

ABSTRACTS

R. A. REINERS, Editor. ABSTRACTORS: J. G. Endres, J. Iavicoli,

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• Fats and Oils

THE BAKING PERFORMANCE OF BUTTER POWDER. P. M. T. Hansen (Div. Dairy Res., C.S.I.R.O., Melbourne). *Australian J. Dairy Technol.* 18, 86-91 (1963). Butter powder was used with satisfactory results as the only source of shortening in cake recipes using various proportions of fat. However, the baking performance suffered if the powder was prepared from fat homogenized at high pressure (100-200 at.) in the presence of much protein. Powders in which some of the non-fat milk solids had been replaced with starch or sugar gave particularly satisfactory results with household flour. It was found that the best baking results were obtained when the temperature conditions favoured some crystallization of the butter fat either in the powder or during the preparation of the batter. Some suggested uses have been given for butter powder in fruit cake, scones and icing.

VAPOUR PHASE CARBONYL ABSORPTION IN THE FAR ULTRA-VIOLET. J. F. Horwood and J. R. Williams (Div. Dairy Res., C.S.I.R.O., Melbourne, Australia). *Spectrochim. Acta* 19, 1351-62 (1963). The usefulness of far ultra-violet absorption spectra in the classification and identification of carbonyl compounds purified by gas chromatography was investigated. From the vapour spectra examined it was concluded that alkan-2-ones (C_3 to C_{10}), alkan-3-ones (C_4 to C_{10}) and symmetrical alkanones (C_3 , C_5 , C_7 , C_9 and C_{11}), alk-1-en-3-ones (C_3 to C_9), alkanals (C_3 to C_{11}), alk-2-enals (C_3 , C_4 , C_6 and C_8) and some cycloalkanones (C_5 to C_8) can be differentiated as classes. Differentiation between homologues was possible for alkan-2-ones (C_3 to C_6), alkanals (C_3 to C_6), and alkanones (C_5 to C_8).

ESTIMATING SOLIDS-NOT-FAT AND PROTEIN IN MILK FROM DIFFERENT SOURCES USING PERCENTAGE MILK FAT, PROTEIN, AND SOLIDS-NOT-FAT. R. E. Erb, U. S. Ashworth, L. J. Manus, and N. S. Golding (Dept. of Dairy Sci., Washington State Univ., Pullman). *J. Dairy Sci.* 46, 1217-1227 (1963). The major components of milk were determined to compare the relative accuracy of indirectly estimating protein and solids-not-fat (SNF). The study included 2,842 monthly test-day samples from Guernsey, Holstein, and Jersey cows, 960 semimonthly composited samples from 40 herds, and 718 bulk milk samples from 64 herds representing one two-day composite monthly. Standard errors of estimate ($S_{y \cdot x}$) for within breed when using per cent milk fat to estimate per cent SNF were 0.53, 0.25, and 0.17% SNF, respectively, for monthly samples from individual cows, lactation averages, and two-day composites of herd milk; similarly compared, when per cent protein was used to estimate per cent SNF, the $S_{y \cdot x}$ were, respectively, 0.47, 0.19, and 0.14% SNF. Percentage milk fat and per cent SNF (gravimetrically determined) were compared for indirectly estimating per cent protein. The within breed $S_{y \cdot x}$ for monthly samples from individual cows, lactation averages, and two-day composites of herd milk were, respectively, 0.32, 0.16, and 0.17 for per cent milk fat and 0.29, 0.14, and 0.15 for per cent SNF.

THE USE OF SEPHADEX FOR THE REMOVAL OF NONLIPID CONTAMINANTS FROM LIPID EXTRACTS. M. A. Wells and J. C. Dittmer (Dept. of Biochem., College of Med., Univ. of Kentucky, Lexington). *Biochemistry* 2, 1259-1263 (1963). When lipid extracts are passed through columns prepared of Sephadex in chloroform-methanol-water, the extracts are freed of non-lipid contaminants. The removal of amino acids, carbohydrates, nucleotides, HCl, and inorganic phosphate from lipid extracts by this procedure is of the order of 99%. Lipid recoveries of 98-100%, as determined by the recovery of total fatty acids and of C^{14} -cholesterol, are obtained with most extracts.

DETERMINATION OF MALONALDEHYDE BY ULTRAVIOLET SPECTROPHOTOMETRY. Tai-wan Kwon and B. M. Watts (Dept. of Food and Nutr., Florida State Univ., Tallahassee). *J. Food Sci.* 28, 627-630 (1963). Malonaldehyde occurs mainly as the enol form ($\text{CHOH}=\text{CHCHO}$) in aqueous solution. The UV absorption spectrum of the compound is pH-dependent. Below pH 3.0, the compound is s-cis, planar, having an intramolecular H bond, with absorption maximum at $245 \text{ m}\mu$ and molar absorptivity (ϵ) = 1.34×10^4 . Above pH 7.0, the compound is completely dissociated and the maximum absorption of the enolate anion occurs at $267 \text{ m}\mu$ with $\epsilon = 3.18 \times 10^4$. The absorbance difference between acidified and basified malonaldehyde solutions at $267 \text{ m}\mu$ can be used as a measure of malonaldehyde even in the presence of other compounds that absorb in this

spectral region, provided their absorption is not pH-dependent. This difference is directly proportional to malonaldehyde concentrations from $5 \times 10^{-6} \text{ M}$ to $3 \times 10^{-5} \text{ M}$. The method has been successfully applied to the assay of malonaldehyde in distillates from rancid foods. Its sensitivity is only about 40% of the 2-thiobarbituric acid (TBA) test, but is sufficient to detect threshold levels of rancidity. The test is simpler, much more rapid, and more specific than the TBA test.

DIRECT QUANTITATIVE ISOLATION OF MONOCARBONYL COMPOUNDS FROM FATS AND OILS. D. P. Schwartz, H. S. Haller, and M. Keeney (Dairy Products Lab., Eastern Utilization Res. and Development Div., Agricultural Res. Svc., U. S. Dept. of Ag., Washington 25, D. C.). *Anal. Chem.* 35, 2191-2194 (1963). A quantitative procedure is described for the direct isolation of carbonyl compounds from fats and oils. Carbonyl compounds in the fat are converted to their 2,4-dinitrophenylhydrazones, subsequently freed of fat, and fractionated by adsorption on activated magnesia and partially deactivated alumina. The fat-free monocarbonyl fraction is then separated into classes on magnesia and the members of each class are obtained by column partition chromatography and identified by supplementary techniques. The procedures are ideally suited for small samples of fat, but can be applied to kilogram quantities. Advantages and limitations of the method are discussed.

IRRADIATION-INDUCED CHANGES IN MILK FAT. E. A. Day and S. E. Papaioannou (Dept. of Food Sci. and Tech., Oregon State Univ., Corvallis). *J. Dairy Sci.* 46, 1201-1206 (1963). Gamma irradiation of milk fat under reduced atmospheric pressure produced free monocarbonyls at a rate proportional to irradiation dose. The 2-thiobarbituric acid number and total carbonyls increased, but not at a linear rate. Small quantities of hydroperoxides were produced; radiation dosages higher than 1.5 Mrad decreased the concentration. The flavor of the irradiated fat appeared to have three components: hydrolytic rancidity, oxidized, and candlelike. The monocarbonyls of irradiated fat were isolated and analyzed by gas chromatography and as 2,4-dinitrophenylhydrazine derivatives. Alkanals predominated and their order of decreasing concentration in the irradiated fat was: C_{16} , C_{15} , C_{14} , C_{13} , C_{10} , C_8 , C_6 , C_7 , C_4 , iso- C_4 , C_5 , C_3 , C_2 , C_1 . Methyl ketones also were produced by irradiation and the following compounds were found: C_{15} , C_{11} , C_9 , C_7 , C_5 , C_4 . Indirect evidence is presented indicating that the long-chain aldehydes are responsible for the candle-like defect. It is suggested that the long-chain aldehydes and methyl ketones are produced via hydrolytic rather than oxidative mechanisms.

CHANGES IN FATTY ACID COMPOSITION DURING PREPARATIVE THIN-LAYER CHROMATOGRAPHY. M. Z. Nichaman, C. C. Sweeley, N. M. Oldham, and R. E. Olson (Dept. of Biochem. and Nutrition, Graduate School of Public Health, Univ. of Pittsburgh, Pittsburgh 13, Pa.). *J. Lipid Res.* 4, 484-485 (1963). Iodine may react chemically with unsaturated lipids, thus invalidating GLC studies of the eluted spots. A partial loss of polyunsaturated fatty acids after staining of the TLC plates with iodine vapor was found. Staining of the plates with iodine vapor, prior to the removal of the lipid and transmethylation, leads to a relative decrease in polyunsaturated fatty acids with a proportional increase in the more saturated fatty acids. It is also apparent that the loss of polyunsaturated fatty acids is roughly proportional to the total number of double bonds, since the relative losses of linoleate, eicosatrienoate, and arachidonate were 7%, 21%, and 29%, respectively, when compared with a sample isolated by column chromatography. The fact that the results obtained with unstained plates or plates prestained with Rhodamine 6G are nearly identical with those obtained by column chromatography may be taken as evidence that little autoxidation of polyunsaturated fatty acids occurs during thin-layer chromatography. Iodine vapor is thus to be avoided in detecting lipids spots on thin-layer plates if the lipids are to be subsequently analyzed by GLC. Rhodamine 6G is a suitable, noninterfering substitute.

A TECHNIQUE FOR QUANTITATIVE RECOVERY OF LIPIDS FROM CHROMATOPLATES. B. Goldrick and J. Hirsch (The Rockefeller Inst., New York 21, N. Y.). *J. Lipid Res.* 4, 482-483 (1963). A simple method was developed for the quantitative recovery of approximately 30 mg of lipid from chromatoplates. A central feature of this method is the use of a simple device to assure quantitative recovery of silicic acid particles. The adsorbent is cleanly separated from the glass by a few firm strokes from a single-edge razor blade and collected into a mound, using a black background to assist in visualizing traces

of uncollected material. The particles of adsorbent are then sucked into a glass aspirator consisting of heavy glass tubing drawn out at both ends with a sintered glass filter (pore size 20–25 μ) in the expanded center portion. Thereafter, the glass aspirator is disconnected from the vacuum, held vertically, and tapped to displace any silicic acid adhering to its mouth. Solvents are slowly introduced from a syringe via teflon tubing to prevent particles blowing back as air is displaced. Elution is then carried out in a few seconds with the aid of moderate pressure from a cylinder of compressed nitrogen. For a total of 40 separate chromatographic separations, the recovery of carboxyl ester averaged 98.7% with a standard deviation of 6.68. Corresponding figures for recovery of C^{14} counts were 99.5% \pm 6.97.

A SIMPLE METHOD FOR THE SEPARATION OF MINUTE AMOUNTS OF TISSUE LIPIDS BY THIN-LAYER CHROMATOGRAPHY AND GAS-LIQUID CHROMATOGRAPHY. M. Dobiášová (Isotope Lab., Inst. for Biol. Res., Czech. Acad. of Sciences, Prague 6, Czech.). *J. Lipid Res.* 4, 481–482 (1963). Lipids of lungs, livers, intestine, and white and brown adipose tissue were extracted with Bloor's solution, and aliquots of 2–15 mg were transferred to 2-ml glass ampoules. Analytical thin-layer chromatography was performed on microscope slides (2.5 x 8 cm) using Silica Gel G. The chromatograms were developed in hexane-diethyl ether-ethyl acetate 40:10:1.5 (v/v) and detected by charring after having been sprayed with 25% perchloric acid in water. Phospholipids, cholesterol, free fatty acids, triglycerides, and cholesterol esters were readily distinguished by this procedure. Diglycerides appear at the same spot as cholesterol. The identified regions were then sucked into conical glass tubes 6 mm in diameter. The lipids were extracted from the silica gel with chloroform-methanol 2:1 and placed in 2-ml ampoules. At least 85% of the lipids was thus eluted. Methanolysis of the various lipid classes was carried out in the same ampoules. The methyl esters were extracted three times with 0.3 ml petroleum ether, bp 25–50°C. The petroleum ether layer was collected in capillary tubes and transferred to clean ampoules. The solvent was evaporated to dryness, the esters analyzed using a Pye argon chromatograph.

