ABSTRACTS

R. A. REINERS, Editor. ABSTRACTORS: R. Aguilar B., J. G. Endres, Kazuo Fukuzumi, J. Iavicoli, K. Kitsuta, F. A. Kummerow, Gladys Macy, Louise R. Morrow, E. G. Perkins, and T. H. Smouse

• Fats and Oils

ANALYSIS OF INDIVIDUAL PHOSPHATIDES IN COMMERCIAL LECITHIN. Noriko Nakao, Hiroshi Enei and Shizuyuki Ota (Ajinomoto Co., Inc., Kawasaki City). *Yukagaku* 14, 278–84 (1965). Determination of components in commercial soybean lecithin was carried out by Hübscher method. It contained 27.3% phosphatidyl choline, 14.3% phosphatidyl ethanolamine, 16.7% phosphatidyl inositol, 17% phosphatidyl serine and 18.3% others (glycerophosphoric acid, polyphosphoric acid, cyclic glycerophosphoric acid, ethanolamine, serine, etc.). Values estimated by the Dawson method were also listed.

BÖHMER NUMBER. III. COMPARISON OF ACETONE METHOD AND ETHER METHOD. Masao Imamura, Isao Niiya, Yoko Kinoshita and Taro Matsumoto (Japan Margarine & Shortening Makers Assoc., Tokyo and Nihon Univ., Tokyo). *Yukagaku* 14, 275–8 (1965). Böhmer numbers of pure lard, that mixed with beef tallow or horse fat were measured by acetone method and ether method. The melting points of glyceride and fatty acid were higher by the ether method and the Böhmer numbers of abdominal and back fat was 0.25 and 0.56, respectively, higher by the ether method than by the acetone method. The Böhmer number of lard mixed with other animal fat showed greater differences between the acetone and ether methods; the difference was 1.12 for a mixture of 20% tallow and 80% lard.

GAS CHROMATOGRAPHY OF C-18 SATURATED AND UNSATURATED FATTY ACID METHYL ESTERS. Satoshi Nakasato, Katsuhiko Higuchi and Nobuyuki Suzuki (Gov. Chem. Research Inst., Tokyo and Chiba Inst. Technology, Narashino City). *Yukagaku* 14, 338–42 (1965). The influence of various factors on the separation of methyl stearate and methyl oleate peaks was investigated using diethyleneglycol succinate polyester as the stationary liquid phase of gas chromatography. Both a thermal conductivity detector and a hydrogen flame ionization detector were employed. The degree of polymerization of polyesters had little effect on the column efficiency in the molecular weight range of 2700–5400. On the other hand, the influence of the procedure for supporting the polyesters on the solid supporters was great and the type of support had the greatest influence on column efficiency.

COLOR DEVELOPMENT OF FRYING OILS DURING HEATING. I. SOME OBSERVATIONS ON DETERMINATION AND MECHANISM OF COLOR DEVELOPMENT OF SOYBEAN OIL. Akira Mukai, Iwao Yamamoto and Shizuyuki Ota (Ajinomoto Co., Ltd., Kawasaki City). *Yukagaku* 14, 292–8 (1965). The intensity of development of color of soybean oil by heating for 7 hours at 200C was estimated by use of Lovibond Tintometer, then highly colored oil was reduced with sodium borohydride, lithium aluminum hydride and hydrazine hydrate. Infrared spectra indicated that carbonyl group and epoxy group were found to partake the development of red color. The reduction product with sodium borohydride was treated with mercuric acetate and observation of infrared spectrum indicated that the carbonyl groups related closely to the red coloration are α,β- and α,α'-unsaturated carbonyls.

CHANGES OF UNSAPONIFIABLE MATTER IN OIL BY HEATING. Goro Kajimoto and Katsunori Mukai. *Yukagaku* 14, 359–63 (1965). Soybean oil, rapeseed oil and olive oil were heated at 100, 190 and 230C, respectively, and the changes in unsaponifiable matter were examined. The decrease in unsaponifiable matter was greater with higher temperatures and longer time of heating; the decrease was about 20% by heating 26 hours at 200C and there was no change in case of autoxidation. The TLC pattern of the steroid fraction in the unsaponifiable matter was changed greatly by heating.

STABILITY OF SOYBEAN PHOSPHATIDES IN STORAGE CONDITION. I. CHANGE OF SOYBEAN PHOSPHATIDES BY HEATING. Hitoshi Enei, Noriko Nakao and Shizuyuki Ota (Ajinomoto Co., Inc., Kawasaki City). *Yukagaku* 14, 352–9 (1965). Soybean lecithin was heated for 5–60 minutes at 120–150C with soybean oil and the effects of heat treatment on the emulsifying properties of soybean lecithin were studied. The result indicated that the concentration of water-soluble compounds, such as glycerophosphoryl compounds and glycerophosphoric acid, and lysophosphatides increased with heating. Heated soybean lecithin easily made a stable emulsion of O/W type.

CHARACTERISTIC AND NUTRITIVE CHANGES OF OILS DURING HEATING. V. COMPOSITION OF DISTILLABLE PRODUCTS. Toshimi Akiya (Food Research Inst., Tokyo). *Yukagaku* 14, 347–52 (1965). The lower molecular weight products from oils heated at 300C were separated by distillation and found to be composed of acidic, carbonyl and neutral fractions. The acidic fraction consisted of free fatty acids mainly. The pattern of fatty acids was similar to that of original fatty acids composition in the early stage of heating but the content of lower fatty acid was increased with an increase of time of heating. The neutral fraction showed more than 15 peaks by gas chromatography. The main components were hydrocarbons with numbers of carbon atoms 8, 9, 10, 11 and 12. Thus, the cleavage of ester linkage was dominant at the early stage of heating at 300C, followed by the cleavage of oxidative products to lower fatty acids.

BLUEPRINT RECORDS OF THIN-LAYER CHROMATOGRAMS. N. S. Radin (Mental Health Res. Inst., Univ. of Mich., Ann Arbor, Mich.). *J. Lipid Res.* 6, 442 (1965). A new source of blueprint paper for recording thin-layer chromatograms is reported.

A REACTION TUBE FOR METHANOLYSIS; INSTABILITY OF HYDROGEN CHLORIDE IN METHANOL. Yasuo Kishimoto and N. S. Radin (Mental Health Res. Inst., Univ. of Mich., Ann Arbor, Mich.). *J. Lipid Res.* 6, 435–6 (1965). A test tube fitted with an O-ring joint closure is described for use in methanolysis of lipids with HCl-methanol. Data on the reaction of HCl with methanol during methanolysis and storage are presented.

A FEED ADAPTER FOR CONTINUOUS AUTOMATIC TRANSFER OF SOLUTIONS INTO VACUUM EVAPORATION SYSTEMS. R. E. Bailey (Dept. of Med., Univ. of Oregon Med. School, Portland, Oregon). *J. Lipid Res.* 6, 436–38 (1965). The design of a simply constructed feed adapter for continuous transportation of solutions into a vacuum evaporation system is described.

SEPARATION OF CHOLESTEROL FROM ITS COMPANIONS, CHOLESTANOL AND Δ^7-CHOLESTENOL, BY THIN-LAYER CHROMATOGRAPHY. A. S. Truswell and W. Derek Mitchell (Med. Res. Council Atheroma Res. Unit, Western Infirmary, Glasgow, Scotland). *J. Lipid Res.* 6, 438–41 (1965). A two-step thin-layer chromatographic procedure is described for separation of cholesterol from its naturally occurring companions. Impregnation of Silica Gel G with silver nitrate retards cholesterol relative to cholestan-3β-ol and Δ^7-cholestenol, which can be separated from each other on a second plate by reversed-phase thin-layer chromatography at low temperature.

BOX FOR APPLICATION OF SAMPLES TO THIN-LAYER CHROMATOGRAMS UNDER NITROGEN. R. L. Cruess and F. W. Seguin (Orthopaedic Res. Lab., Royal Victoria Hosp., Montreal, P.Q., Canada). *J. Lipid Res.* 6, 441–42 (1965). A method is described for the application under nitrogen of samples of phospholipids to thin-layer chromatograms in order to prevent oxidation of the phospholipids and to decrease the influence of atmospheric humidity on R_f values.

COLORIMETRIC ULTRAMICRO METHOD FOR THE DETERMINATION OF FREE FATTY ACIDS. M. Novak (Inst. for Care of Mother and Child, Prague, Czechoslovakia). *J. Lipid Res.* 6, 431–3 (1965). Previously described colorimetric ultramicro methods for the determination of serum and tissue fatty acids have been improved in sensitivity and selectivity by extracting cobalt rather than copper soaps by means of a solvent lighter than water instead of chloroform. The complex of Co^{++} with α-nitroso-β-naphthol is measured at 500 mμ.

RELATIONSHIP BETWEEN MONOCARBONYL COMPOUNDS AND FLAVOR OF POTATO CHIPS. B. D. Mookherjee, R. E. Deck and S. S. Chang (Dept. Food Science, Rutgers, The State Univ., New Brunswick, N.J.). *J. Agr. Food Chem.* 13, 131–34 (1965). Eighteen monocarbonyl compounds have been identified in fresh potato chips and 19 in stale potato chips. The quantitative change of each individual carbonyl compound during storage has been investigated. Among saturated aldehydes the largest increase was in hexanal and next in pentanal; among 2-alkanones the important increase was in 2-pentanone and next in 2-propanone; and among 2-enals the largest increase was in 2-heptenal and 2-octenal. Only one 2,4-dienal—viz., 2,4-decadienal—was found in both fresh and stale potato chips. Its amount was greatly decreased during storage. The mechanism of the formation of these carbonyl compounds and their relationship to the flavor of potato chips are discussed.

SEPARATION AND IDENTIFICATION OF CARBONYL AND SULFUR COM-

POUNDS IN THE VOLATILE FRACTION OF COOKED CHICKEN. L. J. Minor, A. M. Pearson, L. E. Dawson and B. S. Schweigert (Dept. Food Science, Michigan State Univ., East Lansing, Mich). *J. Agr. Food Chem.* 13, 298–300 (1965). Carbonyls, organic sulfur compounds, and hydrogen sulfide were isolated from the volatile fraction resulting when 4.5 kg. of ground chicken from White Leghorn pullets was oxidatively cooked in 5 liters of water for 13 hours at 102C and distilled under nitrogen for 10 hours at 102C. A yield of 1.8 grams of carbonyl-2,4-dinitrophenylhydrazones was obtained. Carbonyl compounds that were identified by three or more methods included derivatives of diacetyl, acetone, methyl ethyl ketone, and normal aliphatic aldehydes containing two, three, four, five, six, and eight carbon atoms. One 2,4-dien-1-al was also tentatively identified. The presence of hydrogen sulfide, mercaptan(s), and organic disulfide(s) in the volatile fraction was demonstrated by separation and identification as their lead derivatives. Results indicate that carbonyl and sulfur compounds are of major importance in chicken flavor.

FATTY ACIDS IN SOME LEAF PROTEIN CONCENTRATES. I. H. Lima, T. Richardson and M. A. Stahmann (Dept. of Biochem. and Dairy and Food Industries, Univ. of Wisconsin, Madison, Wis.). *J. Agr. Food Chem.* 13, 143–45 (1965). The total fatty acid composition of leaf protein concentrates from seven plant species was studied by gas-liquid chromatography of their methyl esters. In general, the concentrates contained from about 3 to 8% fatty acids, more than 75 to 80% of which were linolenic, palmitic, and linoleic acids. Lesser amounts of oleic, palmitoleic, and stearic acids contributed 12 to 17% of the total fatty acids. Small amounts of other saturated and unsaturated fatty acids were detected. There appeared to be no gross qualitative differences in the composition of fatty acids from the leaf protein concentrates from different species.

