ABSTRACTS

R. A. REINERS, Editor. ABSTRACTORS: N. E. Bednarcyk, J. G. Endres, J. Iavicoli, K. Kitsuta, F. A. Kummerow, E. G. Perkins, T. H. Smouse, J. A. Thompson and R. W. Walker

• Fats and Oils

STORAGE AND PRESERVATION OF MARGARINE PRIOR TO DISTRIBUTION. H. Korp (Margarinbalaget AB, Stockholm, Sweden). *Rev. Franc. Corps Gras* 16, 543–549 (1969). Storage, preservation and distribution of the finished product are important steps in margarine processing. Use of satellite warehouses offers advantages of greater flexibility, reduction in the amount of immobilized capital and lower administrative costs. Examples taken from current distribution practice in the Swedish margarine industry are given. Finally, some recent developments in margarine formulation, which affect storage and handling characteristics, are mentioned.

LIPOLYTIC ACTIVITY OF CERTAIN FAMILIES OF MICROORGANISMS GROWN IN MARGARINE. K. Stefanovic *et al. Bilt. Bil. Ulja Masti* 6(1), 10–13 (1969). The lipolytic activities of *Ps. aeruginosa, B. lentus, B. subtilis, Staph. aureus* and *Strep. faecalis* in margarine were studied. The most marked changes occur at 20C, while at 4C, they remain within normal sample limits. The strongest lipolytic activity is manifested by members of the *Alcaligenes* family (*Ps. aeruginosa*), by *B. lentus* and by members of the *Micrococcus* family. (Rev. Franc. Corps Gras)

EFFECT OF SEVERAL FACTORS ON THE BLEACHING OF SUNFLOWER OIL. J. Turkulov *et al. Bilt. Bil. Ulja Masti* 6(1), 13–18 (1969). Bleaching should be carried out at 80–90C with 0.75–1% of BRV earth for 20–30 minutes. Better results are obtained with crude oil than with neutralized oil. Results obtained with Tonsil activated earth are about 20% better than those obtained with BRV earth. The color of the bleached oil should correspond to an extinction of 0.90 at 450 mμ. (Rev. Franc. Corps Gras)

PRODUCTION AND USE OF THE SOAPSTOCK AND GUMS FROM THE PROCESSING OF SUNFLOWER SEEDS. I. ENRICHMENT OF PRESSCAKES WITH SOAPSTOCK AND GUMS. L. Grozdanova *et al. Przemysl Spozywczy* 5(1), 1–15 (1969). The optimal conditions for recovering the soapstock and gums from the refining of sunflower seed oils are reported. These materials are suitable for use in various animal feeds. They may also be mixed with the oilseed presscake for animal feed. Changes occurring during storage of the enriched presscake have been studied and are reported here. (Rev. Franc. Corps Gras)

CHARACTERISTICS OF THE LIPIDS FROM SUNFLOWER SEEDS GROWN IN BULGARIA. L. Nedelceva *et al. Przemysl Spozywczy* 5(1), 17–21 (1969). Qualitative changes in the lipids from Russian sunflower seeds grown in Bulgaria in 1965–1968 are reported. The Peredovik variety adapted very well to growing conditions in Bulgaria. The qualities of linoleic acid and tocopherols were increased considerably in the second growing season. (Rev. Franc. Corps Gras)

NATURALLY OCCURRING DIOL LIPIDS: DIALKOXYPENTANES IN PORPOISE (PHOCOENA PHOCOENA) JAW OIL. U. Varanasi and D. C. Malins (Bureau of Commercial Fisheries, Food Science Pioneer Res. Lab., Seattle, Wash.). *Science* 166, 1158–1159 (1969). Dialkyl ethers of diols (dialkoxyalkanes), naturally occurring lipids, have been isolated from the jaw oil of the porpoise *Phocoena phocoena*. The principal constituents are dialkoxypentanes containing two 18-carbon chains. The alkoxy linkage may play an important role in the metabolism of the diol lipids.

GAS CHROMATOGRAPHIC COLUMN SYSTEMS EXHIBITING TEMPERATURE INDEPENDENCE OF SOLUTE RETENTION. P. F. McCrea and J. H. Purnell (Dept. Chem., Univ. College Swansea, Swansea, Wales). *Anal. Chem.* 41, 1922–29 (1969). Novel gas chromatographic systems manifest temperature independence of solute retention over certain temperature ranges, achieved through use of a conventional solvent in conjunction with a solvent whose sorbing power for vapors increases with temperature. This behavior is characteristic of systems undergoing phase changes or other transitions over an extended range of temperature. The theory of the method is developed and its validity and the practicality of the technique are established by detailed study of the characteristics of oleic acid-stearic acid mixtures used alone and in combination with squalane. Temperature-independent retention is achieved with test solute mixtures over spans of up to 10C in the region 45 to 65C. Extension of the method to incorporate the use of a wide variety of transition sorbents and to achieve wider spans of compensation is discussed, and areas of obvious application are indicated.

POROUS STAINLESS STEEL AS A CARRIER GAS SEPARATOR INTERFACE MATERIAL FOR GAS CHROMATOGRAPHY-MASS SPECTROMETRY. P. M. Krueger and J. A. McCloskey (Inst. for Lipid Res., Baylor College of Med., Houston, Texas 77025). *Anal. Chem.* 41, 1930–35 (1969). Silanized porous stainless steel of 0.1 micron mean pore size has been used as the interface material in an effusion type carrier gas separator for gas chromatography-mass spectrometry. Two interface systems have been constructed, providing effluent introduction to the mass spectrometer either through a conventional direct inlet vacuum lock using a separator-probe assembly, or through a compact, permanently mounted unit. Efficiencies of 40–48% were measured for cholestane (MW 372) with column flow rates of 7 (entire column under vacuum) and 35 ml/min and ion source pressures of $2–4 \times 10^{-5}$ mm Hg. Minimal tailing was observed for fatty acid esters, steroids and nucleoside derivatives. The M-18 peak of cholesterol was 34% of M for a sample introduced through the combination system (separator 250C) compared to 28% for conventional probe introduction at the same ion source temperature.

COMPUTERIZED LEARNING MACHINES APPLIED TO CHEMICAL PROBLEMS. INTERPRETATION OF INFRARED SPECTROMETRY DATA. B. R. Kowalski, P. C. Jurs, T. L. Isenhour and C. N. Reilley (Dept. Chem., U. Washington, Seattle, Wash. 98105). *Anal. Chem.* 41, 1945–49 (1969). Learning machine methods with computer implementation have been applied to 4500 infrared spectra. A learning criterion is presented, and compounds are placed into chemical classes by weight vectors developed from a representative training set. The learning machine method is compared to a classification method based on comparing the spectrum to average spectra and is shown superior in each of the cases tested. Included are studies of training set size, even and uneven training set distributions, a no-decision criterion for detecting patterns difficult to classify, and a method of assigning confidence levels to predictions.

AN INVESTIGATION OF COMBINED PATTERNS FROM DIVERSE ANALYTICAL DATA USING COMPUTERIZED LEARNING MACHINES. P. C. Jurs, R. R. Kowalski, T. L. Isenhour and C. N. Reilly. *Ibid.*, 1949–53. A computerized learning machine is applied to the interpretation of patterns produced by combining mass spectra, infrared spectra, and melting and boiling points. Through a generalized learning procedure using negative feedback, the machine evaluates which data from each source is most relevant to answering a given question. Parameter reduction methods are applied to reduce the number of input parameters and minimize the required data and storage of trained weight components.

CRITIQUE OF SOME CONVENTIONAL EVALUATION METHODS AND A CONTINUOUS FLOW, STEADY-STATE BLENDER FOR EVALUATION OF GAS CHROMATOGRAPHY DETECTER LINEARITY. W. H. King, Jr. and G. D. Dupre (Esso Res. P.O. Box 121, Linden 07036). *Anal. Chem.* 41, 1936–40 (1969). Conventional detector evaluation methods suffer various drawbacks which are thoroughly discussed. A new method using a steady-state flowing gas of known solute concentration is presented. The steady-state method eliminates adsorption problems and unequivocally determines detector linearity on an absolute basis. The technique presented employs a constant temperature saturator and a series of calibrated flowmeters to make blends of clean helium and solute-saturated helium. The lower range of concentration which was normally used was approximately 10^{-9} gram/sec; the upper limit is set by the solute saturation pressure at approximately 10^{-4} gram/sec. The accuracy is ±5%. Several late model flame ionization detectors were evaluated. Some showed reasonable linear response up to

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10^{-5} gram/sec., while others were grossly nonlinear over the entire range.

