ABSTRACTS

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

• Fats and Oils

Trace elements in edible fats. VIII. De-metalisation of soybean oil by cation exchange resins. A. Vioque, M. A. Albi and Maria del P. Villagran (Inst. de la Grasa y sus Deriv.). *Grasas y Aceites* 16, 107–112 (1965). An evaluation of Amberlite CG-120 and Dowex 50WX8 resins, both strong acids, using acetone or hexane as solvents showed that prooxidant metals of crude soybean oils can be effectively removed. Optimal conditions are discussed.

Economic information. Anon. *Lipidos* 25, 70–75 (1965). Statistics are given on prices and world production of oleagenous seeds, fatty materials and vegetable oils.

Neutralizing distillation and simultaneous deodorization of fats and oils. II. A. Millet M. *Lipidos* 25, 51–52 (1965). The "Desoneutrol" installation (Gianazza, Italy) is described for neutralizing and deodorizing oil by quickly circulating a thin layer of oil at about 200C and at residual pressure of one millimeter of mercury.

Notes on the manufacture of fat products by esterification. II. R. Parlan M. *Lipidos* 25, 49–51 (1965). Today it is possible to produce a good esterified oil, even when its components have been artificially prepared. Synthetic glycerol is industrially produced in Europe by sugar fermentation. Synthetic fatty acids are produced by paraffin oxidation. Methods of esterifying fatty acids with glycerol are discussed.

Physical-chemical studies on ground olive pastes. XXIII. Heterogenous distribution of the solids in the pastes. J. M. M. Moreno, C. Gomez H., C. Janer, J. Pereda, J. M. Ferre and V. Flores (Inst. de la Grasa y sus Deriv.). *Grasas y Aceites* 16, 117–127 (1965). The non-homogeneous distribution of the solids in ground olive pastes influences the oil extraction by pressing. A method for isolating pit fragments is described. The results prove that the shearing and pressing of the paste increase the heterogeneity of this distribution. The behavior of mechanical extraction equipment in industrial scale are interpreted according to these results.

Mold phosphatides. I. Thin-layer and column chromatographic investigations on Aspergillus fischeri. A. A. El-Nockrashy, M. M. Hassan, M. Sadek and A. M. Gad (Nat. Res. Center, Dokki, Egypt). *Grasas y Aceites* 16, 113–116 (1965). A method is described for the isolation, characterization and quantitative evaluation of *Aspergillus fischeri* phospholipids by thin-layer chromatography in combination with column chromatography.

Sunflower cultivation and industry. II. I. Mizuno and A. H. Guerreo. *Informaciones sobre Grasas y Aceites (Buenos Aires)* 3 (3-4), 17–30 (1965). This is the second part of a work giving a detailed description of this industry in Argentina.

Argentinian exports of oleaginous seeds, vegetatle oils and by-products in 1964. E. R. Zeni. *Informaciones sobre Grasas y Aceites* 3 (3–4), 13–16 (1965). Exports of seeds, oils, cakes, pellets and flour amounted to $123.5 million in 1964.

Sorbic acid. A preservative agent for margarine production. O. S. Stepanova et al. (Odessa University). *Informaciones sobre Grasas y Aceites* 3 (3–4), 10–12 (1965). Margarine samples kept at 20–25C showed no spoilage after 28 days when 0.02% of sorbic acid was added. Kept at 6–8C the samples retained good taste and odor after 4 months. An addition of 0.03% to the package (wood or cardboard) avoids growth of molds.

Epoxidation of linseed oil. Anon. *Informaciones sobre Grasas y Aceites (Buenos Aires)* 3 (3–4), 63–64 (1965). The employed method consisted of heating the oil with an organic acid (formic 80%), adding a catalyst (sulfuric or phosphoric acid, concentrated) dissolved in a small volume of 35% H_2O_2 and then adding slowly additional H_2O_2 while agitating. The epoxy (oxirane) components were determined by titration with hydrobromic acid dissolved in acetic acid.

Peanut oil. Comparative yields in Tucuman. R. M. Inigo (Tucuman Agr. Center). *Informaciones sobre Grasas y Aceites (Buenos Aires)* 3 (3–4), 57–61 (1965). Yields from several varieties of Argentinian peanuts were measured at the Tucuman Agricultural Experiment Station.

Fat materials in the canning industry. G. Roskis. *Informaciones sobre Grasas y Aceites (Buenos Aires)* 3 (3–4), 31–40 (1965). The canning of fish utilizes by far the largest amount of fat in the industry. In France the main oils used are peanut and olive oil. This paper includes a table showing the physical and chemical characteristics of the oils used in fish canning in several European countries and Algiers.

