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ABSTRACTS OF PAPERS

1

TECHNIQUES OF LIPID ANALYSIS. O.S. PRIVETT and W.L. ERDAHL, The Hormel Institute, University of Minnesota, 801 16th Ave. NE, Austin, MN 55912.

A 16 mm film is presented showing techniques of lipid analysis and associated methodology in sample handling and extraction. Specialized techniques of thin layer chromatography (TLC) and ozonolysis for detection and analysis are demonstrated. Apparatus and techniques are also demonstrated for the analysis of lipids by liquid chromatography via a flame ionization detector (LC-FID), a new liquid chromatography-mass spectrometry computer system (LC-MS-COM), and direct analysis by chemical ionization mass spectrometry via a novel interface-computer system (IF-CIMS-COM).

2

INTERACTION OF HUMAN PLASMA HIGH DENSITY LIPOPROTEIN HDL_2 (d 1.063–1.21 g/ml) WITH SYNTHETIC PHOSPHOLIPIDS. ELAINE L. GONG, ALEX V. NICHOLS, and TRUDY M. FORTE, Donner Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720.

Interaction of HDL_2, a major class of high density lipoproteins in human plasma, with sonicates of saturated synthetic phospholipids dihexadecanoyl- ($DiC_{16}PC$), ditetradecanoyl- ($DiC_{14}PC$), didodecanoyl- ($DiC_{12}PC$), didecanoyl- ($DiC_{10}PC$), dioctanoyl- (DiC_8PC), and dihexanoyl- (DiC_6PC) phosphatidyl choline, was evalulated by gradient gel electrophoresis, preparative and analytic ultracentrifugation, and electron microscopy. Incubation of HDL_2 with PC at PC:apolipoprotein HDL_2 ratios (mM:mg) $<1.5:1$ ($DiC_{14}PC$, $DiC_{12}PC$, and $DiC_{10}PC$) and $<2:1$ (DiC_8PC) resulted in marked dissociation of apolipoprotein A-I (apoA-I) from the HDL_2. At higher ratios of the above PC, we observed less dissociated apoA-I and obtained evidence for its incorporation into new complexes. These complexes contained apoA-I and the PC used in the incubation mixtures and had physical-chemical properties similar to model complexes formed during interaction of apoA-I with the corresponding PC. Release of apoA-I was associated with uptake of PC by HDL_2 and resulted in an apparent increase in HDL_2 particle size at low and high ratios for mixtures containing $DiC_{14}PC$, $DiC_{12}PC$, and $DiC_{10}PC$. With DiC_8PC at ratios $\leq 2:1$, there was little change in HDL_2 particle size, although a substantial amount of apoA-I was dissociated. Above the 2:1 ratio, the electrophoretic and ultracentrifugal properties of the HDL_2 changed drastically, indicating extensive redistribution of both lipid and apolipoprotein components. Comparable studies on the interaction of HDL_2 with $DiC_{16}PC$ and DiC_8PC, at both low and high ratios, showed little change in HDL_2 properties. Our results indicate that, under the experimental conditions used, the interaction of HDL_2 with PC which leads to PC uptake, apolipoprotein dissociation, and complexing of apolipoprotein by PC, is strongly dependent on acyl-chain length of the PC.

3

EFFECT OF PHOSPHATIDYL CHOLINE ON THE HEMOLYTIC ACTIVITY OF BILE SALTS. ICHIRO HARA, MITSUYO OKAZAKI, Department of Chemistry, Tokyo Medical and Dental University, Kohnodai, Ichikawashi, Chiba Prefecture, 272, Japan; MAKOTO HAYASHI, and YAKANORI KOBAYASHI, University of Chiba, Japan.

It is well known that bile salts can solubilize many lipid-like matters by forming mixed micelles with them. The purified phosphatidyl choline (PC) (soybean, egg yolk) and synthetic

dipalmitoyl PC were mixed with cholate (NaC) or deoxycholate (DOC) at various ratios of the respective components. We assayed the hemolytic activity of these mixed micelle solutions by using human O-type blood red cell suspended in 0.85% NaCl solution. Hemolysis occurred abruptly at about 0.80 mM of DOC, which corresponds almost to the CMC of DOC, but it is markedly inhibited by adding PC to the solution. The concentration of DOC showing that the hemolysis increases with increasing amount of PC in it is indicated in the following: PC/DOC (mol/mol): 0.1, 0.33, 0.50, 0.67; concentration of DOC for hemolysis (mM): 0.88, 1.20, 4.20, 8.40. The hemolytic activity of NaCl is weaker than that of DOC, and it is inhibited in the same way as the DOC, but it is likely that the activity of NaCl mostly inhibited only at the ratio of 0.1 (PC/NaCl).

4

ALTERED PHOSPHOLIPID POLYUNSATURATED FATTY ACID PATTERNS IN HEPATIC AND CEREBRAL TISSUES OF VITAMIN B_{12} DEFICIENT RATS. JAMES J. PEIFER, J.N. MORGAN, and R.D. LEWIS, Foods and Nutrition Department, Dawson Hall, University of Georgia, Athens, GA 30602.

Methylmalonyl CoA (MMCoA) mutase and homocysteine methyltransferase (HMT) are both blocked by a deficiency of vitamin B_{12} (B_{12}). The consequences of such metabolic blocks, including an inhibition of acetyl CoA carboxylase, could conceivably interfere with elongation of linoleate (18:2) and the metabolism of polyunsaturated fatty acid (PUFA)-rich phospholipids. Groups of female rats were fed diets that included 3.5% or 0.35% 18:2, 24% soy protein, 0.25% cystine, and 0 or 5 μg B_{12}/100 g diet. After 20 weeks of B_{12} deprivation, hepatic levels of methylmalonic acid were 7 times greater than normal. Ratios of arachidonate (20:4) to 18:2 were, respectively, 2.4 and 5.2 in hepatic phosphatidyl choline (PC) of B_{12} deficient and supplemented groups. Cerebral phospholipids also contained less 20:4 and less total ω6-PUFA. More 18:2, but less 20:4 and 22:5, were found in phospholipids of B_{12} deficient rats. Such observations suggest that a B_{12} deficiency seriously interferes with the conversion of 18:2 → 20:4 → 22:5. Related studies suggest that the altered PUFA patterns are partly due to metabolic blocks of both mutase and HMT. (Supported in part by Ga. Expt. Station S-87 Regional Project funds.)

5

RADICALS FORMED IN AUTOXIDIZING METHYL LINOLEATE DURING THE INDUCTION PERIOD. TOSHIYUKI CHIBA, KENSHIRO FUJIMOTO, TAKASHI KANEDA, Faculty of Agriculture; SHOZO KUBOTA, and YUSAKU IKEGAMI, Chem. Res. Ins. Nonaqueous Solution; Tohoku University, 1-1 Amamiyamachi-Tsutsumidori, Sendai, Japan.

Many workers have studied the autoxidation mechanisms and oxidative products of unsaturated lipids, but little information has been reported on the alterations of lipids during the induction period. Conventional methods for the estimation of lipid oxidation are not useful to study the changes during the induction period because changes in the chemical composition are too small during that period. Reactions during the induction period are supposed to be radical reactions, and several papers have been published on the free radicals in autoxidized oils. The intensities of ESR signals which we measured without a spin trap were very weak and recognized only at 77° K, so it was impossible to analyze the detailed structure of radicals. In this paper the autoxidation mechanism of methyl linoleate during the induction period was investigated by using spin traps that can trap the short-lived free radicals. As substrate oil, methyl linoleate (ML) was prepared and trace peroxides in ML were eliminated by silicic acid column chromatography. Several spin traps were tested, and it was ascertained that 2,3,5,6-trideuteriomethyl nitrosobenzene (DMNB) was suitable for this purpose. ML and DMNB dissolved in benzene were irradiated with UV light, and ESR was measured. The ESR spectrum consisted of a doublet or triplet. This spectrum indicated that the trapped radicals were secondary alkyl radicals. The benzene solutions were irradiated with light passed through several filters. At shorter wavelengths of light greater increase of the signal intensities was recognized. After several minutes the signal intensity increased little. At high concentration (1.5×10^{-2}M), DMNB acted as the initiator of radical production.

6

ANALYSIS OF AUTOXIDIZED AND PHOTOSENSITIZED OXIDIZED FATS BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY. E.N. FRANKEL, W.E. NEFF, and T.R. BESSLER, Northern Regional Research Center, USDA, 1815 N. University, Peoria, IL 61604.

The oxidation products of natural fats include hydroperoxides of oleate, linoleate, and linolenate, and methods to determine their origin are of considerable importance in elucidating precursors of offensive odors and flavor compounds. Quantitative gas chromatography-mass spectrometry (GC-MS) revealed a relatively constant isomeric hydroperoxide composition in samples of pure oleate, linoleate, and linolenate autoxidized to different peroxide values (PV). In contrast, with soybean oil esters the isomeric hydroperoxide distribution varied significantly with different levels of oxidation. At PVs below 50 the proportion of 10- and 12-hydroperoxides was higher than expected by autoxidation of individual fatty esters or their mixtures. Cottonseed and safflower oil esters, free of linolenate, also showed the presence of 12-hydroperoxides when oxidized at low levels. Several lines of evidence support the conclusion that photosensitized oxidation may contribute to the unique hydroperoxide composition of vegetable oil esters at low PVs. A complex multitude of volatile products is expected from decomposition of each of the 12 isomeric hydroperoxides found in oxidized soybean oil.

7

STUDY OF ADDITION COMPOUNDS OF TOCOPHEROL AND LONG CHAIN UNSATURATED COMPOUNDS. HAJIME SEINO and SHOICHIRO WATANABE, Department of Chemistry, Faculty of Hygienic Sciences, Kitasato University, Asamizodai 1, Sagamihara-shi, Kanagawa-ken, 228, Japan.

It was reported that an addition compound of α-tocopherol and linoleic acid was formed when they were heated in air. However, the accurate structure of the addition compound and mechanism of its formation have not yet been clarified. α-Tocopherol and linoleic acid, oleic acid, or methyl esters of these acids were mixed in several ratios and heated under various conditions. The formation of addition compounds of α-tocopherol and linoleic acid or methyl linoleate was confirmed although those of oleic acid or methyl oleate were not observed. It was also found that methyl linoleate could form the addition compound more readily than the acid. 6-Hydroxy-2,2,5,7,8-pentamethyl chroman was then synthesized by the reaction of trimethyl hydroquinone and isoprene, and used as the model compound of α-tocopherol. The model compound was mixed in several ratios with methyl linoleate and heated under various conditions. The reaction mixtures were analyzed periodically by high performance liquid chromatography to examine the formation of the addition compounds.

8

THE LIPIDS OF HUMAN MILK: A REVIEW. ROBERT G. JENSEN and RICHARD M. CLARK, Department of Nutritional Sciences, University of Connecticut, Storrs, CT 06268.

Human milk contains about 3.5% lipid, 98% of which is triacylglycerol with palmitic acid primarily as the secondary ester. Phospholipids, sterols, and other lipids are also present. The cholesterol content ranges from 3.4–25.0 mg per 100 ml of milk. Over 150 fatty acids have been found in human milk lipids; the major fatty acids being palmitic, oleic, stearic, and linoleic. The fatty acid composition responds to changes in diet with linoleic acid contents of from 1.0 to 45.0% having been reported. The average amount of linoleic acid is about 10%. Trans unsaturated fatty acids, 2–18%, have also been detected. Problems in sampling and analysis will be discussed.

9

FATTY ACIDS OF HUMAN MILK AND OF INFANT FORMULAE. J.L. BEARE-ROGERS, L.M. GRAY, and R. HOLLYWOOD, Bureau of Nutritional Sciences, Food Directorate, Ottawa, Ontario K1A 0L2, Canada.

Most of the polyenoic fatty acids in human milk sample reacted with lipoxidase and therefore contained the *cis-cis*-methylene-interrupted system of double bonds. The *trans*-fatty acids occurred mostly in the 18:1 fraction. Lactating women living at home produced milk of a higher content of *trans* fatty acids than did women in hospitals. Compared to human milks, commercially available infant formulae exhibited some particularly high levels of linoleic acid and lacked longer chain polyenoic fatty acids and the phospholipids which usually contain them. Such products might be improved by the addition of a membrane-rich fraction from cow's milk.

10

LIPID COMPOSITION OF NORMAL AND ICTEROGENIC HUMAN MILK. MARY F. PICCIANO, Department of Foods and Nutrition, and EDWARD G. PERKINS, Department of Food Science, 104 Burnsides Research Laboratory, University of Illinois, Urbana, IL 61801.

Prolonged neonatal unconjugated hyperbilirubinemia associated with human milk feeding has been described as a clinical entity. Its exact etiology is unknown. Several investigators have suggested that this form of neonatal jaundice is caused by inhibition of glucuronide conjugating systems essential for bilirubin excretion. It has been reported that the presence of 3α,20-β-pregnanediol is the causative agent in the milk of mothers nursing infants with prolonged jaundice. This compound has been shown to elicit a rise in serum bilirubin levels when administered to normal term infants and to inhibit the excretion of bilirubin glucuronide from rat liver slices. Recent evidence indicates that 3α,20-β-pregnanediol may not be the sole causative factor inducing prolonged hyperbilirubinemia. Some investigators have been unable to confirm its presence in human milk samples from mothers of afflicted infants. The present investigation was undertaken to examine the lipid composition of both the fatty acid and unsaponifiable fraction from icterogenic milk and compare it to normal human milk lipid. The lipid compositions were determined and confirmed by both gas liquid chromatography and gas chromatography-mass spectrometry.

11

HUMAN MILK LIPIDS AS HOST RESISTANCE FACTORS. JON J. KABARA, Department of Biomechanics, East Fee Hall, Michigan State University, East Lansing, MI 48824.

Breast feeding of infants has been replaced by modern prepared formulae. In underdeveloped countries, in particular, formulae feeding has become popular. It is recognized that breast feeding confers on infants a degree of resistance to infection not present in those fed cow or synthetic milk, but the exact cause of this resistance is not clearly understood. Several antibacterial and antiviral factors have been detected in human milk. Of the nonprotein factors, uncharacterized lipids have been implicated as the nonspecific antibacterial and antiviral substances. Likely candidates found in human milk and shown to have moderate to strong biological activity are fatty acids and monoglycerides. Past relationships of structure-function activity for fatty acids and their monoester will be reviewed. Lauric acid, (C_{12}) a saturated acid, palmitoleic acid ($C_{16:1}$), monounsaturated acid, and linoleic acid ($C_{18:2}$), a polyunsaturated acid were the most active members of their respective fatty acid class. The monoesters, however, of these same fatty acids did not follow this same generality, and their exceptions will be discussed. The application of our findings and those of others to food formulators of infant foods will be presented.

12

MAMMARY TRANSFER AND METABOLISM OF HALOGENATED LIPIDS IN THE RAT. H.B.S. CONACHER, R.K. CHADHA, S.M. CHARBONNEAU, and F. BRYCE, Food Research Division, Health Protection Branch, New Research Centre Health and Welfare Canada, Tunney's Pasture, Ottawa, Ontario, K1A 0L2, Canada.

To assess possible incorporation of halogenated fatty acids into the neonate via the milk, lactating Wistar rats were orally

dosed with either brominated olive oil (BOO) (0.6 g/kg body wt/day) or chlorinated olive oil (COO) (0.4 g/kg body wt/day) for 4 days. On days 1-5 inclusive 2 pups per litter were sacrificed and the stomach curd and livers were analyzed for halogenated fatty acids by gas liquid chromatography. On day 5 all dams were also sacrificed and their livers and adipose tissue similarly analyzed. With BOO, brominated fatty acids (bfa) accumulated in both the milk lipids and neonate liver lipids, and appeared to plateau on day 4 at levels of 2% and 5%, respectively. In contrast to the BOO, in which approximately 100% of the bfa was dibromostearic (DBS), the milk bfa comprised 77% (DBS), 8% dibromopalmitic (DBP), and 15% dibromomyristic (DBM) acids, suggesting maternal metabolism to the shorter chain brominated acids. In the neonate liver lipids the bfa composition was 44% (DBS), 12% (DBP), and 44% (DBM), suggesting either further metabolism in the neonate and/or preferential accumulation of the shorter chain brominated acids. The analysis of maternal tissues indicated very low bfa residues, contrary to previous studies in nonlactating rats. Similar results were obtained with COO.

13

DIAGNOSIS OF ESSENTIAL FATTY ACID DEFICIENCY. RALPH T. HOLMAN, The Hormel Institute, University of Minnesota, 801 16th Ave. NE, Austin, MN 55912.

The manifestations of essential fatty acid (EFA) deficiency in man will be reviewed and illustrated. The changes in fatty acid patterns of serum and tissue lipids have been well characterized for man and have been found to be qualitatively similar to those observed in experimental animals. Use of several parameters calculated from analyses of serum lipid fatty acids for the detection and estimation of severity of EFA deficiency in man will be presented. Quantitative methods for estimate of recent intake of EFA will be described.

14

FATTY ACID PATTERNS OF SERUM LIPIDS IN CYSTIC FIBROSIS. SUSAN JOHNSON, The Hormel Institute; JOHN LLOYD-STILL, Children's Memorial Hospital, Chicago, IL; and RALPH T. HOLMAN, The Hormel Institute, University of Minnesota, 801 16th Ave. NE, Austin, MN 55912.

The fatty acid compositions of serum phospholipids (PL), cholesteryl esters (CE), triglycerides (TG), and free fatty acids (FA) were determined on a group of cystic fibrosis (CF) patients. These were compared with similar data from random hospitalized patients of both sexes ranging in age from 0 to 90 years. Fatty acid patterns in all lipid classes were skewed in the direction of essential fatty acid (EFA) deficiency, but the differences were most dramatic in PL. Many calculated parameters useful as indices of EFA status indicated that marginal EFA deficiency exists in CF. The arachidonic acid level in FA was markedly elevated in some of the CF patients. Treatment of 11 CF patients with safflower oil (1 g/kg) failed to correct the aberrations in fatty acid pattern.

15

EVALUATION OF FETAL LUNG MATURITY IN UTERO BY GAS CHROMATOGRAPHY. ERIC J. SING, RICHARD DEPP, MARY ANN FLETCHER, Department of Obstetrics and Gynecology; and DANTE G. SCARPELLI, Department of Pathology; Northwestern University Medical School, 333 E. Superior St., Chicago, IL 60611.

There are various methods for the prediction of fetal lung maturity. Amniotic fluid phospholipid thin layer chromatographic patterns can be used for assessment of fetal lung maturity. The level of phosphatidyl ethanolamine is a good marker for fetal lung maturity in normal human pregnancy. At term pregnancy the level of phosphatidyl ethanolamine is significantly lower when compared with earlier gestation levels. Similarly the level of 20:4 fatty acid is low at term pregnancy. The level of palmitic acid in total lipid of human amniotic fluid is a diagnostic value for the fetal lung maturity. The contamination of meconium and the total palmitic acid in human amniotic fluid will be discussed. The total palmitic/stearic acid ratio at various gestational weeks will be presented, and the results will be correlated with fetal lung maturity.

16

INVOLVEMENT OF PROSTAGLANDINS IN RESPIRATORY DISTRESS SYNDROME IN THE NEWBORN. ERIC J. SING, Department of Obstetrics and Gynecology, Northwestern University Medical School, 333 E. Superior St., Chicago, IL 60611.

Amniotic fluid phospholipids may be important compounds clinically for several reasons. It seems that phosphatidyl ethanolamine and possibly phosphatidyl serine contents of lung play a major role in the synthesis of prostaglandins; however, the phosphatidyl choline contains arachidonic acid. It seems possible that the fatty acids 20:4 and 18:2 might be associated with infants who develop respiratory distress syndrome (RDS). Therefore, it is the purpose of this report to present the data on precursor of prostaglandins in human amniotic fluid at various gestational weeks. A theory is suggested that prostaglandins are involved in RDS and saturated fatty acids are surfactant in human lung. In immature lung the precursor of prostaglandins are high as compared with mature lung. It may be possible that prostaglandin $F_{2\alpha}$ is involved in the process of RDS. It is interesting that the lungs of premature infants are deficient in saturated fatty acids. In newborn infants the RDS is caused by the ability of the fetal or neonatal lung to synthesize adequate quantities of prostaglandin $F_{2\alpha}$ and lack of saturated fatty acids, which act as surfactant. Although prostaglandins are not directly assayed in any of these experiments, it seems likely that they act as local autoregulatory agents. A discussion of the relationship among maturity and prematurity of lung, and RDS will be presented.

17

INVESTIGATION OF THE LOW LEVELS OF LINOLEIC ACID IN THE SKIN LIPIDS OF ACNE PATIENTS. MARY ELLEN STEWART and DONALD T. DOWNING, Dermatology Department, College of Medicine, University of Iowa, Iowa City, IA 52242.

Our previous studies have shown that the linoleic acid content of the skin surface lipids of acne patients (0.19%) is much lower than that of normal subjects (0.56%). Since hyperkeratosis is a symptom of essential fatty acid deficiency, low linoleic acid levels might be responsible for the follicular hyperkeratosis characteristic of acne. However, a group of acne patients was not found to be deficient in circulating essential fatty acids relative to a control group. Since any deficiency in acne might be localized in the skin, we have examined the anatomical origin of the linoleic acid found in skin surface lipids. Preparative separations of wax esters (which originate in the sebaceous glands) from cholesterol esters (coming both from the glands and from the epidermis) have been achieved by thin layer chromatography and gas liquid chromatography. Examination of the fatty acids recovered from the esters indicated that the sebaceous lipids reaching the skin surface may be devoid of linoleic acid, the presence of which in the total surface lipids may be dependent upon the epidermal contribution. Since we have shown that the phosphatidyl choline from isolated sebaceous glands contains 3.1% linoleic acid, this must be recycled before the maturing sebaceous cells disrupt and release their lipid content to the skin surface.

18

DIETARY REQUIREMENTS FOR LINOLENIC ACID IN ANIMALS. J. TINOCO, R. BABCOCK, I. HINCENBERGS, B. MEDWADOWSKI, and P. MILJANICH, Department of Nutritional Sciences, University of California, Berkeley, CA 94720.

Linolenic acid (α-linolenic acid, 9,12,15-octadecatrienoic acid or 18:3n-3) is synthesized by plants but not by vertebrates. It is efficiently digested, absorbed, metabolized, and transported in the animal body. In some species it can be elongated and desaturated to higher homologs. There must be great selectivity for n-3 fatty acids in metabolic and transport processes because n-3 fatty acids are specifically located in particular phospholipid molecules in specific organs or organelles. The fact that these selective processes have survived evolutionary pressures suggests that n-3 fatty acids may play an essential role in animal cells. So far, essentiality of dietary n-3 fatty acid has been proved only in fish. Rainbow trout, for example, require roughly 1% of the diet as either 18:3n-3 or 22:6n-3 for normal growth and development, and, unlike warm-blooded animals, require little or no linoleate (18:2n-6) or other n-6 fatty acids. At least three other species of fish are known to require n-3 fatty acids for good growth. In warm-blooded animals, the dietary requirement, if any, for n-3 fatty acids is unknown. Linolenic acid does have growth-stimulating activity in essential fatty acid-deficient rats, but does not cure the dermatitis and infertility characteristic of EFA deficiency. We have raised rats for two or three generations on diets that provide only about 40 mg/kg diet of 18:3n-3, plus 12.5 g/kg of 18:2n-6. These rats were similar in growth, food intake, organ weight, and appearance to rats that had been raised on a diet containing 2.5 g 18:3n-3 plus 10 g 18:2n-6/kg diet. In lipids of the linolenate-deficient rats, n-3 fatty acids, especially 22:6n-3, were reduced to 10% or less of control values, and were replaced largely by 22:5n-6. Thus the requirement of dietary n-3 fatty acids for survival in rats is no greater than about 40 mg/kg diet. (This work was supported in part by USPHS grant AM 10166 and by Cooperative Regional Project NC-95, California Agricultural Experiment Station.)

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LIPID CHANGES IN PERIPHERAL NERVE MYELIN IN ESSENTIAL FATTY ACID DEFICIENCY. JEFFREY K. YAO, BRUCE A. EVANS, Mayo Clinic; RALPH T. HOLMAN, Hormel Institute; MICHAEL F. LUBOZYNSKI, and PETER JAMES DYCK, Room 812, Guggenheim Building, Mayo Clinic, 200 1st St. SW, Rochester, MN 55901.

The effect of essential fatty acid deficiency (EFAD) on peripheral nerve (PN) function, structure, and myelin composition is largely unknown. The lipid classes and fatty acid composition of isolated myelin from rats fed EFAD diets for 8 months after weaning were compared to those of controls fed conventional diets. Eicosatrienoic acid (20:3ω9), which was not found in control myelin, was present in phospholipids and free fatty acids of EFAD-PN myelin. A lower proportion of 18:2ω6 and 20:4ω6, and a higher proportion of 18:1ω9 was seen in EFAD-PN myelin than in control myelin. The cholesterol, phospholipid, and glycosphingolipid content of EFAD-PN myelin was about one-half that of control myelin. In both EFA and control rats, sciatic nerve of one hind limb was crushed and allowed to regenerate for 18 weeks. The ratio of 20:3ω9 to 20:4ω6 in phospholipids from regenerated myelin was similar to that found in uncrushed myelin. Fatty acid profiles of phospholipids and glycosphingolipids were also similar, which suggests that, during degeneration and regeneration, myelin lipids are sequestered and reused. No significant difference was found in histological, morphological, electrophysiological, and axonal transport studies between deficient and control nerves. A slightly smaller median diameter, however, was found in regenerated EFAD myelinated fibers. These results suggest that changes of lipid composition of myelin, secondary to EFAD, may be pronounced without resulting in a detectable change in function or structure. Nerve regeneration may be slightly impeded by EFA deficiency.

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LOW DIETARY IRON MAY CAUSE A DELAY IN THE ONSET OF ESSENTIAL FATTY ACID DEFICIENCY. G. ANANDA RAO and EDWARD C. LARKIN, Veterans Administration Medical Center, 150 Muir Rd. (151-H), Martinez, CA 94553.

Tissue desaturase activity is enhanced when animals are fed a fat-free diet. Since cytochrome b_5 and the cyanide sensitive factor present in desaturase enzyme contain iron, we theorized that if iron is excluded from the diet, the increase in the activity of the enzyme could be prevented. In the present study, we fed young rats fat-free diets for 2 months. Diets were of a low iron content or were supplemented with iron. Analysis of the fatty acid composition of lipids of tissues (intestinal mucosa, liver, plasma, and erythrocytes) showed that the relative level of 16:1 to 16:0 was decreased by feeding a low iron diet. This indicated that the tissue desaturase activity was depressed by low levels of dietary iron. The levels of 18:2 and 20:4 were appreciably greater in tissue lipids of rats fed a low iron diet than in those from rats fed an iron supplemented diet. Thus, the capacity of tissues to retain essential fatty acids was enhanced by the exclusion of dietary iron. The level of 20:3 was much greater than that of 20:4

in tissues of rats fed the iron supplemented diet, whereas the level of 20:4 was high and that of 20:3 was small on feeding a low iron diet. Therefore, the onset of essential fatty acid deficiency, as indicated by the ratio of 20:3 to 20:4 in tissues, was delayed due to feeding the low iron diet. The packed red cell volumes of rats fed either diet were essentially similar. Thus, upon feeding a fat-free, low iron diet, although desaturase activity was decreased, utilization of iron for red cell synthesis was unaffected.

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RELIABILITY AND OPTIONAL OPERATING CONDITIONS WITH MODERN FLAKERS. JACK A. HOSTETTLER, Oil Milling Division, Buhler-Miag, Inc., Minneapolis, MN.

The design of flakers has been steadily improved over the past decade. The development to high capacity plants has required very reliable machines which must also meet today's high requirements in regard to sanitation and noise emission. Improved feeding systems and product side guides result in an even wear over the entire roll length, which minimizes the roll maintenance.

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EFFECT OF SEED PREPARATION ON OIL QUALITY IN FILTRATION EXTRACTION OF RAPESEED. AKIRA NISHIOKA, Oil & Fat Technical Center, Ajinomoto Co., Inc., 7-14 Daikokucho, Tsurumi-ku, Yokohama 230, Japan; MASAO NAGASHIMA, and TETSUO HINO, Toyo Oil Mills Co., Inc.

The effects of the conditions of seed preparation such as moisture content of seed, cooking temperature, and retention time for pretreatment in filtration extraction of rapeseed on the quality of rapeseed oil, including content of phospholipids, bivalent metal (Mg, Ca), and hydratability of phospholipids are studied. As a result, the elution of phospholipids and Mg increased as moisture of rapeseed increased and cooking conditions became more severe. However, elution of Ca indicated maximum value at 100 C and 30 min. In general, a simple equation is used to represent the relation between content of phospholipids and Mg + Ca contained in extracted oil under normal pretreatment conditions. The hydratability of phospholipids in extracted oil depends on the content of the bivalent metals. Therefore, the content of phospholipids in degummed oil can be estimated accurately by measuring the content of Mg + Ca in extracted oil. In view of the above results, it is desirable to perform seed preparation under low moisture content in order to obtain crude oil which contains hydratable phospholipids. It is reported by Hvolby that phospholipids combined with Ca and Mg are nonhydratable in the case of soybean oil. The same result is also obtained for rapeseed oil.

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EVALUATION OF EXTRACTION PLANT DESIGN AND OPERATION. EDWARD D. MILLIGAN and DAVID C. TANDY, EMI Corporation, 3166 Des Plaines Ave., Des Plaines, IL 60018.

The design criteria and operating techniques for oilseed solvent extraction plants have undergone continual modifications over the past 35 years. The purpose of these modifications has always been to achieve more reliable equipment capable of continuous extended operation without the need for excessive maintenance, and capable of efficient operation with adequate reserve for upsets while still retaining economy of investment and operation; then to operate this equipment safely while still obtaining high quality products at the lowest possible production cost. Experience has shown that a successful installation depends not only on the extractor itself but also on the proper design and operation of other areas including preparation, miscella distillation, meal desolventizing, solvent recovery, material handling, etc. This paper reviews some of these design criteria and operating techniques that are used today, with particular emphasis on the dependency of the design and operation of one part of the plant with the others. Some procedures for evaluating both a proposed plant design and an existing operation are also presented.

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IMPROVEMENTS AND INNOVATIONS IN THE TECHNOLOGY AND EQUIPMENT OF OIL PROCESSING. H.L.S. STAFF, H.L.S. Ltd., Industrial Engineering Company, PO Box 193, Petah Tikva, Israel.

H.L.S. improvements and innovations in the technology and equipment of oil processing are discussed such as a new design of extractor baskets (T.O.M.) which turn over at midway thus inverting the material and dropping it into the basket below in order to avoid formation of an impermeable layer of cake or material. This reduces extraction time and increases the miscella concentration. An overlapping strip of flexible material is attached to each basket and covers the spaces between the baskets, thus eliminating the spilling of material. In the deacidifier-deodorizer operating semi-continuously or continuously, the location of all heat transfer equipment is outside the unit, and a special oil-to-oil heat exchanger ensures a good recuperation of calories. Two kinds of steam introducing devices ensure perfect dispersion of steam in oil and intensive contact with the oil at the same time low consumption of stripping steam. H.L.S. has also developed a new winterizing, dewaxing, and fractionating method in solvent (isopropyl alcohol) without the use of filters and centrifuges. The separation between liquid and solid fractions (crystals) is carried out by a special continuous decantation system.

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SOME STUDIES ON FURTHER IMPROVEMENT OF THE QUALITIES OF EDIBLE OILS. MORIO HAMASHIMA, HISASHI WATANABE, TADASU FUJITA, MASANAO OZEKI, and KOSAKU YASUDA, Research and Development Division, The Nisshin Oil Mills, Ltd., 3,1-Chome, Chiwaka-cho, Kanagawa-ku, Yokohama, 221, Japan.

The improvement of the quality of edible oils is an old yet still new subject which has even more growing importance to the oil industry these days in Japan, as oils obtain a wide range of use in many segments of the food industry. There are many factors which affect the quality of the oils during processing, including selection of raw material origin, method of crushing, refining, and packaging. This paper will review our experiences in some areas of processing of soybean oil and rapeseed oil, which are major and popular sources of liquid oil in Japan. Topics discussed are: (a) crushing condition and quality of the oil and meal, (b) improvement of flavor stability of edible oils by transfer/hydrogenation, and (c) improvement of flavor and oxidative stabilities of edible oils in a plastic bottle with nitrogen gas.

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THE REFINING OF FATTY OILS: THE ROLE OF THE MINOR CONSTITUENTS. MORRIS MATTIKOW, Consultant, New York, NY.

The amount and nature of the minor constituents of a crude vegetable oil often are the determining factors that influence the type of refining and the refining losses incurred. In as much as the phosphatides are present in most crude vegetable oils, as the major component of the minor constituents of crude soy oils, or in much lesser amounts in crude palm oils, the P content of the final refined oil can serve as an index of the degree or extent of refining, a measure of the quality along with other standards. The minor constituents of a crude vegetable oil also play a role in the "ecological factor" that has become of increasing concern in the past ten years. Special processes for the treatment of soapstocks have been proposed to reduce or eliminate waste effluents, and at the same time produce upgraded acid oils. For crude vegetable oils, such as crude palm oils that lend themselves to steam refining, the problems arising from the production of soapstock as in alkali refining, the most common method, are avoided. The problems of acidulation of soapstock are essentially caused by the phosphatides of the crude oil that was alkali refined. The phosphatides, not completely split in the refining with sodium hydroxide solution, and then in the soapstock, further resist decomposition with sulfuric acid. Nondegummed crude fatty oils, for example soy oil or corn oil relatively high in phosphatides, yield soapstocks that are more difficult to split than soapstock from low phosphatide crude oils.

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SOLVENT EXTRACTION OF SPENT BLEACH CLAY. DAVID C. TANDY and RALPH W. BERGER, EMI Corporation, 3166 Des Plaines Ave., Des Plaines, IL 60018.

In the refining of vegetable oils, bleaching to obtain a lighter color is one of the commonly used processing steps. It is done either in batch or continuous units and involves the addition of activated earth bleaching clays to the oil followed by heating and mixing of the slurry for sufficient time to allow the clay to absorb the color from the oil. The slurry is then filtered to remove the clay. To minimize the oil loss, the filter cake is usually steamed and blown with air to recover as much oil as possible before disposing of the spent clay. However, good quality clays have high absorbent activity and, therefore, can retain as much as 45% their weight of oil. This oil can be recovered by solvent extraction. This paper discusses a clay filter cake solvent extraction process recently designed and installed in a large edible oil refinery. The process involves the circulation of hexane through the cake on a pressure leaf filter and the subsequent evaporation of the hexane from the hexane-oil miscella. The operation was integrated into the normal bleach filter cycle and special safety precautions were introduced for handling the flammable solvent.

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DRY-COLUMN METHOD FOR EXTRACTION OF LIPID FROM MUSCLE AND ADIPOSE TISSUES. WILLIAM N. MARMER, ROBERT J. MAXWELL, MARTA P. ZUBILLAGA, and GAIL A. DALICKAS, Eastern Regional Research Center, USDA, 600 E. Mermaid Lane, Philadelphia, PA 19118.

We have developed a dry-column method for the isolation of lipid from muscle and adipose tissues that is a marked departure from procedures currently used. Unlike either the Folch or the Bligh-Dyer method, the new method requires neither tissue homogenization in solvent before extraction nor the removal of nonlipids after extraction. Instead, a weighed sample of tissue is added to a mortar, ground with anhydrous sodium sulfate and then with Celite 545 diatomaceous earth. The resulting dry powder is transferred to a chromatographic column and the lipid—total, or neutral, and polar, in succession—eluted with the proper solvents. We extracted several tissue samples in this manner and found that the results compared favorably with those from parallel Folch extractions. To establish the best conditions for lipid elution and nonlipid retention, we experimented with alterations in the solvent system and the use of postelution adsorbents at the base of the column. We followed the elution of phospholipids by monitoring the eluates by thin layer chromatography (TLC) and colorimetric determination of phosphorus. TLC with visualization by ninhydrin showed the presence or absence of nonlipid, which was also quantified gravimetrically after fractionation using Sephadex G-25 chromatography. Because the new method combines efficient and nondestructive extraction of lipids with simplicity and rapidity of operation and in addition allows a partial resolution of lipid classes, research chemists and food scientists should find it vastly superior to currently used procedures.

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STUDIES ON THE METABOLIC FATE OF ALPHA OLEFIN SULFONATE. SHUNJI INOUE, AKIRA FUKUSHIMA, MASAHIDE IIMORI, Lion Biol. Res. Lab.; J.S. O'GRODNICK, G.D. DUPRE, Bio/dynamics Inc.; and SETSUO TOMISAWA, Department of Pharm., Kitasato University, 202, Tajima, Odawara-Shi, Kanagawa Pref., Japan.

The absorption, distribution, metabolism, and excretion of ^{14}C-alpha olefin sulfonate (AOS) were studied in rats by oral administration of 100 mg (50 μCi) ^{14}C-AOS/kg. After dosing, ^{14}C-AOS was easily absorbed from the gastrointestinal tract. The blood level reached the peak 3 hr after dosing and then rapidly eliminated. Twenty-four hr after dosing, about 2% of the ^{14}C-AOS given was detected in cecum contents, but in other tissues the figures were under 0.1%. Within 24 hr after dosing, 72% of the dose was excreted in the urine and 22% in the feces, while the excretion in the bile was 4.3% within 12 hr. Given radioactivity was almost rapidly disappeared from whole body within 24 hr. AOS and its metabolite were analyzed by several methods: (a) Thin layer chromatography (TLC) with several solvent systems was employed for the separation of metabolites. The results suggested that the Rf

of the ^{14}C-activity was lower than that of standard ^{14}C-AOS. (b) Chromagenic reagents which are specific for functional groups were employed to qualitatively classify the metabolites. (c) Chemical reaction with several reagents of the metabolites was carried out. (d) Binding in vitro of ^{14}C-AOS and its metabolites to bovine or rat serum protein was studied using electrophoresis and equilibrium dialysis techniques. The results suggested that ^{14}C-AOS was bound with albumin, while the metabolite was not. The data obtained from several experiments indicate that the metabolite of ^{14}C-AOS is more polar than intact AOS and may be hydroxylated or polyhydroxylated sulfonic acids with shorter chain.

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LIPID BROMINE CONCENTRATIONS IN TISSUES OF RATS FED BROMINATED FATTY ACIDS. BARBARA JONES, ROBERT R. LOWRY, and IAN J. TINSLEY, Department of Agricultural Chemistry, Oregon State University, Corvallis, OR 97331.

Rats were fed brominated corn oil (0.8% of diet), ethyldibromostearate (0.5%), and ethyltetrabromostearate (0.5%) for periods of 5 and 10 days; and 1, 3, and 6 months. Lipid extracts of heart, liver, muscle, adipose, and kidney tissues were analyzed for bromine by neutron activation analysis. Maximum lipid bromine concentrations were found in the tissues of the 5-day animals and in general decreased with time. Lipid bromine concentrations were highest in animals fed brominated corn oil with lower levels in those animals ingesting ethyldibromostearate and ethyltetrabromostearate, respectively. Liver gave the highest concentrations of lipid bromine with all diets. An initial absorption study indicates differences in absorption among the brominated compounds. Rats were dosed with ca. 50 mg of one of the brominated compounds, and feces and urine were analyzed for bromine. Ethyldibromostearate gave the highest bromine concentration in urine, while the highest fecal bromine concentration was obtained with the tetrabromostearate. Brominated corn oil gave intermediate values in both cases.

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AN INVESTIGATION OF THE METABOLISM OF POLYENOIC ACIDS IN ISOLATED RAT SERTOLI AND GERMINAL CELLS. JEFFREY K. BECKMAN and JOHN G. CONIGLIO, Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37232.

It has become evident in recent years that lipids are of importance to the development and functioning of testicular tissue. Of particular interest is the role of the 22-carbon polyenes (22:5ω6 in the rat, 22:4ω6 in the rooster, and 22:6ω3 in the human) since they accumulate in testes during the time of sexual maturation. Because the testis is an organ composed of many different cell types, we were interested in investigating testicular lipid metabolism at a cellular level. Studies on the lipid composition of spermatids, spermatocytes, and Sertoli cells isolated from rat testes showed that spermatids contained large quantities of 22:5ω6 (≅20% of the total fatty acids), while spermatocytes and Sertoli cells contained much smaller amounts of this polyene (<10% of the total fatty acids). We are presently comparing the capacity of different rat testicular cell types to synthesize 22:5ω6. Enriched fractions of germinal cells and of Sertoli cells were isolated from rat testes at specific intervals following an intratesticular injection of either 1-^{14}C-linoleate or 1-^{14}C-arachidonate. The lipids of these cell fractions were then extracted and the distribution of radioactivity in individual fatty acids was determined following preparative gas chromatography. Sertoli cells contained in their total lipids a much higher specific activity of 22:5ω6 at all time points than did the germinal cells. It is concluded that although germinal cells accumulate most of the testicular 22:5ω6, Sertoli cells are likely the cell type most responsible for its synthesis. (Supported by USPHS Grant no. HD 07694.)

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ON THE ORIGIN OF CAROTENE IN BOVINE MILK FAT GLOBULES. STUART PATTON, Department of Food Science, 111 Borland Lab., The Pennsylvania State University, University Park, PA 16802; and JOHN J. KELLY, Swarthmore College, PA.

There is confusion regarding the origin and location of carotenoids in bovine milk fat globules. A prevalent hypothesis holds that this carotene is contained in the plasma membrane which envelops the milk fat droplet at secretion. To investigate this question we prepared milk fat globule membrane and intracellular fat droplets so that their carotene contents could be assayed. The milk fat globule membrane (4 preparations) was isolated by separating fresh morning milk, washing the cream fraction four times with 0.25 M sucrose, churning the cream, and sedimenting the released membrane from the buttermilk at 49,000 × g for 2 hr. The droplets were isolated as cream layers by homogenizing minced lactating tissue in 0.25 M sucrose and centrifuging the homogenate at 1000 × g for 12 min and 10,000 × g for 20 min (tissue from 3 animals). Lipids were extracted from these membrane and droplet preparations and from their corresponding milks by the Roese-Gottleib procedure. The presence and amounts of carotene in the extracts were determined spectrophotometrically. There was no carotene (<0.5 μg/g of lipid) in any of the milk fat globule membrane preparations, whereas the butter lipids and milk lipids were decidedly yellow colored. The latter (7 samples) ranged from 9.0 to 24.4 μg of carotene per g of lipid. The cell creams tended to have the same or higher concentrations than their corresponding milk lipids. These findings make it unlikely that the plasma membrane is a principal source for the carotene of bovine milk fat globules. A coincidental observation of our study is that the crude mitochondrial fraction (10,000 × g pellet) from lactating tissue has spectacular levels of carotene in its lipids (270–1870 μg/g).