THE SPECIFIC DISTRIBUTION OF UNSATURATED FATTY ACIDS IN THE TRIGLYCERIDES OF PLANTS. F. H. Mattson and R. A. Volpenhein (Procter & Gamble Co., Miami Valley Lab., Cincinnati 39, Ohio). *J. Lipid Res.* 4, 392–396 (1963). The distribution of fatty acids between the primary and the secondary positions of the triglyceride of 28 species of plants was determined. In confirmation of previous results, palmitic and stearic acids were found to be esterified predominantly at the primary positions. An earlier study demonstrated that fatty acids having a chain length of greater than 18 carbon atoms are also esterified predominantly at the primary positions. Examination of the data in the present and previous studies shows oleic, linoleic, and linolenic acids to have a common pattern of distribution. Each of these acids is approximately randomly distributed among the positions on the triglyceride molecule that are not occupied by palmitic or stearic acids or fatty acids having a chain length greater than 18 carbon atoms.

THE EFFECT OF REACTIVE AND UNREACTIVE PEROXIDES ON THE RATE OF OXIDATION OF BLOWN LINSEED OIL. J. Pokorný (Inst. Chem. Technol., Prague). *J. Inst. Chem. Technol. Prague* 6–2, 267–73 (1962). The autoxidation of blown linseed oil is catalyzed more effectively by the presence of hydroperoxides than by other peroxide types. The titanium chloride colorimetric method is considered to give a truer measure of hydroperoxide content than the iodometric method and is therefore to be preferred in studying the behavior of oxidized oils.

PAPER CHROMATOGRAPHY OF PHOSPHATIDES. III. QUANTITATIVE PAPER CHROMATOGRAPHIC DETERMINATION OF PHOSPHATIDES AND PHOSPHATIDIC ACIDS IN RAT ORGANS. H. Wagner, J. Holzl, A. Lissaic and L. Horskammer (Inst. of Pharm., Univ. of Munchen, Ger.). *Biochem. Z.* 339, 34–45 (1963). A simple method is described for the quantitative determination of the phosphatides of animal organs. The separation of the lipid extracts is achieved by two dimensional paper chromatography, using a formaldehyde coated paper and the following solvent mixtures: butanol-glacial acetic acid-water (4:1:5), and tetrahydrofuran-diisobutylketone-water (45:5:6). The substances are quantitatively determined by a spectrophotometric phosphorus analysis after the separation and the ashing of the spots of the chromatogram. The smallest amount of a phosphatide to be detected is about 25 micrograms. The greatest amount of a lipid that can be put on one spot is 6 milligrams.

The quantitative distribution of all known phosphatides and phosphatidic acids has been studied in the whole rat and the following rat organs: brain, heart muscle, lung, liver and kidney. The values found are compared with those of other authors.

SOME SELECTED MINOR INDIAN OILSEEDS AND THEIR OILS. G. Lakshminarayana, R. L. Badhwar and J. G. Kane (Reg. Res. Lab. Hyderabad, India). *Indian Oilseeds J.* 7, 233–37 (1963). An annotated list of 13 minor Indian seeds.

THE ANALYSIS OF HIGH POLYMERS VIA GAS CHROMATOGRAPHS OF THEIR PYROLYSIS PRODUCTS. I. IDENTIFICATION OF POLYMERIZATE PYROLYSIS PRODUCTS. D. Braun. *Farbe Lack* 69, 820 (1963). A description is given of a simple method for preparing gas chromatographs of the pyrolysis products of high polymers from unsaturated monomers. The gas chromatographs thus obtained are not very complicated as highly volatile, uncharacteristic decomposition products are not recorded. Most of the peaks can be attributed to certain substances. The retention times of the substances identified are given and this knowledge facilitates the analysis of unknown polymers and copolymers.

THE ROLE OF THE VANASPATI INDUSTRY IN THE MARKETING OF OILS AND OILSEEDS. K. V. W. Dharan (The Vanaspati Manuf. Assoc. of India, Bombay, India). *Indian Oilseeds J.* 7, 196–8 (1963).

SOME ASPECTS OF MARKETING OF OILSEEDS AND THEIR PRODUCTS IN INDIA. N. P. Chatterji (Agr. Marketing Adv., Nagpur, India). *Indian Oilseeds J.* 7, 179–183 (1963). MARKETING OF OILSEEDS AND OILS IN ANDHRA PRADESH. K. Subba Rao (Dir. of Marketing, Andhra Pradesh, Hyderabad, India). *Ibid.*, 199–202. MARKETING OF OILSEEDS IN MYSORE STATE. C. A. Jamakhandimath (Co-Op Dept. Mysore, Bangalore, India). *Ibid.*, 203–12. MARKETING OF OILSEEDS AND OILS IN MADRAS STATE. V. Srinivasan (Dept. of Marketing, Madras, India). *Ibid.*, 213–19. OILSEEDS DEVELOPMENT IN UTTAR PRADESH. H. K. S. Rana (Marketing Dept., Uttar Pradesh, Lucknow, India). *Ibid.*, 220–22. MARKETING OF OILSEEDS AND OILS IN DELHI. A. P. Sharma (Directorate of Agr. Marketing, Delhi, India). *Ibid.*, 223–25. OILSEEDS IN HIMACHAL PRADESH. O. P. Krishna (Marketing Dept., Himachal Pradesh, Simla, India). *Ibid.*, 226–29.

A SIMPLE METHOD FOR THE QUANTITATIVE MICRO DETERMINATION OF PHOSPHATE COMPOUNDS SEPARATED BY MEANS OF PAPER CHROMATOGRAPHY. E. Gerlack and B. Deuticke (Inst. of Physiology, Univ. of Freiburg, Ger.). *Biochem. Z.* 337, 447–79 (1963). A sensitive method is described for the quantitative micro determination of phosphate compounds separated by means of paper chromatography. The simple and reliable procedure is a modification of the methods of Fisk, Subbarow and Bartlett.

QUANTITATIVE STUDY OF THE OXIDATIVE DISCOLORATION OF ETHYL LINOLEATE. I. OXIDATION IN THE BULK PHASE. F. Franks and B. Roberts (Dept. of Chem. Tech., Inst. of Tech., Bradford 7, Eng.). *J. Appl. Chem. (London)* 13, 302–9 (1963). The atmospheric oxidation of ethyl linoleate has been studied in the bulk phase at 22 and 50°C. The concentration of conjugated dienes, conjugated trienes (diene ketone), and the development of a yellow coloration were obtained as functions of time from extinction coefficients measured at 234, 268, 275 and 350 millimicrons respectively. The energy of activation of the initial hydroperoxidation reaction agrees well with previously published data. The kinetics of hydroperoxide decomposition have been studied. There appears to be a direct relationship between the rate of hydroperoxide decomposition and the rate of yellowing. The conjugated diene ketone which is formed by a second-order hydroperoxide decomposition is itself oxidized, but no simple relationship can be found between the rates of oxidation and the rate of yellowing. It is therefore concluded that the diene ketone is not a colorless precursor of the yellow compounds.

CHROMATIC REACTIONS WITH NITRIC ACID AND THE ADULTERATION OF OLIVE OIL. M. Jacona Grifeo (Exp. Station Fats and Oils, Catania, Italy). *Olearia*, 17, 106–11 (1963). A review is given of the theories concerning the chromatic reaction with nitric acid (Haucheorne reaction) for the detection of adulteration in olive oils. Experiments have shown that the dark brown color induced by nitric acid is not specific for esterified oils, but is found whenever the oil is partially oxidized due to either high temperature processing or natural aging.

REARRANGEMENT OF MILK FAT AS A MEANS FOR ADJUSTING HARDNESS OF BUTTERLIKE PRODUCTS. J. B. Mickle, R. L. Von Gunten and R. D. Morrison (Dept. of Dairying and Mathematics, Okla-

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homa State Univ., Stillwater). *J. Dairy Sci.* 46, 1357-61 (1963). Milk fat was rearranged using selected times, temperatures, and catalyst concentrations, and the resulting fat was made into a semisolid product resembling butter. Hardness of the product was measured at 7-10C and found to decrease with increased catalyst concentrations, but to increase with increased time. A $50 \pm 5\%$ reduction in hardness could generally be accomplished with catalyst concentrations of 1-2%. Statistical analysis indicated that catalyst concentration had more influence on hardness than did reaction time or temperature. This catalyst effect was related to the formation of diglycerides whose emulsifying properties partially explained the decreased butter hardness.

METHOD FOR THE DETERMINATION OF THE FREE FATTY ACIDS OF MILK FAT. D. D. Bills, L. L. Khatri, and E. A. Day (Dept. of Food Sci. and Tech., Oregon State Univ., Corvallis). *J. Dairy Sci.* 46, 1342-47 (1963). Determination of the free fatty acids of milk fat by means of gas-liquid chromatography, employing two internal standards was evaluated. The free fatty acids were isolated from the fat by means of a basic anion exchange resin, converted to methyl esters, and extracted with ethyl chloride. The ethyl chloride solution of methyl esters was then concentrated with a special reflux system prior to chromatography to prevent the loss of the more volatile esters. Appropriate factors were calculated for relating the quantity of added internal standards to that of the naturally occurring free fatty acids.

DETERMINATION OF ORGANIC PEROXIDES BY IODINE LIBERATION PROCEDURES. R. D. Mair and A. J. Graupner (Research Center, Hercules Powder Co., Wilmington, Del.). *Anal. Chem.* 36, 194-204 (1964). Peroxides from the most active to the most stable can be quantitatively determined by means of a coherent group of three methods. Method I (refluxing NaI-isopropyl alcohol) is recommended for all easily reduced peroxides. Method II (refluxing NaI-acetic acid-6% H₂O) is primarily an assay procedure for diaralkyl peroxides, such as dicumyl peroxide. Method III (refluxing NaI-acetic acid-HCl) will determine the most stable di-tert-alkyl peroxides and quantitatively reduce many nonperoxidic compounds. Several interim procedures are discussed. The importance of excess iodide and the key role of H₂O content in the regulation of reducing power are stressed. The scope of the method is demonstrated by their application to the determination of many pure compounds. Relative standard deviations were 0.23% for Method I, 0.30% for Method II, and 0.32% for Method III.

SPECTROPHOTOMETRIC DETERMINATION OF TRACE CONCENTRATIONS OF CARBONYL COMPOUNDS. D. E. Jordan and F. C. Veatch (Continental Oil Co., Research & Development Dept., Ponca City, Okla.). *Anal. Chem.* 36, 120-24 (1964). Substitution of a mixed hydrocarbon-alcohol solvent for the alcohol solvent used by Lappin and Clark permits the accurate determination of trace quantities of carbonyl in complex organic mixtures. The method is based on the condensation of carbonyl compounds with 2,4-dinitrophenylhydrazine to form 2,4-dinitrophenylhydrazones. Addition of alcoholic potassium hydroxide to the 2,4-dinitrophenylhydrazone mixture produces a yellow to wine-red color on which the absorbance is determined at 480 m μ and the carbonyl calculated from a standard curve. The method, developed primarily for carbonyl in alcohols ranging from ethanol to tetracontanol, is also applicable for most hydrocarbons, organic esters and acids, aromatic oxygenates and hydrocarbons, petroleum distillates, and some ethers.

