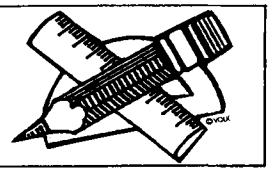
Abstracts



• Biochemistry and Nutrition

LINOLEIC ACID DESATURATION ACTIVITY OF LIVER MICROSOMES OF ESSENTIAL FATTY ACID DEFICIENT AND SUFFICIENT RATS. R.O. Peluffo, A.M. Nervi and R.R. Brenner (Catedra de Bioquimica, Inst. de Fisiologia, Facultad de Ciencias Medicas, Univ. Nacional de La Plata, La Plata, Argentina) *Biochim. Biophys. Acta* 441, 25–31 (1976). Studies were carried out to relate the changes of the fatty acid and lipid composition of rat microsomes with the modification of the activity of the linoleic acid desaturation evoked by an essential fatty acid deficient diet. Two steps were shown in the progression of the essential fatty acid deficiency. The increase of the V of linoleic acid desaturation is considered to be evoked by an increased level of active Δ^6 desaturase. The increased activity of the Δ^6 desaturase in this second period is a secondary and important response of the cell to maintain the unsaturated:saturated acid ratio and fluidity of the membrane.

CONCENTRATION CHANGES OF BILE ACIDS IN SEQUENTIAL SEGMENTS OF PIGEON INTESTINE AND THEIR RELATION TO BILE ACID ABSORPTION. D. Spittell, L.K. Vongroven and M.T. Ravi Subbiah (Mayo Clinic and Mayo Found., Rochester, Minn. 55901) *Biochim. Biophys. Acta* 441, 32–7 (1976). Changes in the concentration of bile acids in sequential segments of pigeon intestine were measured. It was found that the jejunum has the highest concentration of bile acids. When the ratio of bile acids to β-sitosterol (a non-absorbable dietary marker) was examined in contents of various segments, it was found that the ratio showed a marked decrease (86.6%) from the upper to lower jejunum while the subsequent changes in other segments were not as striking. This indicates that the jejunum is the major site of bile acid absorption in the pigeon, unlike in mammals, where the ileum has been shown to be the major site of bile acid absorption. This notion was confirmed by studies of the effect of ileal bypass in these pigeons. This surgical procedure did not significantly change the fecal excretion of bile acids or neutral sterols indicating that the jejunum is capable of maintaining a normal enterohepatic circulation of bile acids in the absence of the ileum in the pigeons.

ISOLATION AND CHARACTERIZATION OF SUBCELLULAR MEMBRANES WITH ALTERED PHOSPHOLIPID COMPOSITION FROM CULTURED FIBROBLASTS. F. Schroeder, J.F. Perlmutter, M. Glaser and P.R. Vagelos (Dept. of Biol. Chem., Div. of Biol. and Biomed. Sci., Washington Univ., St. Louis, Mo. 63110) *J. Biol. Chem.* 251, 5015–26 (1976). Plasma membranes, microsomes, and mitochondria were isolated from mouse fibroblast (LM) suspension cells by modification of several established procedures. Choline analogues such as *N,N'*-dimethylethanolamine, *N*-monomethylethanolamine, or ethanolamine were incorporated *in vivo* into phospholipids of all three cell fractions studied, but to varying degrees depending on the type of analogue used. The *in vivo* incorporation of these bases into membrane phospholipids produced no significant effect on the activities of seven membrane-bound enzymes: (Na^+, K^+)-ATPase, 5'-nucleotidase (plasma membranes); TPNH-cytochrome *c* reductase, glucose-6-phosphatase, inosine diphosphatase (microsomes); and succinate cytochrome *c* reductase (mitochondria). The ratio of zwitterionic phospholipids to acidic phospholipids remained relatively constant in all isolated membrane fractions regardless of analogue supplementation. Neither increase in the degree of unsaturation nor shortening of fatty acid chain length was noted in response to analogue supplementation.

LIPID PEROXIDATION AND ALTERATION OF MEMBRANE LIPIDS IN ISOLATED HEPATOCYTES EXPOSED TO CARBON TETRACHLORIDE. C.C. Weddle, K.R. Hornbrook and P.B. McCay (Biomembrane Res. Lab., Oklahoma Med. Res. Found., Oklahoma City, Okla. 73104) *J. Biol. Chem.* 251, 4973–8 (1976). Lipid peroxidation, determined by malondialdehyde formation, occurs at a low, but detectable, rate in parenchymal cells isolated from livers of fasted rats. Pretreatment of rats with phenobarbital increased malondialdehyde formation about 2-fold, probably because of the increased amount of endoplasmic reticulum. Lipid peroxidation was increased in the cells by the addition of either NADPH or CCl_4, and the effect of the two agents together was more than additive. Phenobarbital pretreatment increased peroxidation due to exposure of the cells to CCl_4 but not that associated with NADPH addition. The amount of CCl_4 producing a 50% increase in malondialdehyde formation was about 3-fold less for cells from phenobarbital-treated rats than for those from control rats. Exposure of liver cells to CCl_4, however, caused major losses in all fatty acids and of protein from the microsomal fraction, but only polyunsaturated fatty acids were decreased in the cellular debris fraction. Incubation with NADPH and CCl_4 together enhanced malondialdehyde formation, but caused no further decrease in fatty acid content in these two fractions. Mitochondrial fatty acids were not decreased by any treatments described.

TRIACYLGLYCEROL CONTENTS AND IN VIVO LIPOGENESIS OF OB/OB, DB/DB AND A^{vy}/A MICE. T.T. Yen, J.A. Allan, P.-L. Yu, M.A. Acton and D.V. Pearson (The Lilly Res. Lab., Indianapolis, Ind. 46206) *Biochim. Biophys. Acta* 441, 213–20 (1976). The triacylglycerol content and the in vivo lipogenesis rates of the liver and the carcass of 12 groups of mice were studied. They were mice of three strains and affected by mutations at three loci: C57BL/6J-ob/ob and normal mice; C57BL/KsJ-db/db and normal mice; and VY/WfL-A^{vy}/a and normal mice. Each type of mice was studied at two body weight levels, before and after the mutants became grossly obese. It was found that the C57BL/6J-ob/ob and the C57BL/KsJ-db/db mice had the characteristics of juvenile type obesity. They had higher lipogenesis rates and accumulated more triacylglycerol when they were young. They gained weight rapidly mainly due to the accumulation of more triacylglycerol as they matured. Their total triacylglycerol content could each 50% of their body weight. At maturity, their lipogenesis rates had decreased to normal. In contrast, the VY/WfL-A^{vy}/a mice had the characteristics of maturity-onset type obesity.

α-HYDROXYLATION OF LIGNOCERIC AND NERVONIC ACIDS IN THE BRAIN. EFFECTS OF ALTERED THYROID FUNCTION ON POSTNATAL DEVELOPMENT OF THE HYDROXYLASE ACTIVITY. S. Murad, G.D. Strycharz and Y. Kishimoto (Eunice Kennedy Shriver Ctr. for Mental Retardation at the Walter E. Fernald State School, Waltham, Mass. 02154) *J. Biol. Chem.* 251, 5237–41 (1976). Rat brain postnuclear preparations catalyzed the α-hydroxylation of nervonic acid with an apparent K_m of 3 μM. Evidence has been presented which suggests that nervonic acid in the brain is hydroxylated by the same enzyme system which hydroxylates lignoceric acid. The hydroxylase activity in brains of normal (euthyroid) rats increased rapidly from a low in the period immediately following birth to a maximum at the 23rd day and then declined to a low level characteristic of the mature brain. Neonatal hypothyroidism retarded the development of the activity and shifted its peak to the 39th day after birth. Conversely, neonatal hyperthyroidism accelerated the entire developmental pattern and shifted the peak to the 16th day after birth. The hydroxylase activity in mouse brain was also increased by thyroid hormone administration from the 13th through the 18th day after birth. Unlike normal mice, the low activity in jimpy mice was not affected by this treatment. It is concluded that thyroid hormones play an important role in the control of brain fatty acid α-hydroxylation. The stimulation of α-hydroxy fatty acid synthesis in response to hyperthyroidism during the early postnatal period may be one of the major effects of thyroid hormones in accelerating myelination of the central nervous system.

CHOLESTEROL SYNTHESIS IN GERMFREE AND CONVENTIONAL RATS. M. Ukai, A. Tomura and M. Ito (Inst. of Germfree Life

Research, Nagoya Univ. School of Med., Nagoya 466, Japan) *J. Nutr.* 106, 1175–83 (1976). The synthesis of cholesterol from labeled acetate and mevalonate by the liver and intestinal tract was investigated in germfree and conventional rats. When a low cholesterol diet was fed, the rates of in vitro synthesis from acetate by the liver, ileum and cecum of germfree rats were 13%, 11% and 25% of those of conventional rats, respectively. Cholesterol feeding markedly inhibited hepatic cholesterol synthesis from acetate in both germfree and conventional rats. Such inhibitions were released by additional cholestyramine feeding. Data indicate that endogenous cholesterol synthesis in the germfree rat may not be responsible for the high cholesterol level in plasma or liver and that the liver cholesterol level may play a major role in the regulation of hepatic cholesterogenesis in the germfree rat by a mechanism similar to that in the conventional rat.

EFFECT OF DILAURYL SUCCINATE ON REPRODUCTION OF THE COCK AND HEN AND PREVENTIVE EFFECT OF VITAMIN E. M. Yoshida and H. Hoshii (Natl. Inst. of Animal Industry, Chiba 280, Japan) *J. Nutr.* 106, 1184–91 (1976). Fertility of White Leghorn cocks fed a diet containing 12% of dilauryl succinate (LS) for 16 weeks was significantly lower than that of cocks fed the control diet. Little difference in fertility and hatchability of fertile eggs was observed between White Leghorn hens fed the control or LS diets, but the percentage of chicks at hatch, which showed signs of encephalomalacia with lesions on the cerebellum, and the percentage of the hens and offspring having fragile erythrocytes were much higher when LS was fed. Offspring of the cocks and hens fed LS died from encephalomalacia earlier than those fed the control diet. These observations with LS feeding were all prevented by oral administration of *dl*-α-tocopheryl acetate. The responses of the cocks and hens to LS were compared with those to linoleic acid in the literature. The direct effect of LS, or more likely of monolauryl succinate, independent of peroxides from unsaturated fatty acids was discussed.

IMMUNOLOGICAL AND COMPOSITIONAL PATTERNS OF LIPOPROTEINS IN CHICKEN (GALLUS DOMESTICUS) PLASMA. J. Y-L. Lu, L.D. Campbell and R.R. Marquardt (Dept. of Animal Sci., Univ. of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2) *Poult. Sci.* 55, 1626–31 (1976). Immunological and compositional patterns of plasma lipoproteins in the laying and non-laying hen (*Gallus domesticus*) and rooster plasmas were compared. Hen plasma contained three immunologically distinct lipoproteins: very low density (VLDL) and low density (LDL); high density (HDL); and lipovitellin (LV). Rooster plasma contained VLDL and LDL; and HDL. Laying hen plasma in comparison to the other two groups of birds contained a high content of VLDL and LV. The plasma lipoprotein pattern of nonlaying hens and roosters were similar except for the relatively high content of LDL and the presence of low levels of LV in nonlaying hen plasma. In general, triglyceride, phospholipid and cholesterol contents were similar for each lipoprotein class in laying hen, nonlaying hen and rooster plasma.

RELATIONSHIPS BETWEEN CIGARETTE SMOKING, ORAL CONTRACEPTIVES, AND PLASMA VITAMINS A, E, C, AND PLASMA TRIGLYCERIDES AND CHOLESTEROL. D.L. Yeung (Dept. of Family Studies, Univ. of Guelph, Guelph, Ontario) *Am. J. Clin. Nutr.* 29, 1216–21 (1976). Plasma vitamins A, E, and C, plasma triglycerides and cholesterol, and leukocyte vitamin C were examined in young healthy adult females who were cigarette and/or oral contraceptive users. It was found that cigarette smoking slightly increased the levels of vitamin A, triglycerides, and cholesterol while oral contraceptives significantly increased these plasma lipids. The effects of cigarette smoking and oral contraceptives on these substances were additive. Neither cigarette smoking nor oral contraceptives had any significant effect on plasma vitamins E and C. Oral contraceptives slightly decreased the level of leukocyte vitamin C in the cigarette smokers. Cigarette smoking did not impart an acute effect on these parameters.

THE LIPID INTERMEDIATES ARISING DURING GLYCOPROTEIN BIOSYNTHESIS IN LIVER MICROSOMES. P. Zatta, D. Zakim and D.A. Vessey (Mole. Biol. Div., Veterans Admin. Hosp., San Francisco, Calif. 94121) *Biochim. Biophys. Acta* 441, 103–14 (1976). Incubation of liver microsomes with GDP[^{14}C]mannose leads to the formation of lipid-linked derivatives of [^{14}C] mannose, a dolichol phosphate monosaccharide and dolichol pyrophosphate oligosaccharides. Standard procedures for separating these two types of compounds from each other were found to be deficient in that fractions thought to contain only dolichol pyrophosphate oligosaccharides are contaminated with dolichol phosphate mannose. This paper presents a column chromatographic procedure which conveniently separates the products of an 8 min labeling experiment into two components; dolichol phosphate [^{14}C]mannose and a [^{14}C]-mannose containing oligosaccharide which is also lipid bound. After brief periods of labeling with GDP[^{14}C]mannose (5 s) an additional oligosaccharide of 3 to 4 sugar residues can be found in the dolichol pyrophosphate oligosaccharides fraction. Incubation of liver microsomes with UDP[^{14}C]glucose or UDP[^{14}C]galactose produces oligosaccharide components containing 7–8 sugar residues. Labeling of microsomes with UDP[^{14}C]acetylglucosamine gives rise to three different components, including a lipid bound oligosaccharide containing 3–5 sugar residues.

EFFECT OF BIOTIN AND NIACIN ON LIPID CONTENT OF LIVERS IN THE LAYING HEN. L.S. Jensen, C.H. Chang and D.V. Maurice (Dept. of Poultry Sci., Univ. of Georgia, Athens, Ga. 30602) *Poult. Sci.* 55, 1771–3 (1976). Single Comb White Leghorn layers maintained in cages were fed a corn-soy diet with a simplified vitamin premix for a 12-week experimental period to determine the effect on liver lipid accumulation. Lipid content was only 29.6% of the liver dry matter and, therefore, was in the normal range and less than that seen in hens with fatty liver syndrome. Adding niacin (44 mg./kg.) or biotin (110 μg./kg.) either alone or in combination failed to significantly alter liver weight or liver lipid content. Liver content of hens fed another diet with a more complex vitamin premix also was not significantly different from that of hens fed the diet with the simplified vitamin premix. None of the diets significantly affected egg production, egg weight, feed consumption or body weight changes observed over the 12-week period.

PHOSPHOLIPID BIOSYNTHESIS IN SARCOPLASMIC RETICULUM MEMBRANE DURING DEVELOPMENT. M.G. Sarzala and M. Pilarska (Dept. of Biochem. of Nervous System and Muscle, Nencki Inst. of Exper. Biol., 3 Pasteur Str., 02–093 Warsaw, Poland) *Biochim. Biophys. Acta* 441, 81–92 (1976). Biosynthesis of phosphatidic acid, phosphatidylcholine and phosphatidylethanolamine in the sarcoplasmic reticulum membrane has been investigated. The results show that sarcoplasmic reticulum, in addition to its main function, i.e. transport and accumulation of Ca^{2+}, is able to synthetize phospholipids by the same pathways as endoplasmic reticulum of other tissues. The changes of activity of enzymes involved in phospholipid biosynthesis during muscle development have been analysed. The extent of *sn*-glycero-3-phosphate and lysophosphatidylcholine acylation by acyl-CoA or free fatty acids in the presence of ATP and CoA is the same at every stage of development. The main product of phosphatidylethanolamine methylation is phosphatidylmonomethylethanolamine. The specific activity of phosphatidylethanolamine methyltransferase increases from the embryonic period to a maximum between the 4th and the 9th day of postnatal life followed by a decrease to the adult value.

IN VITRO INCORPORATION OF ISOMERIC CIS-OCTADECENOIC ACIDS BY RAT LIVER MITOCHONDRIA. D. Sgoutas, R. Jones, P. Befanis and F. Szlam (Dept. of Pathol. and Lab. Med., Emory Univ. School of Med., Atlanta, Ga. 30322) *Biochim. Biophys. Acta* 441, 14–24 (1976). The metabolic fate of positional isomers of *cis*-octadecenoic acids was compared to that of oleic acid, elaidic acid and stearic acid in rat liver mitochondria. The positional isomers as well as elaidic acid and stearic acid were labelled with ^{3}H and they were incubated in pairs with [1-^{14}C]oleic acid. ^{3}H/^{14}C ratios were determined for the administered mixtures and for the isolated lipid classes. The results suggested that all isomers were readily incorporated into the membraneous structure of mitochondria. Those with the double bond in the middle of the acyl chain resembled oleic acid and they were preferentially incorporated in cholesterol esters, triacylglycerols and in the 2-position of triacylglycerols and of phosphatidylcholines. Those with the double bond away from the middle of the chain were metabolically distinct from oleic acid and behaved like *trans* fatty acids. They were rapidly taken up by mitochondria. They were preferentially incorporated in phospholipids and they occupied the 1-position in phosphatidylcholines and the 1- and 3-positions in triacylglycerols.

SEPARATION OF BRAIN PHOSPHATIDYLSERINES ACCORDING TO DEGREE OF UNSATURATION BY THIN-LAYER CHROMATOGRAPHY. N. Salem, Jr., L.G. Abood and Wayne Hoss (Center for Brain

Research and Department of Biochemistry, University of Rochester Medical Center, Rochester, New York 14642) *Anal. Biochem.* 76, 407–15 (1976). Phosphatidylserines (PS) have been prepared from bovine brain using DEAE column chromatography. A method involving $AgNO_3$-impregnated silica gel H thin-layer chromatography is presented for separating intact PS according to the degree of unsaturation of their fatty acids. A detailed analysis was made of the fatty acid composition of the various fractions using gas chromatography. Some data are presented on the composition of molecular species of PS in bovine brain. The two main molecular species found in cerebral cortex are tentatively assigned the structures of 1-octadecanoyl-2-docosahexaenoyl-sn-glycero-3-phosphorylserine and 1-octadecanoyl-2-octadecenoyl-*sn*-glycero-3-phosphorylserine.

RELEASE OF FATTY ACIDS FROM PHOSPHATIDYLCHOLINE BY LECITHIN-CHOLESTEROL ACYLTRANSFERASE. U. Piran and T. Nishida (The Burnsides Research Laboratory, Department of Food Science, University of Illinois, Urbana, Illinois 61801, U.S.A.) *J. Biochem. (Tokyo)* 80, 887–9 (1976). Partially purified lecithin-cholesterol acyltransferase [EC 2.3.1.43] from human plasma released fatty acids from phosphatidylcholine. Heating, sulfhydryl reagents, Ca^{2+}, EDTA, and sodium deoxycholate had similar effects on the lecithin-cholesterol acyltransferase and fatty acid releasing activities of the preparation. A specific cofactor protein for lecithin-cholesterol acyltransferase, apoA-1, also enhanced both activities. Release of fatty acid was due to enzymatic hydrolysis of the ester linkage at carbon-2 of phosphatidylcholine. It is suggested that the two activities are due to a single enzyme.