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FATTY ACID PATTERNS IN SERUM TOTAL LIPIDS FROM FEMALE SWINE DURING ESTRUS AND GESTATION. E.G. HILL, The Hormel Institute; W.L. STOCKLAND, Intl. Multifoods, Courtland, MN; L.G. BLAYLOCK, Intl. Multifoods, Minneapolis, MN; and R.T. HOLMAN, The Hormel Institute, University of Minnesota, 801 16th Ave. NE, Austin, MN 55912.

Serum fatty acid levels were observed from a herd of purebred Yorkshire sows and gilts through the complete gestation cycle and through four successive estrus cycles for nonbred sows. These studies were made to determine the norms for female pigs under these conditions and to observe and document the ranges of the serum fatty acid levels throughout estrus and gestation of the pig. Ten sows and seven gilts of the International Multifoods Supersweet Research Farm were sampled weekly throughout gestation over a 28 week period. Four nonbred sows were sampled twice weekly through several estrus cycles. The animals were maintained on commercial rations. The serum lipid was analyzed for 22 fatty acids (14:0 to 22:6ω3). Major findings show that the total saturated fatty acids remained remarkably constant, with most of the variation in the polyunsaturated fatty acids. The 18:2ω6 and total ω6 fatty acids (primarily the essential fatty acids) tended to decrease after farrowing. Other parameters will be discussed in detail.

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GIANT-RING LACTONES IN THE SKIN SURFACE LIPIDS OF HORSES. SABIN W. COLTON VI and DONALD T. DOWNING, Dermatology Department, College of Medicine, University of Iowa, Iowa City, IA 52242.

Mammalian surface lipids are produced principally by the sebaceous glands of the skin, which secrete lipid mixtures differing dramatically in composition between species. In this study, surface lipids from the sides of male and female horses were collected in acetone and analyzed by thin layer chromatography (TLC) and gas liquid chromatography (GLC). The principal components were cholesterol (14%), cholesterol esters (38%), and a unique fraction (48%) migrating on TLC in the region of diester waxes. This was found to consist of the lactones of 32-, 34-, and 36-carbon ω-hydroxyacids, each having one ethylenic bond and one methyl side chain. Big-ring compounds (muscone and civetone) have been found previously in specialized glands of ma.e musk deer and civet cats, where they function as sex attractants. The lactones from the horse identified in the present study are as large as muscone (C_{16}) and civetone (C_{17}) molecules combined, and include both the methyl side chain and the double bond found, respectively, in those two compounds. The horse lactones might also function as pheromones, but since they are present on the general body surface of both sexes, they possibly act as herding signals rather than in a sexual capacity.

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INFLUENCE OF α-TOCOPHEROL ON TURNOVER OF FUNGAL LIPIDS. MICHELE ADLER and R. CECIL JACK, Department of Biological Sciences, St. John's University, Jamaica, NY 11439.

We have found previously that the addition of α-tocopherol (aT) to growing cultures of the fungus *Glomerella cingulata* led to an increase in the number of double bonds per mole of fatty acid in the triacylglycerols but led to a decrease in the number of double bonds per mole in the phospholipids. In both classes of glycerolipids, the ratios of unsaturated to saturated fatty acids increased in aT-treated cultures. In this study, we report on the labeling and turnover of *G. cingulata* lipids during treatment with α-tocopherol. Samples of mycelium were taken from treated and control cultures at 2, 2½, 3, 4, and 6 days of age. The specific radioactivities of triacylglycerols (TAG), total phospholipids (PL), phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), and phosphatidyl inositol + phosphatidyl serine (PI + PS), were obtained by standard procedures, following incubation of the mycelial samples for 5 min either with 2-^{14}C-acetate or ^3H_2O. Turnover was measured in pulse-chase experiments and was defined as the change in specific radioactivity of the lipids following transfer of acetate-labeled mycelium from labeled to nonlabeled nutrient medium for 60 min. Similar patterns of labeling were obtained with ^{14}C-acetate and ^3H_2O both for aT-treated and control cultures; but, the labeling patterns of the treated cultures were different from those of control cultures in two respects: (a) maximum incorporation into the TAG of control cultures occurred before maximum incorporation into the TAG of treated cultures, (b) incorporation into the TAG of control cultures decreased between 2 and 2½ days but remained constant in the TAG of aT cultures before decreasing from 2½ to 6 days of age. We conclude from the similar labeling patterns from 2-^{14}C-acetate and ^3H_2O that internal pools of acetate did not contribute significantly to the labeling patterns obtained with ^{14}C-acetate. The experiments on lipid turnover showed in general that, both in the TAG and the classes of PL, there were sharp declines in radioactivity from the beginning of the chase up to 45 min after the chase, both in aT and control cultures. Between 45 and 60 min, however, the radioactivities of the aT samples increased (on average) ca. 37% over control values. We conclude from the latter result and previous data that, during the later stages of the chase, the TAG of aT cultures were exchanging relatively saturated fatty acids for more unsaturated fatty acids derived from the phospholipids.

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METABOLISM OF TRIACYLGLYCEROL AND PHOSPHATIDYLGLYCEROL IN FUNGI. GREGORY E. ANEKWE, Department of Biochemistry, College of Medicine of the University of Lagos, P.M.B. 12003, Lagos, Nigeria.

The fungus *Glymerella cingulata* was used in several studies on the patterns of metabolism of triacylglycerol and phosphatidylglycerol. Among these was the determination of the apparent turnover of the glycerides, during fungal aging, their modes of accumulation the type of fatty acids synthesized during aging, and their incorporation of 2-C^{14}-acetate, ^{3}H glucose, and 1-C^{14}-acetate, also during aging. The data from these studies suggested that the patterns of incorporation of the labeled precursors, and the patterns of turnover of the glycerides are similar, as well as the types of fatty acids synthesized. However, marked differences in the patterns of accumulation of the various fractions of triacylglycerols and phosphatidylglycerol were evident. This latter observation was interpreted to correspond with the differing roles of these two classes of glycerides in the aging cell.

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SYNTHESIS AND PROPERTIES OF N-ALKYL MONOAZA

CROWN ETHERS. MITSUO OKAHARA, PING-LIN KUO, MASAKI MIKI, and ISAO IKEDA, Department of Applied Chemistry, Faculty of Engineering, Osaka University, Yamada-kami, Suita, Osaka, 565, Japan.

N-Alkyl monoaza crown ethers were synthesized in good yield by the reaction of ethoxylated alkylamines having suitable ethylenoxy units with equimolar quantities of sulfochlorides such as p-toluenesulfonyl or benzenesulfonyl chloride in the presence of excess sodium or potassium hydroxide in an aprotic solvent. They were also prepared from the more accessible raw materials, N-alkyldiethanolamine and tri- or tetraethylene glycol, by treatment with sulfochloride and alkali metal hydroxide in somewhat lower yield than the above method. Purely isolated N-alkyl monoaza 15-crown-5 and 18-crown-6 with C_1 to C_{12} alkyl group were almost colorless liquids, and their physical and spectral properties were investigated. N-Alkyl monoaza crown ethers with long chain alkyl group are soluble or dispersible in water, and the surface-active properties of the solutions were measured and compared with those of the corresponding open chain ethoxylated amines.

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STUDIES OF ALKYLIMIDAZOLINE DERIVATIVES. MOTOO KOYAMA, NOBORU KASATANI, TSUYOSHI OGINO, and KESAICHI IDE, Nippon Oil and Fats Co., Ltd., Amagasaki Factory Research Laboratory, 56, Ohamacho, 1-Chome Amagasaki, Hyogo, 663, Japan.

The structure and the chemical properties of 1-hydroxyethyl-1-carboxymethyl-2-imidazolinium betaine (I) were investigated. Methods for synthesis of compound (I) are reported in the literature and in patents. Most of them are concerned with the reactions of alkylimidazoline derivatives (II) with sodium monochloroacetate using water as solvent. As (II) is easily hydrolyzed to N-alkanoyl N′-hydroxyethyl ethylene diamine (III), the reaction mixture has a composition involving the carboxymethylated derivatives of (III). In earlier works, the complete isolation of (I) was not reported, so that its correct structure and chemical properties could not be clearly established. We isolated the purified crystals with high melting point (189–192 C) from the reaction mixture of 1-hydroxyethyl-3-undecylimidazoline and sodium monochloroacetate in nonaqueous solvent. The structure of the crystalline compound was established as 1-hydroxyethyl-3-carboxymethyl-2-undecylimidazolinium betaine (IV) by means of instrumental analyses and chemical determinations. In the above reaction, (IV) was obtained dominantly. The chemical stabilities of the imidazolinium betaines are discussed.

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STUDIES ON THE SULFATION OF FATTY ACID ALKANOLAMIDE WITH CHLOROSULFONIC ACID AND ITS BY-PRODUCTS. YOSHIO MAKINO, YASUSHI HAYASAKI, ETSUO SUGI, and KESAICHI IDE, Nippon Oil and Fats Co. Ltd., Amagasaki Factory Research Laboratory, 56, Ohamacho, 1-Chome Amagasaki, Hyogo, 663, Japan.

By-products of sulfation of various fatty acid alkanolamides (I) with chlorosulfonic acid were examined with regard to the influence of alkanolamide structure upon side-reaction. It was found that the reaction products contained several kinds of by-products such as aminoester (II), amidoester (III), and other unidentified substances, and their amount was remarkably dependent upon the structure of (I). For example, in the case of diglycollaurylamide fairly pure sulfated product was obtained even under strong reaction conditions, with only a small amount of 2-laurylamidoethyllaurate. On the other hand, the sulfation of monoethyllaurylamide was a more complicated reaction yielding a large amount of 2-aminoethyllaurate and some other products. It was also established that the pure sulfated alkanolamide (IV) was transformed into (II) and (III) when it was allowed to stand at 60–80 C. These results suggest that (II) was formed in the sulfation process concurrently from both alkanolamide and its sulfated product, by rearrangement via crystalization of these compounds.

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SYNTHESIS OF HIGHER ALCOHOLS BY GAS PHASE CATALYTIC HYDROGENATION OF FATTY ACID METHYL ESTERS UNDER ATMOSPHERIC PRESSURE. KAZUO IKEDA and ATOH MUGISHIMA, Kogakuin University, Faculty of Engineering, Department of Industrial Chemistry, 1-24-2, Nishishinjuku, Shinjuku-ku, Tokyo, 160, Japan.

The synthesis of higher fatty alcohol by gas phase catalytic hydrogenation of fatty acid methyl esters (C_8, C_9, C_{10}, C_{12}, C_{14}, C_{16}, C_{18} fatty acid methyl esters) under atmospheric pressure was studied. In these studies, three methods (A, B, & C) for supplying esters to the hydrogenator under atmospheric pressure were examined. In method (C), circulating hydrogen gas is introduced to the fatty acid methyl esters at 150–300 C and the ester-containing hydrogen gas is passed through hydrogenation catalyst layer ($CuO \cdot Cr_2O_3$) at 250–300 C to give higher fatty alcohols (Jap. Patent Appl.: Japan Kokai: 7608,203). Method (A) is effective on a laboratory scale, but gives a small yield. Method (B) gives an imperfect gaseous reaction and low conversion rate due to incorrect reaction conditions. In method (C), which has practical value, the activation temperature is not critical, but at a reaction temperature of 280–300 C, the influence of combinations of hydrogen flow rate, preheating of hydrogen, and evaporation temperature of esters is distinctly related to the type of esters and characteristics of the apparatus. Among the products from the present gas phase reduction, fatty aldehydes are detected by means of gas chromatography-mass spectrometry, in contrast to the absence of it in the higher pressure hydrogenated products at liquid phase. By-products such as fatty acids, fatty aldehydes, and wax esters were also identified quantitatively in the products by means of thin layer chromatography-flame ionization detector and programmed gas liquid chromatography, etc. Generally, gas phase reduction causes an undesirable side reaction. Because the above by-products are intermediates of higher fatty alcohols, they cause no problems. However, monomers or dimers of hydrocarbons corresponding to the raw material esters are formed as by-products in the present studies, and the appropriate conditions for suppressing formation of these by-products are suggested to increase yields of the main products. The present method could be applied to produce fatty amines such as laurylamine, from the corresponding nitrile.

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FURANOID FATTY ACIDS: SYNTHESIS AND CHARACTERIZATION. MARCEL S.F. LIE KEN JIE, Department of Chemistry, University of Hong Kong, Pokfulam Road, Hong Kong; and F.D. GUNSTONE, University of St. Andrews, St. Andrews, Scotland.

The occurrence of furanoid fatty acids and the chemico-physical characteristics of this novel class of fatty acids will be briefly reviewed. The various methods of preparation of C_{18} furanoid fatty acids from natural fatty acids or by total synthesis will be outlined. A preview of the continuing work on the synthesis, chemico-physical and biological properties of these compounds will also be included.

SESSION I: ANTIOXIDANTS AND FLAVOR STABILITY OF OILS—II

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IMPROVEMENT OF OXIDATIVE STABILITY OF FATS AND OILS. HARUO WATANABE, TOHRU KITAGAWA, MUTSUHITO WATANABE, and HIROKO TAHARA, Central Research Laboratory, Showa Sangyo Co., Ltd., 2-20-2, Hinode Funabashi, Chiba, 273, Japan.

Fats and oils were stabilized by heating them with cereals at temperatures above 150 C. For example, soybean oil mixed with 20% wheat flour by weight was heated for 30 min at 250 C under atmospheric pressure and filtered. AOM stability of this treated oil was over 80 hr compared to 16 hr for the fresh oil. For reference, when the cereals were heated alone and added to untreated fresh fats and oils, their oxidative stability was not improved. The cereals used included wheat, soybean, rice, and corn, grain, grits, and flour. Similar antioxidant effects were shown with defatted cereals. Degree of improvement of oxidative stability by this treatment greatly depended on the amounts of cereals and heating temperature. Increasing the amounts of cereals and elevating temperature gave higher oxidative stability. Similar effects were shown under atmospheric or reduced pressure and under inert gas. When the volatile matters produced by this treatment were added to untreated fresh fats and oils, they showed high stability against oxidative rancidity. Although the well-known antioxidants such as BHA, BHT, PG, and IG, lost much of their effect after heating at deep-frying temperature, these treated fats and oils had high stability toward heating. For example, potato chips fried with this treated oil had remarkably long shelf life.

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STABILITY OF AN INTERESTERIFIED BLEND OF SOYBEAN OIL AND EDIBLE TALLOW. A. PHILIP HANDEL, ROY G. ARNOLD, and YUN CHAN LO, Department of Food Science and Technology, University of Nebraska, Lincoln, NE 68583.

Interesterified blends of soybean oil and edible tallow were produced having physical properties similar to those of tub-type margarines. These blends may be useful as an alternative to hydrogenation for obtaining desirable physical properties. Interesterified blends retain more of the essential fatty acids of the soybean oil and avoid the formation of *trans*-isomers. Peroxide values were determined periodically for oil samples held at 60 C. Interesterified blends, noninteresterified blends, and the oil from commercial margarines were evaluated. Although interesterified blends are not as stable as noninteresterified blends, the interesterified products have a stability which is comparable to commercial margarine oils.

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STANDARDIZATION OF TESTS FOR FAT AUTOXIDATION STABILITY. GORO KAJIMOTO, KAZUO HORIKAWA, SABURO AKASHI, TSUGUO IZUMI, HIROSHI MORISHIMA, TAKAO NAKAYAMA, ETSUJI YUKI, and SATOSHI YONEYAMA, JOCS Fat Stability Committee, Yushi Kogyo Kaikan, 3-13-11, Nihonbashi, Chuo-ku, Tokyo, Japan.

To standardize methods for oxidative stability of edible fats and oils, collaborative experiments were implemented to evaluate the AOM test (AOCS Tentative Method), oven test, organoleptic evaluation, and Olcott's weighing method. The collaborative tests were carried out by 9 to 20 different operators with refined soybean, palm, rapeseed oils, and lard. The values of standard deviation (s) and coefficient of variation (cv) derived from the AOM test were as follows: s 0.74, cv 5.0% for soybean oil; s 1.55, cv 6.4% for palm oil. The results of the weighing method were as follows: s 31.9, and cv 11.5%. The relation between the oxidative stability and the flavor score will be discussed.

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MEASUREMENTS OF OXYGEN UPTAKE AND FLAVOR COMPOUNDS AS INDICATORS OF OIL FLAVOR QUALITY. DAVID B. MIN, Best Foods Research & Engineering Center, 1120 Commerce Ave., Union, NJ 07083.

The flavor stability of oil was measured by the combinations of oxygen uptake, the quantitative and qualitative measurement of volatile flavor compounds, and peroxide values. The amounts of oxygen uptake and volatile compounds in oil indicate that there were positive correlationships. That is, the higher the oxygen uptake in oil, the higher the amount of flavor compounds formed in oil. The amounts of either oxygen uptake or flavor compounds formed increased as the storage periods increased under the normal storage conditions. The combined results of oxygen uptake and flavor compounds formed can be used as good indicators of flavor quality and stability of oil. The detailed chemical mechanisms involved in oxygen and oil reactions will be reported to explain the relationships among the oxygen uptake, peroxide value, and flavor compounds formation. Also discussed is the flavor isolation technique which can selectively eliminate the acidic compounds and water from the isolated flavor compounds by using a precolumn containing potassium carbonate and anhydrous sodium sulfate.

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SUNFLOWERSEED SALAD OIL: CORRELATION OF GAS LIQUID CHROMATOGRAPHY VOLATILES WITH FLAVOR INTENSITY SCORES. W.H. MORRISON, III, B.G. LYON, and J.A. ROBERTSON, R.B. Russell Agricultural Research Center, USDA, PO Box 5677, Athens, GA 30604.

Samples of sunflowerseed salad oil from seed produced in the northern United States containing BHA, BHT, TBHQ, and propyl gallate were stored in flint glass and amber bottles and exposed to light for 16 weeks. Using Dupuy's method for direct gas liquid chromatography (GLC) measurement of volatiles, correlations were made between volatiles and flavor intensity scores. The effects of antioxidant, storage conditions, and containers on intensity scores and volatile profiles will be discussed.

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BITTER TASTE IN OXIDIZED FATTY ACIDS. RIICHIRO USUKI and TAKASHI KANEDA, Faculty of Agriculture, Tohoku University, 1-1 Amamiyamachi-Tsutsumidori, Sendai, 980, Japan.

In the preceding joint meeting between AOCS and JOCS in 1972, we reported that a bitter taste was detected in highly autoxidized soybean oil, especially in the free fatty acid fraction. To elucidate the nature of the bitter substance, autoxidized linoleic acids were separated by silicic acid chromatography, and the fraction with maximum PV (1849) was submitted to treatment with cysteine-ferrous chloride, reduction with sodium borohydride, oxidation with chromic acid, and methyl esterification. The results indicated that a particular structure between the carboxyl group and other functional groups was necessary for bitter taste. To verify this assumption, four oxygenated fatty acids (ricinoleic, 12-oxooctadecenoic, dioxooctadecenoic, and dihydroxyoctadecenoic acid) were synthesized from castor oil, and investigated for correlation with bitter taste. The results showed that ricinoleic acid had a strong bitter taste along with a strong reinforcing taste, and this bitter taste was greatly reduced by methyl esterification. However, 12-hydroxy- and 9,10-dihydroxystearic acids were tasteless. From these results we conclude that it would be essential to have a carboxyl group, hydroxyl group, and unsaturated bond for the appearance of bitter taste in ricinoleic acid.

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FRYING QUALITY OF PALM OILS UNDER SIMULATED DEEP-FAT FRYING CONDITIONS. E. YUKI and K. MORIMOTO, Hiroshima Food Research Institute, 12-ban, 70-go, Hijiyama-honmachi, Hiroshima-shi, Japan.

Frying quality of palm oils was studied under simulated deep-fat frying conditions by means of the continuous water-spraying and heating system. Three samples (palm oil, palm olein A and B) were prepared from the same lot of original oil (Totox value 35.7). Palm olein A was a common product (melting point 20.3 C) and palm olein B was a low melting point one (below 10 C) which was prepared by solvent fractionation. These oils were fully refined in the laboratory, and 20 ppm of citric acid was added after refining. Frying quality was estimated by the changes of acid value, peroxide value, carbonyl value, viscosity increase, and AOM stability, etc. The quality of palm olein was superior to that of palm oil, and that of palm olein A was highest. The effect of methyl silicone for the improvement of frying quality was remarkable in all palm oils, and the addition of γ- and δ-tocopherol was also effective for the increase of fat stability.

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CHANGES IN DIELECTRIC CONSTANT AS A MEASURE OF FRYING OIL DETERIORATION. C.W. FRITSCH, D.C. EGBERG, and J.S. MAGNUSON, James Ford Bell Technical Center, General Mills, Inc., 9000 Plymouth Ave. N., Minneapolis, MN 55427.

Potatoes were fried in soybean oil, hydrogenated vegetable shortening, and an animal-vegetable shortening at 375 F for 8 hr each day for 4 days. The same shortenings were also heated for 32 hr with no frying at 375 F. Samples were taken periodically, analyzed for various changes normally used to measure frying oil deterioration, and changes in the dielectric constant determined with a patented instrument called a Food Oil Sensor. The dielectric constant of all three shortenings increased linearly during the frying of potatoes. The greatest change occurred in the soybean oil and the smallest change in the hydrogenated vegetable shortening. Statistically significant correlations were obtained between instrument reading and total polar materials, decrease in iodine value, color, peroxide value, diene content, and free fatty acids.

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF TRIGLYCERIDES: CONTROLLING SELECTIVITY WITH SILICA AND REVERSE PHASE COLUMNS. R.D. PLATTNER and G.F. SPENCER, Northern Regional Research Center, USDA, 1815 N. University, Peoria, IL 61604.

Rapid separations of triglycerides by chain length and degree of unsaturation have been made by high performance liquid chromatography (HPLC), using both silica columns and reverse phase columns. Surprisingly, on microparticulate silica columns, separations based on chain length were observed. Baseline resolution was observed between triarachidin, tristearin, tripalmitin, trimyristin, and trilaurin, with the longer chain triglycerides eluting before the shorter chain homologs. A mixture of C_{54} triglycerides (tristearin, triolein, trilinolein, and trilinolenin) was separated under the same conditions, with the more saturated components eluting before the more unsaturated analogs. The retentions of triolein and tripalmitin on the silica columns were nearly equal. Better separations were obtained with reverse phase columns. The shorter chain and more saturated triglycerides eluted first on the reverse phase columns. Again the retentions of triolein and tripalmitin were similar. Seven different bonded columns were evaluated for reverse phase HPLC of triglycerides. They were μ-Bondapak-C_{18}, μ-Bondapak-phenyl, and triglyceride analysis (Waters Associates); Zorbax ODS and Zorbax C_8 (DuPont); and Portasil ODS and Portasil ODS-2 (Whatman). All had somewhat different selectivity, and the order of elution of component triglycerides was slightly different. These differences will be discussed. Adding silver ions to the solvent in HPLC analysis markedly changes the selectivity of the solute triglycerides based on the number of double bonds present in the molecule. The retention of the unsaturated triglycerides is significantly decreased with increasing concentrations of silver ion in the solvent system, whereas elution of the fully saturated compounds is not significantly altered.

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF VEGETABLE OILS. ALI EL-HAMDY, M.M. CHAUDRY, and EDWARD G. PERKINS, 104 Burnsides Research Lab., University of Illinois, Urbana, IL 61801.

The analysis of vegetable oils by high performance liquid chromatography (HPLC) has not been extensively investigated due to problems in sample handling, separation, and peak detection. In the present investigation both refractometric and ultraviolet detection were used to detect eluting components. Superior separations were achieved with solvents systems composed of mixtures of tetrahydrofuran, acetone, and acetonitrile, with reversed phase columns. The effects of column carbon content and length of the hydrophobic chain on the separations were investigated with commercially available microparticulate columns. At present, the identification of eluting components is somewhat tenuous, therefore, individual peaks were collected and the fatty acid composition and glyceride structure determined, where feasible, in order to provide detailed information concerning the elution of individual triglycerides where standards were not available.

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APPLICATION OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TO ANALYSIS OF TRIGLYCERIDE COMPOSITIONS OF FATS AND OILS. SHUN WADA, CHIAKI KOIZUMI, AKIHIDE TAKIGUCHI, and JUNSAKU NONAKA, Department of Food Science and Technology, Tokyo University of Fisheries, Konan, Minato-ku, Tokyo, 108, Japan.

In high performance liquid chromatography (HPLC), it was found that triglycerides were eluted through a reverse phase column in the order of their partition numbers (PN), which were defined by the following equation: PN = TC (total acyl carbon number in a triglyceride) $-$ 2 $\times$ DB (number of double bonds in a triglyceride). Moreover, the logarithms of retention times of triglyceride peaks in HPLC increased linearly with their partition numbers. On the basis of the above-mentioned facts, soybean oil triglycerides were separated into six fractions by HPLC. Each triglyceride fraction was collected separately, after which the total carbon numbers of triglycerides in the fractions and their fatty acid compositions were determined by gas liquid chromatography. From the data of DB, TC, and fatty acid composition, the fatty acid combinations of triglycerides and their contents were estimated. Similar estimations were carried out on beef lipid and black cod lipid triglycerides. The results indicated that the highest content of the triglycerides in these lipids were as follows: soybean oil, (3 $\times$ $C_{18:2}$, 17.5%); beef lipids, (1 $\times$ $C_{16:0}$, 2 $\times$ $C_{18:1}$; 33.2%); black cod lipids, (1 $\times$ $C_{16:0}$, 2 $\times$ $C_{18:1}$; 10.0%).

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TRIACYLGLYCEROL COMPOSITION OF RAPESEED OIL. BENGT HERSLÖF, RAGNAR OHLSON, and OLDRICH PODLAHA, AB Karlshamns Oljefabriker, Research Laboratory, S-292 00, Karlshamn, Sweden.

The triacylglycerols of a low erucic acid containing rapeseed oil have been studied by means of different chromatographic techniques. High performance liquid chromatography in combination with gas chromatography and 2-positional analyses provide information about different types of triacylglycerol species existing in the oil. Such a composition allows a comparison with other seed oils in a more accurate way than does fatty acid composition alone.

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY SEPARATION OF MONOUNSATURATED TRANS FATTY ACIDS. R. BATTAGLIO and D. FRÖHLICH, Cantonal Laboratory, PO Box 8030, Zurich, Switzerland.

Abstract not available at press time.

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SEPARATION AND DETERMINATION OF POE TYPE NONIONIC SURFACTANTS BY HIGH SPEED LIQUID CHROMATOGRAPHY. YUKIO KASAI and WATARU YANO, Suzuka College of Technology, Shiroko-cho, Suzuka-shi, Mie-ken, Japan.

A series of nonylphenol ethylene oxide adducts with 1–30 mean oxyethylene units was separated with good resolution by high speed liquid chromatography, using porous micro-spherical silica gel as stationary phase. The recommended conditions are as follows: column, 500 mm $\times$ 2.3 mm or 500 mm $\times$ 4 mm ID; mobile phase, gradient from acetonitrile-ethylacetate (1:4, v/v, water-saturated) to water-methanol (1:9, v/v); flow rate, 0.3–0.8 ml/min; pressure, 50–100 kg/cm^2; detector, ultraviolet detector (277 nm). The separation of various commercial samples with 1–30 oxyethylene units showed the resolution (R_s) above 1 under these conditions. The molecular distribution of the samples was determined by integration of each peak and confirmed to be Poisson distribution.

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DETERMINATION OF SODIUM SULFATE IN DETERGENTS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. MAKOTO YAMANAKA, ATSUO NAKAE, KAZUMI FURUYA, and TSURUO MIKATA, Anal. Chem. Sec., Tochigi Research Laboratories, Kao Soap Co., Ltd., 2606 Akabane, Ichikaimachi, Tochigi, 321–34, Japan.

A method was developed for the determination of sodium sulfate in detergents by high performance liquid chromatography (HPLC). Sulfate ion was separated from other anions (pyrophosphate, tripolyphosphate, etc.) by HPLC on a porous micro-spherical strong anion exchanger (TSK-Gel LS-222®) column with 0.1 mol/l nitric acid and 0.005 mol/l iron (III) nitrate as the eluent and detected with a UV monitor (330 nm) as $FeSO_4^+$ complex. Iron (III) is effective not only as an eluting, but also as a color-developing, agent for sulfate ion. The plot of peak area vs. the weight of sodium sulfate was linear over the range of 8–24 μg. Good recovery and reproducibility were obtained from the samples of standard detergents containing known amounts of sodium sulfate. Sodium sulfate in some commercial detergents was determined by the proposed method with no interference, and the results agreed with those of the conventional chelatometry.

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF SURFACTANTS USED IN DETERGENTS FOR WASHING FABRICS. D. THOMAS, Société Chimiotechnic, 25,27 rue de l'Industrie, 69631, Venissieux, France; and J.L. Rocca, Laboratoire de Chimie Analytique III, Université Lyon, France.

The different surfactant families are analyzed with a high performance liquid chromatography technique using a RP8 stationary phase (Lichrosorb RP8 5 m, 15 cm length column) and mixtures of water-methanol as mobile phases. The mode of detection is UV (254 nm) or refractive index. Two different areas are discussed: 1. Analysis of each family (control of raw materials to be processed). (a) Sulfonate compounds (alkylbenzene, paraffin): each homologue is separated by ion-pair formation with an appropriate counter-ion dissolved in the mobile phase. (b) Soaps are separated with an acid mobile phase or by ion-pair formation with an appropriate counter-ion in mobile phase at pH 9. (c) Nonionic compounds (like fatty alcohols or ethoxylated alkylphenols): these compounds are separated in accordance with the nature of their hydrophobic chain in mobile phase at pH 9. 2. Analysis of surfactant mixtures used in commercial compositions. Appropriate choice of experimental parameters makes it possible to determine rapidly the nature and composition of each surfactant family by means of three successive chromatographic analyses. Besides control of raw materials, the rapidity and simplicity of these separations also allow continuous control of manufacturing and analysis of other commercial detergents.

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ATHEROSCLEROSIS: AN OVERVIEW. DAVID KRITCHEVSKY, Wistar Institute, Bryn Mawr, PA.

Abstract not available at press time.

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DIET AND THE DEVELOPMENT OF ATHEROMA. A. WHITLEY BRANWOOD, College of Physicians and Surgeons, Department of Pathology, 630 West 168 St., New York, NY 10032.

Epidemiologic factors are discussed in relationship to the development of atherosclerosis. The evolution of the atherosclerotic lesion is described from its initial inception to the fully developed advanced disease process. The description of this evolution is discussed critically in relationship to epidemiologic and dietary factors and the theories of atherosclerosis, namely thrombogenic, lipogenic, combined thrombogenic and lipogenic, myointimal, and mutagenic are analyzed, and a hypothesis is suggested to explain the development of atherosclerosis in relationship to race, environment, diet, hypertension, age, sex, and other factors.

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EFFECTS OF DIETARY FAT ON THE CLINICAL AND BIOCHEMICAL FEATURES OF FAMILIAL HYPERLIPOPROTEINEMIA. AKIRA YAMAMOTO, Department of Pathological Science, Research Center, National Cardiovascular Center, 5-125, Fujishirodai, Suita-shi, Osaka-fu, 565, Japan; HIROSHI SUDO, TAKU YAMAMURA, and MASAHARU KUBO, Department of Medicine, Osaka University Medical School, Osaka, Japan.

Patients with different types of familial hyperlipoproteinemia were given (a) a regular diet (fat 50–60 g per day), (b) a low-fat, low-cholesterol diet, and (c) an isocaloric diet supplemented with 30 g of vegetable oil (rice oil 7, safflower oil 3) each for 2 weeks. Serum lipoprotein pattern on electrophoresis, lipid composition of lipoprotein fractions obtained by ultracentrifugation, composition of biliary lipids and bile acids, and the fecal excretion of steroids were estimated. Intake of dietary fat, even vegetable oil rich in polyunsaturated fatty acids, resulted in a significant increase in serum concentration of cholesterol in familial hypercholesterolemia (FH), making a sharp contrast to Type IIb and Type IV hyperlipoproteinemia in which the vegetable oil is effective in decreasing the serum cholesterol. Intravenous alimentation resulted in a marked decrease in concentration of serum cholesterol in a homozygous case of FH. Lipid composition of bile in FH was not affected by dietary changes. A marked increase in concentration of cholesterol in bile was obtained in patients with Type IIb and Type IV hyperlipoproteinemia by supplementation with vegetable oil, even if there were no definite changes in fecal excretion of sterols and bile acids. Cases of hyperlipoproteinemia showing a broad β pattern on electrophoresis were not as scarce among Japanese. However, the value of the chemical index for Type III hyperlipoproteinemia, VLDL-Ch/TG, was usually low compared with the criteria proposed by Fredrickson or Hazzard. Xanthoma was seldom found in our own cases. In this type of hyperlipoproteinemia, a transient change of the lipoprotein pattern between Types III and V was often observed by a change in dietary fat intake. Deficiency in postheparin lipolytic activity, both in lipoprotein lipase and hepatic triglyceride lipase, was detected in a family linked with a trait of xeroderma pigmentosum.

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RELATION OF SERUM LIPOPROTEINS TO ARTERIOSCLEROSIS AND NUTRITION. E. RENNER, Department of Dairy Science, Justus-Liebig-University, Bismarckstr 16, D-6300 Giessen, Federal Republic of Germany.

The different physiological effects of serum lipoproteins cannot be explained on the basis of their cholesterol content. The supposedly "dangerous" very low density lipoproteins (VLDL) and low density lipoproteins (LDL) contain 15% and 45% cholesterol, respectively, while the "useful" high density lipoproteins (HDL) contain 20%. This is an important argument against the assumption that cholesterol is an atherogenic factor. Although the serum lipoprotein fractions can be influenced by diet, the results are often contradictory and clinically of questionable significance. Particularly difficult is the interpretation of the importance of carbohydrates as regards the frequent "type IV" of the hyperlipidemias. These relationships need careful discussion.

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POSTHEPARIN LIPOLYTIC ACTIVITY IN PATIENTS WITH CHYLOMICRONEMIA. HIROSHI SUDO, TAKU YAMAMURA, MASAHARU KUBO, The Second Department of Internal Medicine, Osaka University Hospital, Fukushima-ku, Osaka, 553, Japan; and AKIRA YAMAMOTO, Research Institute, National Cardiovascular Center, Department of Pathogical Science, Osaka, Japan.

Postheparin plasma contains two lipases, lipoprotein lipase (LPL) and hepatic triglyceride lipase (H-TGL). Type I hyperlipoproteinemia is usually recognized as LPL deficiency. Recently it has been shown that some other mechanisms may be involved in the existence of chylomicronemia. The physiological role of H-TGL is unknown. The present study was undertaken to examine the activity of these two lipases in patients with chylomicronemia. Four of the patients were classified as Type I hyperlipoproteinemia and the other four as Type V. Blood samples were obtained at 10, 20, and 30 min after intravenous injection of heparin (0.1 mg = 13 units per kg body weight). Assay mixture of postheparin lipolytic activity consisted of Ediol as a substrate, 0.1 M Tris-HCl buffer, pH 9.0, with 5% bovine albumin and diluted postheparin plasma. Estimation of the LPL activity was based on the selective inhibition by 1 M NaCl. All of the Type I patients showed an extreme deficiency in LPL activity. In contrast to the reports by other authors, there was a slight to moderate decrease in H-TGL activity in our cases. Two of the patients were brother and sister. They had an extreme deficiency in the CII component of VLDL and chylomicron apoproteins. By addition of the normal human serum or purified apo CII to the assay mixture, their LPL activity was recovered above the normal level. One of the Type V patients had a marked decrease in both of the LPL and H-TGL activities and showed a change in lipoprotein pattern following dietary fat restriction into Type III (broad β type) on PAG disc electrophoresis as well as by analysis on ultracentrifugation. His family had a trait of xeroderma pigmentosum, and the case is presumably a variant or peculiar type of LPL deficiency. The other Type V patients showed normal LPL activity with a moderate decrease in H-TGL.

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SOME REGULATORY MECHANISMS OF CHOLESTEROL METABOLISM. HANS KAUNITZ, Columbia University, 630 West 168 St., New York, NY 10032.

Cholesterol is an essential compound because it forms part of cellular membranes and is the mother substance of hormones and bile acids. Yet "lethal" effects have been ascribed to it mainly (but not only) because of the positive correlation between elevated serum cholesterol and arteriosclerotic complications ("Lipid Theory of Arteriosclerosis"). However, it has become evident that different cholesterol containing serum lipoprotein fractions can have either a positive (low density serum lipoproteins [LDL]) or a negative correlation (high density lipoproteins [HDL]) with arteriosclerotic complications. Therefore, explanations on the basis of the lipid theory are unconvincing. Mere correlations can never form the basis of etiological considerations. The presence of increasing amounts of cholesterol in granulomatous tissues (including the atheroma) suggests that it is part of the repair process rather than the cause of the atheroma. The slowly increasing cholesterol content of aging tissues can be similarly interpreted. The behavior of the serum lipid fractions is probably a consequence of the requirement of the tissues for cholesterol under normal and pathological conditions. When diseased tissues need cholesterol for repair, LDL supply it. HDL remove unnecessary cholesterol from normal tissues. Cellular receptors of cholesterol are similarly regulated. Cholesterol never induces tissue damage except when enormous amounts are fed to some species. Caloric restriction occasionally influences abnormal cholesterol metabolism beneficially. Other dietary manipulations have little influence. Serum cholesterol lowering drugs have proved to be harmful.

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LIPOPEROXIDE AND GLUTATHIONE PEROXIDASE IN HUMAN ATHEROSCLEROSIS. FUMIO KUZUYA and NOBORU YOSHIMINE, The 3rd Department of Internal Medicine, Nagoya University School of Medicine, Showa-Ku, Nagoya, Japan.

It has been postulated that lipoperoxide plays an important role in the progression of atherosclerosis. The accumulation of peroxide in tissues of organs has been suggested to cause the disturbance of metabolism. On the other hand, glutathione peroxidase (GSHx), which metabolizes the hydroperoxide in the organs, is also one of the important enzymes working as a scavenger for preventing the increase of peroxides. We, therefore, tried to measure the activity of GSHx and peroxide value in human organs and to observe whether they would be affected by the age and severity of atherosclerosis. Forty cases were sampled from the forensic autopsy, whose ages were 2 years through 81 years without any obvious diseases. Liver, kidney, heart muscle, brain, adrenal glands, lung, and aorta were used in this experiment. Macroscopically, aorta was classified into 5 degrees (e.g., from 0 to 4) according to the severity of atherosclerosis. The intima-media, intima and media of thoracic and/or abdominal aorta were utilized for measurement. The activity of GSHx was measured by the modified method of Little and O'Brien, and the measurement of peroxide in tissues was done by the method of Masugi and Nakamura. Results showed: (a) The activity of GSHx in liver and kidney was significant in higher levels than the others in all age groups. (b) The higher peroxide values were obtained in brain and heart muscle, whereas the lower values were observed in liver and kidney through all ages. (c) GSHx activity seemed to decrease with increasing severity in aorta, in which the significant decrease was observed in stage 3 and 4 in the thoracic aorta. (d) The increasing levels of peroxide in aorta were observed with increasing atherosclerosis. (e) GSHx of intima was always observed in higher levels than that of media, whereas the peroxide value in media was higher than in intima. Therefore, the following was postulated: (a) GSHx was working as a scavenger of peroxides in tissues and maintaining a good metabolic balance. (b) With aging and also with increasing atherosclerosis, the production and accumulation of peroxides were increasing gradually, resulting in the disturbance of balance between GSHx and peroxide. (c) Increase of peroxide in organs, especially in blood vessels, would be one of the accelerating factors for human atherosclerosis.

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SOME ATHEROGENETIC PARAMETERS IN THE PATIENTS HAVING AND/OR BEING PRONE TO ATHEROSCLEROTIC DISEASES. CHIKAYUKI NAITO, HIROKAZU KATO, TSUYOSHI WATANABE, TAKESHI YAMANAKA, HIDETOSHI MEMEZAWA, and TAMIO TERAMOTO, University of Tokyo, Faculty of Medicine, 35–7, Ootsuka 5, Bunkyo-Ku, Tokyo, 112, Japan.

In relation to the "lipid" factor as one of the "risk" factors of atherosclerotic diseases, we examined HDL, LDL, and total cholesterol, so-called extra pre-β, serum thiobarbituric acid (TBA)-reactive chromogens, and fatty acid patterns in phospholipids of platelets in relation to platelet aggregability in the patients with and prone to atherosclerotic diseases: mainly cardiovascular and cerebrovascular diseases and diabetes mellitus. In diabetes mellitus, HDL, LDL, and total cholesterol concentration did not have any correlation to treatment (diet alone, oral antidiabetics, or insulins), to the degree of control of blood sugar level (good or poor), and to the grade of diabetic retinopathies. However, patients with ischemic heart diseases among them had only slightly lower HDL-cholesterol (statistically not significant), significantly higher LDL-total cholesterol, and significantly lower HDL-cholesterol/LDL-cholesterol ratio than those of the rest. The percentage of α-fraction (α-lipoproteins) in a disc electrophoretogram was also significantly lower in the patient group. The results suggested that the HDL-cholesterol/LDL-cholesterol ratio might be clinically more significant than the absolute HDL-cholesterol values to estimate atherosclerosis. TBA-reactive chromogens in the serum, although their biochemical significance is still unknown, were significantly higher in patients with cardiovascular, cerebrovascular, and diabetes mellitus than in the controls. The appearance of extra pre-β was significantly higher in atherosclerotic patients. However, thus far we could not find any special relationship between the food habits of patients and TBA values or extra pre-β appearance. The half-life of platelets was significantly shorter in patients with cerebrovascular diseases and diabetes mellitus than in the controls, although the phospholipase activity in platelets estimated by N-ethylmaleimide stimulation was not significantly different among them. Relative content of arachidonic acid in phospholipids of platelets seemed to be lower in Japanese than that reported in Western literature. However, whether there are any differences in the content of arachidonic acid between controls and atherosclerotic patients among Japanese is now under investigation. In a symposium to follow we will discuss possible relationships of these findings to atherogenesis.