OXIDATIONS WITH LEAD (IV). I. MECHANISM OF THE DECARBOXYLATION OF PENTANOIC ACIDS. J. K. Kochi (Dept. of Chem., Case Inst. Tech., Cleveland, Ohio). *J. Am. Chem. Soc.* 87, 3609–19 (1965). Oxidative decarboxylations of *n*-valeric, isovaleric, and 2-methylbutyric acids with lead tetraacetate in benzene solutions have been examined at 81C. The rates of decomposition are enhanced by pyridine or valeryl peroxide. The decarboxylations are strongly inhibited by oxygen. A free-radical chain mechanism is proposed which includes butyl radicals as transient, and Pb(III) species as metastable intermediates.

METHOD FOR DETERMINATION OF WATER IN BUTTEROIL BY NEAR-INFRARED SPECTROPHOTOMETRY. Phyllis G. Kliman and M. J. Pallansch (Dairy Products Lab., East. Util. Res. and Dev. Div. U.S. Dept. Agr., Washington, D.C.). *J. Diary Sci.* 48, 859–62 (1965). A double beam, near-infrared spectrophotometer has been used to measure quantitatively the amount of water in butteroil. For an analysis, the sample under investigation is dissolved in carbon tetrachloride, split, and one-half of the material dried by addition of calcium hydride. The difference in absorption at 1.9μ between the moisture-containing portion and the dried portion of the sample is equated with the moisture content of the original samples. By use of this relatively fast and accurate method, it was found the butteroil can be rapidly dried by contact with calcium hydride, anhydrous calcium chloride, and Linde Molecular Sieve 4A. Water could also be removed from butteroil by vacuum drying of thin films of the material. In water-saturated atmospheres butteroil absorbs water at a relatively rapid rate, dependent upon temperature.

ISOLATION AND IDENTIFICATION OF THE VOLATILE FATTY ACIDS PRESENT IN HICKORY SAWDUST SMOKE. H. A. Hamid and R. L. Saffle (Food Tech. Dept., Univ. Georgia, Athens, Ga.). *Food Sci.* 30, 697–701 (1965). Acetic, propionic, butyric, *iso*-valeric, *n*-valeric, *iso*-caprioc and *n*-caproic acids were identified in hickory sawdust smoke by gas chromatography, and the relative amounts of each were determined. Formic acid could not be identified, because the flame ionization detector was not sensitive to this acid. A total of eight columns were evaluated, and three of these which gave best separation were used for identification by retention times as well as infrared spectrophotometry.

ETHYL LINOLEATE EMULSIONS FOR PARENTERAL INJECTION. K. Nath Roy (Martin & Harris, Ltd., Calcutta). *U.S. 3,198,704.* Described is a pharmaceutical preparation for parenteral injection which consists of an aqueous emulsion of ethyl linoleate with glycerol monostearate as the emulsifying agent.

INTRAMOLECULAR FATTY ACID DISTRIBUTION IN THE MILK FAT TRIGLYCERIDES OF SEVERAL SPECIES. C. P. Freeman, E. L. Jack and L. M. Smith (Dept. Food Sci. and Technol., Univ. Calif., Davis). *J. Dairy Sci.* 48, 853–58 (1965). The distribution of fatty acids in the triglycerides of some milk fats of nutritional importance was examined by the pancreatic lipase hydrolysis procedure. Milk fats were from the cow, Indian buffalo, goat, sheep, and human. A substantially shortened hydrolysis period was employed, its use validated by a comparison of the original triglyceride composition with the residual monglyceride composition of a randomly interesterified milk fat subjected to such hydrolysis. Certain fatty acids had a similar distribution pattern in all of the milk fats examined, but some differences between the ruminant and human fats were evident in the relative concentration of individual fatty acids esterified at either the 2- or the 1-, 3-positions of the triglycerides. Thus, the short-chain fatty acids C-4:0 and C-6:0 were esterified predominantly, though not exclusively, at the 1-, 3-positions in all species. C-14:0 and C-15:0 acids were preferentially esterified at the 2-position, whereas C-18:0 was concentrated at the 1-, 3-positions in each fat. Human milk fat was notable in having a greater proportion of C-16:0 in the 2-position, and of C-18:0 and C-18:1 in the 1-, 3-position than the milk fats of ruminants.

CHANGES IN LIPID COMPOSITION IN MATURING WHEAT. R. D. Daftary and Y. Pomeranz (Dept. Flour and Feed Milling Ind., Kansas State Univ., Manhattan, Kan.) *Food Sci.* 30, 577–82 (1965). Changes in lipid composition during wheat development were followed by qualitative and quantitative thin-layer chromatography (TLC) and by fractionation on silicic acid columns. Development of the wheats was accompanied by a slight decrease of lipid content on an as is basis, and by almost doubling of lipids on a kernel basis. Free fatty acids in mature wheat were less than half the amount in wheat 21–23 days preceding ripeness. A similar decrease was found in the levels of mono- and diglycerides. No consistent changes were found in phospholipid fractions of lipids extracted from wheat at various stages of maturity. Carotenoid pigments disappeared as the wheat kernel developed and matured.

THE PRINCIPAL MEANS OF OBTAINING SYNTHETIC FATTY ACIDS. C. Matasa and M. Klang (Plant Manager, Bucharest, Rumania). *Oleagineux* 20, 387 (1965). The authors survey the principal synthetic procedures for manufacturing fatty acids from natural gas, petroleum and coal.

• Fatty Acid Derivatives

HIGHER ALCOHOLS. IV. SEPARATION ALCOHOLS BY THIN-LAYER CHROMATOGRAPHY ON ACTIVATED BLEACHING EARTH. Akira Hashimoto, Aiko Hirotani and Katsunori Mukai (Univ. Osaka). *Yukagaku* **14**, 343–7 (1965). Activated bleaching earth was used as an adsorbent for thin-layer chromatography. Good results were obtained in the testing of a mixture of steary alcohol, cholesterol and liquid paraffin, a mixture of sperm whale alcohol and cholesterol, and unsaponifiable matters obtained from woolwax, beeswax, insect wax and sperm whale oil. Hexane-ethyl ether (1:1 vol. ratio) was better developer than other solvents tested.

DIRECT CONVERSION OF 2,4-DINITROPHENYLHYDRAZONE OF PALMITALDEHYDE TO ITS DIMETHYL ACETAL. V. Mahadevan, F. Phillips and W. O. Lundberg (The Hormel Inst., Univ. of Minnesota, Austin, Minn.) *J. Lipid Res.* **6**, 434–5 (1965). A simple procedure is described for the direct conversion of the 2,4-dinitrophenylhydrazone of palmitaldehyde to the corresponding dimethyl acetal, using BF_3-methanol reagent or 10% methanolic HCl in the presence of a keto compound.

SURFACE VISCOSITY OF MONOMOLECULAR FILMS OF LONG-CHAIN ALIPHATIC AMIDES, AMINES, ALCOHOLS AND CARBOXYLIC ACIDS. N. L. Jarvis (U.S. Naval Res. Lab., Wash. D.C.). *J. Phys. Chem.* **69**, 1789 (1965). The surface viscosities of monomolecular films of 14-, 16-, and 18-carbon chain aliphatic alcohols, amines, acids, and amides were determined using a canal viscometer, the viscosities being determined from the rate of flow of the film material through a narrow and relatively deep canal. The surface viscosities were measured as functions of film pressure, substrate pH, and the rate of flow of the film. The surface viscosity data for alcohols and acids were in good agreement with those previously reported viscosity values that were also determined by the canal technique. These surface viscosities determined with a canal viscometer are often an order of magnitude less than the values obtained with other types of viscometers. There are no corresponding data available in the literature with which to compare the surface viscosities of the amide and amine monolayers. Experiments were also carried out to determine which of these monolayers behaved as two-dimensional Newtonian films.

• Biochemistry and Nutrition

SERUM LIPOPROTEIN AND LIPID CHANGES IN ARTERIOSCLEROTIC BREEDER RATS. B. C. Wexler, C. D. Antony and G. W. Kittinger (Univ. of Cincinnati, College of Med., Cincinnati, Ohio). *J. Atheroscler. Res.* **4**, 131–43 (1964). In this study, measurements were made of serum lipoproteins, cholesterol and total lipid in breeder rats. These animals develop arteriosclerosis spontaneously. Histopathologic studies were made of the liver and the aorta of these same animals to determine whether changes in serum lipid chemistry could be correlated with morphological alterations in the liver and aorta. In female breeders with grossly visible arteriosclerosis and in male breeders with microscopic arteriosclerosis, the serum β-lipoprotein and cholesterol level was elevated but total lipids were reduced. These serum lipid changes were accompanied by fatty infiltration of the liver. However, the arterial lesions were relatively free of fat but contained focal subintimal accumulations of acid mucopolysaccharides. The more advanced lesions showed fibrosis, elastosis, calcification and eventual bone formation. This study revealed that although certain serum lipid parameters are elevated, no direct correlation could be established between the morphological changes and the degree of arteriosclerosis, once established, and the changes in serum lipid. These results suggest that arteriosclerosis in breeder rats is accompanied by disturbances in lipid metabolism possibly of hormonal origin, and not reflected histologically in the aorta.

INFLUENCE OF TYPE OF DIETARY FAT ON CHOLESTEROL-INDUCED ATHEROSCLEROSIS IN THE RABBIT. R. O.Vles, J. Buller, J. J. Gottenbos and H. J. Thomasson (Unilever Res. Lab., Vlaardingen; Cardiologic Dept., Wilhelmina Gasthuis, Amsterdam, The Netherlands). *J. Atheroscler. Res.* **4**, 170–83 (1964). The course of atherosclerosis in rabbits induced by feeding cholesterol appears to be influenced by the type of dietary fat. Soyabean oil retards further development of the existing lesion; indeed, the histological picture shows that the process comes to a standstill. Coconut oil, on the other hand, causes an increase in the extent of the lesion, which also showed histologically much activity. An increase in the degree of atherosclerosis is accompanied by an increase in the content of sterol, sterol esters—in particular sterol oleate—and phospholipids in the blood.

RELATIONSHIP OF WHOLE BLOOD CLOTTING TIME TO PHYSIOLOGICAL VARIATIONS IN CIRCULATNG SATURATED FREE FATTY ACIDS. Mary Tompkins and S. Dayton (Med. Service, Wadsworth Hosp., Veterans Administration Center, Los Angeles) *Proc. Soc. Exp. Biol. Med.* **119**, 588–90 (1965). Whole blood clotting times without glass contact and saturated free fatty acid concentrations of serum were determined in 9 normal human subjects in the fasting state and 1½ hours after ingestion of dextrose. The mean concentration of saturated free fatty acids after carbohydrate fell to one-half the fasting value. The mean whole blood clotting times did not change.

ALTERATION OF SEX CHARACTERISTICS OF TURKEY POULTS WITH DIETHYLSTILBESTROL. C. F. Simpson, R. H. Harms and H. R. Wilson (Dept. of Veterinary Sci., Univ. of Florida, Gainesville, Fla.). *Proc. Soc. Exp. Biol. Med.* **119**, 435–8 (1965). Male and female turkeys were started on weekly injections of diethylstilbestrol at 6, 8, and 10 weeks of age. Such treatments caused the development of secondary male characteristics consisting of gobbling, strutting, development of thickenings of the neck region, prolapsed rectums, and precocious mating actions. There were also feminizing effects from DES treatments consisting of atrophy of the testes in males and hypertrophy of the oviduct in females. Increased fat deposition and hypercholesterolemia also occurred in treated birds.

