3,840,676.* The method of binding granular or fibrous foodstuffs comprises intimately contacting the foodstuff with a prolamin and an α-amino acid in a carrier followed by molding and drying the resultant mixture to form the bound product.

BACON-LIKE MEAT ANALOG. H.T. Leidy, J.T. Hayes, Jr. and A.M. Hai (General Foods Corp.). *U.S. 3,840,677.* The product contains a plurality of distinct regions which, when cooked, texturally resemble the fat and lean portions of cooked meat. One region contains 20–39 parts water, 30–50 parts fat, 7–20 parts albumen, 0–5 parts protein isolate, 0–20 parts proteinaceous filler particles, and up to 15 parts flavoring agents. The other region contains 40–65 parts water, 10–25 parts fat, up to 15 parts albumen, 6–24 parts protein isolate, 0–15 parts proteinaceous filler particles, and up to 15 parts flavoring agents. The albumen content of the first region is higher than that of the second.

CREPING PROCESS OF PREPARING AN IMPROVED MEAT ANALOG. A.L. Liepa and T.J. Slone, Sr. (Procter & Gamble). *U.S. 3,840,679.* The process comprises the steps of forming a dry protein mix, adjusting the moisture content to form a dough-like protein wet mix, creping the protein wet mix to form a coherent workable dough sheet, aggregating the sheet by collecting it into a mass, and stabilizing the aggregate by heating it at 155–300F to form a coherent fiber mass resembling meat in appearance, texture and eating quality.

PROTEINACEOUS MATERIAL FOR BEVERAGE USE. G. Puski (Central Soya Co.). *U.S. 3,843,802.* The process for treating a protein material such as soybean protein, sodium caseinate, wheat gluten, fish protein concentrate and microbial protein to produce an acid soluble product involves the following steps for producing an acid protein curd. The curd is first heated to reduce microbiological contamination. Then it is slurried and mixed with a proteolytic enzyme, such as ficin, fungal protease, bacterial protease, pepsin, or papain, to render soluble at an acid pH a major portion of the curd. The pH, temperature and enzyme concentration are adjusted and the protein-enzyme slurry is mixed for two hours, thereby producing a product having an N-terminal free amino group content in the range of 0.125–0.225 per 100 grams of protein. The protein-enzyme slurry is then heated to inactivate the enzyme. Insoluble protein is separated, and the soluble protein is dried. This soluble protein may then be incorporated into beverages having a pH range of 2–5.5 at levels of 1–3%.

PROCESS FOR PRODUCING FIBERS FROM NATURAL PROTEIN OF ANIMAL ORIGIN. N. Yano, H. Takahashi, and Y. Hayasho (Asahi Kasei Kogyo Kabushiki Kaisha). *U.S. 3,843,803.* A

process for producing fibrous protein from a solution of protein of animal origin and vegetable origin involves extruding an aqueous solution of the protein into a coagulating bath. Specifically, the process comprises solubilizing the protein at pH 10–12, adding to the solution an unsaturated fatty acid having 14–22 carbon atoms, or a salt of the fatty acid, and agitating the mixture until a viscous solution is formed. The solution is then extruded into a coagulating bath at pH 2–5 and containing an inorganic salt, thereby obtaining threads of protein. The threads are stretched to 1.5–5 times their original length.

TEXTURIZING PROCESS FOR SINGLE CELL PROTEIN. J.A. Ridgway (Standard Oil Co.). *U.S. 3,843,807.* A process for developing texture in microbial cells comprises the steps of: (a) preparing an aqueous paste of microbial cell material containing 15–40% water and at least 2% ruptured cells; (b) extruding the paste at 70–350F; heat-treating the extrudate at 180–350F for 0.1–30 minutes; and (d) drying the heat treated extrudate to produce a crisp, crunchy, chewy product having a bland or pleasing flavor. The product also resists dispersion in water.

TEXTURIZING OF PROTEIN. A.R. Touba (General Mills, Inc.). *U.S. 3,843,816.* A method for texturizing protein comprises compressing and heating untextured protein material between a pair of parallel opposing heated surfaces for up to 1 minute. The starting material has at least 50% protein, dry weight basis, and 15–35% water. The temperature of the surfaces and the pressure are sufficient to cause the protein to texturize and also cause a portion of the moisture to superheat. The force of the compression entraps a portion of the moisture, and when the pressure is released, the moisture vaporizes and expands the textured protein.