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EFFECT OF NEONATAL MANIPULATION OF CHOLESTEROL HOMEOSTASIS ON SUBSEQUENT RESPONSE TO CHOLESTEROL IN ADULT GUINEA PIG: COMPARISON OF ENHANCING CHOLESTEROL DEGRADATION VERSUS CHOLESTEROL FEEDING. JOB R. LI, LAURIE K. BALE, BRUCE A. KOTTKE, Atherosclerosis Research Unit, Mayo Clinic/Foundation, Rochester, MN 55901; and M.T. RAVI SUBBIAH, Cincinnati Medical Center.

Experiments were designed to determine whether or not the mechanism of handling dietary cholesterol in later life could be influenced by the manipulation of cholesterol homeostasis during neonatal period. The effects of (a) enhancing cholesterol degradation (cholestyramine feeding) and (b) high dietary cholesterol intake during neonatal period of guinea pigs on their subsequent plasma cholesterol (PC) levels and response to later dietary cholesterol challenge were investigated. Male newborn guinea pigs were suckled for 6 days and used for two sets of experiments. In Experiment I, one group was maintained on a 1.1% cholestyramine (CT) diet for 6 weeks and the control group weaned normally. In Experiment II, one group was subjected to 0.25% cholesterol diet for 12 weeks. Normally weaned guinea pigs served as control animals. All groups of guinea pigs were then fed a regular guinea pig chow diet for 6 weeks. Following this period the guinea pigs were then subjected to a 0.25% cholesterol diet for 4 weeks. PC levels (mg/dl) were significantly lower in the group pretreated with CT during the neonatal period when compared to the controls (70.2 ± 5.3 vs. 99.2 ± 10.0). The difference in PC persisted throughout the cholesterol feeding period. PC levels of the group pretreated with high cholesterol (PT) during the neonatal period did not show significant differences from the controls (PT, 89.0 ± 8.8 vs. control, 78.8 ± 11.1). These results suggest that the stimulation of cholesterol catabolism rather than cholesterol feeding during neonatal period can influence the subsequent response to dietary cholesterol.

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EFFECT OF DIETARY CHOLESTEROL AND CORN OIL ON RABBIT GROWTH. T.R. WRENN, JOEL BITMAN, J.R. WEYANT, and D.L. WOOD, Nutrient Utilization Laboratory, Animal Physiology and Genetics Institute, Bldg. 309, Beltsville Agricultural Research Center, USDA, Beltsville, MD 20705.

Mature rabbits fed atherogenic diets have exhibited enhanced lipid mobilization mediated by dietary cholesterol. This experiment was designed to find if the increased circulating lipid would promote growth in young rabbits. Groups of 15 New Zealand White rabbits were fed ad libitum either: commercial chow (CONTROL), chow plus 1% cholesterol (CHOL), chow plus 10% corn oil (CORN), or chow plus 1% cholesterol *and* 10% corn oil (CHOL-CORN). Body weight gains of CHOL and CORN groups were superior to controls. Gains of CHOL-CORN rabbits were less than all others. Much higher plasma cholesterol concentrations occurred with the CHOL-CORN diet (1550 mg/100 ml) than with either CHOL alone (850 mg/100 ml) or CORN alone (50 mg/100 ml). Liver cholesterol reflected the plasma cholesterol concentrations with CONTROL and CORN diets being only 3 mg/g, compared to 10 mg/g for CHOL and 18 mg/g for CHOL-CORN. Some aortic thickening occurred in rabbits on CHOL and CHOL-CORN diets, with 40% of these rabbits showing atherosclerotic plaques when killed after 9 weeks on the diets. The experiment suggests that, while some growth advantage may occur in young rabbits fed cholesterol, it is negated by concurrently feeding high levels of plant lipid, and is no better than feeding lipid alone.

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LONG TERM FEEDING OF PARTIALLY HYDROGENATED HERRING OILS TO CYNOMOLGUS MONKEYS. R.G. ACKMAN, Fisheries and Oceans Canada, Technology Branch, PO Box 550, Halifax, Nova Scotia, B3J 2S7, Canada; F.M. LOEW, Johns Hopkins School of Medicine; B. SCHIEFER, and E.D. OFFERT, University of Saskatchewan.

Over a period of 30 months of continuous feeding of diets containing 25% of either lard-corn oil, or of a partially hydrogenated herring oil containing 20% docosenoic acids, to cynomolgus monkeys, the health of the animals remained good. The primary objective of the study was to determine if the myocardial lesions induced in rats by high fat diets rich in any docosenoic acid could also be induced in the nonhuman primate. Hearts from animals sacrificed at 6, 12, 18, 24, and 30 months failed to show diet-linked differences in myocarditis, but wild-caught animals usually had more lesions than laboratory-bred animals. Foci of myocarditis if present were much smaller than those observed in rats fed the same diets. The lard-corn oil groups histologically showed a transient lipidosis which disappeared in most cases after 12 months, but the corresponding early lipidosis in the partially hydrogenated herring oil groups was apparent for the full 30 months. The relations between diet, serum cholesterol and triglycerides, and findings in the aorta will be discussed.

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CONTINUOUS OIL WINTERIZATION DEVELOPMENT. BASIL T. PAPAHRONIS, Hunt-Wesson Foods, 1645 W. Valencia, Mail Station 501, Fullerton, CA.

This paper presents the series of steps and test procedures utilized in the successful development of a commercial continuous soybean oil winterization process. It describes the initial bench-scale testing, using a one-gallon capacity crystallizer and Buchner funnel vacuum filter. Crystallization was scaled-up directly from these one-gallon crystallizers to 10,000-gallon plant-sized crystallizers. Continuous stearine separation was tested using a pilot size rotary vacuum filter. This test data was then utilized to select a plant-sized rotary vacuum filter, which would match the plant crystallizing capacity. Ultimately, the work herein described provided the basis for commercial continuous soybean oil winterization systems which were successfully put into operation. Crystallization technology relative to rotary vacuum filter operability is discussed, and the advantages of continuous soybean oil winterization are presented.

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WINTERIZATION. Duriron Co.

Abstract not available at press time.

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WINTERIZATION OF PEANUT OIL. R.O. FEUGE and JOHN L. WHITE, SR., Southern Regional Research Center, USDA, PO Box 19687, New Orleans, LA 70179.

Peanut oil solidifies readily above 0 C and does not meet the cold-test specification for salad oil. Fractional crystallization from a solvent and removal of 5% to 7% of the highest melting triglycerides do winterize the oil, but the minute crystals are so difficult to remove that winterization is not economically feasible. Crystal modifiers and changes in the type of solvent were found to be ineffective. The minute, hard-to-filter crystals were attributed partly to the even distribution of 6% to 7% C_{20}–C_{24} saturated fatty acids among the triglycerides. Interesterification to rearrange randomly all fatty acid groups, followed by fractional crystallization from solvents, improved somewhat the ease of winterization, but the crystals still passed through 50-micrometer filters. Directed rearrangement at 20 C further improved ease of winterization but also increased the amount of solids at 0 C to about 23%. Semidirected rearrangements carried to equilibrium at 28 C with the aid of a catalyst that remained in solution at this temperature resulted in the formation of about 7% highly saturated triglycerides, which could be removed with relative ease to yield a well-winterized oil. For the first time, the winterization of peanut oil could be conducted without the aid of a solvent. The C_{20}–C_{24} fatty acids comprised over half of the highly saturated triglycerides formed by semidirected rearrangement.

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NEW PROCESS OF INTERESTERIFICATION OF FATS AND OILS WITH CAUSTIC SODA CATALYST. KATSUYOSHI MIKI, HIROTO SUZUKI, and KOJI ITO, Miyoshi Oil & Fat Co., Ltd., 4-66-1 Horikiri, Katsushika-ku, Tokyo, Japan.

Interesterification with caustic soda catalyst generally requires high reaction temperature (higher than 160 C) and long reaction time (longer than 1 hr). Since the long reaction time at high temperature leads to the formation of various secondary products, the taste and keeping quality of products become worse during reaction. It was confirmed in the large scale actual plant (12 tons reactor) that quick evaporation and dispersion of caustic soda and glycerine water solution in a vacuum reactor in which the fat is filled can lower reaction temperature as well as shorten reaction time. It seems important to accelerate the speed of evaporation of water so that the moisture content in the oil should be reduced to minimum before saponification occurs. With 0.05% caustic soda and 0.15% glycerine, the interesterification reaction of 60–80 ppm moisture contained refined palm oil, lard, or mixtures was substantially completed within 4 min at 100 C. The keeping quality and taste of the products were found at the same level as that of noninteresterified oil and fat products.

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HIGH QUALITY FOOD PRODUCTS FROM BEEF TALLOW BY A TWO-STEP SOLVENT FRACTIONATION PROCESS. FRANCIS E. LUDDY, JAMES W. HAMPSON, and RONALD E. KOOS, Eastern Regional Research Center, USDA, 600 E. Mermaid Lane, Philadelphia, PA 19118.

We have improved the laboratory solvent fractionation process in which three products are obtained from beef tallow: a solid fraction, a confectionery fat, and a "beef oil." The previously described four-step process was reduced to two steps with a simultaneous reduction in the process time requirement and the solvent-to-fat ratio. The new rapid process allows fractionation of 2–4 kilograms of beef fat in the laboratory in one working day, since total crystallization time for both steps is less than 3 hr. The yield of the confectionery fat fraction

was 40% higher by the rapid method than by the four-step procedure. Although the melting characteristics of this fraction by differential scanning calorimetry (DSC) were broader than those of cocoa butter, chocolate coatings made with the fat were acceptable for gloss, flavor, snap, shrinkage, and bloom resistance. The AOM stability of beef oil from the process was unusually high with a value of 70 hr. The same oil with 0.02% of a commercial antioxidant approached 500 hr for AOM stability. In one variation of the process, the costly filtration steps were eliminated, yet the fractions produced were of acceptable quality.

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OPERATION OF H.L.S. PLANT FOR FRACTIONATING PALM OIL BY TRANSESTERIFICATION. H.L.S. STAFF, H.L.S. Ltd., Industrial Engineering Company, PO Box 193, Petah Tikva, Israel.

H.L.S. has built a new palm oil fractionating by transesterification plant in Eilath (Israel) with a capacity of 50 tons/day palm oil. Description of the new plant with photos and slides will be presented. The principle of the new technology is to proceed to a new and more regular distribution of the fatty acids in the triglycerides replacing, on the one hand, in a certain portion of palm oil the unsaturated acid radicals by saturated ones in the mono- or disaturated triglycerides and, on the other hand, replacing in an equal portion of the palm oil the saturated acid radicals by unsaturated ones in the mono- or diunsaturated triglycerides obtaining mostly triunsaturated triglycerides. We obtain a solid fraction (white color) in the ratio of 35 p. 100 with an iodine value of 5 and a melting point of 62–63 C. This fraction contains over 90 p. 100 palmitine. The liquid fraction in the ratio of 65 p. 100 has an iodine value of about 80 and does not contain transisomers. The chilled stability is under 8 C (the oil is kept at 8 C for at least 72 hr and remains clear). Composition of fatty acids in the liquid fraction is as follows: palmitic acid, C16/0, 12.7 parts; oleic acid, C18/1, 66.2 parts; stearic acid, C18/0, 6.4 parts; linoleic acid, C18/2, 13.7 parts. The new system represents a new trend in the fractionating systems of palm oil.

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HYDROGEN PRODUCTION BY AUTOMATION. RONALD G. MINET, KTI Corporation, 201 S. Lake Ave. Suite 713, Pasadena, CA 91101.

Hydrogen can be produced from natural gas, LPG, or naptha interchangeably in a completely automated facility controlled entirely by the hydrogenation demand. Based on proven, fully developed design, a complete hydrogen plant can be delivered on truck transportable skids, completely prepiped for installation and initial operation within 300 hr after delivery. The system delivers 20,000 to 60,000 standard cubic feet of hydrogen per hour at 200 psig and 99.99% purity with a feed and fuel consumption of 400 BTU per standard cubic foot of hydrogen. Computer control permits output to be varied from 10–100% in 2 min without sacrifice of efficiency. The plant can be automatically started up and shut down by remote control. The paper describes the technical details of the plant and its integration into a typical vegetable oil processing facility.

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MILD BASE AND ACID STABLE PHOSPHOLIPIDS FROM CHICKEN EGG YOLK. UN HOI DO and S. RAMACHANDRAN, Applied Science Laboratories, Inc., PO Box 440, State College, PA 16801.

Chicken egg yolk phospholipids were subjected to mild base and acid hydrolysis, and remaining phospholipids were characterized by chemical and enzymatic methods after repeated silica gel column chromatography. Two major lipids, 1-alkyl glycerophosphorylethanolamine (alkyl GPE) and sphingomyelin, and one minor lipid, 1-alkyl glycerophosphorylcholine (alkyl GPC) were identified. Alkyl GPE and alkyl GPC were converted to the corresponding alkyl diacetylglycerols by $LiAlH_4$ reduction followed by acetylation, and the resulting alkyl diacetylglycerols were analyzed by gas liquid chromatography on 3% Silar 10C and 3% SE 30. Major alkyl groups in both ether phospholipids were hexadecyl (16:0), octadecyl (18:0), and 9-octadecenyl (18:1) groups, and minor ones were heptadecyl (17:0) and eicosanyl (20:0) groups. Sphingomyelin was hydrolyzed by the action of phospholipase C from *Clostridium perfringens*. The resulting ceramides were separated into ceramides (99%) containing nonhydroxy fatty acids and ceramides (1%) containing α-hydroxy fatty acids. Fatty acids and long chain bases of ceramides were analyzed by gas chromatography and thin layer chromatography. Fatty acids derived from ceramides containing nonhydroxy fatty acids were predominantly palmitic acid (>90%) and small amounts of myristic, stearic, arachidic, behenic, lignoceric, nervonic, and other minor monoenoic acids. Fatty acids derived from ceramides containing hydroxy fatty acids were predominantly α-hydroxyeicosanoic acid (>98%) and small amounts of α-hydroxypalmitic, α-hydroxybehenic, and α-hydroxystearic acids. Long chain bases of ceramides consisted of sphingosine (97%) and dihydrosphingosine (3%).

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QUANTITATION OF CHOLESTEROL α-OXIDE IN EGGS BY GAS CHROMATOGRAPHY AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. L.S. TSAI, C.A. HUDSON, K. IJICHI, and J.J. MEEHAN, USDA, Albany, CA.

Cholesterol, an intrinsic part of the basic human diet, is present in eggs, red meats, shellfish, organ tissues, milk, and poultry. In solution, colloidal suspension, or crystalline form, cholesterol readily autoxidizes. It is recognized that initial autoxidation products are hydroperoxides, but many secondary products have been isolated and identified. Among them, cholesterol α-oxide, (5α,6α-epoxycholestan-3β-ol) has received much attention due to its potential physiological effects in carcinogenesis and atherogenesis. However, the natural presence of this compound in food systems has not been thoroughly examined. The method described here allows the direct quantitation of free cholesterol α-oxide in four steps with near theoretical recovery and in a relatively short time. Although it was developed using dried eggs, the procedure should be readily adaptable to other food and biological systems. The method involves the extraction of dried eggs with chloroform-methanol (2:1, v/v) to recover the total lipids; enrichment of the cholesterol α-oxide fraction with a 1-g silicic acid column in a 5 ml disposable pipet; and quantitation of cholesterol oxides, both α- and β-isomers, by gas liquid chromatography (GLC). Those samples which showed positive oxide content were further analyzed by high performance liquid chromatography (HPLC). Although GLC is about 100-fold more sensitive, HPLC is the only technique which can resolve the isomers of cholesterol oxide directly. Saponification was not used in the method because only about 25% of the cholesterol α-oxide could be recovered after this treatment. When chloroform-methanol (1:1, v/v), or acetone, was used as solvent for GLC injection, recovery was reduced to 40% of the recovery found using chloroform (containing 1% ethanol as stabilizer), or tetrahydrofuran solvent.

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DIETARY EGGS AND CHANGES IN PLASMA, LIVER, AND BRAIN LIPIDS. SUSAN BONY, CAROLE SUBRAMANIAN, JAMES F. MEAD, and GOVIND A. DHOPESHWARKAR, Laboratory of Nuclear Medicine and Radiation Biology, University of California, 900 Veteran Ave., Los Angeles, CA 90024.

Eggs are considered a good source of many essential nutrients such as proteins, fats, certain minerals, and vitamins. They contain within their shell all the necessary nutrients for the development of embryonic life. However, egg lipids contain relatively large amounts of cholesterol and its role in health and disease is being debated. This work was undertaken to compare the effects of feeding a regular lab chow diet and feeding a diet composed of cooked whole eggs as a sole source of nutrition to pregnant female rats. Tissue analysis of both the mother rats and growing pups showed major differences. In the adult female rats at the end of 60 days on the egg diet there was: (a) only a slight elevation in plasma cholesterol ester level, (b) a threefold increase in liver total lipids, the bulk of it in the form of elevated triglycerides and a highly significant percent increase in cholesterol ester levels. In the growing pups, obvious signs of probable multiple deficiencies were seen. Mortality rates were very high and one of the most striking features of the analytical data was a fivefold increase in brain cholesterol esters in the 12-day-old pups; normal brain lipids at this age are generally devoid of cholesterol esters. The results will be discussed with respect to fatty livers in the adult female rats and the occurrence of cholesterol esters in the brains of growing pups.

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CHOLESTEROL AND CHOLESTEROL ESTERS OF EGGS FROM VARIOUS AVIAN SPECIES. JOEL BITMAN and D.L. WOOD, Nutrient Utilization Laboratory, Bldg. 309, Rm. 211, Beltsville Agricultural Research Center, USDA, Beltsville, MD 20705.

There is little information on the amount or composition of the cholesterol esters of egg yolk. In chicken eggs, estimates of the percentage of cholesterol esters have ranged from 0 to 15%. Data on cholesterol or cholesterol esters in eggs of other avian species are almost nonexistent. We have developed a method to analyze the cholesterol and cholesterol esters of egg yolk by gas liquid chromatography (GLC). After the cholesterol ester fraction was isolated by chromatography on a silica gel (Hi-Flosil) column, individual esters were quantitatively separated and determined by GLC on glass columns packed with SP 2340. The cholesterol content and the nature of the cholesterol esters in eggs from 14 various species of birds were determined. They were classified according to feeding habits as follows: (a) domestic fowl eating grain and plant materials: White Leghorn chicken, Silver-penciled Plymouth Rock chicken, turkey, Japanese quail; (b) wild plant-eating birds: ring-necked dove, grackle, peacock; (c) wild birds eating aquatic plants and animals: mallard duck, black duck; (d) wild aquatic carnivorous birds: laughing gull, brown pelican, great black-backed gull, black-crowned night heron; (e) wild mammal-eating birds: barn owl. Although egg size varied from 7 to 121 grams, total cholesterol content ranged only from 12 to 25 mg per gram yolk. The cholesterol present as ester ranged from 1 to 26% in the 14 species studied. Most of the cholesterol present as ester was esterified to 18:1 or 18:2 fatty acids. Analysis of yolk lipid indicated that the fatty acids consisted mainly of 16- and 18-carbon acids. The predominant order of concentration in all species was 18:1 > 16:0 > 18:2. There was no consistent pattern in either cholesterol content, cholesterol ester composition, or yolk fatty acids that would distinguish one bird species with particular feeding habits from another.

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UPTAKE AND DISTRIBUTION OF *cis*- AND *trans*-12-OCTADECENOIC ACIDS IN HUMAN BLOOD LIPIDS. E.A. EMKEN, H.J. DUTTON, W.K. ROHWEDDER, HENRY RAKOFF, R.O. ADLOF, Northern Regional Research Center, USDA, 1815 N. University, Peoria, IL 61604; R.M. GULLEY, St. Francis Hospital-Med Center; and J.J. CANARY, Georgetown University, Washington, DC.

Triglycerides of deuterium labeled *cis*- and *trans*-octadecenoic acid (12*c*-18:1 and 12*t*-18:1) and *cis*-9-octadecenoic acid (9*c*-18:1) were fed to two young adult male subjects. These fatty isomers each contained a different number of deuterium labels, which allowed mass spectrometric analysis to distinguish between them when they were fed as a mixture. This approach results in a direct comparison of the absorption and distribution of these three monoenoic acids into blood plasma, red blood cells, platelet and lipoprotein lipids. Results from plasma lipid data indicated that all phospholipid fractions selectively incorporated 12*c*-18:1 in preference to 9*c*-18:1. In comparison, 12*t*-18:1 was preferentially incorporated into only the phosphatidyl choline and sphingomyelin fractions. Discrimination against 12*c*-18:1 and 12*t*-18:1 compared to 9*c*-18:1 was found in the plasma neutral lipids, with a strong discrimination against 12*t*-18:1 incorporation into the cholesteryl ester fraction. Low levels of deuterated fatty acids were found in the red blood cell and platelet lipids, but selectivity values were similar to the plasma lipid values. Chylomicron data indicated that all isomers were well absorbed. Variation in the relative distribution of 12*c*-18:1, 12*t*-18:1, and 9*c*-18:1 between the very low density, low density, and high density lipoprotein lipids was found.

Apparently fatty acid uptake and turnover in the various lipoproteins are regulated by different factors.

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DESATURATION OF ISOMERIC *trans*-OCTADECENOIC ACIDS BY RAT LIVER MICROSOMES. M.M. MAHFOUZ and R.T. HOLMAN, The Hormel Institute, University of Minnesota, 801 16th Ave. NE, Austin, MN 55912.

Desaturation of twelve labeled positional isomers of *trans* 18:1 acids was investigated by using liver microsomal enzyme of essential fatty acid deficient rats. The assay system used desaturation of oleic acid as model for comparison. The effects of concentration of microsomal protein, substrate, cofactors, and of time and temperature upon the specific activity of the enzyme reaction were studied to develop an assay system. Each positional isomer desaturated at a unique rate. Some isomers gave mostly *cis-trans* 18:2 ($t\Delta^6$, $t\Delta^{13}$). Others gave *cis-cis* 18:2 ($t\Delta^5$) and some gave a mixture ($t\Delta^4$, $t\Delta^{11}$, Δt^{12}, and $t\Delta^{14}$). Some of these isomers were not measurably desaturated ($t\Delta^8$, $t\Delta^9$, and $t\Delta^{15}$). The products from each isomer were characterized by $AgNO_3$ thin layer chromatography and by gas chromatography. The positions of the double bonds in the newly formed labeled compounds were determined by following the partial reduction with hydrazine hydrate for the labeled free acids, and by reductive ozonolysis of the partially reduced methyl esters. The chain lengths of the labeled aldehyde esters which determine the positions of double bonds were identified by gas chromatography using cold markers of aldehyde esters ranging from C_3 to C_{17}.

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EFFECT OF AUTOXIDIZED OIL ON CARP. KAZUHIKO HATA and TAKASHI KANEDA, Faculty of Agriculture, Tohoku University, 1-1 Amamiyamachi-Tsutsumidori, Sendai, Japan.

It is known that autoxidized oil shows toxic effects in fish as well as in other animals. For example, carp fed autoxidized oil revealed poor growth, high mortality, and characteristic muscular dystrophy known as "Sekoke disease." But it has not been clarified what the main toxic substance in autoxidized oil is, since previous workers used oxidized oil which had not been fractionated. To study the effect of each autoxidized product, pure methyl linoleate hydroperoxide (HP) and secondary oxidized products produced from HP(SP) were prepared and fed to carp for 120 days. The results indicate that both substances induced muscular dystrophy, poor growth, and inactivation of an enzyme, succinate dehydrogenase. SP was found to be slightly more toxic than HP. In the group fed HP, carbonyl compounds increased in both adipose tissue and intestine. No accumulation of peroxide was observed in hepatopancreas or other organs. These results may indicate that absorbed HP was converted to carbonyl compounds in intestine. To elucidate this problem, carp administered HP were killed after 3 or 6 hr, and the amounts of POV and COV of lipids extracted from intestine and other organs were determined. Buffer extract of intestine was incubated with emulsified linoleic acid hydroperoxide in vitro, then decomposed peroxide and produced carbonyl compounds were measured. Inhibitive effects of EDTA or cyanide were also investigated. Consequently, it appears that at least a part of absorbed HP in intestine was converted to carbonyl compounds immediately, and the reaction was catalyzed by a heat unstable factor other than metal salts or heme compounds in intestine.

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THE EFFECT OF PYROPHOSPHATE ON MICROSOMAL NADPH-INDUCED LIPID PEROXIDATION AND CHEMILUMINESCENCE. SANDRA L. GUTHANS, RICHARD H. STEELE, and WILLIAM H. BARICOS, Department of Biochemistry, Tulane University, School of Medicine, 1430 Tulane Ave., New Orleans, LA 70112.

NADPH-induced rat liver microsomal lipid peroxidation, as measured by the thiobarbituric acid (TBA) assay, has been shown by several workers to closely correlate with the production of chemiluminescence (Howes and Steele, *Res. Commun. Chem. Path. Pharmacol.* 2:619 [1971]; and Wright et al., *Fed. Proc.* [Abstr.] 37:767 [1978]). However, we have described a KCN-induced microsomal chemiluminescence that proceeds in the complete absence of TBA assay measurable lipid peroxidation (Guthans et al., *Fed. Proc.* [Abstr.] 37:1722 [1978]). Such chemiluminescence displays only in the presence of pyrophosphate, NADPH, and a sugar or sugar derivative, principally hexose-6-phosphates. Hochstein and Ernster (*Biochem. Biophys. Res. Commun.* 12:388 [1963]) reported that low levels of pyrophosphate stimulated, while high levels inhibited, lipid peroxidation. We find that in the absence of KCN, NADPH-induced microsomal chemiluminescence and lipid peroxidation are inversely proportional to the pyrophosphate concentration in the range 0.05–3 mM. Pyrophosphate concentrations greater than 9 mM completely block both lipid peroxidation and chemiluminescence in these systems. The addition of KCN (15 mM) to such incubations results in a slow but steady buildup of chemiluminescence which is directly proportional to the pyrophosphate concentration in the range 5–40 mM. Such systems are negative for TBA reactive peroxides. When KCN is added at the beginning of the incubation, the total chemiluminescence produced increases linearly with increasing pyrophosphate concentration between 0.5 and 40 mM without the accumulation of TBA reactive material. (This work was supported in part by a grant from Ethyl Corporation and by Pharmacological Sciences training grant 5T32GM07177, NIH Bio. Med. Res. Support grant 5507 RR05377-16.)

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ABSORPTION OF METHYL LINOLEATE HYDROPEROXIDES IN RATS. KENICHI NAKATSUGAWA and TAKASHI KANEDA, Faculty of Agriculture, Tohoku University, 1-1 Amamiyamachi-Tsutsumidori, Sendai, Japan.

In 1953 we reported the toxic nature of hydroperoxides formed during the autoxidation of unsaturated fatty acids. Although many workers have studied the toxicity of autoxidized oils, it is still not clear whether fatty acid peroxides are absorbed directly from the intestinal wall. An attempt has been made in the present study to clarify the absorption mechanism of hydroperoxides. Rats were fasted for 24 hr after anesthesia with Nembutal, and the intestine was taken by ventral middle incision, and the upper half of small intestine was ligated. Methyl linoleate hydroperoxides were emulsified using sodium cholate and injected into the ligated intestine. Thirty minutes later, the ligated intestine was excised and washed with 0.9% NaCl, then the lipids were extracted with $CHCl_3/CH_3OH$ (2:1). The extracts were investigated by high performance liquid chromatography. It was noticed that intact methyl linoleate hydroperoxides were recognized in the extracts, and some of them were incorporated into triglycerides and phospholipids. In the next experiment, lymph was collected from the intestinal lymphatic after 2 hr oral administration of methyl linoleate hydroperoxides, and lipids were extracted with $CHCl_3/CH_3OH$ (2:1). The extracts of lymph were analyzed as described in the first experiment. The results showed the spectral evidence of diene conjugation in the lymph of rats administered hydroperoxide. Three peaks were noticed at the same retention time as methyl linoleate hydroperoxides. These results indicate that some unchanged methyl linoleate hydroperoxide could be absorbed from the intestinal wall. In other experiments, U-^{14}C-methyl linoleate hydroperoxides were also administered, and the lipids obtained from small intestine were analyzed by thin layer chromatography. It was noticed again that U-^{14}C-hydroperoxides were found in triglyceride and phospholipid fractions of intestinal lipids. These results indicate that some unchanged hydroperoxides could be absorbed into intestinal wall and transported to some organs through lymph.

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EFFECT OF GLUTATHIONE PEROXIDASE ON THE AUTOXIDIZED METHYL LINOLEATE. HIRONORI NEGISHI, KENSHIRO FUJIMOTO, and TAKASHI KANEDA, Faculty of Agriculture, Tohoku University, 1-1 Amamiyamachi-Tsutsumidori, Sendai, Japan.

It is known that the feeding of autoxidized unsaturated oils seriously damages animals, and we reported that the decomposed products of hydroperoxides such as hydroperoxyalkenal containing 5 to 9 carbon atoms showed marked toxicity to rats. On the other hand, glutathione peroxidase (GSHpx) appears to be an important factor in controlling endogenous formation of peroxides in cell membranes. To elucidate the protective mechanisms of organisms from exogenous peroxides, the effect of GSHpx on the various autoxidized products of methyl linoleate was investigated. Methyl linoleate (ML) was autoxidized at 60 ± 1 C by bubbling oxygen into the ester. Portions of the ester were withdrawn at the stage of the maximum of POV (hydroperoxide rich ester, AOML-1) and the decreasing stage of POV (secondary oxidized products rich ester, AOML-2). Further, AOML-2 was fractionated into volatile fraction, fatty acid methyl ester fraction, and dimer fraction by molecular distillation. These oils were incubated in vitro with the GSHpx prepared from digestive tracts and liver of mice, and the inhibition of GSHpx by these oils was estimated. Moreover, AOML-1 and AOML-2 were orally administered to the mice, and the effects of these compounds on the GSHpx activities of mice organs were investigated. By the incubation of GSHpx with autoxidized ML in vitro, GSHpx was remarkably inactivated by AOML-1 and the inhibition of AOML-2 was inferior to that of ML hydroperoxides. That is, the correlation between the acute toxicity and the inhibition rate of GSHpx with autoxidized oil was not observed. GSHpx catabolized all peroxides well, and the substrate specificity was not recognized. When mice were administered oxidized oil for 16 days under the low vitamin E level, GSHpx activity levels of internal organs showed a considerable increase, and AOML-2 induced the GSHpx activity more than AOML-1. In spite of the marked toxicity of secondary oxidized products, the inhibition of GSHpx activity depends mainly on hydroperoxides, so it is believed that the toxicity of the secondary oxidized products is not originated by the inhibition of GSHpx activity.

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COLOR IMPROVEMENT IN SURFACTANTS, DETERGENTS, AND SOAPS VIA SODIUM BOROHYDRIDE PURIFICATION. MICHAEL M. COOK and RICHARD A. MIKULSKI, Ventron Corporation, Congress Street, Beverly, MA 01915.

Sodium borohydride purification either prior to or during selected processing or manufacturing operations results in significantly lower color in the final surfactant, soap, or detergent. Subsequent decolorization treatments can be minimized or, in many cases, eliminated altogether or, alternatively, overall product quality improved as the result of the borohydride purification. Detailed applications of the purification procedures and resultant benefits obtained will be reviewed. Color improvements of ethoxylates and propoxylates by borohydride prepurification of the parent alcohols or by treatment during the alkoxylation process will be compared with commonly employed posttreatment methods such as activated carbon or peroxide techniques. Reduction of oxidized impurities in commercial alcohols or polyols with low levels of sodium borohydride can also improve the resultant color of sulfates or esters. In other processes such as saponification, the addition of borohydride with the presently employed alkaline catalysts can minimize color formation due to oxidation or condensation of oxidized impurities. The sodium borate introduced at the parts per million level as the result of this purification presents no problems in the final product and in many cases is removed in subsequent washing or filtration operations. Since minimal additional equipment is needed and only low level of sodium borohydride is required, this purification is both practical in a production environment and cost effective compared to alternative methods.

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MASS BALANCE OF FLUORESCENT BRIGHTENING AGENTS IN BUILT DETERGENTS. MASAKO HAYASHI, The Ochanomizu University, Tokyo; YUKO UENO, Beppu College; MOTOKO KOMAKI, and AKIHIKO YABE, The Ochanomizu University, 1-1, Otsuka 2 Chome, Bunkyo-ku, Tokyo, 112, Japan.

Mass balance of fluorescent brightening agents (FBAs) picked up during the washing process and drained without being utilized has been determined quantitatively under different washing conditions, including different species of FBAs previously applied to the washing cloths. Extraction by cellulose powder and thin layer chromatography separation were the most effective analytical techniques followed by UV spectrophotometry and fluorometry. It was found that as much as 50% of FBAs in the detergents (0.1–0.3% on the weight of detergent) were

drained uselessly in waste suds. Quantitative estimation of the FBAs in river waters in connection with the ABS content will also be reported.

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A STUDY OF MOISTURE DISTRIBUTION IN POWDERED SYNTHETIC DETERGENTS BY THERMOGRAVIMETRY. CLAUDE BENZ and JOSEPH P. SIMKO, JR., Colgate-Palmolive Company, Research and Development Department, 909 River Rd., Piscataway, NJ 08854.

Abstract not available at press time.

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PROPERTIES OF NEW β-ALANINE TYPE AMPHOTERIC SURFACTANTS CONTAINING HYDROXY GROUPS. HISAO HIDAKA, Meisei University, Faculty of Science and Engineering, 337, Hodokubo, Hino-shi, Tokyo, 191, Japan; MASAFUMI MORIYA, and MAKOTO TAKAI, Miyoshi Oil & Fat Co. Ltd., Japan.

The β-alanine type amphoteric surfactants containing one or two hydroxy groups, such as N-(2-hydroxyalkyl)-β-alanine (IIa), N,N-bis-(2-hydroxyalkyl)-β-alanine (IIb), N-(2-hydroxyalkyl)-N-(2-hydroxyethyl)-β-alanine (III) were prepared and their physical-chemical properties compared with N-alkyl-β-alanine (I), where the alkyl group represents a C_{12}, C_{14}, or C_{16} chain. (IIa) and (IIb) were obtained by reaction of epoxyalkane with β-alanine in alkaline aqueous ethanol solution (EtOH; H_2O = 65:35 v/v%). (III) was prepared by adding N-(2-hydroxyalkyl)-ethanolamine to methyl acrylate and subsequent saponification. (III)-C_{12} was highly soluble in water and was soluble even at its isoelectric point (6.5 ∼ 6.7), probably due to two hydroxy groups in the molecule. The surface areas were determined by a monolayer method: (I) 22.06 Å/molecule; (IIa) 25.82; (IIb) 50.11; (III) 36.02 for the C_{12}-derivatives. (IIa) and (III) had thermotropic liquid crystalline properties, while (IIb) did not have such properties. The CMC value, surface tension, emulsion power, foaming power, Krafft point, and calcium ion tolerance, were also examined.

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FUNDAMENTAL STUDIES OF THE PARAMETERS GOVERNING OILY SOIL REMOVAL FROM SYNTHETIC SUBSTRATES. K.W. DILLAN, E.D. GODDARD, and D.A. MCKENZIE, Union Carbide Corporation, Tarrytown, NY 10591.

Using a model detergency system, the parameters governing the removal of oily soils from Mylar and/or Teflon substrates were examined in several built and unbuilt nonionic and selected anionic formulations. The differences in removal times and mechanisms noted for the various soils have been related to the pertinent interfacial energies. The effects of surfactant concentration on roll-up and removal were examined for simple soils on Mylar substrates. At concentrations above the CMC, a linear relationship between log removal time and log concentration was found with several surfactant systems, suggesting that oil roll-up and removal are not controlled strictly by static boundary tension values. An attempt has been made to explain the linear relationship through consideration of such factors as: the dependence of the oil/water interfacial tension on concentration and time, the degree of nonionic surfactant sorption by the substrate, and the partitioning of the surfactant between the oil and water phases. The slopes of the log-log plots were found to vary substantially among several surfactants, and an attempt has been made to relate the slope to relevant surfactant properties, i.e., CMC, interfacial tension lowering, and cloud point. The effects of temperature and other common detergency variables were also examined.

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RECENT DEVELOPMENT IN ALPHA OLEFIN SULFONATE TECHNOLOGY. SADAO TOYODA, Lion Fat & Oil Co. Ltd., 2-1, 7-chome, Hirai, Edogawa-ku, Tokyo, 132, Japan.

Alpha olefin sulfonates (AOS), as reported in several references, have been found to have better biodegradability, less toxicity to organisms, milder skin irritation, and higher safety to human health than linear alkylbenzene sulfonates (LAS) which have been commonly used in most detergent formulations. They are also more economical than alkylether sulfates (AES). Since AOS have good detergency even in hard water, and good pH stability over a wide range, they have recently attracted attention as useful surfactants and are being widely used in household and industrial detergents. For example, they are used in low phosphate heavy duty detergent formulations, dishwashing detergents that are particularly mild to the skin, and in shampoos. Sulfonation technology for producing light colored AOS without bleaching and improved spray drying techniques have been developed recently. This is one of the reasons why AOS have been more widely used in several kinds of detergent systems. The paper will summarize the recent applications of AOS as a surfactant from the point of view of environmental safety, performance properties, and various manufacturing processes.

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DETERGENCY ENHANCEMENTS BY PURE ALCOHOL ETHOXYLATES. H.L. BENSON and Y.C. CHIU, Westhollow Research Center, Shell Development Company, PO Box 1380, Houston, TX 77001.

Oily soil and clay detergencies using a radiolabeled soils' procedure have been compared for a series of high purity dodecanol linear alcohol ethoxylates containing from 3 to 8 moles of ethylene oxide and a corresponding series of C_{12}–C_{13} alcohol ethoxylates with normal, broad polyoxyethylene distributions. Results have been obtained at 75 F and 100 F for a variety of nonpolar soils on permanent press polyester/cotton fabric substrate. Significant detergency enhancements are found for the pure ethoxylates on nonpolar lube oil and triglyceride soils. The maximum benefits occur between 4–5 moles ethylene oxide per mole alcohol (10–11 HLB) depending on the temperature. These results appear to be related not only to lowering of interfacial tensions but also to rates of soil solubilization, which in turn are related to micellar size and composition. Scattered light intensity measurements, an indication of relative micellar size, show strong dependency on the ethylene oxide number and on temperature, which correlate with the detergency data. Similar results have been obtained with blends of the pure ethoxylates containing high and low ethylene oxide contents.

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THERMAL DEGRADATION AND OXIDATION OF THIN FILMS OF UNSATURATED GLYCERIDES. KYOH TAKAOKA, Department of Chemistry, Musashi Institute of Technology, 1-28-1, Chome, Tamazutsumi, Setagaya, Tokyo, Japan; and YOSHIYUKI TOYAMA, Toyo University, Japan.

To understand the oxidation reaction at the surface of oils, we attempted to investigate the thermal oxidation and decomposition of thin films of unsaturated oils by means of thermal analysis and other analytical methods (IR, elementary analysis, and MS). Triolein, trilinolein, and trilinolenin were heated in air in the range of 25 C to 240 C, in order to elucidate the relationship between thickness of sample film (20–1800 μ) and the degree of the oxidative polymerization. Further, thermal polymerization products were measured by means of thermal analysis with a micro-thermo-balance system evacuated to 10^{-4}–10^{-5} torr and heating from 25 C to 500 C at 5 C/min. The compositions of intermolecularly polymerized triolein, trilinolein, and trilinolenin were determined by using the heat loss curve under reduced pressure. Rate constant and apparent activation energy of intermolecular polymerization were calculated in the region of 40 C to 24 C.

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ANTIOXIDANT ACTIVITY OF SEA ALGAE. KENSHIRO FUJIMOTO and TAKASHI KANEDA, Faculty of Agriculture, Tohoku University, 1-1, Amamiyamachi-Tsutsumidori, Sendai, Japan.

In a previous report, the phospholipid fractions of several brown algae were revealed to show considerable antioxidant effects on the autoxidation of methyl linoleate. By successive screening tests for antioxidants in seaweeds, it was noticed that the $CHCl_3$-MeOH fraction of a red alga *Polysiphonia urceolata* showed a marked antioxidant effect. That is, the induction period of the methyl ester of safflower oil, whose induction period at 45 C was 4 days, was extended to 70 days by addition of 1% of this fraction. The antioxidant activity of this alga was the most intense of all seaweeds examined. However the phospholipid fraction of this alga was ineffective and the active ingredient was separated into several fractions by silicic acid column chromatography. Therefore, the synergistic reaction of several components were deduced, as with a brown alga, *Undaria pinnatifida*, which was previously reported. The detailed antioxidation mechanisms of both algae will be discussed.

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EFFECT OF AMINO ACIDS ON AUTOXIDATION OF SAFFLOWER OIL IN EMULSIONS. TOM RIISOM, REX J. SIMS, and JOSEPH A. FIORITI, General Foods Corporation, Pleasantville, NY.

Amino acids were tested as antioxidants in liquid emulsions containing safflower oil and various sugars. The techniques of headspace oxygen absorption and chemiluminescence were used to monitor oxidation rates. The effectiveness of the several amino acids tested was quite variable and dependent upon the level and type of sugar added, the pH of the system, the average oil droplet size, the emulsion stability and its viscosity. These experiments were subsequently expanded to include freeze-dried emulsions containing safflower oil with proteins or gums as the matrix. The antioxidant effects of added amino acids and sugars were determined. Porosity measurements on the dried samples indicated that the diffusion of oxygen may be rate determining.