CHANGES IN MITOCHONDRIAL PHOSPHORYLATIVE ACTIVITY AND ADENYLATE ENERGY CHARGE OF REGENERATING RABBIT LIVER. Y. Kamiyama, K. Ozawa and I. Honjo (Department of Surgery, Kyoto University, Faculty of Medicine, Sakyo-ku, Kyoto, Kyoto 606) *J. Biochem. (Tokyo)* 80, 875–81 (1976). The changes in the cellular concentrations of ATP, ADP, and AMP and in oxidative phosphorylation of mitochondria were investigated in the remaining liver of partially hepatectomized rabbits. The energy charge (defined as half of the average number of anhydride-bonded phosphate groups per adenosine moiety) of the liver remnant decreased from 0.866 to 0.767 ($p<0.01$) within 24 hr after hepatectomy, and then increased to a substantially higher level then normal within 7 days. On the other hand, the mitochondrial phosphorylative activity increased rapidly to 170% of the control within 12 hr and then returned to normal within 7 days. The mitochondrial phosphorylative activity was inversely correlated with the energy charge of the liver remnant ($r=-0.75$, $p<0.01$). The maximal enhancement of mitochondrial phosphorylative activity was found in mitochondria obtained from the liver remnant with the lowest level of energy charge, suggesting a response of mitochondria *in vivo* involving enhanced biosynthetic ATP-utilizing reactions at an early stage of the regenerating process. The enhancement of phosphorylative activity was accompanied by a rise in the respiratory control ratio, P/O ratio and state 3 respiration. The adenylate kinase [EC 2.7.4.3] activity in the liver remnant increased to more than 160% of the control within 2 days after partial hepatectomy, while the pyruvate kinase [EC 2.7.1.40] activity decreased remarkably. However, the changes in the two enzyme activities did not correlate with those of mitochondrial phosphorylative activity or the energy charge of the liver remnant.

EFFECT OF CHOLESTEROL SULFATE AND SODIUM DODECYL SULFATE ON LECITHIN-CHOLESTEROL ACYLTRANSFERASE IN HUMAN PLASMA. M. Nakagawa and S. Kojima (Faculty of Pharmaceutical Sciences, Kumamoto University, Kumamoto, Kumamoto 862) *J. Biochem. (Tokyo)* 80, 729–33 (1976). The effects of cholesterol sulfate and sodium dodecyl sulfate (SDS) on the esterification of cholesterol in sonicated dispersions of lecithin-cholesterol mixtures by lecithin-cholesterol acyltransferase [EC 2.3.1.43] (LCAT) in human plasma were studied *in vitro*. The acyltransferase activity was inhibited at concentrations of cholesterol sulfate higher than 1×10^{-4} M. This inhibition was not eliminated by the addition of bovine serum albumin or $CaCl_2$. On the contrary, the acyltransferase activity was stimulated at concentrations of SDS ranging from 1×10^{-5} M to 1×10^{-3} M, and maximum stimulation was obtained at 5×10^{-4} M. The maximum stimulation disappeared on the addition of bovine serum albumin (30 mg per ml of incubation medium), 1×10^{-3} M $CaCl_2$ or 1×10^{-4} M cholesterol sulfate. On the other hand, the extent of inhibition of the acyltransferase by cholesterol sulfate was not affected by the amount of lecithin in the dispersion added as a substrate, but the maximum stimulation (5×10^{-4} M SDS) of the acyltransferase was interfered with when a large amount of lecithin was present in the dispersion. In addition, the amount of SDS required for maximum cholesterol esterification was not affected by the amount of lecithin present in the dispersion. These results suggest that the action of cholesterol sulfate on the acyltransferase is different from that of SDS.

SOLUBILIZATION OF DIGLYCERIDE ACYLTRANSFERASE FROM THE MEMBRANE OF MYCOBACTERIUM SMEGMATIS. T. Akao and T. Kusaka (Department of Biochemistry, Kawasaki Medical School, Kurashiki, Okayama 701–01) *J. Biochem. (Tokyo)* 80, 723–8 (1976). Diglyceride acyltransferase [acyl-CoA:1,2-diacylglycerol O-acyltransferase, EC 2.3.1.20] was found to be localized in the membrane of *Mycobacterium smegmatis*, and this enzyme could be solubilized from the membrane by treatment with aqueous acetone. The solubilized enzyme required either 1,2-diolein or 1,3-diolein as an acceptor for palmitoyl-CoA. The apparent K_m value for 1,2- or 1,3-diolein and that for palmitoyl-CoA were about 1.4×10^{-5} M and 6×10^{-4} M, respectively. Several sulfhydryl reagents were inhibitory to the enzyme activity, suggesting the existence of a thiol group(s) in its active site. The solubilized enzyme, which was more labile than the membrane-bound one, could be stabilized to some extent with antichaotropic salts such as phosphate, pyrophosphate, and sulfate.

PHOSPHOLIPID METABOLISM IN EHRLICH ASCITES TUMOR CELLS II. TURNOVER RATE OF ETHER PHOSPHOLIPIDS. K. Waku,* Y. Nakazawa* and W. Mori** (*Department of Chemical Toxicology, Medical Research Institute, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo 113, and **Department of Pathology, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo 113) *J. Biochem. (Tokyo)* 80, 711–6 (1976). Radioactive precursors of phospholipids, *i.e.*, $^{32}P_i$, [1-^{14}C]glycerol, [2-^{3}H]glycerol, and [1-^{14}C]acetate, were individually injected into the peritoneal cavity of mice bearing Ehrlich ascites tumor cells and the rates of incorporation were estimated. Although [2-^{3}H]glycerol was not practically incorporated into ether phospholipids, the other three radioactive precursors were incorporated into diacyl, 1-O-alkenyl-2-acyl- and 1-O-alkyl-2-acyl-GPE (GPC). In the experiments on $^{32}P_i$ or [1-^{14}C]acetate incorporation, 1-O-alkyl compounds in the ethanolamine phosphoglyceride fraction showed high specific activities in comparison with 1-acyl compounds. In the case of [1-^{14}C]glycerol incorporation, a high rate of incorporation into 1-O-alkyl compounds was not found. In the choline phosproglyceride fraction, a high rate of incorporation of the above precursors into 1-O-alkyl compounds was not observed. The specific activities of 1-O-alkenyl compounds were fairly low compared with those of 1-acyl- and 1-O-alkyl compounds throughout the incorporation experiments with [1-^{14}C]glycerol and [1-^{14}C]acetate, but in $^{32}P_i$ incorporation, 1-O-alkenyl compounds showed higher specific activities than 1-acyl compounds in ethanolamine phosphoglyceride, suggesting an exchange reaction of the phosphorylethanolamine moiety. From the above findings, it appears that alkyl ether phospholipids of ethanolamine form may have a significant role in ascites tumor cells, based on their rapid turnover.

ISOLATION AND CHARACTERIZATION OF POLY(GLYCOSYL)-CERAMIDES (MEGALOGLYCOLIPIDS) WITH A, H AND I BLOOD-GROUP ACTIVITIES. J. Koscielak, H. Miller-Podraza, R. Krauze and A. Piasek (Department of Biochemistry, Institute of Hematology, Warsaw) *Eur. J. Biochem.* 71, 9–18 (1976). Very complex glycosphingolipids with A, H and I blood-group activities were isolated from human erythrocyte membranes. The membranes were obtained from erythrocytes of blood group A, A_2 and O respectively. A general formula for the antigens is:

$$(Fuc)_{3\text{-}4}(Gal)_n(GlcNAc)_{n\text{-}2}(Glc)_1(Sphingosine)_1$$

(where Fuc is fucose, Gal is galactose, GlcNAc is *N*-acetylglucosamine and Glc is glucose) with values of n ranging from 10–27. A-active preparations contain additionally 2–3 residues of *N*-acetylgalactosamine. In view of the unusual complexity of these compounds they were designated poly(glycosyl)ceramides (formerly megaloglycolipids). Individual poly(glycosyl)ceramide fractions were isolated from A erythrocytes and were found to differ by about 8 glycosyl residues per molecule forming a series of compounds with 22, 30, 38, 51 and 59 glycosyl residues per mole. Structural studies indicate that the main sequence of poly(glycosyl)ceramides consists of the residues of galactopyranose and 2-deoxy-2-acetamidoglucopyranose substituted at 3 and 4 position respectively. These

residues are probably alternating. *N*-Acetylglucosamine substituted at 3 position was not found in poly(glycosyl)-ceramides. Branches of poly(glycosyl)ceramides originate from 3 and 6 position of galactopyranosyl residues. The number of branches is proportional to the degree of molecular complexity. In poly(glycosyl)ceramides isolated from A and A_2 erythrocytes the branches are terminated with the following structures GalNAc α 1 → 3 [Fuc α 1 → 2] Gal; Fuc α 1 → 2 Gal and Gal (presumably Gal β 1 → 4 GlcNAc). In poly-(glycosyl)ceramides from A cells the total number of A and H-active structures per average molecule of 30–35 glycosyl residues amounts to 2.1 and 1.2 respectively while the number of terminal galactose structures is 1.8. For poly(glycosyl)-ceramides from A_2 erythrocytes the corresponding figures are 0.75, 3.5, and 2.1 respectively. Poly(glycosyl)ceramides from O cells comprise about 3.8 H-active structures and 1.8 terminal galactopyranosyl residues. In poly(glycosyl)ceramides with high "*n*" values the number of terminal galactose structures is increased. These fractions display high blood-group I activity. However, the removal of terminal galactose with β-galactosidase affects I-activity only slightly.

STUDIES ON THE INFLUENCE OF FATTY ACIDS ON PYRUVATE DEHYDROGENASE INTERCONVERSION IN RAT-LIVER MITOCHONDRIA. E.I. Walajtys-Rode (Department of Cellular Biochemistry, Nencki Institute of Experimental Biology, Warsaw) *Eur. J. Biochem.* **71**, 229–37 (1976). The effect of fatty acids on the interconversion of pyruvate dehydrogenase between its active (nonphosphorylated) and inactive (phosphorylated) forms was measured in rat liver mitochondria respiring in state 3 with pyruvate plus malate and 2-oxoglutarate plus malate and during state 4 to state 3 transition in the presence of different substrates. The content of intramitochondrial adenine nucleotides was determined in the parallel experiments. Decrease of the intramitochondrial ATP/ADP ratio with propionate and its increase with palmitoyl-L-carnitine in state 3 is accompanied by a shift of the steady-state of the pyruvate dehydrogenase system towards the active or the inactive form, respectively. Transition from the high energy state (state 4) to the active respiration (state 3) in mitochondria oxidizing 2-oxoglutarate or palmitoyl-L-carnitine causes an increase of the amount of the active form of pyruvate dehydrogenase due to the decrease of ATP/ADP ratio in the matrix. No change in ATP/ADP ratio can be observed in the presence of octanoate in mitochondria oxidizing pyruvate or 2-oxoglutarate in state 3 or during state 4 to state 3 transition. Simultaneously, no significant change in phosphorylation state of pyruvate dehydrogenase occurs and a low amount of the enzyme in the active form is present with octanoate or octanoate plus 2-oxoglutarate. Pyruvate abolishes this effect of octanoate and shifts the steady-state of pyruvate dehydrogenase system towards the active form. These results indicate that fatty acids influence the interconversion of pyruvate dehydrogenase mainly by changing intramitochondrial ATP/ADP ratio. However, the comparison of the steady-state level of the pyruvate dehydrogenase system in the presence of different substrates in various metabolic conditions provides some evidence that accumulation of acetyl-CoA and high level of NADH may promote the phosphorylation of pyruvate dehydrogenase. Pyruvate exerts its protective effect against phosphorylation of pyruvate dehydrogenase in the presence of fatty acids of short, medium or long chain in a manner which depends on its concentration. It is suggested that in isolated mitochondria pyruvate counteracts the effect of acetyl-CoA and NADH on pyruvate dehydrogenase kinase.

SUPPLEMENTARY FOODS BASED ON OILSEEDS FOR INFANTS & CHILDREN. M. Swaminathan (Central Food Technol. Res. Inst., Mysore 13, India) *J. Sci. Ind. Res.* **34**, 329–35 (1975). Preparation, chemistry, composition and evaluation of nutritive value of supplementary foods based on oilseeds for infants and children are reviewed. The author concluded that these foods are highly effective in improving the nutritive value of poor diets in the developing countries. 35 references.

VARIATION IN TRYPSIN INHIBITOR ACTIVITY IN SOYBEAN (GLYCINE MAX). A.K. Gupta and A.D. Deodhar *Indian J. Nutr. Diet.* **12**, 81 (1975). Wide variations in protein content (36.41–43.13%) and trypsin inhibitor activity (99.44–272.46 per ml of extract) were observed among 16 soybean varieties each of grain and vegetable types. A few vegetable type varieties containing protein content about 40% showed significantly low trypsin inhibitor activity.

ONION AND GARLIC IN EXPERIMENTAL CHOLESTEROL INDUCED ATHEROSCLEROSIS. R.C. Jain (Dept. of Pathology, Faculty of Medicine, Univ. of Benghazi, Benghazi-Libya) *Indian J. Med. Res.* **64**, 1509–15 (1976). Atherosclerosis was produced experimentally in rabbits by prolonged cholesterol feeding and the effects of onion and garlic were studied on serum and tissue cholesterol. The rabbits were divided into four groups of 10 each. Groups I and II were kept on basal diet and basal diet with cholesterol respectively. Groups III and IV were kept on basal diet to which was added cholesterol with onion and cholesterol with garlic respectively. Serial investigations were performed in all groups for the estimation of serum total, free, ester cholesterol and phospholipids, every four weeks. The severity of aortic atherosclerosis was graded visually and by chemical estimation of cholesterol in the aortic wall. It was found that the administration of onion to rabbits being fed cholesterol did not affect the total, free, ester cholesterol and phospholipids or the development of atherosclerosis in the aorta. However, supplementation of garlic to rabbits fed cholesterol revealed significant lower levels of total, free, ester cholesterol and phospholipids. The cholesterol content of aorta and liver was raised in cholesterol-fed rabbits while onion supplemented rabbits did not show any alterations. However, garlic fed rabbits revealed significantly low cholesterol levels. Visual grading suggested a lower degree of atherosclerosis in the garlic fed rabbits. Although the mechanism of the hypolipaemic activity of garlic is not clear, it is suggested that increased excretion of cholesterol end products in faeces and diminished endogenous synthesis of cholesterol may be a possibility.

EFFECT OF RAGI FEEDING ON SERUM CHOLESTEROL LEVEL. M.S. Pore and N.G. Magar (Dept. of Biochemistry, Inst. of Science, Bombay, India) *Indian J. Med. Res.* **64**, 909–14 (1976). Male albino rats were fed on total ragi flour, where protein was exclusively derived from ragi, for eight weeks. Serum cholesterol and blood haemoglobin levels were found to be decreased in ragi diet group when compared with casein diet group.

CIRRHOSIS OF THE LIVER IN RABBITS INDUCED BY A HIGH CHOLESTEROL DIET—AN EXPERIMENTAL MODEL. P.P. Gupta (Dept. of Veterinary Pathology, Punjab Agric. Univ., Ludhiana, Punjab, India). *Indian J. Med. Res.* **64**, 1516–26 (1976). Hepatic changes induced in rabbits fed on different regiments of high cholesterol diet for a prolonged period of time were studied. The sequence of changes on continuous cholesterol feeding was fatty change, initially centrilobular, later becoming diffuse, with simultaneous accumulation of cholesterol in the parenchymal cells, diffuse portal fibrosis, and eventually 'portal' cirrhosis of the monolobular type. Necrosis of liver cells seemed to have contributed but little to the pathogenesis of cirrhosis. The fibrosis was mainly related to the mesenchymal cell activity in the portal canals. There was no significant regression of changes when cholesterol was withdrawn from the diet after 8 weeks of feeding. One animal out of 4 had developed cirrhosis 20 weeks after withdrawal. The precise mechanism of fibrosis and cirrhosis is not understood but excessive accumulation of cholesterol in the liver probably acts as a cirrhogenic factor. The morphological sequences of the cholesterol-induced portal cirrhosis provides a useful experimental model for a study of the mechanism of cirrhosis, using an endogenous agent, without mediation of nutritional deficiencies.

NUTRITIONAL AND METABOLIC STUDIES OF METHYL ESTERS OF DIMERIC FATTY ACIDS IN THE RAT. A. Hsieh and E.G. Perkins *Rev. Fr. Corps Gras* **24**(1), 19–25 (1977). Methyl esters of dimeric fatty acids were prepared by fractionating a mixture of conjugated linolenic and oleic acids that was heated for 24 hours at 300° C in the absence of air. Rats fed diets containing less than 1% dimers showed no significant difference in the growth rate, feed efficiency, liver/body weight ratio and lipid/liver weight ratio from those fed normal diets. A lymph cannulation study using ^{14}C labeled dimers showed that approximately 0.4% of the dimers fed were absorbed within 12 hours and were transported as free acids in the lymph. Within a 28 hours period, 2 percent of the labeled dimers fed by gastric intubation were oxidized to $^{14}CO_2$ and 1% radioactivity was recovered from the urine. The metabolism of methyl oleate and sodium acetate appeared normal for rats prefed diets containing dimers.

MECHANISM OF LONG CHAIN MONOENOIC FATTY ACIDS ACTING ON THE ENERGY METABOLISM OF HEART. E. Buddecke et al. (Physiol.-Chem. Institut, 4400 Muenster, Germany) Fette, Seifen, Anstrichm. **78**, 196–200 (1976). The oxidation of 1-^{14}C-erucic ($C_{22:1}$) and 1-^{14}C-nervonic ($C_{24:1}$) acids was studied

in comparison to 1-^{14}-C-palmitic and -oleic acids in isolated rat and pig heart mitochondria. After mitochondrial incubation with the albumin-bound fatty acids only small amounts of $^{14}CO_2$ developed from the oxidation of the long chain monoenoic acids as compared to palmitic or oleic acid. The slow down of the oxidation rate was more pronounced in rat than in pig heart mitochondria. The oxidation of palmitic or oleic acid was not found to be inhibited by the C_{20}–C_{24}-monoenoic acids, whereas palmitic or oleic acid inhibited the oxidation of erucic acid competitively. From present findings an idea may be developed of the interference on fatty acid metabolism in heart muscle by erucic and other long chain monoenoic acids.

EFFECT OF HYDROGENATION ON STABILITY AND NUTRITIONAL PROPERTIES OF LOW-ERUCIC RAPESEED OILS. K. Ilsemann et al. (Bundesanstalt f. Fettforschung, 4400 Muenster, Germany) Fette, Seifen, Anstrichm. 78, 181–7 (1976). Low-erucic rapeseed oils, Lesira and Erglu, were converted to more stable edible oils by selective hydrogenation of the linolenic acid moieties while retaining most of the linoleic acid groups. Feeding Lesira oil, hydrogenated Lesira oil, soybean oil and hydrogenated soybean oil to rats did not result in any appreciable differences in growth rates, whereas feeding conventional rapeseed oil caused extensive depression of growth. Among all the groups of animals the group fed conventional rapeseed oil showed the highest weights of heart and liver. The fatty acid patterns of depot and organ lipids did not show any major difference between the groups fed hydrogenated fats and those fed the corresponding unhydrogenated oils. The fatty acid composition of the organ lipids did not reveal deficiency in essential fatty acids. In the groups fed Lesira oil and hydrogenated Lesira oil half of the animals investigated exhibited myocardial lesions of light degree, probably due to the relatively high residual level of long-chain monoenoic fatty acids, whereas in the groups fed soybean oil and hydrogenated soybean oil only one-eighth of the rats examined exhibited such effects. The occurrence and severity of these myocardial lesions are known to be much higher in rats fed conventional rapeseed oils.