International Union for pure and applied chemistry. *Informaciones sobre Grasas y Aceites (Buenos Aires)* 3 (1), 53–54 (1965). Methods of analysis discussed at the Fatty Materials Section of the International Union for Pure and Applied Chemistry in Rotterdam (Netherlands) on September, 1965 are reviewed.

German market for soybeans and their by-products. Anon. *Informaciones sobre Grasas y Aceites (Buenos Aires)* 3 (3–4), 1–3 (1965). Although soybeans are not cultivated in Europe, Germany is a major importer of seeds and meal (from U.S.A., Communist China and Brazil), over 1 million tons in 1963. Regulations and prices are presented.

Commercial standards for animal fats. Anon. *Informaciones sobre Grasas y Aceites (Buenos Aires)* 3 (1), 50–51 (1965). New York Produce Exchange Standards for lards and other fats reviewed.

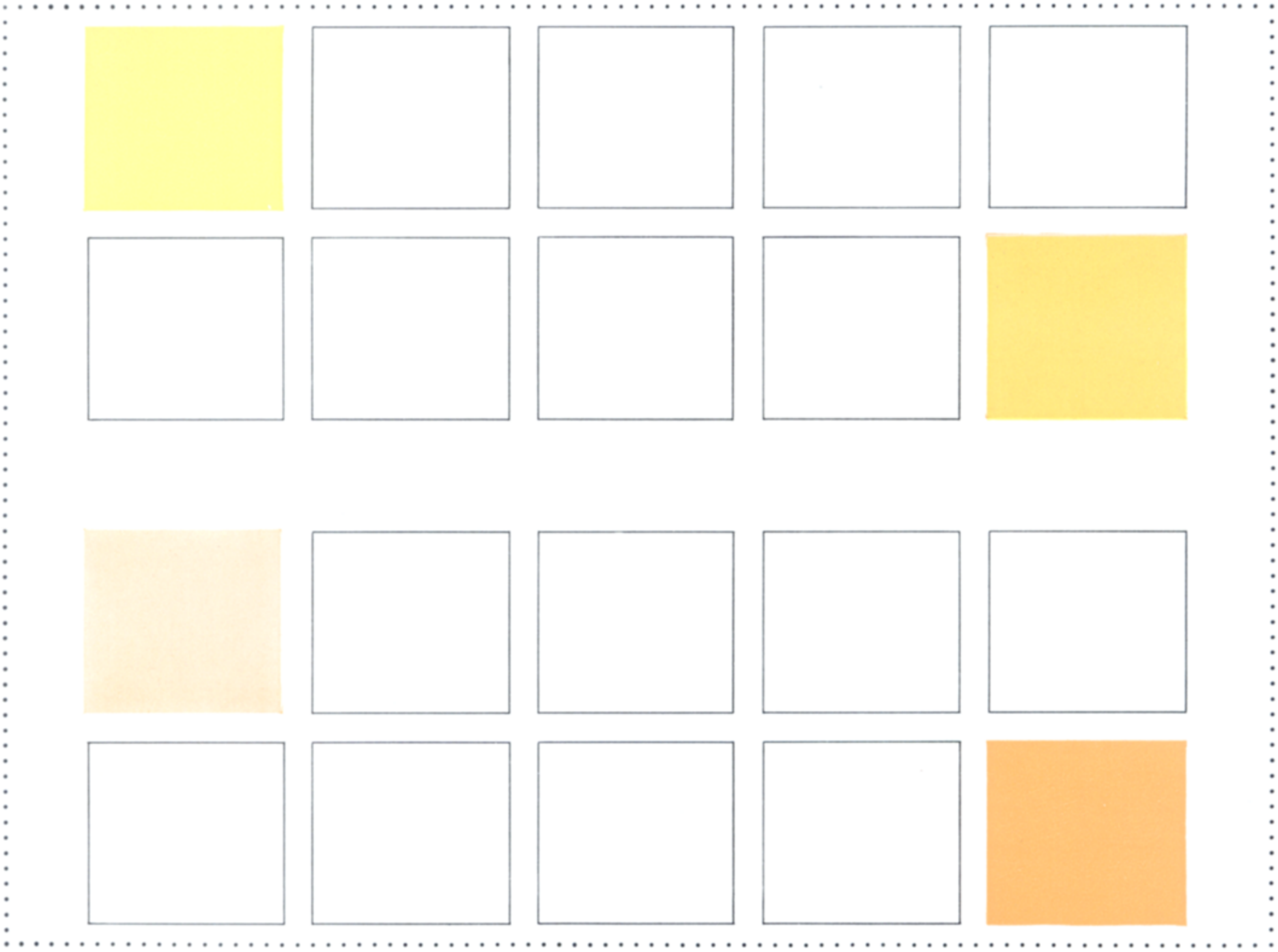
Olive oil. Perspectives of the industry in Spain. J. M. de Sorosa y P. *Informaciones sobre Grasas y Aceites (Buenos Aires)* 3 (1), 42–45 (1965). A review is given of the Spanish olive oil industry today. The trends in machinery are towards the use of improved hydraulic presses, the new type vertical hydraulic presses, better filtration equipment and improved imported integrated equipment for milling, pressing and filtering.