RELATIONSHIP OF AGE AND SEX OF TURKEYS TO AORTIC RUPTURES INDUCED BY DIETHYLSTILBESTROL. C. F. Simpson and R. H. Harms (Dept. of Veterinary Sci., Univ. of Florida, Gainesville, Fla.). *Proc. Soc. Exp. Biol. Med.* **119**, 509–12 (1965). Male and female turkeys were first injected with DES at 6, 8, 10 and 17 weeks of age. A high incidence of aortic ruptures occurred in all birds except those which were injected initially at 17 weeks of age, and these were essentially refractory to the disease. Total serum cholesterol was elevated and systolic blood pressure was lowered by all treatments.

EFFECT OF DEFATTED BRAIN EXTRACT AND SOY STEROLS ON PLASMA CHOLESTEROL LEVELS AND ATHEROGENESIS IN CHOLESTEROL-OIL FED COCKERELS. R. Pick, S. Jain, C. Kakita and P. Johnson (Division of Med., Michael Reese Hosp. and Med. Center, Chicago, Ill.). *Proc. Soc. Exp. Biol. Med.* **119**, 850–4 (1965). Both cerebrosides and soy sterols were very effective in reducing serum cholesterol levels, and thoracic aorta and coronary atherosclerosis in chicks fed an atherogenic diet. This effect of cerebrosides was directly proportional to the dose administered. Soy sterols were without effect on endogenously induced hypercholesterolemia produced by estrogens.

TISSUE EFFECTS OF LYSOLECITHIN INJECTED SUBCUTANEOUSLY IN MICE. G. B. Phillips, P. Bachner and D. G. McKay (College of Physicians and Surgeons, Columbia Univ., New York, N.Y.). *Proc. Soc. Exp. Biol. Med.* **119**, 846–50 (1965). A series of mice was injected subcutaneously with lysolecithin and the tissues examined at measured intervals. Within 10 minutes after injection, striking edema with necrosis of fat tissue was evident at the injection site. Subsequent changes included infiltration with neutrophil leukocytes and histiocytes and hyalinization and necrosis of muscle cells. Despite the marked edema and contrary to previous reports, vascular damage was not demonstrated by light microscopy. The production of lysolecithin could account for much of the local necrosis resulting from snakebite.

PARTICIPATION OF PHOSPHORYLATED INTERMEDIATES IN IN VIVO SYNTHESIS OF TRIGLYCERIDES FROM α-MONOGLYCERIDES IN RAT INTESTINAL MUCOSA. R. Paris and G. Clement (Lab. of Animal Physiol., School of Science, Univ. of Dijon, France). *Proc. Soc. Exp. Biol. Med.* **119**, 591–3 (1965). According to the current concept the β-monoglycerides produced by digestive hydrolysis of the triglycerides are directly acylated. Our results indicate that the α-monoglycerides yield triglycerides with phosphorylated compounds as intermediates whether they are hydrolyzed and the glycerol reutilized after phosphorylation or whether they are directly phosphorylated to lysophosphatidic acids.

FATTY ACID COMPOSITION OF SPHINGOMYELIN AND LECITHIN IN NORMAL HUMAN SERUM. J. S. O'Brien and D. H. Blankenhorn

(Dept. of Med., Univ. of Southern Calif. School of Med., Los Angeles, Calif.). *Proc. Soc. Exp. Biol. Med.* **119**, 862–66 (1965). A procedure is described for the isolation and characterization of the fatty acids of lecithin (plus lysolecithin) and sphingomyelin from blood serum. This procedure was employed for analysis of these fatty acids in 10 normal adult humans. The results indicate that serum lecithin is comprised of an approximately equal proportion of saturated and unsaturated fatty acids while sphingomyelin contains a series of saturated and monounsaturated fatty acids ranging from 14 to 25 carbon atoms. The major fatty acids of lecithin were 16:0, 18:0, 18:1 and 18:2 while the major sphingomyelin acids were 16:0, 18:0, 22:0, 24:1 and 24:0. There were no apparent age-dependent changes in the fatty acid composition of these lipids (16–56 years). The fatty acids of sphingomyelin appeared to vary in composition from subject to subject more than those of lecithin.

PLASMA LIPOPROTEIN LIPASE ACTIVITY IN ISCHAEMIC HEART DISEASE. P. J. Nestel (Dept. of Med., Univ. of Melbourne, Royal Melbourne Hosp., Melbourne, Australia). *J. Atheroscler. Res.* **4**, 193–96 (1964). Plasma lipoprotein lipase activity following an injection of heparin has been measured in 17 men with ischaemic heart disease and 17 age matched healthy men. Activity has been expressed as microequivalents of free fatty acid produced each minute during the incubation of plasma with triglyceride substrate in the presence of albumin. The mean lipolytic activities for the patients and control subjects were 15 μequiv./min/ml and 16 μequiv./min/ml respectively and did not differ significantly. The relationship between fasting plasma triglyceride concentration and lipoprotein lipase activity was found to be not significant.

CARDIOVASCULAR LESIONS, BLOOD LIPIDS, COAGULATION AND FIBRINOLYSIS IN BUTTER-INDUCED OBESITY IN THE RAT. S. Naimi, G. F. Wilgram, M. M. Nothman and S. Proger (Pratt Clinic-New England Center Hosp. and the Dept. of Med., Tufts Univ. School of Med., Boston, Mass.). *J. Nutr.* **86**, 325–32 (1965). A study was made of obesity in the rat, induced by feeding a high butter fat diet over a long period and possible effects of obesity on the development of cardiovascular lesions. A group of 17 male Wistar albino rats was fed a diet that contained 40% butter by weight (providing about 65% of the calories). To evaluate the independent contribution of obesity to the development of cardiovascular disease, repeated measurements were made of other parameters that might be affected by fats, namely, blood lipids, coagulation and fibrinolysis. The animals became grossly obese but did not develop any significant changes in blood lipids, coagulation and fibrinolysis through the period of the experiment (average of 428 days). Nor were there any significant cardiovascular lesions at the end of this period, beyond those normally observed in the aging rat. It was concluded that under the conditions of this experiment where no significant changes occurred in blood lipids, coagulation and fibrinolysis, butter-induced obesity in the rat does not materially predispose to the development of cardiovascular lesions.

EFFECTS OF DIETARY LIPID AND DIETHYLSTILBESTROL UPON LIVER FATTY ACIDS OF CHOLINE-DEFICIENT RATS. G. J. Miller and W. W. Ellis (Div. Biochem., Univ. of Wyoming, Laramie, Wyo.). *J. Nutr.* **86**, 399–405 (1965). The effects of various dietary lipids upon the composition of liver fatty acids in choline-supplemented and choline-deficient rats with and without diethylstilbestrol (DES) treatment, are presented and discussed. Changes in liver fatty acids of choline-deficient rats receiving different dietary lipids appear to be due to specific accumulation of triglycerides with their characteristic fatty acid compositions. In most cases DES treatment resulted in increases in liver monoenoic acids and decreases in stearic and arachidonic acids, particularly in the cholesterol ester fraction. However, these fatty acid effects appeared to be unrelated to the lipotropic action of DES.

MITOCHONDRIAL MEMBRANE GHOSTS PRODUCED BY LIPID PEROXIDATION INDUCED BY FERROUS ION. I. PRODUCTION AND GENERAL MORPHOLOGY. R. C. McKnight, F. Edmund Hunter, Jr. and W. H. Oehlert (Washington Univ. School of Med., Saint Louis, Mo.). *J. Biol. Chem.* **240**, 3439–46 (1965). Treatment of dilute suspensions of rat liver mitochondria in KCl media with 10 to 50 μM Fe^{2+} results in formation of lipid peroxides, an extensive fall in turbidity, and loss of proteins and lipids from the mitochondria. The effects of Fe^{2+} are not inhibited by lowering the temperature to 0C but are abolished by anaerobiosis, by iron-chelating agents, and by antioxidants. KCN, 1 *mM*, and antimycin A, 5*mM*, are partially inhibitory. Over 50% of the protien and 40% of the mitochondrial lipid is released during Fe^{2+}-induced peroxidation, in the form of very small parti-

cles that are not sedimented by centrifugation for 2 hours at 105,500 × g. Observations with phase, interference, and electron microscopy, coupled with information from sedimentation behavior, filtration characteristics, and electronic particle counting and sizing, suggest the formation of mitochondrial membrane ghosts. The membrane ghosts account for 25 to 50% of the original mitochondrial protein. Their general size and shape is the same as that of parent mitochondria, but there is a considerable decrease in their density and dry mass.

LIPOPROTEIN LIPASE ACTIVITY OF RAT AND HUMAN PLACENTA. A. Mallov and A. A. Alousi (Dept. of Pharmacology, State Univ. of New York, Upstate Med. Center, Syracuse, N.Y.). *Proc. Soc. Exp. Biol. Med.* **119**, 301–06 (1965). Lipoprotein lipase activity was found to be present in homogenates of placentas of rats pregnant 18–21 days and of human placentas obtained post partum. In the rat, the LPL activity in placenta was equal to that in heart, but much less than that found in adipose tissue. LPL activity in human placenta was smaller than that in rat placenta. Placental lipase activity was inhibited by DFP, protamine and 1 *M* NaCl, and considerably reduced in the absence of serum in the incubating medium. LPL activity in placenta may be related to the vascularity of this organ and may act to supply the fetus with fatty acids by hydrolyzing lipoproteins obtained from the maternal circulation.

METABOLISM OF ATHEROSCLEROTIC TISSUE OF RABBIT AND DOG, WITH SPECIAL REFERENCE TO ESTERASE AND LIPASE. Nelicia Maier and H. Haimovici (Surgical Division, Montefiore Hosp., New York, N.Y.). *Circulation Res.* **17**, 178–84 (1965). Esterase and lipase were studied in the aorta, liver, and serum of dogs and rabbits on an atherogenic diet. With the exception of dog serum, lipase was not present in any of the tissues studied. At a relatively early stage the atherosclerotic intima-media layer, separated at the cleavage plane, showed increased esterase activity in both animals. At later stages, a reverse trend was noted in the rabbit, whereas in the dog, the esterase activity remained markedly increased. No pre-atherosclerotic enzymatic changes were noted. The liver of both species showed increased esterase activity first and reversal to normal values later. In contrast, rabbit serum displayed no change for several months and decreased activity subsequently whereas in the dog, decreased esterase activity was noted throughout the administration of the atherogenic diet. Lipase activity in dog serum, which was unaffected for several months, decreased later. Our results expand previous findings with other arterial enzymes and indicate further that metabolic alterations of the arterial wall are associated with the development of atherosclerosis.

EFFECT OF AGE AND DIETARY FAT ON SERUM PROTEIN COMPONENTS OF THE RAT. Florence Lakshmanan and Mildred Adams (Human Nutrition Res. Division, Agricultural Res. Service, U.S. Dept. of Agriculture, Beltsville, Maryland). *J. Nutr.* **86**, 337–42 (1965). The effects of age and dietary fat on the concentration of the protein components in the serum of rats were investigated. Two groups of diets were studied: 1) a semipurified diet with the level and kind of fat varied; and 2) the same semipurified diet but with 25% replaced by dried, cooked egg and the kind but not level of fat varied. Of the serum protein components—pre-albumin, albumin + α_1-globulin, α_2-, β- and γ-globulins—pre-albumin concentration was most dependent on diet. Amount and incidence of pre-albumin varied with age and with diet, particularly with the kind and level of fat. Differences observed with the kind of fat did not appear to relate to any specific characteristic of the fat. The age at which high levels of pre-albumin occurred varied with dietary fat. The albumin and α_1-globulin, the β- and the γ-globulin all tended to be influenced by diet with the differences generally related to level of fat. The α_2-globulin was least influenced by diet. At high levels of pre-albumin, concentration of all except α_2-globulin decreased. With age, albumin + α_1-globulin decreased and the other globulins increased.