NUTRITIONAL STATUS OF LOW ERUCIC-ACID RAPESEED OILS. R. Vles et al. (Unilever Research, Vlaardingen, The Netherlands) Fette, Seifen, Anstrichm. 78, 128–31 (1976). Refined rapeseed oils with a high and a low-erucic-acid content were fed to male Wistar rats for six months. Histomorphometric studies of the changes in predetermined heart sections indicated a significantly higher incidence and a greater severity of myocardial lesions in rats fed high-erucic-acid (regular) rapeseed oil than in rats fed either low-erucic-acid rapeseed oil or sunflowerseed oil. After administration of various amounts of Primor oil, a French rapeseed oil containing 0.3% erucic acid, the observed minor changes were indistinguishable in nature, incidence and severity from those observed in control animals.

RECENT RESULTS OF CLINICAL RESEARCH ON LIPID METABOLISM. G. Schettler (Medizinische Universitaetsklinik, 6900 Heidelberg, Germany) Fette, Seifen, Anstrichm. 78, 1–9 (1976). Physiology and pathophysiology of serum lipids are primarily related to lipoprotein metabolism. Physico-chemically, lipoproteins can be separated into four classes which interconvert in plasma: chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL). VLDL originate from liver and are—as LDL—catabolized in liver. LDL are responsible for the development of severe atherosclerosis. Clinically, we distinguish primary hyperlipoproteinemias, which are genetically determined, and secondary forms of hyperlipoproteinemia. Most of these metabolic disorders are risk factors for atherosclerosis and especially for myocardial infarction. This is demonstrated by pathophysiological and clinical data. In addition, possibilities to influence hyperlipoproteinemia are discussed. There exist a great number of studies of primary and secondary prevention which are discussed with regard to their clinical and epidemiological relevance. In this connexion, the effects of dietary and drug treatment are discussed, and the role of polyunsaturated fatty acids is critically evaluated.

COMPOSITIONS FOR TREATING HYPERCHOLESTEROLEMIA. R.J. Jandacek (Procter & Gamble). *U.S. 4,005,195.* The compositions comprise (a) an edible, nonabsorbable, nondigestible liquid polyol fatty acid polyester having at least four fatty acid ester groups, the polyol being selected from the group consisting of sugars and sugar alcohols containing 4–8 hydroxyl groups and each fatty acid group having 8–22 carbon atoms; and (b) a sufficient quantity of an agent to prevent leakage of the liquid polyester through the anal sphincter.

VITAMINIZED COMPOSITIONS FOR TREATING HYPERCHOLESTEROLEMIA. R.J. Jandacek and F.H. Mattson (Procter & Gamble). *U.S. 4,005,196.* The compositions comprise those which are described in *U.S. 4,005,195* together with sufficient fat soluble vitamins to prevent abnormally low levels in animals ingesting the composition.

ANTITHROMBOGENETIC EFFECTS OF LOW ERUCIC ACID RAPESEED OILS IN RATS. Renaud and L. McGregor (INSERM, U. 63, Lyon-Bron (Rhône). *Rev. Fr. Corps Gras* 23, 393–6 (1976). The protecting effects on thrombosis by a bacterial endotoxin, of three different in erucic acid content rapeseed oils, are studied in male rats fed on a hyperlipemic diet during 10 weeks. These three rapeseed oils reduce, significantly and similarly, the severity of thrombosis caused by a diet consisting of butter alone. The antithrombogen effect of the corn oil is shown more efficient; however, the hypolipemic effect of low erucic acid oils is identical with that of the corn oil.

PASSAGE THROUGH THE INTESTINAL MUCOSA OF "NEW CHEMICAL SPECIES" CONTAINED IN A HEATED OIL. N. Combe et al. (Inst. de Chimie Biologique, Univ. de Provence, Place Victor Hugo, 13331 Marseille Cédex 3). *Rev. Fr. Corps Gras* 23, 399–408 (1976). The ingestion of a heated soybean oil results in oxidized or polymerized compounds occuring in the lymph. That has been shown for rats having a fistula of the thoracic duct. The absorption rates of oxidized acids have been determined by tritiated triolein present in the diet.

LIPID REQUIREMENT OF MEMBRANE-BOUND 3-OXOSTEROID Δ^4–Δ^5-ISOMERASE: STUDIES ON BEEF ADRENOCORTICAL MICROSOMES. P. Geynet, C. DePaillerets and A. Alfsen (Laboratoire des Etats Liés Moléculaires, Equipe de Recherche 64 du Centre National de la Recherche Scientifique, Unité d'Enseignement et de Recherches Biomédicale des Saint-Pères, Université de Paris V) *Eur. J. Biochem.* 71, 607–12 (1976). The role of phospholipid in the beef adrenal microsomal 3-oxosteroid Δ^4–Δ^5-isomerase (EC 5.3.1.1) has been investigated with the use of phospholipase A to alter the microsomal phospholipids. The byproducts of phospholipase A digestion have been removed with a wash solution containing bovine serum albumin. Removal of 80–85% of the phospholipid leads to loss of 80–90% of the 3-oxosteroid Δ^4–Δ^5-isomerase activity. Reconstitution experiments have been performed by introduction of lipid aqueous dispersions in the enzymatic assay. Asolectin, a commercially available preparation of soy phosphatides, is able to stimulate the enzymatic activity but does not restore the 3-oxosteroid Δ^4–Δ^5-isomerase activity in phospholipase-A-treated membranes. In contrast, the introduction of aqueous dispersions of microsomal total lipid mixtures in the enzymatic assay brings about a complete restoration of the 3-oxosteroid Δ^4–Δ^5-isomerase activity in the lipid-depleted membranes. It is concluded that the bovine adrenal microsomal 3-oxosteroid Δ^4–Δ^5-isomerase requires phospholipid(s) to exhibit its full catalytic activity.

CO_2-MEDIATED CONTROL OF FATTY ACID METABOLISM IN ISOLATED HAMSTER BROWN-FAT CELLS DURING NOREPINEPHRINE STIMULATION. B. Petterson (The Wenner-Grens Institute, University of Stockholm) *Eur. J. Biochem.* 72, 235–40 (1977). Addition of norepinephrine to isolated hamster brown-fat cells suspended in Krebs-Ringer phosphate buffer induces a pronounced, but temporary, increase in respiratory rate. If Krebs-Ringer phosphate buffer is bubbled with CO_2 prior to the addition of cells and norepinephrine, the respiratory capacity of the cells is further potentiated and most important, the respiration is maintained at a high rate until the medium becomes depleted of oxygen. This respiratory pattern cannot be obtained in CO_2-bubbled Krebs-Ringer bicarbonate buffer. The results indicate that CO_2 has a regulatory effect on fatty acid metabolism in isolated hamster brown-fat cells.

PURIFICATION AND CONTROL OF BOVINE ADRENAL CORTICAL CHOLESTEROL ESTER HYDROLASE AND EVIDENCE FOR THE ACTIVATION OF THE ENZYME BY A PHOSPHORYLATION. G.J. Beckett and G.S. Boyd (Department of Biochemistry, University of Edinburgh Medical School) *Eur. J. Biochem.* 72, 223–33 (1977). A procedure for the purification of cholesterol ester hydrolase from bovine adrenal cortical 105,000 × g supernatant is described. Preincubation of a crude enzyme extract with [γ-^{32}P]ATP followed by purification resulted in the isolation of a phosphorylated preparation of cholesterol ester

hydrolase. The phosphorylated cholesterol ester hydrolase appeared to be composed of 4 subunits, each having a molecular weight of 41,000 ± 280, only one of which may be phosphorylated. Preincubation of the crude enzyme preparation with [α-^{32}P]ATP followed by purification did not produce a phosphorylated preparation of cholesterol ester hydrolase. Cyclic-AMP-dependent protein kinase, cyclic AMP, ATP and magnesium ions were required for activation of purified cholesterol ester hydrolase *in vitro* and the time course of activation closely paralleled the time course of phosphorylation of the enzyme. The addition of ATP, cyclic AMP and magnesium ions to the bovine adrenal cortical 105,000 × g supernatant produced a 2.5-fold stimulation in cholesterol ester hydrolase activity. This stimulation was abolished if protein kinase inhibitor was added prior to the addition of ATP, cyclic AMP and magnesium ions. The addition of magnesium ions or calcium ions to a crude preparation of cholesterol ester hydrolase was found to inhibit activity; however the same additions made to a purified preparation of cholester ester hydrolase were not inhibitory. The decrease in cholesterol ester hydrolase activity on incubation with magnesium ions was accompanied by a loss of ^{32}P radioactivity from the protein. Preincubation of a crude preparation of cholesterol ester hydrolase with alkaline phosphatase resulted in a deactivation of cholesterol ester hydrolase. It is suggested that bovine adrenal cortex cholesterol ester hydrolase is activated by a phosphorylation catalysed by a cyclic-AMP-dependent protein kinase. Deactivation of cholesterol ester hydrolase is accomplished by dephosphorylation catalysed by a phosphoprotein phosphatase, dependent on magnesium or calcium ions.

LIGNOCERIC ACID BIOSYNTHESIS IN THE DEVELOPING BRAIN. ACTIVITIES OF MITOCHONDRIAL ACETYL-CO-A-DEPENDENT SYNTHESIS AND MICROSOMAL MALONYL-COA CHAIN-ELONGATING SYSTEM IN RELATION TO MYELINATION. COMPARISON BETWEEN NORMAL MOUSE AND DYSMYELINATING MUTANTS (QUAKING AND JIMPY). J. Bourre, M.Y. Paturneau-Jouas, O.L. Daudu, and N.A. Baumann (Laboratoire de Neurochimie, Unité 134 de l'Institut National de la Santé et de la Recherche Médicale, Hôpital de la Salpêtrière, Paris) *Eur. J. Biochem.* 72, 41–7 (1977). Age-related changes in the activities of microsomal and mitochondrial elongating systems have been determined in mouse brain from birth to maturity. In microsomes, the components necessary for behenyl-CoA (docosanoyl-CoA) elongation have been found to be NADPH and malonyl-CoA. In mitochondria, both NADH and NADPH are used and acetyl-CoA is the only donor of two-carbon-atoms unit. The synthesized fatty acids were identified by thin-layer and gas chromatography. The specific activity is higher in microsomes than in mitochondria. In microsomes, the specific activity for malonyl-CoA incorporation reached a maximum at 15–20 days of age; this peak was not obtained in the Quaking and Jimpy mutants. The increase in enzyme activity (specific activity and total activity per brain) paralleled the myelin deposition. The activity of the mitochondrial system increases regularly during development: it is not correlated to myelination and it is not affected in the Quaking mutant. The interplay between microsomal and mitochondrial elongation systems is studied.

BIOCHEMISTRY OF DEVELOPMENT IN INSECTS. TRIACYLGLYCEROL AND PHOSPHOGLYCERIDE BIOSYNTHESIS BY SUBCELLULAR FRACTIONS. A. Megias, A.M. Municio, and M.A. Perez-Albarsanz (Departamento de Bioquimica, Facultad de Ciencias, Universidad Complutense, Madrid) *Eur. J. Biochem.* 72, 9–16, (1977). A different biochemical behaviour has been previously shown during several stages of the development of the insect *Ceratitis capitata;* thus, the acyl transferase activity has a different participation in the pathways of synthesis of triacylglycerols and phosphoglycerides by either larval or pharate adult stages of development. The results reported here are concerned with the behaviour of mitochondria and microsomes from larvae and pharate adults for the synthesis of triacylglycerols and phosphoglycerides. In all preparations of either mitochondrial or microsomal fractions the optimum protein amount, previously determined, has been used. The highest incorporation of [^{14}C]palmitate into triacylglycerols and phosphoglycerides was achieved by the microsomal fraction from the larval stage of development of the insect. However, mitochondrial fractions from pharate adults utilized higher levels of [^{14}C]palmitate than the microsomal preparation. These differences were not correlated with the palmitoyl-CoA synthetase activity. Glycerol 3-phosphate influenced the fatty acid incorporation in a different manner depending on the stage of development of the insect. Increasing concentrations of glycerol 3-phosphate stimulated the synthesis of triacylglycerols by microsomal and mitochondrial preparations. This effect was not exhibited by the subcellular preparations of pharate adults. Double-label experiments using [^{14}C]glycerol 3-phosphate and [^{3}H]palmitate showed qualitative differences in the synthesis of triacylglycerols by mitochondrial preparations from either larvae or pharate adults. Incorporation of the labelled fatty acids to endogenous triglycerides was preferentially shown by the mitochondrial larval preparations. The pathways of synthesis of triacylglycerols and phosphoglycerides by microsomal preparations showed only quantitative differences depending on the stage of development of the insect. Acylation of ^{14}C-labelled lyso-phosphatidylcholine and ^{14}C-labelled lyso-phosphatidylethanolamine by [^{3}H]olate was assayed in the presence of mitochondrial and microsomal preparations from the different stages of development of the insect. Microsomal fractions showed an efficient acyl transferase activity mainly when lyso-phosphatidyl-ethanolamine was used as substrate.

COMPARISON OF THE SUPPRESSION OF INTERFERON PRODUCTION AND INHIBITION OF ITS ACTION BY VITAMIN A AND RELATED COMPOUNDS. J.E. Blalock and G.E. Gifford (Dept. of Immunol. and Med. Microbiol., Univ. of Fla., College of Med., Gainesville, Fla. 32610) *Pro. Soc. Exp. Biol. Med.* 153, 298–300 (1976). We have investigated the structural requirements for vitamin A suppression of interferon production and inhibition of interferon action. Inhibition of interferon action seems to be most dependent on the side chain of the vitamin A molecule. The side chain apparently requires a conjugated double bond system and a hydrophilic terminal group to inhibit interferon action. In contrast, the ring portion of the vitamin A molecule seems to be most important for the suppression of interferon production.

RAT INTESTINAL GLYCOLIPIDS. II. DISTRIBUTION AND BIOSYNTHESIS OF GLYCOLIPIDS AND CERAMIDE IN VILLUS AND CRYPT CELLS. J. Bouhours and R.M. Glickman (Thorndike Lab. and Dept. of Med., Harvard Med. Sch. and Beth Israel Hosp., Boston, Mass.) *Biochim. Biophys. Acta* 441, 123–33 (1976). Intestinal epithelial cells were isolated from rat intestine and grouped into villus and crypt cell fractions. Glycolipids were purified from each cell fraction and quantitated by fluorimetric determination of glycolipid sphingosine. Significant quantities of ceramide were found in all cell fractions and accounted for approximately 15% of total glycolipid sphingosine. While villus and crypt cell fractions quantitatively contained differing amounts of sphingosine, all cell fractions contained proportionally similar quantities of sphingosine when compared to cellular cholesterol or phospholipid. Individual glycolipids, however, showed significant differences in distribution between villus and crypt cells. Hematoside and glucosylceramide were proportionally increased in villus cells, while crypt cells showed an increase in trihexosylceramide and ceramide content.

INTERACTION OF CONCANAVALIN A WITH MEMBRANE-BOUND AND SOLUBILIZED LIPOPROTEIN LIPASE OF RAT HEART. T. Chajek, O. Stein and Y. Stein (Lipid Res. Lab., Dept. of Med., B, Hadassah Univ. Hosp., Jerusalem, Israel) *Biochim. Biophys. Acta* 431, 507–18 (1976). Concanavalin A was used to study the configuration of lipoprotein lipase at the surface of capillary endothelium. Incubation of heart homogenates with increasing concentrations of concanavalin A for 5–60 min. resulted in inhibition of up to 50% of enzyme activity. The inhibition was related to the concentration of lectin and the time of incubation and was fully reversible by postincubation with α-methyl-D-mannoside. It is concluded that mannose residues of lipoprotein lipase in heart homogenates and at the endothelial surface of heart capillaries are available to interact with a specific lectin. Such an interaction renders the enzyme less releasable by heparin during perfusion and causes a significant inhibition of enzyme activity in homogenates.

INTERACTIONS BETWEEN PANCREATIC LIPASES, CO-LIPASE, AND TAURODEOXYCHOLATE IN THE ABSENCE OF TRIGLYCERIDE SUBSTRATE. J. Donner, C.H. Spink, B. Borgstrom and I. Sjoholm (Dept. of Physiol. Chem. and the Dept. of Thermochem., Univ. of Lund, Lund, Sweden) *Biochemistry* 15, 5413–7 (1976). We have studied the interactions between lipase, co-lipase, and taurodeoxycholate by calorimetry and circular dichroism determinations. Porcine pancreatic lipase binds to co-lipase to form a 1:1 complex with an association constant of 2×10^6 M^{-1}. The binding is exothermic and proceeds with an increase of entropy and the ΔC_p of the reaction is highly negative,

-1.31 kJ K^{-1} (mol of lipase)$^{-1}$ at 25°C. This is interpreted to be due to hydrophobic interactions between lipase and co-lipase. The binding of taurodeoxycholate to lipase and co-lipase is an endothermic process, which proceeds without any gross conformational changes as judged by circular dichroism measurements, although spectral changes are observed in the aromatic region. No binding between taurodeoxycholate and co-lipase is detected until concentrations near the critical micellar concentration of the former are reached. It is suggested that taurodeoxycholate and co-lipase form mixed micelles.

PHOSPHOLIPID PHASE TRANSITIONS. EFFECTS OF N-ALCOHOLS, N-MONOCARBOXYLIC ACIDS, PHENYLALKYL ALCOHOLS AND QUATERNARY AMMONIUM COMPOUNDS. A.W. Eliasz, D. Chapman and D.F. Ewing (Chem. Dept., Chelsea Col., Univ. of London, London, SW3 6LX) *Biochim. Biophys. Acta* **448**, 220–30 (1976). The interactions of a series of alcohols, acids and quaternary ammonium salts with a phosphatidylcholine-water model biomembrane (dipalmitoyl phosphatidylcholine) system have been studied using differential scanning calorimetry. In particular the effects of these molecules upon the lipid endothermic phase transitions were investigated over a range of concentrations. The range of different effects obtained with the compounds studied offers a means for introducing various degrees and types of perturbation into membrane systems.

HYDRATION SITES OF EGG PHOSPHATIDYLCHOLINE DETERMINED BY MEANS OF MODULATED EXCITATION INFRARED SPECTROSCOPY. U.P. Fringeli and H.H. Gunthard (Lab. for Phys. Chem., Swiss Fed. Inst. of Tech., Univ.-strasse 22, C H-8006 Zurich, Switzerland) *Biochim. Biophys. Acta* **450**, 101–6 (1976). Conventional and modulated excitation infrared spectra of egg phosphatidylcholine are measured in the spectral range of 4,000–900 cm^{-1} by modulation of relative humidity with an amplitude of $\pm < 5\%$ at a mean value of 75% ($T = 30°$ C). From the modulated-excitation spectrum, hydration sites are found to be the $> PO^-_2$ group, the $> C = O$ group and the choline group.

POSITIONAL DISTRIBUTION OF FATTY ACIDS IN RABBIT LUNG PHOSPHOLIPIDS AND TRIACYLGLYCEROLS AND EFFECT OF PROLONGED HYPEROXY. G. Georgiev, G. Dimitrov, K. Koumanov and T. Neicheva (Cen. Lab. of Biophys., Bulgarian Aca. of Sci., 1113 Sofia, Bulgaria) *Biochim. Biophys. Acta* **450**, 1–7 (1976). Investigations have been carried out for the determination of the effect of high oxygen concentration in inspired gas mixture on the positional distribution of fatty acids in rabbit lung phospholipids and triacylglycerols. The following results were obtained: In the phosphatidylcholine fraction, the high oxygen concentration caused a quantitative increase of palmitic acid (16:0) at the α-position, and of myristic (14:0), heptadecenic (17:1) and arachidonic (20:4) acids at the β-position. In the phosphatidylethanolamine fraction, the high oxygen concentration caused an increase of oleic acid (18:1) at the α-position, and of palmitoleic (16:1) and heptadecenic (17:1) acids at the β-position. In the triacylglycerol fraction such changes were not observed. In connection with these effects of oxygen, its possible influence on membrane structures in the lung has been discussed.