• Fatty Acid Derivatives

USE OF P-PHENYLAZOBENZOYL CHLORIDE FOR CHROMATOGRAPHIC ANALYSIS OF FATTY ALCOHOLS. I. Katz and M. Keeney (Dept. of Dairy Sci., Univ. of Maryland, College Park, Md.). *Anal. Chem.* 36, 231-34 (1964). Methyl esters and naturally occurring glycerides were reduced to alcohols with LiAlH₄. Conditions are described for the conversion of the alcohols to p-phenylazobenzoates and the chromatographic isolation of the

colored derivatives. The molar absorptivity of fatty p-phenylazobenzoates was determined to be 28,600 at 322 m μ in n-hexane. Reversed-phase thin-layer and reversed-phase column chromatographic systems are described for the separation of the long chain fatty alcohol derivatives. The formation of p-phenylazobenzoates, in conjunction with the gas liquid chromatographic separation of aliphatic alcohols, can be used for the micro determination of absolute specific activities of radioactive fatty acids.

FATTY DERIVATIVES OF AMINOALKYL AND HYDROXYALKYL HETEROCYCLIC AMINE BASES AS ASPHALT ADDITIVES. J. Katz. *U.S.* 3,114,649. A method is described for preparing a high temperature stabilized coating composition and enhancing the bonding, wetting and anti-stripping properties of asphalt, tar and bituminous coating compositions for basic aggregates, including limestone and dolomite, which will be stable at temperatures of above 300F and between 350F and 450F. An additive (0.5 to 1.5% by weight) consists of a high molecular weight piperazine compound having at least 2 aliphatic side chains attached to at least one nitrogen of the ring, one side chain including a fatty acid residue having 14-22 carbon atoms and the other side chain containing a polymethylene linkage having 2 to 3 carbon atoms and terminating in an amino group carrying a fatty acid residue having 14 to 22 carbon atoms.

OMEGA CONVERSION OF FATTY ACID DERIVATIVES. M. A. Mitz (Armour Pharmaceutical Co.). *U.S.* 3,116,215. An unbranched omega-oxo fatty acid, salt or ester having at least 8 carbon atoms is contacted with an enzyme system concentrated in the soluble fractions of visceral organ tissue cells in the presence of reduced diphosphopyridine nucleotide. The temperature is maintained between 0 and 50C and the pH of the system is adjusted to a range between 6.5 and 8.48. The enzyme system is extracted from a cell homogenate by adding a solvent to the homogenate to attain a final volumetric concentration of about 20-40% solvent while the homogenate is held at a pH of 6-8. The solvent treated homogenate is separated and the resultant supernatant is collected.

• Biology and Nutrition

BIOSYNTHESIS OF FATTY ACIDS AND CHOLESTEROL AS RELATED TO DIET FAT. R. Reiser, Mary C. Williams, Mary F. Sorrels and Nadelia L. Murty (Texas A & M College). *Arch. Biochem. Biophys.* 102, 276-85 (1963). The influence of a series of simple triglycerides and natural fats on cholesterogenesis and lipogenesis in the rat was studied. The fats fed included tributyrin, tricaproin, tricaprolylin, tricaprln, trilaurin, trimyristin, tripalmitin, triolein, trilinolein, lard, butter oil, safflower oil, and a synthetic triglyceride made of 1 part palmitic and 2 parts oleic acids.

ASYMMETRICAL INCORPORATION OF C¹⁴ ACETATE INTO β -CAROTENE BIOSYNTHESIZED BY PHYCOMYCES BLAKESLEEANUS. F. J. Lot-speich, R. F. Krause, V. G. Lilly and H. L. Barnett (Depts. of Biochem. and Plant Pathology, West Virginia Univ. Med. Center, Morgantown). *Proc. Soc. Exp. Biol. Med.* 114, 444-447 (1963). β -Carotene biosynthesized by *Phycomyces blakesleeanus* was purified by column chromatography over basic silicic acid. Degradation studies on the ring system of this radioactive β -carotene indicate that the carboxyl carbon of acetate is incorporated into positions 1,1',3,3',5 and 5' of the ring system whereas the methyl carbon is incorporated into all positions of the ring system. Results indicate that the ring carbons are more radioactive than the side chain carbon atoms.

DIETARY BILE ACIDS AND LIPID METABOLISM. I. INFLUENCE ON LIPIDS AND LIVER SIZE OF CHICKS. G. A. Leveille, H. E. Sauerlich and R. D. Hunt (U.S. Army Med. Res. and Nutrition Lab, Fitzsimons General Hosp., Denver, Colo.). *Proc. Soc. Exp. Biol. Med.* 114, 334-337 (1963). Dietary lithocholic acid elevated plasma cholesterol and lipid phosphorus levels, and increased liver size of growing chicks. In cholesterol-fed chicks, lithocholic acid depressed liver lipid and cholesterol levels. The effects of lithocholic acid were partially reversed by cholic acid in chicks fed a cholesterol-free diet and almost completely reversed in chicks fed cholesterol. The plasma cholesterol level of cholesterol-fed chicks was increased by cholic acid feeding.

FATTY ACIDS OF ACANTHAMOEBA SP. E. D. Korn (Lab of Biochem., Sec. on Cellular Physiology, Nat'l Heart Inst., NIH, Bethesda 14, Md.). *J. Biol. Chem.* 238, 3584-3587 (1963). The fatty acids of *Acanthamoeba* sp., a soil ameba

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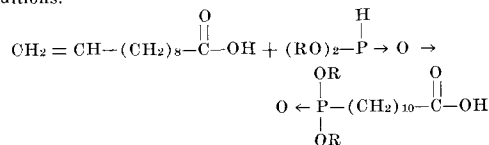
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PHOSPHORUS DERIVATIVES OF FATTY ACIDS. ADDITION OF DIALKYL PHOSPHONATES TO UNSATURATED ACIDS

Richard Sasin, R. A. De Mauriac, Eric Leopold, H. B. Gordon and G. S. Sasin

A series of dialkylphosphonoundecanoic acids was prepared by the reaction of dialkyl phosphonates with undecenoic acid under free radical conditions.



R = CH₃, C₂H₅, C₄H₉, 2-Ethylhexyl

An analogous series of compounds was prepared from oleic acid and dialkyl phosphonates also under free radical conditions. Amides and substituted amides of the phosphono acids were also prepared.

EVALUATION TEST FOR SCOURING AGENTS: FATTY SOIL ON A HARD SURFACE

J. A. Monick

A method has been developed to rate the scouring ability of abrasive cleaners on a tough fatty film which is baked onto an aluminum panel. Results are obtained on a comparative basis, and are best determined by one person using as few panels as possible for each investigation. The soiled surface was prepared by spreading 5 ml lard-chicken fat mixture on one side of the panel, and baking for 5 min at 575F. Three classes of scouring agents were studied: steel wool with soap, silex which was resin-bonded to nylon fiber, and silex on a dish cloth. Results can be expressed as scouring efficiency, and related to an arbitrary standard designated as 100%.

AN AMPEROMETRIC TITRATION METHOD FOR BLEACH EVALUATION

D. J. Sargent

An analytical method has been devised as rapid screening procedure which predicts how effective an active chlorine-containing compound will be as a bleaching agent. The method is based on an amperometric titration which indicates bleaching performance from both the increase in whiteness and fabric tendering standpoints. Excellent correlation was obtained between amperometrically determined data and practical bleaching data for several extensively used bleaching agents. In connection with this amperometric titration method, a polarographic method is also presented which provides good correlation between the polarographically determined data and practical bleaching data.

CONSUMER EVALUATION OF LIQUID SYNDETS II

K. L. Johnson and H. P. Andrews

Six proposed candidates for nonionic biodegradable surfactants have been evaluated using a consumer panel. The formulations studied explore the performance of these materials in the presence of a fatty-based alkylolamide foam stabilizer and biologically "soft" alkyl aryl sulfonate at two levels of nonionic concn. The samples were distributed in pairs accompanied by a facial expression data sheet requesting evaluation of the foaming, general performance and dermatological properties of each. Every sample was compared with every other sample twice in a duplicated balanced incomplete block design evaluating each of the twelve combinations for the 2 x 6 factorial experiment. The nonionic detergents evaluated are all polyoxyalkylated alcohols, including products of natural and synthetic origin. Products based on unsaturated and secondary alcohols are included. Laboratory data were also obtained covering foaming, wetting and surface tension. A full statistical interpretation of the main and interactive effects is presented.

DETERIORATION OF CORN OIL UNDER ASSIMILATED RESTAURANT DEEP FAT FRYING CONDITIONS

R. G. Krishnamurthy and S. S. Chang

One of the major difficulties in the study of the effect of heated oil upon the metabolic pattern of lipids is to obtain oil samples produced under laboratory conditions which are comparable to commercial operations of deep fat frying of foods. After a number of preliminary experiments, a laboratory apparatus has been built which can be used to study the chemical reactions involved in the deep fat frying under assimilated restaurant operations. The volatile decomposition products evolved can be collected by this apparatus for chemical characterizations.

In order to first study the chemical reactions of the frying oil without its interaction with the food fried, cotton balls containing 75% by weight of water were fried every 30 min in corn oil maintained at 185C. Fresh corn oil was used to replenish the oil absorbed by the cotton balls every 12 hr.

The chemical characterization of the volatile decomposition products thus collected and the physical and chemical changes of the frying oil under these conditions will be presented.

CARBONYL COMPOUNDS FORMED DURING DEEP FRYING

L. A. Wishner and Mark Keeney

Fresh commercial corn oil, fresh commercial lard, and hydrogenated vegetable shortening were analyzed for carbonyl compounds before and after the deep frying of 3400 g potatoes at 200C in 300-400 g batches.

(Continued on page 48)

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Program Abstracts . . .

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of temp, pressure, mole ratio of reactants, catalyst concn, and inert gas sparging upon the rate of reaction were studied. Analytical procedures were developed using IR and near-IR spectroscopy and gas chromatography as well as some wet chemical methods of analysis to follow the course of the reactions. Results of these studies and the analytical procedures will be discussed.

DISODIUM 2-SULFOHEXADECYL AND 2-SULFOOCTADECYL SULFATES

A. J. Stirton, F. D. Smith and J. K. Weil

1-Hydroxy-2-alkanesulfonic acids from the metal borohydride reduction of esters of α -sulfolpalmic and α -sulfostearic acid were sulfated with chlorosulfonic acid to give disodium 2-sulfoalkyl sulfates RCH(SO₃Na)CH₂OSO₃Na. The solubility, surface and interfacial tension, critical micelle concn, calcium stability, metallic ion stability, stability to hydrolysis, wetting, foaming, detergent and lime soap dispersing properties were measured.

The 16 and 18 carbon disodium 2-sulfoalkyl sulfates were found to be similar to the isothionate esters of α -sulfolpalmic and α -sulfostearic acid RCH(SO₃Na)CO₂CH₂CH₂SO₃Na in general structure and surface active properties. The general structure, a long hydrophobic chain which terminates in two bulky adjacent hydrophilic groups, is evidently related to the excellent lime soap dispersing agent properties which both disodium 2-sulfooctadecyl sulfate and disodium 2-sulfoethyl α -sulfostearate have in common.

Compared to sodium octadecyl sulfate, the presence of the sulfo group in disodium 2-sulfooctadecyl sulfate greatly increases solubility and critical micelle concn, and thereby affects all surface active properties with some loss in detergency but with greatly improved metallic ion stability and lime soap dispersing properties. Like the sodium alkyl sulfates, the disodium 2-sulfoalkyl sulfates are stable to alkaline hydrolysis but can be hydrolyzed at about the same rate in acid solution; disodium 2-sulfoethyl α -sulfostearate was stable to acid hydrolysis under the same conditions.