EFFECT OF LIVER DAMAGE ON PLASMA FFA RESPONSE TO EPINEPHRINE. J. Kabal and Estelle Ramey (Dept. of Physiology and Biophysics, Georgetown Univ. Med. Center, Washington, D.C.). *Proc. Soc. Exp. Biol. Med.* **119**, 708–10 (1965). Three groups of male Sprague-Dawley rats, control, partially hepatectomized and chronically poisoned with CCl_4, were injected with 1 mg/kg epinephrine in oil. Plasma FFA and blood glucose were measured over a 4-hour post-injection period. Control animals showed the usual hyperglycemic and hyperlipemic response to epinephrine within the first hour after injection. Chronic CCl_4 induced liver damage elicited a different kind of epinephrine response. There was an abnormally sustained hyperglycemia during the 4-hour post-injection period but the plasma FFA never showed any elevation above pre-injection levels.

HYPERCHOLESTEROLEMIC EFFECT OF MENHADEN OIL IN THE PRESENCE OF DIETARY CHOLESTEROL IN SWINE. E. G. Hill, C. L. Silbernick and W. O. Lundberg (The Hormel Inst., Univ. of Minnesota, Austin, Minn.). *Proc. Soc. Exp. Biol. Med.* **119**, 368–70 (1965). Thirty male miniature swine were fed a beef tallow basal ration supplemented with crude soybean phosphatides and menhaden oil with and without 0.5% cholesterol for a period of 48 weeks. The animals that received no cholesterol supplement all maintained normal plasma cholesterol values (75–85 mg %). The swine fed soybean phosphatides plus cholesterol showed no change in plasma cholesterol values. The swine fed beef tallow showed, on cholesterol supplementation, a rise in plasma cholesterol, but returned to normal in 12 weeks. The swine fed menhaden oil showed a 100% increase in plasma cholesterol when supplemented with 0.5% cholesterol and, although this level decreased, it remained substantially above the remaining groups throughout the experiment. Fecal analyses of cholesterol showed much less cholesterol in the feces of fish oil-fed pigs supplemented with cholesterol, indicating that the fish oil (or the highly unsaturated fatty acids in fish oil) may have facilitated the absorption of the dietary cholesterol. The heart and liver analyses showed much lower levels of cholesterol than the other groups, suggesting that the polyunsaturated fatty acids of the fish oil may have aided in transport of the cholesterol to prevent an accumulation in these tissue sites.

UNIQUE STEROL IN THE ECOLOGY AND NUTRITION OF DROSOPHILA PACHEA. W. B. Heed and H. W. Kircher (Dept. of Zoology, Univ. of Arizona, Tucson, Ariz.). *Science* **149**, 758–62 (1965). *Drosophila pachea*, which breeds only in the stems of senita cactus (*Lophocereus schottii*) throughout the Sonoran Desert, requires the cactus as a dietary supplement when reared on laboratory media. Δ^7-Stigmasten-3β-ol, isolated from the cactus or synthesized, can replace the cactus in the diet of flies reared nanaseptically or axenically. Δ^7-Cholesten-3β-ol and $\Delta^{5,7}$-cholestadien-3β-ol could be substituted for the cactus sterol; $\Delta^{5,7}$-stigmastadien-3β-ol produced infertile females. Cholesterol, 4α-methyl-Δ^7-cholesten-3β-ol, β-sitosterol, stigmasterol, ergosterol, and Δ^7-ergosten-3β-ol did not support larval growth.

METABOLISM OF L-ALPHA-TOCOPHEROL BY THE VITAMIN E-DEFICIENT RABBIT. C. D. Fitch and J. F. Diehl (Depts. of Biochem. and Med., School of Med., Univ. of Arkansas, Little Rock, Ark.). *Proc. Soc. Exp. Biol. Med.* **119**, 553–7 (1965). Nutritional muscular dystrophy in the rabbit can be cured by l-alpha-tocopheryl acetate administered either orally or intravenously. Based on duration of survival after a single 50 mg oral dose the average estimated daily requirement was 2.0 mg per kg of body weight for 3 rabbits that received d-alpha-tocopheryl acetate and 9.5 for 2 rabbits that received l-alpha-tocopheryl acetate. After intravenous treatment with the same dose of these compounds, using 2 rabbits in each group, the estimated requirements were 1.2 and 8.0 for the d- and l-epimers, respectively. Liver and serum concentrations of tocopherol decreased more rapidly after treatment with l-alpha-tocopheryl acetate than after treatment with the same amounts of d-alpha-tocopheryl acetate. These observations suggest that the biological inferiority of l-alpha-tocopherol is due, at least in part, to a faster rate of loss from the body.

CHEMICAL AND METABOLIC CHANGES OF HEPATIC LIPIDS FROM RATS EXPOSED TO CHRONIC RADIAL ACCELERATION. D. D. Feller, E. D. Neville, J. Oyama and E. G. Averkin (Physiology Branch, Nat. Aeronautics and Space Admin., Ames Res. Center, Moffett Field, Calif.). *Proc. Soc. Exp. Biol. Med.* **119**, 522–5 (1965). From weaning until 1 year of age female Sprague-Dawley rats were centrifuged at 3.6 and 4.7 g. Liver slices of these rats were incubated with C^{14}-labeled acetate and its incorporation into fatty acids, nonsaponifiable lipids, and CO_2 was measured. When compared with tissue from control rats, liver slices of centrifuged rats exposed to 4.7 g showed an increased formation of C^{14}-nonsaponifiable lipids. Comparable changes were not observed in rats exposed to 3.6 g. No significant alteration was noted for incorporation of acetate into fatty acids or CO_2. The total lipid content of the liver was decreased significantly in rats exposed to 4.7 g. The response evoked from long-term exposure affected synthesis of non-saponifiable lipids whereas short-term exposure affected fatty acid synthesis.

THE DISTRIBUTION OF PHOSPHOLIPID WITHIN MACROPHAGES IN HUMAN ATHEROMATOUS PLAQUES. M. G. Dunnigan (Glasgow Royal Infirmary, Glasgow, Scotland). *J. Atheroscler Res.* **4**, 144–50 (1964). The distribution of phospholipid in predominantly fibrous and predominantly fatty human aortic atherosclerotic plaques was studied using three standard histochemical methods for phospholipid. Lesions complicated by haemorrhage, thrombosis or ulceration were not examined. Non-protein-bound (sudanophilic) phospholipid was found in a variable proportion of the macrophages of fibrous and fatty plaques, a site not previously described in human atherosclerotic lesions. Phospholipid was not seen extracellularly, the extracellular lipid of the fatty plaque reacting as hydrophobic lipid. It is suggested that this intraphagocytic phospholipid may be concerned in the metabolism of plaque hydrophobic lipid.

THE INFLUENCE OF AN INHIBITOR OF CHOLESTEROL BIOSYNTHESIS (TRIPARANOL) ON THE CONCENTRATION OF STEROLS IN RAT TISSUES. J. M. DeOliveira (Res. Dept., Prof. Cruz Lima Service, School of Med., Guanabara, Brazil). *J. Atheroscler. Res.* **4**, 161–69 (1964). The concentrations of cholesterol, desmosterol and total sterols (cholesterol plus desmosterol) were determined in the tissues of rats treated with an inhibitor of cholesterol biosynthesis, triparanol. The results were compared with those obtained from non-treated rats (controls). A colorimetric method was applied to separate cholesterol from desmosterol. As a rule, it was observed that triparanol produced a decrease in cholesterol and total sterol concentration, as well as an increase in desmosterol. The greatest reduction in cholesterol concentration was observed in the aortic wall (46%) and the smallest in the brain and intestinal wall (9%). The increase in desmosterol concentration was most marked in the brain (15 times) while the minimal elevation was found in the aortic wall (1.6 times). In the kidneys, desmosterol was not detected in both control and triparanol-treated rats. The possible consequences of cholesterol depletion in the brain and adrenals have been considered. Finally, the changes in sterol concentrations in the aortic wall are discussed in relation to possible influences on the pathogenesis of atherosclerosis.

METABOLISM OF β-CAROTENE. F. D. Crain, F. J. Lotspeich and R. F. Krause (Dept. of Biochem., Med. Center, West Virginia Univ., Morgantown, West Virginia). *Proc. Soc. Exp. Biol. Med.* **119**, 606–8 (1965). Evidence has been presented through the use of doubly-labeled β-carotene and singly-labeled retinol that β-carotene is converted to product(s) other than retinol.

THE MIGRATION AND ELIMINATION OF HYDROGEN DURING BIOSYNTHESIS OF CHOLESTEROL FROM SQUALENE. J. W. Cornforth, R. H. Cornforth, C. Donninger, G. Popjak, Y. Shimizu, S. Ichii, E. Forchielli and E. Caspi (Shell Res. Limited, Milstead Lab. of Chem. Enzymology, Sittingbourne, Kent, England). *J. Am. Chem. Soc.* **87**, 3224–28 (1965). Lanosterol and cholesterol biosynthesized from 4R-4-H^3-mevalonic acid retain, respectively, five and three of the six labeled atoms present in the intermediate squalene. The cholesterol was degraded and, of the three H^3 atoms retained, one is at the 17α-position. The remaining two tritium atoms are most probably located at C-20 and C-24. This pattern of loss, retention, and distribution of H^3 is in complete harmony with the theoretical mechanisms of squalene cyclization. The absence of tritium in the steroidal nucleus confirms the intermediate oxidation of the C-3 hydroxyl during biosynthesis and shows that when the double bond of cholesterol is formed the 5α-hydrogen atom is eliminated, not rearranged.

FATTY ACID COMPOSITION OF TISSUES OF PIGS FED WHOLE PEANUTS. R. A. Chung, C. L. Ramey, C. C. Lin, J. A. Walls, S. H. Settler, W. H. Farley and E. T. Miles (School of Agr., Tuskegee Inst., Tuskegee, Alabama). *Food Sci.* **30**, 632–35 (1965). The liver of pigs fed a whole peanut supplemented diet significantly increased in fatty acid means in 18:1 (carbon chain length:number of double bonds) and 18:2, and significantly decreased in 18:0, compared with the liver of pigs on a control diet. The kidney decreased significantly in 14:0 and 17:0, and the heart decreased significantly in 16:1. These changes were primarily the result of an increased total lipid consumption since the fatty acid compositions of both dietary lipids were very similar. Of all the tissues studied (liver, heart, kidney, ham, shoulder, skin, bacon fat and chop) the liver contained the largest amount of 18:0, 18:2 and 20:4, and the least amount of 14:0, 16:0, 16:1, 17:1 and 18:1.

LIPOPROTEIN LIPASE ACTIVITY (LLA) IN ADIPOSE, MUSCLE AND AORTIC TISSUE FROM RATS OF DIFFERENT AGE IN HUMAN SUBCUTANIOUS TISSUE. C. Chlouverakis (Dept. of Med., Guy's Hosp., London Bridge, S.E. 1). *Proc. Soc. Exp. Biol. Med.* **119**, 775–8 (1965). When the lipoprotein lipase activity was expressed in enzyme units per gram of wet weight of tissue, only the LLA of the adipose tissue differed between the young and old animals, that of the young animals being almost 4 times that of the old animals ($p < 0.0002$). The LLA of the two other tissues tested was virtually the same. When LLA was expressed not in relation to the wet weight of the tissue but referred to 100 mg of tissue protein, the adipose tissue LLA was still greater in the young animals although only twice that in the old animal ($p < 0.05$). There was no difference in the muscular and aortic tissue LLA between the two groups of rats.