COMPARATIVE STUDY OF ADSORPTION ISOTHERMS AND GAS-CHROMATOGRAPHIC PROPERTIES OF POWDERED ETCHED GLASS. G. Alberini, F. Bruner and G. Devitograncesco (Istituto di Chimica, Univ. Rome, 00185 Rome, Italy). *Anal. Chem.* **41**, 1940–44 (1969). The continuous flow system is used to determine the nitrogen adsorption isotherms of glass powders etched with hot alkaline solutions. The effect of alkali concentration on surface area, isotherm shape and pore volume distribution is compared with the gas-chromatographic properties of the glass powder. Etching conditions are found to be critical. Water vapour is used as surface deactivant and its effect on surface characteristics is related to the change on retention volumes and column efficiency.

REMOVAL OF TRACES OF NICKEL FROM HYDROGENATED FATS BY MEANS OF CATION EXCHANGERS. M. Rac *et al. Bilt. Bil. Ulja Masti* **6**(1), 7–10 (1969). The authors determined the optimum working temperature, compared a cation exchanger of the gel type with one of the macroreticular type, and determined their capacities. This method worked very well, but success depended to a large degree on the quality of the exchangers and on the operating procedure. (Rev. Franc. Corps Gras)

APPLICATIONS OF SPECTROSCOPY TO STRUCTURE DETERMINATION. PART IV. IR SPECTRA OF α,β-UNSATURATED CARBONYL COMPOUNDS. P. S. Kalsi (Dept. of Chem. and Biochem., Punjab, Agr. Univ., Ludhiana, India). *Perfumery Essent. Oil Record* **60**, 316–321 (1969). The use of IR for the structure determination of α,β-unsaturated carbonyl compounds is reviewed.

DETECTION OF ANIMAL BODY FAT IN BUTTER FAT (GHEE). A SUMMARY OF THE RESEARCH WORK DONE AT C. A. L. J. S. Pruthi and Y. R. Gupta (Central Agmark Lab., Nagpur, India). *Indian Oil Soap J.* **25**(1), 8–12 (1969). Eight analytical techniques for the detection of animal body fat in ghee were studied. The methods included paper and thin-layer chromatography, determination of unsaponifiable matter, solubility of ghee in different solvents, spectrophotometric examination, critical temperature of dissolution, fractionation of ghee with ethanal and then determining the refractive indices of the two fractions, and use of the hydroxamic acid: iron complex method. None of the methods could detect animal body fat in ghee at the low levels of 1 to 5%.

THE FATTY ACID COMPOSITION OF SOME SOAPMAKING FATS AND OILS. PART 2. COCONUT AND PALM KERNEL OILS. A. Allen, G. H. Padley and G. R. Whalley. *Soap Perfumery Costmetics* **42**, 372–378 (1969). A short presentation of the qualitative fatty acid composition of coconut and palm kernel oil as used in the United Kingdom.

THE DEVELOPMENT OF THE PAT METHOD FOR THE DETERMINATION OF LINSEED OIL FOOTS. J. D. von Mikusch (Unilever Res. Lab., Hamburg, W. Ger.). *Farbe Lack* **75**, 847–853 (1969). The efforts of the IUPAC and IASC committees to develop a more reliable method for the determination of foots in raw linseed oil is reviewed. A method more reliable than the current British Standard method is desired. Some of the cooperative work leading to the proposed gravimetric Phosphoric Acid Test is presented, along with results covering more than 800 oil shipments analyzed during two trial periods. The data shows that 61% of the oils tested comply with a maximum 0.25% gravimetric foots clause.

PRODUCTION OF FACTICE-LIKE PRODUCTS. Henkel and Cie. *Brit. 1,153,557*. A process for the production of factice-like products comprises reacting esters, which are derived from unsaturated fatty acids and/or resin acids and polyhydric alcohols (*e.g.* castor oil) and which, if desired, have been polymerised and/or treated with an oxidising agent, with phosphorus halides and disulphur dichloride. The substances are suitable as additions to rubber mixtures, lacquers, plastics, adhesives and lubricants. They have a very high decomposition point and, in contrast to pure S factices, possess excellent flameproofing properties as well as an increased resistance to ageing, owing to their P content. (World Surface Coat. Abs. No. 328)

• Biochemistry and Nutrition

FECAL NEUTRAL STEROIDS AND BILE ACIDS FROM GERMFREE RATS. T. F. Kellogg and B. S. Wostman (Lobund Lab., Univ. of Notre Dame, Notre Dame, Ind. 46556). *J. Lipid Res.* **10**, 495–503 (1969). The amount and composition of fecal neutral sterols and bile acids excreted by adult male germfree and conventional rats have been determined. The amounts of neutral sterols excreted were 12.8 (germfree) and 19.5 (conventional) mg/kg of body wt per day. The germfree rats excreted cholesterol and lathosterol (methostenol was not assayed); the conventional rats excreted coprostanol and coprostanone in addition. The amounts of bile acids excreted were 11.3 (germfree) and 21.4 (conventional) mg/kg of body wt per day. The bile acids excreted by the rats were tentatively identified as tauro-β-muricholate, tauro-α-muricholate and taurocholate, besides an unidentified component. The conventional rats excreted the corresponding unconjugated acids as well as many other unconjugated bile acids. No significant correlation was found between the amount of coprosterols and the total amount of neutral sterols excreted by the conventional rats. This suggests that bacterial reduction of cholesterol is not an important mechanism of increasing neutral sterol excretion of conventional rats as compared to germfree rats. Evidence is presented that suggests that this difference in neutral sterol excretion is due to changes in intestinal secretion and sloughing between the two types of animal. The factors responsible for the differences in bile acid excretion have not been identified.

EFFECT OF CARBON TETRACHLORIDE ADMINISTRATION ON THE SYNTHESIS OF TRIGLYCERIDES AND PHOSPHOLIPIDS IN RAT LIVER. Y. Shimizu (Dept. of Exper. Toxicology, Nat. Inst. of Ind. Health, 2051 Kizukisumiyoshi-cho, Kawasaki, Kanagawa, Japan). *J. Lipid Res.* **10**, 479–86 (1969). *In vivo* incorporation of choline-methyl-^{14}C into liver lecithin and its biosynthetic precursors was studied in CCl_4-treated rats. Radioactivity in cytidine diphosphoryl (CDP-)choline and lecithin was reduced to one-third of control levels, whereas that of phosphorylcholine was increased to 4.7 times control levels. Incorporation of phosphorylcholine-^{32}P into lecithin by homogenates prepared from livers of CCl_4-treated animals was reduced, but conversion of CDP-choline-^{32}P to lecithin by the isolated microsomal fraction did not show any significant depression. A block in the synthesis of CDP-choline is indicated. The *in vivo* utilization of methionine for lecithin synthesis was not affected. After intravenous injection of palmitic acid-1-^{14}C, radioactivity of triglycerides from microsomal and mitochondrial fractions was markedly lower than the controls, whereas radioactivity of triglycerides in the soluble fraction was greatly increased. Radioactivity of diglycerides changed from 0.5% of total lipids in the control to 10% of total lipids in CCl_4-treated animals. Incorporation of palmitic acid into phospholipids was also suppressed. The results demonstrate that synthesis of both phospholipids and triglycerides is inhibited in rats 4–5 hr after CCl_4 administration.

SYNTHESIS OF LIPIDS FROM ACETATE BY HUMAN PREPUTIAL AND ABDOMINAL SKIN IN VITRO. H. E. Vroman, R. A. Nemecek and S. L. Hsia (Depts. of Derm. and Biochem., Univ. of Miami School of Med., Miami, Fla. 33136). *J. Lipid Res.* **10**, 507–14 (1969). Lipogenesis *in vitro* from acetate-1-^{14}C was studied in human preputial skin and abdominal skin. Radioactive lipids were separated by column chromatography on Florisil and by thin-layer chromatography on silica gel. Radioactivity was incorporated chiefly into the triglyceride, sterol, and polar lipid fractions, while lesser amounts of ^{14}C were found in the hydrocarbon, wax, diglyceride, monoglyceride and fatty acid fractions; labeling of steryl esters was minimal. On thin-layer chromatography, the radioactive polar lipids had mobilities similar to lysolecithin, phosphatidyl choline, phosphatidyl ethanolamine and phosphotidic acid. The radioactive fatty acids of the different lipid fractions were separated by gas-liquid chromatography. The major ^{14}C-

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labeled acids were 16:0 and 18:0. Radioactivity was also detected in acids 14:0, 15:0, 16:1, 18:1, 18:2, 20:0, 20:1, 22:0, 24:0, 24:1 and 26:0. No radioactivity could be detected in arachidonic acid, although this fatty acid comprises 9% of the chromatographed fatty acids. The pattern of incorporated ^{14}C was different from the percentage mass composition of the fatty acids. Skin is therefore active in the biosynthesis of a wider variety of lipids than previously demonstrated.