Disodium 2-sulfooctadecyl sulfate and disodium 2-sulfoethyl α -sulfostearate are biodegradable in the River Water Die-Away test, but not as easily as sodium octadecyl sulfate since the product of enzymatic hydrolysis is surface active.

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Program Abstracts . . .

(Continued from page 45)

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Analysis of the fats and distillates showed a carbonyl pattern in essential agreement with the classical autoxidation mechanism for the different fats, i.e., the typical alkanals, alk-2-enals, and alk-2,4-dienals. The dominance of this process over that of thermal oxidation was also indicated in the carbonyl pattern by the absence of alk-1-ene-3-ones and other thermal oxidation products. The comparison of the concn of the carbonyl compounds in the fats before and after frying and in their distillates indicates that the deodorization process which accompanies deep frying may be efficient in preventing the accumulation of toxic concn of the oxidation products in fats which have been in continued use.

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H. Kaunitz, R. E. Johnson and L. Pegus

Matching groups of male albino rats were fed purified diets containing fresh or oxidized cottonseed oil, olive oil, chicken fat, or beef fat for two years. The oxidized fats were prepared by aeration at 60C for 40 hr. This mild oxidation was selected in order to approximate what might occur to the fats under unfavorable conditions of storage and use. The rats were observed for wt gain, food intake, and survival rate. Periodically, six rats were sacrificed from each group and examined for organ wt and pathology. Their sera and tissues were examined for cholesterol, total lipid, and fatty acid composition of the latter; the depot fat triglycerides of those rats sacrificed after 70 weeks on the diet were examined for their molecular structure, as were those of the dietary fats. Mineral analyses were also carried out on serum and tissues.

Several of these parameters were affected by the kind of fat fed and/or its state of oxidation.

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NUTRITIVE VALUE OF HEAT POLYMERIZED VEGETABLE OILS

N. V. Raju, M. Narayana Rao and R. Rajagopalan

Peanut, sesame and coconut oils were heated at 270C for 8 hr in an open iron pan. These fats were fed to albino rats at 15% level in otherwise adequate diets. All rats fed heated fats showed a growth depression. Livers of rats receiving the heated oil were congested and showed extensive periportal fatty infiltration. Rats on heated peanut oil showed 1) reduced B-vitamin storage in the liver, 2) increased glucose and cholesterol levels in the blood, and 3) a disruption of digestion and absorption of carbohydrate.

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CHROMATOGRAPHIC STUDIES ON OXIDATIVE AND THERMAL FATTY ACID DIMERS

C. D. Evans, D. G. McConnell, E. N. Frankel and J. C. Cowan

A chromatographic study was carried out to investigate the nature and possible estimation of nonvolatile polymeric products in edible oils. This information may be helpful in any evaluation of changes that result from oxidation and thermal treatment of oils during processing and storage.

Dimers obtained by low-temp oxidation of methyl linoleate were compared in their chromatographic behavior to thermal dimers prepared by high-temp polymerization of conjugated methyl linoleate. Distilled dimers of each were subjected to liquid-partition chromatographic separations on silicic acid columns as methyl esters, as free acids, and as methyl esters prepared by saponification and re-esterification. Chromatographically isolated dimers were also rechromatographed before and after each treatment.

When thermal dimer esters are saponified and re-esterified, chromatographic recoveries are quantitative, and the expected changes in polarity result; whereas, with oxidative dimers esters, gross changes in polarity occur. All chromatographic separations of dimer esters or their acids show four distinct areas of increasing polarity. Fractionations will be discussed on the basis of these changes in polarity for the monomers, dimers, and trimers of both the thermally and oxidatively prepared polymers.

Distilled oxidative dimers do not give highly resolved chromatographic fractions, but show a large peak (ca. 30%) of the same polarity as the thermal dimer, and have a major peak (ca. 50%) in an area of much higher polarity. Saponification and re-esterification of these two chromatographically isolated fractions show that they are not composed of homogeneous material because fractions of various polarities are recovered. When mixtures of oxidatively and thermally prepared methyl linoleate dimers are chromatographed, the experimental results agree well with those calculated from the fractionation of the component dimers. Results indicate that the nonvolatile oxidative products contain material that behaves chromatographically like a thermal dimer.

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STUDIES ON HEATED FATS

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Interest has increased in studies on the effect of heat on edible fats. Recent reports indicate that during deep frying over extended periods, a significant portion of the oil is polymerized. Results of a study on corn oil heated at 180C and 200C will be reported. The heated oil was fractionated into 8 fractions. The first 4 fractions constituting ca. 60-80% were found to be triglycerides, the remaining 4 fractions constituted degraded and polymeric products of high mol wt.

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homa State Univ., Stillwater). *J. Dairy Sci.* 46, 1357-61 (1963). Milk fat was rearranged using selected times, temperatures, and catalyst concentrations, and the resulting fat was made into a semisolid product resembling butter. Hardness of the product was measured at 7-10C and found to decrease with increased catalyst concentrations, but to increase with increased time. A $50 \pm 5\%$ reduction in hardness could generally be accomplished with catalyst concentrations of 1-2%. Statistical analysis indicated that catalyst concentration had more influence on hardness than did reaction time or temperature. This catalyst effect was related to the formation of diglycerides whose emulsifying properties partially explained the decreased butter hardness.

METHOD FOR THE DETERMINATION OF THE FREE FATTY ACIDS OF MILK FAT. D. D. Bills, L. L. Khatri, and E. A. Day (Dept. of Food Sci. and Tech., Oregon State Univ., Corvallis). *J. Dairy Sci.* 46, 1342-47 (1963). Determination of the free fatty acids of milk fat by means of gas-liquid chromatography, employing two internal standards was evaluated. The free fatty acids were isolated from the fat by means of a basic anion exchange resin, converted to methyl esters, and extracted with ethyl chloride. The ethyl chloride solution of methyl esters was then concentrated with a special reflux system prior to chromatography to prevent the loss of the more volatile esters. Appropriate factors were calculated for relating the quantity of added internal standards to that of the naturally occurring free fatty acids.

DETERMINATION OF ORGANIC PEROXIDES BY IODINE LIBERATION PROCEDURES. R. D. Mair and A. J. Graupner (Research Center, Hercules Powder Co., Wilmington, Del.). *Anal. Chem.* 36, 194-204 (1964). Peroxides from the most active to the most stable can be quantitatively determined by means of a coherent group of three methods. Method I (refluxing NaI-isopropyl alcohol) is recommended for all easily reduced peroxides. Method II (refluxing NaI-acetic acid-6% H₂O) is primarily an assay procedure for diaralkyl peroxides, such as dicumyl peroxide. Method III (refluxing NaI-acetic acid-HCl) will determine the most stable di-tert-alkyl peroxides and quantitatively reduce many nonperoxidic compounds. Several interim procedures are discussed. The importance of excess iodide and the key role of H₂O content in the regulation of reducing power are stressed. The scope of the method is demonstrated by their application to the determination of many pure compounds. Relative standard deviations were 0.23% for Method I, 0.30% for Method II, and 0.32% for Method III.

SPECTROPHOTOMETRIC DETERMINATION OF TRACE CONCENTRATIONS OF CARBONYL COMPOUNDS. D. E. Jordan and F. C. Veatch (Continental Oil Co., Research & Development Dept., Ponca City, Okla.). *Anal. Chem.* 36, 120-24 (1964). Substitution of a mixed hydrocarbon-alcohol solvent for the alcohol solvent used by Lappin and Clark permits the accurate determination of trace quantities of carbonyl in complex organic mixtures. The method is based on the condensation of carbonyl compounds with 2,4-dinitrophenylhydrazine to form 2,4-dinitrophenylhydrazones. Addition of alcoholic potassium hydroxide to the 2,4-dinitrophenylhydrazone mixture produces a yellow to wine-red color on which the absorbance is determined at 480 m μ and the carbonyl calculated from a standard curve. The method, developed primarily for carbonyl in alcohols ranging from ethanol to tetracontanol, is also applicable for most hydrocarbons, organic esters and acids, aromatic oxygenates and hydrocarbons, petroleum distillates, and some ethers.

• Fatty Acid Derivatives

USE OF P-PHENYLAZOBENZOYL CHLORIDE FOR CHROMATOGRAPHIC ANALYSIS OF FATTY ALCOHOLS. I. Katz and M. Keeney (Dept. of Dairy Sci., Univ. of Maryland, College Park, Md.). *Anal. Chem.* 36, 231-34 (1964). Methyl esters and naturally occurring glycerides were reduced to alcohols with LiAlH₄. Conditions are described for the conversion of the alcohols to p-phenylazobenzoates and the chromatographic isolation of the

colored derivatives. The molar absorptivity of fatty p-phenylazobenzoates was determined to be 28,600 at 322 m μ in n-hexane. Reversed-phase thin-layer and reversed-phase column chromatographic systems are described for the separation of the long chain fatty alcohol derivatives. The formation of p-phenylazobenzoates, in conjunction with the gas liquid chromatographic separation of aliphatic alcohols, can be used for the micro determination of absolute specific activities of radioactive fatty acids.

FATTY DERIVATIVES OF AMINOALKYL AND HYDROXYALKYL HETEROCYCLIC AMINE BASES AS ASPHALT ADDITIVES. J. Katz. *U.S.* 3,114,649. A method is described for preparing a high temperature stabilized coating composition and enhancing the bonding, wetting and anti-stripping properties of asphalt, tar and bituminous coating compositions for basic aggregates, including limestone and dolomite, which will be stable at temperatures of above 300F and between 350F and 450F. An additive (0.5 to 1.5% by weight) consists of a high molecular weight piperazine compound having at least 2 aliphatic side chains attached to at least one nitrogen of the ring, one side chain including a fatty acid residue having 14-22 carbon atoms and the other side chain containing a polymethylene linkage having 2 to 3 carbon atoms and terminating in an amino group carrying a fatty acid residue having 14 to 22 carbon atoms.

OMEGA CONVERSION OF FATTY ACID DERIVATIVES. M. A. Mitz (Armour Pharmaceutical Co.). *U.S.* 3,116,215. An unbranched omega-oxo fatty acid, salt or ester having at least 8 carbon atoms is contacted with an enzyme system concentrated in the soluble fractions of visceral organ tissue cells in the presence of reduced diphosphopyridine nucleotide. The temperature is maintained between 0 and 50C and the pH of the system is adjusted to a range between 6.5 and 8.48. The enzyme system is extracted from a cell homogenate by adding a solvent to the homogenate to attain a final volumetric concentration of about 20-40% solvent while the homogenate is held at a pH of 6-8. The solvent treated homogenate is separated and the resultant supernatant is collected.

• Biology and Nutrition

BIOSYNTHESIS OF FATTY ACIDS AND CHOLESTEROL AS RELATED TO DIET FAT. R. Reiser, Mary C. Williams, Mary F. Sorrels and Nadelia L. Murty (Texas A & M College). *Arch. Biochem. Biophys.* 102, 276-85 (1963). The influence of a series of simple triglycerides and natural fats on cholesterogenesis and lipogenesis in the rat was studied. The fats fed included tributyrin, tricaproin, tricaprolylin, tricaprln, trilaurin, trimyristin, tripalmitin, triolein, trilinolein, lard, butter oil, safflower oil, and a synthetic triglyceride made of 1 part palmitic and 2 parts oleic acids.