TRANSFER OF LABELLED CHOLESTEROL ACROSS THE AORTIC INTIMAL SURFACE OF NORMAL AND CHOLESTEROL-FED COCKERELS. S. Christensen (Dept. of Physiol., Univ. Aarhus, Denmark). *J. Atheroscler. Res.* **4**, 151–60 (1964). Citrate blood from normal and cholesterol-fed birds was incubated with 4-C^{14}-cholesterol and then reinjected to the donor birds, which were killed 10 min, 1, 2, 4, 5 or 24 hours later. Plasma elimination curves for the labelled cholesterol were consistent with the concept that, at the time of injection, about 75% of the label in plasma was part of plasma lipoproteins. The thoracic aorta was split into two layers of approximately equal thickness. These layers were studied for the uptake of labelled cholesterol. In some experiments free and esterified cholesterol were separated. The uptake of label by the inner layer was used in calculations of the rate of transfer of plasma total cholesterol across the intimal surface. In normal birds a value of 0.10μg/cm²/h was obtained. In cholesterol-fed birds an average value of 0.23μg/cm²/h was calculated for the period of cholesterol feeding. At the time of experimentation the rate was calculated at 0.40μg/cm²/h. A co-variation was present between the rate of transfer of plasma cholesterol across the intimal surface and the aortic intima-media cholesterol concentration in 25 birds (P 0.001), and also, in these birds, between the plasma and intima-media cholesterol concentrations (P 0.001). The rate of transfer of plasma cholesterol into intima-media was 6 times as big as the rate of accumulation of cholesterol in intima-media during cholesterol feeding.

METABOLISM OF LABELLED STEROID PRECURSORS BY NORMAL BOVINE ADRENAL MEDULLA LN VITRO. A. Carballeira, A. Mehdi and Eleanor Venning (Depts. of Investigative Med. and Experimental Med., McGill Univ., Montreal, Canada). *Proc. Soc. Exp. Biol. Med.* **119**, 751–56 (1965). Normal bovine adrenal medulla can effect the following reactions *in vitro*: a) Transformation of a straight-chain precursor (acetate) into steroid tetracyclic structures; b) Double bond shifting from Δ^5 to Δ^4 with concomitant oxidation at C_3; c) Introduction of —OH groups at specific sites of the pregnane nucleus and d) Conversion of C_{21} to C_{19} steroids. It has consistently failed to metabolize cholesterol to corticosteroids. It has shown in several steroid biosynthetic reactions a greater responsiveness to diphospho- and triphosphopyridine nucleotides than the cortex.

SQUALENE IN PISUM SATIVUM. ITS CYCLIZATION TO β-AMYRIN AND LABELING PATTERN. E. Capstack, Jr., N. Rosin, G. A. Blondin, and W. R. Nes (Dept. of Chem. Clark Univ., Worcester, Mass.) *J. Biol. Chem.* **240**, 3258–63 (1965). A cell-free preparation from peas was found to be capable of cyclizing squalene to β-amyrin. The labeling pattern of squalene biosynthesized in peas was found to correspond to the labeling pattern of squalene isolated from animal tissues. A postulate based on the stereochemistry of equilibration of the Δ^2-and Δ^3-isopentenyl pyrophosphates is offered as an explanation for the observed labeling pattern of squalene. It states that the hydrogen atom removed from or attacking C-2 of the isopentenyl pyrophosphate will be cis-oriented with respect to the terminal carbon atom which receives or loses a hydrogen atom.

SERUM LIPIDS OF MEN FED DIETS DIFFERING IN PROTEIN QUALITY AND LINOLEIC ACID CONTENT. Ada Campbell, M. E. Swendseid, W. H. Griffith and S. G. Tuttle (School of Public Health, Univ. of California, Los Angeles, Calif.). *Am. J. Clin. Nutr.* **17**, 83–87 (1965). When wheat gluten was substituted for a casein-lactalbumin mixture as the chief source of nitrogen in an experimental diet, less nitrogen was retained in four of five subjects tested. With six subjects studied and under the experimental conditions employed, this treatment had no significant effect on the serum content of total lipids, sterol esters, glyce-

erides, phospholipids or unesterified fatty acids. This situation pertained whether the diet contained 12% linoleic acid or 40% linoleate as fat. However, serum cholesterol levels were lower and the per cent of linoleate in the sterol ester, glyceride and phospholipid serum fractions was increased when the subjects were fed diets containing the larger amount of the polyunsaturated fatty acid with either type of protein.

COENZYME Q_9 (UBIQUINONE (45)) AND ERGOSTEROL IN CRITHIDIA FASCICULATA. J. P. Kusel and M. M. Weber (Dept. of Microbiology, St. Louis Univ. School of Med., St. Louis, Mo.). *Biochim Biophys. Acta* 98, 632–39 (1965). A quinone has been isolated from the hemoflagellate *Crithidia fasciculata* and characterized as a coenzyme Q_9 (ubiquinone (45)) by its ultraviolet absorption spectrum, melting point, and chromatographic behavior. Ultraviolet absorption spectra of solvent extracts of the organism indicated the presence of a sterol. It was identified as ergosterol by its characteristic ultraviolet and infrared absorption spectra, as well as by the melting points of the compound and its benzoate and acetate derivatives. Kinetic studies on the lysis of the organism by digitonin suggest that the sterol is located in the cell membrane.

STUDIES ON THE INHIBITORY MECHANISM OF SOME HYPOCHOLESTEROLEMIC AGENTS ON 7-DEHYDROCHOLESTEROL Δ^7-BOND REDUCTASE ACTIVITY. R. Niemiro and R. Fumagalli (Inst. of Pharmacology, Univ. of Milan, Milan, Italy). *Biochim. Biophys. Acta* 98, 624-31 (1965). The reduction of 7-dehydrocholesterol in cell-free rat-liver homogenates has been studied. The Michaelis constant (K_m) was calculated from 11 experiments to be $1.14°10^{-3}$. Three hypocholesterolemic agents inhibited 7-dehydrocholesterol conversion by liver homogenate. *Trans*-1,4-bis (2-dichlorobenzylaminoethyl) cyclohexane dichloride (AY-9944) and Triparanol acted as non-competitive inhibitors, 20, 25-diazacholesterol as a competitive one. AY-9944 had nearly 10 times the potency of 20,25-diazacholesterol and about 100 times that of Triparanol.

GAS-LIQUID CHROMATOGRAPHIC ANALYSIS OF THE TISSUE STEROL FRACTION IN WOLMAN'S DISEASE AND RELATED LIPIDOSES. A Rosowsky, A. C. Crocker, Dorothy Trites and E. J. Modest (Harvard Med. School at the Children's Hosp., Boston, Mass.). *Biochim. Biophys. Acta* 98, 617–23 (1965). Sterol fractions from tissues of patients with Wolman's disease and other lipidoses were analyzed by gas-liquid chromatography in an effort to determine whether any structurally abnormal analogues of cholesterol are produced in these disorders. Retention times relative to cholestane as an internal standard were first established for cholesterol and nine related sterols as trimethylsilyl ethers, both individually and in mixtures. The results of this study indicate that no meaningful quantities of any sterol other than cholesterol itself are present in the tissues examined. It is therefore concluded that although the inborn error in lipid metabolism associated with Wolman's disease might involve a malfunction in the biochemical mechanism regulating the rate of cholesterol production or disposal, it does not involve a metabolic block in the normal biosynthetic pathway to cholesterol.

FUNCTION OF SPECIFIC BILE ACIDS IN CHOLESTEROL ESTERASE ACTIVITY IN VITRO. G. V. Vahouny, S. Weersing and C. R. Treadwell (Dept. of Biochem. George Washington Univ., School of Med., Washington, D.C.). *Biochim. Biophys. Acta* 98, 607–16 (1965). Substrates for synthetic and hydrolytic pancreatic juice cholesterol esterase (sterol ester hydrolase, EC 3.1.1.13), activities were solubilized in micelles of phosphatidylcholine alone or mixed micelles of phosphatidylcholine and a bile acid. The comparative effectiveness of various bile acids in solubilizing the substrates, cholesterol and oleic acid, or cholesterol oleate, was measured turbidimetrically, and the effects of the same bile acids on enzymatic activity were quantitatively determined. In esterification studies, dihydroxy bile acids solubilized substrates as effectively as trihydroxy bile acids. However, only with the latter group, cholic acid and its conjugates, was there significant cholesterol esterification. Comparable results were obtained in hydrolysis studies; essentially no splitting of cholesterol oleate occurred in the absence of trihydroxycholanic acids. Even when cholesterol oleate was effectively solubilized in mixed micelles of glycodeoxycholate and phospholipid, no enzymatic hydrolysis of the substrate occurred until taurocholate was added. Thus, cholic acid and its conjugates appear to be cofactors for pancreatic juice cholesterol esterase. Data obtained by microtitration of fatty acids and thin-layer silicic acid chromatography of the enzyme digests indicated that the bile acid did not complex with either the fatty acid or cholesterol substrates. The presence of taurocholate effectively prevented tryptic (trypsin, EC 3.4.4.4) and chymotryptic (chymotrypsin, EC 3.4.4.5) inactivation of cholesterol esterase, even though general proteolysis of pancreatic proteins was not effected. In the absence of taurocholate, trypsin addition resulted in complete loss of cholesterol esterase activity within 10 min. It appears, therefore, that the specific requirement of trihydroxy bile acids for cholesterol esterase activity, and the protective effect of taurocholate against proteolytic inactivation of this enzyme, are due to the formation of a specific bile acid-enzyme complex.

INVESTIGATION OF THE LIPID CONTENT OF NORMAL AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENT RED CELLS. A. Szeinberg, J. Zaidman and L. Clejan (Government Hosp., Tel-Hashomer, Israel). *Biochim. Biophys. Acta* 98, 598-606 (1965). Normal and glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate: NADP oxidoreductase, EC 1.1.1.49) deficient red cells have been compared in regard to their lipid content and its stabiltiy during incubation with hemolytic compounds. In fresh samples no significant differences in cholesterol or total lipid phosphorus were detected between the two kinds of red cells. Slight differences were detected in the composition of phosphatides. The mean sphingomyelin content was lower, and the phosphatdiylserine content higher, in the enzyme-deficient than in the normal cells. The differences were statistically significant at the 5% level in a material consisting of about 20 normal and 20 enzyme-deficient blood samples. However, most of the single values fell within overlapping regions, not permitting individual differentiation between the two kinds of cells. Incubation with nitrofurantoin or acetylphenylhydrazine did not lead to any alterations in the cholesterol or total lipid phosphorus. On the other hand such incubation resulted in a significant decrease of phosphatidylserine and increase of phosphatidylethanolamine in the majority of the enzyme-deficient red cell samples. The possible mechanisms and significance of these changes are discussed.

PRECURSORS OF N-ACYLETHANOLAMINES IN HEN'S EGG-YOLK LIPID. J. J. Wren and Danuta Merryfield (The Lyons Lab., London, Great Britain). *Biochim. Biophys. Acta* 98, 589-97 (1965). Long-chain N-acylethanolamines can be isolated from the non-saponifiable fraction of hen's egg-yolk lipid, but a portion of them are artifacts. The yields of artifacts obtained by applying several saponification and alcoholysis procedures to cephalins, and to mixtures of ethanolamine and triglycerides, have been determined. So-called mild aqueous saponification has given the highest yield. Base-catalyzed aminolysis is implicated as the mechanism of artifact formation. N-acylethanolamines have not been detected in yolk lipid in free state, but precursors have been detected from which the amides (10 parts/million of yolk lipid) are liberated under basic conditions. Deamination with ninhydrin permits liberation from the precursors without simultaneous artifact formation.