METABOLISM OF D- AND L-GLYCERALDEHYDE IN ADIPOSE TISSUE: A STEREOCHEMICAL PROBE FOR GLYCEROKINASE ACTIVITY. G. Antony, L. W. White and B. R. Landau (Dept. of Pediatries, Univ. of Vermont Coll. of Med., Burlington, Vt. 05401). *J. Lipid Res.* 10, 521–27 (1969). Distributions of ^{14}C have been determined in free glycerol, in glycerol from triglycerides, in glucose from glycogen, and in lactate after incubation of D-glyceraldehyde-3-^{14}C and L-glyceraldehyde-3-^{14}C with rat adipose tissue. The distributions are interpreted in terms of presently accepted possible reactions for the initial metabolism of glyceraldehyde. Formation of glycerol-1-^{14}C from D-glyceraldehyde-3-^{14}C indicates that in adipose tissue glyceraldehyde is reduced to glycerol. Incorporation of ^{14}C from D-glyceraldehyde-3-^{14}C into carbon 3 of the glycerol of triglyceride indicates that D-glyceraldehyde is either phosphorylated or oxidized to D-glyceric acid, or both, in its initial metabolism. Incorporation of ^{14}C from L-glyceraldehyde-3-^{14}C into carbon 3 of glycerol indicated that L-glyceraldehyde is reduced to glycerol, which is phosphorylated and (or) converted to D-glyceric acid via L-glyceric acid. Some ^{14}C from L-glyceraldehyde-3-^{14}C is incorporated into carbon 1 of glycerol to triglycerides and carbon 4 of glycogen; the explanation for this incorporation is uncertain.

TISSUE DISTRIBUTION OF GLYCOSPHINGOLIPIDS IN A CASE OF FABRY'S DISEASE. J. M. Schibanoff, S. Kamoshita and J. S. O'Brien (Dept. of Pathol., Univ. S. Cal., School of Med., Los Angeles, Cal. 90033). *J. Lipid Res.* 10, 515–20 (1969). A survey was made of the glycolipid composition of various tissues, including liver, spleen, kidney (cortex and medulla), lymph node, pancreas, prostate gland, heart muscle, thenar muscle, gastrointestinal smooth muscle, frontal cerebral cortex, anterior thalamus, brain stem, a peripheral automonic ganglion and renal arterial intima and media, from a patient who died with Fabry's disease. The tissues had been fixed in formalin for 3 yr. Analytical data on trihexosyl ceramide from heart muscle and pancreas indicate a structure identical to trihexosyl ceramide from kidney: galactosylgalactosylglucosyl ceramide. Fatty acid compositions of trihexosyl ceramide and dihexosyl ceramide revealed a wide range of fatty acids, with 16:0, 18:0, 20:0, 24:0 and 24:1 predominating. These glycolipids comprised 10–41% of the total lipid in the formalin-fixed organs studied. Trihexosyl ceramide predominated in all tissues and was the only glycolipid found in muscle tissues, lymph node and arterial tissues. Dihexosyl ceramide was found in kidney, pancreas, liver, spleen and cerebral tissues. The accumulation of trihexosyl ceramide in cardiac muscle and arterial tissues may account in part for the cardiovascular complications so prominent in Fabry's disease.

LONG-CHAIN BASES IN THE SPHINGOLIPIDS OF ATHEROSCLEROTIC HUMAN AORTA. R. V. Panganamala, J. C. Geer and D. G. Cornwell (Depts. of Physiol. Chem. and Path., The Ohio State Univ., Columbus, Ohio 43210). *J. Lipid Res.* 10, 445–455 (1969). Long-chain bases were liberated from human aorta sphingomyelin by a combined enzymatic hydrolysis-alkaline hydrolysis procedure and these bases were isolated by thin-layer chromatography. Human aorta sphingomyelin contained significant amounts of 4-hexadecasphingenine, 4-heptadecasphingenine, sphinganine, 4-sphingenine, and 4, *x* 14-sphingadienine. Small amount of hexadecesphinganine, 4-tetradecasphingenine, a sphingadienine isomer, an unknown sphinganine, and two unknown diene long-chain bases were also found in sphingomyelin. The presence of a branched-chain 4-sphingenine was tentatively established and the possible presence of a sphingenine isomer was suggested. The major sphingenines were the same in the sphingomyelin, sulfatide, and cerebroside-sulfatide fractions of human aorta.

THE EFFECT OF AGE UPON THE LIPIDS OF THE LONG BONES OF THE RAT. T. Sakai, R. L. Cruess and K. Iida (Royal Victoria Hosp., Montreal 2). *Proc. Soc. Exp. Biol. Med.* 132, 100–104 (1969). An analysis of the lipids of all regions of rat bones at varying ages has been carried out. There was a precipitous drop in the total lipid of the epiphysis, metaphysis and diaphysis with this fall being paralleled by a drop in the phospholipid, cholesterol, triglyceride and fatty acid. The phospholipid value was higher in the metaphysis than the other two regions of the bone during the active growth period, and it is felt that this is associated with the extremely active bone formation being seen in the metaphysis.

LIPOLYTIC ACTIVITY OF RIBONUCLEOTIDE AND DEOXYRIBONUCLEOTIDE-3′,5′-CYCLIC MONOPHOSPHATES IN ISOLATED RAT FAT CELLS. T. Braun, O. Hechter and H. P. Bar (Inst. Biomedical Res., Amer. Medical Assoc. Educ. and Res. Found., Chicago 60610). *Proc. Soc. Exp. Biol. Med.* 132, 233–236 (1969). The lipolytic activity of a series of ribonucleotide and deoxyribonucleotide,3′,5′-cyclic monophosphates was investigated in fat cells incubated in a saline-phosphate medium, from which Ca^{2+}, Mg^{2+} and K^+ were omitted. It was found that the effect of 3′,5′-AMP to stimulate lipolysis is not specific; 3′,5′-UMP, 3′,5′-IMP, 3′,5′-CMP, 3′,5′-GMP, and 3′,5′-dAMP also exhibit lipolytic activity. Quantitatively, however, the lipolytic potency of 3′,5′-AMP is considerably greater than all of the other nucleotides tested. The results indicate the importance of the nucleotide base and the 2′-hydroxyl group of the ribose moiety for lipolytic activity.

THE ALPHA AND THE OMEGA OF VITAMIN A METABOLISM. J. A. Olson (Dept. of Biochem., Faculty of Sciences, Mahidol Univ., Rana VI Road, Bangkok, Thailand). *Am. J. Clin. Nutr.* 22, 953–62 (1969). Since isomerization, transesterification, and lactone formation occur so readily with vitamin A metabolites, some of these biologically active derivatives may well contain the basic structure of vitamin A. Admittedly, the possibility that decarboxylation may lead to a biologically active molecule must still be seriously considered. Nonetheless, when small doses of retinol are administered to rats, the major form found in the tissues is the undegraded molecule.

ABSORPTION OF VITAMIN A. J. Ganguly (Dept. of Biochem., Indian Inst. of Sci., Bangalore, India). *Am. J. Clin. Nutr.* 22, 923–33 (1969). Higher fatty acids are mostly absorbed through the lymphatic system as esters. Retionic acid is a higher fatty acid with 20 carbons (no doubt it is an unusual fatty acid having one β-ionone ring and several conjugated double bonds), but it is not absorbed through the lymphatic system; all of it is apparently absorbed through the portal circulation and excreted through the bile.

RETINOL TRANSPORT IN HUMAN PLASMA. D. S. Goodman (Dept. of Med., Columbia Univ. Col. of Physicians and Surgeons, New York, N.Y. 10032). *Am. J. Clin. Nutr.* 22, 911–12 (1969). The retinol-binding protein was homogeneous in the analytical ultracentrifuge, with a sedimentation velocity ($S_{20,w}$) of approximately 2.2 S. Solutions of RBP are fluorescent (characteristic of retinol) and have ultraviolet absorption spectra with peaks at 330 mμ (resulting from

The Problem of Moldy Feeds and Foods

It has long been known that the growth of molds and other fungi can have both desirable and undesirable consequences. For example, the distinctive character of roquefort and camembert cheeses results from the use of the molds *Penicillium roqueforti* and *Penicillium camemberti.*

On the other hand, the spoilage of many foods, such as bread, meat, fruits and vegetables, can result from the growth of undesirable molds. Molds not only cause spoilage, they can also cause diseases in plants, animals and humans. When allowed to develop in feed and food products, some molds can produce toxins which may be extremely harmful.