INHIBITION OF VITAMIN D-STIMULATED ACTIVE TRANSPORT OF CALCIUM OF RAT INTESTINE BY DIPHENYLHYDANTOIN-PHENOBARBITAL TREATMENT. Helen C. Harrison and H.E. Harrison (Depts. of Pediatrics, Johns Hopkins Univ.-School of Med., Baltimore, Md. 21205) *Pro. Soc. Exp. Biol. Med.* **153**, 220–4 (1976). The effect of combined diphenylhydantoin and phenobarbital administration on the response of vitamin D-depleted rats to ergocalciferol, cholecalciferol, and 25-hydroxycholecalciferol (25-OHCC) was measured by determinations of serum calcium and phosphate concentrations and of intestinal transport of calcium *in vitro* by everted loops of small intestine. The major action of this anticonvulsant drug treatment in the rat was to inhibit the action of the various forms of vitamin D including 25-OHCC in increasing active transport of calcium by the everted intestine. The increase of calcium diffusibility across the intestine by vitamin D was not blocked nor was the action of vitamin D in increasing serum calcium concentrations. A specific inhibitory effect of the anticonvulsant drugs on an energy-dependent calcium transport system in the intestinal mucosa is suggested.

EFFECTS OF LOCAL ANAESTHETICS ON PHOSPHOLIPASES. H. Kunze, N. Nahas, J.R. Traynor and M. Wurl (Dept. of Biochem. Pharmacol., Max-Planck-Inst. for Exper. Med., Hermann-Rein-Strasse 3, 34 Gottingen, G.F.R.) *Biochim. Biophys. Acta* **441**, 93–102 (1976). The effects of six local anaesthetics have been studied on the activities of soluble phospholipases A_2 (EC 3.1.1.4) and lysophospholipase (EC 3.1.1.5). Phospholipase A_2 activity in human seminal plasma towards sonicated radioactively-labelled phosphatidylethanolamine was slightly stimulated at low and inhibited at high concentrations of all anaesthetic compounds employed. The order of decreasing potency was chlorpromazine, dibucaine, tetracaine, lidocaine, cocaine and procaine. In line with previous findings, the mode of inhibition was seen to be competitive with respect to Ca^{2+}. Lysophospholipase activity in rat liver cytosol towards radioactively labelled lysophosphatidylcholine was inhibited by all local anaesthetics used; the order of decreasing potency was chlorpromazine, dibucaine, tetracaine, cocaine, lidocaine and procaine. The inhibition was un-competitive with respect to substrate. The inhibitory and stimulatory potencies of the local anaesthetics employed closely parallel their lipid solubilities and anaesthetic potencies.

A COMPARATIVE STUDY OF THE GLYCOLIPIDS OF HUMAN, BIRD AND FISH TESTES AND OF HUMAN SPERM. M. Levine, J. Bain, R. Narashimhan, B. Palmer, A.J. Yates and R.K. Murray (Depts. of Biochem., Med. and Pathol., Univ. of Toronto Faculty of Med., Toronto, Ontario, Canada) *Biochim. Biophys. Acta* **441**, 134–45 (1976). The glycolipids of human testis and sperm have been compared. Both adult testis and the sperm exhibited remarkably complex, but generally similar, patterns of glycolipids. In particular, both contained appreciable amounts of the sulfogalactosylmonoalkylmonoacylglycerol, recently shown to be the principal glycolipid of the testis and sperm of a number of animals. In contrast, immature (prepubertal) human testis did not contain this compound. To extend knowledge on the possible distribution of sulfogalactosylmonoalkylmonoacylglycerol in the testes of other chordates, we have also analysed the glycolipids of the testes of a number of birds and fish.

INTERACTIONS BETWEEN PHOSPHOLIPIDS AND BARBITURATES. A.G. Lee (Dept. of Physiol. and Biochem., Univ. of Southampton, Southampton SO9 3TU, U.K.) *Biochim. Biophys. Acta* **455**, 102–8 (1976). The effects of a number of barbiturates on the temperature of the lipid phase transition have been studied using chlorophyll *a* as a fluorescence probe. The barbiturates cause a reduction in the temperature of the phase transitions of dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidylethanolamine, the effects being greatest at lower pH values where more of the barbiturate is present in the uncharged form. There was no significant interaction between the barbiturates and dipalmitoyl phosphatidylserine. These and other observations on the actions of local anaesthetics are used to develop a model for local anaesthesia. It is suggested that the sodium channel is surrounded by an annulus of lipid in the gel state, this rigid microenvironment preventing the sodium relaxing from its active configuration to an inactive one. Local anaesthetics, which reduce the temperature of lipid phase transitions, trigger a change of the annular lipid from the gel to the liquid-crystalline state, with a consequent relaxation of the sodium channel to an inactive configuration, in which the sodium current is reduced or blocked.

EVIDENCE FOR PHASE BOUNDARY LIPID. PERMEABILITY OF TEMPO-CHOLINE INTO DIMYRISTOYLPHOSPHATIDYLCHOLINE VESICLES AT THE PHASE TRANSITION. D. Marsh, A. Watts and P.F. Knowles (Max-Plank-Inst. fur Biophysikalische Chemie, D-3400 Gottingen, West Germany) *Biochemistry* **15**, 3570–8 (1976). The existence of distinct regions of mismatch in molecular packing at the interfaces of the fluid and ordered domains during the phase transition of dimyristoylphosphatidylcholine vesicles has been demonstrated by measuring the temperature dependence of the permeability to a spin-label cation and comparing this with a statistical mechanical calculation of the fraction of interfacial lipid. The relatively high intrinsic permeability of the interfacial regions ($P \sim 0.2–1.0 \times 10^{-8}$ cm/s) is attributed to the mismatch in molecular packing of the lipid molecules at the ordered-fluid boundaries, which could have important implications not only for permeability in natural membranes, but also for the function of membrane-bound enzymes and transport proteins.

EFFECT OF GLUTATHIONE PEROXIDASE ACTIVITY ON LIPID PEROXIDATION IN BIOLOGICAL MEMBRANES. P.B. McCay, D.D. Gibson, K.L. Fong and K.R. Hornbrook (Biomembrane Res. Lab., Okla. Med. Res. Foundation, Oklahoma City, Okla. 73190) *Biochim. Biophys. Acta* **431**, 459–68 (1976). Results are presented indicating that, although glutathione peroxidase

activity inhibits lipid peroxidation in membranes, it does not appear to do so by reducing membrane lipid peroxides to lipid alcohols, as has been shown by others to be the case for free fatty acid peroxides in solution. Lipid peroxidation was studied in an enzymic system (microsomal NADPH oxidase) and in a non-enzymic system (mitochondria plus ascorbate). A study of the fatty acids in the phospholipids of microsomes and mitochondria demonstrated that detectable amounts of hydroxy fatty acids were not formed in the membranes when the latter were incubated in the presence of the glutathione peroxidase system even under conditions known to have generated significant levels of lipid peroxides in the membrane. Fatty acid analyses of the microsomal and mitochondrial particles indicated that glutathione peroxidase activity inhibited loss of polyunsaturated fatty acids when these organelles were exposed to peroxidizing conditions. It appears, therefore, that glutathione peroxidase activity must exert its effect on this system by preventing free radical attack on the polyunsaturated membrane lipids in the first place. A possible mechanism for the interruption of a free radical attack on the lipids is proposed.

SECRETION OF LECITHIN:CHOLESTEROL ACYLTRANSFERASE FROM ISOLATED RAT HEPATOCYTES. G. Nordby, T. Berg, M. Nilsson and K.R. Norum (Inst. for Nutr. Res., School of Med., Univ. of Oslo, Oslo, Norway) *Biochim. Biophys. Acta* 450, 69–77 (1976). Lecithin:cholesterol acyltransferase is secreted from isolated rat hepatocytes. The secretion is stimulated when serum is added to the incubation medium. Optimal conditions for secretion are: $5 \cdot 10^6$ hepatocytes per ml, 5 h incubation, pH 7.3–7.4 and 25% serum in the incubation medium. Concomitantly with the secretion of lecithin:cholesterol acyltransferase there is a secretion of unesterified cholesterol and triacylglycerol. Colchicine or cycloheximide in the incubation medium inhibits secretion of lecithin:cholesterol acyltransferase.

LIPID-MEDIATED PROTEIN INTERACTION IN MEMBRANES. S. Marcelja (Inst. za fiziku Sveucilista, Zagreb, Yugoslavia) *Biochim. Biophys. Acta* 455, 1–7 (1976). This study describes the effects ensuing from a non-specific interaction between membrane integral proteins and the surrounding lipids. The results are obtained using an appropriate molecular field theory to describe the ordering of membrane lipids. The modification of the lipid structure near a protein molecule, while most pronounced within the annulus of the first neighbour molecules, extends two or three layers beyond the annulus. The ordering of lipids within the annulus has a modified temperature dependence, and becomes a continuous function of temperature for low lipid/protein ratios. The change in order of lipid molecules surrounding a protein leads to an indirect, lipid-mediated interaction between membrane integral proteins. This interaction depends sensitively on the bulk lipid order. Under favourable circumstances, it gives rise to protein aggregation.

MOLECULAR CONTROL OF MEMBRANE PROPERTIES DURING TEMPERATURE ACCLIMATION. FATTY ACID DESATURASE REGULATION OF MEMBRANE FLUIDITY IN ACCLIMATING TETRAHYMENA CELLS. C.E. Martin, K. Hiramitsu, Y. Kitajima, Y. Nozawa, L. Skriver and G.A. Thompson, Jr. (Dept. of Botany, The Univ. of Texas, Austin, Tex. 78712) *Biochemistry* 15, 5218–27 (1976). This is a study of the molecular mechanisms employed by *Tetrahymena pyriformis* to change the lipid composition and thereby the fluidity of its various membranes during temperature acclimation. By quantitatively measuring the intramembrane particle aggregation using freeze-fracture electron microscopy, membrane physical properties in 39.5°C grown cells shifted to 15°C were found to be correlated with the degree of phospholipid fatty acid desaturation. Alteration of the phospholipid polar head group distribution from that of 39.5°C-grown cells to the significantly different pattern of 15°C grown cells appeared not to be of critical importance in the acclimation process. Changes in fatty acid desaturation during acclimation from high to low temperatures and vice versa were analyzed using normal cells and cells fed large amounts of polyunsaturated fatty acids. Fatty acid desaturase activity corresponded to the degree of membrane fluidity but not to the cell temperature. All evidence was compatible with the hypothesis that membrane fluidity is self-regulating, with the action of fatty acid desaturases being modulated by the physical state of their membrane environment.

STUDIES ON MEMBRANE FUSION. II. INDUCTION OF FUSION IN PURE PHOSPHOLIPID MEMBRANES BY CALCIUM IONS AND OTHER DIVALENT METALS. D. Papahadjopoulos, W.J. Vail, W.A. Pangborn and G. Poste (Dept. of Experi. Pathol., Roswell Park Memorial Inst., Buffalo, N.Y. 14263) *Biochim. Biophys. Acta* 448, 265–83 (1976). The effect of divalent metals on the interaction and mixing of membrane components in vesicles prepared from acidic phospholipids has been examined using freeze-fracture electron microscopy and differential scanning calorimetry. Ca^{2+}, and to a certain extent Mg^{2+}, induce extensive mixing of vesicle membrane components and drastic structural rearrangements to form new membranous structures. Different phospholipids also vary markedly in their relative responsiveness to Ca^{2+} and Mg^{2+}, with certain phospholipids being much more susceptible to fusion by Ca^{2+} than Mg^{2+}. Vesicle fusion induced by divalent cations also requires that the lipids of the interacting membranes be in a "fluid" state ($T > T_c$). Fusion of vesicle membranes by Ca^{2+} and Mg^{2+} does not appear to be due to simple electrostatic charge neutralization. Rather the action of these cations in inducing fusion is related to their ability to induce isothermal phase transitions and phase separations in phospholipid membranes.

STUDIES ON MEMBRANE FUSION. I. INTERACTIONS OF PURE PHOSPHOLIPID MEMBRANES AND THE EFFECT OF MYRISTIC ACID, LYSOLECITHIN, PROTEINS AND DIMETHYLSULFOXIDE. D. Papahadjopoulos, S. Hui, W.J. Vail and G. Poste (Dept. of Experi. Pathol., Roswell Park Memorial Inst., Buffalo, N.Y. 14263) *Biochim. Biophys. Acta* 448, 245–64 (1976). The interaction and mixing of membrane components in sonicated unilamellar vesicles and also non-sonicated multilamellar vesicles prepared from highly purified phospholipids suspended in NaCl solutions has been examined. Electron microscopy and differential scanning calorimetry were used to characterize the extent and kinetics of mixing of membrane components between different vesicle populations. No appreciable fusion was detected between populations of non-sonicated phospholipid vesicles incubated in aqueous salt (NaCl) solutions. Our results indicate that while these agents enhance mixing of vesicle membrane components, in most cases mixing probably proceeds via diffusion of phospholipid molecules rather than by fusion of entire vesicles. Increased mixing of vesicle membrane components was also produced when vesicles were prepared containing a purified hydrophobic protein (myelin proteolipid apoprotein) or were incubated in the presence of dimethylsulfoxide.

RETARDING EFFECTS OF DNA ON THE AUTOXIDATION OF LIPOSOMAL SUSPENSIONS. D.D. Pietronigro, M.L. Seligman, W.B.G. Jones and H.B. Demopoulos (Dept. of Pathol., New York Univ. Med. Center, New York, N.Y.) *Lipids* 11, 808–13 (1976). Deoxyribonucleic acid (DNA) is associated with the cell membrane of prokaryotes and the inner nuclear membrane of eukaryotes. The unsaturated fatty acids of phospholipids, which constitute the bilaminar structure of membranes, undergo autoxidation in the presence of O_2. Calf thymus DNA was incubated with methyl archidonate-enriched phosphatidyl choline liposomes in order to study the effect of DNA upon the oxidation of phospholipids while present in their natural in vivo bilayer configuration. DNA retarded the rate of lipid oxidation as monitored by both diene conjugation and the TBA test, but it did not alter the induction period. These results suggest that DNA is scavenging free radicals produced within the phospholipid bilayer.

SELECTIVE UTILIZATION OF ENDOGENOUS UNSATURATED PHOSPHATIDYLCHOLINES AND DIACYLGLYCEROLS BY CHOLINEPHOSPHOTRANSFERASE OF MOUSE LUNG MICROSOMES. M.G. Sarzala and L.M.G. Van Golde (Lab. of Vet. Biochem., St. Univ. of Utrecht, Biltstraat 172, Utrecht, the Netherlands) *Biochim. Biophys. Acta* 441, 423–32 (1976). In the presence of CMP, cholinephosphotransferase of mouse lung microsomes catalyzes the conversion of endogenous phosphatidylcholines into 1,2-diacyl-*sn*-glycerols and CDP-choline. In this conversion cholinephosphotransferase shows a distinct preference for those molecular species of phosphatidylcholine which contain an unsaturated fatty acid. The enzyme hardly utilizes endogenous dipalmitoylglycerophosphocholine as a substrate. The low affinity of cholinephosphotransferase for either dipalmitoylglycerophosphocholine or 1,2-dipalmitoyl-*sn*-glycerol strongly endorses the concept that the CDPcholine pathway alone cannot be responsible for the production of pulmonary dipalmitoylglycerophosphocholine.

INVESTIGATION OF PHOSPHATIDYLETHANOLAMINE BILAYERS BY DEUTERIUM AND PHOSPHORUS-31 NUCLEAR MAGNETIC RESONANCE. J. Seelig and H.-U. Gally (Dept. of Biophys. Chem., Biocenter

of the Univ. of Basel, CH-4056 Basel, Switzerland) *Biochemistry* 15, 5199–204 (1976). The motion of the ethanolamine head group in unsonicated lipid bilayers above and below the phase transition is studied by means of deuterium and phosphorus magnetic resonance. For this purpose, dipalmitoyl-3-*sn*-phosphatidylethanolamine is selectively deuterated at the two ethanolamine carbon atoms. The deuterium quadrupole splittings of the corresponding bilayer phases are measured at pH 5.5 as a function of temperature. In addition, the phosphorus-31 chemical shift anisotropies of planar-oriented and randomly dispersed samples of dipalmitoyl-3-*sn*-phosphatidylethanolamine are measured at pH 5.5 and 11 by applying a proton-decoupling field. The knowledge of the static chemical shift tensor provides the basis for a quantitative analysis of the head-group motion. The nuclear magnetic resonance data are consistent with a model in which the ethanolamine group is rotating flat on the surface of the bilayer with rapid transitions occurring between two enantiomeric conformations.

HOW VALINE DEPRIVATION AND ITS REVERSAL AFFECT FATTY ACID METABOLISM IN HeLa CELLS. J.R.B. Slayback and I.M. Campbell (Dept. of Biochem., Faculty of Arts and Sci., Univ. of Pittsburgh, 605 Parran Hall, 130 DeSoto Street, Pittsburgh, Pa. 15261) *Biochim. Biophys. Acta* 450, 33–44 (1976). A protocol based on radio gas chromatography demonstrates that when HeLa cells are deprived of valine for short periods of time (6–7.5 h), their overall fatty acid biosynthetic activity is depressed after a latency of a few hours. The transfer of newly synthesized fatty acyl units to phospholipids is curtailed much faster than their transfer to triacylglycerols. Despite the cut-back in fatty acid biosynthesis, valine deprivation causes a lipid accumulation in the cells. Valine deprivation appears to affect de novo synthesis of fatty acid units from acetate more rapidly than desaturation and elongation. When valine is returned to the valine-deprived culture, overall fatty acid biosynthesis is resumed well within 2 h. Newly synthesized fatty acyl units are transferred to both the phospholipids and the 1,2-diacylglycerols of the cells but not initially to the triacylglycerols.

HORMONAL REGULATION OF LIVER MITOCHONDRIAL PYRUVATE CARRIER IN RELATION TO GLUCONEOGENESIS AND LIPOGENESIS. M.A. Titheradge and H.G. Coore (Dept. of Biochem., Univ. of Birmingham, P.O. Box 363, Birmingham B15 2TT, England) *FEBS Letters* 71, 73–8 (1976). By diminishing the phosphorylation of pyruvate dehydrogenase, insulin would be expected to promote synthesis of fatty acids from pyruvate in liver as well as in adipose tissue. Our results suggest that the mechanism of insulin action on liver pyruvate dehydrogenase does not involve persistent alteration of the mobility of the mitochondrial pyruvate carrier.

BLOOD SAMPLING TECHNIQUES FOR STUDYING RAPIDLY TURNING OVER METABOLIC FUELS IN MICE. N. Baker, D. Morris and C. Sandborg (Tumor-Lipid Lab., Res. Service, Veterans Admin. Wadsworth Hosp. Center, Los Angeles, Calif. 90073) *Lipids* 11, 818–20 (1976). Experiments were carried out in control and Ehrlich ascites carcinomatous mice to determine whether orbital venous sinus blood could be used to reflect blood in the systemic circulation (decapitation blood) in the case of a rapidly turning over metabolic fuel such as free fatty acids. The early time course of intravenously injected, labeled free fatty acids was measured using [9,10-^{3}H]palmitic acid and [1-^{14}C]linoleate complexed to mouse serum. No significant differences between decapitation and orbital sinus blood were found at early times in either group of mice. The orbital sinus clearly contains blood that is not stagnant and is replaced so rapidly that it is suitable for studying very rapidly turning over, circulating metabolites.