STUDIES ON THE PRODUCTION OF SPHINGOLIPID BASES BY THE YEAST, HANSENULA CIFERRI. M. L. Greene, T. Kaneshiro and J. H. Law (J. B. Conant Lab., Harvard Univ., Cambridge, Mass.). *Biochim. Biophys. Acta* 98, 582–88 (1965). The acetylated sphingolipid bases produced by *Hansenula ciferri* have been examined by chromatographic and degradative methods. Mixtures of fully and partially acetylated bases were extracted both from the extracellular medium and from the cells. Whole cell experiments with labeled precursors showed that phytosphingosine is derived from serine and palmitic acid, apparently by a pathway analogous to that found in animal tissues. Labeled hexadec-2-enoic acid was incorporated equally as well as palmitic acid into the sphingolipid bases, while 2-hydroxypalmitic acid was incorporated much less well than palmitate.

ISOLATION AND SEPARATION OF INOSITOL PHOSPHATES FROM HYDROLYSATES OF RAT TISSUE. U. B. Seiffert and B. W. Agranoff (Mental Health Res. Inst., Univ. of Mich., Ann Arbor, Mich.). *Biochim. Biophys. Acta* 98, 574–81 (1965). An electrophoretic method for the rapid separation of the phosphate esters of inositol is presented. It is used, together with ion-exchange chromatography, to establish the presence of inositol di- and triphosphates in hydrolysates of trichloroacetic acid residues of rat brain, liver, heart, kidney, and lung. With the aid of a P^{32} marker, inositol di- and triphosphates were also found in hydrolysates of rat erythrocyte stroma. Glycerol diphosphate

was found in hydrolysates of rat liver. Inositol tetraphosphate was not found in the hydrolysates of any of the tissues studied. Residues remaining after extraction of rat brain by established lipid extraction procedures retained substantial amounts of bound inositol di- and triphosphates.

SYNTHETIC LECITHINS CONTAINING ONE SHORT-CHAIN FATTY ACID AND THEIR BREAKDOWN BY PHOSPHOLIPASE A. P. R. Bird, G. H. DeHaas, C. H. T. Heemskerk and L. L. M. VanDeenen (Dept. of Biochem., Lab of Organic Chem., The State Univ., Utrecht, The Netherlands). *Biochim. Biophys. Acta* **98**, 566–73 (1965). The synthesis is described of two structurally isomeric L-α-lecithins containing as fatty acid residues butyric acid and oleic acid. Phospholipase A (EC 3.1.1.4) from *Crotalus adamanteus* hydrolysed the β-fatty acid linkage irrespective of the nature of the fatty acid constituent, though γ-oleoyl-β-butyryl)-L-α-lecithin was found to be much more readily attacked than (γ-butyryl-β-oleoyl)-L-α-lecithin.

THE ACTION OF PHOSPHOLIPASE A ON LIPOPROTEINS. G. V. Marinetti (Dept. Biochem., Univ. of Rochester School of Med. and Dentistry, Rochester, N.Y.). *Biochim. Biophys. Acta* **98**, 554–65 (1965). Snake venom has been shown to cause a clearing of a suspension of egg yolk. This clearing is due to phospholipase A (phosphatide acylhydrolase, EC 3.1.1.4) acting on the lipoproteins to produce lysolecithin (and lysophosphatidylethanolamine). The lysolecithin produced is capable of solubilizing the egg yolk suspension. The clearing of egg yolk suspension under controlled conditions of pH, ionic strength, and type of salt used, can be the basis for a rapid assay method for phospholipase A activity. A variety of venoms were tested and a wide range of phospholipase A activity was observed. The most active venoms were *Agkistrodon p.p., Naja naja, Ophiophagus hannah, Vipera russelli, and Micrurus fulvius.* The activity of phospholipase A was found to be inhibited to varying degrees by certain cations (Cu^{2+}, Fe^{3+}, Zn^{2+}), EDTA, iodoacetate, solvents (dioxan), formaldehyde, heating, freezing, sonication, ultraviolet irradiation, and exposure to high pH. The activity of phospholipase A was enhanced by trypsin (EC 3.4.4.4) treatment and mild HCl treatment, and by addition of ethyl ether to the egg yolk suspension. Column chromatography of a variety of snake venoms on Sephadex G-75 gave different distribution profiles for each venom. Of the venoms studied only that from *Vipera russelli* was resolved into two distinct phospholipase A containing peaks. Paper chromatography serves as a rapid check for confirming the presence of phospholipase A. The confirmation is necessary since certain agents (Al^{3+}, high pH, high salt concentration) cause a clearing of the egg yolk suspension in the absence of the enzyme.

THE ACTION OF PHOSPHOLIPASE C ON MUSCLE MICROSOMES: A CORRELATION OF ELECTRON MICROSCOPE AND BIOCHEMICAL DATA. J. B. Finean and A. Martonosi (Dept. of Med. Biochem. and Pharmacology, and Dept. of Biochem., Univ. of Birmingham, Birmingham (Great Britain)). *Biochim. Biophys. Acta.* **98**, 547–53 (1965). Electron microscope studies of muscle microsome preparations which have been inactivated by treatment with phospholipase C (phosphatidylcholine cholinephorphohydrolase EC 3.1.4.3) indicate that the diglyceride product of the hydrolysis of lecithin separates from the microsomal membrane as uniformly dense droplets. They are dispersed during reactivation of the system with a lysolecithin or a synthetic lecithin. Analyses of profile diameters indicate that the total area of membrane in the system is reduced almost in proportion to the loss of lipid during the process of hydrolysis and that it is not increased during the reactivation process. It is suggested that the reactivating lecithin does not effect a structural reconstitution of the microsomal membrane but the exact nature of the interaction remains uncertain.

TRIGLYCERIDE BIOSYNTHESIS FROM MONOGLYCERIDES IN ISOLATED SEGMENTS OF INTESTINAL MUCOSA. UTILIZATION OF AN ETHER ANALOGUE OF 2-MONOSTEARIN. S. I. Sherr and C. R. Treadwell (Dept. Biochem., George Washington Univ., School of Med., Washington, D.C.). *Biochim. Biophys. Acta* **98**, 539–46 (1965). Triglyceride synthesis from monoglycerides was investigated during uptake by everted segments of rat small intestine. 1-Monoglycerides are converted into triglycerides via a 1,3-diglyceride intermediate. 1,2-Diglycerides are also formed during this conversion in varying amounts depending on the experimental conditions. This indicates the existence of an equilibrium between the 1,2- and 1,3-diglyceride isomers. It could not be ascertained whether 1,3-diglycerides are directly acylated to form triglycerides or are first isomerized to a 1,2-diglyceride. 2-Monoglycerides are converted to triglycerides via a 1,2-diglyceride intermediate. Indirect proof of this was achieved by using the ether analogue of 2-monostearin which was also taken up by the mucosa and esterified.

METABOLISM OF PLASMALOGEN. III. RELATIVE REACTIVITIES OF ACYL AND ALKENYL DERIVATIVES OF GLYCEROL-3-PHOSPHORYLCHOLINE. W. E. M. Lands and Priscilla Hart (Dept. of Biological Chem., Univ. of Mich., Ann Arbor, Mich.). *Biochim. Biophys. Acta* **98**, 532–38 (1965). The alkenyl ether derivatives of phospholipids (plasmalogens) react at slower rates than the acyl analogs in several enzyme-catalyzed reactions. Alkenylglycerol-3-phosphorylcholine is essentially inert as a substrate for acyl-CoA:phospholipid acyltransferase. This result suggests that *in vivo* the 2-acyl substituent may be present before the alkenyl ether group is formed in the molecule. Alkenyl acylglycerol 3-phosphorylcholine is essentially inert as a substrate for cabbage phospholipase D (EC 3.1.4.4.). This lack of reactivity allows a convenient separation of alkenyl acylglycerol 3-phosphorylcholine from its diacyl analog in naturally occurring mixtures.

QUANTITATIVE STUDY OF THE PATHWAYS OF TRIGLYCERIDE SYNTHESIS BY HAMSTER INTESTINAL MUCOSA. K. Kern, Jr. and B. Borgstrom (Dept. Physiological Chem., Univ. of Lund, Lund, Sweden). *Biochim. Biophys. Acta* **98**, 520–31 (1965). The relative quantitative significance of the α-glycerophosphate and the monoglyceride pathways for triglyceride biosynthesis by the intestinal mucosa has been evaluated. Hamster intestinal rings were incubated in bile salt micellar solution with oleic acid and either 1-monoolein or the stable ether analogues of 1- and 2-monoolein. The lipids were isotopically labelled facilitating measurement of their uptake and distribution in the tissue. There was a clear-cut preference for the monoglyceride pathway. When monoglyceride or monoether was available, 80–100% of the higher glycerides were synthesized via this pathway. Both the 1- and 2- monoethers served as suitable acceptors of fatty acid. The major end product was always triglyceride. These findings indicate that the monoether was absorbed intact into the cell and that there was no positional specificity in its acylation.

ALTERATIONS IN PHOSPHOLIPID METABOLISM INDUCED BY ETHANOL ADMINISTRATION. H. J. Fallon, L. A. Pesch and G. Klatskin (Dept. of Internal Med., Yale Univ. School of Med., New Haven, Conn.). *Biochim. Biophys. Acta* **98**, 470–75 (1965). Previous investigations have shown that ethanol administration increases the requirements for choline in rats, and in large quantities may cause infiltration of the liver with lipids. The present study measures the incorporation of isotopically labeled methyl groups into the choline-containing phospholipids by liver slices obtained from normal rats and rats receiving 15% ethanol in drinking water. An increase in the incorporation of radioactive methyl groups into lecithin was noted in animals receiving ethanol for 7 days. This occurred when either methionine-Me-C^{14} or formate-C^{14} was the precursor but not when serine-3-C^{14} was employed. The incorporation of isotope into the various phospholipid fractions was determined by thin-layer chromatography. The data exclude an ethanol-induced block in the synthesis *de novo* of lecithin by the sequential methylation pathway as a cause of the previously observed findings and suggest that a decrease in the intrahepatic pool of methyl groups may occur during ethanol metabolism.

DIRECT INCORPORATION OF OCTANOATE INTO LONG-CHAIN FATTY ACIDS BY SOLUBLE ENZYMES OF MYCOBACTERIUM TUBERCULOSIS. Y. Kanemasa and D. S. Goldman (Inst. for Enzyme Res., Univ. of Wisc., Madison, Wisc.). *Biochim. Biophys. Acta* **98**, 476–85 (1965). The incorporation of acetate into long-chain fatty acids by cell-free extracts of the $H_{37}Ra$ strain of *Mycobacterium tuberculosis* is markedly stimulated by the presence of other fatty acids; the stimulation is maximal when octanoate is added to the system. A partially-purified enzyme system catalyzes the avidin-insensitive elongation of octanoyl-CoA by acetyl-CoA. In the absence of acetyl-CoA, acyl-CoA's such as octanoyl-CoA or decanoyl-CoA are incorporated directly into fatty acids by what appears to be intermolecular condensations. Thus, octanoyl-1-C^{14}-CoA yields C-16 and C-24 fatty acids with one-half and one-third respectively, of the C^{14} in the carboxyl carbon. We suggest the possibility that, in *M. tuberculosis*, synthesis of long-chain fatty acids may be accomplished through

direct condensation of shorter chain length acyl-CoA's rather than only through a stepwise elongation mechanism.