Attention was sharply focused on this last undesirable consequence as a result of the loss of about 100,000 turkey poults in England in 1960. These mysterious deaths were finally traced to a particular mycotoxin which has since been identified and named aflatoxin. The turkeys had been fed with a feed which contained moldy peanut meal. The meal, in turn, had contained aflatoxin.

So far, four species of the molds of the genus *Aspergillus* have been said to produce this toxin and four species of the genus *Penicillium.* However, the prime offender is *Aspergillus flavus.*

Aflatoxins have been shown to be toxic to rainbow trout, ducklings (the most susceptible of all species), turkeys, rats, guinea pigs, dogs and monkeys. Generally, coho salmon and channel catfish are resistant to aflatoxin and sheep and pigs are comparatively resistant. Studies with monkeys have shown that those on low protein diets are less resistant to aflatoxin than those receiving better protein nutrition.

Two types of toxicity are involved. When large amounts of aflatoxin are ingested in a short time, abnormal production of giant bile duct cells, anemia, organ damage and death are the result. When small amounts of aflatoxin are ingested over a long period of time, liver cancer is the result.

It is the latter type of toxicity that may be most significant for humans. As yet, there is no conclusive direct evidence that the aflatoxins have caused liver cancer in humans. However, epidemiological data on the incidence of human liver cancer in Africa seems to support the mycotoxin theory. The *Aspergillus flavus* mold grows extremely well in areas with very high relative humidity (exceeding 80%). Those areas of Africa which have high relative humidities also have the highest incidence of liver cancer. In drier regions, the incidence is lower.

It is interesting to note that in the semi-arid climate in Israel, no significant amounts of aflatoxin have been found in peanuts grown in that country, presumably because the growth of *Aspergillus flavus* is not favored by the low humidity.

Measures should be taken which will prevent the growth of mold and the formation of aflatoxin in farm commodities. For instance, failure to avoid the following faulty procedures can encourage aflatoxin production in peanuts: shell damage and kernel splitting; delays between harvesting and drying; drying beds which are too deep; slow drying; excessively fast drying; improper storage with inadequate aeration of the post-dried peanuts.

Using techniques which prevent these conditions has been very effective in the U.S.A. Peanuts have been kept a wholesome food, essentially free from aflatoxins.

Preventive measures can also be taken with other commodities. Cotton should not be picked after a heavy dew because it will encourage aflatoxin production in the cottonseed. Using low aeration rates in drying green rice also will promote aflatoxin production.

Many procedures have been studied for removing or reducing the aflatoxin content of oilseeds, such as peanuts or cottonseed. Extraction with certain solvents can result in virtually complete removal of aflatoxins. However, applying such procedures in the developing countries seems somewhat remote.

Although simple heating is relatively ineffective against aflatoxin, roasting of peanuts does result in a significant reduction of the aflatoxin content that may be present. This technique may be of great practical importance in relation to such things as the production of peanut butter.

For developing countries, fermentation procedures may well deserve the most attention as a means of preventing or reducing aflatoxin in edible products. It has been shown that *Flavobacterium aurantiacus* (NRRL B-184) can completely detoxify aflatoxin-contaminated milk, corn oil, peanut butter, peanuts and corn. Soybeans were also partly decontaminated by this organism.

ABSTRACTS: BIOCHEMISTRY AND NUTRITION

the bound retinol) and at 280 mμ. The molar extinction of retinol bound to RBP and in aqueous solution appears to be the same as that of retinol itself in solution in benzene. There seems to be one binding site for one molecule of retinol per molecule of RBP. There are no fatty acid or fatty acyl chains present in purified RBP. The amino acid composition of RBP differs from that of all previously characterized plasma proteins, including the plasma lipoproteins. The usual concentration of RBP in plasma is of the order of 3–4 mg/100 ml.

BIOSYNTHESIS OF VITAMIN A FROM β-CAROTENE. *Ibid.* 963–5. The biosynthesis of vitamin A from β-carotene takes place mainly in the intestinal mucosa, during the absorption of dietary β-carotene. The normal *in vivo* reaction sequence involves two steps: first, the cleavage of β-carotene to form two molecules of retinal; second, the reduction of retinal to retinol. The newly formed retinol is then mainly esterified with long-chain fatty acids, and the retinyl esters are incorporated into lymph chylomicrons and transported from the intestine via the intestinal lymphatics.

FACTORS AFFECTING ABSORPTION, TRANSPORT AND STORAGE OF VITAMIN A. S. R. Ames (Distillation Products Ind., Div. of Eastman Kodak Co., Rochester, N.Y.). *Am. J. Clin. Nutr.* 22, 934–5 (1969). Vitamin A absorption is markedly impaired in vitamin E-deficient animals. Oral supplementation with d-*alpha*-tocopherol increased the utilization of orally administered vitamin A about sixfold. Even when vitamin A was administered intramuscularly in emulsified form, utilization in the vitamin E-deficient animal was low. With simultaneous injection of vitamin E, vitamin A utilization was markedly increased. Thus, the influence of vitamin E in promoting high vitamin A utilization is not limited to improving intestinal absorption. Vitamin E is essential for the normal *in vivo* utilization of vitamin A.

ISOLATION AND CHARACTERIZATION OF POLYPEPTIDES OF HUMAN SERUM LIPOPROTEINS. B. Shore and V. Shore (Div. of Biology and Med., Lawrence Radiation Lab., Univ. of Calif., Livermore, Calif. 94550). *Biochemistry* 8, 4510–16 (1969). Two or more different polypeptides were isolated by DEAE-cellulose chromatography from each of the protein moieties of several fractions of human serum lipoproteins. The polypeptides were characterized by amino acid composition, carboxyl-terminal analysis and polyacrylamide gel electrophoresis. The high-density lipoproteins of density 1.083–1.124 g/cc (HDL_2) were more heterogeneous than those of density 1.126–1.195 g/cc (HDL_3) with respect to polypeptide content and contained several polypeptides in addition to the two which comprise most of the protein of the HDL_3 fraction. The low-density lipoprotein fraction of density 0.98–1.006 g/cc (S_f 20–100) also contains several polypeptides, two of which are similar to if not identical with peptides found as minor components in the high-density lipoproteins. The protein of the low-density lipoprotein fraction of density 1.029–1.039 g/cc (S_f 4–8 lipoproteins) yielded two polypeptides, which were different from the peptides of high-density lipoproteins

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and the S_i 20–100 fraction of low-density lipoproteins. Multiple forms, differing slightly in amino acid composition, of some of the peptides were found. The high-density lipoproteins and the low-density lipoproteins may be structurally and metabolically related by their content of lipid complexes of two polypeptides, one of which has carboxyl-terminal R-Ala-Val-Ala-Ala and one of which has an unusually high content of glycine, serine and glutamic acid, which are major components of S_i 20–100 lipoproteins and minor components of HDL_2 lipoproteins.

VITAMIN A ACTIVITY OF CORN CAROTENES FOR SWINE. R. H. Wellenreiter, D. E. Ullrey, E. R. Miller and W. T. Magee (Dept. of Animal Husbandry, Mich. State Univ., E. Lansing, Mich.). *J. Nutr.* **99**, 129–36 (1969). Three trials, using 204 weanling pigs, were conducted to estimate the vitamin A potency of corn carotenes for the pig. The pigs were depleted of their vitamin A stores by feeding a wheat- or milo-based diet until serum vitamin A levels approached or fell below 10 μg/100 ml. After depletion the pigs were assigned at random to one of the following seven repletion diets: 1) basal diet; 2) basal diet supplemented with 3 levels of carotene from corn or corn gluten meal, or both, with carotene concentrations from 1.04 to 10.3 mg/kg of diet; 3) basal diet supplemented with 3 levels of all-*trans*-retinyl palmitate, with retinyl palmitate concentrations from 73 to 654 μg/kg diet. At higher levels of carotene intake, when corn gluten meal was included in the repletion diets, 1 mg of carotene had a vitamin A potency of 123 to 174 IU. When corn was the only source of carotenes and the concentration was more typical of corn-soy swine diets, 1 mg of carotene had a vitamin A potency of 261 IU. It would appear that the NRC relationship, between 1 mg of dietary carotene and 500 IU of Vitamin A activity, exaggerates the usefulness of corn carotenes for swine when liver vitamin A storage is used as the criterion.