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ANTIOXIDANT ACTIVITY OF AMINO ACIDS BOUND TO TROLOX-C. M.J. TAYLOR and T. RICHARDSON, Department of Food Science, University of Wisconsin, Madison, WI 53706.

Trolox-C is an analogue of α-tocopherol (vitamin E), differing by having a carboxyl group in place of the isoprenoid side-chain of vitamin E. Manufactured by Hoffman-LaRoche, Trolox-C has greater antioxidant activity than many commercial food antioxidants and is relatively nontoxic (LD_{50} similar to BHA and BHT). We have synthesized a number of antioxidants more potent than Trolox-C by covalently attaching various amino acids to Trolox-C. Methionine, tryptophan, histidine, and cysteine were attached to Trolox-C through the formation of an amide bond between the carboxyl group of Trolox-C and the amino group of the amino acid. The amide would presumably be hydrolyzed by enzymes in the gastrointestinal tract, releasing amino acid and relatively nontoxic Trolox-C during digestion. Antioxidant activity was evaluated by measuring the time for removal of dissolved oxygen from an emulsion of linoleic acid methyl ester after addition of hemoglobin. Antioxidant activity is expressed as a protective index (PI), which is the ratio of time for oxidation of emulsion with antioxidant to that of a blank emulsion. For example, the PI of Trolox-C is 1.94; of Troloxyl-tryptophan-OMe, 13.7; of Troloxyl-methionine-OMe, 10.1; of Troloxyl-cysteine-OH, 4.89; and of Troloxyl-histidine-OMe, 3.18 (pH 7.2, 10^{-3} *M*). The methyl ester of the Troloxyl-amino acids had greater antioxidant activity than the corresponding free acid form. Antioxidant activity at 6.7, and a comparison to BHA, BHT, and α-tocopherol will be presented.

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DIVERSITY OF SYNERGISM BETWEEN PHENOLIC ANTIOXIDANTS AND AMINO COMPOUNDS. YUKIHIRO ISHIKAWA, Faculty of Education, Tottori University, Koyama-cho, Tottori, 680, Japan.

Recently, a possible mechanism of synergism between tocopherols (mainly γ- and δ-Toc) and trimethylamine oxide (TMAO) in the inhibition of autoxidation of methyl linoleate was proposed (Y. Ishikawa et al., *Agric. Biol. Chem.* 42:703 [1978]). It was found that Toc reducing dimers played an important role in synergism, and methyl keto-octadecadienoate (keto acid) was characteristically formed. We have studied the diversity of synergism by considering the characteristic phenomena described above. γ-Toc showed strong synergism with tri-n-octylamine (TOA) in the inhibition of autoxidation of methyl linoleate. After the disappearance of γ-Toc, a large amount of γ-Toc reducing dimers was maintained in a reaction mixture for a long time, and TOA acted as a peroxide decomposer to give keto acid. However, a mechanism of synergism between them differs much from that between Toc and TMAO in the oxidation pattern of methyl linoleate. A mechanism of synergism between BHA and TMAO seems to be similar to

that between Toc and TMAO. However, phenolic antioxidants such as 6-hydroxychroman-2-carboxylic acid and tert-butylhydroquinone did not show synergism with TMAO. The former showed synergism with TOA, but the latter did not. This diversity of synergism between phenolic antioxidants and amino compounds is likely to be closely related to steric environment of OH group(s) in antioxidants, formation of their reducing dimers, and activities of derivatives of amino compounds as antioxidant or synergist.

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BEHAVIOR OF CITRIC ACID IN EDIBLE OILS. MAMORU KOMODA, KOUJI MIYAKOSHI, and SHIGEZO MATSUBARA, Sugiyama Chemical and Industrial Laboratory, 11, Kagetori-cho, Totsuka-ku, Yokohama, Japan.

A quantitative gas liquid chromatographic method for citric acid and its decomposed products in refined oils and fats was developed. At 20 and 30 ppm levels, recoveries of the acids from the oil varied from 95 to 100% except for itaconic acid. When soybean oil containing 50 ppm citric acid was treated at reduced pressure under various deodorizing conditions, no other acid but citric acid was found to decrease as the deodorizing temperature increased and the time prolonged. When 1,000 ppm of citric acid was used, a considerable amount of unreacted citric acid, a small amount of aconitic and itaconic acids were found. Soybean oil comprising 50 or 1,000 ppm of citric acid was heated at atmospheric pressure under various conditions. The results were similar to those of deodorizing conditions. The formation of citraconic acid together with aconitic and itaconic acids was observed only when the oil comprising 1,000 ppm of citric acid was heated at the higher temperature. These findings indicate that the synergistic effect of these acids derived from citric acid are negligible in commercial edible oils. Citric acid did not protect the oil against fluorescent light but markedly improved flavor and oxidative stability when the oils were deteriorated under spontaneous autoxidation conditions. However, its efficiency was independent of its concentration. Citric acid solubility in the soybean oil was measured. At least 50 ppm citric acid could be dissolved in the oil after 4 weeks of storage.

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A RAPID METHOD FOR ANALYSIS OF REFINED VEGETABLE OILS FOR TBHQ BY GAS CHROMATOGRAPHY. RICHARD E. AUSTIN and DAVID M. WYATT, Technical Service and Development Division of Eastman Chemical Products, Inc., Kingsport, TN 37662.

A rapid, practical analytical method for determining TBHQ at the 50–150 ppm level in refined vegetable oils is described. The method utilizes a simple acetonitrile extraction of 1 ml samples followed by gas chromatographic measurement of the TBHQ and internal standard. The method has shown quantitative recoveries at the 90% level from soybean, sunflowerseed, and cottonseed oils. The gas chromatographic technique employs injection of the acetonitrile extract without the use of pre-columns required by other methods. Total operator time is about 15 min per sample, and the method is applicable to modern laboratory automation systems. The least squares calibration of TBHQ/internal standard response shows a correlation coefficient of 0.98. Other facets of gas chromatographic measurement of TBHQ, such as silyl derivatization techniques, will be discussed.

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A COMPARISON OF THE VOLATILITIES OF TBHQ, BHA, AND BHT ANTIOXIDANTS FROM SOYBEAN OIL UNDER SIMULATED FRYING CONDITIONS. DAN F. BUCK, Technical Service and Development Division, Eastman Chemical Products, Inc., Kingsport, TN 37662.

TBHQ, BHA, and BHT are used in the food industry as antioxidants for frying oils. One of the problems of phenolic antioxidants is that they are removed from oils by heat and steam distillation. However, little work has been done comparing the volatilities of these antioxidants. This work describes a comparison of the volatilities of TBHQ, BHA, and BHT from soybean oil under simulated frying conditions. Loss rates for each antioxidant were determined under conditions of heat (180 C) and a combination of heat (180 C) and steam distillation. Regression analysis of the data indicated that a linear relation exists between antioxidant loss and time. Results of this work indicate very little difference in loss rates for each antioxidant. In addition, the regression equations show that steam distillation contributes little to loss of antioxidants from frying oil. AOM tests substantiate this conclusion as the oils subjected to both heat and steam distillation have the same stability as oil subjected to heat only. AOM testing also showed the superior properties of TBHQ in protecting soybean oil.

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF STEROIDS. ERICH HEFTMANN, Western Regional Research Center, USDA, 800 Buchanan St., Berkeley, CA 94710.

After a brief discussion of the merits and limitations of high performance liquid chromatography (HPLC) relative to other chromatographic methods, special problems in the application to steroids are discussed. Solutions are presented, using examples of procedures currently in use for various classes of steroids, particularly those encountered in fats and oils.

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QUANTITATIVE DETERMINATION OF FERULATES IN RICE BRAN OIL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. K. TANABE, A. TANAKA, A. KATO, and T. HASHIMOTO, National Chemical Lab. for Industry, 1-1-5, Honmachi, Shibuya-ku, Tokyo, 151, Japan.

Steryl esters of ferulic acid are characteristic minor components of rice bran oils. A new liquid chromatographic method for measuring the content of ferulates that is sensitive and precise is reported. A DuPont Model 830 high performance liquid chromatograph equipped with a fluorescence detector with a 0.25 m × 4.6 mm ID stainless steel column containing Zorbax sil (DuPont) was used. The mobile phase was a mixture of ethanol-hexane (1:50) at a flow rate of 2.0 ml/min. Under these conditions, the retention time of ferulates was 5.9 min. The minimum detectable amount of ferulates was 0.01 μg. The assay was used in the determination of ferulates in sixteen samples of rice bran oils. The contents of ferulates were 0.73–1.70% in crude oils and 0.02–0.24% in commercial oils.

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ANALYSIS OF AUTOXIDATION PRODUCTS OF CHOLESTEROL BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. L.S. TSAI and C.A. HUDSON, USDA, Albany, CA.

Autoxidation products of cholesterol were analyzed by high performance liquid chromatography (HPLC), using a μPorasil column and various mixtures of hexane and isopropanol as eluting solvents. The relative elution volumes of 12 cholesterol oxidation derivatives were obtained. Cholestan-3β,5α,6β-triol; 5α-cholestan-3β,6β-diol; 5-cholesten-3β,7α-diol; 5-cholesten-3β,7β-diol; 5-cholesten-3β,25-diol; 5-cholesten-3β,20-diol; and 5-cholesten-3β-ol-7-one were resolved from cholesterol and from each other. The 3,5-cholestadiene and 3,5-cholestadien-7-one were resolved from cholesterol and the above compounds, but not from each other. Another possible oxidation product, 5,24-cholestadien-3β-ol, was not resolved from cholesterol. A phenomenon of drastic changes in eluting strength of solvent with small changes in isopropanol content (0.5–5.0% in hexane) was observed, which cannot be explained simply by solvent polarity index. Effects of solvent isopropanol content on the elution volume of a number of compounds will be discussed. The advantages of HPLC over thin layer chromatography in this study were high resolution, rapid analysis, and direct quantitation with high sensitivity. The detectable level of all compounds for accurate quantitation by differential refractometer was about 1.0 μg. For those compounds which absorb significantly at 210 nm, such as cholesterol, the sensitivity was increased.

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REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF HEATED SOYBEAN OIL. M.M. CHAUDRY and E.G. PERKINS, 104 Burnsides Research Lab., University of Illinois, Urbana, IL 61801.

High performance liquid chromatography (HPLC) has become a widely used analytical tool. The availability of columns to allow controlled nondestructive separations of complex mixtures of lipids has increased its attraction to lipid chemists. Recent work in our laboratory and others have shown the applicability of octadecylsilane bonded microparticulate packings to the separation of complex triglyceride mixtures by HPLC. We have extended this work into the separation of heated and oxidized fat components. Production run 107 IV soybean oil was heated in a deep fryer with simultaneous frying of potatoes until the oil showed signs of deterioration. The heated oil was separated on a Zorbax ODS HPLC column using mixtures of acetonitrile and tetrahydrofuran as the mobile phase. The component peaks which separated and contained either triglyceride or changed components were collected and subjected to further analyses by gas liquid and thin layer chromatography. The results were compared with those obtained by subsequent analysis of unheated oil and the use of available heated fat components as standards to obtain basic elution data.

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF HEATED FATS AND OILS: OLIVE OIL. ALI EL-HAMDY and EDWARD G. PERKINS, 104 Burnsides Research Lab., University of Illinois, Urbana, IL 61801.

The thermal oxidation of fats and oils such as occurs in a deep fat fryer results in a complex mixture of oxidation products of intermediate and high molecular weight. The use of classical and conventional methods of separation such as urea adduction, distillation, and column chromatography are very time consuming and exhibit inferior separation power. For these reasons a combination of high performance liquid chromatography (HPLC), high performance gel permeation chromatography, and gas liquid chromatography were employed. In the present work, olive oil was heated in a commercial deep fryer at 215 C for 10 days. During 8 hr of each day french fries were prepared every 30 min. The resultant heated oil samples were first fractionated according to molecular weight with high performance gel permeation chromatography on a 500 Å microstyragel column, using tetrahydrofuran as the mobile phase. Fractions of components of molecular weights of less than 2000 were collected and subsequently separated on a reverse phase HPLC column. The components which were separated on this column were individually collected, converted to their methyl esters, and analyzed by gas chromatography. The higher molecular weight fractions were collected and separated by the reverse phase column. Individual components were also collected and analyzed by mass spectrometry and nuclear magnetic spectroscopy for structural characterization.

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NATURALLY OCCURRING VITAMIN D_3 IN FISH PRODUCTS ANALYZED BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY USING VITAMIN D_2 AS AN INTERNAL STANDARD. ELIANN EGAAS and GEORG LAMBERTSEN, Directorate of Fisheries, Bergen, Norway.

A chemical method for the analysis of naturally occurring vitamin D is proposed. The unsaponifiable matter of oils and tissues is prepared; cholesterol is partly removed by double precipitation at low temperature in methanol. The vitamin D fraction is collected on an adsorption column by high performance liquid chromatography (HPLC). The fraction is further purified, and the vitamins D_2 and D_3 are separated on a partition column (reverse phase) by HPLC. Recovery was 89 to 93%, standard deviation 3%. The only vitamin D analogue found in fish oils, livers, and fillets, was cholecalciferol (D_3). Hence, ergocalciferol (D_2) could be used as an internal standard. The provitamins ergosterol and 7-dehydrocholesterol, as well as the previtamins, were separated from the vitamin D-fraction on the adsorption column. Results in the range 0.050 to 134 μg D_3 per gram (2 to 5360 I.U. per gram) are given. One cod liver oil was analyzed in a rat bioassay, giving supporting results.

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DETERMINATION OF TOCOPHEROL HOMOLOGUES IN FOODSTUFFS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. HIROYASU FUKUBA, TORUKO MIYOSHI, and TOSHIE TSUDA, Research Institute of Food Chemistry, Ochanomizu University, Otsuka. Tokyo, 112, Japan.

By the combination of high performance liquid chromatography (HPLC) and spectrofluorometric analysis α-, β-, γ-, and δ-tocopherols in foodstuffs were successfully separated and determined. The experimental conditions were as follows: column, Shimadzu-DuPont Zorbax Sil; mobile phase, n-hexane-isopropyl ether (98:2); flow rate, 0.9 ml/min; detector, Shimadzu Spectrofluorospectrophotometer RF-500 (Ex. 298 nm, Em. 325 nm). As the internal standard, 2,2,5,7,8-pentamethyl-6-hydroxy chroman was employed. The peak of this compound appeared between those of α- and β-tocopherols. In some cases fluorescent substances interfered with the method. In the case of sesame oil, these substances were found to be sesamolin and sesamin. Some spices, especially white and black paper, allspice, and clove, interfered with the method. Clove extract showed seven fluorescent groups on the chromatogram. The first group and α-tocopherol, and the second group and β-tocopherol overlapped each other. Allspice contained two fluorescent groups that resembled those of the first and the second groups of clove extract. With samples containing these spices the interfering substances had to be separated by silicic acid column chromatography before the HPLC operation.

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY ANALYSIS OF SEVERAL PRESERVATIVES IN OILS AND FOODS. WILLIAM P. VAN ANTWERP, Agri-Science Laboratories, Inc., Los Angeles, CA 90025.

Reverse phase high performance liquid chromatography (HPLC) is an extremely efficient tool for the separation of many kinds of organic molecules. Suitable choice of both column-type and mobile phase composition allows great versatility in separation and excellent resolution. We have developed a method for the analysis of several common preservatives in oils and finished foods. The method involves a simple extraction, pre-injection clean-up, and HPLC separation, followed by both absorbance and fluorescence detection. Butylated hydroxy anisole, butylated hydroxy toluene, benzoic acid, sorbic acid, and sodium citrate are analyzed by this technique with part per million detection limits for the aromatic preservatives. A study of several foods and oils is included.

SESSION Q: SYMPOSIUM: LOW DENSITY AND VERY LOW DENSITY LIPOPROTEINS: STRUCTURE, COMPOSITION, AND PROPERTIES

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VERY LOW DENSITY LIPOPROTEIN AND LOW DENSITY LIPOPROTEIN METABOLISM: AN OVERVIEW. DANIEL STEINBERG, Division of Metabolic Disease, Department of Medicine, University of California, San Diego, CA 92093.

It is becoming increasingly clear that lipoprotein fractions as conventionally defined in terms of density ranges are to varying degrees heterogeneous and complex in their metabolic origin. For example, the very low density lipoprotein (VLDL) fraction includes molecules covering a wide range of size and composition. Most VLDL is normally synthesized and secreted by the liver, but a significant fraction has its origin from the intestine, either directly secreted or derived from chylomicrons. In normal man, most low density lipoprotein (LDL) is derived from VLDL. However, patients with familial hypercholesterolemia secrete LDL directly into the plasma in large quantity. In normal man, most VLDL apoprotein B is converted to LDL before disappearing from the plasma compartment. However, in patients with hypertriglyceridemia a large fraction of VLDL apoprotein B is removed from plasma without prior conversion to LDL. These newer findings considerably complicate interpretation of steady state patterns or phenotypes; eventually they should clarify the mechanisms underlying the hyperlipoproteinemias. It is now clear that a significant amount of LDL is degraded by extrahepatic tissues. Uptake of LDL by cells in culture is largely mediated by the high affinity LDL receptor defined by Brown and Goldstein. In addition, however, LDL can be taken up by low affinity mechanisms and this may be particularly significant with respect to atherogenesis and xanthoma formation. The uptake of LDL imposes a "burden" of cholesterol that must be returned to the liver for excretion. Glomset has proposed that HDL may play a key role in this process although evidence for the operation of this mechanism in vivo is limited. Interest in this proposal is heightened by the recent findings that risk of atherosclerosis correlates negatively with HDL levels. Although cell binding of HDL occurs largely by mechanisms distinct from those responsible for binding of LDL, certain subfractions of HDL (those containing apoprotein E) compete with LDL binding. This interaction may also contribute to the "protective" effect of high HDL levels.

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PHYSICAL-CHEMICAL INVESTIGATIONS OF LOW DENSITY LIPOPROTEIN AND VERY LOW DENSITY LIPOPROTEIN. DONALD M. SMALL, Biophysics Institute, Boston University Medical Center, Boston, MA 02118.

A variety of techniques have been used to probe the structure of low density lipoprotein (LDL) and very low density lipoprotein (VLDL) particles. The phase equilibrium of the major lipid classes of LDL and VLDL were used to predict the number of phases and the distribution of the different molecules in LDL and VLDL particles. These studies predict that both LDL and VLDL are particles composed of lipids in two separate phases. One phase is a surface phase made up of phospholipids, most of the cholesterol, and a small amount of triglyceride. The other is a core phase made up of triglyceride, cholesterol ester, and small amounts of free cholesterol. Lipid partition studies from LDL show that these two phases can be isolated and characterized. Using microcalorimetry, it was demonstrated that LDL from man and many other species undergo a transition related to the ordering of cholesterol esters within the core. VLDL does not show this transition because of the high concentration of triglyceride which abolishes cholesterol ester transitions. Using lipoproteins from different animals made hypercholesterolemic (pig, monkey), it was observed that specific lipoprotein classes (HDL_c, and LDL) usually had transition temperatures above the body temperature of the animal. The higher the transition temperature, the greater the chance of atherosclerosis in the animal. It is shown that the transition temperature, in the absence of triglyceride contaminants in the core, correlates very well with the fatty acid composition. In the presence of core triglyceride both fatty acid composition of the cholesterol esters and the quantity of triglyceride correlate with the transition. However, the correlation with triglyceride content within the lipoproteins is not very strong which suggests that other factors may be present which alter transition temperature. Finally, X-ray scattering studies indicate that the ordered structure within the lipoprotein consists of a radially oriented, quasi-layered cholesterol ester molecule below the transition and a less ordered system above the transition. NMR studies show that the fatty acyl chains are fairly mobile, but the cholesterol ring systems become relatively immobilized below the transition. The putative structures of HDL, HDL_c, human LDLs, swine LDL, monkey LDL, and VLDL, will be given, and the relation of the core transitions to atherosclerosis will be discussed. (The work discussed was performed with Drs. D. Atkinson, R. Deckelbaum, R. Mahley, L. Rudel, G. Shipley, and A. Tall.)

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SPHERICAL SYMMETRY AND STRUCTURE OF SERUM LOW DENSITY LIPOPROTEIN. ANGELO M. SCANU, The University of Chicago, Division of The Biological Sciences and The Pritzker School of Medicine, Department of Medicine, 950 E. 59th St., Chicago, IL 60637.

From data on size and chemical composition and from considerations on the surface area and volume occupied by its constituents, low density lipoprotein (LDL) can be described as a spherical particle having cholesteryl esters and triglycerides contained in a core of radius = r-20.2Å covered by the closely packed hydrophobic ends of unesterified cholesterol and phospholipids. Such a model is compatible with early solution X-ray scattering studies and neutron scattering analyses which, by postulating spherical symmetry, assigned the LDL constituents to locations predicted from the electron density distribution. However, the concept of spherical symmetry as applied to LDL structure has received a challenge from the more recent structural studies. By freeze-etching electron microscopy employing a novel rapid freezing technique, LDL has been reported to have a morphology deviating from a perfect sphere due to a small number of surface globules arranged in a tetrahedral symmetry. Similarly, solution X-ray scattering experiments at variable densities, although recognizing an almost spherical core, consider the surface of LDL as having four protein globules located at tetrahedral positions with capacity of structural remodeling at least as a function of the two temperatures studied (21 C and 41 C). Thus, the area of LDL structure remains unsettled. A major drawback in the interpretation of both early and recent scattering studies has been the lack of knowledge of the chemical and solution properties of the LDL protein (apo LDL) and of its behavior at the water-lipid interface. Until this knowledge is established the validity of the proposed models cannot be assessed. It is reasonable to presume that apo LDL plays a role in the organization of the surface and overall LDL structure. Progress is expected from the integration of chemical and physical studies.

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SIZE DETERMINATION OF HUMAN LOW DENSITY LIPOPROTEIN (LDL) SUBFRACTIONS. MASON M.S. SHEN, RONALD M. KRAUSS, FRANK T. LINDGREN, TRUDY M. FORTE, and THORNTON W. SARGENT IV, Donner Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720.

The heterogeneity of human serum low density lipoprotein (LDL) has been demonstrated by several techniques. In this study, analytical ultracentrifugation and electron microscopy were used to determine particle size in subfractions of LDL separated by equilibrium density gradient ultracentrifugation. LDL (d 1.019–1.063) were isolated from serum of twelve healthy subjects, six men and six women aged 25–56. Each sample was centrifuged in a sodium bromide gradient (d 1.020–1.054), and six subfractions were collected from each gradient at equilibrium. The individual subfractions were submitted to standard analytical ultracentrifugation at density 1.061 and 1.200 g/ml, and hydrated densities were calculated from the $\eta F°$ versus ρ plots. The linearity of this plot was verified in other studies using six data points rather than the standard two. The Stokes' radius was determined for each subfraction assuming spherical particles. The mean results showed a progressive increase in Stokes' radius from 85 to 99 Å with decreasing hydrated density of the six fractions. In six of the subjects, the particle sizes were also determined by electron microscopy after negative staining. Particles generally appeared round and mean radius increased from 107 to 132 Å with decreasing density. While the results of the two methods were strongly correlated ($r = 0.87$, $p < 0.001$) in each fraction the radius measured by electron microscopy was 20–30% greater than the calculated Stokes' radius. The discrepancy between the results of these two methods suggests that the assumption that the particles are spherical may not be correct.

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CONFORMATION OF THE PROTEIN MOIETY OF LOW DENSITY LIPOPROTEIN TREATED WITH TRYPSIN. J.P. KANE, G.C. CHEN, Cardiovascular Research Institute and Department of Medicine, University of California, San Francisco, CA 94143; and M.J. CHAPMAN, INSERM, Creteil, France.

Low density lipoprotein (LDL) was prepared from fresh human serum by repetitive ultracentrifugation at densities 1.024 g/cm^3 and 1.045 g/cm^3. The LDL were subjected to limit hydrolysis for 5 hr at 37 C with trypsin treated with L-1 (tosylamido-2-phenyl) ethyl chloromethyl ketone. Stable particles containing ca. 80% of the original protein mass (T-LDL) were separated from small peptides by gel permeation chromatography on Sephadex G-75. The circular dichroic spectrum of T-LDL showed a trough at 222 nm and a peak at 195-196 nm, but unlike native LDL the second minimum ca. 208 nm was absent. — $[\Theta]_{222}$ was always smaller at all temperatures for

T-LDL than for native LDL (10,623 ± 124 vs. 12,290 ± 171 $\frac{\text{deg cm}^2}{\text{dmol}}$ at 25 C), corresponding to helical fractions of 27 and 33%, respectively. Both preparations showed a progressive, linear decrease in ellipticity of about 15% as the temperature was increased from 20 to 60 C. This progression was completely reversible. In the presence of SDS (25 mM) both spectra showed a substantial blue shift with deep troughs at 206–207 nm but little change in $[\Theta]_{222}$, suggesting that SDS changes the dispersion of both T-LDL and native LDL without substantially altering helicity. The modest reduction in helicity observed with T-LDL suggests that certain helical regions in apo B are accessible to tryptic attack.

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THE B PROTEINS OF HUMAN SERUM LIPOPROTEINS. KAREN S. KUEHL and ROBERT G. LANGDON, Department of Biochemistry, University of Virginia School of Medicine, Charlottesville, VA 22908.

Apo B, a major protein of very low density (VLDL) and of low density lipoproteins (LDL) has been determined by the measurements of Smith, Dawson, and Tanford to have a molecular weight of 255,000 ± 5%. SDS PAGE, however, demonstrates heterogeneity of this protein with two major and one minor components being regularly present, having apparent molecular weights of 2.6×10^5, 2.4×10^5, and 1.4×10^5. This heterogeneity is present in VLDL as well as in LDL. Peptide mapping demonstrates the two high molecular weight species to be identical in VLDL and in LDL. Cyanogen bromide cleavage of the two high molecular weight components of apo B with subsequent separation of the peptides by isoelectric focusing, high voltage electrophoresis, paper chromatography, SDS PAGE, and ion exchange chromatography all demonstrate a limited number (four or five) methionine peptides, rather than the 36 met peptides predicted by amino acid analysis and molecular weight; this finding is consistent with highly repetitive structure of apo B. The met peptides which contain cysteine are found in only two fractions, another observation consistent only with highly repetitive structure of apo B. Amino terminal analysis of apo B yields blocked amino terminal glutamic acid in excess of the predicted one mole per 2.4×10^5 g. Analyses of the blocked peptides suggest that more than one peptide is present and that at least one of these contains two residues of glutamic acid. Carboxy terminal analyses of apo B by hydrazinolysis yield serine and glycine; both these amino acids are released by carboxy peptidase A or Y digestion of apo B. Analysis of apo B using chemical techniques demonstrate that the protein has a highly repetitive structure and may consist of multiple polypeptide subunits joined by covalent bonds.

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THE PROPERTIES OF SOLUBILIZED APOLIPOPROTEIN B. WALDO R. FISHER, RACHEL B. SHIREMAN, and PAUL W. CHUN, Department of Medicine, JHMHC Box J-226, University of Florida, Gainesville, Florida 32610.

Human plasma low density lipoprotein (LDL) differs in molecular weight from one individual to the next over a range of 2.4 to 3.6 million, with these differences resulting from different quantities of lipid associated with the apolipoprotein of LDL, apoB. To determine whether differences in LDL molecular weight result from chemical differences in apoB, a detailed study of the properties of apoB has been undertaken. Delipidated apoB may be solubilized in the absence of detergents by extraction of LDL with ether-ethanol in the presence of 6 M guanidine and at low temperature. The physical properties of apoB may be studied in various denaturing solvents. In agreement with the findings of Smith et al., the minimum molecular weight of apoB appears to be 250,000; however, the apoprotein readily undergoes self-association. The apoprotein retains the biological property of binding to the LDL receptor on fibroblasts and of being internalized and degraded by these cells in a manner analagous to that of LDL. Since apoB is a glycoprotein, the role of the carbohydrate in the binding of apoB to the plasma membrane receptor was assessed. The carbohydrate was largely removed from LDL by digestion with glycosidases from *Diplococcus pneumoniae*, and such LDL bound to the fibroblast receptor in a manner indistinguishable from native LDL. Alternately, delipidated apoB was digested extensively with trypsin plus Pronase. If the resulting glycopeptides had any measurable affinity for the fibroblast receptor, then their presence should cause an inhibition of the binding of radioiodinated LDL to the fibroblast. The absence of such inhibition is further evidence that the carbohydrate moiety of apoB does not participate in the binding of this protein to its receptor. As a consequence of the binding of LDL to the fibroblast and of its subsequent internalization, there is "down regulation" of receptors, a suppression of HMG CoA reductase activity, and an enhancement of ACAT activity within the cell. By contrast, incubation of fibroblasts with apoB results in an enhancement of cholesterol synthesis from ^{14}C-acetate and an increase in the number of plasma membrane receptors for this apoprotein. An associated depletion of intracellular cholesterol apparently results from a net transfer of cholesterol out of the cell where it presumably is bound by apoB in the medium. Thus, in contrast to most peptide hormones which directly regulate the number of their plasma membrane receptors, apoB indirectly regulates the number of its receptors through its delivery to (or removal from) the cell of cholesterol. ApoB may also be proteolytically cleaved into peptide fragments of varying size by the action of trypsin on native LDL. Partially tryptic digested LDL may be delipidated, the apoB peptides solubilized in 6 M urea and chromatographed on the Sepharose 6B. A partial resolution of peptides in accordance with size results. Among these peptides are some which bind to the LDL receptor, as well as peptides which retain certain of the immunologic properties of intact apoB. A further characterization of these peptides hopefully will help in understanding the properties of apolipoprotein B.

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GLUTATHIONE AND NITROGEN PROTECT APOLIPOPROTEIN B FROM AGGREGATION. DIANA M. LEE, WU H. KUO, and HEDIO MAEDA. Oklahoma Medical Research Foundation, 825 NE 13th St., Oklahoma City, OK 73104.

LDL_2 (d 1.030–1.040 g/ml) preparations(C) were isolated from fresh human plasma to which EDTA, penicillin, streptomycin, NaN_3 and DFP had been added. LDL_2 preparations(T) were also isolated from fresh plasma to which glutathione (0.05%) was added in addition to the other preservatives. The latter LDL_2(T) were kept under N_2. During storage at 4 C the solution of LDL_2(C) became cloudy and gradually formed precipitate. In contrast, the solution of LDL_2(T) remained transparent for many months. ApoLDL_2(T) was completely soluble in 6 M guanidine·HCl-Tris buffer containing dithiothreitol in contrast to partial solubility of apoLDL_2(C). The reduced ApoB solutions were chromatographed on a calibrated Sepharose CL-6B using guanidine-containing buffer (saturated with N_2) for elution. Only a small amount of ApoB(T) was eluted at the void volume; two major peaks were eluted with distribution coefficients of 0.076 and 0.18 which corresponded to MW 108,000 and 93,000. In contrast, the ApoB from LDL_2(C) was eluted either predominantly or completely at the void volume depending on the storage time of its parent LDL_2. On SDS-polyacrylamide (10%) gel electrophoresis, the reduced, carboxymethylated, and Cu^{2+}-treated ApoB(T) (Huang and Lee, *Circulation* 57:III-94 [1977]) as well as LDL_2(T) and apoLDL_2(T) migrated into the region of an albumin dimer for ApoB, whereas ApoB from LDL_2(C) or apoLDL_2(C) did not enter the gel. All preparations of ApoB(T and C) could be converted into a water-soluble form by Cu^{2+} treatment, but this did not alter their distribution coefficients on gel filtration or their electrophoretic mobilities. These results suggest that oxidation of lipids and/or protein of LDL_2(C) may be occurring slowly during the isolation process, even in the presence of EDTA, and creating higher molecular weight artifacts of ApoB of lower solubility. The use of glutathione and N_2 on LDL minimized the aggregation and enhanced the solubility of ApoB later in denaturing agents; Cu^{2+} treatment increased the solubility of ApoB in aqueous buffers but did not decrease its molecular weight.

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CHARACTERIZATION OF THE LP(a) LIPOPROTEIN. JOHN J. ALBERS, Northwest Lipid Research Clinic, University of Washington, 326 9th Ave., Seattle, WA 98104.

The Lp(a) lipoprotein [Lp(a)], also called the sinking pre-B lipoprotein, was initially considered to be a genetic variant of B or low density lipoprotein (LDL) present in ca. 35% of the caucasian population determined by a single autosomal dominant gene. Quantitation of Lp(a) by a sensitive radioimmunoassay indicates that Lp(a) is present in nearly all individuals though its concentration varies widely among individuals (0.3–110 mg/dl). Quantitation of Lp(a) in essentially all first degree relatives in 42 families suggests that the concentration of Lp(a) is determined by a polygenic mechanism. Cholesterol feeding in four subjects resulted in an increase in LDL and apoB whereas Lp(a) remain essentially constant. Thus Lp(a) appear metabolically independent of B lipoprotein even though it shares the same structural protein, apoB. Subjects who had a mycardial infarction at age <50 had higher levels of Lp(a) than the normal population. Thus, high levels of Lp(a) appear to be associated with premature coronary disease. Consistent with this hypothesis ^{125}I-Lp(a) is avidly taken up by human arterial smooth muscle cells in culture.

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PLASMA AND URINARY LIPOPROTEINS AND APOLIPOPROTEINS IN HUMAN NEPHROTIC SYNDROME. V.G. SHORE, Lawrence Livermore Laboratory, University of California, PO Box 5507, Livermore, CA 94550; T.M. FORTE, Lawrence Berkeley Laboratory, University of California; H. LICHT, and S.B. LEWIS, Naval Regional Medical Center, Oakland, CA.

Plasma and urinary lipoproteins were characterized to elucidate possible abnormalities associated with elevated plasma very low density (VLDL) and/or low density (LDL) lipoproteins and decreased high density lipoproteins (HDL) in nephrotic humans. Lipiduria is not related quantitatively to concentration or distribution of plasma lipoproteins. At low and high output, urinary lipids are associated in part with soluble lipoproteins, of which HDL_2, HDL_3, and LDL are major and VLDL and IDL minor species. Urinary lipoproteins, the corresponding plasma lipoproteins, and normal lipoproteins are very similar in particle size and morphology as seen in electron micrographs. They are similar also in electrophoretic mobility, except that the mobility of urinary VLDL is slow, and in reaction with antisera to normal LDL and HDL. The intact protein moieties and apolipoproteins isolated by DEAE-cellulose chromatography and isoelectric focusing were examined by analytical polyacrylamide gel electrophoresis and isoelectric focusing with densitometric scanning, amino acid analysis, and immunodiffusion. Plasma VLDL are not deficient in apoC's, and the apoCII to apoCIII ratio is within the normal range. ApoB is present in urinary VLDL, IDL, and LDL. Plasma HDL_2 and HDL_3 contain a higher-than-normal proportion of minor apolipoproteins CI, CII, and CIII. Urinary HDL_2 and HDL_3 contain apoAI, apoAII, and a high proportion of three acidic proteins that react with antisera to normal HDL and are similar to apoCII and apoCIII in molecular weight and isoelectric points, but very different in amino acid composition. These results indicate that large as well as small lipoproteins are filtered and lost in the urine in nephrosis, that changes in lipoprotein composition occur in the process, and that the hyperlipoproteinemia is not due to loss of the lipase activator apoCII. (This work was performed under the auspices of the U.S. DOE by LLL, under contract no. W-7405-ENG-48.)

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RAPID INSTRUMENTAL TECHNIQUES FOR THE ANALYSIS OF VOLATILES IN SALAD DRESSINGS. M.G. LEGENDRE, E.T. RAYNER, W.H. SCHULLER, and H.P. DUPUY, Southern Regional Research Center, USDA, PO Box 19687, New Orleans, LA 70179.

Simple techniques are described for the analysis of neutral volatiles in salad dressings by direct gas chromatography or by a combination of direct gas chromatography and mass spectrometry. For gas chromatographic analysis, a glass inlet

liner of the gas chromatograph is prepared with a salad dressing sample and placed in the injection port, where the combined action of heat, water, and carrier-gas flow sweep the volatiles from the sample to the gas chromatographic column. Acidic volatiles are retained by potassium carbonate in the liner below the sample. The spent liner is removed, and the column oven is temperature programmed to resolve the volatiles. For mass spectrometric identification of volatiles, a liner is placed in an external closed-inlet assembly equipped with a sodium sulfate condenser that removes moisture from the volatiles and permits effective identification of the neutral volatile compounds. With this technique, the aldehydes, ketones, alcohols, esters, and sulfur-containing compounds of fresh and aged salad dressings are identified. Certain volatiles such as aldehydes and ketones increase during storage and may be indicative of product stability.

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GAS CHROMATOGRAPHY-MASS SPECTROMETRY OF C_{60-80} MYCOLIC ACIDS FROM MYCOBACTERIA AND RELATED ACID-FAST BACTERIA. SEIKO TORIYAMA, IKUYA YANO, MASAMIKI MASUI, EMI KUSUNOSE, and MASAMICHI KUSUNOSE, Department of Bacteriology, Osaka City University Medical School, Asahimachi-1, Abenoku, Osaka, Japan.

Mycolic acids are unusually high molecular weight 3-hydroxy fatty acids possessing a long chain alkyl branch at the 2-position and known to be a specific constituent of glycolipids or cell wall skeletons of Mycobacteria, Nocardia, and Corynebacteria. The physiological properties of the cell walls in Mycobacteria are reported to relate profoundly to the structures of mycolic acids. To obtain precise information about the functions of the individual molecular species of mycolic acids, analysis by gas chromatography seemed to be essential. Recently, we have developed gas chromatography-mass spectrometry (GC-MS) analysis of such types of fatty acids ranging from C_{20} to C_{68} from various acid-fast bacteria. However, the GC-MS analysis of longer chain mycolic acids than C_{70} was extremely difficult, owing to their instability at high temperatures. More recently, we have succeeded in the GC-MS analysis of C_{70-80} mycolic acids from the various strains of true Mycobacteria. By this method, the molecular species of methyl mycolate were separated as their trimethylsilyl (TMS) derivatives, according to their total carbon and double bond numbers. The total carbon and double bond numbers were determined from the molecular ions $[M]^+$, ions due to $[M-15]^+$, $[M-43]^+$, $[M-47]^+$, and $[M-90]^+$, etc. On the other hand, the straight chain structures were identified by the mass fragment ions $[A]^+$ due to C_2–C_3 cleavage; $[R{\cdot}CH{-}O{-}Si{\cdot}(CH_3)_3]^+$ for monocarboxy mycolic acids or $[CH_3O{-}CO{-}R{\cdot}CH{-}O{-}Si{\cdot}(CH_3)_3]^+$ for dicarboxy mycolic acids, and the branched chain structures were identified by the mass fragment ions $[B]^+$ due to C_3–C_4 cleavage $[(CH_3)_3{\cdot}SiO{\cdot}CH{\cdot}CH(R'){\cdot}COOCH_3]^+$. On the basis of the molecular species composition, each species of mycobacteria was shown to possess a characteristic profile in mono- and dicarboxy mycolic acid composition. This GC-MS analysis of mycolic acid molecular species gives one of the most powerful informative tools for the chemotaxonomy of mycobacteria. We also describe the change in mycolic acid composition during growth, temperature change, and the presence of antituberculous isoniazide.

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ANALYSIS OF CURED MEAT FLAVOR VOLATILES BY DIRECT-SAMPLING GAS CHROMATOGRAPHY-MASS SPECTROMETRY. M.E. BAILEY, Department of Food Science and Nutrition, University of Missouri, Columbia, MO 65211; M.G. LEGENDRE, and H.P. DUPUY, Southern Regional Research Center, USDA, PO Box 19687, New Orleans, LA 70179.

The rapid gas chromatographic food-sampling procedure of Dupuy and co-workers was used with mass spectrometry to study volatiles from cooked bacon and ham cured with varying levels of sodium nitrite and without nitrite. The chromatographic profile of cooked, lean bacon (*L. dorsi* muscle) processed without nitrite was qualitatively similar to that of bacon cured with 1,000 ppm sodium nitrite. Quantitative profiles were different, and low molecular weight volatiles from bacon without nitrite were more concentrated than those treated with nitrite. During storage, hexanal and 2-pentylfuran increased and nonanal decreased in samples not cured with nitrite. After storage at 4 C for 1 week, nitrite did not affect volatiles from bacon drip. Results from ham were similar to those from bacon, indicating that nitrite retarded oxidation in both products. The rapid GC food-sampling procedure can be used for measuring the flavor quality of bacon and similar cured meats during processing, storage, and distribution.

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IDENTIFICATION OF ENZYMIC REACTION PRODUCTS BY RAPID UNCONVENTIONAL METHODOLOGY. ALLEN J. ST. ANGELO and MICHAEL G. LEGENDRE, Southern Regional Research Center, USDA, PO Box 19687, New Orleans, LA 70179.

Peanut and soybean lipoxygenase were used to catalyze the oxidation of linoleic acid and methyl linoleate in aqueous reaction mixtures to form hydroperoxides. Aliquots of the reaction mixtures, without prior time-consuming extractions or chemical modifications, were placed directly into the heated or nonheated injection system, and monitored by rapid unconventional direct gas chromatography and combined gas chromatography/mass spectrometry. When the reaction mixtures of peanut enzyme and substrate were analyzed at room temperature, only hexanal was found. However, at elevated temperatures, several major and minor components were identified. Similar results were found when the mixtures of the soybean enzyme and substrate were analyzed at elevated temperatures. These data show that this unique approach can be used as an efficient, accurate, and rapid technique for direct examination of volatile reaction products catalyzed by enzymes under varying conditions.

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A SIMPLE PROCEDURE FOR THE QUANTITATIVE EXTRACTION OF LIPIDS FROM ANIMAL TISSUES. F. PHILLIPS and O.S. PRIVETT, The Hormel Institute, University of Minnesota, 801 16th Ave. NE, Austin, MN 55912.