TEXTURIZING PROCESS FOR SINGLE CELL PROTEIN. S.R. Tannenbaum (Standard Oil Co.). *U.S. 3,845,222.* A process for imparting texture to microbial cells comprises the steps of (a) heating an aqueous paste containing 10–50% water of microbial cell material to 150–400F for 10–300 seconds; (b) simultaneously applying a shearing force to the cell paste, the shearing force corresponding to a shear rate of 10–60 rpm and a torque of 200–2000 metergrams; (c) extruding the heated and sheared cell paste through a die to provide a shaped extrudate; and (d) exposing shaped extrudate to an oxygen containing gas stream to produce a product which is chewy, crunchy, crispy and resists dispersion in water.

PROCESS FOR EXTRUDING OILSEED PROTEIN MATERIAL. W.T. Atkinson (Archer Daniels Midland Co.). *U.S. 3,845,228.* An expanded food product is prepared by extrusion of a mixture of solvent extracted oilseed proteinaceous material, containing 40–80% protein, with 20–60% water. The improvement consists of adding 0.1–2% of lecithin, based on the dry weight of the mixture.

PREPARATION OF SOYBEAN PRODUCTS FREE OF ANTITRYPSIN. M. Rambaud (Societe Industrielle des Oleagineux). *U.S. 3,845,229.* The method comprises crushing unextracted soybeans containing urease, the moisture content of the crushed beans being at most 12%, spraying the crushed beans with an aqueous solution of urea until 0.1–0.4% urea has been added, flaking the beans, and then cooking the flakes at 100–110C in steam, with the moisture content of the flakes being 18–20%, until destruction of the antitrypsin factor is obtained.

PREPARATION OF A BASE FOR PROTEIN BEVERAGES. W.L. Hempenius, J. Valenti and R.E. Moser (Quaker Oats Co.). *U.S. 3,846,560.* The process for making an acidic aqueous solution of polypeptides for use as a base for an acidic protein beverage comprises (a) heating an aqueous slurry of a defatted protein, as from soya, cotton, or corn seeds at 150–375F for a time sufficient to increase the yield of the end product but insufficient to adversely affect the flavor of it; (b) subjecting the slurry to enzymatic hydrolysis to produce polypeptides; (c) adjusting the pH to 2.5–6.0; and (d) removing the precipitated material from the slurry to leave a clear, acidic solution of polypeptides suitable for use as a base for preparing the beverage.

EDIBLE MIXTURE. D.J. Malin. *U.S. 3,846,564.* An edible mixture includes textured protein mixed with an amount of comminuted animal glandular tissue sufficient to mask the flavor of the protein.

RECOVERY OF PROTEIN. C.T. Egger and R.T. Olson (Grain Processing Corp.). *U.S. 3,849,391.* A process for recovering protein from a vegetable or microbial source comprises forming an aqueous slurry of the starting material at a pH other than the isoelectric point of the protein, rapidly heating the slurry under pressure to 250–350F for not more than 6 minutes, flash cooling the heated slurry under reduced pressure to separate the vapor therefrom, and recovering the cooled homogeneous slurry containing the protein in solution.

PREPARATION OF EDIBLE PROTEIN FIBERS. T. Sakita (Nisshin Oil Mills). *U.S. 3,951,079.* The process comprises (a) dissolving a protein curd separated from defatted soybeans with aqueous alkali to form a spinning dope; (b) extruding the dope through fine holes into a coagulating bath to form protein fibers; and (c) subsequently washing the fibers. The coagulating bath consists of an aqueous solution of 3–5% of organic acids, 3–7% of neutral inorganic salts, and 0.5–2% of hydrogen peroxide. The bath has a pH of at least 2.5.

• Drying Oils and Paints

COATINGS UPDATE. ALKYD RESIN TECHNOLOGY. W. Brushwell. *Paintindia* 24(7), 17–20 (1974). Review of recent publications.