Animal fats. Manufacturing procedures. C. P. Botta. *Informaciones sobre Grasas y Aceites (Buenos Aires)* 3 (1), 18–41 (1965). Rendering of fatty materials by the classical method, by a method using a colloid mill, by the Titan process, by the Sharples method and by the Super-D-Canter is described in detail. Manufacturing methods are given for oleo oil, hog edible fat, lards, bone fat (caracu) and hoof oil.

Sunflower seed in American markets. Anon. *Informaciones sobre Grasas y Aceites (Buenos Aires)* 3 (1), 10–13 (1965). Imports of sunflower seed by U.S.A. are discussed. Amounts, prices and fluctuations between 1957–1964 are given as well as perspects for the future.

Liquid oils. Current situation and perspectives. *Informaciones sobre Grasas y Aceites (Buenos Aires)* 3 (1), 1–9 (1965). Price fluctuations of vegetable oils from 1954–1964 are analyzed. World vegetable oil production in 1964 was of about 14.3 million tons (over 6% more than 1963), due mainly to the good olive crops in the Mediterranean countries. The olive oil production is notably higher than the world consumption (the carry-over of this oil for 1964 is estimated in 600,000 tons). For 1965 a reduction in the world olive oil production of about 700,000 tons is expected.

Gas chromatographic determination of campesterol, β-sitosterol and stigmasterol. A. Rozanski (Res. Labs., The Upjohn Co., Kalamazoo, Mich.). *Anal Chem.* 38, 36–40 (1966). An accurate gas chromatographic method for the determination of campesterol, β-sitosterol, and stigmasterol is described. The sterols are separated as trimethylsilyl ethers on a stainless steel column, packed with 0.75–1.0% silicone gum rubber on an acid-washed, silanized support, having about 4000 theoretical plates. The quantification is carried out by measuring ratios of peak areas before and after addition of a standard amount of one of the sterols to the sample, or by using cholesterol as an internal standard. For the first time, the relative responses in the flame ionization detector of the trimethylsilyl ethers of these three sterols and cholesterol have been accurately determined and found to be significantly different . The method is easily used on a routine basis with precision and accuracy



hitherto unattainable, and has been applied to the analysis of soybean sterols.

Pyrolysis for structure determination of cyclopropene and cyclopropene fatty acids. Joanne Gellerman and H. Schlenk (Univ. of Minnesota, The Hormel Institute, Austin, Minn.). *Anal. Chem.* **38**, 72–76 (1966). Pyrolysis of cyclopropane fatty esters at 350C with SiO_2 as catalyst yields numerous olefinic isomers. The structure of the original ester can be concluded from the products of ozonization-hydrogenation of the olefinic mixture. The same method has been applied to sterculic ester. The cyclopropene ring requires heating to 160C with SiO_2 for cleavage. Dienoic esters result and the products of oxidative fragmentation are again characteristic for the original structure. Hydrogenation with Pd-Pb catalyst in methyl acetate reduces sterculic ester selectively to dihydrosterculic ester while common unsaturated fatty esters remain unchanged. The latter are reduced selectively by PtO_2 in methanol without concurrent hydrogenolysis of the cyclopropane ring. Proper combination of hydrogenation with pyrolysis enables determining the structure of a cyclic acid without isolating it in purity.

An improved method for the colorimetric determination of volatile fatty acids separated by chromatography. S. Zmarlicki. *Przem. Spoz.* **19**, 37–41 (1965). The short chain fatty acids are complexed with ninhydrin. The colored complexes are eluted from a column with ethylamine. The method is reported to be quantitative for the C-2 to C-8 fatty acids. (Rev. Franc. Corps Gras).

Factors determining the consistency of margarine. J. Hannewijk. *Tl. Sr. Pior.* **9**, 137–48 (1965). A comprehensive discussion is given of the effect of triglyceride composition and structure upon the consistency of shortenings as well as margarines. (Rev. Franc. Corps Gras).