A method is described for the quantitative extraction of lipid from animal tissues with chloroform-methanol (C/M) that eliminates secondary purification of the lipid extract by dextran gel chromatography or aqueous washing of the organic extract. Nonlipid substances that contaminate C/M extracts are separated by pre-extraction of the tissue with dilute, ca. 0.25%, aqueous acetic acid. The concentration of acetic acid should be such to give a final extract with a pH between 4 and 4.4. Hot acetic acid is used with some tissues to prevent enzymatic hydrolysis. The residual tissue is extracted twice with 40 volumes of C/M (1:1, v/v). Approximately 97% of the lipid is recovered in these extractions. A third extraction with C/M (1:1, v/v) is performed if the procedure is discontinued at this stage in a shortened version of the method, which gives yields of ca. 98%. The remainder of the lipid is recovered after treatment of the tissue with 1 N HCl by two additional extractions, the first with 40 volumes of C/M (1:2, v/v), and the second with 40 volumes of methanol. The extended procedure gives complete extraction of the lipid including the gangliosides, free of nonlipid substances.

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SOME RESULTS FROM AN IUPAC STUDY ON THE LIPOXYGENASE CATALYZED DETERMINATION OF POLYUNSATURATED FATTY ACIDS. OSTEN LEVIN, Margarinbolaget AB, Fack, S-104 25 Stockholm, Sweden.

Within the Commission on Oils, Fats and Derivatives of IUPAC the feasibliity of quantitative determination of fatty acids with *cis-cis*-1,4-pentadienoic structure has been studied by collaborative testing. A number of enzyme preparations ranging in activity from a few thousand units up to half a million units per mg have been studied. With the exception for the preparation of lowest specific activity, they have all been found suitable. It has been found necessary to use a calibration curve at the determinations, rather than to adopt a fixed value for the specific absorbance of the hydroperoxide formed. It has also been noticed that the measured absorbance around 235 nm generally is influenced by the mechanical handling of the enzyme containing solution of a saponified oil sample. Also, the molar absorbance of the hydroperoxide formed at the enzymatic reaction usually seems lower than expected according to earlier literature data.

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EVALUATION OF OXIDATIVE DETERIORATION OF OILS AND FOODS BY MEASUREMENT OF ULTRAWEAK CHEMILUMINESCENCE. RIICHIRO USUKI, TAKASHI KANEDA, AKIO YAMAGISHI, CHOICHI TAKYU, and HUMIO INABA, Faculty of Agriculture, Tohoku University, 1-1 Amamiyamachi-Tsutsumidori, Sendai, Japan.

It has been known for several years that the oxidation of organic materials is accompanied by emission of weak chemiluminescence. However, it was difficult to detect this emission quantitatively. The present study was undertaken to evaluate the oxidative deterioration of oils and foods by using the single photoelectron counting system, which was designed for the measurement of ultraweak chemiluminescence. Measurement procedure of chemiluminescence is as follows: a quartz cell (50 × 10 × 43 mm) is filled with sample oil and set in an oven at controlled temperature. The output pulses of the photomultiplier, the number of which corresponded to the intensity of the chemiluminescence, are processed by several electronic circuits, then measured by a digital counter and a digital printer. Several thermally oxidized soybean oils were prepared, and those chemical characteristics and chemiluminescence were measured. The results showed that the increase of emission counts was closely correlated to the oxidative deterioration of oils. For example, oil heated for 32 hr showed AV of 2.01, COV of 304, IV of 96, and emission counts of 22193, while nonheated oil showed 0.046, 3, 129, and 728, respectively. A similar relationship was observed for soybean oil in which potatoes were fried, autooxidized soybean oil, instant Chinese noodles, and milk powder. This new method has a great advantage for the measuring of food quality by unskilled persons in a short time without pretreatment. Accordingly, various applications to other foods, food additives, and antioxidants are expected.

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QUANTITATIVE ESTIMATION OF LIPIDS BY THIN LAYER-FLAME IONIZATION DETECTOR CHROMATOGRAPHY. TOSHIHIRO ITOH, MASAMICHI TANAKA, and HIROSHI KANEKO, Division of Chemistry, School of General Studies, Kitasato University, 1-Asamizodai, Sagamihara, Kanagawa 228, Japan.

Various neutral and polar lipid samples were separated on the silica gel-sintered quartz rod by thin layer chromatography. These developed rods were scanned automatically through the Iatroscan analyzer (Model TFG-10, Iatron Lab., Tokyo) which was equipped with a hydrogen flame ionization detector. Neutral lipid mixtures (sterol, free fatty acid, triglyceride, and sterol ester) could be well resolved to the respective component on a silica gel-sintered rod by developing with petroleum ether: ethyl ether:glacial acetic acid mixture. Detector response of each component was found to be linear over a range of 2–10 μg loads with the maximum SD of about 5%. Polar lipid mixtures (cardiolipin, phosphatidyl ethanolamine, phosphatidyl choline, sphingomyelin, and so on) could be analyzed with practically the same efficiency as described for neutral lipids using chloroform:methanol:water mixtures. The method was conveniently applied to analyzing the relative amount of nonpolar lipid, phospholipid, and glycolipid in a mixture of total lipid extract. In addition, it was found that a reagent-impregnated silica gel-sintered rod could greatly facilitate the estimation of certain lipid mixtures. The silver nitrate impregnated silica gel rod was powerful for separation of molecular species of triglyceride and phosphatidyl choline. Boric acid impregnated silica gel rod enhanced resolution of neutral lipid mixtures containing 1-monoglyceride, 2-monoglyceride, 1,2-diglyceride, 1,3-diglyceride, triglyceride, as well as free fatty acid. Finally, the application of alumina- and florisil-sintered quartz rod for quantitative analysis of lipid composition was also investigated.

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A SIMPLE MATHEMATICAL CORRECTION FOR NONLINEARITY OF OPTICAL DENSITY IN THE QUANTITATION OF THIN LAYER CHROMATOGRAMS OF LIPIDS BY PHOTODENSITOMETRY. DONALD T. DOWNING and ANNA M. STRANIERI, Department of Dermatology, College of

Medicine, University of Iowa, Iowa City, IA 52242.

Several previous investigators have shown that the light-scattering characteristics of thin layer chromatographic (TLC) media produce a nonlinear relationship between the quantities of material in chromatographic spots and their optical density. With photodensitometers calibrated against a linear optical density standard, accurate quantitation by TLC requires either extensive use of reference compounds or adjustment of the photodensitometer response electronically or mathematically. Published mathematical corrections based on the Kubelka-Munk expression for light-scattering media are complex and require a computer facility. In the present study it was found that mathematical correction is readily achieved by a form of triangulation in which the height parameter is adjusted exponentially before calculation of peak area. This manipulation, performed with an inexpensive electronic calculator, provided a linear relationship between amount of lipid chromatographed and the adjusted areas of peaks in the photodensitometric scans of the acid-charred chromatograms. Furthermore, when expressed in terms of the carbon content of the lipids, the calibration lines of all of the lipids were coincident.

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THE DETERMINATION OF MICROAMOUNTS OF IRON IN FATS AND OILS BY ATOMIC ABSORPTION SPECTROPHOTOMETRY. HARUO HIRAYAMA, ZENYA SHIMODA, and MASAHIKO HIGUCHI, JOCS Committee of Fats and Oils Analysis, c/o Yushi Kogyo Kaikan, 3-13, 3-chome Nihonbashi Edobashi, Chuo-ku, Tokyo 103, Japan.

A rapid and precise method for the determination of iron in fats and oils has been studied. The sample is decomposed by dry-ash procedure with filter paper-wick. The ash is dissolved in hydrochloric acid and directly atomized into the air-acetylene flame, and the absorbance was measured at 248.3 nm. The effect of the ashing technique on the recovery of iron from representative stocks is discussed. The proposed method was evaluated with respect to recovery and reproducibility by carrying out collaborative studies on the samples which were prepared by adding known amounts of iron to soybean oil. The results thus obtained showed good agreement with the theoretical values and also agreement with flameless atomic absorption spectrophotometry results. The limit of detection with this procedure is 0.10 ppm.

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POLYUNSATURATED FATTY ACIDS OF HUMAN NEOPLASTIC TISSUES. EIJI ARAKI, NOBUO OKAZAKI, National Cancer Center, Tsukiji 5-1-1, Chuo-ku, Tokyo, 104, Japan; and TOSHIO ARIGA, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan.

Polyunsaturated fatty acids of human neoplastic tissues and the corresponding normal tissues were analyzed by combination gas liquid chromatography and chemical ionization mass spectrometry-computer system. Chemical ionization mass spectrometry has been demonstrated to be useful not only for the molecular weight determination but also for the structural elucidation of polyunsaturated and/or long chain fatty acids. Applied to the polyunsaturated fatty acids of hepatocellular carcinoma, these methods have given characterization of unphysiological isomers of such fatty acids as $C_{14:2}$, $C_{16:2}$, $C_{18:2}$ (3), $C_{18:3}$, $C_{19:2}$, $C_{19:3}$ (2), $C_{20:3}$, $C_{20:4}$, and $C_{22:3}$. Among those fatty acids, $C_{20:3}$n-9 was detected in ca. 70% of the tissue of hepatocellular carcinoma. This unphysiological isomer was only detected in patients with hepatocellular carcinoma, who had alpha fetoprotein in their sera, and was demonstrated to be preserved in those hepatoma tissues transplanted in athymic nude mice over 25 transplant generations, while the concentration of linoleic acid in the liver was higher in nude mouse than in humans. It appears, therefore, that the synthesis of $C_{20:3}$n-9 is one of the essential metabolic aberrations of human hepatoma, quite independent of nutritional conditions.

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COMPOSITION OF DIETARY FAT AND TUMOR INCIDENCE. IAN J. TINSLEY, JOHN A. SCHMITZ, and PHILIP D. WHANGER, Department of Agricultural Chemistry, Oregon State University, Corvallis, OR 97331.

The effect of the composition of dietary fat on tumor incidence and development is somewhat uncertain despite the common conclusion that polyunsaturated fats tend to enhance tumor incidence and/or development. In this study, statistical techniques will be used in an attempt to establish the contribution of the constituent fatty acids in the development of spontaneous mammary tumors in the C_3H mouse. The lipid (10%) of the 20 different diets is comprised of either one of eleven different fats and oils (lard, beef tallow, butter, coconut, olive, cottonseed, corn, safflower, linseed, rapeseed, span) or combinations of these fats and oils. These combinations were selected such that the levels of the major fatty acids (12:0, 14:0, 16:0, 18:0, 18:1, 18:2, 18:3, 20:1, and 22:1) varied over a reasonable range and were not significantly correlated with one another. At this stage in the study (all animals at least 12 months on experiment) tumor incidence varies from 11.5% in mice fed a diet containing 10% rapeseed oil (high erucic acid) to 43.2% in mice fed a diet containing 10% cottonseed oil. Time to tumor is longest in mice fed the diet containing 10% olive oil. (Supported by P.H.S. grant no. CA 20998.)

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EFFECT OF DIETARY PROTEIN AND FAT ON FECAL STEROLS AND BILE ACIDS IN THE RAT AND HAMSTER. N. TOTANI, M.E. MOMSEN, and J.R. CHIPAULT, The Hormel Institute, University of Minnesota, 801 16th Ave. NE, Austin, MN 55912.

The higher incidence of colon cancer in western populations that eat meat, compared to vegetarian groups, is believed to be due to the higher amounts of more highly degraded sterols and bile acids found in the feces of meat eating groups. These fecal steroids are structurally related to the carcinogenic aromatic polycyclic hydrocarbons. Although analysis of feces from humans on meat or vegetarian diets tends to support this hypothesis, no experimental data using well defined and controlled diets have been presented. Rats and hamsters were placed on fat-free diets containing either extracted beef protein, purified soy protein, or casein as the sole source of protein. After 4 months each diet was modified by substituting isocalorically either beef fat or soybean oil for a portion of the carbohydrate. All the modified diets contained 15% of fat. Fecal sterols and bile acids were determined periodically by thin layer and gas chromatography. Dietary fat had little effect on the excretion of dietary sterols but it increased fecal copro-sterols derived from dietary sterols by intestinal bacteria. Fecal 7-dehydrocholesterol which has been reported to be carcinogenic was unaffected or slightly decreased by dietary fat. Bile acids in rats' feces were increased when beef fat or soybean oil was added to the beef protein diet. In the other groups, bile acids in both rats' and hamsters' feces were decreased when the fats were in combination with protein. The same was true for lithocholic acid which is regarded as a carcinogen. Sterols, including 7-dehydrocholesterol, and bile acids, including lithocholic acid, were highest in the feces of animals on the completely vegetarian diets containing soybean protein.

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LIPID METABOLISM IN AFLATOXIN- AND/OR ORAL CONTRACEPTIVE-TREATED RATS. LILLA AFTERGOOD, ANNE ROGEL, ROSLYN B. ALFIN-SLATER, School of Public Health, University of California, Los Angeles, CA 90024.

The effect of an oral contraceptive (OC), with and without aflatoxin (A), on tumor formation and lipid metabolism has been studied in female rats. OC was introduced either during the induction of the tumor by A or during the tumor development following the discontinuation of A. Analyses were performed at 3, 6, 9, and 12 months. Food consumption was slightly decreased when A was present in the diet and also following the discontinuation of A. This effect was at times enhanced by the presence of OC. Body weight was decreased during the OC treatment but returned to control values after OC was discontinued. Liver weight expressed as percent of body weight was the highest at 12 months in the A-treated rats without OC. Plasma cholesterol and tocopherol levels generally increased following A treatment, whereas they were decreased by OC administration. Cholesterol was increased in livers containing hepatomas, particularly during OC treatment. Hepatic cholesterol biosynthesis was increased, whereas fatty acid biosynthesis was decreased in A-treated rats. In general, the administration of OC either during the initiation or during the development of tumors diminished the extent and severity of carcinogenesis. (Supported by grant no. 1 R01 CA 20938 from National Cancer Institute.)

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EFFECT OF AFLATOXIN, INGESTED WITH CORN OIL, ON HEPATIC MICROSOMAL CYTOCHROME P-450 LEVELS IN RATS. ANAHID CRECELIUS, Department of Foods and Nutrition, California State Polytechnic University, Pomona, 3801 W. Temple Ave., Pomona, CA 91768; L. AFTERGOOD, and R.B. ALFIN-SLATER, University of California, Los Angeles, CA 90024.

Female Wistar strain rats were placed at weaning on 5% corn oil diet (C) or on the same diet with 1.7 ppm of aflatoxin B (CA) for 3 months. After 3 months aflatoxin supplementation was discontinued and each group was divided into four dietary subgroups, namely, (a) continuing on 5% corn oil (C or CA), (b) corn oil replaced with 5% lard (L or LA), (c) corn oil replaced with 2.5% lard and 2.5% corn oil (LC or LCA), and (d) fat-free diet (FF or FFA). At 12–13 months of age the animals were killed and hepatic microsomal protein, cytochrome P-450, and plasma and liver cholesterol levels were determined. It was expected that both dietary aflatoxin and type of fat will influence hepatic cytochrome P-450 levels significantly. The calculated values show no statistically significant difference between the groups fed corn oil, lard, or a combination of the two or when aflatoxin was added to the diet. Hepatic microsomal protein levels were low when compared with published values for normal rats. The low protein levels may be due to fatty infiltration of the liver. Plasma cholesterol levels were high in all the groups but highest in those on the corn oil and corn oil with aflatoxin diets. The incorporation of aflatoxin to the diet made a statistically significant difference in animals given lard in the diet. Liver cholesterol levels were higher in animals fed lard and fat-free diets. The incorporation of aflatoxin to the diet affected the liver cholesterol levels of animals on corn oil and fat-free diets significantly.

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LIPID MOBILIZATION IN LYMPHOMA-BEARING MICE. SHINICHI KITADA, JAMES F. MEAD, and ESTHER F. HAYS, Laboratory of Nuclear Medicine and Radiation Biology, 900 Veteran Ave., Los Angeles, CA 90024.

1-^{14}C-Linoleic acid was fed to healthy AKR mice, and the resulting labeled adipose tissue was transplanted into the peritoneal cavity of age-matched lymphoma-bearing and control AKR mice. Significant amounts of radioactivity were found after 3 days in liver, thymus, and lymph nodes in lymphoma-bearing mice, whereas only negligible amounts of radioactivity were found in liver and thymus of control mice. In thymus, which was almost totally replaced by lymphoma cells, the radioactivity was found to be about 90% in the phospholipid fraction. These results are consistent with the idea that lipid mobilization is taking place in the lymphoma-bearing mice and that triglyceride fatty acids from adipose tissue are furnishing fatty acids for membrane synthesis and possibly for energy in the growing tumor cells.

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MOLECULAR DISORGANIZATION OF PLASMA MEMBRANE LIPID BILAYER OF CANCER CELLS. KINYA KOIZUMI, NAOKI YAMANAKA, and KAZUO OTA, Laboratory of Chemotherapy, Aichi Cancer Center Research Institute, Nagoya, 464, Japan.

Recently, lipid components in membrane were reported to be important to the understanding of cell abnormalities. Therefore, we focused on the demonstration of the molecular disorganization of lipid bilayer of isolated plasma membranes (PM) from cancer cells (rat hepatomas, L1210 leukemia cell). The results were as follows. (a) Microviscosity of tumor PM determined using DPH probe was lower than those of the corresponding normal cells. The decrease in microviscosity was related to molar ratio of cholesterol to phospholipid (PL) and/or to phasmalogen content. (b) The analysis of PL composition

was as follows: (i) increases in % of sphingomyelin and ethanolamine phospholipid (EP) in total PL-P; (ii) a decrease in % of choline phosphoglyceride (CP); (iii) a decrease in ratio of CP/choline phosphosphingoside and an increase in EP/choline phospholipid. (c) Phospholipase A, lysophospholipase, and lyso-PL acyltransferase activities which relate to PL metabolism were found to be altered. These results indicate that PL metabolisms of PM are important to understanding the molecular disorganization of malignant tumor PM.

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INCREASE OF ANTITUMOR EFFECT OF DRUGS BY CELL MEMBRANE MODIFICATIONS. NAOKI YAMANAKA, TAKETOSHI KATO, and KAZUO OTA, Laboratory of Chemotherapy, Aichi Cancer Center Research Institute, Nagoya, 464, Japan.

Extensive studies are now in progress to kill cancer cells specifically through cell membrane. We tried to improve the effect of cancer chemotherapy by membrane modifications, and the results of our studies revealed the following facts. (a) By modifying lipid components in membrane, affinity of drugs against cancer cells was improved; when the content of cholesterol ester in membrane was increased, the sensitivity against Amphotericin B was accelerated. (b) Vitamin A was effective in modifying cell membrane; the combination of Vitamin A with lysolecithin or biscoclaurin alkaloid, cepharanthine was useful in labilizing cell membrane and was effective in increasing the sensitivity against cancer drugs such as 5-Fu. (c) Liposome was effective as a carrier of drugs, (d) Antitumor antibiotics such as neocarzinostatin, macromomycin, and sporamycin were effective in damaging cells through cell membrane modification. The above-described findings are also important in clarifying the specificity of cancer cells.

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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF DERIVATIVES OF POLYOXYETHYLENE ALKYLPHENYL ETHER. SHOICHIRO WATANABE, TSUYOSHI UCHIBORI, and KAZUO KAWADA, Department of Chemistry, Faculty of Hygienic Sciences, Kitasato University, Asamizodai 1, Sagamihara-shi, Kanagawa-ken, 228, Japan.

Some derivatives of polyoxyethylene nonylphenyl ether (POE) were prepared by introduction of certain functional groups (i.e., amino, nitro, formyl, carboxyl, hydroxymethyl, etc.) into the aromatic nucleus. Though the derivatives (I) containing functional groups such as amino or carboxyl are structurally ionic, their surface activity comes from their nonionic moieties namely the polyoxyethylene and alkylphenyl groups. The derivatives (II) having a nitro, formyl, or hydroxymethyl group are nonionic. Surface tensions of the aqueous solutions of the derivatives (I and II) were as low as those of the starting POE. Some of the derivatives (I) showed high antimicrobial activity. The activity of the derivatives (II) was not as high as of derivatives (I). It was observed that the derivatives which showed antimicrobial activity were much more adsorbed on bacteria than the starting POE. It was concluded that polyoxyethylene alkylphenyl ethers can show antimicrobial activity when certain functional groups, such as a formyl group, are introduced; this seemed to facilitate the adsorption of the surfactant on bacteria.

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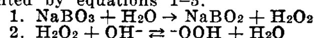
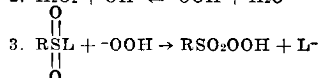
ORGANOSULFUR COMPOUNDS AS PEROXYGEN ACTIVATORS. JOSEPH H. FINLEY, FRED SCHOLER, JOHN BLUMBERGS, BURT BAUM, GAYLEN BRUBAKER, CHARLES LUTZ, and ANDY GALLOPO, FMC Corporation, PO Box 8, Princeton, NJ 08540.

Activators are organic compounds that enhance bleaching by peroxygen compounds in low temperature (<60 C) laundering. Because of the current worldwide trend toward lower laundering temperatures, there is a growing need for activators, particularly in Europe. In response to this need, FMC has developed several novel classes of activators having the general structure: RSO_2L, where R is an organic radical and L is a labile group. Included in these classes are: sulfonyl fluorides, sulfonic anhydrides, carboxylic-sulfonic anhydrides, sulfonyl azides, alpha disulfones, phenylsulfonates, and N-sulfonylimidazoles. This paper summarizes the structures and properties of these novel activator compounds and discusses the performance testing program used at FMC to determine their commercial potential. Included in this program were stain removal tests involving a variety of common stains, assessment of activator stability in formulations, and various other tests. Several of the compounds have exhibited excellent stain removal efficacy and stability in dry bleach formulations. The presumed mode of activation by sulfonyl activators will also be discussed. It is thought that persulfonic acids (RSO_2OOH) are formed as the initial products in bleaching solutions by a sequence of reactions represented by equations 1–3.



1. $NaBO_3 + H_2O \rightarrow NaBO_2 + H_2O_2$
2. $H_2O_2 + OH^- \rightleftarrows {}^-OOH + H_2O$



3. $RS(=O)_2L + {}^-OOH \rightarrow RSO_2OOH + L^-$

Persulfonic acids cannot be detected chemically in bleaching solutions at normal U.S. laundering temperatures (40 C). However, results of several experiments, including the finding that azide ion (N_3^-) is formed much more rapidly in alkaline solutions containing perborate than in its absence, supply indirect evidence that persulfonic acids are formed.

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ADSORPTION OF A SURFACTANT ON THE BUBBLE SURFACE BY FOAM FRACTIONATION. ISAO MARUISHI, Osaka Prefectural Industrial Research Institute, 2-1-53, Enokojima, Nishi-ku, Osaka, 550, Japan.

The adsorption of p-nonyl phenyl poly (oxyethylene) ether (EO 5 to 50 moles) from the aqueous solution containing the surfactant in various concentrations was studied by a foam fractionation method. Air was bubbled through the solution in a glass column (30φ × 500 mm) at a constant flow rate (150 cm^3/min), and the concentration of the surfactant in the collapsed foam was measured. The results were as follows: (a) The adsorption of the surfactant on the bubble surface was found to come to equilibrium in 2 or 3 sec, and the adsorption isotherm was confirmed to be in good agreement with the Langmuir equation. (b) The adsorption amount showed a maximum value when the number of ethylene oxide added was about 10 moles, and decreased slightly in the range of 10 to 50 moles.

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SOAP-BASED DETERGENT FORMULATIONS: XXVI. FORMULATIONS OF SOAP-LIME SOAP DISPERSANT COMBINATIONS WITH BUILDERS AND INORGANIC SALTS. W.R. NOBLE and W.M. LINFIELD, Eastern Regional Research Center, USDA, 600 E. Mermaid Lane, Philadelphia, PA 19118.

The effects of various types of builders and electrolytes on the detergency of blends of tallow soap and lime soap dispersing agents (LSDA) were studied. The LSDAs were an anionic surfactant (sulfated tallow alkanolamides) and amphoteric surfactants of the sulfobetaine and betaine types. Builders were trisodium nitrilotriacetate (NTA), sodium tripolyphosphate (STPP), trisodium 2-oxa-1,1,3-propanetricarboxylate (OPT), sodium silicate, and a commercial synthetic sodium zeolite. The amphoteric LSDAs were vastly superior to anionics. They offered greater flexibility in choice of builder, amounts of LSDA and inorganic filler in detergent formulations, and performed as well as or better than commercial phosphate detergents. Amphoteric blends in which the only builder was a sodium silicate were superior to commercial phosphate detergents. A reduction in the amount of amphoteric LSDA from 10–15% to 4–5% was possible when either NTA, OPT, or STPP was used as a builder. While incorporation of large amounts of sodium sulfate into these formulations reduced detergency, performance was still close to that of commercial phosphate detergents. NTA or OPT was a more effective builder for anionic LSDA formulations than for amphoteric formulations. Anionic LSDA formulations built with these materials performed almost as well as amphoteric LSDA formulations. Generally, the amphoteric formulations were more tolerant of inert fillers and very high concentrations of hardness ions than were the anionics. The synthetic sodium zeolite was not an effective builder in soap-LSDA type detergents.

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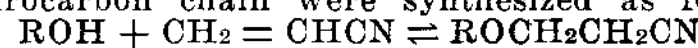
PROPERTIES OF AQUEOUS SOLUTIONS OF ALKOXYPROPYLAMINE SURFACTANTS. YOSHIO NEMOTO and HIROYUKI FUNAHASHI, Nagoya Municipal Industrial Research Institute, 3–24 Rokuban-cho, Atsuta-ku, Nagoya, Japan.

Alkoxypropylamines (II) having a homologous series of alkoxy hydrocarbon chain were synthesized as follows:



$$ROH + CH_2 = CHCN \rightleftarrows \underset{(I)}{ROCH_2CH_2CN}$$

$$(I) + 2H_2 \rightarrow \underset{(II)}{ROCH_2CH_2CH_2NH_2}$$

II is characterized by the existence of one oxygen atom in the hydrocarbon chain. Nonionics (III) and cationics (IV) derived from II were more hydrophilic than those from straight chain alkylamine; the hydrocarbon chain having one oxygen atom corresponded to a hydrocarbon chain containing approximately 3 ~ 4 fewer carbon atoms. This fact was confirmed by the measurement of surface tension, wetting, foam height, solubilization, etc. The hydrophilic nature of these surfactants might be important especially in special industrial applications. For example IV (e.g., methoxy dimethyl benzyl ammonium chloride) was found to be an excellent migrating agent for the dyeing of acrylic fiber.

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ULTIMATE BIODEGRADABILITY OF ALCOHOL ETHOXYLATES: SURFACTANT CONCENTRATION AND POLYOXYETHYLENE CHAIN LENGTH EFFECTS. L. KRAVETZ, K.F. GUIN, W.T. SHEBS, and H. CHUNG, Shell Development Company, PO Box 1380, Houston, TX 77001.

An ultimate biodegradability shake flask study of a detergent range alcohol ethoxylate has been made at realistic levels (2 mg/l) found in municipal waste treatment and at higher loadings (15 and 1000 mg/l) such as might be found in a spill situation. Radiolabeled alcohol ethoxylates were used to facilitate following their fate to biodegradation products. Unlabeled linear alkylbenzene sulfonates (LAS) and ^{14}C-labeled glucose were also included for comparison. Alcohol ethoxylates were found to biodegrade rapidly and extensively to CO_2 and water at 2, 15, and 1000 mg/l levels at rates which were only slightly dependent on concentration. The polyoxyethylene chains were found to degrade as extensively as the alcohol ethoxylates as a whole although slightly slower. C_{13} LAS biodegraded more slowly than the alcohol ethoxylates particularly at the higher concentrations studied where cell growth studies indicated that C_{13} LAS was bacteriostatic. Radiotracer studies in which CO_2 and O_2 uptake were monitored simultaneously provided evidence which suggests that the initial microbial attack on alcohol ethoxylates occurs near the hydrophobe-hydrophile junction.

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ACYLGLUTAMATE (AGS) AND ITS APPLICATION FOR ALLERGEN CONTROL. H. NAKAYAMA, Saiseikai Central Hospital, 1-4-17, Mita, Minatoku, Tokyo, Japan; and M. TAKEHARA, Ajinomoto Co., Inc., Yokohama, Japan.

Sodium N-acylglutamate (AGS) is a newly developed detergent derived from glutamic acid and mainly C_{16} and C_{18} fatty acids. Safety evaluation tests on animals including LD_{50}, subacute toxicity, skin irritation in different vehicles, skin sensitization, phototoxicity, photosensitization, irritation on injured skin and eye irritation tests were performed in comparison with other kinds of detergents, such as sodium lauryl sulfate (SLS), linear alkylbenzene sulfonate (LAS), soap, etc. The results showed that AGS was significantly less irritating than SLS and LAS. Similar results were obtained by closed patch tests on human skin and cytotoxicity tests using cultured human epidermal cell line established as JTC-17. In addition, AGS is free of ordinary contact allergens, and is weakly acidic (pH 5–6), similar to normal human skin; as a result, AGS seems not to help allergens penetrate into the skin even when contaminated with allergens. The properties mentioned above have made AGS suitable for the dermatological patients who had been suffering from recurrent dermatitis induced by cosmetics and toiletries. As the dermatitis was not expected to relapse as long as the patients used the detergents and cosmetics

containing no responsible contact allergens, such detergents and cosmetics were designated Allergen Control System (ACS), and they were evaluated clinically as to the effectiveness in stopping the recrudescence of dermatitis. AGS was selected as the detergent for ACS, and 75% of pigmented cosmetic dermatitis, which had been regarded as almost incurable, was proved to be curable or greatly improved by this new treatment.

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ANALYSIS FOR CARBOXYMETHYLOXYSUCCINATE (CMOS) IN ENVIRONMENTAL SAMPLES. EUGENE MONES and JOAN G. BARROWS, Lever Brothers, Edgewater, NJ.

CMOS was introduced into a package contact stabilization treatment plant in a trailer park in Lake Elmo, Minneapolis, by supplying CMOS-containing laundry product to the residents. The study was run for a period of ten weeks, from June to September. Samples were taken throughout the plant and analyzed by a gas liquid chromatographic (GLC) method (Aue et al., *J. Chromatogr.* 72:259) adapted for detection of CMOS from environmental samples in the ppb range. Sewage samples (100 ml) were acidified, filtered, and absorbed onto an ion exchange column (AG1X2 adjusted to pH 6). CMOS was eluted with 2M formic acid and evaporated to dryness with low heat. Conversion to the tributyl ester was accomplished by the addition of butanol-HCl. After removal of the butanol, the samples were diluted in chloroform and analyzed on a Hewlett-Packard 5840 gas chromatograph using 6 ft glass columns with 10% Dexsil 300 on Chromosorb WHP. The columns were temperature programmed from 200–250 C. A GLC run is completed in 16 min. Modification of the GLC method allows one to distinguish between tributyl citrate and tributyl CMOS. Influent levels of CMOS reached a maximum of 4.7 ppm, while effluent levels reached a maximum of ca. 0.4 ppm, indicating 90% or better degradation.

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ENGINEERING AND DESIGN OF OVERSEAS PLANTS. C. LOUIS KINGSBAKER, Chemical Plants Div., Dravo Corp., Pittsburg, PA.

The engineering and design of overseas plants require a detailed study of the country and its prevailing laws and methods to be used by the engineering contractor to perform the work. The background of the engineers selected for these projects must be carefully evaluated to fit the needs of the country and project. Construction of oilseed plants will also be reviewed.

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BIDDING AND EXPORTING EQUIPMENT FROM THE UNITED STATES. WILLIAM A. BARGER, French Oil Mill Machinery Co., Piqua, OH.

Abstract not available at press time.

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BUDGETING AND PURCHASING IN FOREIGN COUNTRIES. JOHN E. HEILMAN, Continental Grain Co., New York, NY.

The effect of high inflation, devaluating local currencies, government import restrictions and incentives, duties, etc., on costs and purchasing will be discussed. The "atmospherics" of purchasing will be discussed.

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MODERN OILSEED PROCESSING PLANTS OVERSEAS. WILLIAM FETZER, Oil Milling Division, Buhler Bros., Uzwil, Switzerland.

There are a variety of multiseed plants in operation besides plants built exclusively for the processing of one specific oilseed. Large plants have been built mainly for the processing of soybeans, whereas multiseed plants are still of rather small scale. The layout for a soybean plant has undergone a steady movement in order of scope with new technology and environmental requirements. Multiseed plants are located mainly overseas and are of growing importance in developing countries. The layout for the seed preparation is discussed in regard to single and multiseed plants, considering reliability and maintenance aspects.

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PHYSICAL CONSTRUCTION OVERSEAS. HAROLD J. SANDVIG, Cargill, Inc., Minneapolis, MN.

Topics discussed include: selection of contractors and suppliers; adjusting to local building codes, customs, and practices; adjusting to building materials available, maintaining quality standards; types of construction contracts most workable; and honoring contracts, honoring time table/completion dates.

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START-UPS OUTSIDE THE USA. LESLIE WATKINS, Anderson, Clayton & Co., Houston, TX.

Abstract not available at press time.

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OCCURRENCE AND STRUCTURE OF NOVEL GLYCOLIPIDS IN RICE BRAN OIL. Y. FUJINO, M. OHNISHI, and T. MIYAZAWA, Department of Agricultural Chemistry, Obihiro University, Obihiro, Hokkaido, Japan.

Systematic studies were carried out on neutral lipids, phospholipids, and glycolipids of rice bran oil. This paper reports on the occurrence and structure of some novel glycolipids. *Glycoglycerolipids*: In addition to the commonly occurring monoglycosyl-, sulfoquinovosyl-, and diglycosyldiglycerides, two new compounds were identified as 3-trigalactosyl(β1'-3)- and 3-tetragalactosyl(β1'-3)-1,2-diacyl-*sn*-glycerol. *Glycosterols*: Cellobiosyl(β1'-3)-β-sitosterol and cellotriosyl(β1'-3)-β-sitosterol were identified among the commonly occurring sterylglycosides, such as the monoglycosyl- and acylmonoglycosyl sterol. *Glycosphingolipids*: mono-, di-, tri-, and tetraglycosylceramides were detected and the unique structure established as glucosyl(β1'-1)-N-hydroxyarachidoyl- and cellobiosyl(β1'-1)-N-hydroxylignoceroyl-octadecasphinga-4-*trans*-8-*cis*-dienine. Distribution of these novel glycolipids in plant lipids and their functional significance need further study.

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AN IMPROVED METHOD FOR THE ACID VALUES OF RICE BRAN OIL. YASUHIKO TAKESHITA, Kokushikan University, Department of Engineering, 4-28-1, Setagaya, Setagaya-ku, Tokyo, 154, Japan.

The official method (JAS) for acid value (AV) specifies phenolphthalein (PP) as indicator. Other indicators such as alkaliblue-6B (AB) are permitted as alternates. In the case of rice bran oil a large difference was noted between the use of the two indicators, viz., AV of 0.20 with PP and 0.03 with AB. Wheat and corn germ oils also gave a nearly double value with PP. No differences between PP and AB were noted with refined soybean oil or rapeseed oil. The discrepancy was found to be due to the occurrence of minor phenolic compounds such as ferrulic acid triterpenoid in rice bran oil. Refining reduced the difference to 0.1. On the basis of these observations JAS has adopted AB as the indicator for rice bran oil.

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LIPID COMPOSITION OF OATS (*AVINA SATIVA* L.). MADHU R. SAHASRABUDHE, Food Research Institute, Agriculture Canada, Horticulture Building, #55, C.E. FARM, Ottawa, Ontario K1A 0C6, Canada.

Compositions of lipids extracted from a sample of Hinoat oat by seven solvent systems and that extracted with chloroform-methanol (2:1 v/v) from six selected cultivars representing high and low lipid contents are reported. Lipid components (steryl esters, triglycerides, partial glycerides, free fatty acids, glycolipids, and phospholipids) were separated by silicic acid column chromatography and thin layer chromatography and quantitated by gas liquid chromatography analysis of fatty acids and phosphorus determinations. Twelve oat cultivars were examined for the fatty acid composition of lipid extracted with n-hexane. Lipids extracted from Hinoat by different solvent systems ranged from 5.6 to 8.8%. Quantitative distribution of lipid components extracted with chloroform-methanol from six cultivars containing 4.6 to 11.6% lipid showed a significant correlation ($\gamma = 0.99$) between the total lipid and the neutral lipid content. Phospholipid content was similar in all cultivars, but glycolipids showed a twofold increase in high lipid oats. Triglycerides contained less palmitic and more oleic acid than the glycolipids or phospholipids. Nine glycolipids and eleven phospholipids have been identified, and the polar lipid composition of Hinoat oat is presented.

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EXTRACTABILITY OF WHEAT FLOUR LIPIDS BY SOLVENTS THAT VARY IN SOLUBILITY PARAMETERS. O.K. CHUNG, Y. POMERANZ, U.S. Grain Marketing Research Laboratory, USDA, 1515 College Ave., Manhattan, KS 66502; and R.M. JACOBS, Department of Biochemistry, Kansas State University.

Lipids were extracted with hexane, acetone, and 2-propanol (and their aqueous binary azeotropes) and with benzene from a flour that contained 1.2, 7.2, or 13.8% moisture. Total extracted lipids were highly correlated ($r = 0.970$) with the solubility parameter (δ) of the extractant. Extractability increased little from increase in flour moisture content. The high correlation was primarily from an increase in polar lipids from 21.9 to 91.2 mg/10 g flour. The increase in nonpolar lipids from 63.4 to 83.1 mg/10 g flour was less consistent. Among the nonpolar components, amounts of extracted free fatty acids increased with increase in flour moisture and of extracted monoglycerides increased with increase in the δ value of extractants. Among the polar components, extraction of digalactosyldiglycerides increased most significantly and consistently with increase in δ. Lysophospholipids and trigalactosyldiglycerides were unextracted by hexane or benzene but were extracted by acetone, 2-propanol, or their aqueous azeotropes.

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LIPID-PROTEIN CONCENTRATES FROM SAFFLOWER EXPELLER CAKE. C.K. LYON and G.O. KOHLER, Western Regional Research Center, USDA, Berkeley, CA 94710.

Safflower seed is usually processed by pre-pressing and hexane extraction. Lipid-protein concentrates were prepared from the pre-press expeller cake, which contains about 15% oil and 20% protein, by extraction with aqueous alkali, then co-precipitation of oil and protein in the extract with acid. In the laboratory, using hammer mills or high-shear homogenizers, concentrates were obtained containing up to 47% fat and 46% protein. In the pilot plant, the best extraction, using an attrition mill, yielded concentrates containing 42% fat and 46% protein. Fibrous residue after extraction of the expeller cake contained as low as 4% fat and 7% protein, in the laboratory, and 12% fat and 7% protein, in the pilot plant. Phenolic glucosides which contribute to bitterness and cathartic activity in safflower meal were absent in the concentrates. Loaves of bread with 10% of the wheat flour replaced by safflower lipid-protein concentrates had acceptable properties and contained up to 40% more protein than the controls.

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NUTRITIONAL EVALUATION OF RICE BRAN AND RICE BRAN FRACTIONS USING RAT AND CHICK BIOASSAYS. A.A. BETSCHART, R.M. SAUNDERS, M.R. GUMBMANN, Western Regional Research Center, USDA, Berkeley, CA 94710; and F.H. KRATZER, Department of Avian Sciences, University of California, Davis, CA.

Full-fat and defatted rice bran and various fractions were evaluated by male weanling Sprague-Dawley rats, and chicks. In 28-day studies with rats in which rice bran was fed at levels to provide 10% protein in the diet, the Protein Efficiency Ratios (PER) of unheated and heat-treated rice bran were 1.59 and 1.66, respectively. Neither PER nor pancreas weights were significantly affected by heat treatment. Fractionation of rice bran by various methods produced fractions with improved nitrogen (N) digestibility and PER. Rice bran fractions containing 16–70% protein (N × 5.95), 11–50% crude fat, and 3–5% ash were prepared by various procedures. PER of these fractions ranged from 2.2–2.5 (casein corrected to 2.5) with N digestibility ≧87% as opposed to ≅50% for rice bran. Growth feed efficiency of chicks, determined as body weight gain, was significantly improved with heat treatment of rice bran. Body weight of chicks, determined in 24-day experiments

with diets containing 60% heat-treated or autoclaved rice bran were equivalent to those of chicks consuming the control, corn-based diet.

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SULFUR AMINO ACID-OXIDIZED LIPID INTERACTIONS: A POSSIBLE CAUSE OF LYSINOALANINE FORMATION.
J.W. FINLEY, E.L. WHEELER, and H.G. WALKER, Western Regional Research Center, USDA, Berkeley, CA 94710.

Several different proteins (soy, safflower, alfalfa leaf protein, and lactalbumin) were treated with hydrogen peroxide prior to alkaline treatment in a study of the effect of oxidation on the formation of lysinoalanine (LAL). The oxidation state of cysteine appeared to be the determining factor in the production of LAL. When cysteine was oxidized to either the sulfinic acid or cysteic acid, no LAL formation was observed. LAL formation was observed, however, at intermediate oxidation states. The relationship of the oxidation state of cysteine, as induced by lipid hydroperoxides, to the tendency to form LAL in oilseed products will be discussed. Various reducing agents, such as bisulfite and mercaptoamino acids, prevented the effect of the hydrogen peroxide. The practicality of adding reducing agents will be considered.

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CONVERSION OF SOYBEAN EXTRACTION PLANT IN BOLIVIA TO PRODUCTION OF FLOURS FOR HUMAN CONSUMPTION.
G.C. MUSTAKAS, Northern Regional Research Center, USDA, 1815 N. University, Peoria, IL 61604; E.D. MILLIGAN, EMI Corp., Des Plaines, IL; J. TABORGA A., Sociedad Aceitera del Oriente; and D.A. FELLERS, Western Regional Research Laboratory, USDA, Albany, CA.