CONTRIBUTION TO THE STUDY OF POLYENIC COMPOUNDS AUTOXIDATION AND DRYING PHENOMENON. IV. R. Poisson. *Double Liaison* 20 No 219, 421–7 (1973). An analytical study of the products from the autoxidation of crotonal and citral indicates that hydroxylated polyesters are formed during the autoxipolymerisation of polyenic compounds contained in drying oils. Main observations previously shown are collected and conclusions about the whole work drawn. (World Surface Coatings Abs. No. 384)

NEW COATINGS FROM CHEMICALLY MODIFIED LINSEED OIL AND HYDROXYL-BEARING BUTADIENE AND ACRYLIC RESINS. T.H. Khoe and L.E. Gast (USDA, N. Reg. Res. Lab., Peoria, Ill. 61604). *J. Paint Technol.* 46(598), 53-5 (1974). New vehicles for coatings were prepared from polybutadiene, butadiene-styrene, butadiene-acrylonitrile and acrylic resins with pendent hydroxyl groups on the polymer backbones, and either hydroformulated or maleated linseed oils. Most of these resins showed better film properties than linseed alkyd resins. The films air-dried faster and had better hardness; xylene resistance and acid resistance were excellent. The resins from maleated linseed oil may have potential as water-dispersible coatings.

APPLICATIONS OF DICARBOXYLIC ACIDS OBTAINED BY METATHESIZING FATTY ACID ESTERS. J.M. Van Thiel and C. Boelhouwer (Chem. Technol. Lab., U. of Amsterdam). *Farbe u. Lack* 80(11), 1028–9 (1974). A polyester was synthesized from 1,2-propanediol and 1,18-dimethyl-9-octadecene dioate, the latter obtained by metathesizing methyl oleate. The polyester did not possess air drying properties; it could, however, be cured using sulfur or dibenzoyl peroxide. The copolymerization with styrene was not possible. The acid amide, 9-octadecene diamide-1,18, was prepared from 9-octadecene diacid-1,18. The diamide reacted with an epoxide resin. With paraformaldehyde the poly(methylene amide) was synthesized from the diamide.

TECHNOLOGY OF ALKYD RESINS. W. Brushwell. *Farbe u. Lack* 80(11), 1053–6 (1974). Alkyd resins have been a varnish raw material for decades which offers possibilities for numerous combinations. The present state of the development is reported quoting 37 references and numerous patents.

THIXOTROPIC COATING COMPOSITION. D.R. Brothers. *U.S. 3,852,228.* A sprayable thixotropic coating composition comprises 3–15% of butyl rubber, 3–15% polybutene having a molecular weight of at least 50,000, 2–30% drying oil, 13–25% aromatic solvent, 4–23% pigment, and up to 2% dryer.

• Detergents

DYNAMICAL ASPECTS OF SOLUBILIZATION DISCLOSED BY ANALYZING ESR SPECTRA OF SOLUBILIZED RADICALS. II: EFFECT OF ALKYL-CHAIN LENGTH OF SURFACTANT. T. Nakagawa and H. Jizomoto (Res. Lab., Shionogi & Co. Ltd., Osaka, Japan). *Colloid and Polymer Sci.* 252(6), 482–5 (1974). A stable and slightly water-soluble radical, t-butyl-(1,1-dimethylpentyl)-nitroxide, was solubilized up to saturation into series of aqueous surfactant solutions of various concentrations. The surfactants examined were sodium octyl, decyl, dodecyl and tetradecyl sulfates. Comparisons of ESR spectra obtained experimentally with computer-generated theoretical spectra revealed that the average stay-time of a radical molecule in a micelle is insensitive to the change of surfactant concentration, and that an increase in the alkyl-chain length of the

surfactant brings about a slight but steady increase of the stay-time.

STEPWISE ASSOCIATION PROPERTIES OF SOME SURFACTANT AQUEOUS SOLUTIONS. Y. Zimmels and I.J. Lin (Dept. of Mineral Eng., Technion, Israel Inst. Technol., Haifa, Israel). *Colloid & Polymer Sci.* 252(7/8), 594–612 (1974). The subject of pre- and postmicellar association is reviewed. Conductivity and surface tension results (in conjunction with literature data) show that each homolog of the sodium fatty-acid soap series has at least three definite association concentrations, collectively termed "multi-CMC." Hydrolysis of the soaps was shown to be compatible with association and to vary with it. The variation of the equivalent conductivity with concentration is discussed theoretically, the former shown to increase with premicellar association. A modified version of the Onsager equation, based on stepwise association model, is presented. Theory and experiment were in good agreement. Stepwise bulk association produces discontinuities in the solution-vapor interfacial tension vs. concentration curve. The discontinuity points coincide with the defined CMCs. The enthalpy of micellization is of the same order for all CMC points, with the peak corresponding to the conventional CMC. At least three sets of log CMC vs. chain length relations are defined for the C_8–C_{18} homologs of the sodium fatty-acid soap series. The above results, in conjunction with reinterpreted literature data, suggest that association is discontinuous, and that there is a central CMC with secondary association concentrations distributed on both sides.