EFFECTS OF THE ADDITION OF BENTONITE TO HIGH-GRAIN DAIRY RATIONS WHICH DEPRESS MILK FAT PERCENTAGE. R. B. Rindsig, L. H. Schultz and G. E. Shook (Dept. of Dairy Sci., Univ. of Wisc., Madison, Wisc. 53706). *J. Dairy Sci.* **52**, 1770–75 (1969). Twelve lactating Holstein cows were placed on an experiment consisting of three periods: four-week normal control, six-week fat depression, and six-week treatment. In the last period the cows were divided into three groups and allotted to three treatments: I, fat-depressing ration; II, fat-depressing ration + 5% bentonite and III, fat-depressing ration + 10% bentonite. A highly significant increase in milk fat percentage was noted in both bentonite treatments versus the fat-depressing ration, but there was no significant difference between the two levels of bentonite. The cows in Treatments I, II, and III maintained milk fat percentage at 86, 144 and 144% of the fat-depressing period, and 60, 87, and 87% of the normal control period, respectively. Milk production was significantly higher for Treatment II. A significant increase in the proportion of rumen acetate, along with a decrease in propionate and valerate, was noted in the two bentonite treatments compared to the fat-depressing ration. There was a significant increase in the arteriovenous difference of blood acetate in the two bentonite treatments compared to the fat-depressing ration. Arteriovenous differences for blood glucose and ketones and for plasma free fatty acids and triglycerides were not altered significantly.

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LIPID COMPOSITION OF RAT LIVER PLASMA MEMBRANES. K. Ray, V. P. Skipski, M. Barclay, E. Essner and F. M. Archibald (Divisions of Exp. Chemotherapy and Cytology, Sloan-Kettering Inst. for Cancer Res., Rye, N.Y. 10580). *J. Biol. Chem.* **244**, 5528–36 (1969). Rat liver plasma membranes were isolated by discontinuous sucrose gradient centrifugation. The purity of membrane preparations was checked by electron microscopy and enzyme assays. A new glycolipid, tentatively called the S-lipid, is proposed as a marker to indicate the purity of plasma membranes. It is present in most subcellular particulates (mitochondria, microsomes, lysosomes, etc.) but is absent from plasma membranes. The plasma membrane lipids represent 39.8% of the total dry weight of the membranes. Plasma membrane total lipid contains 39% "neutral" lipids of which free cholesterol (18.1%) and free fatty acids (7.9%) are the major components. The phospholipids constitute 55.4% of membrane total lipids. The phospholipid fraction is composed mainly of phosphatidylcholine (19.3% of the total lipids), phosphatidylethanolamine (10.3%) and sphingomyelin (9.8%).

THE EFFECT OF CROTON OIL PRETREATMENT ON SKIN TUMOR INITIATION IN MICE. H. Hennings, G. T. Bowden and R. K. Boutwell (McArdle Lab. for Cancer Res., Univ. of Wisc. Med. Ctr., Madison, Wisc. 53706). *Cancer Res.* **29**, 1773–80 (1969). A single application of 0.5% croton oil to mouse skin stimulates nucleic acid and protein synthesis in the treated area. The maximum rates of synthesis were attained at 6 hours for RNA, 12 hours for protein and 18 hours for DNA. By 48–72 hours, the rate of synthesis of RNA and protein was returning to normal, but the rate of DNA synthesis was still 2–3 times the control value. In order to test the hypothesis that cells synthesizing DNA are more susceptible to initiation of skin tumor formation, mice were given a single, preliminary application of croton oil either 18 or 48 hours before initiation with 7,12-dimethylbenz(a) anthracene (DMBA), β-propiolactone (BPL), or urethan. Tumors were subsequently elicited by multiple applications of croton oil. Croton oil pretreatment did not affect initiation by BPL, caused a slight increase in tumor yield after initiation by DMBA, but clearly increased tumor incidence in mice initiated with urethan. The overall binding of DMBA-^{3}H to mouse skin DNA, RNA and protein or to RNA fractionated on a methylated albumin kieselguhr column, was not greatly affected by the croton oil pretreatment.

ADDITIVE NATURE OF SODIUM BICARBONATE AND MAGNESIUM OXIDE ON MILK FAT CONCENTRATIONS OF MILKING COWS FED RESTRICTED-ROUGHAGE RATIONS. J. W. Thomas and R. S. Emery (Dairy Dept., Mich. State Univ., E. Lansing, Mich. 48823). *J. Dairy Sci.* **52**, 1762–69 (1969). Sodium bicarbonate (272 or 363 g/day) and MgO (136 or 181 g/day) alone or combined were added to the diet of 20 cows given a low-roughage-high-grain ration in a Latin-square trial. Responses were the same for the second, third, and fourth weeks of each four-week period. Both supplements increased fat test and rumen pH, but decreased molar proportion of valerate and propionate in the rumen. These effects were additive when both were fed together. Sodium bicarbonate increased daily milk and fat production, but MgO caused a slight decrease, probably due to its more pronounced effect on decreasing grain intake. Cows with the greatest level of milk and fat production had the greatest mammary uptake of glucose and acetate. Mammary uptake of plasma glucose, acetate, cholesterol, cholesterol esters and triglycerides were positively related to rumen valerate and propionate proportions, but, when supplements were fed, valerate assumed much more importance than propionate.

SERUM CHOLESTEROL REDUCTION BY CHROMIUM IN HYPERCHOLESTEROLEMIC RATS. H. W. Staub, G. Reussner and R. Thiesen, Jr. (Gen. Foods Technical Ctr., Tarrytown, N.Y. 10591). *Science* **166**, 746–47 (1969). The addition of chromium(III) to the drinking water of rats in a normal laboratory environment on a hypercholesterolemic diet resulted in lower serum cholesterol concentrations whether the dietary carbohydrate was either sucrose or starch. However, rats fed the sucrose diet with chromium in drinking water had serum cholesterol concentrations similar to those of rats fed the starch diet without chromium in drinking water.

METABOLISM OF STEROIDS BY TRANSPLANTABLE MOUSE INTERSTITIAL CELL TUMOR. O. J. Lucis and R. Lucis (Dept. of Pathol., Dalhousie Univ., Halifax, Nova Scotia). *Cancer Res.* **29**, 1647–52 (1969). Biotransformation of steroid hormones

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by a spontaneous interstitial cell tumor originating from a BALB/cJ mouse has been studied *in vitro*. Tumor tissue grown in a female host converted estrone predominantly to estradiol-17β. The same tissue transformed estradiol-17β to estrone to a considerably lesser extent. Incubation of the tumor tissue with androstenedione yielded testosterone as the principal conversion product and several other metabolites with chromatographic characteristics of 11β-hydroxytestosterone, 11β-hydroxyandrostenedione and 11-ketotestosterone. Interstitial cell tumors grown in male and female hosts transformed progesterone to testosterone, 20α-hydroxypregn-4-en-3-one, androstenedione and 11-deoxycorticosterone. The tumor tissue *in vitro* showed the presence of enzyme systems which are commonly found in testis tissue as well as steroid 11β- and 21-hydroxylases which are normally localized in adrenocortical cells. Testis tissue from animals bearing interstitial cell tumor formed *in vitro* less testosterone from progesterone than testes from normal animals.

CONTROL OF CHOLESTEROL SYNTHESIS IN HEPATOMAS: THE EFFECT OF BILE SALTS. J. R. Sabine (Dept. Animal Physiol., U. Adelaide, Glen Osmond, S.A. 5064, Australia). *Biochim. Biophys. Acta* **176**, 600–04 (1969). Other authors have suggested that bile salts may be the cholesterol metabolite responsible *in vivo* for the feed-back regulation of cholesterol synthesis in liver. Since the liver tumor lacks this feed-back control, the role of bile salts have been examined in 2 transplantable hepatomas-5123C in Buffalo rats and BW7756 in C57 L/J mice. When bile salts were removed *in vivo* by feeding the bile-salt adsorbent, cholestyramine, then cholesterol synthesis was increased in the liver but not in the hepatomas. When a bile salt (sodium deoxycholate, 0.5 mM) was added *in vitro* to a homogenate preparation, then cholesterol synthesis was inhibited in both liver and hepatoma. When sodium deoxycholate was added to incubation medium with tissue slices, then by contrast to the results with homogenates, cholesterol synthesis was inhibited in liver but not hepatoma. This is further evidence in support of the hypothesis that the defective control of lipid synthesis shown by hepatomas, which defect has been suggested as perhaps fundamental to the neoplastic process, may be due to an inability of metabolic regulators to reach the active sites of enzyme action.