Relation of the refractive index and the molecular weight of fatty acids. F. S. Omel'cenko. *Piscev. Technol.* **4**, 30–1 (1965). With knowledge of the refractive index of fatty acids, one can establish their purity and determine different physiochemical properties and their molecular structure. After determining the refractive index by experiment, it is easy to calculate the molecular weight of a mixture of fatty acids. (Rev. Franc. Corps Gras).

Oxygen molecule as isomerizing agent during hydroperoxidation of methyl oleate. N. A. Kahn (Div. of Food and Fruit Res., East Reg. Lab., Dacca, Pakistan). *Oleagineux* **20**, 523–526 (1965). The similarity of the oxygen molecule to various isomerizing agents (nitrogen dioxide, sulphur, selenium and iodine) has been established. The specific behavior of nitrogen dioxide and the physico-chemical properties of the oxygen molecule has left no doubt that the latter can easily form transition complexes facilitating *cis-trans* interconversion. Results of experimentation on the hydroperoxidation of methyl oleate has lent support to these ideas and made possible the proposition of suitable mechanisms for *cis-trans* interconversion.

The manufacture of dispersions. K. Marshall (Indus. Pharmacy Unit, Bradford Inst. of Tech., Bradford, Eng.). *Soap, Perfumery, Cosmetics*, **38**, 759–68 (1965). Liquid-liquid emulsification, homogenisation and solid dispersion are a part of many manufacturing procedures, yet remain largely empirical in nature. This review is primarily concerned with production aspects. Elementary theoretical concepts, where generally accepted, and suggestions for assessing process efficiency are included to demonstrate probable means of realizing optimum manufacturing conditions.

A survey of the current position of the rice bran oil industry in various countries. S. D. Thirumala Rao and K. S. Murti (Oil Tech. Inst., Anantapur, India). *Oils Oilseeds J. (Bombay)* **17**, 6–9 (1965).

Quantitative determination of stigmasterol in rice bran oil. Nobuharu Azagami and Shugo Morita (Toyo Koatsu Inds., Sunagawa-shi, Hokkaido). *Yukagaku* **14**, 497–9 (1965). Sigmasterol contents in 5 samples of mixed sterols isolated from rice bran oil were determined by infrared spectrophotometry and gas chromatography and the values were found to be 13.0–14.5% by the former method and 14.3–15.8% by the latter. The infrared method is simple and a combined determination with the GLC method seems to be useful.

Kapok seed oil. 1. Cyclopropenoid fatty acid in kapok seed oil. Yoshiyuki Kawase, Kiyomi Niizuma, Kosaku Yasuda and Kazuhiko Yoshitomi (Nissin Oil Mills, Ltd., Yokohama). *Yukagaku* **14**, 506–9 (1965). The determination of cyclopropenoid fatty acid in kapok seed oil was caried out in a benzene-acetic acid solution of the oil. Hydrogen bromide in acetic acid was added and the excess hydrogen bromide back titrated with 0.1N sodium carbonate in acetic acid. The content of cyclopropenoid fatty acid in crude kapok seed oil was usually at 12–14%. Both bleaching and deodorization processes tended to decrease the amount of cyclopropenoid fatty acid with an increase in conjugated diene. Large amount of earth in the bleaching process resulted in extensive opening of cyclopropenoid ring.

Problem of fat oxidation in fried instant noodles. Taro Yamashita (Japan Oil and Vitamin Inspection Inst., Tokyo). *Yukagakau* **14**, 754-66 (1965). A review with 20 references.

Problems arising from the oil oxidation in the marine products and its prevention. Kenzo Toyama (Tokyo Univ. Fisheries, Tokyo). *Yukagaku* **14**, 760–6 (1965). A review with 110 references.

Stability of soybean phosphatides in storage conditions. II. Change of soybean phosphatides suspension by heating. Hitoshi Enei, Noriko Nakao and Shizuyuki Ota (Ajinomoto Co., Inc., Kawasaki-shi, Japan). *Yukagaku* **14**, 522–8 (1965). The changes of phosphatide components and emulsifying properties in heated soybean phosphatide suspensions were studied. Soybean phosphatide suspensions (10%) were heated at 95C for 0, 15, 30, 60 and 120 minutes, respectively, then freeze-dried. The content of water-soluble compounds and lysophosphatide was increased in the heated samples. Heated phosphatide suspension easily made O/W type emulsion in the system of soybean oil with water and they were more stable than unheated sample. Emulsifying properties of lysolecithin and glyceryl-phosphorylcholine prepared from lecithin were determined; both of them formed O/W type emulsion easily but former gave a stable emulsion while that of the latter was unstable.