EFFECT OF DIETARY SULFUR UPON THE FATTY ACID PRODUCTION IN THE RUMEN. P. D. Whanger and G. Matrone (Dept. of Animal Science, North Carolina State, Univ. of North Carolina, Raleigh, N.C.). *Biochim. Biophys. Acta* **98**, 454-61 (1965). Sheep fitted with a ruminal tube were fed purified diets with and without sodium sulfate. The volatile fatty acids and lactic acid content was determined on the rumen fluid taken from these sheep. Also acetate-C^{14} was incubated with a washed cell suspension taken from these sheep and the incorporation into butyrate and higher acids was determined. The levels of propionate, butyrate and higher fatty acids were higher in the rumen fluid from the sulfur-fed animal than from the rumen fluid of the sulfur-deficient sheep. There were only traces of lactic acid produced in the rumen fluid of the sulfur-fed animal, but there was a large amount of lactic acid produced in that of the sulfur-deficient animal. More labeled acetate was incorporated into the higher fatty acids by the microorganisms obtained from the sulfur-fed animal than by the microorganisms from the sulfur-deficient animal. The results indicate that the microorganisms from the sulfur-fed animal can synthesize butyrate and higher fatty acids from acetate whereas the microorganisms from the sulfur-deficient animal cannot.

METABOLISM OF PHOSPHOLIPIDS. VIII. BIOSYNTHESIS OF PHOSPHATIDYLCHOLINE IN THE INTESTINAL MUCOSA. M. I. Gurr, D. N. Brindley and G. Hubscher (Dept. of Med. Biochem., Univ. of Birmingham, Birmingham Great Britain). *Biochim. Biophys. Acta* **98**, 486–501 (1965). The occurrence of the enzyme cholinephosphotransferase (CDPcholine: 1,2-diglyceride cholinesphosphotransferase, EC 2.7.8.2) has been demonstrated in the intestinal mucosa of the cat, guinea pig and rabbit. The characteristics of the enzyme with respect to substrate concentrations, pH optimum, Mg^{2+} requirement, inhibition by Tween 20 and stability to freezing and thawing were studied, using a mixed particulate subcellular fraction of cat intestinal mucosa as source of the enzyme and 1,2-diglyceride and CMPcholine-P^{32} as substrates. A fractionation of homogenates of the small intestinal mucosa of the cat and guinea pig showed that the enzyme is largely concentrated in the microsomal fraction, though comparison using enzyme markers indicated that it is also a true mitochondrial constituent. Further subfractionation of the microsomal fraction from cat small intestinal mucosa into two smooth-vesicle and one rough-vesicle fractions revealed that the cholinephosphotransferase was almost exactly divided between the rough-vesicle fraction and one of the smooth-vesicle fractions, whereas the second smooth-vesicle fraction contained negligible amounts of this enzyme. The enzyme cholinephosphate cytidyltransferase (CTP: phosphorylcholine cytidyltransferase, EC 2.7.7.15) also occurs in the intestinal mucosa of the cat indicating that the CDPcholine-dependent biosynthetic pathway for phosphatidylcholine is operating in the intestinal mucosa. The possibility of alternative pathways for the biosynthesis of phosphatidylcholine in the small intestinal mucosa were examined in studies *in vivo* using cats injected with inorganic P^{32}. From activity-time curves of the individual phospholipids it was concluded that neither the methylation of pohsphatidylethanolamine nor the Ca^{2+}-activated exchange reaction of free choline with preformed phospholipid could be a major pathway for the formation of phosphatidylcholine. The results are discussed in relation to fat absorption and formation of chylomicrons.

THE FATTY ACIDS OF INDIVIDUAL LIVER PHOSPHATIDES FROM RATS GROWN ON A FAT-FREE DIET. K. Tischer and J. L. Glenn (Dept. of Biochem., Albany Med. College of Union Univ., Albany, N.Y.). *Biochim. Biophys. Acta* **98**, 502–11 (1965). The purpose of the present study was to analyze the fatty acid composition of individual liver phosphatides from rats fed a fat-free diet for a 12-week period and commencing at the time of weaning. The effects of supplementation of either linoleic acid or linolenic acid to such a diet were also recorded. The level of 5,8,11-eicosatrienoic acid rose sharply in all phosphatides as a result of the fat-free diet, with the exception of cardiolipin, where large increases in oleic acid and palmitoleic acid were observed. Daily supplementation of either linoleic acid or linolenic acid were effective in suppressing the synthesis of 5,8,11-eicosatrienoic acid. The administration of linoleic acid with the fat-free diet resulted in the appearance of docosatetraenoic acid in some of the phosphatides, while linolenic acid supplementation led to the occurrence of eicosapentaenoic acid in all of the phosphatides.

THE ACYLATION OF LYSOPHOSPHATIDES WITH LONG-CHAIN FATTY ACIDS BY RAT BRAIN AND OTHER TISSUES. F. R. Webster (Dept. of Chem. Pathology, Guy's Hosp. Med. School, London). *Biochim. Biophys. Acta* **98**, 512–19 (1965). The acylation of lysolecithin with various long-chain fatty acids by rat-brain homogenates has been investigated. Palmitoleic and linoleic acids showed the greatest incorporation; saturated acids with chain lengths between C_{12} and C_{20} and oleic, linolenic and arachidonic acids were less effectively utilized. Lysolecithin was more actively acylated than lysophosphatidylethanolamine by brain and liver. Some acylation of lysophosphatidylserine was brought about by liver but not by brain. The lysolecithin-acylating activity of a number of rat tissues and of human brain has been studied.

THE UTILIZATION OF 1- AND 2-MONOGLYCERIDES FOR INTESTINAL TRIGLYCERIDE BIOSYNTHESIS. J. L. Brown and J. M. Johnston (Dept. of Biochem., Univ. of Texas, Southwestern Med. School, Dallas, Texas). *Biochim. Biophys. Acta* **84**, 448–57 (1964). Evidence for the intact utilization of 2-monopalmitin for triglyceride biosynthesis by the intestine has been obtained. The enzymes catalyzing the conversion of both the 1- and 2-monoglycerides to triglycerides reside primarily in the microsomes. One enzyme, monoglyceride transacylase (acyl-CoA: monoglyceride acyltransferase), accepts both the 1- and 2-isomers. The enzyme demonstrates a preferential utilization of the 2-monoglyceride. The interrelationships of the reported finding to the absorption of fats is presented.

STUDIES ON THE GANGLIOSIDE MICELLE. R. E. Howard and R. M. Burton (Dept. of Pharmacology and the Beaumont-May Institute of Neurology, Washington Univ. School of Med., St. Louis, Mo.). *Biochim. Biophys. Acta* **84**, 435–40 (1964). The molecular weight of β-ganglioside was measured by vapor pressure depression in a number of different solvents. The molecular weight was found to be near 1665, corresponding to a structure containing sphingosine, stearic acid, glucose, galactose, N-acetylgalactosamine, and two N-acetylneuraminic acid residues. In aqueos solution, the ganglioside associated to form a micelle structure with an aggregate weight of 200,000 or greater. The critical micelle concentration was found to be $1.0 \cdot 10^{-5}$M. A discussion of these properties is presented.

GANGLIOSIDES AND ACETYLOCHOLINE OF THE CENTRAL NERVOUS SYSTEM. R. M. Burton, R. E. Howard, S. Baer and Yvonna Balfour (Beaumont-May Institute of Neurology, Washington Univ. School of Med., St. Louis, Mo.). *Biochim. Biophys. Acta* **84**, 441–47 (1964). Both gangliosides and bound acetylcholine are higher in grey matter than in white. The subcellular distributions of these two compounds appear to be parallel in rat brain, occurring primarily in the crude mitochondrial fraction, and upon further fractionation both gangliosides and bound acetylcholine appear in the pinched-off nerve ending fraction. The synaptic vesicle fraction isolated after disruption of the nerve endings by osmotic shock contains both acetylcholine and gangliosides. This parallel distribution and the physical properties of gangliosides suggest a functional role for gangliosides in the transport of acetylcholine from synaptic vesicles through the presynaptic membrane.

THE OCCURRENCE AND METABOLISM IN VITRO OF UNESTERIFIED FATTY ACID IN MOUSE BRAIN. C. E. Rowe (The Med. School, Univ. of Birmingham, Birmingham, Great Britain). *Biochim. Biophys. Acta* **84**, 424–34 (1964). A method has been developed for the isolation and estimation of unesterified fatty acid in tissue extracts. This is based on treatment of extracted lipid with diazomethane followed by isolation of the methyl esters by chromatography on a thin layer of alumina. It has been found that mouse brain contains appreciable quantities of unesterified fatty acid. The principal aceids were palmitic, stearic, oleic and arachidonic acids. When mouse brain was incubated with sodium acetate-I-C^{14} for periods up to 6 hours, 28%–66% of the incorporation into lipid was into unesterified fatty acid. Of the principal acids acetate was incorporated into palmitic acid only.

THE INTESTINAL ABSORPTION AND METABOLISM OF MICELLAR SOLUTIONS OF LIPIDS. J. M. Johnston and B. Borgstrom (Dept. of Physiological Chem., Univ. of Lund, Lund, Sweden). *Biochim. Biophys. Acta* **84**, 412–23 (1964). The absorption of micellar solutions of conjugated bile salts and labeled fatty

acids and/or monoglycerides have been demonstrated employing intestinal slices and brush border preparations. The absorption of the lipid from these solutions appears to be enzymatically and energetically independent and occurs at a faster rate than corresponding solutions bound to albumin or in the emulsified state. The resynthesis of the absorbed micellar lipids into triglycerides has been demonstrated and the relationship of these findings to the mechanism of the absorption of fats *in vivo* discussed.

THE UPTAKE OF CHYLOMICRON FATTY ACIDS BY ISOLATED LIVER CELLS. C. Green and Joan Webb (Biochem. Dept. Univ. of Liverpool, Liverpool, Great Britain). *Biochim. Biophys. Acta* 84, 404–11 (1964). Rats were fed tripalmitin-C^{14} and guinea-pigs injected with C^{14}-labelled chylomicrons and the livers examined at various time intervals. Most of the C^{14} was associated with the individual liver cells but up to 43% of the total in the tissue could be free of the cells. Isolated rat-liver cells can bind chylomicrons to the extent of 40 μg lipid per mg tissue N. The bound chylomicrons are hydrolysed to give free fatty acids. The fatty acids produced are firmly bound but can be removed by fatty acid-free albumin, suggesting that hydrolysis occurs at the cell surface. The hydrolysis of chylomicron triglyceride is not inhibited by protamine sulphate or by heating the cells at 60C; nor is it stimulated by taurocholate.

SUSCEPTIBILITY OF ERYTHROCYTES OF VARIOUS ANIMAL SPECIES TO THE MENOLYTIC AND PHOSPHOLIPID SPLITTING ACTION OF SNAKE VENOM. E. Condrea, Z. Mammon, S. Aloof and A. De-Vries (The Rogoff Med. Research Inst., Dept. of Experimental Biology of the Tel Aviv Univ.) *Biochim. Biophys. Acta* 84, 365–75 (1964). A parallelism was found between hemolysis and erythrocyte phospholipid splitting induced by the action of cobra venom on washed erythrocytes of various species: guinea-pig, dog, human, rabbit. No significant phospholipid splitting was produced by cobra venom in camel and sheep erythrocytes, which are resistant to the hemolytic action of the venom. Isolated cobra venom phospholipase A (phosphatide acylhydrolas, EC 3.1.1.4) had no or slight hemolytic and phospholipid splitting action on the various erythrocytes, including those of the guinea-pig and dog, which are the most sensitive to the action of the whole venom. The different sensitivity of the various erythrocytes to the cobra venom is a reflection of their susceptibility to the action of venom direct lytic factor, a basic protein, which has hemolytic but no phospholipase activity. Isolated cobra venom phospholipase readily hydrolyzed the phospholipids of osmotic ghosts derived from both sensitive and resistant erythrocytes. *Vipera palestinae* venom which did not lyse the erythrocytes of any of the species tested, was also unable to hydrolyse the phospholipids in their osmotic ghosts. Cobra-venom direct lytic factor rendered the phospholipids of osmotic ghosts derived from sensitive erythrocytes available to the action of *V. palestinae* venom, but was not able to do so in the case of osmotic ghosts derived from resistant erythrocytes.