ASYMMETRICAL INCORPORATION OF C¹⁴ ACETATE INTO β -CAROTENE BIOSYNTHESIZED BY PHYCOMYCES BLAKESLEEANUS. F. J. Lot-speich, R. F. Krause, V. G. Lilly and H. L. Barnett (Depts. of Biochem. and Plant Pathology, West Virginia Univ. Med. Center, Morgantown). *Proc. Soc. Exp. Biol. Med.* 114, 444-447 (1963). β -Carotene biosynthesized by *Phycomyces blakesleeanus* was purified by column chromatography over basic silicic acid. Degradation studies on the ring system of this radioactive β -carotene indicate that the carboxyl carbon of acetate is incorporated into positions 1,1',3,3',5 and 5' of the ring system whereas the methyl carbon is incorporated into all positions of the ring system. Results indicate that the ring carbons are more radioactive than the side chain carbon atoms.

DIETARY BILE ACIDS AND LIPID METABOLISM. I. INFLUENCE ON LIPIDS AND LIVER SIZE OF CHICKS. G. A. Leveille, H. E. Sauerlich and R. D. Hunt (U.S. Army Med. Res. and Nutrition Lab, Fitzsimons General Hosp., Denver, Colo.). *Proc. Soc. Exp. Biol. Med.* 114, 334-337 (1963). Dietary lithocholic acid elevated plasma cholesterol and lipid phosphorus levels, and increased liver size of growing chicks. In cholesterol-fed chicks, lithocholic acid depressed liver lipid and cholesterol levels. The effects of lithocholic acid were partially reversed by cholic acid in chicks fed a cholesterol-free diet and almost completely reversed in chicks fed cholesterol. The plasma cholesterol level of cholesterol-fed chicks was increased by cholic acid feeding.

FATTY ACIDS OF ACANTHAMOEBA SP. E. D. Korn (Lab of Biochem., Sec. on Cellular Physiology, Nat'l Heart Inst., NIH, Bethesda 14, Md.). *J. Biol. Chem.* 238, 3584-3587 (1963). The fatty acids of *Acanthamoeba* sp., a soil ameba

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mal and vegetable fats for this market is keen. Discussions were held with Italian renderers about the possible development of mobile units which could demonstrate fat-in-feed benefits and usage throughout the country.

An interesting development was learned in a visit with Dr. Nobile of Ledoga. Their research has now produced a sucrose ester product which is competitive with petroleum based detergents. Of all the fats tested in its manufacture, tallow produced the best results. While the price is somewhat higher, it requires only one-fifth as much material to do the same job as petroleum-based detergents. Steps were taken to speed up the availability and knowledge of this product to potential users in the U.S.

SPAIN. A visit to the NRA Technical Office in Barcelona revealed an impressive amount of work being done, with informative material of many types being translated into several languages and circulated throughout Europe. In Madrid, it was estimated that we would soon see an increase of about 10,000 tons in the usage of fat for feed manufacturing.

NRA sponsored feeding tests have shown very good results from tallow additives and the milk replacer market also offers excellent opportunities. Large, new broiler operations are being constructed, which will offer us still more market potential.

FRANCE. Since France has a fat surplus, our main objective here is to increase internal utilization so that French production will not compete with our exports to other European markets.

The French know about the use of fats in feeds but limit their use to very low levels, largely because they feel that the suppliers do not know how to properly handle fats or to stabilize them. Our efforts should therefore be directed to educational and technical assistance of our counterparts in this country.

SWITZERLAND. High energy, high protein rations are well understood in this country, but they have not gotten into this field as heavily as might be expected. This was discussed in a conference at Geneva with CRINA, an organization of feed manufacturers from eight different countries which have formed a joint research facility in this country. A seminar on fats was conducted and preliminary discussions were held which may lead to a research project on the use of fats.

BELGIUM. Two research institutes and several farms were visited to evaluate the progress of the joint feeding tests being conducted for NRA, the Feed Grains Council and the Soybean Council. This program is designed to demonstrate to the Belgian farmer that he can make more money feeding corn including fat than he can by pasture grazing methods.

In conferences relative to the Common Market, we were advised that we have no cause to worry that our encouragement of the use of fats in feeds would be hampered by any restrictive regulations.

HOLLAND. This country is a substantial tallow producer and a large majority of their production is now going into fat for feed. Renderers in this country are interested in working with us on experimental projects in the field of protein meals and further discussions will be held on this subject.

ENGLAND. Discussions were held with Lever and Colgate in connection with tallow sueroglyceride.

that can be grown in axenic culture on soluble nutrients, have been characterized. The principal fatty acids are myristate, palmitate, 9-hexadecenoate, stearate, 9-octadecenoate, 9,12-octadecadienoate, 11-eicosenoate, 11,14-eicosadienoate, 8,11,14-eicosatrienoate, and 5,8,11,14-eicosatetraenoate. The relative concentrations of several of the fatty acids are significantly influenced by the temperature of growth.

EFFECT OF CHOLESTYRAMINE, A BILE ACID BINDING POLYMER ON PLASMA CHOLESTEROL AND FECAL BILE ACID EXCRETION IN THE RAT. J. W. Huff, J. L. Gilfillan and V. M. Hunt (Merck Inst. for Therapeutic Res., Rahway, N.J.). *Proc. Soc. Exp. Biol. Med.* 114, 352-355 (1963). Administration of cholestyramine, a bile acid binding polymer to rats on a normal diet was without effect on plasma or liver cholesterol concentration, but produced a rise in fecal bile acid excretion. These effects were accompanied by an increase in hepatic *de novo* cholesterol synthesis. Cholestyramine prevented the rise in plasma cholesterol resulting from administration of cholesterol and cholic acid. The fecal bile acids were increased slightly with a marked elevation in the dihydroxycholic to cholic acid ratio.

SHORT-TERM AORTIC LIPOIDOSIS IN THE RAT: EVALUATION OF SOME FACTORS RELATED TO THE EXPERIMENTAL PROCEDURE. R. Hess and P. Loustalot (Res. Lab., Pharmaceutical Dept., CIBA Ltd., Basle, Switzerland). *J. Atheroscler. Res.* 3, 280-287 (1963). In an endeavor to obtain, in a 4-week experiment, a maximum degree of macroscopically visible aortic lipoidosis in the rat, various modifications of an existing experimental procedure were tested. This experimental procedure consisted in feeding the rats with a semi-synthetic diet containing supplements of cholesterol, peanut oil, cholic acid, thiouracil, and a high or low level of protein (basic diet). Additional treatment with lactose, sucrose, NaCl, neomycin, and Triton, as well as withdrawal of choline or interrupted administration of the basic diet for a limited period of time, failed to increase significantly the degree of lipoidosis in the aorta. A significant decrease in aortic lipoidosis was found after the animals had been fed with a basic diet containing no chloride but rich in protein. A clear-cut increase in the degree of lipoidosis was obtained by combining the lipoidosis-producing diet with measures designed to cause medical sclerosis.

EFFECT OF DIHYDROTACHYSTEROL (AT 10) AND EPINEPHRINE ON SERUM AND TISSUE CHOLESTEROL AND CALCIUM LEVELS. R. G. Herrmann and R. J. Parker (Lilly Research Lab., Indianapolis, Ind.). *Proc. Soc. Exp. Biol. Med.* 114, 331-333 (1963). A hypercholesteremic diet was fed to rats for up to 28 weeks. Additional dosing of vitamin D, epinephrine or dihydro-tachysterol (AT 10) had no pronounced effect on serum or liver cholesterol levels.

FREE AND TOTAL CHOLESTEROL IN HUMAN BLOOD FRACTIONS. B. E. Hawthorne, E. Smith and J. O. Pescador (Foods and Nutr. Dept., Oregon State Univ., Corvallis, Oregon). *J. Nutr.* 81, 241-248 (1963). The micromodification of the method of Sperry and Webb by Galloway and co-workers has been adapted to the determination of cholesterol in red cells and white cells plus platelets separated by differential centrifugation from 0.4 ml of blood. Using these procedures, free and total cholesterol were determined in the serum and cell fractions isolated from the blood of 33 subjects, men and women, ranging in age from 22 to 91 years. Values for total cholesterol range from 122 to 338 mg/100 ml in sera, from 82 to 151 mg/100 ml in red cells, and from 536 to 5124 mg/100 g in the white cells plus platelets. The concentrations of cholesterol in the white cells plus platelets are higher than values previously reported. The amount of free cholesterol in white cells plus platelets varies widely among individuals, whereas cholesterol present in the free form in sera and red cells is quite constant from person to person. White cell analyses may prove valuable in investigations of lipid metabolism in humans.

THE CONDENSATION REACTION OF FATTY ACID SYNTHESIS. III. IDENTIFICATION OF THE PROTEIN-BOUND PRODUCT OF THE REACTION AND ITS CONVERSION TO LONG CHAIN FATTY ACIDS. P. Goldman, A. W. Alberts, and P. R. Vagelos (Enzyme Sec., Lab. of Biochem., Nat'l Heart Inst., NIH, Bethesda 14, Md.). *J. Biol. Chem.* 238, 3579-3583 (1963). Enzyme-bound acetoacetate formed from the condensation of malonyl coenzyme A and acetyl coenzyme A in a fatty acid-synthesizing system obtained from *Escherichia coli* has been shown to be an intermediate in the synthesis of long chain fatty acids. Evidence is presented that the acetoacetate is bound to Enzyme II,

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EGYPT. There is no question that Egypt could use more tallow, along with a variety of fatty acids. Soap production is only 50% of capacity and would increase automatically if they had more raw material. The Ministry of Supply estimates that they can use 50% more tallow than they currently import, but face the problem of hard currency. It was suggested that if we would allow 12 months rather than six-month credit, tallow imports could be increased 20,000 tons/year.

Feed industry potential is good. The government has a plan which is geared for production of 20,000 broilers a day, which would require the import of at least 35,000 tons conen.

TURKEY. The Turkish soap industry has converted to tallow in the last year and a half, finding that it makes good economic sense to free larger amounts of their olive oil for export. They are badly in need of technical assistance, however, to help the soap manufacturers take full advantage of tallow's potential. (Following Mr. Haugh's visit, Juan Amiehgali, NRA's European Technical Consultant, and a Spanish soap expert spent two weeks in Turkey for this purpose.)

ISRAEL. Both the feed and soap industries have expressed an interest in continuing their initial use of tallow. For example, a representative of one of the leading feed organizations recently returned from a visit to the U.S. convinced of the real value of tallow and plans to start adding it to his feed. Its regular use should soon be well established in this industry.

Economics create some problems in that vegetable oil foots can be imported for considerably less than tallow. The users recognize the extra value of tallow, but there is a limit to how much more they will pay for it. Much of our future here will depend upon the price-establishing variations of supply and demand, along with the interest of the Israeli government in using its PL 480 allocation of tallow.

GREECE. The Greeks have traditionally used by-products from the production of olive oil for soap manufacture. Until these products are upgraded and move into other channels, there does not appear to be much prospect of significant tallow imports for soap manufacture. Technical advice, along with some U.S. know-how and investment, could change this picture, but it is doubtful that the Greeks will do it themselves.

ITALY. A revolution in agricultural feeding is taking place in this country and prospects for increased usage of products such as ours are excellent. For example, Italy is importing thousands of calves and is using large amounts of milk replacers to feed them. Competition between ani-



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mal and vegetable fats for this market is keen. Discussions were held with Italian renderers about the possible development of mobile units which could demonstrate fat-in-feed benefits and usage throughout the country.

An interesting development was learned in a visit with Dr. Nobile of Ledoga. Their research has now produced a sucrose ester product which is competitive with petroleum based detergents. Of all the fats tested in its manufacture, tallow produced the best results. While the price is somewhat higher, it requires only one-fifth as much material to do the same job as petroleum-based detergents. Steps were taken to speed up the availability and knowledge of this product to potential users in the U.S.

SPAIN. A visit to the NRA Technical Office in Barcelona revealed an impressive amount of work being done, with informative material of many types being translated into several languages and circulated throughout Europe. In Madrid, it was estimated that we would soon see an increase of about 10,000 tons in the usage of fat for feed manufacturing.