Gas chromatographic studies on the mechanism of catalytic hydrogenation of conjugated polyenoic acid. Toru Takagi (Nagoya Univ.). *Yukagaku* **14**, 559–62 (1965). Catalytic hydrogenation of methyl eleostearate and conjugated octadecadienoate were carried out by using palladium black and platinum black. Only octadecenoate and stearate were

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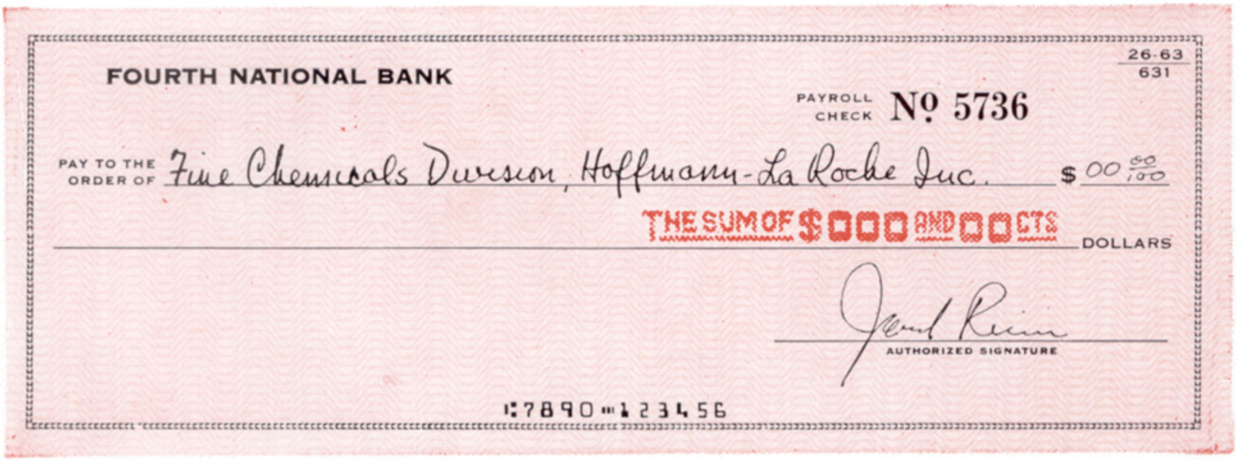

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detected in the product from each ester. Oxidative scission of octadecenoates produced from eleostearate indicated the formation of nearly equimolar amounts of 9, 10, 11, 12 and 13-octadecenoate. Conjugated octadecadienoate was selectively hydrogenated in the presence of linoleate. One step hydrogenation mechanism from conjugated polyenoic acid to monoenoic acid is adequate to explain the results obtained.

HYDROXY FATTY ACIDS IN SEED OILS. I. Maromi Takeda and Yoshiyuki Toyama. *Yukagaku* 14, 576–86 (1965); II. *Ibid.* 638–46. A review.

ONE METHOD OF STUDYING ON THE CRYSTAL TRANSFORMATIONS OF PLASTIC FATS. Keiji Sumi and Yoshiro Abe. *Yukagaku* 14, 606–12 (1965). Crystal transformation of plastic fats such as tallow, hydrogenated fish oil, the binary system of tallow and hydrogenated fish oil, and cocoa butter was studied by x-ray diffraction. Four crystal forms α, β', intermediate and β were identified. As tallow and hydrogenated fish oil take the crystal structure of β' and intermediate form, it is possible to determine the intensity of diffraction band at 3.8 A which is characteristic. Cocoa butter takes α and β form, and the α directly transforms to β. The proportion of β form, therefore, can be determined in similar manner. The relation between transformation of polymorph and tempering of the plastic fats can be graphically presented within 4 hours.

CULINARY MIX UTILIZING LIQUID OIL SHORTENING AND PROCESS FOR PREPARING SAME. R. M. Weiss, J. M. Sinner, and W. F. Bloch (Pillsbury Co.). *U.S. 3,222,184.* A prepared package cake mix for producing a cake having at least 13% shortening basis dry ingredients comprises: a container containing a dry admixture comprised of sugar, flour, leavening and an oil-emulsifier concentrate. The ratio of liquid oil and emulsifier in the concentrate is about 1 part by weight oil to 1 part emulsifier. The total quantity of liquid oil in the concentrate does not exceed about 25% of the total shortening necessary to shorten the cake. A method of preparing the cake mix comprises the following steps: preparing a composition comprised of sugar, flour, leavening, 1–3 parts emulsifier and 1–3 parts of a quantity of liquid shortening in the range of 10–50% of the total shortening used in the cake, as the principal ingredients by thoroughly admixing the ingredients; placing the composition in an outer container together with a second container containing liquid oil shortening in an amount to provide the balance of the shortening needed to produce a cake; and closing the outer container.