ON THE OCCURRENCE OF DIPHOSPHOINOSITOL IN THE LIPIDS OF LIVER AND PANCREAS. G. A. Kfoury and S. E. Kerr (Dept. of Biochem., Amer. Univ. of Beirut, Beirut, Lebanon). *Biochim. Biophys. Acta* 84, 391–403 (1964). The lipids of liver were reexamined for polyphosphoinositide, previously reported absent. The presence of a diphosphoinositide (DPI) was revealed by the isolation of inositol diphosphate (IP_2) from an acid hydrolysate, and its glyceryl derivative from an alkaline hydrolysate of the concentrated phospholipid fraction. Starting with lipids extracted from liver and pancreas by azeotropic dehydration with 1,2-dichloroethane, materials soluble in acetone and alcohol were removed. After partition of the alcohol-insoluble phospholipids in CCl_4-light petroleum-methanol-H_2O (31:31:35:3, v/v), the non-polar fraction was assayed for inositol polyphosphate by chromatographic fractionation of the acid hydrolysate on Dowex-2 X8 Cl^-. A diphosphate mixture eluted by NaCl or LiCl was separated by refractionation with a formate system yielding IP_2 and glycerol diphosphate. The mixed glyceryl derivatives of IP_2 and glycerol diphosphate were secured by chromatographic fractionation of the alkaline hydrolysate. Based on the recovery of IP_2 and its glyceryl derivative, the content of DPI in the original pork liver lipid is 0.28 mmoles/kg. DPI could not be extracted by ether from acetone-dehydrated fresh beef liver, but was extracted with neutral chloroform-methanol (2:1, v/v). Most of the IP_2 found in pancreatic lipid occurred in material insoluble in $CHCl_3$ and in the CCl_4-light petroleum solvent.

THE "FREE LIPIDS" OF BRUCELLA ABORTUS BANG. I. INVESTIGATION OF THE PHOSPHATIDES. W. Wober, O. W. Thiele and B. Urbaschek (Inst. of Physiology and Chem., Univ. of Gottingen,

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Germany). *Biochim. Biophys. Acta* **84**, 376–90 (1964). *Brucella abortus* Bang was grown aerobically on glycerol-thionin-agar. The "free lipids" extracted from these bacteria (yielding 1% of dry wt.) inhibit the Schultz-Dale reaction in the brucellose system. The lipid mixture was separated by acetone yielding 81.2% acetone-insoluble fraction. The acetone-insoluble part was separated by column chromatography under thin-layer chromatographic control. According to appearing or disappearing of a component in the thin-layer chromatogram the fractions obtained by column chromatography were combined to form 30 collective fractions. Each of them consists of several components. By preparative thin-layer chromatography 7 aminophosphatides were isolated from the column-chromatographic fractions. Among the 7 phosphatides phosphatidylethanolamine, phosphatidylserine, lecithin and lysolecithin were identified. One of the remaining 3 phosphatides contains ethanolamine, two contain choline among their hydrolysis products. Moreover, they contain phosphate esters other than glycerophosphate. Among the component fatty acids of the 7 phosphatides, saturated acids predominate, especially palmitic acid. Monoethenoic acids and octadecadienoic acid are also present. There are also odd-numbered straight-chain (n-C_{15}, n-C_{17}, n-C_{19}) and hydroxy fatty acids in low concentrations and perhaps saturated methyl-branched C_{17}, C_{19} and C_{21} acids. All phosphatides contain a C_{19}-cyclopropane acid which was reported recently.

EFFECT OF DIET ON THE CHOLESTEROL ESTER COMPOSITION OF LIVER AND OF PLASMA LIPOPROTEINS IN THE RAT. L. I. Gidez, P. S. Roheim and H. A. Eder (Albert Einstein College of Med., Yeshiva Univ., New York, N.Y.). *J. Lipid Res.* **6**, 377–82 (1965). The relationship between the cholesterol ester composition of the liver and the plasma lipoproteins was studied in groups of rats maintained for 5–11 weeks on the following diets: (I) rat pellets, (II) rat pellets with added olive oil and cholesterol, and (III) fat-free diets containing 0.4% cholesterol. In the control animals (Group I), the cholesterol esters of liver and $d < 1.019$ lipoproteins had nearly identical compositions and consisted mainly of oleate and linoleate. The $d > 1.063$ lipoprotein cholesterol esters were mainly linoleate and arachidonate. In the livers of rats fed olive oil and cholesterol (Group II), the cholesterol esters contained largely oleic acid and the d 1.006–1.019 lipoproteins had a very similar cholesterol ester composition. The $d > 1.063$ lipoproteins had a high proportion of esters of polyunsaturated acids and oleate. The livers of rats on the fat-free diet contained no linoleate and increased amounts of monoenoate esters as compared to the Group I control animals, and the $d < 1.019$ lipoproteins had a similar cholesterol ester composition. The $d > 1.063$ lipoproteins contained a high proportion of monoenoic acids, relatively small amounts of linoleate and arachidonate, and significant amounts of eicosatrienoate. These results suggest that different mechanisms are involved in the formation of the cholesterol esters of the various lipoproteins.

IMPROVED PROCEDURE FOR THE EXTRACTION OF LIPIDS FROM HUMAN ERYTHROCYTES. H. G. Rose and M. Oklander (General Med. Res. Lab., V.A. Hospital, Bronx, N.Y.). *J. Lipid Res.* **6**, 428–31 (1965). A procedure for the extraction of human erythrocyte lipids using chloroform-isopropanol 7:11 (v/v) is described. It is simple and reproducible, affords almost quantitative extraction of cholesterol and phospholipid, uses a single extraction tube, and yields an extract only slighty contaminated by heme.

INTERACTION OF DEXTRAN SULFATE WITH LOW-DENSITY LIPOPROTEINS OF PLASMA. M. Janado and T. Nishida (Burnsides Res. Lab., Univ. of Illinois, Urbana, Ill.). *J. Lipid Res.* **6**, 331–4 (1965). The interaction between dextran sulfate and low-density lipoproteins of the S_f0–10 class in phosphate buffer of pH 7.4 and ionic strength 0.1 was studied by means of analytical ultracentrifugation. The sedimentation pattern of the dextran sulfate-lipoprotein mixture at high dextran sulfate/lipoprotein weight ratios showed a boundary indicative of a soluble complex with a high sedimentation rate, and a free dextran sulfate boundary with a lower sedimentation rate. The amount of free dextran sulfate seemed to determine the distribution of various transition states of polymers, ranging from disintegrated units to large insoluble aggregates.

DETECTION OF PHOSPHOLIPIDS ON PAPER CHROMATOGRAMS BY NEUTRON ACTIVATION. P. Johnson, E. J. Weber, H. E. Carter and M. S. Krober (Biochem. Div., Noyes Lab. of Chem., Univ. of Ill., Urbana, Ill.). *J. Lipid Res.* **6**, 425–6 (1965). Improvements in the detection of phospholipids on paper chromatograms by neutron activation (with the formation of P^{32}-compounds) have been made. Formaldehyde-treated papers were

subjected to low neutron dosage after chromatographic development and the resulting radioactive spots were detected by automatic scanning and autoradiography.

BINDING OF METAL IONS TO MONOLAYERS OF LECITHINS, PLASMALOGEN, CARDIOLIPIN, AND DICETYL PHOSPHATE. D. O. Shah and J. H. Schulman (Stanley-Thompson Lab, School of Engineering, Columbia Univ., New York, N.Y.) *J. Lipid Res.* 6, 341–49 (1965). The surface pressure-area curves of synthetic (dipalmitoyl), egg, and yeast lecithins showed that the limiting areas depend on the degree of unsaturation of the fatty acid residues. The surface potential of phosphatidal choline (240 mv) was lower than that of the dipalmitoyl lecithin (380 mv), both at 60 A^2/molecule. This difference in surface potentials is attributed to the presence of an additional induced dipole in the double bond of the vinyl ether linkage of the plasmalogen. The binding of metal ions to lecithin and phosphatidal choline resulted in an increase in the surface potential. With different lecithins, the binding of calcium varied with the degree of unsaturation, suggesting that steric characteristics of fatty acid residues significantly influence the phospholipid-metal ion interaction. The surface pressure-area curves of lecithin, phosphatidal choline, and dicetyl phosphate were not affected by the presence of the divalent metal ions in the subsolution whereas the surface pressure-area curve of cardiolipin showed 10–13% contraction of the film in their presence. The component dipoles of the dicetyl phosphate and that of the fatty acid are explained. The increase in the surface potential of phospholipids and the contraction-expansion effect of the cardiolipin monolayer is accounted for by postulating a position for the divalent metal ion in the dipole lattice.

POST-HEPARIN PHOSPHOLIPASE AND FATTY ACID TRANSESTERIFICATION IN HUMAN PLASMA. W. C. Vogel, W. G. Ryan, J. L. Koppel and J. H. Olwin (Presbyterian-St. Luke's Hosp., College of Med., Univ. of Illinois, Chicago, Ill.). *J. Lipid Res.* 6, 335–40 (1965). The incubation of post-heparin plasma and egg phosphatidyl ethanolamine in the presence of methanol, ethanol, or glycerol resulted in the synthesis of fatty acid esters of these alcohols. This synthesis requires fatty acids derived from the degradation of phosphatidyl ethanolamine. The fatty acid transesterification activity and the post-heparin phospholipase activity were found to involve the fatty acid at the 1-position of egg phosphatidyl ethanolamine.

MODE OF ATTACK OF HYDRIODIC ACID ON UNSATURATED GLYCERYL ETHERS. D. J. Hanahan (Dept. of Biochem., Univ. of Washington, Seattle, Wash.). *J. Lipid Res.* 6, 350–55 (1965). The mode of action of hydriodic acid (HI) on a sample rich in selachyl alcohol (1-0-9(*cis*)-octadecenyl glycerol) has been studied. HI attacked the olefinic bonds as well as the ether bonds, with the formation of at least two diiodides as well as the expected primary iodide. Subsequent conversion of these iodides to the acetates yielded a mixture of saturated diacetates and monounsaturated monoacetates. The unsaturated acetates consisted of a mixture of positional isomers and showed the presence of a *trans* double bond. On the basis of these results it was concluded that HI can attack on either side of the double bond, with the resultant formation of isomeric products in the subsequent dehydrohalogenation reaction. A reaction scheme for formation of these products is presented. The use of HI in studies of the structure of unsaturated glyceryl ethers is not recommended.

ALTERATIONS IN THE LIPIDS OF BONE CAUSED BY HYPERVITAMINOSIS A AND D. R. L. Cruess and I. Clark (Royal Victoria Hospital, Montreal, and College of Physicians and Surgeons of Columbia Univ.). *Biochem. J.* 96, 262–5 (1965). The total lipid, phospholipid, total and free fatty acid, free and esterified cholesterol contents of the long bones of normal, hypervitaminotic A, D and A plus D rats were determined. Toxic amounts of vitamin A decreased the total fatty acid content; toxic amounts of vitamin D increased triglycerides, esterified cholesterol and in particular the phospholipids of bone. An interaction occurred between toxic amounts of vitamins A and D, which prevented, to a large extent, the alterations in bone lipids that occur in hypervitaminosis D. Only 50–65% of the $CHCl_3$-MeOH extractable material could be accounted for by the phospholipids, free fatty acids, triglycerides and total cholesterol. The identification of the remaining 35–50% is under investigation. According to the authors, the studies suggest an involvement of vitamin D in lipid metabolism and tend to support the idea that lipids are involved in ossification.