NRA sponsored feeding tests have shown very good results from tallow additives and the milk replacer market also offers excellent opportunities. Large, new broiler operations are being constructed, which will offer us still more market potential.

FRANCE. Since France has a fat surplus, our main objective here is to increase internal utilization so that French production will not compete with our exports to other European markets.

The French know about the use of fats in feeds but limit their use to very low levels, largely because they feel that the suppliers do not know how to properly handle fats or to stabilize them. Our efforts should therefore be directed to educational and technical assistance of our counterparts in this country.

SWITZERLAND. High energy, high protein rations are well understood in this country, but they have not gotten into this field as heavily as might be expected. This was discussed in a conference at Geneva with CRINA, an organization of feed manufacturers from eight different countries which have formed a joint research facility in this country. A seminar on fats was conducted and preliminary discussions were held which may lead to a research project on the use of fats.

BELGIUM. Two research institutes and several farms were visited to evaluate the progress of the joint feeding tests being conducted for NRA, the Feed Grains Council and the Soybean Council. This program is designed to demonstrate to the Belgian farmer that he can make more money feeding corn including fat than he can by pasture grazing methods.

In conferences relative to the Common Market, we were advised that we have no cause to worry that our encouragement of the use of fats in feeds would be hampered by any restrictive regulations.

HOLLAND. This country is a substantial tallow producer and a large majority of their production is now going into fat for feed. Renderers in this country are interested in working with us on experimental projects in the field of protein meals and further discussions will be held on this subject.

ENGLAND. Discussions were held with Lever and Colgate in connection with tallow sueroglyceride.

that can be grown in axenic culture on soluble nutrients, have been characterized. The principal fatty acids are myristate, palmitate, 9-hexadecenoate, stearate, 9-octadecenoate, 9,12-octadecadienoate, 11-eicosenoate, 11,14-eicosadienoate, 8,11,14-eicosatrienoate, and 5,8,11,14-eicosatetraenoate. The relative concentrations of several of the fatty acids are significantly influenced by the temperature of growth.

EFFECT OF CHOLESTYRAMINE, A BILE ACID BINDING POLYMER ON PLASMA CHOLESTEROL AND FECAL BILE ACID EXCRETION IN THE RAT. J. W. Huff, J. L. Gilfillan and V. M. Hunt (Merck Inst. for Therapeutic Res., Rahway, N.J.). *Proc. Soc. Exp. Biol. Med.* 114, 352-355 (1963). Administration of cholestyramine, a bile acid binding polymer to rats on a normal diet was without effect on plasma or liver cholesterol concentration, but produced a rise in fecal bile acid excretion. These effects were accompanied by an increase in hepatic *de novo* cholesterol synthesis. Cholestyramine prevented the rise in plasma cholesterol resulting from administration of cholesterol and cholic acid. The fecal bile acids were increased slightly with a marked elevation in the dihydroxycholic to cholic acid ratio.

SHORT-TERM AORTIC LIPOIDOSIS IN THE RAT: EVALUATION OF SOME FACTORS RELATED TO THE EXPERIMENTAL PROCEDURE. R. Hess and P. Loustalot (Res. Lab., Pharmaceutical Dept., CIBA Ltd., Basle, Switzerland). *J. Atheroscler. Res.* 3, 280-287 (1963). In an endeavor to obtain, in a 4-week experiment, a maximum degree of macroscopically visible aortic lipoidosis in the rat, various modifications of an existing experimental procedure were tested. This experimental procedure consisted in feeding the rats with a semi-synthetic diet containing supplements of cholesterol, peanut oil, cholic acid, thiouracil, and a high or low level of protein (basic diet). Additional treatment with lactose, sucrose, NaCl, neomycin, and Triton, as well as withdrawal of choline or interrupted administration of the basic diet for a limited period of time, failed to increase significantly the degree of lipoidosis in the aorta. A significant decrease in aortic lipoidosis was found after the animals had been fed with a basic diet containing no chloride but rich in protein. A clear-cut increase in the degree of lipoidosis was obtained by combining the lipoidosis-producing diet with measures designed to cause medical sclerosis.

EFFECT OF DIHYDROTACHYSTEROL (AT 10) AND EPINEPHRINE ON SERUM AND TISSUE CHOLESTEROL AND CALCIUM LEVELS. R. G. Herrmann and R. J. Parker (Lilly Research Lab., Indianapolis, Ind.). *Proc. Soc. Exp. Biol. Med.* 114, 331-333 (1963). A hypercholesteremic diet was fed to rats for up to 28 weeks. Additional dosing of vitamin D, epinephrine or dihydro-tachysterol (AT 10) had no pronounced effect on serum or liver cholesterol levels.

FREE AND TOTAL CHOLESTEROL IN HUMAN BLOOD FRACTIONS. B. E. Hawthorne, E. Smith and J. O. Pescador (Foods and Nutr. Dept., Oregon State Univ., Corvallis, Oregon). *J. Nutr.* 81, 241-248 (1963). The micromodification of the method of Sperry and Webb by Galloway and co-workers has been adapted to the determination of cholesterol in red cells and white cells plus platelets separated by differential centrifugation from 0.4 ml of blood. Using these procedures, free and total cholesterol were determined in the serum and cell fractions isolated from the blood of 33 subjects, men and women, ranging in age from 22 to 91 years. Values for total cholesterol range from 122 to 338 mg/100 ml in sera, from 82 to 151 mg/100 ml in red cells, and from 536 to 5124 mg/100 g in the white cells plus platelets. The concentrations of cholesterol in the white cells plus platelets are higher than values previously reported. The amount of free cholesterol in white cells plus platelets varies widely among individuals, whereas cholesterol present in the free form in sera and red cells is quite constant from person to person. White cell analyses may prove valuable in investigations of lipid metabolism in humans.

THE CONDENSATION REACTION OF FATTY ACID SYNTHESIS. III. IDENTIFICATION OF THE PROTEIN-BOUND PRODUCT OF THE REACTION AND ITS CONVERSION TO LONG CHAIN FATTY ACIDS. P. Goldman, A. W. Alberts, and P. R. Vagelos (Enzyme Sec., Lab. of Biochem., Nat'l Heart Inst., NIH, Bethesda 14, Md.). *J. Biol. Chem.* 238, 3579-3583 (1963). Enzyme-bound acetoacetate formed from the condensation of malonyl coenzyme A and acetyl coenzyme A in a fatty acid-synthesizing system obtained from *Escherichia coli* has been shown to be an intermediate in the synthesis of long chain fatty acids. Evidence is presented that the acetoacetate is bound to Enzyme II,

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the heat-stable protein required in the condensation reaction of fatty acid synthesis.

TRANSFER RATE OF SERUM CHOLESTEROL INTO THE RABBIT AORTIC WALL IN VARIOUS PHASES OF ATHEROSCLEROSIS AND AFTER APPLICATION OF CORTISONE OR THYROXINE. V. Felt, R. Röhling, J. Hladovec and S. Vohout (Res. Inst. of Endocrinology and Res. Inst. of Pharmacology and Biochemistry, Prague, Czechoslovakia). *J. Atheroscler. Res.* 3, 301-308 (1963). The transfer rate of serum cholesterol into the aortic wall was increased 14 times in the progressive phase, and 9 times in the stationary phase of atherosclerosis as compared to aortae of normal rabbits. In the regressive phase there was no significant difference from the controls. The application of cortisone to normal rabbits and of L-thyroxine to atherosclerotic rabbits was without significant influence on this process of cholesterol transfer.

EFFECT OF INDOOR HOUSING CONDITIONS ON SERUM CHOLESTEROL LEVELS OF WHITE CARNEAU PIGEONS. C. H. Eades, Jr., V. B. Solberg, I. C. Hsu and G. E. Philips (Dept. of Biochem., Warner Lambert Res. Inst., Morris Plains, N.J.). *Proc. Soc. Exp. Biol. Med.* 114, 515-517 (1963). Old white carneau pigeons maintained under regulated light (9 hr. fluorescent light, 15 hr. darkness), temperature ($76 \pm 2^\circ\text{C}$), and humidity (about 40%) conditions and in groups of 6 in cages that do not permit flying for a period of over 18 months do not have the seasonal cyclic variations in serum cholesterol possessed by pigeons that have free access to flying areas and are exposed to yearly weather conditions.

BIOHYDROGENATION IN GERM-FREE RATS. R. Blomstrand, G. A. Dhopeswarkar and B. E. Gustafsson (Dept. of Clinical Chemistry, Serafimerlasarettet; Dept. of Symbiosis; Karolinska Institutet, Stockholm, Sweden). *J. Atheroscler. Res.* 3, 274-279 (1963). Elaidic acid ($1\text{-}^{14}\text{C}$) was fed to 5 germ-free rats and 2 control rats. Per cent distribution of radioactivity determined by degradation of stearic acid isolated from liver lipids of germ-free animals clearly showed that biohydrogenation of elaidic acid to stearic acid occurred in the germ-free rats. Thus the biohydrogenation process was attributed to cellular enzymes and not to the intestinal microbial flora.

EFFECT OF BENZYL N-BENZYL CARBETHOXYHYDROXAMATE (W-398) ON EXPERIMENTAL ATHEROSCLEROSIS AND HYPERCHOLESTEREMIA. F. M. Berger, J. F. Douglas, B. J. Ludwig and S. Margolin (Wallace Laboratories, Division of Carter Products, Inc., Cranbury, N. J.). *Proc. Soc. Exp. Biol. Med.* 114, 337-341 (1963). Benzyl N-benzyl carbethoxyhydroxamate (W-398) given orally to rabbits caused disappearance of atherosclerotic lesions previously established by administration of a diet containing 1% cholesterol. W-398 also prevented the occurrence of atherosclerotic lesions when administered jointly with a cholesterol containing diet. Elevated blood cholesterol levels in adult rabbits or weanling rats fed a hypercholesteremic diet were also reduced by administration of W-398. The drug was effective in doses that were well tolerated by the animals.

LIPID-PROTEIN PARTICLES: ISOLATION FROM SEEDS OF GOSSYPIUM HIRSUTUM. L. Yatsu and A. M. Altschul (Seed Protein Pioneering Research Lab., U.S. Dept. of Agriculture, New Orleans, Louisiana). *Science* 142, 1062-1064 (1963). Subcellular particles were isolated from cottonseed by tanning of the cell contents followed by differential centrifugation. The particles, high in protein content and containing approximately 28 and 44 percent lipids, are thought to be the site of oil storage and lipid synthesis.

SYNTHESIS OF α -LINOLENIC ACID BY LEISHMANIA ENRIETTII. E. D. Korn and C. L. Greenblatt (Nat'l Heart Inst., and Nat'l Inst. of Arthritis and Metabolic Diseases, Bethesda 14, Md.). *Science* 142, 1301-1303 (1963). The zooflagellate *Leishmania enrietti* synthesizes α -linolenic acid as determined by experiments with stearic acid uniformly labeled with C^{14} . This is the first demonstration of the synthesis of α -linolenic acid by a non-photosynthetic organism.