IN VIVO STUDIES OF CHOLINE-ME-^{3}H AND CHOLINE-1,2-$^{14}C_2$ INCORPORATION INTO LUNG AND LIVER LECITHINS. H. L. Spitzer, J. R. Norman and K. Morrison (Dept. Medicine, Univ. Alabama Med. Center, Birmingham). *Biochim. Biophys. Acta* **176**, 584–90 (1969). A comparison has been made of choline-Me-^{3}H and choline-1,2-$^{14}C_2$ incorporation *in vivo* into the lecithins and the sphingomyelin of lung and liver. The data suggest that in liver, but not in lung, there is a significant reincorporation of choline methyl groups into arachidonoyllecithin; there is choline incorporation into linoleoyllecithin not involving CDP-choline; and a disproportionate amount of the choline incorporated into sphingomyelin is endogenously synthesized and not derived from the free choline pool.

PHOSPHOLIPASES IN ARTERIAL TISSUE. III. PHOSPHATIDE ACYL-HYDROLASE, LYSOPHOSPHATIDE ACYL-HYDROLASE AND SPHINGOMYELIN CHOLINE PHOSPHOHYDROLASE IN RAT AND RABBIT AORTA IN DIFFERENT AGE GROUPS. S. Eisenberg, Y. Stein and O. Stein (Lipid Res. Lab., Hebrew Univ.-Hadassah Med. School, Jerusalem). *Biochim. Biophys. Acta* **176**, 557–69 (1969). Aortae of rats and rabbits 1 to 24 months of age were examined for phospholipid content and the activity of different phospholipases. In both species there was a fall in DNA content/wet weight and a rise in the phospholipid to DNA ratio with age. Out of the total phospholipids, sphingomyelin showed the greatest increase. The various phospholipases investigated in aortic homogenates displayed a behaviour which varied with age. In both species there was no change in sphingomyelinase activity, a slight increase in lysolecithinase and a remarkable rise in the phosphatide acyl-hydrolase. In the rat the rise in phosphatide acyl-hydrolase is much more marked than in the rabbit aorta. Incorporation of labelled choline into lecithin and sphingomyelin by rat and rabbit whole aortae *in vitro* was increased with age. The following chain of events for the ageing rat aorta was postulated: the increase in phosphatide acyl-hydrolase activity, which follows the rise in phospholipid synthesis, prevents the accretion of lecithin, while the lack of increase in sphingomyelinase activity results in a rise in cellular sphingomyelin. The possible correlation between the biochemical, enzymic and morphological changes occurring during ageing of aortic medial cells is discussed.

UTILIZATION OF MOLECULAR SPECIES OF DIGLYCERIDES IN THE SYNTHESIS OF LECITHIN. J. B. Mudd, L. M. G. Van Golde and L. L. M. Van Deenen (Biochem. Lab., State Univ., Utrecht, Netherlands). *Biochim. Biophys. Acta* **176**, 547–56 (1969). The synthesis of lecithin by rat liver microsomes was measured in the presence of ^{14}C-CPO-choline and three molecular species of diglycerides derived from rat liver lecithin containing four, two and one double bond. The rate of synthesis of lecithin was the same regardless of the fatty acid composition of the diglyceride. Similar results were obtained when different molecular species of diglycerides derived from egg lecithin were used as acceptor molecules. When ^{3}H-labelled monounsaturated and ^{14}C-labelled diunsaturated diglycerides were incubated in different ratios with unlabelled CDP-choline the lecithins produced reflected an identical alteration in isotopic ratio. These results demonstrate that cholinephosphotransferase from rat liver microsomes shows no specificity with respect to the fatty acid composition of the diglycerides, at least for the molecular species investigated so far.

LIPID METABOLISM IN CELLS GROWN IN TISSUE CULTURE: O-ALKYL, O-ALK-1-ENYL AND ACYL MOIETIES OF L-M CELLS. R. E. Anderson, R. B. Cumming, M. Walton and F. Snyder (Med. Div., Oak Ridge Assoc. Universities, Oak Ridge Nat. Lab., Oak Ridge 37830). *Biochim. Biophys. Acta* **176**, 491–501 (1969). The lipid composition of L-M cells, a variant of L-cells that can be grown in a lipid-free, chemically defined medium in the absence of serum, has been determined. The effect of 10% horse serum, a requirement for growth of regular L-strain fibroblasts, on the lipid composition of the L-M cell has also been investigated. The lipid classes of the L-M cells grown on horse serum are quantitatively similar to those of the L-M cells grown on the chemically defined medium; the lipid composition of the cells grown under both conditions is different from that of the horse serum. However, L-M cells utilize fatty acids from the serum since large levels of linoleic acid are found in the lipid classes of those cells grown on horse serum. The L-M cells and the tumors induced by injecting L-M cells into mice are rich sources of glyceryl ethers. Glyceryl ether diesters are most abundant in the L-M cells grown on the chemically defined medium (20% of the neutral lipids), whereas the tumors contain approx. 8% glyceryl ether diesters in the neutral lipid fraction. The phospholipids of L-M cells grown with and without serum and of L-M tumors contain near equal percentages of glyceryl ethers (12.3–15.9%). The O-alkyl and O-alk-1-enyl moieties are saturated or monounsaturated and have chain lengths of 16 and 18 carbon atoms.

SYNTHESIS OF LONG-CHAIN FATTY ACIDS IN MITOCHONDRIA. E. M. Wit-Peeters (Lab. of Biochem., B. C. P. Jansen Inst., Univ. of Amsterdam, Amsterdam, The Netherlands). *Biochim. Biophys. Acta* **176**, 453–62 (1969). NADH is much more effective than NADPH in promoting the incorporation of acetate into the long-chain acids of sonicated guinea-pig heart mitochondria, aged in order to remove endogenous nicotinamide nucleotides. In the presence of sufficient NADH, NADPH has no effect and NADP+ inhibits. Added Krebs-cycle intermediates stimulate the incorporation of acetate in intact mitochondria, but have no effect on the incorporation of acetyl-CoA in sonicated mitochondria, in the presence of sufficient NADH. Fractionation of rat-liver mitochondria with digitonin with the use of marker enzymes showed that the system responsible for fatty acid synthesis is bound to the inner membrane. In mitochondria treated with digitonin the incorporation of malonyl-CoA is about as rapid as that of acetyl-CoA. In some submitochondrial fractions, however, acetyl-CoA is much the better substrate, indicating that it is unlikely that malonyl-CoA is an intermediate in the incorporation of acetate. The incorporation of malonyl-CoA is probably preceded by decarboxylation to acetyl-CoA. ATP is an absolute requirement for the incorporation of acetyl-CoA or malonyl-CoA by heart submitochondrial preparations, indicating that *de novo* synthesis does not take place in these preparations.

A SEMIMICRO METHOD FOR THE STEREOSPECIFIC ANALYSIS OF TRIGLYCERIDES. W. W. Christie and J. H. Moore (Hannah Dairy Res. Inst., Ayr, Great Britain). *Biochim. Biophys. Acta* **176**, 445–52 (1969). The precision of the methods for the stereospecific analysis of triglycerides devised by Brocker-

hoff has been investigated. A modified procedure has been developed which is capable of considerable accuracy with 10–40 mg of triglyceride or less. α,β-Diglycerides are prepared by reaction of the triglycerides with ethyl magnesium bromide in diethyl ether. These are converted synthetically to phospholipids which are hydrolysed by the stereospecific phospholipase A of snake venom. The fatty acid composition of the resulting lysophosphatide accurately represents that of the 1-position of the original triglyceride. The fatty acid composition of the 2-position must be determined independently by pancreatic lipase hydrolysis so that the composition of the 3-position can then be calculated. Alternative methods for determining the composition of the 2- and 3-positions are available. Application of the method to synthetic 2-oleo-distearin, two pig depot fats and maize oils is described.

5α-CHOLEST-8(14)-EN-3β-OL, A POSSIBLE INTERMEDIATE IN THE BIOSYNTHESIS OF CHOLESTEROL. W. H. Lee, B. N. Lutsky and G. J. Schroepfer, Jr. (Div. of Biochem., Dept. of Chem., U. Illinois, Urbana 61801). *J. Biol. Chem.* 244, 5440–48 (1969). (3α-^{3}H)-5α-Cholest-8(14)-en-3β-ol has been prepared by chemical synthesis. The incorporation of the label of this compound into liver cholesterol by intact rats has been demonstrated. The convertibility of 5α-cholest-8(14)-en-3β-ol to 5α-cholest-7-en-3β-ol and cholesterol by rat liver homogenates has been established. Upon incubation of (3α-^{3}H)-5α-cholest-8(14)-en-3β-ol with a rat liver homogenate preparation under anaerobic conditions, the added substrate was recovered unchanged. The presence of a sterol with the expected properties of 5α-cholest-8(14)-en-3β-ol in rat skin has been demonstrated.