Bolivia, in cooperation with the Western Regional Research Center and the Agency for International Development (AID), is determining the feasibility of a soy-fortification program for wheat products. During the past year, Bolivian soybean production has increased to nearly 30,000 metric tons. Soybeans provide an effective means for protein enhancement of the Bolivian diet. All existing soybean extraction plants in Bolivia were constructed to produce oil and animal feed. For the soy-fortification program, one or more of these plants must be converted to have the capability of producing edible-grade soybean flour. During August 1978, two engineers representing USDA and EMI Corporation, a U.S. private engineering firm, visited the soybean processing plant Sociedad Aceitera del Oriente (SAO) at Santa Cruz to develop a plan for the plant conversion. Those items which were considered in that plan were (a) seed quality and cleaning operations, (b) bacteriological data relating to the existing plant design and equipment, (c) seed tempering and new dehulling facilities, (d) bird, insect, and rodent contamination, (e) disposition of millstock and millfeed by-products, and (f) addition of new facilities for extracted flake denaturation control and flour grinding. The information gained from this study should be useful and directly applicable to other soybean crushing plants in the world. As future shortages develop in human diets due to the scarcity and high cost of animal proteins, more plant conversions to edible soy protein products will be required.

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DEVELOPMENT OF A COMMERCIALLY PRODUCIBLE LOW CALORIE, HIGH PROTEIN HAMBURGER BUN.
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A formulation was developed that can produce a low calorie, high protein bun in a commercial bakery by conventional means. The dough contains normal ingredients plus 25% alpha cellulose (C), 3% carboxy methyl cellulose (CMC), 8% defatted soy flour (SF), 3 to 5% wheat gluten, sodium stearoyl-2-lactylate (SSL), and ethoxylated monoglycerides (all concentrations based on flour). Initial results showed that surfactants such as SSL are necessary to making an acceptable low calorie bun. SF (6 to 12%) increased bun volume and gave desired crust color to bun. C (100 and 200 mesh) gave better bun volume than 50 or 300 mesh C. Wheat gluten (up to 30%) significantly increased bun volume and toughness. Of 12 gums tested, only carrageenan, CMC, and guar gum (GG) significantly increased bun volume. CMC at 4.44% or GG at 4.18% gave greatest bun volume of all concentrations tested. A one-third replication of a 3^4 factorial with CMC and GG levels at 1.97, 3.95, or 5.92%; C at 22.37, 23.68, or 25.00%; and SF at 7.89, 10.53, or 13.16% was run. The effect of these factors at these concentrations was determined on bun volume, compressibility, proximate analyses, and crust and crumb color. Pilot tests at bakeries showed that doughs with 115–120% water were too sticky to scale properly, but with water at 105–110%, the doughs machined and scaled correctly. One pilot test during formulation development gave a bun which was liked moderately by a consumer panel (n = 150). The bun currently undergoing further pilot testing has 33 to 35% fewer calories and 30% more protein than a regular bun and will have 38 to 40% H_2O, 10 to 12% undigestible fiber, 2.0% fat, and 2.0% ash.

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SERUM LIPOPROTEIN CHANGES IN BABOONS AFTER REMOVAL OF A CHOLESTEROL SUPPLEMENTED DIET.
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Three male and three female baboons, which had been fed a standard monkey chow plus 20% lard and 0.4% cholesterol for 4 months, were transferred to a noncholesterol supplemented diet. Subsequently serum lipoproteins (LP) were measured after 0, 1, 3, 5, 7 or 0, 1, 3, 6, 8 days in the males or females, respectively. The serum LPs were separated and isolated by an isopycnic density gradient ultracentrifugal method and analyzed for total cholesterol (TC). Two major peaks, corresponding to low density (LDL) and high density lipoproteins (HDL), were observed. Serum LP-TC concentrations began to decrease even after only one day on the noncholesterol supplemented diet and showed a progressive decline with time. After one week, the LDL-TC manifested a larger decrease (74.8%) than the HDL (9.3%) compared to the pre-diet transfer values in male baboons. Similarly the LDL-TC decrease (53.5%) was greater than the HDL-TC decrease (30.9%) in female animals, although the latter change was 3 times as great as in males. There were proportional decreases in the cholesterol ester, phospholipid, protein, and apolipoprotein concentrations of both LDL and HDL in both sexes. Removal of a cholesterol containing diet caused a rapid decrease of serum lipoproteins during the first week, although that of LDL was more pronounced than HDL in baboons. Whereas the HDL decrease in males was modest (9.3%), that in female was more pronounced (30.9%).

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DIET INDUCED ALTERATIONS OF PLASMA LOW DENSITY LIPOPROTEIN IN NONHUMAN PRIMATES.
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Experiments have been carried out to evaluate the influence of dietary cholesterol on plasma low density lipoprotein (LDL) of several species of nonhuman primates, including *Macaca fascicularis, Macaca nemestrina, Macaca mulatta,* and *Cercopithecus aethiops.* LDL size (molecular weight), LDL molar concentration (particle number), and LDL mass concentration were all increased in response to dietary cholesterol, but significant individual animal variation was found in the type and extent of these modifications, both within and among species. In all species, LDL total mass and molar concentrations were increased, although the extent of increase was not the same for each species. In some species, primarily those of the genus Macaca, the increase in LDL total mass was primarily due to an increase in LDL size, whereas in other animals the primary change was due to an increase in number of LDL particles. The factors which control this differential response are unknown but may be related to cholesterol ester metabolism, as there was a highly significant proportionality between the cholesterol ester fatty acid (CEFA) pattern and LDL molecular weight, i.e., the more saturated and monounsaturated the CEFA, the higher the molecular weight. There was also a significant inverse relationship between plasma LDL molecular weight and HDL concentration, and the CEFA pattern of HDL paralleled that of LDL. It appears that factors involved in control of cholesteryl ester transport determine, at least in part, the extent of LDL molecular weight enlargement and, simultaneously, the HDL concentration. The importance of these observations relates to the fact that the lipoprotein measurements were shown to be highly significant predictors of the extent of coronary artery atherosclerosis in these animals.

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LIPOPROTEINS OF FETAL AND NEWBORN CALVES.
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Fetal calf serum, and to a lesser degree, newborn calf serum is frequently used to supplement tissue culture media; however, the lipoprotein composition of both sera are ill defined. We have studied the physical and chemical properties of the various lipoproteins of both fetal and newborn calf. Analytic ultracentrifugation indicated that there was no significant concentration of very low density lipoproteins in any of the bovine sera studied. Fetal lipoprotein concentration was extremely low, 77.5 ± 18.8 mg/ml with high density lipoprotein (HDL) and low density lipoprotein (LDL) present in almost equivalent amounts (ratio of HDL/LDL = 0.80). Polyacrylamide gel electrophoresis of apolipoproteins revealed that fetal calf LDL contained only apoB protein while HDL contained two bands, one similar to apoA-I (80%) and another similar to apoC-II (20%). Newborn calf serum was substantially different from fetal calf serum in that there was a fivefold increase in HDL level with little or no change in LDL. The HDL/LDL ratio in newborn calf was 5.45 which is similar to that of mature steer (4.97). The lipid moiety of newborn calf HDL contained 18% less phospholipid and 38% more cholesteryl ester than fetal HDL. The apoproteins of newborn calf serum differed from fetal calf in that HDL apoC-II decreased while there was a concomitant appearance of apoC-III proteins. Electron microscopy revealed that fetal calf LDL and HDL were round particles 24.5 ± 2.5 and 9.9 ± 1.1 nm diameter, respectively, while newborn calf LDL and HDL were 24.7 ± 2.9 and 13.4 ± 1.4 nm, respectively. Prenatally it appears that LDL and HDL are synthesized in low, approximately equivalent, quantities. Immediately after birth there is a preferential increase in HDL concentration which may relate to dietary or other metabolic changes in the neonate.

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CHOLESTEROL METABOLISM IN HUMAN MONOCYTE-MACROPHAGES.
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Of the circulating blood cells only one is consistently found in the atherosclerotic reaction—the monocyte-macrophage. In the atherosclerotic reaction and in tendon xanthomas, these cells are filled with lipid, particularly cholesteryl esters. We have developed techniques for the rapid isolation of human monocytes and have demonstrated their unique sterol synthesizing abilities. In normal human monocytes cholesteryl esters account for less than 3% of the total cholesterol. Incubating normal monocytes for 24 hr with 100 μM mevalonate did not increase the cholesteryl ester content of the cells. When normal monocytes were converted into macrophages by cultivation in 10% autologous fasting serum on a plastic surface, the cholesteryl ester content did not increase over the period of observation (14 days). Heat inactivation of lecithin:cholesterol acyl transferase activity had no effect on the cholesteryl ester content of the cells. These results will be compared to those obtained with 10% autologous fasting serum from heterozygote familial hypercholesterolemics and normal autologous postprandial serum.

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HORMONAL REGULATION OF THE LOW DENSITY LIPOPROTEIN RECEPTOR.
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The increased cholesterol required by cells during their proliferation may be derived either from enhanced cholesterol synthesis or by stimulation of receptor-mediated low density lipoprotein (LDL) transport. Since stimulators of cellular proliferation, such as insulin and platelet factor, previously have been shown to enhance cholesterol synthesis, the effect of these factors on LDL receptor activity was tested using cultured human skin fibroblasts. When deprived of cholesterol by exposure to lipoprotein-deficient serum, insulin resulted in enhanced binding, internalization, and degradation of ^{125}I-LDL. Maximal transport capacity (V_{max}) of LDL rather than the affinity of LDL for its receptor (K_m) was stimulated by insulin; these changes were blocked by cycloheximide. Cholesterol synthesis was stimulated by insulin under identical conditions. LDL receptor activity was stimulated by insulin to the same extent in the presence or absence of platelet factor, despite the striking difference in the proliferative response to insulin under these conditions. This suggests that the insulin-induced changes in LDL receptor activity occur independently of cellular proliferation. Platelet factor in the medium resulted in stimulation of LDL receptor activity when compared with matched serum devoid of platelet factor. The effect of insulin and platelet factor on the LDL receptor was synergistic. L-triiodothyronine (T3) also stimulated LDL receptor activity by stimulating V_{max} of LDL transport without influencing K_m. T3 and insulin also synergistically stimulated LDL receptor activity. Thus, certain hormonal factors may influence the supply of cellular cholesterol by stimulating LDL receptor-mediated transport, thereby providing the additional cholesterol required for cellular proliferation.

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SECRETION OF VERY LOW DENSITY LIPOPROTEIN AND THE DEGRADATION OF LOW DENSITY AND HIGH DENSITY LIPOPROTEINS BY CULTURED RAT HEPATOCYTES. DAVID B. WEINSTEIN, SHARON PANGBURN, ROGER A. DAVIS, and DANIEL STEINBERG, Division of Metabolic Disease, Department of Medicine, University of California, San Diego, CA 92093.

Adult rat hepatocytes, isolated by collagenase digestion and maintained for several days in culture in arginine-deficient medium, retain liver-specific functions such as the synthesis and secretion of very low density lipoprotein (VLDL), high density lipoprotein (HDL), albumin, and bile acids. Triglyceride (TG) synthesis and VLDL secretion were measured by following ^{3}H-glycerol incorporation into cell and medium components. Greater than 95% of the medium ^{3}H-TG was in a VLDL fraction which contained apoB, apoE, and very little apoC. In the absence of exogenous lipids in the medium the lipid composition of the particles was similar to that of VLDL from perfused livers and the rate of secretion was about one-third that of perfused livers. Cells from sucrose-fed rats and cells exposed to fatty acid-albumin mixtures secreted ^{3}H-TG at rates 4–5 times that of cells from normal rats. Orotic acid inhibited VLDL secretion, but not the synthesis of triglyceride, and this effect was reversed by adenine. Macromolecules (dextrans, fatty acid-free albumin, γ-globulin) added to the medium reduced the secretion of all VLDL components while albumin secretion was unimpaired. Inhibition of VLDL secretion was dependent on the concentration but not the molecular weight of the macromolecule and was readily reversed by removal of the macromolecule. Studies on uptake and degradation of ^{125}I-labeled low density lipoprotein (LDL) and HDL at low lipoprotein concentration showed that total uptake (internalized plus degraded) was 10 times (LDL) and 43 times (HDL) the fluid endocytosis rate. At equimolar concentrations HDL degradation was 4 times that of LDL. Hepatocyte cultures which retain functional control in a defined environment provide an excellent model for the study of liver metabolism.

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ABNORMAL HMG-CoA REDUCTASE SUPPRESSION BY HYPERTRIGLYCERIDEMIC VERY LOW DENSITY LIPOPROTEIN SUBCLASSES. S.H. GIANTURCO, C.J. PACKARD, J.S. SHEPHERD, S.G. ESKIN, L.C. SMITH, and A.M. GOTTO, JR., The Methodist Hospital and Baylor College of Medicine, 6516 Bertner Blvd., Mail Station A-601, Houston, TX 77030.

To test if the suppression observed with the zonally isolated hypertriglyceridemic (HTG) VLDL were due to a subpopulation of smaller suppressive particles, more homogeneous subclasses of VLDL—$VLDL_1$ (S_f 100–400), $VLDL_2$ (S_f 60–100), and $VLDL_3$ (S_f 20–60)—were obtained by flotation through a discontinuous salt gradient and tested for suppression. The $VLDL_1$ subclass contained only large particles with a mean diameter of 47.5 nm ± 12.4; $VLDL_2$ particles averaged 36.4 nm ± 7.5; and the S_f 20–60 particles were 30.5 nm ± 6.8, for both normal and hypertriglyceridemic VLDL. $VLDL_1$ and $VLDL_2$ from normolipemic subjects failed to suppress HMG-CoA reductase activity in normal fibroblasts. $VLDL_3$ (S_f 20–60) from normal plasma suppressed HMG-CoA reductase, but only one-third as effectively as LDL. Smaller size does indeed appear to be an important factor in suppression. $VLDL_1$, $VLDL_2$, and $VLDL_3$ from patients with hypertriglyceridemia were highly effective in suppression. Suppressive $VLDL_1$ and $VLDL_2$ subclasses with potencies comparable to LDL were from HTG patients with hyperlipoproteinemia Types IIb, III, IV, and V. Variability was noted in Type IV hyperlipoproteinemia preparations. VLDL subclasses from plasma of two patients with a Type IV pattern with moderately elevated plasma triglycerides (150–300 mg/dl) produced effects similar to normal VLDL, whereas all VLDL subclasses from two other Type IV patients with plasma triglycerides in this range were moderately suppressive. All three VLDL subclasses obtained from two additional Type IV patients with plasma triglycerides greater than 300 mg/dl were as suppressive as LDL. The VLDL abnormality appears to be associated only with hypertriglyceridemia—and not hypercholesterolemia—since zonally isolated VLDL from a homozygous patient with a Type IIa pattern failed to suppress. In contrast, each of the VLDL subclasses from a Type IIb patient suppressed. As with the zonally isolated VLDL, there were no consistent compositional differences among the normal and the abnormal VLDL which could account for differences in suppression. Small particle diameter alone cannot account for suppression, since HTG $VLDL_1$ and $VLDL_2$—which contain no small particles—are effective in suppression. To show that the protein portion of the HTG VLDL is involved in suppression, HTG $VLDL_1$ were treated with 0.1 M 1,2-cyclohexanedione to block arginyl residues. This chemical modification abolished the ability of the particles to suppress at levels where unmodified VLDL suppressed maximally; removal of the cyclohexanedione with hydroxylamine restored the suppressive activity of the HTG VLDL. These experiments provide evidence that suppression by HTG VLDL is a consequence of interaction of the protein portion of the VLDL with the specific LDL cell surface receptor. In cultured vascular endothelial cells, the $VLDL_1$ from a hypertriglyceridemic patient suppressed HMG-CoA reductase activity; normal $VLDL_1$ did not. Hypotheses based on these data will be presented.

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DETERMINATION OF MONO- AND DISULFONATES IN ALPHA OLEFIN SULFONATES BY CAPILLARY TUBE ISOTACHOPHORESIS. TOYOKI SUGIYAMA, KENZO SAKURAI and TOSHIO NAGAI, Central Research Laboratories, Lion Fat and Oil Co., Ltd., 7-13-12, Hirai, Edogawa-ku, Tokyo, 132, Japan.

In the production of detergent grade alpha olefin sulfonates (AOS) a rapid and relatively simple method for analysis is desirable. High performance liquid chromatography and thin layer chromatography methods have been reported for the analysis of mono- and disulfonates in AOS. However, the determination of mono- and disulfonates is still laborious and time consuming. This paper describes isotachophoresis a technique for the simultaneous separation and determination of mono-, and disulfonates, and inorganic sulfates. The system uses 0.005M HCl and 0.01M histidine in water/acetone solvent as leading electrolyte and 0.01M caproic acid aqueous solution as terminator. Each component is separated and determined using a calibration curve. Analysis time is about 30 min for one sample.

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DETERMINATION OF OIL CONTENT OF SUNFLOWERSEED BY NUCLEAR MAGNETIC RESONANCE. J.A. ROBERTSON and W.H. MORRISON, III, Russell Research Center, Field Crops Laboratory, USDA, PO Box 5677, Athens, GA 30604.

The wide-line nuclear magnetic resonance (NMR) analyzer is routinely used to determine the oil content of sunflowerseed by plant breeders, and the technique is now under consideration as the official oil method for the domestic trading of sunflowerseed. Since NMR analysis for oil in soybeans has been found to be more reproducible and statistically more reliable than the AOCS extraction method, the effect of several variables such as optimum r.f. level, sample size, and temperature on NMR analysis of sunflowerseed and oil was evaluated. As the temperature of the sample was increased 1 C, the instrument response decreased by about 0.4%. The effect of iodine value of different edible oils on instrument response also was studied. Increasing iodine value by 10 units produced a decrease in response by about 1%. Based on these results, a study was conducted to determine the effect of unsaturation of sunflowerseed oil on the instrument response. The significance of these results on the use of NMR as a tentative official AOCS method will be discussed.

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STRUCTURE DETERMINATION OF NONIONIC SURFACTANTS USING ^{13}C NUCLEAR MAGNETIC RESONANCE. THOMAS R. OAKES, Economics Laboratory, Inc., St. Paul, MN.

^{13}C nuclear magnetic resonance (NMR) has proven to be a valuable tool in determining the structure of a variety of nonionic surfactants. Block surfactants of ethylene oxide (EO) and propylene oxide (PO) have been studied. EO vs. PO termination is readily determined. The presence of heteric regions can easily be detected. The structure of alcohol alkoxylates can also be determined. Again, ^{13}C NMR can readily distinguish between EO or PO terminated materials or determine if the EO and PO were added randomly. The nature of the R group may also be inferred. The combination of ^{13}C and ^{1}H NMR will be demonstrated as a powerful tool in determining surfactant structure.

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SEPARATION AND DETERMINATION OF ISOMERIC OCTADECENOATES BY GAS LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY. KUNIHIKO SAITO and KIYOSHI SATOUCHI, Department of Med. Chem., Kansai Medical School, Moriguchi, Osaka, 570, Japan.

The separation and relative quantification of a mixture of oleic (*cis*-9-octadecenoic) and *cis*-vaccenic (*cis*-11-octadecenoic) acids were carried out in a gas liquid chromatograph-mass spectrometer system by selected ion retrieval. The derivatives were pyrrolidides and the liquid phase was cyanopropylsiloxane (Silar 10C). This method was applied to analysis of these two fatty acids from choline and ethanolamine glycerophospholipids prepared from various normal rat livers (adult, fetus, and regenerating livers) and hepatomas (Morris. 7316 A and Yoshida ascites, AH 7974). The results obtained were similar to those reported by Wood et al. This method was found to be simpler than other chemical methods such as ozonolysis. which requires isolation of the $C_{18:1}$ fraction. It was possible to analyze ca. 10^{-1} g.

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QUANTITATIVE MICROANALYSIS OF MIXTURES OF POSITIONAL ISOMERS IN ETHYLENIC FATTY ACID METHYL ESTERS. DONALD T. DOWNING, Dermatology Department, College of Medicine, University of Iowa, Iowa City, IA 52242.

A previous report demonstrated that rapid and accurate analysis of double bond positional isomers in fatty acid methyl esters could be achieved by a modification of the periodate/permanganate oxidation technique of von Rudloff followed by gas chromatographic analysis of the oxidation products. The monomethyl esters of the dicarboxylic fragments, as well as the volatile monocarboxylic portions, were readily extractable from the acidified oxidation mixture with hexane. Loss of the volatile acids was avoided by subsequent handling of the products as their tetramethylammonium salts, which ultimately were

pyrolyzed to methyl esters within the gas chromatograph. The method, originally applicable to 0.1 to 1.0 mg quantities of methyl esters, has now been adapted to the quantitative analysis of mixtures of positional isomers totaling less than 10 μg, using the same stable reagents and inexpensive solids injector for the gas chromatograph. To maintain efficient conversion of the dicarboxylic fragments to dimethyl esters it was necessary to reduce the pH of the solution of tetramethylammonium salts by addition of acetic acid and then to concentrate the solution. It was then possible to quantitate as little as 1 μg of a positional isomer.

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AUTOMATIC RETRIEVAL OF COSMETIC MATERIALS BY METHYLENE UNIT VALUES. RYUJIRO NAMBA, AKIRA SHIBAMOTO, SACHI HASHIMOTO, and ISAO MATSUMOTO, Shiseido Laboratories, 1050, Nippa-cho, Kohoku-ku, Yokohama-shi, 223, Japan.

In order to develop an on-line program and speed up routine work, a method of rapid identification of cosmetic materials such as fatty oils and surfactants by gas chromatography (GC) was investigated using methylene unit (MU) values. Temperature programmed GC was applied to analyze compounds having a wide range of carbon numbers. When peaks of a sample and the standard n-alkanes overlapped, the calculation of MU values was not possible. To avoid this, the separation from two runs was determined. These repetitions were carried out on every sample using an automatic sampler. The measurement of retention times and the calculation of MU values from retention times were carried out by a mini-computer. Good reproducibility of MU values was obtained by the above method. About 200 MU values of the standard compounds for the analyses of cosmetic materials were filed as a data base to information retrieval, and the program for automatic identification was developed. On the other hand, the delta MU values, the difference in values between two stationary polar and non-polar phases, were said to yield better precision for sample identification. The relationships among different kinds of functional groups such as alcohols, acids, esters, etc., were also studied.

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SELECTIVE HYDROGENATION OF SOYBEAN OIL. X. ULTRA HIGH PRESSURE AND COPPER-CHROMITE CATALYST. S. KORITALA, J.P. FRIEDRICH, and T.L. MOUNTS, Northern Regional Research Center, USDA, 1815 N. University, Peoria, IL 61604.

Soybean oil was hydrogenated at 170 C up to 30,000 psig hydrogen pressure. A selectivity ratio (K_{Le}/K_{Lo}) of 5.5 was achieved at this high pressure. This value is somewhat lower than the selectivity at 1000 psig, but much higher than that obtained with nickel catalysts. At 15,000 psig, part of the linoleate in soybean oil was hydrogenated directly without prior conjugation, whereas at low pressures conjugation precedes hydrogenation. This difference in mechanism might explain the lower selectivities obtained at high pressures. More *trans* isomers are formed at 100 psig than at 50 psig. *trans* Isomer content decreased when the pressure was increased to 30,000 psig. Geometric isomerization of linoleate was greater at 1000 psig and 15,000 psig than at 50 psig. No significant differences were found in the double bond distribution of *trans* monoenes, even though the amount of *trans* monoene formed decreased as pressure was increased to 30,000 psig.

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SOME ASPECTS ON ADSORBENT TREATMENT AND HYDROGENATION OF FISH OIL. ERNST H. GOEBEL, QUIMICA SUMEX, S.A. de C.V., Av. Insurgentes sur 1700–4° Piso, Mexico 20, D.F. Mexico; MIGUEL ROMERO, and GERMAN PAUL, Investigacion y Desarrollo Industrial, S.A., Mexico.

Since crude fish oil contains a large variety of undesirable toxic metal traces and catalyst poisoning impurities, adsorbent treatments before hydrogenation are being discussed. The effects of such treatments on hydrogenation efficiency and catalyst performance are explored for several types of hydrogenation, including selective, nonselective, and touch hydrogenation. Results of introductory tests on crude and refined oils with respect to changes of metal traces measured by atomic absorption spectrophotometry will be presented. Main emphasis is placed upon sulfur removal from the fish oil to be hydrogenated since sulfur is a catalyst poison most frequently found in measurable amounts in fish oil, and the one with deleterious effects on catalyst. Several adsorbents are evaluated.

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SELECTIVE HYDROGENATION OF SOYBEAN OIL. XI. CONJUGATION AND CATALYTIC ACTIVITY WITH COPPER-CHROMITE AT PRACTICAL PRESSURES. S. KORITALA, J.P. FRIEDRICH, and T.L. MOUNTS, Northern Regional Research Center, USDA, 1815 N. University, Peoria, IL 61604.

Soybean oil was hydrogenated with copper-chromite catalyst at 170 C, and pressure was varied between 50 and 500 psig. The linolenate selectivity remained essentially unchanged over the pressure range. Geometric isomerization increased as pressure increased. Conjugated diene isomers were found in the products up to 200 psig. Above this pressure, conjugated diene was not measurable. The rate of hydrogenation was directly proportional to pressure over the pressure range studied.

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SULFUR-PROMOTED NICKEL HYDROGENATION CATALYSTS. D.A. SCARPIELLO, SCM Corporation, Dwight P. Joyce Research Center, PO Box 8827, Strongsville, OH 44136.

The hydrogenation of triglycerides is normally accompanied by a parallel reaction—isomerization, both geometrical and positional. The relative rates of hydrogenation and isomerization can be controlled by varying the concentration of hydrogen on the catalyst surface. Experimentally this is accomplished by adjusting temperature, agitation rate, catalyst concentration, and hydrogen pressure. However, not much attention has been directed toward the type of catalyst used. Recently, Ni catalysts have become available which, when promoted by sulfur, result in enhanced rates of *trans*-isomerization during hydrogenation. Concomitantly, a more selective hydrogenation occurs than with nonsulfided catalysts, with respect to decreased formation of saturates. The activity and selectivity of several sulfur-promoted nickel catalysts are compared. The effect of various sulfur/nickel ratios on hydrogenation activity and *trans*-isomers formation is also discussed. The kinetics of cottonseed hydrogenation for two sulfided nickel catalysts are compared. For soybean, hydrogenation rates were determined for fresh and used sulfided nickel catalysts.

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SELECTIVE HYDROGENATION WITH COPPER CATALYSTS. V. KINETICS AND MECHANISM AT HIGH PRESSURE. S. KORITALA, Northern Regional Research Center, USDA, 1815 N. University, Peoria, IL 61604.

Previous work at this center indicated that, at high pressures, *trans* isomer formation was increased, whereas conjugated diene was eliminated as a measurable product. The mechanism of hydrogenation at 1000 psi was therefore investigated with pure methyl esters as well as their mixtures. A comparison of double bond distribution in *trans* monoenes obtained during hydrogenation of linoleate and alkali-conjugated linoleate showed that 95% of the double bonds in linoleate conjugate prior to hydrogenation. The mode of hydrogen addition to conjugated triene and diene at high pressure is similar to that at low pressure; but positional and geometric isomerizations of unreacted conjugated triene and diene were considerably less at high pressure. Geometric isomerization of methyl linoleate and linolenate was considerable at high pressure, whereas it was negligible at low pressure. Competitive reaction rate ratios were determined using mixtures of fatty esters. Conjugated esters were much more reactive than polyunsaturated esters with methylene interrupted double bonds. For example, conjugated dienes are 12 times more reactive than the triene methyl linolenate and 31 times more reactive than methyl linoleate.

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ARSENOLIPIDS IN MARINE DIATOMS. ROBERT V. COONEY, University of California, San Diego, and A.A. BENSON, Marine Biology Research Division, Scripps Institution of Oceanography, La Jolla, CA 92093.

Our laboratory has been concerned with the biological transformation of arsenic by marine diatoms. In addition to various methylated arsenicals, we have identified the major arsenic-containing lipid as O-phosphatidyltrimethylarsoniumlactate. This novel phospholipid is present in the majority of organisms surveyed to date. In addition, thin layer chromatography revealed several lipid-soluble compounds whose chemical characteristics have been investigated. The arsenolipid differs from other known phospholipids with respect to the ease of hydrolysis in acid of the phosphodiester bond. This lability, due to the interaction between the phosphate and carboxyl groups, may serve as a model of the mechanism by which various phosphodiesterases work. Preliminary evidence indicates that these arsenolipids are passed through the food chain, and apparently some organisms are able to convert the lipids to arsenobetaine. Arsenic-containing compounds accumulate in fish oils as well. The use of ^{74}As labeled arsenolipids from diatoms has allowed us to follow the path of arsenic through the food chain. Also, we have observed that the occurrence of arsenolipids may be more significant in tropical regions where nutrients are more limiting to growth than in nutrient-rich areas.

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ANALYSIS AND QUANTITATION OF MOLECULAR SPECIES OF PHOSPHOLIPIDS FROM SEA SQUIRTS INCLUDING ETHER ANALOGUES. AKIRA HAYASHI, MASANORI MORITA, and FUMITO MATSUURA, Department of Chemistry, Faculty of Science and Technology, Kinki University, Kowakae 3-4-1, Higashiosaka, 577, Japan.

Complex lipids (3.7 g) obtained from visceral tissues (560 g) of *Pyura michaelseni* consisted mainly of choline (25%) and ethanolamine (30%) glycerophospholipids, and of sphingomyelin (15%). Diglycerides were prepared from both glycerophospholipids by treatment with phospholipase C (*Bacillus cereus*) and separated by chromatography on Lipidex-5000 into three classes: 1-alkyl-2-acyl-, 1-alk-1'-enyl-2-acyl-, and 1,2-diacylglycerols. Molecular species of each diglyceride class were analyzed by gas chromatography-mass spectrometry (GC-MS) as its trimethylsilyl (TMS) ether or acetyl derivatives. For more detailed analyses, diglyceride monoacetates of each class were fractionated according to degree of unsaturation by argentation thin layer chromatography (TLC) and analyzed by GC-MS. Alkenyl-acyl (64%) and alkyl-acyl (27%) types were major classes in ethanolamine phospholipids, and alkyl-acyl (51%) and diacyl (41%) types in choline lipids. Analyses of molecular species of each class revealed that there were 10 to 20 species with total carbon numbers ranging from 28 to 39, and with from 0 to 6 ethylenic bonds. It was noticed that saturated chains (16:0 and 18:0) are linked to position 1 of glycerol in both the ether or ester bonds, in contrast to the high content of polyunsaturated acids such as 20:5 and 22:6 in position 2. Molecular species of sphingomyelin were also analyzed for the ceramide moiety as the TMS ether derivative by GC-MS. Discussion will emphasize the connection between the structure and function of membrane in marine animals.

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CHARACTERIZATION OF LONG CHAIN BASES FROM STARFISH. TARO HORI, MUTSUMI SUGITA, CHIAKI NISHIMORI, and NAOMI NISHIO, Department of Chemistry, Faculty of Liberal Arts and Education, Shiga University, Otsu, Shiga Prefecture, 520, Japan.

Long chain bases were liberated from a crude mixture of sphingolipids from whole tissue of the starfish, *Asteria pectinifera*. The bases were converted into N-acetyl-O-trimethylsilyl derivatives. The derivatives were analyzed by gas chromatography-mass spectrometry (GC-MS). They were all phytosphingosine analogues, but many peaks in gas liquid chromatography (GLC) did not correspond to any of the usual long chain bases, indicating paraffin chain branching. For conclusive identification of the branched bases, the long chain fraction was oxidized with lead tetraacetate, followed by silver oxide, and the fatty acids produced were analyzed as their

methyl esters by GLC. Through these analyses eight long chain bases: *iso*-hexadeca (6%), *n*-hexadeca- (15%), *iso*-heptadeca- (34%), *anteiso*-heptadeca- (13%), *n*-heptadeca (8%), *iso*-octadeca- (14%), *anteiso*-octadeca- (3%), and *n*-octadecaphyto-sphingosine (7%), were determined.

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QUANTITATION OF STEROIDS IN MOLLUSCS. DENNIS T. GORDON, Oregon State University Seafoods Laboratory, 250–36th St., Astoria, OR 97103.

The quantitative steroid composition of molluscs and, in particular, oysters *(Crassostrea gigas)* can be highly variable depending upon the method of analysis. Use of the colorimetric Liebermann-Burchard (LB) reaction has indicated a range of 148–241 (mean 196) mg steroid per 100 g oyster; the range dependent on individual oyster size and time of harvest. Digitonin precipitation of the nonsaponified total lipid extract followed by steroid determination using the LB reaction gives values that are ca. 25% lower in oysters. Total steroid levels determined in other molluscs after saponification followed with or without digitonin precipitation are not significantly different. Over-estimation of the steroid levels in molluscs employing the LB reaction results from the presence of $\Delta 7$ steroids which have a higher extinction molar coefficient ($\epsilon = 4290$; 14 min) compared to $\Delta 5$ steroids ($\epsilon = 1860$; 14 min). Simultaneous determination of the $\Delta 7$ and $\Delta 5$ steroid classes (modified LB) in oysters indicates mean levels of 30 mg % and 130 mg %, respectively. Quantitative results obtained by gas liquid chromatography (GLC) ranged from 70–110% (mean 87%) of levels determined via the modified LB procedure. Variations in steroid quantitation of molluscs due to individual steroids via both colorimetric and GLC procedures will be discussed. The steroid composition of molluscs common to the Pacific Northwest will be presented.

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LIPIDS OF DEEP BENTHIC ANIMALS. JUDD C. NEVENZEL, Scripps Institution of Oceanography, La Jolla, CA 92093.

Detailed mechanisms for the transfer of the n-3 family of essential fatty acids and carotenoids from the zone of primary production at the surface to the deep benthic environment at the water/sediments interface are not known. Indeed, photosynthetic plants in the photic zone may not be the only source of 18:3(n-3) in the benthos. In preliminary work the lipids of organisms collected in the deep trenches (down to 10,900 m) have been analyzed for polyunsaturated C_{18}-C_{22} acids. The amounts of 20:5(n-3) and 22:6(n-3) in *Hirondellea gigas*, a hyperid amphipod from the Philippine Trench, were low (1.2% and 3.2%, respectively); the major acids were 16:0 (10.8%), 16:1 (14.0%), and 18:1 (about 60%). The lipid content was high (21% wet wt). As usual for all benthic animals analyzed to date, triacylglycerols were the major neutral lipids; no wax esters were detected.

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DISTRIBUTION AND BIOSYNTHESIS OF WAX ESTERS IN DEEP-SEA ANIMALS. MITSU KAYAMA, Department of Fisheries, Faculty of Fisheries and Animal Husbandry, Hiroshima University, 2–17 Midori-machi, Fukuyama, Hiroshima, 720, Japan.

It has been found that marine animals as diverse as the sperm whale and the castor oil fish contain wax esters as their major lipid type. Interest in deep-sea organisms and improved survey techniques have extended our knowledge of the occurrence of wax esters in marine animals. Fifteen species of micronektonic fish belonging to four families, Gonostomatidae, Sternoptychidae, Chauliodontidae, and Myctophidae, were collected at night between the surface and depths down to 2000 m in Sagami and Suruga Bays. Five species of micronektonic shrimp belonging to the order Decapoda were also caught in the same bays. As representative marine zooplankton, two species of the order Amphipoda, three species of the order Euphausiacea, and five species of the order Calanoida were collected. The results of lipid class analyses of these marine animals indicated that one of the most characteristic constituents was wax esters. A reciprocal relationship has been observed between the triglycerides and wax esters. The former is the main lipid class in surface-living animals, whereas the latter becomes the major class in the meso- and bathypelagic animals. In order to study the biosynthesis of wax esters by marine animals, in vivo and in vitro experiments were carried out. Enzymatic studies were carried out using carp hepatopancreas and copepod preparations. The results indicate that the enzyme for hydrolysis of wax esters is apparently different from the one for synthesis. Moreover, the effects of plus and minus loads and alloxan-induced diabetes on the wax esters of carp were tested. The relationship of wax esters and triglycerides seems to be regulated by the hydrostatic conditions and is mediated through the metabolism of glucose for synthesis of alcohol moiety of fatty acids.

SESSION AA: POSTER SESSION—I

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SIMPLIFIED METHOD FOR THE DETERMINATION OF TOCOPHEROLS AND STEROLS BY GLASS CAPILLARY GAS CHROMATOGRAPHY. HAL T. SLOVER, RAYMOND H. THOMPSON, JR., and GEORGE V. MEROLA, Nutrition Institute, USDA, Building 264, BARC-East, Beltsville, MD 20705.

Methods are needed for the specific, rapid determination of tocopherols and sterols in foods. This paper describes in detail a procedure for determining individual members of both types of compounds in oils, fats, and lipid extracts of foods by quantitative glass capillary gas chromatography using the total unsaponifiable fraction. Samples containing ca. 100 mg of lipid are saponified in a capped tube with aqueous KOH by heating for 8 min at 80 C, the unsaponifiable fraction extracted with cyclohexane, freed of solvent, derivatized to form the trimethylsilyl ethers of both tocopherols and sterols, and chromatographed on a 50 m × 0.25 mm glass capillary column coated with Dexsil 400. Most of the individual tocopherols and commonly occurring sterols are well separated from one another, although interfering peaks have been observed with some samples. A significant improvement in tocopherol and sterol methodology has been achieved by simplification of sample preparation, the elimination of the need for preparative TLC, and the use of highly efficient glass capillary columns. These columns were obtained commercially and have proven to be long lasting, operating over several months for several hundred samples without deterioration in performance. Information on the recovery, precision, and practical application of the method will be presented.

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USE OF RESPONSE SURFACE METHODOLOGY TO STUDY GAS CHROMATOGRAPHIC VARIABLES RELATING TO INSTRUMENT PERFORMANCE AND VERSATILITY. THOMAS R. FERGUSON and LLOYD M. SMITH, Department of Food Science and Technology, 3450 Chemistry Annex, University of California, Davis, CA 95616.

Response surface methodology was used to develop an empirical model describing a gas chromatographic system. Responses measured were analysis time, resolution of methyl octanoate from methyl decanoate, and column efficiency. Experimental variables for isothermal runs were column temperature and column head pressure in a 3 × 3 central composite design. Experimental variables for the temperature programmed run were initial column temperature, program rate, and column head pressure in a 3 × 3 × 3 central composite design. The chromatographic systems explored were a capillary column gas chromatograph, using both nitrogen and helium as carrier gases, and a packed column gas chromatograph using helium carrier gas. Fitted regression equation analyses were performed using both analysis of variance and canonical analysis. Overlay contour plots of analysis time and resolution together with contour plots of column efficiency showed differing degrees of interaction between the experimental variables depending on the chromatographic system. Overlay plots allow the investigator to choose chromatographic conditions that optimize both analysis time and column resolution.

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THE USE OF LIPO-FRAX COLUMN CHROMATOGRAPHY FOR THE PURIFICATION OF NEUTRAL LIPID EXTRACTS FOR ANALYSIS ON THE AUTOANALYZER—II. HERBERT K. NAITO, VIOLET MILETIC, Lipid-Lipoprotein Laboratories, Division of Laboratory Medicine, The Cleveland Clinic Foundation, 9500 Euclid Ave., Cleveland, OH 44106; and LAURANCE F. FERRERI, Virginia Polytechnic Institute and State University.

Chromatographic columns (Lipo-Frax® from Analytical Products, Inc.) containing activated alkaline metal oxides can retain water and polar compounds from isopropanol extracts of serum or plasma. The convenience of these pre-packed microcolumns (method I) was compared to the traditional method of extracting serum neutral lipids using the zeolite mixture (method II). In method I, the need for careful attention to making of the zeolite mixture, as well as the centrifugation or phase-separation step can be eliminated. The isopropanol extracts of both methods were assayed for total cholesterol (TC) and triglycerides (TG) using the Technicon AutoAnalyzer-II according to the procedures used by the Lipid Research Clinics. The TC assay is based on the Liebermann-Burchard colorimetric reaction, while the TG determination is based on the fluorescent method of Kessler and Leaderer. The original method of adding the serum to the isopropanol and immediately passing the mixture through the Lipo-Frax® column gave irreproducible results (c.v. >15%). We modified and improved the Lipo-Frax® extraction procedure by slowly adding the serum to the isopropanol and shaking the mixture for 30 min before adding it to the columns. While this time-consuming step defeated the speed and convenience of method I, it insured increased accuracy and precision of the lipid analyses. One hundred fifty patient samples were randomly selected (range: TC = 122–359 mg/dl, TG = 43–359 mg/dl) and extracted by methods I and II. The correlation coefficient of methods I and II was 0.97 for TC and 0.99 for TG. Within-day reproducibility studies show that both methods had a c.v. of <0.35% for TC and <0.75% for TG. Day-to-day variation studies of both extraction procedures demonstrate a c.v. of <1.0% for TC as well as for TG. Our study indicates that the Lipo-Frax® columns provide an alternative means of extracting serum TC and TG. The method can be as reliable as the conventional zeolite method providing that some modifications are adopted.

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A FURTHER STUDY ON THE TRITERPENE ALCOHOLS OF THEACEAE AND SOME OTHER VEGETABLE OILS. TOSHIHIRO ITOH, TOSHITAKE TAMURA, and TARO MATSUMOTO, Nihon University, Tokyo, Japan.

Triterpene alcohol and sterol constituents of three Theaceae and some other vegetable oils were previously studied in this laboratory (*Lipids* 9:173 [1974]; 10:454, 808 [1975]; 11:434 [1976]). However, because the composition of triterpene alcohol fractions of these oils is very complicated, many minor components remained unidentified. Therefore, a further study was undertaken here on the triterpene constituents of two Theaceae oils, tea seed oil from *Thea sinensis* L. and camellia oil from *Camellia japonica* L.; pokeweed seed oil from *Phytolacca americana* L. (Phytolaccaceae), and shea butter from the kernels of *Butyrospermum parkii* (Sapotaceae). Among a number of triterpene alcohols present in these oils, nineteen components could be identified or tentatively identified in this study: cycloartenol, 24-methylenecycloartanol, parkeol, 24-methylene-24-dihydroparkeol, lanosterol, euphol, butyrospermol, tirucallol, tirucalla-7,24-dienol, dammaradienol, 24-methylenedammarenol, α-amyrin, β-amyrin, lupeol, germanicol, taraxasterol, ψ-taraxasterol, taraxerol, and myricadiol (taraxer-14-en-3β,28-diol). Tirucalla-7,24-dienol and butyrospermol are the predominant components of the two Theaceae and pokeweed oils. Shea butter, on the other hand, contains α-amyrin followed by butyrospermol and lupeol as the major triterpene constituents.