GLYCEROL ESTERS IN LEADED GASOLINE. E. N. Case, S. H. Patinkin and D. R. Carlson (Sinclair Research Inc.). *U.S. 3,222,146.* A leaded gasoline consists of base hydrocarbon gasoline containing at least about 10 volume per cent unsaturated hydrocarbons, a small amount of lower-alkyl lead anti-knock agent sufficient to reduce knock and about 1 to 50 moles per mole of lead compound of a gasoline-compatible ester containing up to 20 carbon atoms and comprising a glycerol triester of an aliphatic carboxylic acid of 1–9 carbon atoms in an amount sufficient to increase the octane number of the leaded gasoline.

PROCESS FOR RENDERING ANIMAL FAT. M. E. Blumberg and G. J. Gould (Pennsalt Chemicals Corp.). *U.S. 3,222,384.* In a process for rendering animal hide fleshings including the steps of heating the fleshings to at least 160F in the presence of added water, comminuting the heated fleshings, centrifugally separating the fleshings into tissue and melted fat containing residual tissue, screening the residual tissue and melted fat and then centrifugally separating the residual tissue from the melted fat, the improvement comprises screening and separating the residual tissue from the melted fat in the presence of at least one compound having the formula $R{-}S{-}R'{-}O(R'{-}O)_nH$ serving as a surface active agent. R represents a tertiary alkyl radical containing 12 carbon atoms, R′ is an ethylene radical, and n represents the number 9.

AERATING COMPOSITION FOR FATS AND SHORTENINGS CONTAINING SAME. J. V. Luck and R. H. Ellinger (Glidden Co.). *U.S. 3,224,882.* An aerating composition for triglyceride fats consists of a blend of about 2.3–4 parts of stearyl-1 and palmityl-1 lactylic acids with one part of free stearic and palmitic acids, the stearyl-1 predominating over the palmityl-1 lactylic acid. The composition contains, on the basis of the acyl-1 lactylic acids, not more than about 10% acylated polylactic acids.

AEROSOL TOPPING. M. Pader and S. D. Gershon (Lever Brothers Co.). *U.S. 3,224,883.* An edible whippable topping composition with excellent storage stability confined under pressure in an aerosol dispensing container consists of a base fat, a casein material and a phosphoric acid ester composition selected from the group consisting of (1) those obtained by reacting a derivative of phosphorus with a mono- and/or diglyceride mixture obtained by super-glycerinating a fat having an iodine value within the approximate range of 30–70 and (2) those obtained by combining phosphoric acid esters prepared by separately reacting a derivative of phosphorus with a saturated and with an unsaturated mono- and/or diglyceride. In the latter case, the saturated glyceride results from super-glycerination of a fat having an iodine value below 30, and the unsaturated mixture results from super-glycerination of a fat having an iodine value above 70.

TOPPING MIX. M. Pader and S. D. Gershon (Lever Brothers Co.). *U.S. 3,224,884.* A whippable topping mix which has improved stability for preparing a whipped topping that is similar to whipped cream comprises a base fat, a water dispersible protein, a phosphoric acid ester of a glyceride and at least one emulsifier selected from (1), (2) and (3) where (1) consists of a partial ester of a glycol and a higher saturated fatty acid; (2) consists of a mixture of a lactylated glycerol ester of a saturated fatty acid and an ester of a polyhydric alcohol and an unsaturated fatty acid; and (3) consists of a mixture of a lactylated glycerol ester of a saturated fatty acid and a lactylated ester of a polyhydric alcohol and an unsaturated fatty acid.

PROCESS FOR ACID AND THEN ALKALINE REFINING FATTY OILS. L. O. Bergman (Malmo, Sweden). *U.S. 3,226,407.* In a process for refining crude fatty oil in which the oil is degummed with acid, neutralized with aqueous alkali solution and subsequently treated with acid, the improvement involves: (1) the degumming is effected under reduced pressure with orthophosphoric acid and (2) the neutralization is effected by passing the

degummed oil up through alkaline aqueous solution, the oil being fed into the solution in a plurality of separate streams and rising through the solution due solely to the buoyancy forces existing between the oil and aqueous solution. The streams, while in contact with the solution, break at their upper ends into droplets of 0.3 to 3 mm in diameter. The droplets then continue to rise through the solution to form an oil layer thereon, whereby the oil is intimately contacted with the alkali solution to neutralize the fatty acids while concomitantly avoiding substantial saponification and emulsification.