DOES A CYTOPLASMIC FACTOR STIMULATE THE TRANSFER OF PHOSPHATIDYLSERINE FROM LIPOSOMES TO MITOCHONDRIA? J. Baranska and L. Wojtczak (Dept. of Cellular Biochem., Nencki Inst. of Experi. Biol., Pasteura 3, 02–093 Warsaw, Poland) *FEBS Letters* 71, 83–6 (1976). It is clearly evident that liver cytoplasmic fraction stimulates a transfer of labelled phosphatidylserine to mitochondria only under conditions when this phospholipid can be immediately decarboxylated to phosphatidylethanolamine and that only the accumulation of phosphatidylethanolamine is increased by the cytoplasmic fraction.

EFFECT OF ANTI-INSULIN SERUM ON THE HEPATIC LIPID METABOLISM OF BHE RATS. C.D. Berdanier and E.T. Koh (Carbohydrates Lab., Nutr. Inst., Agric. Res. Service, USDA, Beltsville, Md. 20705) *Am. J. Clin. Nutr* 29, 1190–5 (1976). The effects of daily injections of anti-insulin serum (AIS) on the hepatic synthesis of lipids was studied in young male BHE rats after 3 weeks of feeding either a 45% carbohydrate-40% protein diet, a 65% sucrose diet, or a 65% protein diet. One-half of the animals in each diet group received injections daily with AIS, and the remaining animals in each group received injections with isotonic saline solution. After 3 weeks the animals were killed, and levels of serum insulin, cholesterol, and triglycerides were determined as were the levels of liver lipid, cholesterol, fatty acid synthetase, and the conversion of acetate ^{14}C to cholesterol ^{14}C. AIS treatment lowered serum insulin levels, serum triglyceride levels, caloric intake, weight gain, liver weight, acetate ^{14}C incorporation into cholesterol ^{14}C, and the percentage of liver lipid that was cholesterol. Diet affected serum and liver lipid levels, fatty acid synthetase activity, and the incorporation of acetate ^{14}C into cholesterol ^{14}C. The results of this study show that the lipogenic characteristic of the BHE rat is diet dependent, but that this characteristic can be modified to a limited extent by AIS treatment. Further, the results also suggest that the hyperlipemic characteristic of these rats may be independent of the hyperinsulinemic characteristic.

LIPID ACCUMULATION IN CELLS DERIVED FROM PORCINE AORTA AND GROWN UNDER ANAEROBIC CONDITIONS. R.G. Briggs and J.L. Glenn (Dept. of Biochem., Albany Med. College, Albany, N.Y. 12208) *Lipids* 11, 791–7 (1976). Fibroblast-like cells, derived from porcine aorta, were cultured under aerobic and anaerobic conditions. Light and electron microscopic examinations, lipid composition measurements, and incorporation of radioactive precursors into lipids of these cells were performed. Anaerobically grown cells accumulated oil red 0 stainable droplets and within 6 hr the triacylglycerol content increased to 4 times the level determined in cells grown under aerobic conditions. This ratio remained constant throughout an additional 12 hr of growth. The fatty acid composition of the triacylglycerols which accumulated under anaerobic conditions differed from the composition of fatty acids in the triacylglycerols present in the growth medium. The cellular unesterified fatty acids of the anaerobically grown cells differed only slightly in composition from the fatty acids in the growth medium, while the unesterified fatty acids of aerobically grown cells differed to a greater extent from those of the growth medium.

PALMITATE INCORPORATION IN THE LUNGS OF DOGS WITH GRANULOMATOUS DISEASE. P.R.B. Caldwell, R.D. Wigle, T.S. Cottrell, and H.O. Heinemann (Dept. of Med., Col. of Phys., and Surgeons, Columbia Univ., New York, N.Y. 10032) *Proc. Soc. Exp. Biol. Med.* 152, 685–90 (1976). Moderate hypoxia did not influence the pulmonary incorporation of an intravenous dose of [1-^{14}C]palmitate either in dogs with experimentally produced granulomatous disease or in normal controls. The lung weight in the diseased animals, was, on the average, double that of the controls. There was a proportionate increase in uptake of the radioactive label at 1 hr after infusion in the diseased lungs, hence the specific activity of labeled palmitate (counts per minute per gram of phospholipid) was no different in the two groups. Moreover, half the radioactivity of the phospholipids was recovered in palmitate separated from the phosphatidyl choline fraction in both diseased and normal lungs. Anatomic studies demonstrated increased numbers of Type II pneumocytes lining all alveolar air spaces in the diseased lung. Autoradiographic studies indicated the presence of labeled palmitate in the Type II cells, but not in the inflammatory cells of the granulomata. We conclude that the increased palmitate uptake in this disease is accounted for by the metabolic activity of the Type II pneumocytes.

VITAMIN K-DEPENDENT CARBOXYLASE. SOLUBILIZATION AND PROPERTIES. C.T. Esmon and J.W. Suttie (Dept. of Biochem., College of Agri. and Life Sci., Univ. of Wisconsin-Madison, Madison, Wis. 53706) *J. Biol. Chem.* 251, 6238–43 (1976). Vitamin K is required for an enzymatic carboxylation of glutamyl residues in a microsomal protein precursor of plasma prothrombin to form γ-carboxyglutamic acid. The enzyme system (carboxylase) which catalyzes this reaction has now been solubilized by extraction of the microsomes with Triton X-100 and has been shown to fix $H^{14}CO_3^-$ as γ-carboxyglutamic acid residues in biologically active prothrombin. Enzyme activity requires O_2 and vitamin K hydroquinone or vitamin K + NADH. Unlike the microsomal-bound carboxylase, soluble carboxylase activity is independent of either ATP or MG^{2+} addition and is unaffected by either the ATP analog,

adenyl-5'-yl imidodiphosphate (AMP-P(NH)P, or EDTA. These observations suggest that the energy required to drive the carboxylation reaction is derived from the oxidation of the reduced form of vitamin K. Although the membrane-bound carboxylase is inhibited by Warfarin, this anticoagulant is ineffective as an inhibitor of the soluble enzyme. A second anticoagulant, 2-chloro-3-phytyl-1,4-natpthoquinone (chloro-K), differs from Warfarin in that it effectively inhibits both the membrane-bound and soluble carboxylases.

EVIDENCE FOR CARRIER PROTEINS IN BILE ACID SYNTHESIS. THE EFFECT OF SQUALENE AND STEROL CARRIER PROTEIN AND ALBUMIN ON THE ACTIVITY OF 12α-HYDROXYLASE. G.A. Grabowski, K.E. McCoy, G.C. Williams, M.E. Dempsey, and R.F. Hanson (The Gastroenterology Unit, Dept. of Internal Med., Univ. of Minn., Minneapolis, Minn. 55455) *Biochim. Biophys. Acta* 441, 380–90 (1976). The possibility that carrier proteins are involved in bile acid synthesis was investigated using rat liver homogenates. The 105,000 *X g* supernatant fraction was found to contain heat stable proteins that bound the bile acid precursor, 7α-hydroxy-4-cholesten-3-one, and increased the amount of 7α, 12α-dihydroxy-4-cholesten-3-one formed by the microsomal enzyme, 12α-hydroxylase. Subsequent studies were carried out to determine if squalene and sterol carrier protein or albumin, two lipid binding proteins present in the 105,000 *X g* supernatant fraction of rat liver homogenates, may be responsible for the effects seen with this fraction. Kinetic analysis indicated that the apparent stimulation of 12α-hydroxylase by squalene and sterol carrier protein and albumin was due to increased solubilization of the substrate, 7α-hydroxy-4-cholesten-3-one.

THE INFLUENCE OF POSTNATAL NUTRITIONAL DEPRIVATION ON THE PHOSPHOLIPID CONTENT OF DEVELOPING RAT LUNG. I. Gross, I. Ilic, C.M. Wilson and S.A. Rooney (Div. of Perinatal Med., Depts. of Pediatrics, Obstetrics and Gynecol. and Lung Res. Center, Yale Univ. School of Med., New Haven, Conn. 06510) *Biochim. Biophys. Acta* 441, 412–22 (1976). It has been previously reported that fasting may result in decreased lung surfactant production. In order to investigate this relationship and the role of nutrition in lung phospholipid synthesis, 21-day-old rats were exposed for 60 h to one of five dietary regimens: standard rat chow (controls), fasting, pure glucose, pure fat, or pure protein. Pulmonary cholinephosphotransferase (EC 2.7.8.2) activity was decreased in the fasted animals and those fed the protein diet, but not in the glucose or fat-fed animals. The activities of acetyl-CoA carboxylase (EC 6.4.1.2) and microsomal fatty acid elongation were decreased in all the experimental groups except for the glucose-fed group. It is concluded that fasting results in a decrease in lung cell size but not in lung cell number. Total phospholipid and phosphatidylcholine content in lung tissue and lung lavage is decreased per cell but not per unit cell mass.

SELENIUM AND VITAMIN E AND INCIDENCE OF RETAINED PLACENTA IN PARTURIENT DAIRY COWS. II. PREVENTION IN COMMERCIAL HERDS WITH PREPARTUM TREATMENT. W.E. Julien, H.R. Conrad and A.L. Moxon (Dept. of Dairy Sci., Ohio Agric. Res. and Develop. Center, Wooster, Ohio 44691) *J. Dairy Sci.* 59, 1960–2 (1976). In a series of field experiments in Ohio involving 193 parturient cows of the Holstein and Guernsey breeds, the prophylactic efficacy of selenium and vitamin E was tested under field conditions. Herds initially were chosen because of a chronic problem with retained placenta which could not be related to a known etiological factor. Each herd was divided into three groups. Group A received an injection of 50 mg of sodium selenite 40 days prepartum and 680 units of alpha tocopherol acetate followed by the same treatment 20 days prepartum. Group B received a single injection of 50 mg of sodium selenite 20 days prepartum, and 680 IU of vitamin E. Group C served as the control. Incidence of retained placenta was reduced from a mean of 51.2% in control cows to 8.8% for animals injected with selenium and vitamin E. No differences in efficacy were between Group A and B, and it appears that the single 20 day prepartum injection of 50 mg sodium selenite and 680 IU of alpha tocopherol acetate is an effective prophylactic for prevention of retained placenta.

ALTERATIONS IN PHOSPHATIDYLCHOLINE SPECIES AND THEIR REVERSAL IN PULMONARY SURFACTANT DURING ESSENTIAL FATTY-ACID DEFICIENCY. E.C. Kyriakides, D.A. Beeler, R.H. Edmonds and J.A. Balint (Dept. of Med., Biochem., and Anatomy Neil Hellman Med. Res. Building, Albany Med. College, Albany, N.Y. 12208) *Biochim. Biophys. Acta* 431, 399–407 (1976). Previous studies have indicated that essential fatty-acid deficiency in rats resulted in significant reduction of palmitate content of lung tissue and lavage phosphatidylcholines. Experiments were, therefore, undertaken to confirm and further characterize these changes and to examine the reversal of these alterations when essential fatty acid deficient rats were fed fat-free diets supplemented with linoleate for 1–14 days. On feeding the diet containing linoleate to the deficient rats, a reversal of these changes began after one day and was nearly complete by 7–14 days.

ACYL GROUP COMPOSITION OF MEMBRANE PHOSPHOLIPIDS IN MAMMARY TISSUES AND CARCINOMA INDUCED BY DIMETHYLBENZ(A)ANTHRACENE. B.S. Leung and G.Y. Sun (Dept. of Surgery and Clinical Res. Centr., Univ. of Oregon Med. Sch., Portland, Oregon 97201) *Proc. Soc. Exper. Biol. Med.* 152, 671–6 (1976). Phospholipids and their acyl group composition in mammary adenocarcinomas and mammary tissue of the same tumor-bearing animals were investigated. Breast adenocarcinoma induced by dimethylbenz(*a*)anthracene exhibited a phospholipid pattern which was different from that of the mammary tissue. Tumor phospholipids had higher proportions of diacyl-GPI, diacyl-GPE, and alkenylacyl-GPE and a lower proportion of diacyl-GPC than the controls. The acyl groups of most phospholipids in tumors showed a marked increase in the proportion of 18:1 and a decrease in the proportion of 18:2. The fatty acid composition of plasmalogen and triglyceride, however, remained unchanged. In spite of the decrease in the proportion of 18:2, there was no apparent difference in the proportion of 20:4 in most of the phosphoglycerides; however, a significant decrease in this fatty acid was noted in diacyl-GPI. Results of this study demonstrated that the membrane phospholipids of mammary adenocarcinoma were altered in respect to acyl group composition. Changes in physical properties of the cell membrane, in turn, could lead to abnormal manifestation of membrane regulated events in tumor cells.

THE METABOLISM IN VIVO AND IN VITRO OF PLASMA LOW-DENSITY LIPOPROTEIN FROM A SUBJECT WITH INHERITED HYPERCHOLESTEROLAEMIA. N.B. Myant, D. Reichl, G.R. Thompson, M.J.P. Higgins and D.J. Galton (Med. Res. Council Lipid Metabolism Unit, Hammersmith Hosp., London) *Clin. Sci. Mole. Med.* 51, 463-5 (1976). The metabolism *in vivo* and *in vitro* of an abnormal low-density lipoprotein (LDL) obtained from a patient with an inherited form of hypercholesterolaemia was compared with that of LDL obtained from a normal subject. The rates of turnover of the apoprotein of the two types of LDL in a normal subject, and their uptake and catabolism by normal lymphocytes *in vitro*, were similar. It is concluded that the abnormal behaviour of the patient's LDL may not be due to an abnormality in the apoprotein component.

PARTIAL PURIFICATION AND CHARACTERIZATION OF A TRIGLYCERIDE LIPASE FROM PIG ADIPOSE TISSUE. S. Matsumura, M. Matsuo and Y. Nishizuka (Dept. of Biochem., Kobe Univ. School of Med., Kobe 650, Japan) *J. Biol. Chem.* 251, 6267–73 (1976). A triglyceride lipase was extracted from defatted pig adipose tissue powder with dilute ammonia and purified about 230-fold by a combination of ammonium sulfate fractionation, heparin-Sepharose 4B, DEAE-cellulose, and Sephadex G-150 column chromatographies and isoelectrofocusing electrophoresis. The enzyme was distinguishable in physical and kinetic properties from the two previously defined lipases in adipose tissue, lipoprotein lipase, and hormone-sensitive lipase. The purified enzyme was fully active in the absence of serum lipoprotein and was not stimulated by adenosine 3':5'-monophosphate-dependent protein kinase. In marked contrast to the already defined lipases, the enzyme was strongly inhibited by serum albumin. The enzyme had a molecular weight of about 43,000, a pI of 5.2, and pH optimum of 7.0. The enzyme hydrolyzed triolein to oleic acid and glycerol, and did not exhibit esterase activity. The apparent K_m for triolein was 0.05 mM. Physiological roles of this new species of lipase remained to be explored.

ACTIVITY OF CHOLINEPHOSPHOTRANSFERASE, LYSOLECITHIN: LYSOLECITHIN ACYLTRANSFERASE AND LYSOLECITHIN ACYLTRANSFERASE IN THE DEVELOPING MOUSE LUNG. V. Oldenborg and L.M.G. Van Golde (Lab. of Vet. Biochem., St. Univ. of Utrecht, Biltstraat 172, Utrecht) *Biochim. Biophys. Acta* 441, 433–42 (1976). The present study presents the activity profiles of cholinephosphotransferase, lysolecithin: lysolecithin acyltransferase and lysolecithin acyltransferase at different stages of development of the mouse lung. The specific activity of

cholinephosphotransferase, a key enzyme in the de novo synthesis of phosphatidylcholine, increases during the later stages of fetal development until it reaches a maximal value at a gestational age of 17 days, i.e. 2 days before term. Thereafter, the activity of the enzyme declines again until around term. The specific activity of lysolecithin acyltransferase, which catalyzes the direct acylation of 1-acyl-*sn*-glycero-3-phosphocholine, does not change significantly during the prenatal development and is lower than that of either lysolecithin: lysolecithin acyltransferase or cholinephosphotransferase at all stages of development.

EFFECTS OF GLYCEROL IN VLDL SECRETION BY THE ISOLATED RAT LIVER. D. Petit, A. Raisonnier, M.E. Bouma and R. Infante (Unite de Recherches d'Hepatologie de l'I.N.S.E.R.M. (U-9) and Lab. de Biochim., Faculte de Med. St. Antoine, 75571 Paris Cedex 12, France) *Biochim. Biophys. Acta* **431**, 481–92 (1976). Stimulation of VLDL production by increasing fatty acid availability is now well established. However, a possible regulatory role of glycerol, another lipid precursor, in VLDL synthesis by the liver has not yet been substantiated. The present experiments investigate this problem using the isolated perfused rat liver. Incorporation of radioactive fatty acids into liver or plasma lipids was lower than in the control group. Significant differences were observed between saturated and unsaturated fatty acids used as lipid precursors. Production of VLDL as assessed by radioactive leucine and fatty acid incorporation in the VLDL of the perfusate was depressed by glycerol. Glycerol partly inhibits the normal stimulation of VLDL production by plasmatic fatty acid overload.

STUDIES ON NUCLEOTIDE DIPHOSPHATE DIACYLGLYCEROL SPECIFICITY OF ACIDIC PHOSPHOLIPID BIOSYNTHESIS IN RAT LIVER SUBCELLULAR FRACTIONS. B.J.H.M. Poorthuis and K.Y. Hostetler (Dept. of Med., Div. of Metabolic Disease, The Univ. of Calf., San Diego, Calif.) *Biochim. Biophys. Acta* **431**, 408–15 (1976). Cytidine diphosphate diacylglycerol, uridine diphosphate diacylglycerol, adenosine diphosphate diacylglycerol and guanosine diphosphate diacylglycerol were synthesized chemically and their purity assessed. The activity of these compounds in acidic phospholipid synthesis was examined in rat liver mitochondria and microsomes. Although considerable phosphatidylglycerol formation was observed with UDPdiacylglycerol and ADPdiacylglycerol, it is unlikely that these compounds are of physiologic importance, at least in rat liver, since CTP:phosphatidic acid cytidyltransferase in microsomes and mitochondria was shown to be specific for cytidine triphosphate. The lack of specificity of phosphatidylglycerol synthesis for CDPdiacylglycerol is currently unexplained but may be of some importance in other tissues or in other organisms.

IN VITRO UPTAKE OF VITAMIN A FROM THE RETINOL-BINDING PLASMA PROTEIN TO MUCOSAL EPITHELIAL CELLS FROM THE MONKEY'S SMALL INTESTINE. L. Rask and P.A. Peterson (Inst. of Med. and Physiol. Chem., Biomed. Center, Univ. of Uppsala, Uppsala, Sweden) *J. Biol. Chem.* **251**, 6360–6 (1976). The *in vitro* uptake of retinol from its plasma carrier protein, the retinol-binding protein (RBP), to the cells of the monkey's small intestine has been studied. [^{3}H]Retinol was readily delivered from the RBP to the cells without a concomitant cellular uptake of the RBP. The [^{3}H]retinol accumulation in the cells was dependent on the temperature and was virtually abolished at 0°. Several metabolic inhibitors could not impede the uptake process. The cellular [^{3}H]retinol accumulation was linear for about 45 min and exhibited characteristic saturation kinetics. The uptake of [^{3}H]retinol by the cells could be inhibited by RBP containing unlabeled retinol A-depleted RBP, and Fab' fragments against RBP. In contrast, free, unlabeled retinol and the metabolite form of RBP, lacking retinol and affinity for prealbumin, were inactive. It is therefore suggested that there is a receptor for vitamin A on the cell surface which recognizes the protein part of the protein · ligand complex.