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TOTAL LIPID, PROTEIN, STARCH, SUGAR, AND FATTY ACID KERNEL COMPOSITION OF 39 FILBERT (*CORYLUS AVELLANA*, L.) CLONES. DARYL G. RICHARDSON and MAXINE M. THOMPSON, Department of Horticulture, Oregon State University, Corvallis, OR 97331.

Filbert kernels (*Corylus avellana*, L.) of 39 breeding clones were analyzed for total lipid, protein, starch, free sugars, mois-

ture, insoluble residue, and fatty acid composition. Total lipid ranged from 54 to 68%, protein (11.5 to 20%), starch (0.2 to 6.9%), sugars (4 to 13%), and moisture (2.2 to 4.3%). The predominant fatty acids were oleic (69 to 80%), linoleic (12 to 24%), palmitic (3.7 to 7.3%), stearic (0.4 to 2.8%), and linolenic (trace to 1.5%). Insoluble residue ranged from 8 to 13%. The relationship of these factors to nut quality will be discussed.

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NUTRIENT COMPOSITION OF THE FATS AND OILS. JOHN L. WEIHRAUCH, Consumer and Food Economics Institute, USDA, Federal Building, Hyattsville, MD 20782.

Data on the nutrient composition of the fats and oils have been compiled for publication in a separate section of the revised and expanded U.S. Department of Agriculture Handbook No. 8, "Composition of Foods—Raw-Processed-Prepared." The new handbook section will contain information on the proximate composition, minerals, vitamins, lipids (fatty acids, cholesterol, and total phytosterols), and amino acids for animal fats, vegetable oils, margarines, spreads, shortenings, and salad dressings. Described are the problems and procedures of compiling the data via computer through the Nutrient Data Bank. The utility and limitations of the reference tables are discussed. Research needs for the expansion of the section on fats and oils will be presented.

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OPERATING A 500 KW ORGANIC RANKINE CYCLE SYSTEM UTILIZING LOW TEMPERATURE HEAT. S. ICHIKAWA, R.H. SAWYER, AFI Energy Systems, 110 South Orange Ave., Livingston, NJ 07039; and H.R. TYLER, Allied Chemical Corp., Morristown, NJ.

The first commercial scale application in the United States of an organic rankine cycle to recover energy from low temperature waste heat is installed at Allied Chemical's plant in Claymont, Delaware. This rankine cycle system utilizes a fluorocarbon refrigerant as a working fluid and a stream of 220–240 F sulfuric acid as a heat source to produce 500 KW of electrical power. This paper will discuss the design installation, start-up, operation, and performance of the system. The actual operational experience of the unit, its control and safety systems, and its maintenance requirements will also be included.

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STEAM EJECTOR DESIGN AND OPERATION IN TODAY'S WORLD OF HIGH STEAM COSTS. D. RICHARD O'CONNOR, Kinema Inc., PO Box 176, Elizabeth, PA 15037.

One of the largest classes of energy users in your refineries is the steam ejector system. Now that energy conservation is the name of the game, ejector design engineers have suggestions relating to this problem. This presentation deals with the proper selection of steam ejector staging, suggested operating methods, and detailed reasons to achieve optimum energy costs without adversely affecting process operations. Also presented is a scheme to utilize a long known, but little used, characteristic of steam ejectors to produce significant steam savings with nominal capital expenditure.

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NEW DEVELOPMENTS IN WASTE HEAT RECOVERY USING HEAT PIPES. DENNIS A. LITTWIN, Q-dot Corporation, 726 Regal Row, Dallas, TX 75247.

With fuel prices skyrocketing and fuel availability decreasing, waste heat recovery is becoming more attractive to energy intensive industries. This paper describes various waste heat recovery devices currently available for air-to-air applications and new devices developed to generate steam from wasted hot airstreams. Four general classes of applications of waste heat exchangers can be identified: (a) using energy recovered from process exhaust to regenerate the process, (b) using energy from process exhaust to generate steam, (c) using energy from process exhaust to heat comfort make-up air during the winter months, and (d) using comfort exhaust to preheat comfort make-up air during the winter months and/or precool comfort make-up air during the summer months. Descriptions and/or installations are described.

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ENERGY CONSERVATION IN THE EDIBLE OIL REFINERY. PHILIP BOLLHEIMER, JR. and WILLIAM B. CAMPBELL, PSI Process Systems, Inc., 2930 Airways Blvd., Suite 208, Memphis, TN 38116.

In order to meet the Federal Energy Administration's 1980 goal of 12% energy saving, edible oil processors must make both engineering design changes in their plants as well as changes in operating procedures. Both old and new methods to conserve energy from an engineering design standpoint are discussed. In the face of rapidly rising energy costs, old design criteria and rules-of-thumb must be reevaluated. The rules which governed pipe insulation thickness, steam trap selection, and pump and motor sizing are no longer applicable. Equipment such as waste heat boilers, economizers, and mechanical vacuum pumps, which in the past did not show sufficient payback, are now economical. New plants should be designed and older plants retrofitted with heat recovery systems in the refining, hydrogenation, and deodorization processes. Electrical distribution systems should be designed to minimize power losses and to allow load shedding.

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MECHANISM OF ACTION OF LIPOPROTEIN LIPASE CHRISTOPHER J. FIELDING, Cardiovascular Research Institute, University of California Medical Center, San Francisco, CA 94143.

Lipoprotein lipase (LPL) located at the vascular endothelial surface, catalyzes the hydrolysis of 1(3)-position fatty acids in the triglycerides of the plasma triglyceride-rich lipoproteins. The enzyme is activated for triglyceride hydrolysis by formation of a stoichiometric complex with a specific lipoprotein apoprotein (apo C-2), and recent research has indicated that such activation is mediated via a salt-labile charge-charge interaction. The reaction is probably terminated as apo C-2 dissociates from the substrate lipoprotein as its triglyceride content is depleted and as the product lipoprotein remnant is generated and becomes increasingly noncompetitive with newly secreted particles (chylomicrons or very low density lipoproteins). Several aspects of the LPL reaction are incompletely understood: the relationship between LPL species in different tissues, the regulation of endothelial levels of LPL, and the possible role of other apolipoproteins in the LPL reaction. Recent developments in these areas will be reviewed.

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USE OF THREE ISOTOPIC LABELS (^{3}H, ^{131}I, ^{125}I) TO MEASURE TURNOVER OF PLASMA TRIGLYCERIDE AND APOLIPOPROTEIN B IN VERY LOW DENSITY LIPOPROTEINS AND LOW DENSITY LIPOPROTEINS. NGOC ANH LE, JOHN TURNER, University of California, San Diego, CA; JOHN MELISH, University of Hawaii; HENRY GINSBERG, and W. VIRGIL BROWN, Mount Sinai School of Medicine, Department of Medicine, Annenberg 24–100, New York, NY 20029.

Very low density lipoproteins (VLDL) are secreted into plasma by liver and intestine and transport triglyceride (TG) to organs of utilization and storage. Apolipoprotein B (apo-B) is an essential component of this lipoprotein. Apo-B is partially retained in plasma as lipolytic processes convert VLDL to intermediate density (IDL) and low density (LDL) lipoproteins. LDL apo-B may also be secreted directly into plasma, probably by liver. Methods have been developed to monitor the specific activity of VLDL-TG after in vivo labeling with ^{3}H-glycerol as well as VLDL apo-B and IDL-apo-B after in vitro labeling with ^{131}I and ^{125}I, respectively. ^{131}I-VLDL (50 μCi) and ^{3}H-glycerol (300 μCi) are simultaneously injected intravenously followed later by injection of ^{125}I-LDL (50 μCi). Three ml plasma samples (18 in the first 48 hr) are used to isolate VLDL, IDL, and LDL in the ultracentrifuge. The lipid is removed by acetone and isopropanol extractions leaving a residual protein pellet. The triglyceride is separated from phospholipids in these extracts with zeolite and hydrolyzed. Fatty acids are removed with chloroform and the remaining aqueous phase used for determination of glycerol and ^{3}H radioactivity. The protein pellet is solubilized in 9 M tetramethylurea and apo-B selectively precipitated by addition of an equal volume of H_2O. This step is repeated after which the apo-B precipitate (5–40 μg) is washed with H_2O and solubilized in Na carbonate, $CuSO_4$ solution for determination of radioactivity (^{131}I) and protein (Lowry). ^{131}I and ^{125}I-LDL apo-B specific activity is monitored for a further 14 days following the ^{125}I-LDL injection. These techniques have been used in six studies to estimate the synthetic rate of each labeled component and to relate triglyceride turnover to the interconversion and clearance of lipoprotein apo-B.

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IMMUNOCHEMICAL STUDIES OF APOLIPOPROTEIN B (ApoB) IN TRIGLYCERIDE (TG)-RICH LIPOPROTEINS. GUSTAV SCHONFELD, Lipid Research Center, Washington University School of Medicine, Box 8046, 4566 Scott Ave., St. Louis, MO 63110.

Measurements of ApoB levels in human whole plasma and in plasma lipoproteins have been useful in studies of the kinetics of ApoB, and in characterizing the composition of lipoproteins under a variety of conditions. For example, it has been established that lipoprotein compositions are altered in subjects with the hyperlipidemias, and by dietary and drug treatments. These alterations probably reflect the differing kinetics of lipids and apoproteins under those conditions. The alteration in composition may change the interactions between lipoproteins and cells. In addition to "metabolic" information, immunoassays for ApoB also yield interesting information on the immunoreactivity of ApoB in various lipoproteins. For these assays, antisera are produced against holo-LDL or ApoB. Some of the antisera can distinguish between the ApoB in different density subfraction of TG-rich lipoproteins suggesting that the chemistry, disposition, and/or conformation of ApoB on these particles differ with their sizes.

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POLYDISPERSITY: AN ALTERED STRUCTURAL STATE OF HUMAN PLASMA LOW DENSITY LIPOPROTEIN. WALDO R. FISHER, Department of Medicine, JHMHC Box J-226, University of Florida, Gainesville, FL 32610.

In most human subjects without hypertriglyceridemia, low density lipoprotein (LDL) exists as a spectrum of macromolecules existing over a narrowly defined density range and differing little in their molecular weight. The relative homogeneity of such monodisperse LDL differs from the structurally heterogeneous LDL found in approximately two-thirds of subjects with hypertriglyceridemia. Sf 20 LDL, Sf 10 LDL, and Sf 4 LDL, the major components of polydisperse LDL, may be readily isolated and have molecular weights of 4.9, 3.2, and 2.5 million, respectively. These molecular weights reflect the amount of lipid associated with the apoprotein, and compositional analysis reveals that each of these lipoproteins contains the same weight of apoprotein per particle, with apoB the predominant apoprotein. The metabolism of polydisperse LDL has been studied in five subjects, using tritiated leucine as a metabolic tracer, and kinetic analysis of apolipoprotein B metabolism was performed in collaboration with Drs. Berman, Phair, and Zech at NIH. ApoB is metabolized in a sequential fashion from large VLDL particles to small Sf 4 LDL, with a discontinuity in LDL metabolism yielding Sf 20, Sf 10, and Sf 4 LDL. Further, there is a striking increase in the synthesis of apoB as compared to normals. In part, this synthesis feeds into large VLDL where apoB enters the VLDL "delipidation chain;" a second major input of apoB is into small VLDL and Sf 20 LDL, thus in large part bypassing VLDL. The removal of apoB from plasma proceeds through two catabolic pathways. The major pathway removes apoB from small VLDL or Sf 20 LDL; a second pathway removes Sf 4 LDL from plasma. Thus, in these subjects a major overproduction of apoB appears to exceed the capacity to transport apoB through LDL, and much apoB is removed from plasma as small VLDL or Sf 20 LDL. The kinetic analysis appears to establish the metabolic relationship of Sf 20, 10, and 4 LDL as precursor to product but leaves unclear the mechanism by which these

lipoproteins are generated from each other. In this conversion, there is a loss from all of the major constituent lipids of LDL. In vitro there is no exchange of apoB between Sf 4 and Sf 20 LDL, nor is there an exchange of delipidated, solubilized apoB in native LDL, a finding consistent with the kinetic data showing a unidirectional flow of apoB from large VLDL to small LDL. The metabolic fate of these LDL is unclear. Sf 20, 10, and 4 LDL all bind to the fibroblast receptor; however, this observation does not clarify the fate of apoB which is cleared from plasma, on the one hand as small VLDL and Sf 20 LDL, and on the other as Sf 4 LDL.

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FORMATION AND CATABOLISM OF PLASMA CHOLESTERYL ESTERS IN THE RAT. RICHARD J. HAVEL, Cardiovascular Research Institute and Department of Medicine, University of California, San Francisco, CA 94143.

Among mammals, cellular cholesterol homeostasis can be maintained at widely differing concentrations of low density lipoproteins (LDL). As in other mammals, the protein component of rat LDL arises primarily from catabolized very low density lipoproteins (VLDL). By contrast, most of the cholesteryl esters (CE) of rat LDL are produced by lecithin-cholesterol acyltransferase (LCAT), presumably acting upon high density lipoproteins (HDL), from which the esters are transferred to LDL. If CE of LDL are taken up into cells by pinocytotic mechanisms, the rate of transport of these CE can be estimated from the observed rate of transport of the protein component of LDL. This rate is small in the rat, as compared with transport of LCAT-derived CE in HDL. Experiments with perfused rat livers suggest that most LDL, as well as HDL, are catabolized in extrahepatic tissues. By contrast, CE in rat chylomicrons and VLDL are synthesized in the tissues of origin and are catabolized primarily in the liver. These considerations lead to the conclusion that whereas triglyceride-rich lipoproteins in the rat participate in an enterohepatic cycle of cholesterol transport, HDL and LDL participate in a substantially separate extrahepatic cycle. The latter cycle may have a major role in cholesterol homeostasis by transporting cholesterol from sites where cells are degraded (i.e., spleen) to sites of cellular proliferation (i.e., bone marrow and lymph nodes). In the rat, HDL containing the arginine-rich apoprotein could substitute in part for LDL in the efferent arc of this transport system. If similar pathways apply in humans, most LCAT-derived CE must be catabolized as a component of LDL.

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HEPATIC BIOSYNTHESIS OF PLASMA LIPOPROTEINS DURING CHOLESTASIS. T.E. FELKER, R.L. HAMILTON, J.L. VIGNE, and R.J. HAVEL, Cardiovascular Research Institute, University of California, San Francisco, CA 94143.

A segment of common bile duct was resected in rats 42 hr before study. Isolated livers from normal controls, sham-operated controls, and cholestatic animals were perfused for 6 hr by recirculating 40 ml of Krebs-Ringer bicarbonate buffer containing washed rat erythrocytes (25%), 150 mg/dl glucose and ^{3}H-lysine. Perfusates from cholestatic livers had about tenfold less very low density lipoprotein (VLDL) protein than sham-operated controls although VLDL from cholestatic livers were richer in protein (16% vs. 9% of mass) and poorer in triglycerides (59% vs. 67.7% of mass). Low density lipoprotein (LDL) ($1.015 < d < 1.075$ g/ml) protein increased twofold in perfusates of cholestatic livers but total lipoprotein increased >tenfold owing to the presence of LP-X and a separate pseudomicellar particle that contained more triglyceride than cholesteryl esters (*Proc. Natl. Acad. Sci.* 75:3459 [1978]). There was no change in the amount of high density lipoprotein (HDL) ($1.075 < d < 1.175$ g/ml) protein recovered from perfusates of cholestatic livers. However, nascent HDL from cholestatic perfused livers contained one-half as much cholesteryl ester (10% of mass) and by electron microscopy appeared more discoidal than control HDL, suggesting reduced LCAT activity in cholestatic perfusates. By radioimmunoassay arginine-rich apolipoprotein (ARP) predominated in perfusate HDL from control livers (ARP/A-I = 1.7:1.0), whereas A-I was the major apoprotein in HDL from cholestatic livers (ARP/A-I = 0.7:1.0). A-I contained one-half of the total apoprotein radioactivity in HDL from cholestatic livers, but only 25% in controls. The specific activity of A-I was two to fourfold higher in HDL from perfusates of cholestatic livers than in normal and sham-operated controls. The amount of A-I that accumulated in perfusates of cholestatic rats was twofold higher (112 µg/g liver/6 hr) than in sham-operated controls (44 µg/g liver/6 hr). Accumulation of ARP did not change significantly in cholestasis.

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STIMULATION OF LOW DENSITY LIPOPROTEIN SECRETION BY PERFUSED LIVERS OF GUINEA PIGS FED CHOLESTEROL. R.L. HAMILTON, Department of Anatomy and Cardiovascular Research Institute, University of California, San Francisco; R. OSTWALD, Nutritional Science, University of California, Berkeley; L.S.S. GUO, R.J. HAVEL, Cardiovascular Research Institute, University of California, San Francisco, CA 94143.

Lipoproteins secreted from livers of guinea pigs fed standard chow were compared with those secreted by fatty livers induced by feeding diets containing 1% cholesterol and 5% cottonseed oil. Very low density lipoprotein (VLDL) ($d < 1.015$ g/ml), a major secretory lipoprotein from control livers, contain, as % mass: 0.5% cholesteryl esters (CE), 70% triglycerides (TG), 3% free cholesterol, 17% phospholipids, and 9.5% proteins. Trace amounts of small triglyceride-rich particles are recovered in the low density lipoprotein (LDL) ($1.015 < d < 1.050$). The apoproteins of both triglyceride-rich fractions are similar by SDS gel electrophoresis: B-apoprotein predominates, with small amounts of ARP and C apoproteins. Major changes occur in these fractions after 10 and 14 days of cholesterol diet. Both perfusate VLDL and LDL contain a larger amount of ARP. CE content of VLDL increases to 10–15% of mass although total core (CE + TG) is unchanged. Significantly more lipoprotein is recovered in LDL, which differs greatly from controls. This fraction migrates as a broad-beta band on agarose gel electrophoresis, contains 45–55% CE and only 6–14% TG and, by electron microscopy, contains particles with angular surfaces. These changes are accentuated after 1–3 months of cholesterol diet. ARP becomes a more prominent component of both VLDL and LDL and CE content of VLDL reaches a maximum of 32%. LDL becomes a major and VLDL a minor lipoprotein recovered from perfusates of these fatty livers. Closely similar changes occur in the plasma lipoproteins of these cholesterol-fed guinea pigs.

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LYMPH CHYLOMICRON SUBPOPULATIONS. ALAN VOST, McGill University Medical Clinic, The Montreal General Hospital, 1650 Cedar Ave., Montreal, Quebec, Canada.

Possible heterogeneity of lymph chylomicrons was investigated in thoracic duct lymph of male rats. Equimolar emulsions of triolein, trilinolein, and trilinolenin in sodium taurocholate solution were fed, as pulse doses, via gastrostomies. Lymph, from indwelling thoracic duct cannulas, was collected only during post absorptive chylomicron production. To separate discrete fractions of triglyceride-rich lipoproteins (chylomicrons and very low density lipoproteins) a method had been devised for pH gradient electrophoresis (APHGE) on thin layers of agarose gel beads. This technique permits direct visualization or densitometry of unstained triglyceride-rich lipoproteins in the thin layers. Defibrinated lymph and chylomicrons showed major heterogeneity during APHGE. Chylomicrons were separated into five or six discrete bands. In these subpopulations the distribution of volume and frequency of chylomicrons at serial diameter intervals, measured by e.m., indicated coalescence of most particles in Band 1 (origin) and 40% of particles in Band 6 (front) but <10% in the four intermediate bands. >90% of subpopulation triglyceride fatty acids were 18-unsaturated and triglyceride fatty acid composition did not differ significantly between subpopulations. These findings suggest (a) that numerous subpopulations of chylomicrons exist and that contaminating preparative artefact (coalescence) can be identified specifically by APHGE and (b) that the chylomicron subpopulations transport triglyceride derived from a common precursor pool. These results are not consistent with the view that intestinal lymph contains only two homogeneous populations of VLDL and chylomicrons.

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EFFECT OF A CHOLESTEROL DIET ON CHICKEN SERUM LIPOPROTEINS AND APOLIPOPROTEINS. ARTHUR W. KRUSKI, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78284.

Fifteen cockerels were fed regular chow (R) and fifteen R + 1% cholesterol (C). At 6 weeks, the diet groups were exchanged; serum lipoproteins (LP) and apolipoproteins (apo LP) were examined 0, 1, 3, 7, and 14 days later by isopycnic density gradient ultracentrifugation. After crossover from R to C, serum total cholesterol (TC) increased from 130 to 201 mg/dl at 1 day and to 478 mg/dl at 14 days. After the 1st and 3rd days the increase in TC was almost completely due to a previously absent very low density lipoprotein (VLDL) fraction. Between the 3rd and 7th days, low density lipoprotein (LDL) shifted to a less dense species which, during the 7th to 14th days, overlapped the broadening VLDL band. These changes were accompanied by the appearance of a new apo-LP (19,000 daltons). Trace amounts of apo-LP between 30,000 and 40,000 daltons were observed. When R replaced C, changes in LP and apo-LP reversed as the serum TC decreased from 452 to 148 mg/dl. By the 14th day, VLDL had almost vanished, the LDL concentration and density had become normal, and the 19,000 dalton apo-LP disappeared. These results indicate that feeding cholesterol to chickens sequentially changes the LP profile and stimulates the appearance of a new, 19,000 dalton apo-LP.

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QUANTITATIVE GAS LIQUID CHROMATOGRAPHY DETERMINATION OF METHYL LINOLENATE IN THE PRESENCE OF CONJUGATED DIENES. S. KORITALA, Northern Regional Research Center, USDA, 1815 N. University, Peoria, IL 61604.

Catalytic hydrogenation of polyunsaturated fatty acid esters, particularly with copper catalysts, produces conjugated dienes; these interfere with gas liquid chromatography analysis of methyl linolenate on the polar columns used for separation of methyl esters. A similar problem is encountered during partial conjugation of soybean oil methyl esters. A new column of intermediate polarity made from a mixture of OV-17 and OV-225 separated these critical pairs and enabled quantitative determination of methyl linolenate in partially hydrogenated or conjugated esters. The proposed method is quicker than the alkali-isomerization method.

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THE MEASUREMENT OF FAT DILATION. KAZUAKI SUZUKI, EIJI NAKAI, and YUKINOBU MURASE, Asahi Electrochemical Co., Ltd., 1, 7-Chome, Higashi-Ogu Arakawaku, Tokyo, 116, Japan.

On studying the physical properties of the plastic fats, it is important to know the liquid/solid fat ratio or the solid fat content at various temperatures. There are many methods such as spectrometry (NMR), colorimetry, and dilatometry, by which the solid fat content is measured. Among these methods, dilatometry is the most convenient method because of simplicity of procedures and apparatus, providing significant numerical values. The numerical value obtained by dilatometry generally is called the solid fat index (SFI). There are many defects in the usual procedures used for SFI such as AOCS or JOCS (Part 1) tentative methods. For instance, the fat sample may not be solidified below 0 C and reliable data cannot be obtained at low temperature by the AOCS method, while mercury used by JOCS (1) method is toxic to man and has the disadvantage of wastewater pollution. We have investigated a method to be able to obtain reliable data at low temperature without toxicity to man or wastewater pollution. The method investigated by us consists of a $CaCl_2$ aqueous dye solution (30.4% by weight) as sealing liquid and the dilatometer as used by JOCS (1). The disadvantages using water or mercury as sealing liquids are eliminated and the advantages of the usual procedures are retained by this modified method. In addition, other methods

were compared with dilatometry to measure the solid fat content. The values obtained by this method were similar with spectrometry (NMR method) below 50% solid in fat. Furthermore, tempering temperature and time were investigated in order to measure the SFI of fat rapidly.

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QUANTITATIVE ANALYSIS OF STEROLS IN FOODS BY GAS LIQUID CHROMATOGRAPHY. T. KANEDA, K. FUJIMOTO, and A. YUSA, Department of Food Chemistry, Faculty of Agriculture, Tohoku University, 1-1 Amamiyamachi, Tsutsumidori, Sendai, 980, Japan.

Japanese Resources Bureau, Science and Technology Agency has requested that we develop a simple and exact analytical method for cholesterol determination in foods. The determination of cholesterol is usually carried out by colorimetric methods, however, these methods are unsuitable for cholesterol in foods, because the presence of several sterols is known in some foods such as shellfish. We decided to estimate the cholesterol content by gas liquid chromatography (GLC). For the extraction of lipids from foods, a number of organic solvents were tested, and it was observed that chloroform-methanol (2:1) extraction is the most suitable procedure. The direct hydrolysis method also gives good recovery. Several papers have been published on the pretreatment of unsaponifiable matter by thin layer chromatography, (TLC), florisil column chromatography, and others. However, according to our data, these pretreatments reduce the recovery of cholesterol and we observed that the pretreatment is not necessary to get good results. It is known that trimethylsilyl ether derivatives and acetate are useful for GLC, but we could get good results using the free sterols. 5-α-Cholestane is used as an internal standard. Before and after the hydrolysis of lipids, the internal standard was added and the recovery of cholesterol was tested. The results indicate that if a suitable amount of internal standard is added, similar results are obtained, regardless of addition time.

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SILVER NITRATE COATING OF THIN LAYER CHROMATOGRAPHY PLATES FOR STEROL ACETATE SEPARATIONS. HENRY W. KIRCHER and JAN MRGUDITCH, Department of Nutrition and Food Science, University of Arizona, Tucson, AZ 85721.

Thin layers of silica gel on aluminum sheets (E. Merck) were coated with silver nitrate by dipping the sheets into solutions of the salt in various organic solvents. Sheets prepared from various concentrations of silver nitrate in aqueous ethanol and methanol-acetonitrile mixtures were used with methylene chloride, chloroform, and 1:1 chloroform-carbon tetrachloride to separate steryl acetates. Examples of Δ^{0}, Δ^{5}, Δ^{7}, $\Delta^{8(14)}$, Δ^{22}, $\Delta^{5,7}$, $\Delta^{5,22}$, $\Delta^{7,22}$, $\Delta^{5,7,22}$, and $\Delta^{5,24(28)}$ steryl acetates of the cholestane, campestane, ergostane, stigmastane, and poriferastane series as well as several $3\beta,6\alpha$-sterol diol diacetates were used as illustrations of the methodology.

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DETERMINATION OF STEROLS IN FATS AND OILS. SATOSHI YONEYAMA, TOSHITAKE TAMURA, and TAKENORI MARUYAMA, JOCS Committee of Fat and Oils Analysis, c/o Yushi Kogyo Kaikan, 3-13-11, Nihonbashi, Chuoku, Tokyo, Japan.

Methods for the determination of sterols in edible oils and fats were studied. The sterols were isolated and analyzed by thin layer chromatography or digitonin precipitation, coupled with gas liquid chromatography, respectively, by using cholesterol or β-sitosterol as an internal standard. Quantitative determination of sterols was made by cutting off the peaks of cholesterol and phytosterol on the recorder charts from the gas chromatograms recorded with and without the presence of the internal standards, and followed by the calculation of the changes in the peak weight caused by the addition of the standard. Recovery experiments were carried out by 10 collaborators with samples of medium chain triglyceride added to a known concentration of cholesterol and phytosterol. Mean recovery rate was over 81.4%, and it was found that even 10 mg of cholesterol contained in 100 g of oil or fat was determined with a relatively good reproducibility. Both the methods of the thin layer chromatography and the digitonin precipitation coupled with gas liquid chromatography gave satisfactory results.

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APPLICATION OF NEAR-INFRARED REFLECTANCE SPECTROSCOPY TO QUALITY CONTROL IN OIL PLANT. Y. MACHIDA, T. WATANABE, Central Research Laboratory; and K. AONUKI, Kashima Plant, Showa Sangyo Co., Ltd., Hinode 2-20-2, Funabashi-shi, Chiba, 273, Japan.

A near-infrared reflectance (NIR) instrument (Technicon InfraAlyzer) was used for quality control of soybean meal in the oil plant. Each sample of soybean meal was ground to particles of smaller than 60 mesh by using a sample mill with a 1 mm screen. This sample was analyzed for total-N, oil, and moisture. The results obtained by InfraAlyzer were compared with the data obtained by the standard analytical procedures, i.e., Kjeldahl method for total-N, diethyl ether extraction method for oil, and oven method for moisture. Standard errors of difference (SE) between the data obtained by both methods were 0.073, 0.152, and 0.111 for protein, oil, and moisture, respectively. The results suggest that the NIR spectroscopic analysis can be applied to the determination of protein and oil in the quality control process of soybean meal because there is little deviation between the results by both methods. Also, in the case of ground sample, a close agreement in moisture content was found between the data obtained by both methods. However, when the original meal was tested by the oven method for commercial use, its moisture content was significantly different (SE: 0.260) from that obtained by InfraAlyzer in the ground sample, since a certain degree of moisture loss occurred during the grinding process.

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QUANTITATIVE ANALYSIS OF HYDRONAPHTHALENE DERIVATIVES CONTAINED IN HEATED TUNG OIL. NAGAO TOTANI, The Hormel Institute, Austin, MN; SETSUKO HARA, YOICHIRO TOTANI, and NOBORU MATSUO, Department of Industrial Chemistry, Faculty of Engineering, Seikei University, Kichijoji-Kitamachi 3 Musashino-shi, Tokyo, 180, Japan.

Since C. Chin (*J. Chem. Soc. Jpn.* 53:281 [1950]) suggested the formation of cyclic derivatives on heating tung oil, there exist extensive works on the structure of the dimeric acid. Among these, the structure and formation of the hydrobenzene derivatives have appeared in literature, whereas little has been reported on that of the hydronaphthalene derivatives. The present work is concerned with the quantitative determination of the hydronaphthalene derivatives, produced on heating tung oil, by means of colorimetry. It is known that polycyclic aromatic compounds form a dark greenish complex with sodium. Therefore, at first, various amounts of purified naphthalene were reacted with sodium dispersed in solid paraffin and the absorbance at 420 nm of the mixture was measured and plotted vs. the concentration of naphthalene. The resulting calibration curve showed a good linearity in the range of 12–35 mg of naphthalene in 25 ml THF. In a stainless steel autoclave, photoisomerized tung oil was heated with sodium hydroxide in ethylene glycol at 290 C for 15 min. The reaction mixture was then acidified, esterified, and distilled under vacuum to isolate the dimeric ester, which was subsequently aromatized by heating at 280 C for 5 hr with 10% Pd-C as a catalyst. This aromatized dimeric ester was then reacted with sodium and, from the measured absorbance at 420 nm of the mixture with the calibration curve, the content of naphthalene derivatives was determined. As a result, it was found that about 1.2% of naphthalene derivatives was present in heated tung oil.

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MODERN ANALYTICAL METHODS FOR SUNFLOWERS. ART CARNRICK, A&L Plains Agricultural Labs, Lubbock, TX.

At the present time there are several methods of sunflower analysis in common use. The differences between these methods can affect the analytical results. The major discrepancies are in the areas of foreign matter or dockage and oil content to an "as is" basis. Depending on the method, results may vary from 1–2%. The specific methods for each type of analysis as well as pertinent calculations will be discussed. The report will also make suggestions based on laboratory research for a uniform method such that results may be consistent among laboratories.

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COLOR CONTROL BY AUTOMATED TINTOMETER. D.G. CHAMBERLIN, Tintometer USA, 49 Cannonball Rd., Pompton Lakes, NJ 07442.

Since the early years of this century, the edible oil industry has chosen to specify colors by means of the Lovibond Tintometer and color scales. Lovibond chose three color scales, Red, Yellow, and Blue in order to match any sample. Each scale was originally graded by depth into more than two hundred steps. The industry found it convenient to express colors in terms of Red and Yellow units only, as the red content of an oil is a suitable measure of the refining needed and, therefore, of its value. The interest of the U.S. Bureau of Standards was early drawn to this system, and they purchased a complete set of the glasses in 1912. In 1926, they undertook a major project sponsored by the Cotton Seed Crushers Association to study and standardize both instrument and scales. This work lead to the definition of the N" (now AOCS) red scale and to a description of an instrument adapted for this field. The use of different instruments in Europe and the United States and the possibility of confusion between two red scales has caused confusion over the years. Major buyers, faced with the need for agreement between distant laboratories, have wished for an instrument less dependent on the skill and training of an individual. The U.K. firm of Unilever recently designed a three-filter colorimeter which can be calibrated to reproduce the values found by visual measurement. Interference filters measure the transmittance of the oil in the blue and green regions of the spectrum, while a third reading is taken in the red, thus allowing for suspended matter. This development represents a new departure from earlier attempts to specify Lovibond color by spectrophotometric measurement at single wavelengths. The Food Research Association in England studied the correlation found between this and the standard visual instrument, and their findings will be discussed in this paper.

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LOSS OF ADDED FATTY ACID ESTERS FROM GRAPE SURFACES DURING DRYING TO RAISINS. A.E. STAFFORD, G. FULLER, and H.R. BOLIN, Western Regional Research Center, USDA, 800 Buchanan St., Albany, CA 94710.

Fatty acid esters have been shown to reduce the drying time of grapes by interacting with the waxy surface of the grapes. Laboratory scale procedures were developed to determine the fate of esters during dehydration. Thompson seedless grapes were dipped in a water emulsion containing 2% fatty acid esters, 2% potassium carbonate, and an emulsifier. Grapes were dried at temperatures ranging from ambient to 160 F. Drying curves vs. fatty acid ester concentration on the grape surfaces were obtained. Multiple dips during drying increased the rate significantly. The fate of fatty acid esters during dehydration was determined by running the exhaust through cold traps. These studies showed a considerable loss of esters during drying which did affect the drying rate. The ester losses appeared to be caused by the co-evaporation of water and esters during drying.

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USE OF OIL IN BAKED PRODUCTS: I. BACKGROUND AND BREAD. DEBORAH HARTNETT and WILLIAM THALHEIMER, ICI Americas Inc., Wilmington, DE 19897.

The benefits of fat systems in yeast-raised and chemically leavened baked goods are reviewed. The effects of rising ingredient costs and competition on ingredient technology are described, leading to a discussion concerning the trend in the United States to switch from the standard shortenings, lard and partially hydrogenated vegetable shortening, to oil in baked products. Problems associated with the incorporation of oil in bread formulations follow. Test work has shown that surfactants are effective additives in overcoming these negatives and in promoting the production of quality bread. A comparison of oil vs. lard as the shortening medium in bread is described. Evaluation of emulsifiers with oil in bread are discussed.

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USE OF OIL IN BAKED PRODUCTS: II. CAKES AND SWEET GOODS. DEBORAH HARTNETT and WILLIAM THALHEIMER, ICI Americas Inc., Wilmington, DE 19897.

The discussion on the current trend in the United States to switch from the conventionally accepted lard or partially hydrogenated vegetable shortening to oil as the shortening medium in baked goods is continued. Problems encountered when oil is used to replace the plastic shortenings in sweet doughs and cakes are described. Test work has shown that surfactants are useful tools in counteracting the resultant negative aspects associated with the use of oil. A review of data describing various surfactant systems with oil and their effects on finished sweet dough and cake quality are discussed.

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THE FUNCTIONS OF SILICONE OIL IN FRYING OIL. SHIZUYUKI OHTA and HYOJI KUSAKA, School of Fisheries Science, Kitasato University, Sanriku-machi, Kesen-gun Iwate-ken, 022-01, Japan.

It is generally well known that silicone oil (SiO) increases thermal stability of frying oil. However, the mechanism of its stabilizing ability is not well defined. To elucidate this mechanism, the effect of SiO on the stability of oils was investigated by various heating procedures. The oil (refined but not deodorized soybean oil and/or linseed oil) was treated by stirring the surface in a beaker and air bubbling through a glass-tube inserted into a test tube with varying temperatures (120, 150, 180, and 240 C) and SiO content (1, 10, 100, and 1000 ppm), respectively. Further, a frying test was carried out at 240 C using a Tofu-Namaage. The extent of deterioration of these heated oils was evaluated mainly by the viscosity measurements. In heating procedures, it was shown from the above experiments that the thermal deterioration of the frying oil was suppressed with addition of SiO. On the other hand, when linseed oil or its methyl esters were heated for a long time under a stream of nitrogen, the protective effect of SiO was not demonstrated. At room temperatures, SiO suppressed slightly the increase of POV of linseed oil or ethyl ester of their fatty acid, but not as effectively as BHA or TDPA. The results of measurements of surface tension of several oils with SiO suggested the behavior of SiO on the air-to-oil surface but could not explain the protective effect of SiO.

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EFFECTS OF MICROCLIMATE ON COMPOSITION AND MELTING CHARACTERISTICS OF BAHIA COCOA BUTTER. DOUGLAS W. LEHRIAN, Hershey Foods Corporation, 925 Reese Ave., Hershey, PA 17033; PHILIP G. KEENEY, The Pennsylvania State University; and DAVID R. BUTLER, Oversea's Development Ministry.

Studies directed toward elucidation of a chemical explanation of the softness of some Brazilian cocoa butters are reported. The microclimate provided by positioning heaters near developing cocoa fruits resulted in an internal fruit temperature increase of 7.5 C in the seed area closest to the heat source and 3.0 C in the backside seed section. The increased temperature was maintained throughout the period of active lipid synthesis and until the fruits ripened and were harvested. Fruits from an unheated area of the same tree and from adjacent trees of the same cultivar were collected as controls grown under cooler conditions. Lipid pressed from shell-free portions of heat-treated and control seeds were found to have slightly different fatty acid compositions. Butter from heat-treated seeds had slightly more palmitate and stearate and 3% less oleate than butter from control seeds. The melting characteristics of these butters were obviously different. Cocoa butter from seeds grown at temperatures 7.5 C and 3.0 C higher than control seeds were considerably harder as determined by differential scanning calorimetry. Triglycerides of cocoa butters from heat-treated and control seeds were separated by high performance liquid chromatography (HPLC) into three major and two minor fractions which were present in different proportions in heated and control butters. Triglycerides in each HPLC fraction were determined by lipolysis, methyl ester analysis, and calculation according to Coleman (1961). Statistically significant differences ($p = 0.05$) were found relative to the triglycerides of Fractions 2, 3, and 4 (major peaks). Of greatest interest regarding the softness of cocoa butter was the composition of Fraction 2. Major constituents were the triglycerides POP and POO (P = palmitate, O = oleate). Butter from heated seeds had POP and POO in the ratio 4:1 while in control butters the ratio was 2:1. This indicates that environmental conditions (temperature) during seed development affect lipid composition and that the ratio of S_2U and SU_2 triglycerides is important in determining melting behavior of cocoa butter (S = saturated, U = unsaturated).

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FATS AND OILS STATUS IN FRANCE. A. UZZAN, Institut des Corps Gras, Paris, France.

Abstract not available at press time.

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METABOLISM OF (1-^{14}C) LINOLENIC ACID IN COHO SALMON, *ONCORHYNCHUS KISUTCH*. R.S. PARKER and R.O. SINNHUBER, Department of Food Science and Technology, Oregon State University, Corvallis, OR 97331.

Juvenile coho salmon were injected intraperitoneally with (1-^{14}C) linolenic acid, and sampled at 24, 120, and 240 hr. Liver, heart, and gill lipids were extracted, analyzed, and half-lives of individual liver phosphoglycerides and n-3 fatty acids determined from rates of loss of radioactivity. Incorporation of label into gill was much less than into either heart or liver. Inositol phosphoglycerides had the shortest half-life of all hepatic phospholipids. Total acyl half-life was shorter for the choline glycerophospholipids than for the ethanolamine glycerophospholipids, as were the half-lives of all individual n-3 fatty acids. Eicosapentaenoic acid (20:5n-3) had the shortest half-life in both phospholipids (50–60 hr), while docosapentaenoic acid (22:5n-3) and docosahexaenoic acid (22:6n-3) had much longer half-lives. Specific activities of the shorter chain n-3 fatty acids were much greater than the longer, more unsaturated homologs at all times, suggesting possible differences in their mechanisms of incorporation into phospholipids. Diacylglycerol analysis indicated that de novo synthesis could be responsible for the incorporation of only a small portion of the labelled long chain n-3 fatty acids found in phospholipids. The fatty acid half-lives reported here for salmon are in general agreement with those found previously in mammals, indicating that poikilotherms do not necessarily have reduced levels of lipid metabolism.

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MODIFIED METHODOLOGY FOR DETERMINATION OF FREE FATTY ACIDS AND IODINE VALUE OF SOLVENT-EXTRACTED FISH OILS AND LIPIDS. JEANNE D. JOSEPH and GLORIA T. SEABORN, National Marine Fisheries Services, Southeast Fisheries Center, Charleston Laboratory, PO Box 12607, Charleston, SC 29412.

Iodine value (IV) and free fatty acid (FFA) content are widely used to respectively evaluate the unsaturation and indicate the quality of fats and oils. In our study of lipids of Southeast Coastal Atlantic pelagic fishes, we were unable to obtain sufficient lipid from a single solvent extraction to carry out these determinations by official AOCS methods and we considered multiple or scaled-up extractions to be wasteful of time and expensive organic solvents. Our modified methodology is based on the chloroform-methanol extraction system of Bligh and Dyer, but incorporates the FFA titration method developed by Ke et al., and permits duplicate determinations of total lipid content, IV and FFA with as little as 3 g of lipid in chloroform solution. A sufficient amount of solution remains for the preparation of fatty acid methyl esters. The method was tested satisfactorily on a variety of model mixtures and natural oils and lipids.

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LIPID CLASSES AND FATTY ACIDS OF ATLANTIC HERRING ROE. J. KAITARANTA, C.A. EATON, and R.G. ACKMAN, Department of Fisheries and the Environment, Technology Branch, PO Box 550, Halifax, Nova Scotia, B3J 2S7, Canada.

On the Pacific coast of Canada a lucrative herring roe export industry has recently developed. On the Atlantic coast the poorly synchronized spawning periods hinder development of an efficient industry producing a quality product. An understanding of the development of lipid components as the roe matures and of lipid degradation patterns during storage, would permit better utilization of the Atlantic coast resource. The lipid content of herring roe ranges from 1.5 to 5.1% of wet weight. In the phospholipid, which is an important lipid fraction, the pentaenoic and hexaenoic PUFA total about 40% of the fatty acids. These acids are primarily responsible for the rancidity arising from autoxidation, one of the major problems in the roe industry. In this study the lipid and fatty acid compositions of Canadian Atlantic herring roe will be compared with those of herring roe from the low salinity Baltic Sea. The methodology includes lipid class determinations by Chromarod thin layer chromatography on the Iatroscan and fatty acid analysis by gas liquid chromatography on glass WCOT columns.