DIETARY FATS AND PLASMA LIPIDS IN CHICKS. A. Bandyopadhyay and S. Banerjee (Dept. of Physiology, Sardar Patel Med. College, Bikaner, India). *Proc. Soc. Exp. Biol. Med.* 114, 222-224 (1963). Chicks were fed mustard oil, sesame oil, coconut oil and hydrogenated groundnut oil mixed with the basal diet with or without the addition of cholesterol. The level of oil was 10% and that of cholesterol 1% of the basal diet. The different diets were fed for 8 weeks and plasma contents of different lipids were estimated. Addition of oils alone in the diet increased the plasma NEFA values without producing any change in other fractions of plasma lipids. When the different oils were fed in addition to cholesterol, there was a rise in total plasma contents if different lipids were estimated. Addition of oils alone in the diet increased the plasma NEFA values without producing any change in other fractions of plasma lipids. When the different oils were fed in addition to cholesterol, there was a rise in total plasma cholesterol, β -lipoprotein cholesterol, β -lipoprotein percentage, triglycerides and NEFA values of plasma. The rise in plasma cholesterol was maximum in chicks fed cholesterol along with hydrogenated oil or sesame oil and minimum in chicks fed cholesterol along with mustard oil, with intermediate rise after coconut oil. The solubility of cholesterol was highest in coconut oil and least in mustard oil. Hypercholesteremia does not seem to depend on saturation or unsaturation of the oils or on the solubility of cholesterol in the oils.

EFFECT OF SELENIUM ON MUSCULAR DYSTROPHY IN VITAMIN E-DEFICIENT RATS AND GUINEA PIGS. E. Bonetti and F. Stirpe (Istituto di Patologia Generale dell' Università de Siena, Siena, Italy). *Proc. Soc. Exp. Biol. Med.* 114, 109-115 (1963). Administration of sodium selenite reduced the severity of muscular dystrophy in rats fed vitamin E-free diets, with adequate or low protein content. The effect of selenite was more evident when dystrophic lesions were not severe. Selenite was without effect on muscular dystrophy in vitamin E-deficient guinea pigs.

PROVITAMIN A_2 FROM LUTEIN. P. Budowski, I. Ascarelli, J. Gross, I. Nir (National and Univ. Institute of Agriculture, Rehovot, Israel). *Science* 142, 969-971 (1963). Alfalfa lipids were treated with *p*-toluenesulfonic acid in benzene under reflux; after saponification, chromatography of the unsaponifiable portion yielded a carotenoid having a single absorption maximum at $460 \text{ m}\mu$ in ethanol and hexane. Its properties correspond to those of the dehydration product of lutein. 3'-hydroxy-3,4-dehydro- β -carotene, for which the name "anhydrolutein" is proposed. Vitamin A depleted chicks convert this pigment to vitamin A_2 , as shown by the high ratio of absorbancies at 693 and $620 \text{ m}\mu$ in a mixture of CHCl_3 and SbCl_5 and the absorption maximum at $350 \text{ m}\mu$ in hexane exhibited by the liver lipids. The possible role of this pigment in the biogenesis of vitamin A_2 is discussed.

THE STRUCTURE OF A DIMERIC METABOLITE OF D- α -TOCOPHEROL ISOLATED FROM MAMMALIAN LIVER. A. S. Csallany and H. H. Draper (Div. of Animal Nutrition, Univ. of Illinois, Urbana). *J. Biol. Chem.* 238, 2912-2918 (1963). The structure of a dimeric metabolite of α -tocopherol isolated from mammalian liver and synthesized by oxidation of the vitamin with alkaline $\text{K}_2\text{Fe}(\text{CN})_6$ has been elucidated. Spectroscopic and chemical characterization showed that the dimer consists of two tocopherone moieties joined by a C=C bond between the heterocyclic rings at the 7,7'-position. Dimer formation entails a demethylation of α -tocopherol at the 7-position of the chroman ring; the remaining methyl substituents of the benzene ring as well as the phetyl side chain remain intact. Reduction with LiAlH_4 yields a ditocopherol containing two hydroxychroman rings and reoxidation with FeCl_3 leads to the formation of the corresponding tocopherol diquinone. The metabolite has been assigned the trivial name di- α -tocopherone.

THE BIOSYNTHESIS OF FATTY ACIDS IN THE CELLULAR SLIME MOLD, DICTYOSTELIUM DISCOIDEUM. F. Davidoff and E. D. Korn (Lab. of Biochem., Nat'l Heart Inst., NIH, Bethesda 14, Md.). *J. Biol. Chem.* 238, 3210-3215 (1963). An aggregateless mutant of the cellular slime mold *Dictyostelium discoideum* has been demonstrated to synthesize unsaturated fatty acids efficiently from exogenous, labeled precursor fatty acids. Exogenous fatty acids of chain length less than C_{20} were degraded to acetate. Acetate was used predominantly for chain elongation of fatty acids of intermediate chain length. Laurate and myristate served partially as acceptors of acetate, and were partially degraded to acetate. Palmitate appears to be the common intermediate in the biosynthesis of all the long chain unsatu-

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rated acids. It was converted by direct desaturation to palmitoleic acid, 16:1, and a 16 carbon diunsaturated acid, 16:2; by elongation of 16:1 to *cis*-vaccenic acid, 18:1; and, by direct elongation, to stearate and thence to oleic acid, 18:1, and a diunsaturated acid, 18:2. Stearate was converted to oleate, 18:1, and then metabolized further by a second desaturation to 18:2. Margarate (17:0) was also actively transformed into the unsaturated fatty acids, 17:1 and 17:2. The common pathways are (a) specific and sequential desaturation of long chain fatty acids at positions 9 and 5, and (b) chain elongation of saturated and mono-unsaturated 16 carbon acids by addition of a 2 carbon unit.

CHLORINATED INSECTICIDES IN THE BODY FAT OF PEOPLE IN THE UNITED STATES. W. E. Dale (Communicable Disease Center, U. S. Public Health Service, Atlanta, Ga.) and G. E. Quinby. *Science* 142, 593-595 (1963). Benzene hexachloride and dieldrin are present in the body fat of people in the general population of the United States. The mean concentration of dieldrin is 0.15 ± 0.02 parts per million, which is in good agreement with the concentration reported for southern England. The mean concentration of benzene hexachloride is 0.20 ± 0.04 ppm, which is considerably lower than the mean concentration reported for France. Paired analyses of DDT by gas chromatographic and colorimetric methods show that the results of the latter may give incorrectly high results when applied to human fat.

FATTY ACID AND PHOSPHOLIPID COMPOSITION OF THE CELLULAR SLIME MOLD, *Dictyostelium discoideum*. THE OCCURRENCE OF PREVIOUSLY UNDESCRIBED FATTY ACIDS. *Ibid.*, 3199-3209. The fatty acid and phospholipid composition of an aggregateless mutant of *Dictyostelium discoideum* has been analyzed in detail. The composition of the phospholipids is similar to that of many animal tissues, containing phosphatidylcholine, 34%; phosphatidylethanolamine, 32%; lysophosphatidylethanolamine, 10%; phosphatidyl ethanolamine, 8%; bis-phosphatidylglycerol, 8%; and phosphatidylserine, 7%. Evidence is presented both for considerable molecular heterogeneity within each class of phosphatide and for the presence of phosphatides containing the same fatty acid at both the α and β positions. The percentage of unsaturated fatty acids in the lipids of the slime mold amoeba is unusually high, about 90% of the fatty acids of the phospholipids and 50% of the fatty acids of the neutral lipids. Among these acids are previously undescribed dienoic and monoenoic acids.

LIPOPROTEIN MOVEMENT THROUGH CANINE AORTIC WALL. L. E. Duncan, Jr., K. Buck, A. Lynch (Clinical Endocrinology Branch, National Heart Inst., Bethesda 14, Md.). *Science* 142, 972-973 (1963). Low-density (1.019 to 1.063) lipoprotein labeled with radioiodine enters the inner layer of the canine aortic wall directly from the aortic lumen. Its rate of entry is greatest in the proximal aorta and decreases progressively down the length of the aorta. A similar gradient was observed previously for the accumulation of cholesterol early in experimental atherosclerosis.

SYNTHESIS OF FATTY ACIDS IN ANIMAL TISSUES. I. INCORPORATION OF C^{14} -ACETYL COENZYME A INTO A VARIETY OF LONG CHAIN FATTY ACIDS BY SUBCELLULAR PARTICLES. W. R. Harlan, Jr. and S. J. Wakil (Dept. of Biochem., Duke Univ. Medical Center, Durham, N. C.). *J. Biol. Chem.* 238, 3216-3223 (1963). Subcellular particles of mammalian liver incorporated acetyl-CoA into a variety of saturated and unsaturated fatty acids. Relative requirements can be demonstrated for ATP, NADH, and NADPH. A mechanism unique to particulate fractions elongates added acyl-CoA derivatives by addition of acetyl-CoA units. This synthesis is avidin-insensitive and apparently not dependent on malonyl-CoA as an intermediate. The incorporation of the synthesized fatty acids into complex lipids is dependent upon the particle carrying out the synthesis. However, a major portion of the acids synthesized by mitochondria or microsomes is incorporated into phospholipids.

RESTORATION OF PHOTOREDUCTASE ACTIVITIES IN ACETONE-EXTRACTED CHLOROPLASTS BY PLASTOQUINONES AND TOCOPHERYL-

QUINONES. M. D. Henninger and F. L. Crane (Dept. of Biol. Sci., Purdue Univ., Lafayette, Indiana). *Biochemistry* 2, 1168-1171 (1963). Identification of a new member of the plastoquinone group in spinach chloroplasts brings the total number of quinones in these chloroplasts to eight. These include plastoquinones A, B, C, and D; vitamin K₁; and α -, β -, and γ -tocopheryl-quinones. Evidence is presented that each of these quinones functions with a different pattern of specificity for restoration of photoreductase or for stimulation of photooxidase activities in acetone-extracted spinach chloroplasts.

A RESPIRATORY DISEASE SYNDROME IN CHICKENS FED ESSENTIAL FATTY ACID DEFICIENT DIETS. D. T. Hopkins, R. L. Witter and M. C. Nesheim (Dept. of Poultry Husbandry and Avian Disease, Cornell University, Ithaca, N. Y.). *Proc. Soc. Exp. Biol. Med.* 114, 82-86 (1963). A respiratory disease syndrome characterized by formation of bronchial exudate and lung consolidation was observed in chicks fed diets very low in essential fatty acids. These lesions were not observed in chicks receiving similar diets containing 10% of soybean oil.

METABOLISM AND FUNCTION OF BACTERIAL LIPIDS. I. METABOLISM OF PHOSPHOLIPIDS IN *ESCHERICHIA COLI* B. J. Kanfer and E. P. Kennedy (Dept. of Biol. Chem., Harvard Med. School, Boston 15, Mass.). *J. Biol. Chem.* 238, 2919-2922 (1963). Some general features of the metabolism of phosphatides in *Escherichia coli* B have been investigated. Phosphatidylserine and phosphatidylethanolamine have been identified amongst the phosphatides of the lipid fraction readily extractable with chloroform and methanol. The content of phosphatidylglycerol was shown to be much higher in rapidly growing cultures than in cultures in stationary phase. Pulse labeling experiments with orthophosphate- P^{32} revealed that phosphatidylethanolamine, once formed, is completely stable, undergoing no detectable turnover in rapidly growing cultures of *E. coli* B. In contrast, radioactivity present as phosphatidylglycerol after pulse labeling is rather rapidly lost under identical conditions.

FATAL SYNDROME ASSOCIATED WITH VITAMIN E STATUS OF PREGNANT RATS. M. A. Kenney and C. E. Roderuck (Food and Nutrition Dept., Iowa State Univ. of Science and Tech., Ames). *Proc. Soc. Exp. Biol. Med.* 114, 257-261 (1963). Female albino rats, depleted of vitamin E for at least 5 weeks before mating, were given total oral supplements of 0, 2, 6, or 20 mg dl- α -tocopherol on the 8th and 9th days of gestation. Their reproductive performance was compared with controls fed stock ration. The casein-cod liver oil diet (CClo) caused fetal resorption, lowered concentrations of tocopherol in serum, and induced susceptibility to hemolysis in the presence of dialuric acid. Oral supplements of tocopherol on the 8th and 9th days of gestation permitted fetal development to continue, but did not insure normal birth. Gestation was prolonged and most of the young were stillborn. Maternal mortality at parturition was 53%, 27%, and 8% for groups given 2, 6, and 20 mg tocopherol. Death followed lethargy, pallor, dyspnea, and vaginal hemorrhage. Small spleen, large hemorrhagic kidneys, and large adrenals (relative to body weight), accumulation of pleural fluid, and high serum concentration of non-protein nitrogen were characteristic of animals which died at parturition. Hepatic fat of animals fed CClo and given 2 doses of tocopherol to maintain pregnancy was higher than of stock controls or of resorbing rats fed CClo.