THE EFFECT OF TWO ISOMERIC OCTADECENOIC ACIDS ON THE LIPID METABOLISM AND GROWTH OF NOVIKOFF HEPATOMA CELLS. D.E. Wennerstrom and H.M. Jenkin (The Hormel Inst., Univ. of Minn., Austin, Minn. 55912) *Biochim. Biophys. Acta* **431**, 469–80 (1976). The origin and metabolism of octadecenoic acid was examined in intact Novikoff rat hepatoma cells by using labeled precursors and two isomeric octadecenoic acids which differed in their abilities to stimulate cell growth in a serum-free medium. The isomers were measured in the cellular lipid by ozonolysis and reduction of the ozonides. The results indicate that the 18:1 fatty acid accumulated in the cell lipid by uptake of the preformed acid from the medium. The cis-6-18:1 was more extensively metabolized than the cis-18:1 to 16:1 and 20:1 fatty acids by chain shortening and chain elongation. Both isomers inhibited de novo fatty acid synthesis from acetate by cells suspended in a serum-free medium. The isomers did not exert coordinate control of both fatty acid and cholesterol biosynthesis in the Novikoff cells.

EFFECT OF EARLY POSTNATAL DIETARY STERCULATE ON THE FATTY ACID COMPOSITION OF RAT LIVER AND BRAIN LIPIDS. R.K. Pullarkat, J. Maddow and H. Reha (Dept. of Neurochem., Inst. for Basic Res. in Mental Retardation, 1050 Forest Hill Road, Staten Island, N.Y. 10314) *Lipids* **11**, 802–7 (1976). Pregnant rats were fed a high carbohydrate diet containing either 1% trilinolein or 1% trilinolein with 0.2% methyl sterculate from 18 day gestation to 21 day postpartum. The pups were weaned at 21 days and continued on the same diet for an additional 10 days. The microsomal stearyl CoA desaturase activities of the liver were effectively inhibited. Liver triglycerides showed increases in the saturated fatty acids concentrations at the expense of the corresponding monoenes. The concentration of *cis* 6–7 octadecenoic acid was elevated. In liver phospholipids, the concentration of stearic acid was increased without a corresponding decrease in the oleic acid content. A drastic decrease in the nervonic acid (24:1, n-9) concentration of liver sphingomyelin was observed. The lipids of the brain did not contain sterculic acid, and brain desaturase activity was unaffected. There was no significant change in the concentration of monoenoic acids from 16:1 to 22:1. However, nervonic acid was decreased by 32%. These results suggest that brain nervonic acid may be derived from a precursor other than oleic acid.

EFFECTS OF TREATMENT OF WHOLE FAT SOYBEANS OR SOY FLOUR WITH FORMALDEHYDE TO PROTECT THE POLYUNSATURATED FATTY ACIDS FROM BIOHYDROGENATION IN THE RUMEN. B.A. Ackerson, R.R. Johnson and R.L. Hendrickson (Dept. of Animal Sci. and Industry, Oklahoma State Univ., Stillwater, Okla. 74074) *J. Nutr.* **106**, 1383–90 (1976). Full-fat, ground soy flour (GSF) was treated with 37% formaldehyde (HCHO) and evaluated by in vitro and in vivo criteria to determine the protection afforded linoleic acid against ruminal biohydrogenation when the materials described above were fed as a protein supplement to rations for growing lambs. The supplements compared were soybean meal (SBM), untreated GSF and GSF treated with 10.2 ml HCHO/100 g GSF and soaked for 2 hours. Organoleptic evaluations were conducted to determine if any flavor differences in meat from lambs fed these supplements could be detected. No significant differences were noted in daily feed intake, feed efficiency or average daily gain for lambs fed growing-finishing rations containing any of the products tested as the protein supplement. A taste panel could not detect any differences in flavor of ground loin among any of the treatments.

MEMBRANE ASSOCIATED PHOSPHOLIPOPROTEINS OF BACILLUS LICHENIFORMIS 749. P.S. Aiyappa and J.O. Lampen (Waksman Inst. of Microbiol., Rutgers Univ., The State Univ. of New Jersey, New Brunswick, N.J. 08903) *Biochim. Biophys. Acta* **448**, 401–10 (1976). The membrane-bound penicillinase of *Bacillus licheniformis* 749/C is a phospholipoprotein that differs from the hydrophilic exoenzyme in that its polypeptide chain carries an additional 25 residues (mostly hydrophilic) with phosphatidylserine as the NH_2-terminus. To determine if other phospholipoproteins are present in the plasma membrane, the penicillinase-inducible strain 749 was grown without inducer in the presence of [2-^{3}H]glycerol. Electrophoretic separation of the membrane proteins (after removal of free lipids) showed an association of ^{3}H-activity with certain of the proteins which could not be broken by lipid solvents and strongly denaturing conditions. Penicillinase should be considered as the first observed example of a group of phosphatidylserine-containing proteins present in the plasma membrane *B. licheniformis* 749 and 749/C.

METABOLISM OF DIFFERENT MONOACYLPHOSPHOLIPIDS IN ISOLATED HEPATOCYTES AND THE INTACT RAT. B. Akesson, A. Arner and R. Sundler (Dept. of Physio. Chem. 3, Univ. of Lund, P.O. Box 750, S-220 07 Lund 7, Sweden) *Biochim. Biophys. Acta* **441**, 453–64 (1976). The 1-[^{3}H]palmitoyl, 2-[^{3}H]oleoyl, and 2-[^{14}C]linoleoyl derivatives of *sn*-glycero-3-phosphoethanolamine and the corresponding derivatives of *sn*-glycero-3-phosphocholine were injected intraportally to rats and their incorporation into liver lipids was studied 15 min. thereafter. Both the uptake by the liver and the degree of

acylation was higher for the unsaturated compounds. The uptake of lysophosphatidylethanolamine was higher than that of lysophosphatidylcholine. The metabolism of 1-lysophosphatidylethanolamine was also studied in isolated hepatocytes. The degree of hydrolysis was much more prominent than in vivo. It can be concluded that the monoacylphospholipid acyltransferase reactions operating at positions 1 or 2 yield different saturated acyl chain profiles in phosphatidylethanolamine and phosphatidylcholine of a specific unsaturation. This may be important in the regulation of the fatty acid composition of the membrane phospholipids.

EFFECT OF HYPERLIPEMIC SERUM ON CHOLESTEROL ACCUMULATION IN MONKEY AORTIC MEDIAL CELLS. S.R. Bates and R.W. Wissler (Dept. of Pathol. and Specialized Ctr. of Res. in Athero., Univ. of Chicago, Chicago, Ill. 60637) *Biochim. Biophys. Acta* **450**, 78–88 (1976). The effect of hyperlipemic monkey serum on cholesterol ester formation and accumulation in monkey aortic medial cells grown in tissue culture was studied. The cellular incorporation and esterification of free cholesterol was followed using the specific activity of serum labeled with free [^{14}C]cholesterol while the cellular sterol content was analyzed by gas-liquid chromatographic techniques. The effects produced by hyperlipemic monkey serum (HMS) and normal monkey serum (NMS) were evaluated at both comparable percentage levels in the media and at equivalent exogenous cholesterol concentrations. Under these conditions the total cholesterol content of the HMS-grown cells was 35% greater than that of NMS-grown cells, due to an elevation in free cholesterol of approximately 3 μg/mg cell protein and a 2- to 4-fold increase in esterified cholesterol. At similar percentage levels, the hyperlipemic serum stimulated a greater incorporation of free cholesterol into the monkey medial cells, accompanied by a 2-fold increase in the cellular esterification of this free cholesterol.

PARTIAL PURIFICATION AND PROPERTIES OF THE FATTY ACID ELONGATION SYSTEMS IN THE OUTER AND INNER MEMBRANES OF BEEF LIVER MITOCHONDRIA. L.W. Bond and T.I. Pynadath (Dept. of Chem., Kent St. Univ., Kent, Ohio 44242) *Biochim. Biophys. Acta* **450**, 8–20 (1976). Purified outer membrane of beef liver mitochondria was found to elongate medium chain fatty acyl-CoA primer by the incorporation of [1-^{14}C]acetyl-CoA. This enzymic activity, extracted by Triton X-100, was purified 8-fold by ammonium sulfate fractionation followed by chromatography on a Sephadex column. About 42% of the radioactivity in the fatty acids synthesized by the outer membrane enzyme system, from myristoyl-CoA and [1-C^{14}] acetyl-CoA, was in palmitic acid. Of the remaining activity, 41% was in stearic acid and about 38% in longer-chain acids. Hence, the elongation of the primer fatty acid by one C_2 unit appeared to be the predominant process in this synthesis. In the elongation of myristoyl-CoA by the inner membrane enzyme system, palmitic acid which constituted nearly 78% of the fatty acids synthesized, was the primary product.

TRANSFORMATION OF ARACHIDONIC ACID AND HOMO-γ-LINOLENIC ACID BY RABBIT POLYMORPHONUCLEAR LEUKOCYTES. MONOHYDROXY ACIDS FROM NOVEL LIPOXYGENASES. P. Borgeat, M. Hamberg and B. Samuelsson (Dept. of Chem., Karolinska Inst., S-104 01 Stockholm, Sweden) *J. Biol. Chem.* **251**, 7816–20 (1976). Addition of arachidonic acid and homo-γ-linolenic acid to a suspension of rabbit peritoneal neutrophils led to the synthesis of 5-L-hydroxy-6,8,11,14-eicosatetraenoic acid and 8-L-hydroxy-9,11,14-eicosatrienoic acid, respectively. Both hydroxy acids were found to be the main metabolites of their respective unsaturated C-20 fatty acid precursor, constituting more that 50% of the total substrate conversion. The formation of the two metabolites was not inhibited by indomethacin, indicating that the enzymes involved were unrelated to the prostaglandin synthetase system. The presence in the two compounds of a hydroxyl group α to a pair of conjugated *cis/trans* double bonds suggested that they were formed by action of lipoxygenase(s).

IN VITRO PARTIAL RELIPIDATION OF APOLIPOPROTEINS IN PLASMA. B.E. Cham and B.R. Knowles (Dept. of Med., Univ. of Queensland, Royal Brisbane Hosp., Brisbane, 4029 Australia) *J. Biol. Chem.* **251**, 6367–71 (1976). *In vitro* recombination of lipids with apolipoproteins is achieved when a concentrated solution of plasma lipids in petroleum ether is mixed with delipidated plasma. Combination of phospholipids and unesterified fatty acids are observed in amounts comparable with those originally present in the native unextracted plasma; triglycerides combine partially and cholesterol only slightly. The apparent concentrations of proteins reacting with high density and low density lipoprotein antibodies decrease when no lipid is present in plasma on assay by single radial immunodiffusion and immunoelectrophoresis, using commercially available lipoprotein antibodies. On relipidation, full immunochemical properties of high density lipoprotein are restored, but relipidated low density lipoprotein exhibits only partial immunochemical restoration.

MECHANISM OF PANCREATIC LIPASE ACTION. 2. CATALYTIC PROPERTIES OF MODIFIED LIPASES. C. Chapus and M. Semeriva (Centre de Biochim. et de Biol. Moleculaire du C.N.R.S. 31, Chemin Joseph-Aiguier, 13274 Marseille Cedex 2, France) *Biochemistry* **15**, 4988–91 (1976). Reaction of lipase with diethyl pyrocarbonate results in the modification of three histidine residues. One is highly reactive, although without affecting the activity, while the two others react more slowly with a concomitant loss of activity on both dissolved and emulsified substrates. In the three cases, the activity on emulsified substrates is abolished.

INFLUENCE OF FAT LEVEL AND TYPE OF CARBOHYDRATE ON THE CAPACITY OF PECTIN IN LOWERING SERUM AND LIVER LIPIDS OF YOUNG RATS. M.L.W. Chang and M.A. Johnson (Nutr. Inst., Agric. Res. Service, U.S. Dept. of Agr., Beltsville, Md. 20705) *J. Nutr.* **106**, 1562–8 (1976). The effect of pectin on lowering cholesterol and fat concentration in tissues of the rat was investigated in relation to the level of fat and the type of carbohydrate. Groups of rats were fed nutritionally complete diets containing equal protein:energy, varied with 5% (low-fat, 2% corn oil plus 3% beef tallow) or 20% (high-fat, 2% corn oil plus 18% beef tallow) fat and cornstarch or sucrose as carbohydrate for 2 weeks. Although there were no interactions, the feeding of pectin reduced the cholesterol level in liver and serum from dietary cholesterol to a greater degree in rats fed the low-fat than in those fed the high-fat diet. This effectiveness also varied with type of carbohydrate. The size of perirenal fat pads and the concentrations of total liver lipid and cholesterol were less in rats fed the high-fat pectin diet than in those fed the high-fat diet without pectin, indicating that pectin also had an ability to lower fat absorption. Dietary pectin increased the rate of removal of serum cholesterol, and a similar trend was shown in liver cholesterol which had accumulated from the pre-fed hypercholesterol diet.

PARTICIPATION OF A TRISACCHARIDE-LIPID IN GLYCOSYLATION OF OVIDUCT MEMBRANE GLYCOPROTEINS. W.W. Chen and W.J. Lennarz (Dept. of Physiol. Chem., The Johns Hopkins Univ. School of Med., Baltimore, Md 21205) *J. Biol. Chem.* **251**, 7802–9 (1976). Preincubation of a hen oviduct membrane preparation with UDP-*N*-acetyl-[^{14}C]glucosamine and bacitracin, followed by incubation with GDP-mannose, leads to formation of a chloroform/methanol (2/1)-extractable glycolipid. Treatment of the lipid with mild acid results in release of a trisaccharide shown to have the structure β-mannosyl-*N*-acetylglucosaminyl-*N*-acetylglucosamine. Incubation of purified trisaccharide-lipid with oviduct membranes in the presence of sodium deoxycholate, Mn^{2+}, and GDP-mannose leads to formation of a labeled glycoprotein with an apparent molecular weight of 25,000. Under these conditions no oligosaccharide-lipid is formed from the trisaccharide-lipid. Structural studies revealed that the labeled glycoprotein contained a trisaccharide side chain. These results provide direct evidence for the enzymatic formation of a lipid-linked trisaccharide containing a β-mannosyl unit, and its subsequent participation in the glycosylation of membrane glycoprotein(s).

SHORT TERM STUDY OF SUCROSE POLYESTER:A NONABSORBABLE FAT-LIKE MATERIAL AS A DIETARY AGENT FOR LOWERING PLASMA CHOLESTEROL. R.W. Fallat, C.J. Glueck, R. Lutmer and F.H. Mattson (General Clin. Res. Ctr. and Lipid Res. Lab., Univ. of Cincinnati, Cincinnati, Ohio 45247) *Am. J. Clin. Nutr.* **29**, 1204–15 (1976). The efficacy, safety, and acceptability of sucrose polyester (SPE), a fat-like material that is neither digested nor absorbed, were assessed in 13 normal and seven hypercholesterolemic subjects for its potential as a cholesterol-lowering agent. Addition or substitution of SPE for culinary fats in the diets of the normocholesterolemic individuals produced a mean reduction of total and low-density lipoprotein cholesterol of 14 and 17%, respectively ($P < 0.001$), despite the daily ingestion of a diet containing 800 mg of cholesterol and of dietary fat with a P/S ratio of 0.4. Total and low-density lipoprotein cholesterol were not significantly reduced by similar 10-day feeding periods of SPE in seven subjects with familial hypercholesterolemia. High-density lipoprotein cholesterol and triglycerides were not changed in normal or

hypercholesterolemic subjects receiving SPE. SPE was easily incorporated into routine foodstuffs in addition to, or in substitution for, conventional dietary fats. On the basis of this short term evaluation in humans and other investigations with the rat and dog, SPE appears to have potential as a cholesterol-lowering agent.

THE MECHANISM OF HYPOGLYCEMIA DUE TO SEMISTARVATION IN THE RAT. G. Gacs (Second Dept. of Paediatrics, Semmelweis Med. Univ., Budapest, 1094. Tuzolto u. 7. Hungary) *J. Nutr.* 106, 1557–61 (1976). A quantity of diet providing 50% of the energy and protein requirement was fed to 4 week old rats. After 3 weeks, the amount of food was reduced to 25% and resulted in hypoglycemia in most of the rats within 4 to 7 days. The rats were divided into three groups: normal controls; malnourished, exhibiting blood sugar levels more than 40 mg/100 ml; malnourished with blood sugar concentration less than 40 mg/100 ml. The plasma concentrations of energy providing substrates: free fatty acids, glycerol, pyruvic acid, lactic acid and alanine were significantly lower in the hypoglycemic group than in the other two groups, while the first two groups displayed no significant differences. Plasma insulin concentrations were reduced in the undernourished groups and the lowest levels were found in the hypoglycemic rats. It was concluded that in semistarvation, fatal hypoglycemia is due to the depletion of fat stores accompanied by impaired gluconeogenesis from alanine.

A SHIFT FROM PHOSPHOLIPID TO TRIGLYCERIDE SYNTHESIS WHEN CELL DIVISION IS INHIBITED BY TRANS-FATTY ACIDS. G. Graff and W.E.M. Lands (Dept. of Biol. Chem., The Univ. of Mich., Ann Arbor, Mich. 48109) *Chem. Phys. Lipids* 17, 301–14 (1976). The yeast mutant *Saccharomyces cerevisiae* (KD 46) requires added unsaturated fatty acid for growth. When cell growth was inhibited by the presence of *trans*-acids there was a marked inhibition of oleate esterification into phospholipids accompanying continued incorporation into triglycerides. Apparently some control point in phospholipid synthesis associated with the cell cycle occurs after the stage of phosphatidate biosynthesis.

CHOLESTEROL-LOWERING EFFECT OF COLESTIPOL HYDROCHLORIDE GIVEN TWICE DAILY IN HYPERCHOLESTEROLEMIC PATIENTS. K. Gundersen, E.E. Cooper, G. Ruoff, T. Nikolai and J.R. Assenzo (Dept. of Diabetes and Athero. Res., The Upjohn Com., Kalamazoo, Mich. 49001) *Atherosclerosis* 25, 303–10 (1976). In a randomized design study in 66 hypercholesterolemic patients, dosages of 10 g of colestipol HCl twice daily lowered serum cholesterol an average of 19% more than placebo therapy. These results are comparable to those in other studies in which the same total daily dose was given in three or four doses. The most common side effect was constipation, reported by 6 patients on colestipol HCl and 3 patients on placebo. No untoward systemic reaction or abnormal laboratory data were seen except for a slight rise in serum alkaline phosphatase during colestipol HCl therapy. The drug was well accepted by most patients.

ANNULAR LIPIDS DETERMINE THE ATPASE ACTIVITY OF A CALCIUM TRANSPORT PROTEIN COMPLEXED WITH DIPALMITOYLLECITHIN. T.R. Hesketh, G.A. Smith, M.D. Houslay, K.A. McGill, N.J.M. Birdsall, J.C. Metcalfe and G.B. Warren (Dept. of Biochem, Univ. of Cambridge, Cambridge CB2 1QW, United Kingdom) *Biochemistry* 15, 4145–50 (1976). Pure complexes of dipalmitoyllecithin (DPL, 16:0, 16:0) with Ca^{2+}, Mg^{2+} dependent ATPase from sarcoplasmic reticulum are unusual in retaining significant ATPase activity down to about 30 C, well below the transition temperature of the pure lipid at 41 C. A minimum of about 35 lipid molecules per ATPase is required to maintain maximal ATPase activity, but the complexes are progressively and irreversibly inactivated at lower lipid to protein ratios. Complexes containing more than the minimum lipid requirement show very similar temperature profiles of activity above 30 C over a wide range of lipid to protein ratios, up to 1,500:1. Spin-label studies indicate that, at lipid to protein ratios of less than about 30 lipids per ATPase, no DPL phase transition can be detected, but at all higher ratios, a phase transition occurs at about 41 C. In all of these complexes there are breaks in the Arrhenius plots of ATPase activity at 27–32 C and at 37.5–38.5 C. These results are interpreted as evidence for a phospholipid annulus of at least 30 lipid molecules which interact directly with the ATPase and cannot undergo a phase transition at 41 C.