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A COMPARISON OF THE FATTY ALCOHOLS AND ACIDS OF COPEPOD LIPIDS WITH THOSE OF ATLANTIC HERRING, MACKEREL, AND CAPELIN. W.N. RATNAYAKE, and R.G. ACKMAN, Fisheries and Oceans Canada, Technology Branch, PO Box 550, Halifax, Nova Scotia, B3J 2S7, Canada.

The fatty alcohols and acids found in commercial oils or muscle and skin lipids of Atlantic herring, mackerel, and capelin have been compared with lipid from a mixture of the copepods presumed to be the major dietary source of the lipids in these fish. The proportions of the positional isomers of the 22:1 alcohols and acids were studied by oxidative fission and open-tubular gas liquid chromatography. This study demonstrated that the unusual ω11 structure dominating the 22:1 fatty acid isomer mixture in most marine fish and mammals is actually derived from the corresponding 22:1ω11 fatty alcohol of the copepod. The fatty alcohols included many minor components structurally similar to those found in marine animal fatty acids (e.g., *iso* and *anteiso* 15:0 and 17:0), and the basic PUFA of plant origin, 18:2ω6 and 18:3ω3, were represented by only about 1% of the corresponding fatty alcohols, proportions also found in most mixtures of marine fatty acids. The C_{20} and C_{22} polyunsaturated alcohols were present at very low levels except for erratic proportions of 22:6ω3 at up to 11%. The fatty acids of the wax esters of the copepods were dominated by 14:0 at 36%, but the capelin wax ester acids had exceptionally high levels (40% for body origin) of 20:5ω3, but only normal levels (2–5%) of 14:0.

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ALTERATIONS IN LONGER CHAIN FATTY ACIDS OF FISH OIL DURING PARTIAL HYDROGENATION. J-L. SEBEDIO, R.G. ACKMAN, and M.F. LANGMAN, Department of Fisheries and the Environment, Technology Branch, PO Box 550, Halifax, Nova Scotia, B3J 2S7, Canada.

Samples were collected at intervals during the commercial hydrogenation, with nickel catalyst, of fish oil from an IV of 119 to an IV of 79. The methyl esters of the C_{20} and C_{22} fatty acids were recovered from preparative gas liquid chromatography (GLC) and were fractionated by $AgNO_3$-thin layer chromatography (TLC). All stages of processing and isolation were monitored by quantitative open-tubular GLC. *Cis* and *trans* monoethylenic C_{20} and C_{22} fatty acids were further examined for ethylenic bond position by oxidative fission. The C_{20} and C_{22} natural PUFA with 4, 5, and 6 ethylenic bonds were quickly eliminated in the course of the reduction. Artifact trienes (mostly nonmethylene-interrupted) increased to 4.0% at IV 101 and then decreased to 2.7% at IV 79, while artifact dienes increased to 4.3% at IV 101 and to 11.5% at IV 79. There was only a marginal increase in the C_{20} and C_{22} saturated and total monoethylenic acids during the hydrogenation. At IV 79 36% and the 20:1 and 29% of the 22:1 isomers had been converted to *trans*. The original oil (IV 119) contained 85% of total 20:1 as *cis* ω9, but at IV 79 there remained only 50% *cis* 20:1ω9 with 15% *trans* 20:1ω9. Similarly for the 22:1, the original oil contained 92% *cis* ω11, while at IV 79

there remained only 61% *cis* with 15% *trans* ω11. During *trans* monoethylenic isomer formation, extensive positional isomerization occurred. At IV 79, the major C_{22} monoethylenic isomers formed were *cis* and *trans* 22:1ω10, respectively, 2.7 and 5.8% of total 22:1, and 22:1ω12, respectively, 2.6 and 5.0% of total 22:1. A similar migration of the ethylenic bonds was observed for the 20:1 isomers, where *cis* and *trans* ω8 and ω10 were formed in amounts corresponding to those observed for the 22:1 isomers. Extensive formation of new 20:1 and 22:1 does not take place during the partial hydrogenation of clupeid oils under the conditions employed in Canada, and in the product, proportions of 20:1 and 22:1 are close to those of the initial composition, for example, 14 and 21%, respectively, in the oil discussed above. In menhaden oil the proportions of 20:1 and 22:1 are much lower, usually in the range 1–4 and 1–2%, respectively. Correspondingly, the proportions of 20:5 and 22:6 are usually about 10–12 and 5–10%, respectively, or twice those of clupeid oils. The physical properties of the partially hydrogenated menhaden oil will therefore be determined by the artifact fatty acids derived from 20:5 and 22:6 and their position in the triglyceride molecule. Comparisons of the compositions of menhaden oils from the Atlantic coast and from the Gulf of Mexico will be made for the years 1976, 1977, and 1978, and will provide a basis for selecting artifact fatty acid groups for detailed study.

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ALKOXY-ACYL COMBINATIONS IN THE WAX ESTERS FROM WINTERIZED SPERM WHALE OIL. GAYLAND F. SPENCER, Northern Regional Research Center, USDA, 1815 N. University, Peoria, IL 61604.

Wax esters from commercial, winterized, sperm whale oil were separated by degree of unsaturation. The specific combinations of fatty alcohol and fatty acid moieties making up each of the wax esters were determined by gas chromatography-mass spectrometry. Saturated wax esters made up 6% of the oil, and hexadecanyl dodecanoate (1.3%) was the most abundant of these components. Among the monoethylenic wax esters, which totalled 44% of the oil, hexadecanyl octadecenoate (6%) and hexadecanyl hexadecenoate (5%) were prevalent. Dienoids were the principal wax ester class (50%) with octadecenyl octadecenoate (14%), octadecenyl hexadecenoate (10%), and octadecenyl icosenoate (6%). Over 240 different wax esters were detected and quantitated.

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JOJOBA OIL AS A REPLACEMENT FOR SPERM WHALE OIL. THOMAS K. MIWA, Northern Regional Research Center, USDA, 1815 N. University, Peoria, IL 61604.

Jojoba (*Simmondsia chinensis* or *S. californica*) oil is the only known natural source of large quantities of liquid wax esters other than whale oil from the endangered species *Physeter macrocephalus* (*P. catodon*). Primarily composed of esters of long chain C_{20} and C_{22} acids and alcohols, jojoba oil resembles winterized sperm whale oil in physical and chemical constants. Jojoba oil contains little or no triglyceride as compared to 30% triglycerides in sperm whale oil. Jojoba acids and alcohols are linear and uniformly ω-9 in their *cis* unsaturation, whereas the acids and alcohols in sperm whale oil are diversified in structure, including methyl-branched structures and a number of positionally isomeric ethylenic bonds. Simulated in-use lubricant tests on sulfurized jojoba and sperm whale oils indicate the great potential for jojoba as an industrial oil for use in extreme-pressure lubricants.

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THE EFFECT OF IN VIVO ALTERATION OF ADIPOSE TISSUE LIPID COMPOSITION UPON THE IN VITRO LIPOLYTIC RESPONSE TO NOREPINEPHRINE. ATIF B. AWAD and E. ANDREW ZEPP, Department of Biochemistry, Kirksville College of Osteopathic Medicine, Kirksville, MO 63501.

The present study clearly shows that, by feeding rats a semisynthetic diet of known composition enriched with saturated fatty acids, the epididymal fat pad responsiveness to norepinephrine in vitro can be abolished relative to fat pads from animals fed a similar diet but enriched with polyunsaturated fatty acids. Addition of varying concentrations of norepinephrine to the incubation medium produced a clear dose-response relationship in fat pads from animals fed diet enriched with polyunsaturated fatty acids while no effect of norepinephrine was apparent at any dose level in fat tissue from animals fed saturated fatty acids. These changes in lipolytic responsiveness were concurrent with alterations in fatty acid compositions of adipose tissue phospholipids and triglycerides as well as in total tissue contents of phospholipids and cholesterol.

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RESPONSE OF LINOLENIC ACID-DEFICIENT RATS TO DIETARY CHOLINE DEFICIENCY: STUDIES ON THE ROLE OF LINOLENIC ACID IN CHOLINE BIOSYNTHESIS IN FEMALE RATS. J. TINOCO, G. ENDEMANN, B. MEDWADOWSKI, and P. MILJANICH, Department of Nutritional Sciences, University of California, Berkeley, CA 94720.

In livers of choline-deficient rats, there is often an accumulation of 22:6n-3 phosphatidyl ethanolamines (PE), perhaps due to reduced transmethylation of PE to form phosphatidyl cholines (PC). If so, it would mean (a) that 22:6n-3 PE are the preferred substrates for PC formation by this path, and (b) that 22:6n-3 PE are required precursors for the biosynthesis of choline in rats. If this essential substrate were removed, then a secondary choline deficiency should be induced. Therefore, we reduced the content of 22:6n-3 in liver PE by feeding rats for two generations on a diet that contained 1.25% methyl linoleate as the only source of lipid (linolenate-deficient group). Control rats were given diets with 0.25% methyl linolenate plus 1.0% methyl linoleate (linolenate-supplemented group). These rats were then transferred to low methionine, choline-deficient diets (or choline-supplemented diets) in which the lipid compositions remained the same. Lipids of liver, plasma, brain, and muscle were fractionated and analyzed. Linolenate-deficient and linolenate-supplemented rats responded equally to the low methionine, choline-deficient diets in that both groups accumulated approximately equal concentrations of triglyceride and esterified cholesterol in their livers. The similarities of the responses suggested that 22:6n-3 does not have a specific role in the biosynthesis of choline in female rats. Unexpectedly, we found that dietary linolenic acid deficiency did reduce plasma triglyceride concentrations in both choline-deficient and choline-supplemented rats. (This work was supported in part by USPHS grant AM 10166 and by Cooperative Regional Project NC-95, California Agricultural Experiment Station.)

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THE EFFECTS OF PEROXIDE AND THYROID HORMONES ON ERYTHROCYTE MEMBRANES. SUSHIL K. JAIN and PAUL HOCHSTEIN, University of Southern California, School of Medicine, 2025 Zonal Ave., Los Angeles, CA 90033.

The vital role of lipids as components of biological membranes and their contribution to cellular integrity at both structural and functional levels are well known. In the present experiments we have examined the effects of thyroxine (T_4) in potentiating red blood cell (RBC) membrane lipid damage in the presence of low level, steady state amounts of hydrogen peroxide. For these purposes an H_2O_2 generating system (0.1 μmoles/min) consisting of xanthine oxidase and hypoxanthine was employed. Using these conditions there was no measurable alteration in the peroxidation of membrane phospholipids as determined by the appearance of fluorescent polymers, in the activities of ATPase and acetylcholine-esterase associated with phospholipids or in the volume of treated cells. However, if T_4 was present during the incubations at a concentration of 0.1 μM, lipid peroxidation and the partial inactivation of the membrane-bound enzymes was observed. These changes were accompanied by increase in cellular volume which lead to hemolysis in isotonic media. Of the membranes phospholipids only phosphatidyl serine was markedly decreased. Treatment of cells with T_4 alone caused no changes in any of the above parameters. The alterations were also not observed when 6.0 μm butylated hydroxyanisole was included in the medium along with the H_2O_2-generating system and T_4. The data obtained are consistent with the view that the cytotoxic effects of H_2O_2 are potentiated by thyroid hormones through reactions which involve their deiodination at the lipid sites, the formation of iodide radicals, and the initiation of peroxidation reactions in endogenous membrane phospholipids. Such a series of reactions may account for the loss of specific membrane lipids (e.g., phosphatidyl serine), the inactivation of membrane enzymes and, hence, the volume changes observed. (Supported by NIH grant no. 19615.)

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BIOLOGICAL, QUANTITATIVE, ULTRASTRUCTURAL, AND BIOCHEMICAL STUDIES ON ISOLATED RAT HEARTS FED WITH ERUCIC ACID. H.M. MANZ, H.H. HILLER, Institut für Physiologie, Freie Universität Berlin, FB 01, WE 02, Arnimallee 22, D-1000 Berlin 33, West Germany; K. STAHL, and H. THEMANN, Univers. Münster, West Germany.

Under the various documented effects of erucic acid, the most attention is drawn to the histological and biological changes of animal rat hearts. Feeding high erucic acid concentration leads to a growth retardation in those animals. These observations led to a limitation of erucic acid in foodstuff (e.g., margarines, vegetable oils) to 5%. To investigate the effect of 4% erucic acid in a diet on growth rate, histology, and function of rat heart cell membranes, we fed a control and an erucic acid diet. After 14 weeks feeding period we measured the body weight; the surface density of mitochondrias, the numerical density of mitochondrias, the volume fraction of cardiac muscle cell nuclei, capillaries and myocardial fibers were estimated by stereological methods. To determine the functional integrity of the heart cell membranes, we measured the CPK (Creatinphosphokinase) enzyme release of the isolated rat heart 20 min after a total ischeamia. The enzyme release indicated the myocardial cell damage. All animals did not show any difference in growth rate and body weight after 14 weeks. The quantitative ultrastructural studies show an increase of the mitochondrial volume fraction and an increase in the surface density per cm^3 heart muscle cell. The area of cristae related to the unit myocardial cell volume amounted to 2.6 m^2/cm^3 for the control group and to 4.3 m^2/cm^3 for the diet fed group. Related to the unit mitochondrial volume the cristae membrane concentration is increased from 10.4 to 13.4 m^2/cm^3. The biochemical data show a marked increase of CPK enzyme release after an ischeamic period of 20 min of those hearts fed with erucic acid indicating an increased fragility of cell membranes and an impaired ATP/energy supply. In spite of various investigations, it is not clear whether an increase of the surface density of the mitochondrial cristae coincides with an increase in enzyme activity. Our results show a negative correlation between increased surface density of mitochondrial cristae and energy supply by the mitochondrias. Addition of erucic acid to food, even at a low concentration of 5%, could be harmful to the mitochondrial function and should be carefully reinvestigated. (H.H.H. supported by Deutsche Forschungsgemeinschaft.)

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SURFACE VISCOSITIES OF PHOSPHOLIPID AND CHOLESTEROL MONOLAYERS AS MEASURED BY THE OSCILLATING PENDULUM METHOD. RHOBERT W. EVANS, J. TINOCO, and M.A. WILLIAMS, Department of Nutritional Sciences, University of California, Berkeley, CA 94720.

The viscosity of a membrane or membrane model system is a measure of the mobility of the molecules in the system. Therefore we have measured, by the oscillating pendulum method, surface viscosities in monolayers of various phospholipids and of cholesterol. Viscosities were expressed as the rate of damping of oscillations of a pendulum by a lipid monolayer relative to the rate of damping produced by a clean water surface. Viscosities were measured as a function of surface pressure and molecular area at 22 ± 2 C. Lipids investigated included cholesterol, 1-palmitoyl-2-arachidonoyl-phosphatidyl choline (16:0–20:4n-6 PC), 1-palmitoyl-2-stearoyl-phosphatidyl choline (16:0–18:0 PC), and the dipalmitoyl species of phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl dimethylethanolamine (PDME), phosphatidyl monomethylethanolamine (PMME), phosphatidyl glycerol (PG), phosphatidic acid (PA), and bis-phosphatidic acid (BPA). Results are summarized as follows: (a) Surface viscosities (increased damping of oscillations) of cholesterol and of 16:0–20:4n-6 PC monolayers were low and barely measurable under our conditions. (b) All fully saturated phospholipid monolayers had high surface viscosities which increased with increasing surface pressure.

Surface viscosities decreased approximately in order: di 16:0 PA $>$ di 16:0 PE $>$ di 16:0 PG $>$ di 16:0 PMME $>$di 18:0 PC $>$ di 16:0 PDME $>$ 16:0 BPA $>$ 16:0–18:0 PC $>$ di 16:0 PC. (c) Addition of 1 mole % or more of cholesterol to di 16:0 PC profoundly reduced its surface viscosity. (This work was supported by USPHS grant AM 12024.)

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BLOOD PLASMA LIPOPROTEIN AND TISSUE CHOLESTEROL OF CALVES FED SOYBEAN OIL, CORN OIL, VEGETABLE SHORTENING, OR TALLOW. MARLENE RICHARD, NORMAN JACOBSON, KENNETH WIGGERS, JEANNE STEWART, and THOMAS HEEG, 313 Kildee Hall, Iowa State University, Ames, IA 50011.

Soybean oil, corn oil, vegetable shortening (Crisco®), and beef tallow were fed to four respective groups of 2-week-old suckling calves in reconstituted milk formulae which supplied 30% of calories from fat. Plasma cholesterol was monitored weekly. At 0, 5, 10, and 15 weeks of the experiment, plasma lipoproteins were separated into VLDL, LDL, HDL, and VHDL fractions by ultracentrifugation; cholesterol concentration was determined in each fraction. After 15 weeks the animals were slaughtered and samples of liver, muscle, fat, and brain were removed for cholesterol analysis. The blood plasma cholesterol concentration increased in all groups over time. The increase was least for the tallow group; and increases were much greater for the soybean oil and corn oil groups than for the Crisco® and tallow groups. Increases in plasma cholesterol concentration were associated with the LDL fraction. Data from plasma lipoprotein and tissue analyses will be presented. (Supported in part by the Iowa Beef Industry Council.)

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EFFECTS OF DIETARY LIPID ON THE LEVELS OF FREE AND ESTERIFIED PLASMA CHOLESTEROL IN THE MONGOLIAN GERBIL. NINA J.H. MERCER and BRUCE J. HOLUB, Department of Nutrition, University of Guelph, Guelph, Ontario N1G 2W1, Canada.

Various governmental agencies have recommended dietary changes which include lowering the total fat intake and increasing the percentage of polyunsaturated fatty acids in the fat with the expectation that these modifications would result in a reduced plasma cholesterol level. In order to confirm the suitability of the Mongolian gerbil as a test animal for examining the effects of these recommended changes on plasma cholesterol levels, the response of this animal to different types of dietary lipid was studied. After feeding a basal diet, animals were maintained for 4 weeks on semipurified diets containing 40 calorie % in fat, 14% in protein, and 46% in carbohydrate (starch:sucrose ratio of 2:1). Precautions were taken to normalize the content of cholesterol and plant sterol in all diets which contained lard, safflower oil, or a mixture of tallow: safflower oil (39:1) as the dietary lipid. At all sampling times (1, 3, and 4 weeks), the mean plasma levels of total, free, and esterified cholesterol were lowest in the animals fed diets containing safflower oil; the corresponding values were 12–85% greater in gerbils fed lard and 60–151% greater in those ingesting tallow:safflower oil (39:1). The free cholesterol levels consistently exhibited a more dramatic response to dietary lipid than the esterified cholesterol. These and other results support the suitability of this particular experimental model for studying the regulatory effect of dietary lipid on plasma cholesterol levels. (Supported by the Ontario Heart Foundation.)

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DETERMINANTS OF PLASMA TRIGLYCERIDES AND VERY LOW DENSITY LIPOPROTEINS CHOLESTEROL IN THE MRFIT STUDY. LEWIS H. KULLER, ARLENE CAGGIULA, University of Pittsburgh, Graduate School of Public Health, 130 DeSoto St., Pittsburgh, PA 15261; W. MCFATE SMITH, Institutes of Medical Sciences; NORMAN LASSER, College of Medicine and Dentistry of New Jersey; STEPHEN HULLEY, Stanford University; and RICHARD GRIMM, University of Minnesota.

The participants in the Multiple Risk Factor Intervention Trial (MRFIT) have had plasma triglycerides measured at baseline and yearly thereafter. Plasma lipoproteins and triglycerides were measured after a 12 hr fast. If the triglycerides were less than 300 mg % (91% of cases) very low density lipoprotein (VLDL) cholesterol was estimated by dividing the triglycerides by 5, triglycerides $>$300 mg (9%) by ultracentrifugation. The mean plasma triglyceride at baseline was 193 mg %, median 158 mg %. The plasma triglyceride levels were directly correlated with the body mass index, wt/ht^2 increasing from a mean of 149 (BMI 23) to 225 (BMI 32). The plasma triglycerides also increased with the number of alcohol drinks per day. Individuals who were nonalcohol drinkers had a plasma triglyceride of 179, those who had more than 35 drinks per week 219 mg %. There was no consistent relationship between any other nutrients and baseline triglyceride levels. There was no consistent change in triglyceride levels between baseline and the year 2 examination. Individuals who gained weight had an increase and those that lost weight a decrease in triglyceride levels. Participants who were on thiazide diuretics at baseline had higher triglyceride levels. About half of the special care participants were placed on thiazide diuretics between baseline and year 2. Those who were placed on thiazide had an increase in triglycerides and VLDL cholesterol as compared to those not on thiazide diuretics. The effect was present even after adjustment for weight change. Changes in alcohol consumption were only modestly related to change in triglycerides.

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CONTRIBUTIONS OF WEIGHT LOSS TO CHANGES IN LIPOPROTEIN CHOLESTEROL LEVELS IN THE MULTIPLE RISK FACTOR INTERVENTION TRIAL (MRFIT). NORMAN L. LASSER, College of Medicine and Dentistry of New Jersey, 100 Bergen St., Newark, NJ 07103; STEPHEN B. HULLEY, Institutes of Medical Sciences; ELIZABETH D. MUNVES, College of Medicine and Dentistry of New Jersey; JEREMIAH STAMLER, Northwestern University Medical School; and ROGER SHERWIN, University of Maryland.

The Multiple Risk Factor Intervention Trial (MRFIT) is a randomized clinical trial in 12,866 men designed to test the hypothesis that lowering the three major risk factors for coronary heart disease (CHD) will reduce the incidence of CHD deaths in the coronary-prone men participating in the study. All of the 6000+ Special Intervention (SI) men in MRFIT were advised to follow a cholesterol-lowering diet in which the lipid composition was modified and, in those overweight, the total calories reduced. Plasma lipids were measured at baseline and annually thereafter, while cholesterol levels in lipoprotein fractions were measured at the baseline and 2nd annual examinations. Data from both the 1st and 2nd annual follow-up examinations in SI men show an association between weight change and each of the lipid and lipoprotein cholesterol classes. Over the 2-year period, HDL cholesterol (C-HDL) change correlated negatively with weight change, ranging from a ΔC-HDL of -0.9% (weight gain $\geq$4 lb) to one of $+6.6\%$ (weight loss $\geq$10 lb). ΔC-VLDL was positively correlated with Δweight, ranging from -16.2% (weight loss $\geq$10 lb) to $+17.2\%$ (weight gain Δ4 lb); and ΔC-LDL was similarly related, ranging from -3.4% (weight stable) to -8.3% (weight loss $\geq$10 lb). There was a strong association between weight change and serum cholesterol response, regardless of initial cholesterol level. The fall in cholesterol was also associated with better adherence to the fat-modified diet as judged from the 24-hour dietary recall data, but the observed cholesterol fall exceeded that predicted from reported compositional change by more than 50% in those losing $>$10 lb, regardless of baseline triglyceride. In multivariate analysis, this effect of weight loss was also found to be independent of alcohol use, smoking habit, blood pressure, and use of antihypertensive drugs. These data support the conclusion that weight change is an important determinant of changes in lipoprotein cholesterol levels for men on a fat-modified diet. Moderate weight loss contributes to serum cholesterol and C-LDL reduction in men following a fat-modified eating pattern, even when they are normotriglyceridemic.

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ABNORMALITIES OF SERUM LIPOPROTEINS IN ABETALIPOPROTEINEMIA. HERBERT J. KAYDEN, Department of Medicine, New York University School of Medicine, 550 First Ave., New York, NY 10016.

Abetalipoproteinemia is a rare autosomal recessive disease with multiple system clinical manifestations. Observations in many of the affected patients document that some of these clinical features are due to malabsorption of essential nutrients, and supplementation with fat soluble vitamins appears to alter a number of the described symptoms. The importance of tocopherol to musculo-skeletal and central nervous system development can be identified. Although the absence of β apoprotein is a cardinal feature of the disease, with the absence of chylomicrons, very low density lipoprotein (VLDL) and low density lipoprotein (LDL) in the plasma, there are also abnormalities in high density lipoproteins (HDL). Apolipoprotein E is present in the plasma of several patients, and may account for the suppressive role of HDL in the activity of HMG-CoA reductase in lymphoid cell lines. The plasma lipoproteins in patients with the rarer form of abetalipoproteinemia, i.e., where both parents have the autosomal dominant disorder of hypobetalipoproteinemia, are to be compared with the more frequently described cases of abetalipoproteinemia, where both parents are apparently normal.

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NORMOTRIGLYCERIDEMIC ABETALIPOPROTEINEMIA: A CLINICAL SYNDROME ASSOCIATED WITH ABNORMAL APOLIPOPROTEIN B. MARY J. MALLOY, JOHN P. KANE, D.A. HARDMAN, Cardiovascular Research Institute, University of California, San Francisco, CA 94143; and KANU B. DALAL, Heart Research Institute, Presbyterian Medical Center, San Francisco, CA.

A nine-year-old female was found to have serum cholesterol levels from 23 to 37 mg/dl. Chylomicrons and lipoproteins of alpha and prebeta mobility were seen on electrophoresis in agarose gel, but no beta lipoproteins have been noted during one year's observation. Serum triglycerides, fasting, vary from 20 to 35 mg/dl. The composition of lipoproteins separated from postprandial serum by preparative ultracentrifugation was:

		Percent by mass					
Fraction[a]	mg/dl	PL	FC	CE	TG	X	Protein
$d < 1.006\ g/cm^3$	96	9.2	3.6	5.3	70.4	4.0	7.8
$1.006 < d < 1.063$	1	22.4	4.0	44.0	7.2	2.4	20.0
$1.063 < d < 1.21$	74	18.1	2.6	21.4	2.0	2.8	53.1

[a] PL, phospholipid; FC, free cholesterol; CE, cholesteryl ester; TG, triglyceride; X, unidentified polar lipid, probably hydroperoxidized. Increased sphingomyelins were noted in all fractions.

Following 100 g oral fat loads serum triglycerides rose as high as 90–190 mg/dl and serum contained spherical lipoprotein particles of 400–2000 Å diameter, but no increase in protein or lipid occurred in the $1.006 < d < 1.063\ g/cm^3$ (LDL) interval. No malabsorption of TG occurred and biopsied intestinal mucosa was normal 24 hr after fat load. Serum triglyceride levels also increased with carbohydrate feeding suggesting normal secretion of very low density lipoproteins (VLDL). The patient is obese, has ataxia, mild acanthocytosis, and moderate developmental retardation. Serum tocopherol levels are immeasurably low. Vitamin A levels are normal and serum carotenes moderately low. Electron microscopy of the lipoproteins of the $1.006 < d < 1.063$ interval revealed sparse 300 Å spheres showing core and surface regions and cuboidal forms of 200 Å diameter. Total immunoreactivity of plasma corresponds to only 1 mg/dl of apo B although 35% of the $d < 1.006$ apoprotein is insoluble in 4.2 M tetramethylurea. The $d < 1.006\ g/cm^3$ lipoproteins are precipitated by heparin and Mn^{++}. Apo B from that fraction is completely soluble in 8 M urea, differs in amino acid composition from normal apo B, and appears as a single band on SDS gel electrophoresis corresponding to a MW of 2.5×10^5, clearly smaller than that of the principal band of normal apo B as determined by the Ferguson technique. These abnormalities could represent either a partial deletion mutation or absence of an apo B subunit protein. In the presence of this defect secretion of VLDL and chylomicrons proceeds, but the formation of normal LDL from these precursors is virtually abolished.

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TYPE III HYPERLIPOPROTEINEMIA: IMPLICATIONS FOR THE ROLES OF ISOAPOLIPOPROTEIN E_3 AND ESTROGENS IN NORMAL LIPOPROTEIN HOMEOSTASIS. WILLIAM R. HAZZARD, 465 Harborview Hall, 326 Ninth Ave., Seattle, WA 98104.

Type III hyperlipoproteinemia is characterized by increased levels of cholesterol-rich chylomicrons (chylos) and β-migrating very low (VLDL) and intermediate density lipoproteins (IDL), while low density lipoproteins (LDL_2, d 1.019–1.063) are decreased, implying defective catabolism of chylos and VLDL. These VLDL are abnormally rich in total apo-E, but lack isoapolipoprotein E_3 (with a relative increase in E_2). This E_3 lack is genetically determined by an autosomal co-dominant mechanism; expression of this deficiency as type III requires co-inheritance of an independent cause of hyperlipidemia, usually familial combined hyperlipidemia. I*-VLDL apo-B turnover studies have confirmed retarded, incomplete conversion of both autologous and normal VLDL (from type IV donors) to LDL in untreated subjects with type III. Triglyceride (TG) removal related to heparin-releasable enzymes is moderately retarded, kinetic analysis suggesting inefficient lipase-TG interaction (increased apparent Km) but normal maximal removal capacity. The latter has been confirmed by direct measurement of adipose tissue lipoprotein lipase (LPL) which was normal; furthermore, postheparin hepatic and extrahepatic TG lipases were normal. Estrogen exerts a paradoxical, hypolipidemic effect in type III, tending to normalize VLDL composition, catabolic conversion to LDL, and TG removal; however, while total apoE declines, E_3-deficiency persists. Clofibrate also facilitates VLDL-LDL interconversion while markedly reducing lipid levels. Finally, two family members with coincident E_3 deficiency and familial hypercholesterolemia (FH) (and presumptive apo-B receptor deficiency) became normal with clofibrate, while those members with FH who were heterozygous for E_3 deficiency did not. Hence, estrogens and clofibrate may facilitate the clearance of E_3-deficient remnants by a mechanism independent of both E_3-dependent and apo-B receptors.

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THE METABOLISM OF VERY LOW DENSITY LIPOPROTEINS AND LOW DENSITY LIPOPROTEINS IN NORMAL AND DYSLIPOPROTEINEMIC MAN. ERNST J. SCHAEFER, LOREN A. ZECH, and H. BRYAN BREWER, JR., Molecular Disease Branch, National Heart, Lung & Blood Institute, Bethesda, MD.

We investigated the plasma kinetics of radiolabeled very low density lipoprotein (VLDL) and low density lipoprotein (LDL) apolipoprotein (apoB) in four normal subjects (N) and in four patients with homozygous familial hypercholesterolemia (FH). FH is characterized by premature atherosclerosis, tendinous xanthomas, fibroblast lipoprotein receptor abnormalities, and markedly elevated plasma cholesterol levels. Plasma, urine, and whole body radioactivity were monitored for 14 to 21 days. Mean LDL apoB synthesis was 22.51 mg/kg/day in FH and 8.53 mg/kg/day in N. The mean fractional catabolic rate (FCR) for LDL apoB was .19/day in FH and .46/day in N. In N almost all LDL apoB was derived from VLDL while in FH over 50% of LDL apoB synthesis was not derived from VLDL. In addition we studied VLDL apoB metabolism before and during estrogen administration (ethinyl estradiol 0.1 mg/day) (E) in five normal females. During E administration plasma cholesterol and triglyceride increased 17% and 85%, respectively, and VLDL apoB and VLDL triglyceride increased 16% and 75%, respectively. No significant change in VLDL apoB FCR was noted during E administration. These data are consistent with the concept that E administration causes increased VLDL apoB synthesis, and that FH homozygotes have increased and aberrent apoB synthesis as well as decreased catabolism.

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OXANDROLONE CAUSES DISAPPEARANCE OF ApoE FROM VERY LOW DENSITY LIPOPROTEINS IN TYPE V HYPERLIPOPROTEINEMIA. JOSEF R. PATSCH, TSUMOTU HARA, and ANTONIO M. GOTTO, JR., Baylor College Medicine and The Methodist Hospital, 6516 Bertner Blvd., Mail Station A601, Houston, TX 77030.

We have previously demonstrated that clofibrate can normalize plasma triglyceride (TG) levels in hypertriglyceridemic individuals by decreasing very low density lipoprotein (VLDL) plasma concentrations without altering significantly their lipid and apoprotein composition. This effect of clofibrate is accompanied by increased levels of high density lipoprotein (HDL_2) which has been shown in vitro to originate from HDL_3 and degradated VLDL. These effects of clofibrate can be reasonably explained by the documented fact that this drug enhances lipolysis of VLDL. Because reports on the mode of the hypotriglyceridemic action of the synthetic steroid, oxandrolone, are much more conflicting, we studied the effects of this drug on a patient with marked hypertriglyceridemia. Before drug therapy was initiated, a 38-year-old male type V hyperlipoproteinemic had a plasma cholesterol (Ch) level of 233 mg/dl and a plasma triglyceride (TG) level of 608. Of seven VLDL subfractions isolated by zonal ultracentrifugation, those of $S_f > 200$ were the most abundant species. Each of the subfractions was unusually rich in apoE but contained apoB, apoC-I, apoC-II, and apoC-III as well. Intermediate density lipoprotein (IDL) and low density lipoprotein (LDL) had normal chemical compositions, they contained apoB but only traces of apoE and apoC. HDL-Ch was 28 mg/dl with the HDL_2 subfraction entirely absent and the HDL_3 containing an abnormally high proportion of TG (10%) and a low proportion of cholesteryl ester (CE) (3%). HDL_3 exhibited an abnormal absence of apoC proteins. After administration of 2.5 mg oxandrolone per day for one week, plasma-TG changed to 447 and the plasma-Ch to 295. All VLDL subfractions dropped significantly, with those of S_f 60–100 becoming the dominant ones. Similarly, IDL and LDL were decreased. HDL-Ch was unchanged and HDL_2 remained absent. HDL_3 increased slightly in density due to the increase of its protein content to 57%. Subsequent administration of 7.5 mg oxandrolone per day for one week produced even further changes. Plasma-TG dropped to 222 and plasma-Ch remained unchanged. With this therapy, all VLDL subfractions dropped drastically with no apparent changes in their chemical composition. Of the VLDL still present, those fractions of S_f 50–150 were the most abundant. A remarkable drop of apoE to undetectable levels in each of the VLDL subfractions was observed, while apoB- and apoC-proteins remained present. IDL and LDL levels and composition were not remarkably altered. HDL-Ch decreased to 21 mg/dl, HDL_2 remained undetectable, and HDL_3 protein content increased further to 61%, resulting in increased hydrated density of the particle. This increase in apoprotein content of HDL_3 was not caused by the appearance of apoE. The previous abnormally high TG content of HDL_3 fell to normal levels and was accompanied by a rise of the CE to its normal level. In summary, the oxandrolone caused a sharp decrease of VLDL levels with a drastic alteration of VLDL apoprotein composition. However, this was not accompanied by a normalization of HDL levels and distribution, particularly the appearance of HDL_2. We conclude that the action of oxandrolone on VLDL metabolism is fundamentally different from that of clofibrate and may involve altered hepatic secretion or rapid removal by a mechanism other than intravascular TG hydrolysis.

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DETOXIFICATION OF ZEARALENONE-CONTAMINATED CORN. G.A. BENNETT, O.L. SHOTWELL, and C.W. HESSELTINE, Northern Regional Research Center, USDA, 1815 N. University, Peoria, IL 61604.

Several chemical and physical treatments were investigated as possible methods to remove or destroy zearalenone contamination in corn. An ammoniation process, which significantly lowers aflatoxin levels in corn, had no effect on zearalenone contamination in yellow corn. Also, treatments of propionic acid, acetic acid, hydrochloric acid, sodium bicarbonate, and hydrogen peroxide failed to reduce zearalenone levels. High temperature treatment (150 C) had no effect on zearalenone in ground corn. Formaldehyde, in vapor form from paraformaldehyde crystals or as aqueous solutions, destroyed significant quantities of zearalenone in naturally contaminated yellow corn and spiked animal feed. Samples treated with aqueous formaldehyde (0.75% w/w) should be dried at 50 C or more to cause effective destruction of zearalenone. Levels as high as 10.0 ppm zearalenone in animal feed and 8.0 ppm in ground yellow corn were reduced to less than 0.50 ppm with formaldehyde. Zearalenone contamination in whole kernel corn was not significantly reduced with formaldehyde.

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A SENSITIVE SCREENING METHOD FOR ZEARALENONE IN CORN. CHARLES E. HOLADAY, National Peanut Research Laboratory, USDA, PO Box 637, Dawson, GA 31742.

A sensitive screening method for zearalenone in corn based on minicolumn chromatography has been developed. The sample is extracted with methanol and water. The extract is cleaned up by precipitating most of the interfering materials out of the extract with a heavy metal salt. The zearalenone is extracted from the salt solution with toluene. A portion of the toluene layer is pipeted onto the minicolumn, and the zearalenone is washed down onto the interface of the florisil and alumina adsorbents in the minicolumn with a hexane-acetone solution. The column is placed under a high intensity long-wave UV light source, and if zearalenone is present, a blue fluorescent band at the interface of the florisil and the alumina can be seen. Sensitivity of the method is about 40 μg/kg. The presence of aflatoxin does not cause false positives because it is held up on the florisil layer. Time for completion of a test is about 10 min and costs in chemicals and equipment are minimal.

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CRYSTALLIZATION BEHAVIOR OF HIGH ERUCIC ACID RAPESEED OIL. KINICHI KAWAMURA, Best Foods Research and Engineering Center, 1120 Commerce Ave., Union, NJ 07083.

DSC isothermal analysis was used to investigate the crystallization behavior of high erucic acid rapeseed oil (HEAR oil), in conjunction with usual cooling/heating methods. The crystallization of HEAR oil was found to be two-staged: (a) the crystallization of the α-form and (b) its transformation to the β-form under isothermal cooling conditions. The α-form can also be transformed to the β-form under constant heating rate conditions, e.g., +10°K/M, but this transformation is governed by the rate of heating. The crystallization behavior of HEAR oil appears to be dominated by its characteristic triglyceride composition as the transformations were completely altered by random interesterification. Further, when HEAR oil was blended with soybean oil (SBO), the change in crystallization behavior was proportional to the increased amount of SBO. It may be possible to apply this method to investigate HEAR oil crystallization in oil and water emulsions. Preliminary studies indicated that the α-form did not cause emulsion breakdown but the transformed β-form, which might be the expanded crystal form, did.

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SYNTHESIS OF 7-, 8-, 9-, AND 10-OXO-16-HYDROXYHEXADECANOIC ACIDS AND OF 7,16-, 8,16-, 9,16-, AND 10,16-DIHYDROXYHEXADECANOIC ACIDS. A.P. TULLOCH, Prairie Regional Laboratory, National Research Council of Canada, Saskatoon, Sask. S7N 0W9, Canada.

The cutin of plants consists of polyesters formed from oxygenated C_{16} and C_{18} acids. The C_{16} acids consist of mixtures of 7,16-, 8,16-, 9,16-, and 10,16-dihydroxyhexadecanoic acids and of 7-, 8-, 9-, and 10-oxo-16-hydroxyhexadecanoic acids. These C_{16} acids have now been synthesized by routes which use common intermediates. Methyl 7-oxo-16,17-dihydroxyheptadecanoate was converted by oxidative cleavage followed by a series of selective reductions and oxidations to 7-oxo(or hydroxy)-16-hydroxyhexadecanoic acid; reduction followed by cleavage and selective oxidation gave 10-oxo(or hydroxy)-16-hydroxyhexadecanoic acid. A similar series of reactions applied to methyl 9-oxo-16,17-dihydroxyheptadecanoate gave 8-oxo-(or hydroxy)- and 9-oxo(or hydroxy)-16-hydroxyhexadecanoic acids.

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METHANOL AND PREPARATIVE LIPID CHEMISTRY. B. RAMESH and C.V. VISWANATHAN, Lipid Research Laboratories, No. 8, Indrayani Flats 'A', Prabhat Road Lane 15, Poona 411004, India.

Earlier, this laboratory reported on the selective extraction of phospholipids free of accompanying triglycerides from egg yolk by methanol (communicated to the *J. Am. Oil Chem. Soc.* on 15–9–78). In this meeting, data will be presented which emphasizes further the usefulness of this solvent in the fractionation of other naturally occurring complex lipid mixtures. Brain tissue, on repeated extractions with methanol alone, yielded lipids in quantitative amounts (comparable with the amounts obtained by the Folch extraction procedure). Further, crude concentrates (purity around 90%) of sphingoglycolipids, cholesterol, and phospholipids were obtained from total brain lipids by simple low temperature (5 C) fractionation in methanol. This was possible because of the vast solubility differences of various lipid classes in methanol. Fractionation of the alkaline hydrolysis products of beeswax into fatty alcohols (mono- and diols) and fatty acids (normal and hydroxy) was achieved at room temperature by precipitation of the former in methanol. This was possible due to the vast solubility differences between the fatty alcohols on the one hand, whose average chain length varied mainly between C_{26} and C_{32}, and fatty acids on the other, whose chain length was largely restricted to C_{16}. Selective aqueous methanol extraction of phosphatidyl choline was achieved from naturally occurring mixtures of phosphatidyl choline and phosphatidyl ethanolamine (phospholipids from egg yolk, brain, and soya). The various lipid concentrates so obtained in the above experiments are useful as the starting materials in the preparation of high purity lipids by the conventional chromatographic methods, wherein one can simultaneously achieve substantial increase in yield of them per unit column load.

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CRITICAL ANALYSIS OF SODIUM STEAROYL LACTYLATE BY GAS LIQUID CHROMATOGRAPHY. RICHARD R. SUCHANEC, Hercules Incorporated, Research Center, Wilmington, DE 19899.

A convenient sample work-up has led to the development of a more generally applicable gas liquid chromatographic (GLC) procedure for the analysis of sodium stearoyl lactylate (SSL). Reliable calibrations can be made based on the use of in situ derivative-forming reactions that offer increased volatility and thermal stability to individual sample components. Lot-to-lot variation in composition can be monitored with the proposed GLC method in less than 30 min. By taking into account independently determined levels of water and sodium, total quantitation is possible. Identification of fatty acid feedstocks has also been demonstrated. The derivatization techniques, which do not depend on any fractionation steps (e.g., distillation or extraction), have been successfully applied to other polyester systems as well.