COATINGS UPDATE. ALKYD RESIN TECHNOLOGY. W. Brushwell. *Paintindia* 24(7), 17–20 (1974). Review of recent publications.

CONTRIBUTION TO THE STUDY OF POLYENIC COMPOUNDS AUTOXIDATION AND DRYING PHENOMENON. IV. R. Poisson. *Double Liaison* 20 No 219, 421–7 (1973). An analytical study of the products from the autoxidation of crotonal and citral indicates that hydroxylated polyesters are formed during the autoxipolymerisation of polyenic compounds contained in drying oils. Main observations previously shown are collected and conclusions about the whole work drawn. (World Surface Coatings Abs. No. 384)

EFFECT OF SURFACTANT CONCENTRATION ON THE CRITICAL THICKNESSES OF LIQUID FILMS. E. Manev, A. Scheludko and D. Exerowa (Dept. Phys. Chem., Sofia U. and Inst. Phys. Chem. Bulgarian Acad. Sciences, Sofia, Bulgaria). *Colloid & Polymer Sci.* 252(7/8), 586–93 (1974). Is shown that an increase in surfactant concentration at first causes the critical thicknesses of rupture on microscopic films to decrease but gradually a nearly constant value is reached. With films of aqueous solutions of fatty acids (valeric, caproic, caprylic and capric acids), this dependence correlates well with the effect of the surfactant concentration on the damping of capillary waves. With surfactants of the detergent type (OPE-7, OPE-20) in addition to the change of the critical thickness, the transition from rupture to formation of black spots is described, as the surfactant concentration exceeds C_{b1}. The remarkable fact in the latter case is the independence of the critical thickness of the final state, be it rupture or formation of first or second black films. On the basis of the experimental data the assumption is put forward that the critical thickness of rupture or the critical thickness of formation of black spots is substantially affected by macroscopic non-uniformities in the film thickness. Thus the conclusion is reached that the critical thickness of an ideally plane parallel film which is the object of the theory, must be obtained by extrapolation of the measured value toward extremely small radii.

DETERMINATION OF SIZE AND SHAPE OF MICELLES BY GEL FILTRATION CHROMATOGRAPHY. K.S. Birdi (Fysisk-Kemisk Inst., Tech. U. of Denmark, Lyngby, Denmark). *Colloid & Polymer Sci.* 252(7/8), 551–4 (1974). The size and shape of a series of nonionic surfactants (polyoxyethylene nonylphenols with number of ethylene oxide groups 10, 13 and 18) were determined by gel filtration chromatography. The Stokes radius (R) was determined from the retention time. The hydration of micelles was estimated from the frictional ratios. The number of hydrating water molecules per oxyethylene unit was found to be 3–5, which agrees with the results reported by other investigators using other methods. It is thus shown that gel filtration chromatography can be applied to micellar systems.

THE SURFACE ACTIVITY OF THE LOWER HOMOLOGUES OF N-ALKYLAMMONIUM CHLORIDES IN AQUEOUS SOLUTIONS. EFFECT OF HYDROPHOBIC HYDRATION. K. Tamaki (Dept. Chem., Yokohama City U., Kanazawa-ku, Yokohoma, Japan). *Colloid & Polymer Sci.* 252(7/8), 547–50 (1974). The surface tensions of aqueous solutions of n-alkylammonium chlorides (methyl to hexyl) have been measured at 25C. With methylammonium chloride the surface tension increased with concentration, whereas with ethylammonium chloride and higher homogues, the surface tension decreased with concentration. The results suggest that this transition is attributed to the effect of hydrophobic hydration. For propylammonium chloride and higher homologues, the relation between the surface tension lowering $\Delta\gamma$ and the concentration C in the range of 0–10 dyne/cm, can be expressed by the equation; $\Delta\gamma = A\sqrt{C} + BC$, where A and B are constants. The surface tension coefficient B has been found to increase approximately twofold for each additional CH_2 group of the alkyl chain.

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