COMPARISON OF PATTERNS OF FECAL BILE ACID AND NEUTRAL STEROL BETWEEN CHILDREN AND ADULTS. C.T.L. Huang, J.T. Rodriguez, W.E. Woodward and B.L. Nichols (Sec. of Nutr. and Gastroenterol., Dept. of Pediatrics, Baylor College of Med., Houston, Tex.) *Am. J. Clin. Nutr.* 29, 1196–203 (1976). The patterns of bile acids and neutral sterols in the feces of five infants under 1½ years of age, five children 4 years of age, and nine adult subjects without history of gastrointestinal diseases were studied by gas-liquid chromatography. Progressive changes in both bile acid and neutral sterol profiles were observed with maturation in infants and children. The patterns in the 4 year olds approached those observed in adults. Daily excretion of cholesterol metabolites, when expressed as mg/day increased with age: 139 ± 38, 278 ± 54 and 719 ± 211 (for five adult subjects only) for the respective groups. The corresponding values after correction by body weights were 15.4 ± 4.5, 17.9 ± 3.0 and 10.3 ± 3.0 mg/kg per day for the respective groups. The production of coprostanol was correlated with 7α-dehydroxylation of cholic acid and chenodeoxycholic acid.

DIFFERENTIAL DISTRIBUTION OF LIPOSOME-ENTRAPPED [^{3}H]-METHOTREXATE AND LABELLED LIPIDS AFTER INTRAVENOUS INJECTION IN A PRIMATE. H.K. Kimelberg (Div. of Neurosurgery and Dept. of Biochem., Albany Med. College, Albany, N.Y. 12208) *Biochim. Biophys. Acta* 448, 531–50 (1976). Positive liposomes consisting of phosphatidylcholine, cholesterol and stearylamine and negatively charged liposomes consisting of phosphatidylcholine, cholesterol and phosphatidylserine, were double labelled with either ^{3}H-labelled dipalmitoyl phosphatidylcholine and [^{14}C]cholesterol or with [^{14}C]cholesterol and [^{3}H]methotrexate entrapped in the aqueous phase. The plasma levels and urinary excretion of radioactivity from sonicated and non-sonicated liposomes were then compared with the levels of radioactivity from free [^{3}H]-methotrexate during a 4 h experimental period after an initial intravenous injection in cynomolgous monkeys. Tissue uptake at the completion of the 4 h experimental period was also measured. It was found that plasma radioactivity from [^{3}H]methotrexate and [^{14}C]cholesterol in sonicated positive liposomes was cleared more slowly than from comparable non-sonicated liposomes, and considerably slower than from free [^{3}H]methotrexate.

A COMPARATIVE STUDY OF HUMAN TISSUE AND POST-HEPARIN PLASMA TRIGLYCERIDE LIPASES. G. Klose, R. De Grella and H. Greten (Klinishces Inst. fur Herzinfarktforschung, Med. Univ. Heidelberg, Heidelberg, West-Germany) *Atherosclerosis* 25, 175–82 (1976). Human post-heparin plasma contains at least two different triglyceride lipases (TGL). The plasma lipolytic activity has been attributed to extra-hepatic and hepatic origin. Both post-heparin triglyceride lipases were partially purified and characterized. With heparin-Sepharose 4 B affinity chromatography it was possible to partially purify human adipose tissue lipoprotein lipase (LPL) as well as a lipase from human liver. The effects of NaCl, pre-heparin plasma, pH and temperature on these two tissue lipases and plasma lipases were studied in parallel. Antibodies were produced against plasma hepatic triglyceride lipase (plasma H-TGL) that did not cross react with LPL. TGL activity of human liver was completely inhibited by antibodies against plasma H-TGL. From these results it appears that human post-heparin plasma contains two-triglyceride lipase activities which originate from liver and extra-hepatic tissues such as adipose tissue.

ABSORPTION OF DIETARY β-SITOSTEROL IN LAYING HENS AND ITS INCORPORATION INTO THE EGG. B.J. Kudchodkar, L. Horlick and J.B. O'Neil (Depts. of Med. and Poultry Sci., Univ. of Saskatchewan, Saskatoon, Saskatchewan, S7N OWO, Canada) *J. Nutr.* 106, 1629–36 (1976). Studies were undertaken to determine the dietary β-sitosterol absorption in laying hens and its incorporation into the egg. Hens were divided into four groups and fed a commercial low-fat laying diet. Group 1 served as controls; the diet of group 2 was supplemented with 10% corn oil; group 3 with 4% plant sterols (emulsion in carboxymethylcellulose); group 4 with 10% corn oil and 4% plant sterols. The daily β-sitosterol intake of hens in groups 1, 2, 3, and 4 was 0.036 g, 0.095 g, 2 g and 2.56 g, respectively. After consuming the diets for 30 to 40 days, cholesterol and β-sitosterol contents of the eggs were estimated by gas liquid chromatographic and mass spectrometric methods. Feeding corn oil with and without plant sterols increased cholesterol content of the eggs, while feeding plant sterols alone had no effect on egg cholesterol levels. Failure to detect any significant amount of β-sitosterol in the eggs may be due to the lack of absorption of β-sitosterol in these hens. At the peak radioactivity deposition in the eggs, only

2% of the absorbed β-sitosterol radioactivity was found in the eggs of group 4 compared to 5% found in group 1, while cholesterol radioactivity was nearly 4 to 5 times in both groups.

EFFECT OF GLYCEROL ON LIPOGENIC ENZYME ACTIVITIES AND ON FATTY ACID SYNTHESIS IN THE RAT AND CHICKEN. M.H. Lin, D.R. Romsos and G.A. Leveille (Dept. of Food Sci. and Human Nutr., Mich. State Univ., East Lansing, Mich. 48824) *J. Nutr.* 106, 1668–77 (1976). The influence of glycerol on the rates of fatty acid synthesis in liver slices from rats and chickens and in pieces of adipose tissue from rats was first studied. Then the effect of dietary glycerol on lipid metabolism in rats and chickens was examined. Media containing 3 or 10 mM glycerol depressed the rate of glucose conversion to fatty acids in rat liver slices. However, media containing up to 25 mM glycerol did not influence the rate of fatty acid synthesis in chick liver slices. The activities of citrate cleavage enzyme, fatty acid synthetase and malic enzyme in livers of rats fed the glycerol-containing diets were dramatically increased. However, this stimulation of enzyme activity occurred without a concomitant increase in the in vivo rate of fatty acid synthesis in the rat liver. In the chicken, unlike the rat, dietary glycerol did not stimulate but instead decreased hepatic malic enzyme and fatty acid synthetase activities. No significant differences in adipose tissue lipogenic enzyme activities or in the rates of fatty acid synthesis were observed in rats fed glycerol-containing diets. The lipogenic response to glycerol feeding depends on the species as well as the organ.

PHOSPHOLIPID MEMBRANE STABILIZATION BY DIMETHYLSULFOXIDE AND OTHER INDUCERS OF FRIEND LEUKEMIC CELL DIFFERENTIATION. G.H. Lyman, D. Papahadjopoulos and H.D. Preisler (The Depts. of Med. A and Exper. Pathol., Roswell Park Memorial Inst., 666 Elm St., Buffalo, N.Y. 14263) *Biochim. Biophys. Acta* 448, 460–73 (1976). A large number of low molecular weight polar cryoprotective agents have recently been found to induce erythroid differentiation of Friend leukemic cells in vitro. The effect of these agents on membrane fluidity in phospholipid vesicles was studied by determining the solid-to-liquid crystalline phase transition using differential scanning calorimetry. Some of the inducing agents studies were found to raise the normal transition temperature (T_c) by a few degrees. All of these agents were found to produce a separate transition at a much higher temperature. Changes in the head group of the phospholipid, the pH, the presence of divalent cations, and the addition of other membrane-active compounds were found to significantly influence the inducing agent's effects on the T_c of phospholipid membranes.

EFFECTS OF THE THYROID STATE ON CHOLESTEROL METABOLISM IN THE RAT. D. Mathe and F. Chevallier (Lab. de Physiol. de la Nutr., Univ. Paris XI, Batiment 447, F 91405, Orsay, France) *Biochim. Biophys. Acta* 441, 155–64 (1976). An isotopic equilibrium method which permits the in vivo measurements of cholesterol turnover processes was applied to different groups of rats: radiothyroidectomized, low-iodine fed, and L-thyroxin fed. Plasma cholesterol concentrations were enhanced after thyroidectomy and reduced by large dose of L-thyroxin. A low-iodine diet decreased plasma thyroxin level but did not affect plasma cholesterol. The proportion of de novo biosynthesized cholesterol eliminated into the feces (external secretion) was reduced while thyroxin levels were low. The fecal excretion of cholesterol and the in vitro exchange of cholesterol between erythrocytes and plasma were increased by L-thyroxin ingestion. The rate of cholesterol biosynthesis was decreased after thyroidectomy and enhanced by L-thyroxin feeding. In fact, changes in thyroid state modified indirectly the biosynthesis of cholesterol by its effects on metabolism and on the coefficient of intestinal absorption of cholesterol.

CHANGES IN THE CHOLESTEROL AND PHOSPHOLIPID CONTENT OF MOUSE SPLEEN AFTER RAUSCHER LEUKEMIA VIRUS INFECTION. A. Montfoort, W.A.M. Boere and L.J.L.D. Van Griensven (Dept. of Exper. Pathol., Med. Faculty, Erasmus Univ., Rotterdam, The Netherlands) *Lipids* 11, 798–801 (1976). The effect of Rauscher Leukemia Virus (MuLV-R) infection on the lipid composition of mouse spleen from BALB/c mice was investigated. Drastic changes in the lipid composition of the spleen as a result of tumor growth induced by the virus could be demonstrated at 21 days after infection. The molar ratio of cholesterol to phospholipids was found to be low, while a shift within the choline containing phospholipid classes resulted into a lower sphingomyelin and a higher phosphatidyl choline content of the MuLV-R infected spleen. The cholesterol ester content increased more than two-fold during tumor growth, and shifts in the fatty acid patterns of the lipids were demonstrated.

DISTINCT TESTICULAR 17-KETOSTEROID REDUCTASES, ONE IN INTERSTITIAL TISSUE AND ONE IN SEMINIFEROUS TUBULES. DIFFERENTIAL MODULATION BY TESTOSTERONE AND METABOLITES OF TESTOSTERONE. E.P. Murono and Anita H. Payne (Steroid Res. Unit, Depts. of Obstet. and Gynecol. and Biol. Chem., The Univ. of Michigan, Ann Arbor, Mich. 48109) *Biochim. Biophys. Acta* 450, 89–100 (1976). The final step in the biosynthesis of testosterone is the reduction of androstenedione, which is catalyzed by the microsomal enzyme 17-ketosteroid reductase. At the optimum pH, a 70-fold difference in K_m values was observed, 17 μM for the interstitial tissue enzyme and 0.25 μM for the enzyme from seminiferous tubules. Testosterone and metabolites of testosterone have very different effects on each of these enzyme activities. The interstitial tissue enzyme activity is inhibited by testosterone and several 5α-reduced metabolites of testosterone and by estrogens. In contrast, it was demonstrated that among the above steroids, only dihydrotestosterone inhibits the 17-ketosteroid reductase activity of seminiferous tubules and this inhibition was only observed at very high concentrations of inhibitor. Testosterone stimulated the 17-ketosteroid reductase activity of seminiferous tubules. 5α-Androstane-3α,17β-diol at low concentrations stimulated the enzyme activity from seminiferous tubules, while it had no effect at high concentrations. The remainder of the steroids tested had no effect on the 17-ketosteroid reductase activity of seminiferous tubules.

HYDROLYSIS OF TRIPHOSPHOINOSITIDES BY A SOLUBLE FRACTION OF CRITHIDIA FASCICULATA. F.B.St. C. Palmer (Dept. of Biochem., Dalhousie Univ., Halifax, Nova Scotia B3H 4H7, Canada) *Biochim. Biophys. Acta* 441, 477–87 (1976). Homogenates of *Crithidia fasciculata* (a species of Trypanosomidae) were shown to contain a phosphatase (EC 3.1.3.36) and a phosphodiesterase (EC 3.1.4.11) which hydrolyse triphosphoinositides. Approximately 30% of the diesterase and most of the phosphatase are present in the soluble fraction. The triphosphoinositide phosphatase is specifically dependent upon Mg^{2+} and is stable to storage with or without freezing. The triphosphoinositide phosphodiesterase requires Ca^{2+} and is inactivated during storage. Both activities are maximal in the presence of cetyltrimethylammonium bromide and require protection or reactivation by GSH or dithiothreitol. Unlike similar mammalian enzymes the protozoal triphosphoinositide phosphatase does not hydrolyse diphosphoinositides. The two enzymes may be separated by $(NH_4)_2SO_4$ fractionation and gel filtration on Sephadex G-200.

LIPID METABOLISM IN CULTURED AORTIC SMOOTH MUSCLE CELLS AND COMPARISON WITH OTHER CELL TYPES. PART 2. REVERSIBILITY OF LIPID ACCUMULATION CAUSED BY HYPERLIPEMIC SERUM. J.D. Pearson (Sir William Dunn School of Pathol., Univ. of Oxford, Oxford, Great Britain) *Atherosclerosis* 25, 205–12 (1976). The lipid compositions of cultured rabbit aortic smooth muscle cells and skin fibroblasts were determined for cells grown in media containing either normolipemic or hyperlipemic sera. Both cell types accumulated cholesteryl esters and triglycerides after treatment with hyperlipemic serum. Within 4 days of returning cells that had accumulated these neutral lipids to medium containing a low percentage of normolipemic serum, their concentrations in both cell types had returned to levels similar to those found in cells cultured in standard growth medium. Thus the accumulation of cholesteryl esters and triglycerides in smooth muscle cells, as in fibroblasts, may be completely reversed in vitro.

IMPAIRED GANGLIOSIDE SYNTHESIS IN RAT LIVER AFTER D-GALACTOSAMINE ADMINISTRATION IN VIVO. E. Rupprecht, C. Hans, G. Leonard and K. Decker (Biochem. Inst. der Univ. Freiburg, D-7800 Freiburg, G.F.R.) *Biochim. Biophys. Acta* 450, 45–56 (1976). D-Galactosamine reduces the hepatic content of uridine phosphates, UDP-glactose, and UDP-glucose due to an accumulation of UDP-amino sugars; this deficiency can result in severe hepatocellular damage. Alterations of glycosphingolipid synthesis in the early phase of this pathogenic process were studied by measurements of the incorporation of labeled galactose into glycosphingolipids of rat liver. [^{14}C]Galactose was injected 2 h after galactosamine administration and the specific radioactivities of the glycosphingolipid precursors, UDPgalactose and UDPglucose, were determined. The specific radioactivity of UDPgalactose, when integrated

over the whole period of radioactive synthesis, was four times higher in the galactosamine-treated animals than in the controls; the corresponding ratio of UDPglucose was 0.85.

HYDROLYSIS OF CHYLOMICRON PHOSPHATIDYLCHOLINE IN VITRO BY LIPOPROTEIN LIPASE, PHOSPHOLIPASE A_2 AND PHOSPHOLIPASE C. R.O. Scow and T. Egelrud (Sec. on Endocrinol., Lab. of Nutr. and Endocrinol., Nat'l. Inst. of Arthritis, Metabolism and Digestive Diseases, Bethesda, Md. 20014) *Biochem. Biophys. Acta* 431, 538–49 (1976). The effects of lipoprotein lipase, phospholipase A_2 and phospholipase C on chylomicron phosphatidylcholine and triacylglycerol were studied with rat lymph chylomicrons containing phosphatidylcholine labeled with [^{32}P]phosphate and [^{3}H]palmitic acid and triacylglycerol labeled with [^{14}C]oleic acid. Phospholipase A_2 and phospholipase C hydrolyzed chylomicron phosphatidylcholine, >92% in 10 min, but not triacylglycerol. The resultant phosphatidylcholine-deficient chylomicrons, which could be concentrated by ultracentrifugation and resuspended in incubation medium, were readily depleted of triacylglycerol when incubated with lipoprotein lipase. The finding indicate that phosphatidylcholine can be removed from the surface film of chylomicrons without disrupting the particles or blocking the action of lipoprotein lipase on the core triacylglycerol.

EVIDENCE THAT VITAMIN A IS NOT REQUIRED FOR THE BIOSYNTHESIS OF OVALBUMIN IN CHICKS. W.D. Sneider and G. Wolf (Dept. of Nutr. and Food Sci., Mass. Inst. of Tech., Cambridge, Mass. 02139) *J. Nutr.* 106, 1515–26 (1976). Four-day-old pullets fed a vitamin A-deficient diet were stimulated daily with 1 mg 17β-estradiol-3-benzoate/day for 6 to 19 days. The onset of vitamin A deficiency had no effect on oviduct growth in these chicks; even though vitamin A-deficient chicks showed a severe decline in growth rate while controls (fed the same diet supplemented with retinyl palmitate) continued to grow, estrogen stimulation resulted in similar oviduct size. Ovalbumin concentrations of estrogen-stimulated chicks were determined by immunoprecipitation of the soluble protein supernatant fraction of oviduct. The concentration of ovalbumin in oviducts of chicks fed a vitamin A-supplemented diet was similar to the concentration in oviducts of chicks fed a vitamin A-deficient diet.

LIPID-SACCHARIDE INTERMEDIATES IN GLYCOPROTEIN BIOSYNTHESIS. I. FORMATION OF AN OLIGOSACCHARIDE-LIPID BY THYROID SLICES AND EVALUATION OF ITS ROLE IN PROTEIN GLYCOSYLATION. M.J. Spiro, R.G. Spiro and V.D. Bhoyroo (Depts. of Med. and Biol. Chem., Harvard Med. Schl., the Elliot P. Joslin Res. Lab., and the Peter Bent Brigham Hosp., Boston, Mass. 02215) *J. Biol. Chem.* 251, 6400–8 (1976). Thyroid slices were found to incorporate radioactivity from ^{14}C-labeled sugars into the carbohydrate moiety of a polar lipid soluble in chloroform/methanol/water, 10/10/3. This radiolabeled glycolipid was purified by chromatography on DEAE-cellulose and was shown to have as its monosaccharide constituents mannose, glucose, and glucosamine. This compound could also be labeled by incubation of thyroid slices with [^{3}H]mevalonic acid or [^{32}P]phosphate as demonstrated by the coincidence of elution profiles upon DEAE-cellulose chromatography. By means of pulse-chase experiments in slices a relationship was demonstrated between the disappearance of radioactivity from the lipid-bound oligosaccharide and its appearance in protein-bound form. When protein synthesis was inhibited by the addition of puromycin during the chase period of the experiment transfer of oligosaccharide from the lipid to protein appeared to be blocked and the level of radiolabeled oligosaccharide-lipid increased.

ASYMMETRY OF THE LIPID-BILAYER OF SINDBIS VIRUS. W. Stoffel and W. Sorgo (Inst. fur Physiol. Chemie der Univ. Koln, Germany) *Chem. Phys. Lipids* 17, 324–35 (1976). The organization of the lipid bilayer of the enveloped Sindbis virus has been studied. In the model membrane which consists only of two virus specific glycoproteins and host derived lipids the latter were radioactively labelled with ^{14}C-palmitic acid by prelabelling their BHK 21 host cell lipids. The purified virus particles were submitted to neuramidase, bromelain and combromelain-neuraminidase treatment. It could be demonstrated that the N-acetyl neuraminic acid residue of the total hematoside present in the virion is hydrolyzed by neuraminidase leaving the particles fully intact. Proteolysis of the spikes leads to particle aggregation yet an unchanged hematoside content. This was fully transformed into ceramidelactoside by subsequent neuraminidase treatment. The analyses of the ceramide species present in hematoside of the control particles and ceramidelactoside derived thereof by neuraminidase hydrolysis are in very close agreement. From these experiments it is concluded that all hematoside molecules are organized in the outer half of the bilayer of the envelope.