ENZYMATIC SYNTHESIS OF THE ANTIGEN CARRIER LIPID. J. G. Christenson, S. K. Gross and P. W. Robbins (Dept. of Biology, MIT, Cambridge 02139). *J. Biol. Chem.* 244, 5436–39 (1969). Two enzyme systems which catalyze polyprenol synthesis from Δ^3-isopentenyl pyrophosphate and farnesyl pyrophosphate have been extracted from *Salmonella newington*. One system, which is soluble, yields products of chain length shorter than the 55-carbon isoprenoid antigen carrier lipid. The other, which is found in particulate fractions, yields a product that after acid hydrolysis is identical with the hydrolyzed antigen carrier lipid. This latter product is able to react with the sugar nucleotides UDP-galactose and TDP-rhamnose to form a disaccharide-diphosphate-polyprenol identical with disaccharide diphosphate antigen carrier lipid on diethylaminoethyl cellulose. It is proposed that the antigen carrier lipid is formed by the particulate enzyme through successive additions of Δ^3-isopentenyl pyrophosphate to farnesyl pyrophosphate.

STUDIES OF THE PROTEINS IN HUMAN PLASMA VERY LOW DENSITY LIPOPROTEINS. W. V. Brown, R. I. Levy and D. S. Fredrickson (Molecular Disease Branch, NHI, NIH, Bethesda 20014). *J. Biol. Chem.* 244, 5687–94 (1969). The protein components of human plasma very low density lipoproteins ($S_f > 20$) were studied following partial and total delipidation. After the neutral lipids were extracted with heptane, the resulting phospholipid-protein complexes contained at least one immunochemical reactant different from the major apoproteins of high density and low density lipoproteins. Purification required total delipidation with ethanol-ether, gel filtration and diethylaminoethyl cellulose chromatography. Two proteins were then isolated that differed from the proteins of high density or low density lipoproteins, and their purity was established by immunochemical analysis and polyacrylamide gel electrophoresis. One of these had γ mobility, NH_2-terminal threonine, COOH-terminal valine, and no tyrosine, histidine, cysteine or cystine. The second, α_2-migrating protein had NH_2-terminal serine, COOH-terminal alanine, and no isoleucine, cysteine or cystine. These two proteins constituted approximately half of the total protein in very low density lipoprotein.

VITAMIN E DEFICIENCY AND FAT STRESS IN THE DOG. K. C. Hayes, S. W. Nielsen and J. E. Rousseau, Jr. (Inst. of Nutr. and Food Science, U. of Connecticut, Storrs). *J. Nutr.* 99, 196–209 (1969). Male beagle puppies (32) were fed vitamin E-deficient diets with four levels (1,5,10, and 15%) of safflower oil with or without a vitamin E supplement for a 15-week period. The unsupplemented dogs developed a vitamin E deficiency which was correlated with increased dialuric acid hemolysis of red cells and decreased plasma tocopherol values. Both hemoglobin and packed cell volume were depressed by increasing fat consumption, unrelated to tocopherol supplementation and attributed to *in vivo* red cell disruption. Creatine phosphokinase values were elevated in tocopherol-deficient dogs and were correlated with fat consumption. Terminal plasma vitamin A concentrations were lower in dogs receiving more than 1% supplementary fat. At necropsy, browning of the intestinal muscularis in the tocopherol-deficient dogs was related to the consumption of polyunsaturated fats (PUFA). Microscopically, lipofuscin was seen in smooth muscle of gut, urinary bladder and small arterioles. Neuroaxonal dystrophy and myodegeneration were also found in the vitamin E-deficient dogs. The requirement for tocopherol was directly related to PUFA consumption, apparently associated with the metabolism of the fat and not with an antioxidant role of the vitamin.

METABOLITES OF VITAMIN D_3 AND THEIR BIOLOGIC ACTIVITY. G. Ponchon and H. F. Deluca (Dept. of Biochem., U. Wisconsin, Madison). *J. Nutr.* 99, 157–67 (1969). The metabolism of vitamin D_3 has been studied after intravenous injection of 10 IU (1,2-^{3}H)-vitamin D_3 to vitamin D-deficient rats. A new chromatographic system achieved the separation of unchanged vitamin D and 11 radioactive metabolites of which only one (25-hydroxycholecalciferol) exhibits intense antirachitic activity. An important proportion of the radioactivity in bones and small intestine is represented by this active metabolite. By way of contrast, liver and kidneys accumulate selectively unchanged D_3. Plasma as expected contains large amounts of 25-hydroxycholecalciferol.

COMPLEMENT-DEPENDENT DAMAGE TO LIPOSOMES PREPARED FROM PURE LIPIDS AND FORSSMAN HAPTEN. S. C. Kinsky, J. A. Haxby, D. A. Zopf, C. R. Alving and C. B. Kinsky (Dept. Pharmacol., Washington School of Med., St. Louis 63110). *Biochemistry* 8, 4149–58 (1969). The extent and rate of marker loss from Forssman-sensitized liposomes was dependent upon the amount of hapten incorporated in the lipid mixture used for generation of the liposomes, and upon the concentration of antiserum and complement present during assay. The extent of glucose release was essentially the same from liposomes prepared with either sphingomyelin or lecithin, and was not affected by the charge on the liposomal membrane. The available data suggest that lipids alone (perhaps in bilayer configuration) may serve as "substrate" for complement, and that cell membranes may not contain any unique and specific endogenous receptor sites (protein and/or carbohydrate) for components of the complement sequence.

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EFFECT OF CARBON MONOXIDE AND PHENOBARBITAL ON HYDROXYLATION OF BILE ACIDS BY RAT LIVER MICROSOMES. K. Einarsson and G. Johansson (Dept. of Chemistry, Karolinska Inst., Stockholm). *FEBS Letters* 4, 177–80 (1969). The results of the present work show differences between the hydroxylation reactions in the biosynthesis and metabolism of bile acids. Carbon monoxide was found to affect differently the three hydroxylations stimulated by phenobarbital administration.

STUDIES ON THE LYSIS OF RED CELLS AND BIMOLECULAR LIPID LEAFLETS BY SYNTHETIC LYSOLECITHINS, LECITHINS AND STRUCTURAL ANALOGS. F. E. Reman, R. A. Demel, J. De Gier, L. L. M. Van Deenen, H. Eibl and O. Westphal (Biochem. Lab., Univ. of Utrecht). *Chem. Phys. Lipids* 3, 221–33 (1969). The results obtained in this study show a great influence of the acyl chain on the lytic behavior of lysolecithins and lecithins. The lysolecithins studied ranged in acyl chain length from 10 to 18 carbon atoms. The palmitoyl- and stearoyl derivatives were found to be the most active, whereas the short chain compounds like decanoyl-lysolecithin showed no activity at all. It appears that the action of lysolecithins, desoxy-lysolecithins and lecithins toward red cells and lipid bilayers shows reasonable similarity with the exception of some unsaturated compounds.

INFLUENCE OF DIETARY FAT MIXTURES OF PLATELET ADHESIVENESS, ATHEROSCLEROSIS AND PLASMA CHOLESTEROL CONTENT IN RABBITS. J. Kloeze, U.M.T. Houtsmuller and R. O. Vles (Unilever Res. Lab., Vlaardingen, Netherlands). *J. Atheroscler. Res.* 9, 319–34 (1969). The results of the present experiment with rabbits are in accordance with those from previous experiments with rats; different types of dietary fat (even those containing linolenic acid) administered over a prolonged period do not influence platelet adhesiveness. Evidently, even a diet without essential fatty acids and polyunsaturated fatty acids fed for 62 weeks to rabbits does not induce a higher degree of platelet adhesiveness than an adequate diet.

EFFECT OF N-γ-PHENYLPROPYL-N-BENZYLOXY ACETAMIDE AND OF CLOFIBRATE ON THE LIPIDS OF NORMAL AND HYPERCHOLESTEREMIC RATS. F. M. Berger, J. F. Douglas, G. G. Lu and B. J. Ludwig (Wallace Lab., Cranbury, N.J. 08512). *Proc. Soc. Exp. Biol. Med.* 132, 293–97 (1969). N-γ-Phenylpropyl-N-benzyloxy acetamide and clofibrate were given to weanling rats in the diet for 2 weeks. The hypocholesteremic and other effects of the drugs differed greatly depending on the kind of diet which was provided. Clofibrate reduced serum cholesterol of animals on the usual, normocholesteremic diet, but had much less effect in animals on a hypercholesteremic, high fat diet. N-γ-Phenylpropyl-N-benzyloxy acetamide, in contrast, was much more effective in lowering blood cholesterol levels in animals fed a high fat diet. The two drugs also produced different effects on liver cholesterol, liver triglycerides, the serum liver cholesterol pool and the protein content of the livers. Both drugs produced liver enlargement but did not affect plasma SGOT, SGPT and alkaline phosphatase. These findings suggest that different mechanisms are involved in the hypocholesteremic action of these compounds.