• Fatty Acid Derivatives

HYDROCARBONS GENERATED IN CATALYTIC REDUCTION OF FATTY ESTERS. Isao Ikeda, Shozo Hattori and Saburo Komori (Osaka Univ.). *Yukagaku* **14**, 510–16 (1965). Methyl palmitate, cetyl alcohol and cetyl acetate were treated with hydrogen at high pressure (initial pressure at 100–200 atm.) in the presence of various catalysts, such as Cu-Cr-O, Zn-Al-O, Fe-Al-O, Fe-Cu-Al-O, Fe_2O_3, reduced Fe, ZnO, Al_2O_3, CuO Cr_2O_3, and Raney Ni. The amount and type of hydrocarbon generated depended on the catalyst used and the reaction temperature. In the case of reduced Fe and the metal oxides, generation of a C-16 hydrocarbon was considered to be produced by dehydration of alcohol and it was preferential to the generation of hydrocarbon having 15 carbon atoms which was considered to be produced by elimination of hydroxymethyl group from the alcohol. The order of its priority was as follows: Fe-Cu-Al-O $>$ ZnO, CuO, reduced Fe, $Al_2O_3 >$ Zn-Cr-O, Fe-Al-O $> Cr_3O_2 >$ Cu-Cr-O. In case of Cu-Cr-O catalyst, there was no appreciable difference in the amount of either hydrocarbon generated. In case of Raney Ni, the generation of C-15 hydrocarbon was predominant and the generation of C-16 hydrocarbon was almost the same as that of C-14 hydrocarbon or less. In spite of high pressure of hydrogen, the content of olefins in the hydrocarbon formed was increased in the order Fe-Al-O $<$ Zn-Al-O $<$ Zn-Cr-O $<$ $CR_2O_3 <$ ZnO $< Al_2O_3 < Fe_2O_3$. At the same reaction temperature (around 300C) the ease of generation of hydrocarbon was in the order of Raney Ni $>$ Cu-Cr-O $>$ Fe-Cu-Al-O $>$ reduced Fe $>$ Fe-Al-O $>$ Zn-Al-O, ZnO $> Cr_2O_3 >$ CuO $> Fe_2O_3 >$ Zn-Cr-O $> Al_2O_3$.

RUST-PREVENTIVE PROPERTIES OF LONG-CHAIN ALKYLPHOSPHONIC ACID DERIVATIVES. II. RUST-PREVENTIVE PROPERTIES OF 9(10)-PHOSPHONOSTEARIC ACID COMPARED WITH THE OTHER 9(10)-SUBSTITUTED STEARIC ACIDS. Yuzo Machida, Yoshiki Okamono and Hiroshi Sakurai (Osaka Univ.). *Yukagaku* **14**, 563–5 (1965). 9(10)-Phosphonostearic acid had better properties than the others, such as 9(10)-carboxy, 9(10)-mercapto and 9(10)-aminostearic acid by the potassium ferricyanide test, salt water immersion test and humidity cabinet test.

CHLORINATED FATTY MATERIALS. I. HEAT-STABILITIES OF CHLORINATED FATTY ACIDS AND SOME OF THEIR DERIVATIVES. Hiromu Miya and Tetsuya Yokokawa (Ind. Res. Inst., Kanagawa Pref.). *Yukagaku* **14**, 630–5 (1965). Chlorinated fatty materials were heated for 4 hours in nitrogen, air or in a sealed tube *in vacuo* (1 mm. Hg). 9- (or 10-) monochlorostearic acid, 9,10-dichlorostearic acid and their derivatives were more stable than random chlorinated materials. No difference was observed between fatty acids and their derivatives. They showed most color development in sealed tube.

WAX ESTER SUBSTITUTE FOR JOJOBA OIL FROM THE SEED OF LIMNANTHES DOUGLASSI. T. K. Miwa and I. A. Wolff (Secretary of Agr. U.S.A.). *U.S. 3,226,406.* A liquid wax ester product is produced by the method comprising: (a) solvent extracting the glyceride oil present in ground seed of *Limnanthes douglasii*; (b) removing the solvent; (c) separately preparing the mixed constituent fatty acids by saponification and the mixed corresponding fatty alcohols from separate portions of the glyceride oil by reduction; and (d) reacting 1.10 mole equivalent of the constituent fatty acids with 1.00 mole equivalent of the fatty alcohols in the presence of 100 and 0.015 mole equivalents, respectively, of xylene and *p*-toluene-sulfonic acid monohydrate to form the desired liquid wax ester product.