SULFATED GLYCOPROTEINS, GLYCOLIPIDS, AND GLYCOSAMINOGLYCANS FROM SYNAPTIC PLASMA AND MYELIN MEMBRANES: ISOLATION AND CHARACTERIZATION OF SULFATED GLYCOPEPTIDES. D.L. Simpson, D.R. Thorne and H.H. Loh (Langley Porter Neuropsychiatric Res. Inst. and the Dept. of Pharmacol., Univ. of Calif. School of Med., San Francisco, Calif. 94143) *Biochemistry* 15, 5449–57 (1976). In this report we provide biochemical evidence that a highly purified synaptic plasma membrane fraction derived from rat brain, after intraventricular injection of ^{35}S-labeled sodium sulfate, is enriched in a number of large sulfated glycoproteins compared with a purified myelin fraction studied concurrently. A fraction of the detergent-solubilized sulfated glycoprotein bound specifically to concanavalin A-Sepharose. In addition, we have identified the ^{35}S-labeled lipid-soluble material in these membrane fractions as cerebroside sulfate.

INTRACELLULAR PHOSPHOLIPID TRANSFER AND EXCHANGE. P. Jill Stewart-DeHaan and W.C. McMurray (Dept. of Biochem., Univ. of Western Ontario, London, Ontario, Canada) *Chem. Phys. Lipids* 17, 290–300 (1976). The intracellular transfer of phospholipids in rat liver was studied. The factors affecting the transport and the role of phospholipid transfer proteins in this process were investigated. The procedure was based upon the labelling of microsomal phospholipids with either ^{14}C or ^{32}P, and incubation with unlabelled mitochondria in an in vitro system. The re-isolated mitochondria became labelled demonstrating an exchange of phospholipid between the two membranes. The transfer was stimulated by the addition of high-speed supernatant, was unaffected by the addition of ATP, but did not occur at 0°C. The conditions required were found to be similar, and the high-speed supernatant promoted transfer to the same extent as when labelled microsomes acted as the donor.

THE SYNTHESIS OF LIPIDS FROM [1-^{14}C]ACETATE BY HUMAN VENOUS ENDOTHELIUM IN TISSUE CULTURE. D.N. Slater (Div. of Pathol., Univ. of Sheffield, Sheffield, Great Britain) *Atherosclerosis* 25, 237–44 (1976). Endothelial cells from human umbilical cords were harvested, using a trypsin technique, grown in tissue culture and lipid synthesis studied using [1-^{14}C]acetate as a precursor. Radiosubstrate was incorporated into fatty acids, mono-, di- and triglycerides, cholesterol esters and phospholipids. Radioactivity was also present in the culture medium in the mono- and diglyceride fractions and in the phospholipids running with the solvent front.

FEED-BACK CONTROL OF CHOLESTEROL SYNTHESIS IN PARTIALLY HEPATECTOMIZED RATS. N. Takeuchi, Y. Katayama, K. Matsumiya, K. Uchida and Y. Yamamura (The Third Dept. of Internal Med., Osaka Univ. Hosp., Fukushima-ku, Osaka 553, Japan) *Biochim. Biophys. Acta* 450, 57–68 (1976). Partial hepatectomy caused a marked stimulation of cholesterol and fatty acid syntheses without affecting serum total cholesterol, total phospholipid and triacylglycerol concentrations of rats so far examined 48 h after the operation. The lipid content in the liver, especially triacylglycerol and ester cholesterol, was increased markedly by the operation. Feeding of a high cholesterol diet which elevated serum cholesterol and phospholipid levels to the partially hepatectomized rats, accelerated the accumulation of hepatic triacylglycerol and ester cholesterol by the partial hepatectomy. The increase of cholesterol synthesis after partial hepatectomy was inhibited by cholesterol feeding. Therefore, it is conceivable that the negative feed-back control of cholesterol synthesis is induced by cholesterol feeding under the stimulated cell divisions of the liver after partial hepatectomy.

ENZYMATIC SYNTHESIS OF (15S)-[15-^{3}H]PROSTAGLANDINS AND THEIR USE IN THE DEVELOPMENT OF A SIMPLE AND SENSITIVE ASSAY FOR 15-HYDROXYPROSTAGLANDIN DEHYDROGENASE. H.H. Tai (Dept. of Med., The Genesee Hosp. and the Univ. of Rochester Sch. of Med. and Dent., Rochester, N.Y. 14607) *Biochemistry* 15, 4586–92 (1976). The stereospecificity of swine renal NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase has been determined. It was found that the enzyme is a B-side specific dehydrogenase. The amount of prostaglandin oxidized is determined by the radioactivity of labeled glutamate present in the supernatant after charcoal precipitation of labeled prostaglandin. Concurrent assays with the

present tritium release method and the thin-layer chromatography method indicated excellent correlation. The distribution pattern indicated high levels of enzyme activity in gastrointesttinal tract, lung, kidney, and spleen. The assay method may prove to be valuable for studying enzyme turnover and enzyme regulation by hormonal and pharmacological agents.

POSTNATAL PATTERNS OF BRAIN LIPIDS IN PROGENY OF VITAMIN B-6 DEFICIENT RATS BEFORE AND AFTER PYRIDOXINE SUPPLEMENTATION. M.R. Thomas and A. Kirksey (Dept. of Foods and Nutr., Purdue Univ., West Lafayette, Ind. 47907) *J. Nutr.* 106, 1404–14 (1976). The influence of deficient and adequate maternal intakes of pyridoxine on lipid profiles in brains of progeny at 5, 10, 15, 25 and 50 days of age was studied. The effects of supplementing deficient dams at two different times with pyridoxine on the brain development of progeny were also examined. Three groups of weanling, female rats were fed diets deficient in pyridoxine (1.2 mg pyridoxine · HCl/kg diet) and another group received a control diet (30.0 mg pyridoxine · HCl/kg diet). One deficient group and the control group were fed their diets throughout growth, gestation and lactation. Two groups of dams were fed the deficient diet through growth, gestation and until 5 or 10 days postpartum when pyridoxine was supplemented by feeding the control diet. Supplementation of dams fed a low level of pyridoxine (1.2 mg/kg diet) with the vitamin beginning at 5 days postpartum reversed all observed effects of the low vitamin intake on brain lipids in progeny.

POSTNATAL PATTERNS OF FATTY ACIDS IN BRAIN OF PROGENY FROM VITAMIN B-6 DEFICIENT RATS BEFORE AND AFTER PYRIDOXINE SUPPLEMENTATION. M.R. Thomas and A. Kirksey (Dept. of Foods and Nutr., Purdue Univ., West Lafayette, Ind. 47907) *J. Nutr.* 106, 1415–20 (1976). The influence of deficient and adequate maternal intakes of pyridoxine on fatty acid profiles in brains of progeny at 5, 10, and 15 days of age was studied. The effects of two different times of initiating rehabilitation of deficient dams on the brain development of progeny at 5, 10, 15, 25 and 50 days of age were also examined. Three groups of weanling, female rats were fed diets deficient in pyridoxine (1.2 mg pyridoxine · HCl/kg diet) and a fourth group received a control diet (30.0 mg pyridoxine · HCl/kg diet) throughout growth, gestation and until 5 and 10 days postpartum. Supplementation with 30.0 mg pyridoxine · HCl/kg was begun in two deficient groups at 5 and 10 days postpartum. Supplementation of deficient dams with vitamin B-6 at 5 and 10 days postpartum prevented the reduction of ω6 fatty acids found in deficient progeny.

DIETARY FAT LEVEL AS AFFECTING RUNNING PERFORMANCE AND OTHER PERFORMANCE-RELATED PARAMETERS OF RATS RESTRICTED OR NON-RESTRICTED IN FOOD INTAKE. D. Tollenaar (Nutr. Group, Food Sci. Lab., U.S. Army Natick Development Ctr., Natick, Mass. 01760) *J. Nutr.* 106, 1539–46 (1976). The effect of food energy density on certain physical performance characteristics of rats was studied during ad libitum and weight-restricted feeding. The rate of performance increased with decreasing body weight during food restriction was significantly higher for the low fat than for the high fat group. Water consumption was considerably lower during restricted than during ad libitum feeding. Plasma glucose was lower when the 70% fat diet was fed than when compared to the other fat diets at both levels of food intake, lower from day 8 on of restricted than during ad libitum food intake, and lower on day 15 than on day 8 of the restricted intake.

EFFECT OF DIETARY LIPID ON DRUG-METABOLIZING ENZYMES. A.E. Wade and W.P. Norred (Dept. of Pharmacol., School of Pharmacy, Univ. of Georgia, Athens, Ga. 30602) *Fed. Proc.* 35, 2475–79 (1976). Male rats fed diet containing 3% corn oil for 3 weeks metabolized hexobarbital, aniline and heptachlor significantly faster than those fed fat-free diet. Half-maximal changes in aniline hydroxylation occurred in rats fed corn oil at approximately 0.1% of calories, whereas half-maximal changes in hexobarbital oxidase and heptachlor epoxidase occurred in rats fed corn oil at 1 to 1.5% of calories. Kinetic measurements of the drug-metabolizing enzyme system in washed microsomes revealed that maximal rate of aniline and ethylmorphine metabolism in male rats occurred with 3% corn oil diet, whereas maximal rate for hexobarbital occurred with 10% corn oil diet.

PURIFICATION AND CHARACTERIZATION OF ADRENAL CORTEX MITOCHONDRIAL CYTOCHROME P-450 SPECIFIC FOR CHOLESTEROL SIDE CHAIN CLEVAGE ACTIVITY. H-P. Wang and T. Kimura (Dept. of Chem., Wayne State Univ., Detroit, Mich. 48202) *J. Biol. Chem.* 251, 6068–74 (1976). Cytochrome P-450 was purified from bovine adrenal cortex mitochondria by affinity chromatography using an octylamine-substituted Sepharose column. The resulting optically clear preparation was stable at −20° for months. The specific concentration of cytochrome P-450 in the preparation was about 5 nmol of heme per mg of protein. The preparations were free of adrenodoxin, adrenodoxin reductase, phospholipids, and other heme contaminations. Polyacrylamide gel electrophoresis of the purified cytochrome P-450 preparation treated with sodium dodecyl sulfate and mercaptoethanol showed a single major band with a molecular weight of about 60,000. Fluorescence spectra showed an excitation maximum at 285 nm and an emission maximum at 305 nm with a shoulder at 330 nm as the cytochrome P-450 molecule is excited at 285 nm, or an emission maximum at 335 nm when the cytochrome molecule is excited at 305 nm. After reconstitution with adrenodoxin and its reductase, this cytochrome P-450 was highly active for cholesterol desmolase with an NADPH-generating system as electron donor but was not active for steroid 11β-hydroxylase.

COLON CANCER AND DIET, WITH SPECIAL REFERENCE TO INTAKES OF FAT AND FIBER. A.R.P. Walker (Human Biochem. Res. Unit, South African Inst. for Med. Res., P.O. Box, 1038, Johannesburg, 2000, South Africa) *Am. J. Clin. Nutr.* 29, 1417–25 (1976). Colon cancer, rare in the past, and in developing populations, currently accounts for 2 to 4% of all deaths in Western populations. Evidence suggests the primary cause to be changes in diet, which affect the bowel milieu intérieur. It is possible that in sophisticated populations, the higher concentrations of fecal bile acids and sterols, and longer transit time, favor the production of potentially carcinogenic metabolites. Of secular changes in diet, evidence suggest that the following may have etiological importance: the fall in intake of fiber-containing foods with its effects on bowel physiology, and the decreased fiber but increased fat intakes, in their respective capacities to raise concentrations of fecal bile acids, sterols, and other noxious substances. For possible prophylaxis against colon cancer, recommendations for a lower fat intake, or a higher intake of fiber-containing foods (apart from fiber ingestion from bran) are extremely unlikely to be adopted. For future research, western populations with considerably lower than average mortality rates, e.g., Seventh Day Adventists, Mormons, the rural Finnish population, as well as developing populations, demand intensive study. Also requiring elucidation are the respective roles of diet and of genetic constitution on concentrations of fecal bile acids, etc., and on transit time, in prone and nonprone populations.

THE MANIPULATION OF THE FATTY ACID COMPOSITION OF DICTYOSTELIUM DISCOIDEUM AND ITS EFFECT ON CELL DIFFERENTIATION. G. Weeks (Dept. of Microbiol., Univ. of British Columbia, Vancouver, British Columbia, V6T 1W5, Canada) *Biochim. Biophys. Acta* 450, 21–32 (1976). The fatty acid composition of *Dictyostelium discoideum* has been modified by growing the axenic strain, Ax-2, in media containing long chain polyenoic fatty acids. Large amounts of linoleic and linolenic acids are incorporated into the cellular lipids and further desaturated to two unusual fatty acids, 5,9,12-octadecatrienoic acid and 5,9,12,15-octadecatetraenoic acid, respectively. Arachidonic acid is also extensively incorporated but not further desaturated. *D. discoideum* normally contains none of the above polyenoic fatty acids, and the amount incorporated depends upon the concentration of the fatty acid in the growth media.

CHOLESTEROL CONTENT OF POLYUNSATURATED MEAT. J.R. Weyant, T.R. Wrenn, D.L. Wood and J. Bitman (USDA Nutrient Utilization Lab., Animal Physiol. and Genetics Inst., ARS Beltsville Agr. Res. Ctr., Beltsville, Md. 20705) *J. Food Sci.* 41, 1421–5 (1976). The possibility that the high blood cholesterol accompanying protected lipid feeding would cause deposition of cholesterol in body tissues and counteract advantages due to higher polyunsaturated fatty acid content was studied by comparing tissue cholesterol from cows, calves and steers fed polyunsaturated fats with those on conventional diets. Chuck, round, heart, liver, fat, plasma and milk were compared from cows fed either protected safflower oil-casein-formaldehyde (SOC-F), unprotected safflower oil-casein (SOC) or a conventional (control) hay-grain ration for 2½ yr. Ground beef of steers fed SOC-F or SOC for 6 wk had cholesterol levels of 67.1 and 65.0 mg/100 g and

C18:2 levels of 14% and 2%. Our data show that the cholesterol content of these polyunsaturated meats was not greater than in conventional products.

THE EFFECT OF CHOLESTEROL ON THE VISCOSITY OF PROTEIN-LIPID MONOLAYERS. M. Blank and Lily Soo (Dept. of Physiol., Columbia Univ., New York, N.Y. 10032) *Chem. Phys. Lipids* 17, 416–22 (1976). The addition of cholesterol to a layer of lipids in a membrane structure is generally believed to result in an increase in the viscosity of the layer. We have shown that cholesterol and two other monolayer-forming lipids markedly decrease the viscosity of a serum albumin monolayer at the decane-water interface, a model membrane system. However, when the protein monolayer already has a surface active lipid component present, the effect of added cholesterol depends upon the other substance. When the albumin monolayer contains tristearin, added cholesterol increases the viscosity. When the lipid is octadecanol, cholesterol decreases the viscosity. The dependence of the change in interfacial viscosity due to cholesterol upon the original composition of the interfacial layer may be useful for characterizing the composition of layers of unknown composition, e.g. some natural membranes.

PREPARATION OF 1-ACYL-2-SUCCINYL GLYCERO-3-PHOSPHORYLCHOLINE AND EVIDENCE AGAINST ITS INVOLVEMENT IN SUCCINATE DEHYDROGENASE ACTION. F.H. Brain, A.N. Davison, V. Natarajan and P.S. Sastry (Biochem. and Chem. Dept., Guy's Hosp. Med. School, London SE1 9RT, England) *Chem. Phys. Lipids* 17, 407–15 (1976). 1-Acyl-2-succinylglycero-3-phosphorylcholine (GPC) was synthesized and its properties described. Although 1-acyl-2-succinyl GPC is a good substrate for succinate dehydrogenase, experiments on the incorporation of [2,3-^{14}C]succinate into mitochondrial lipids gave no evidence to indicate that it is an intermediate in the enzymic oxidation of succinate to fumarate, as has been suggested earlier.

STEADY-STATE KINETICS OF LIPOXYGENASE OXYGENATION OF UNSATURATED FATTY ACIDS. M.J. Gibian and R.A. Galaway (Dept. of Chem., Univ. of Calif., Riverside, Calif. 92502) *Biochemistry* 15, 4209–14 (1976). The oxygenation of linoleate and arachidonate catalyzed by soybean lipoxygenase is subject to competitive product inhibition. For normal conditions, there is an additional inhibition due to product that causes the reaction to cease before completion. This process is reversible upon addition of further substrate and is proposed to be a chemical change of the enzyme. At very low enzyme concentrations, inactivation or adsorption of enzyme on the vessel surface is significant, leading to even lower rates and percent completions. In the very early stages of a typical catalyzed reaction, a lag, or induction period, occurs. It was previously known that this lag is eliminated by product hydroperoxide—and not by the corresponding alcohol. The hydroperoxide elimination of the lag is inhibited by the alcohol. It is proposed that this is a chemical activation of the enzyme to produce a catalytically functional form.

LIPOPROTEIN LIPASE FROM BOVINE MILK. ISOLATION PROCEDURE, CHEMICAL CHARACTERIZATION, AND MOLECULAR WEIGHT ANALYSIS. P.-H. Iverius and Ann-Margret Ostlund-Lindqvist (Dept. of Med. and Physiol. Chem., Univ. of Uppsala, Biomed. Ctr., S-751 23 Uppsala, Sweden) *J. Biol. Chem.* 251, 7791–5 (1976). Lipoprotein lipase of high purity has been isolated from bovine milk by affinity chromatography on heparin-Sepharose, adsorption to C_7-aluminum hydroxide gel, and intervent dilution chromatography on heparin-Sepharose. Chemical analysis shows that the enzyme is a glycoprotein containing 8.3% carbohydrate. The monomer molecular weight, determined under reducing conditions in 6.6 M guanidine HCl by sedimentation equilibrium ultracentrifugation and analytical gel chromatography, is 48,300 and 50,800, respectively. Analyses of the sedimentation coefficient ($s^0_{20,w}$ = 5.40 S) and the diffusion coefficient ($D^0_{20,w}$ = 48.8 $\mu m^2/s$) in a buffer of physiological pH and ionic strength yield a molecular weight of 96,900. In solution, the native enzyme thus appears to be a dimer of presumable identical subunits.

CHOLESTEROL SULFATE IN RAT TISSUES. TISSUE DISTRIBUTION, DEVELOPMENTAL CHANGE AND BRAIN SUBCELLULAR LOCALIZATION. M. Iwamori, H.W. Moser and Y. Kishimoto (Eunice Kennedy Shriver Ctr. for Mental Retardation at Walter E. Fernald State School, Waltham, Mass. 02154) *Biochim. Biophys. Acta* 441, 268–79 (1976). A reliable micromethod for the determination of the tissue level of cholesterol sulfate has been developed. Cholesterol sulfate was separated from the bulk of the free cholesterol by silica gel column chromatography, and the cholesterol sulfate fraction subjected to benzoylation. A small amount of contaminating free cholesterol and other lipids remaining in this fraction were converted to benzoyl esters while the cholesterol sulfate remained unreacted. The cholesterol sulfate was then separated from the benzoylated contaminants by a second silica gel chromatography column and subjected to solvolysis. The liberated cholesterol was determined by gas-liquid chromatography. The highest level of cholesterol sulfate in subcellular fractions of rat brain occurred in a fraction rich in nerve endings. The level here was 10 times higher than that in the mitochondrial fraction, which contained the lowest levels of this steroid sulfate. ●