IN VITRO STUDIES OF VITAMIN D-INDUCED AORTIC CALCIFICATION. R. Eisenstein, H. Ellis and J. Rosato (Div. Pathol., Presbyterian-St. Luke's Hospital, Chicago 60612). *Proc. Soc. Exp. Biol. Med.* 132, 58–62 (1969). Arterial segments incubated in a culture system containing serum from rats with hypervitaminosis D accumulate more calcium than segments incubated in a system containing normal rat serum. If such arterial segments are incubated in serum, they accumulate more calcium from normal serum than rachitic serum and more from serum from rats with hypervitaminosis than from normal serum. Addition of either vitamin D or calcium alone to normal serum does not result in excess calcium accumulation by arteries incubated in such serum, but addition of both does. Different aortic segments accumulate different amounts of calcium, apparently in relation to the proportion of elastic tissue in the segments.

EFFECT OF POLYENE MACROLIDES ON CHOLESTEROL METABOLISM OF THE CHICK. H. Fisher, P. Griminger and C. P. Schaffner (Dept. Nutr., Rutgers Univ., New Brunswick 08903). *Proc. Soc. Exp. Biol. Med.* 132, 253–5 (1969). It was shown that small concentrations of certain dietary polyene macrolides, and in particular the aromatic heptaenes, effectively reduce plasma cholesterol of cholesterol-fed chicks, presumably through a mechanism which involves binding of lipids, particularly cholesterol, in the digestive tract.

HEPATIC AND SPLANCHNIC UPTAKE AND OXIDATION OF FREE FATTY ACIDS. J. J. Spitzer, H. Nakamura, S. Hori and M. Gold (Dept. Physiol., Hahnemann Med. College, Philadelphia, Pa. 19102). *Proc. Soc. Exp. Biol. Med.* 132, 281–6 (1969). The uptake and oxidation of free fatty acids by the liver and by the non-hepatic splanchic area (NHSA) were investigated in anesthetized, control and diabetic dogs. The animals received a constant infusion of albumin-bound 1-^{14}C-palmitate. The liver of the control dogs removed 67 μmoles of FFA/min, the NHSA took up 32 μmoles/min. Hepatic extraction of the labeled FFA was 35%, splanchnic extraction 22% of the arterial level. About 13% of the removed FFA was oxidized both by the liver and by the NHSA. The liver released 13% of the removed labeled carbon as TG, and 7% of the fatty acid carbon as β-OHB. Hepatic FFA uptake accounted for 37%, NHSA uptake for 22% of the total body FFA flux. NHSA removed glucose in the control, but not in the diabetic animals. Uptake and oxidation of FFA, both by the liver and by the NHSA, were considerably elevated in the diabetic dogs. However, the ratios of FFA uptake to total FFA flux were not significantly different. The fractional release of TG from the liver was not significantly changed. A direct correlation was shown between the arterial FFA level and either the uptake or oxidation of FFA by the NHSA. Since neither the pancreas nor the spleen exhibited a more active FFA metabolism than did the intestine, it seemed that most of the metabolic changes noted in the NHSA were due to the gastrointestinal tract.

MECHANISM AND IDENTIFICATION OF THE TRIGLYCERIDE ALTERATION CAUSED BY A PLASMA FACTOR. B. A. Sachs and L. Wolfman (Endocrine Service, Medical Div., Montefiore Hospital, Bronx 10467). *Proc. Soc. Exp. Biol. Med.* 132, 256–7 (1969). The previously described plasma factor capable of producing an increase in the R_f value of triolein in TLC was shown to be bicarbonate acting as a catalyst in the production of ethyl esters of oleic acid from triolein.

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EFFECT OF THE ETHYL ESTER AND SODIUM SALT OF α-P-CHLOROPHENOXYISOBUTYRIC ACID ON CHOLESTEROL OXIDATION BY RAT LIVER MITOCHONDRIA. D. Kritchevsky, S. A. Tepper, P. Sallata, J. R. Kabakjian and V. J. Cristofalo (Wistar Inst. Anatomy, Philadelphia, Pa. 19104). *Proc. Soc. Exp. Biol. Med.* 132, 76–82 (1969). Mitochondrial preparations from livers of rats fed 0.3% ethyl α-*p*-chlorophenoxyisobutyrate (CPIB) show an increased capacity to oxidize cholesterol-26-^{14}C to $^{14}CO_2$. When oxidation is calculated per milligram of mitochondrial nitrogen, oxidation of cholesterol by mitochondria from CPIB-fed rats is the same as that observed in normal rat liver mitochondria. In the absence of the boiled supernatant cofactor (cytosol) the mitochondria from CPIB-treated rats show a significantly higher capacity for cholesterol oxidation than do normal mitochondrial preparations. Mitochondria prepared from livers of rats fed sodium α-*p*-chlorophenoxyisobutyrate also oxidize more cholesterol than do control preparations. Fasting of the rats for 18 hr prior to mitochondrial preparation does not affect the extent of oxidation either in the presence or absence of cytosol. Mitochondrial preparations from CPIB-fed mice also oxidize more cholesterol than do controls. Studies of mitochondrial acid phosphate and β-glucuronidase activity suggest that the increased oxidation of cholesterol is not due to lysosomal activity. Feeding of either the ethyl ester or sodium salt of α-*p*-chlorophenoxyisobutyric acid causes increase in liver size and usually an increase in liver triglyceride levels, confirming results of others.

HEPATIC ACETOACETYL-CoA DEACYLASE ACTIVITY IN RATS FED ETHYL CHLOROPHENOXYISOBUTYRATE (CPIB). R. E. Burch and G. L. Curran (Dept. of Med., College of Physicians and Surgeons, Columbia Univ., New York 10032). *J. Lipid Res.* 10, 668–73 (1969). Intact or sonicated mitochondria from the livers of rats fed a diet containing 0.2% ethyl chlorophenoxyisobutyrate (CPIB) for 3 wk showed acetoacetyl-CoA deacylase activity enhanced 26 and 39%, respectively, over that shown by comparable fractions from rats fed the same diet without CPIB. The corresponding supernatant fractions did not differ in activity. The enhanced activity of mitochondrial acetoacetyl-CoA deacylase in the livers of the CPIB-treated rats could effectively decrease the amount of acetoacetyl CoA available within the cell for synthetic processes.

INTERACTIONS OF LIPIDS WITH A MEMBRANE STRUCTURAL PROTEIN FROM MYELIN. P. E. Braun and N. S. Radin (Mental Health Res. Inst., Univ. of Mich., Ann Arbor, Mich. 48104). *Biochemistry* 8, 4310–18 (1969). A water-soluble, delipidated membrane protein from bovine brain myelin combines with anionic lipids to form insoluble complexes. The minimal amount of phosphatidylserine, phosphatidylinositol, cerebroside sulfate and oleic acid required to precipitate completely the protein corresponds to 39 mole % of the total basic amino acid groups in the protein. Complexes precipitate optimally near neutrality and are stabilized by divalent cations. Nonionic lipids (cholesterol and cerebroside) and lecithin form nonprecipitating complexes with the protein which can be demonstrated by centrifugation in sucrose density gradients. These lipids also bind to protein-anionic lipid complexes. Succinylation of the protein greatly reduces interaction with lipids and abolishes the capacity to form insoluble complexes. These observations are discussed with respect to the structural role which different kinds of lipids might assume in myelin.

ANALYSIS OF THE DISTRIBUTION OF LIPOPROTEIN PATTERNS IN HEALTHY MEN AND IN PATIENTS WITH MYOCARDIAL INFARCTION. G. L. Mills, C. E. Taylaur and P. A. Wilkinson (Middlesex Hosp. Med. Schl. London). *Clin. Chim. Acta* 26, 67–70 (1969). An analysis has been made of the frequency distribution of serum lipoprotein patterns in a population of healthy men and in patients with myocardial infarction. From this it is possible to make a semi-quantative estimate of the degree of abnormality of a given pattern and the success of treatment in returning it to normal. The analysis also suggests that the triglyceride-rich lipoproteins of high flotation rate may be less closely associated with atherosclerotic heart disease than the substances of lower flotation rate, of which cholesterol is the major component.