LIPASE ACTIVITY IN BLOOD FROM THE HEPATIC AND PERIPHERAL VASCULAR BEDS FOLLOWING HEPARIN. V. S. LeQuire, R. L. Hamilton, R. Adams and J. M. Merrill (Vanderbilt Univ. School of Medicine, Nashville, Tenn.). *Proc. Soc. Exp. Biol. Med.* 114, 104-107 (1963). Clearing factor lipase, an enzyme with the ability to clear a lipemic substrate and to cause hydrolysis of triglyceride, is released during a single passage of heparin through the vascular beds of the dog liver, dog hindlimb and human forearm.

LIPOYL DEHYDROGENASE FROM BEEF LIVER MITOCHONDRIA. C. J. Loyl (Esdel B. Ford Inst. for Med. Res., Henry Ford Hosp., Detroit 2, Mich.). *J. Biol. Chem.* 238, 3443-3452 (1963). Lipoyl dehydrogenase has been isolated from beef liver mitochondria. The enzyme was found to have a molecular weight of 102,000 and to contain 2 moles of flavin adenine dinucleotide per mole of enzyme. This preparation showed the presence of six enzymically active flavoprotein components when examined by chromatography on ion exchange resins and by starch gel electrophoresis.

THE PREPARATION OF H³-VITAMIN D₂ AND D₃ AND THEIR LOCALIZATION IN THE RAT. A. W. Norman and H. F. DeLuca (Dept. of Biochem., Univ. of Wisconsin, Madison). *Biochemistry* 2,

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1160-1168 (1963). A convenient procedure for the preparation of tritium-labeled vitamin D₂ and D₃ is described. The distribution of a physiological dose of H³-vitamin D₃ as a function of time has been studied in the various tissues of the rat. Evidence is presented for the involvement of the ileum in the absorption of vitamin D. The liver rapidly accumulated the largest amount of radioactivity whether the dose was administered orally or intracardially. No evidence was found for the involvement of the adrenals in the metabolism of vitamin D.

THE EFFECT OF AGE ON THE CLEARANCE OF C¹⁴-LABELED TRIPALMITIN IN DOGS AFTER ORAL FEEDING. H. Sobel, K. Parsa, and R. Masserman (Aging Research Lab., Veterans Admin. Hosp., Sepulveda, Calif.). *J. Gerontol.* **18**, 340-342 (1963). C¹⁴-labeled tripalmitin was fed to dogs of four age groups in a meat patty containing lard. Samples of blood were drawn periodically up to 96 hours. Some dogs were sacrificed at 24, 48, 72, and 96 hours, and the aortas were removed. After 2 hours the radioactivity was highest in the serum of dogs 4-8 years of age. It was lowest in those 9 years and older. After 18 hours, the C¹⁴ counts differed only slightly within the age groups; thereafter, particularly at 96 hours, dogs less than one year of age exhibited apparently greater counts than old dogs. Radioactive lipids accumulated in the aorta, and the values were greatest at 96 hours. However, no age differences were observed.

OBSERVATIONS ON NATURALLY OCCURRING PHOSPHOLIPID SYSTEMS. P. D. S. Wood and L. W. Kinsell (Institute for Metabolic Res., Highland-Alameda County Hosp., Oakland, Calif.). *Proc. Soc. Exp. Biol. Med.* **114**, 225-226 (1963). Thin-layer chromatography has been used to gain a roughly quantitative impression of the phospholipid distribution within the human chylomicron and of the phospholipid mixture present in various animal materials. The phospholipids of chylomicrons obtained following a fat meal were similar in distribution to those of total human plasma, and comprised phosphatidyl choline, sphingomyelins, phosphatidyl ethanolamine and lysophosphatidyl choline. Hen egg-yolk phospholipids showed a higher content of phosphatidyl ethanolamine but lower amounts of sphingomyelins. The sera of the sheep, steer and hog were examined, and of these the phospholipids of the last were closest in composition to those of the chylomicron. It is suggested that hog serum phospholipids may provide a physiologically acceptable emulsifying system for use in intravenous fat emulsions.

EFFECTS OF PITUITARY GROWTH HORMONE AND CORTICOTROPIN ON FAT METABOLISM IN ISOLATED RAT DIAPHRAGM. K. L. Manchester (Dept. of Biochem., Univ. of Cambridge, Cambridge, Great Britain). *Biochim. Biophys. Acta* **70**, 531-537 (1963). The effect of hypophysectomy and of some pituitary hormones *in vitro* on the incorporation of C¹⁴ from acetate and palmitate into lipid and protein of isolated rat diaphragm has been studied. The most striking effects observed were: a diminution of incorporation into lipid as a result of hypophysectomy, a reduction in incorporation into lipid by growth hormone and corticotropin in diaphragm from normal animals, but not by growth hormone in diaphragm from hypophysectomized animals, and a stimulation of incorporation into protein by both hormones in diaphragm from hypophysectomized animals.

METABOLISM OF FATTY ACIDS IN THE ISOLATED PERFUSED RAT HEART. O. Stein and Y. Stein (Depts. of Experimental Med. and Cancer Res. and Biochem., Hebrew Univ.—Hadassah Med. School, Dept. of Med. "B" Hadassah Univ. Hosp., Jerusalem, Israel). *Biochim. Biophys. Acta* **70**, 517-530 (1963). Rat hearts were perfused with a medium containing two different radioactive fatty acids, labeled with C¹⁴ and H³, respectively. No preference was found for the extraction from the medium of the following acids tested: palmitic, stearic, oleic and linoleic acid. However, the distribution of these acids in the heart lipids differed. In the neutral lipids of the heart, over 96% of the fatty acids incorporated were found in the triglyceride fraction. In this fraction the incorporation of palmitic acid exceeded that of its competitors: stearic, oleic and linoleic acids. In the phospholipids of the heart, the lecithin fraction contained over 70% of the fatty acids incorporated. Here, stearic and linoleic acid incorporation exceeded that of palmitic acid, while oleic acid was incorporated to a similar extent.

THE ROLE OF LIPIDS IN MITOCHONDRIAL ELECTRON TRANSFER AND OXIDATIVE PHOSPHORYLATION. D. E. Green and S. Fleischer (Institute for Enzyme Research, Univ. of Wisconsin, Madison, Wisconsin). *Biochim. Biophys. Acta* **70**, 554-582 (1963). The

mitochondrion has been fragmented into a fraction containing the elementary particles (the seat of the electron-transfer chain) and a fraction containing the structural protein. On the basis of high-resolution electron microscopy and detailed chemical studies, the mitochondrion is pictured as a structural protein-phospholipid matrix to which are affixed many thousands of elementary particles. Phospholipids can be oriented in water to form water-clear micelles. The micelle is the key to an understanding of the role of phospholipid and of the interaction of phospholipid with mitochondrial proteins. Two types of interaction have been characterized. The first is ionic, and occurs between acidic phospholipids and basic proteins like cytochrome c. The second, mainly hydrophobic in nature, involves the interaction of structural protein with all phospholipids tested, acidic as well as basic.

OXIDATION OF CARBON-14-LABELED ENDOGENOUS LIPIDS BY ISOLATED PERFUSED RAT HEART. J. C. Shipp, J. M. Thomas and L. Crevasse (Dept. of Medicine, Univ. of Florida School of Medicine, Gainesville, Fla.). *Science* **143**, 371-73 (1964). Lipids of the rat heart were labeled with carbon-14 *in vivo*. The production of C¹⁴O₂ during the subsequent perfusion of these glycogen-depleted hearts with buffer, but without added substrate, provided direct evidence of the oxidation of endogenous heart lipids. The net decrease in phospholipid content alone could account for 75 percent of the total metabolic CO₂ formed.

FATTY ACID COMPOSITION OF PLASMA AND LIVER LIPID COMPONENTS AS INFLUENCED BY DIET IN THE GROWING CHICK. G. A. Leveille, J. A. Tillotson and H. E. Sauberlich (U. S. Army Med. Res. and Nutrition Lab, Fitzsimons Gen. Hosp., Denver, Colo.). *J. Nutr.* **81**, 357-62 (1963). The influence of dietary protein and cholesterol on plasma and liver lipids and on their component fatty acid composition was studied in growing chicks. Increased dietary protein decreased plasma and α -lipoprotein lipid levels and increased liver cholesterol. Cholesterol supplementation elevated plasma and liver lipids but decreased α -lipoprotein lipids. In general, feeding a low-protein or a cholesterol-supplemented diet resulted in similar fatty acid changes. Both treatments resulted in significantly increased oleic and decreased arachidonic acid levels in the plasma cholesterol esters, glycerides and phospholipids. The oleic acid level of the liver lipids was also higher in chicks fed the low-protein or cholesterol-supplemented diets. The dietary treatments tended to decrease linoleic acid levels particularly in the cholesterol ester fraction. The cholesterol ester fraction showed the most consistent changes.

ADENOSINE TRIPHOSPHATASE AND ATP-P_i EXCHANGE IN MITOCHONDRIA OF ESSENTIAL FATTY ACID-DEFICIENT RATS. R. M. Johnson (Inst. of Nutrition and Food Tech., Ohio State Univ., Columbus, Ohio). *J. Nutr.* **81**, 411-14 (1963). ATPase, Mg⁺⁺-ATPase, DNP-ATPase, and ATP-P_i exchange was measured in liver mitochondria from normal and essential fatty acid-deficient rats. Little difference was noted in these systems when freshly prepared mitochondria were used. When the mitochondria were aged at 30C for various times, however, differences between the normal and deficient mitochondria became apparent. Uncoupling of oxidative phosphorylation occurred relatively rapidly in deficient mitochondria that were aged, and this appeared to be linked to a loss of ATP-P_i exchange activity, but not to ATPase activity.

VITAMIN E CONTENT OF VEGETABLE OILS AND FATS. D. C. Herting and E. J. E. Drury (Distillation Products Industries, Div. of Eastman Kodak Co., Rochester, N. Y.). *J. Nutr.* **81**, 335-42 (1963). Analyses of vegetable oils or fats from 17 different plant types showed as low as zero μ g of α -tocopherol/g of castor bean and linseed oils and as high as 1276 μ g/g of wheat germ oil. Contents of total tocopherol ranged from 2 μ g/g of coconut oil to 1896 μ g/g of wheat germ oil. In individual types, the tocopherol levels appeared to be influenced by source of plant, time of harvest, stability after harvest, refining procedure, and perhaps by commercial hydrogenation procedures. Estimates of man's requirement for vitamin E as related to the dietary level of polyunsaturated fatty acids suggest that among the common edible polyunsaturated oils (cottonseed, corn, safflower, and soybean), only cottonseed supplies sufficient vitamin E to counter-balance the effect of its polyunsaturated fatty acids.

THE CONVERSION OF CHOLESTEROL AND 20- α -HYDROXYCHOLESTEROL TO STEROIDS BY ACETONE POWDER OF PARTICLES FROM BOVINE CORPUS LUTEUM. P. F. Hall and S. B. Koritz (Dept. of Physiology and Biochemistry, Univ. of Pittsburgh School of Med., Pittsburgh 13, Pa). *Biochemistry* **3**, 129-34 (1964).