• Biochemistry and Nutrition

INFLUENCE OF SEX AND GONADAL HORMONES ON RAT-LIVER AND CARCASS LIPIDS DURING THE DEVELOPMENT OF AN ESSENTIAL FATTY ACID DEFICIENCY. Rosemarie Ostwald, Pauline Bouchard, P. Miljanich and R. L. Lyman (Univ. of California, Berkeley). *Biochem. J.* **97**, 485–99 (1965). Groups of intact male and female rats and castrated rats injected with estradiol or testosterone were fed a diet containing hydrogenated coconut oil for 9 weeks, and at intervals the amounts and fatty acid compositions of the carcass and liver lipids were determined. Male rats grew faster and larger, and exhibited typical external essential fatty acid deficiency symptoms sooner than did females. Testosterone-treated castrated males were similar to males, and estradiol-injected castrated males remembled females. Intact females maintained a higher linoleic acid concentration in their carcass than did males. Results indicated that the greater growth rate of the male rats caused them to require and synthesize more phospholipids than did the females. In the absence of adequate amounts of arachidonic acid, eicosatrienoic acid was substituted into the additional phospholipid. The earlier symptoms of EFA deficiency in the male rat could therefore be ascribed to the higher tissue concentrations of this unnatural phospholipid and its inability to perform the normal metabolic functions of phospholipids.

LOW CALORIE DIET. H. W. Howard, W. B. Jacobsen, Jane Hsueh, R. C. Selby and T. W. Workman (Borden Co.). *U.S. 3,219,454.* Described is a liquid food for human consumption which consists of an aqueous dispersion of mixed protein, carbohydrates and fat. The protein is water dispersible and is selected from the group consisting of milk, meat, egg, and degraded and amino acid fortified soy and peanut proteins; the fat is selected from the group consisting of milk fat, soybean oil and corn oil. The proportions by weight are about 650–1,000 parts of carbohydrates and 300–600 parts of protein for 100 parts of the fat, the carbohydrates being in proportion to provide at least 475 calories for an amount of the liquid food providing a total of 900 calories. The carbohydrate includes a mixture of 5–50 grams of soluble dextrin, 60–90 grams lactose, and 2–20 grams total of dextrose and maltose for the indicated amount of the liquid food. The protein is at least half milk protein.

AUTOGENOUS, VITAMIN E ACTIVE BEADLET PRODUCT AND PROCESS. C. H. Benton, Jr. (Eastman Kodak Co.). *U.S. 3,221,029.* The described product consists of beadlets of d-α-tocopheryl acid succinate having a bulk density in a range from about 24 to 38 pounds per cubic foot and a particle size in a range from about 20 to 100 mesh U.S. screen size. A quantity of d-α-tocopheryl acid succinate is melted and the molten mass is mixed with an inert liquid dispersion medium in which the tocopherol compound is insoluble and which has a viscosity selected in accordance with the desired average beadlet size. The droplets are solidified and hardened by removing the heat until the temperature of the droplets is below the solidifying temperature of the d-α-tocopheryl acid succinate, whereby beadlets are formed.

SIMULTANEOUS OXIDATION OF TOCOPHEROL AND CAROTENE BY OXYGEN FROM AIR IN THE PRESENCE OF SATURATED AND UNSATURATED FATTY ACID ESTERS. M. Loncin and B. Jacobsberg (C.E.R.I.A., Brussels). *Fette Seifen Anstrichmittel* **66**, 910–11 (1964). α-Tocopherol is not attack by atmospheric oxygen at 50C if other double bonds susceptible to oxidation are present. Such bonds may originate from fatty acids or carotene.

THE FLAVOUR VOLATILES OF FATS AND FAT-CONTAINING FOODS. II. A GAS CHROMATOGRAPHIC INVESTIGATION OF VOLATILE AUTOXIDATION PRODUCTS FROM SUNFLOWER OIL. P. A. T. Swoboda and C. H. Lea (Low Temp. Res. Station, Cambridge). *J. Sci. Food Agr.* **16**, 680–9 (1965). Volatile compounds formed during the autoxidation of freshly refined sunflower oil at 37C, as well as those produced during subsequent heating of the oxidized oil, were concentrated by vacuum distillation and separated by gas chromatography. A total of 22 individual aliphatic compounds of carbon chain length greater than 5 were identified. Semi-quantitative analysis, using a flame ionization detector demonstrated a marked preponderance of hexanal in the volatile products of low temperature oxidation; the much greater quantity of volatiles resulting from thermal decomposition of the first-formed hydroperoxides contained major proportions of the deca-2,4-dienals. According to the authors, the formation of the identified compounds (saturated and unsaturated aldehydes, ketones, hydrocarbons and an alcohol) can be explained in large part by accepted mechanisms for the free radical decomposition of linoleate and oleate hydroperoxides, though it is not easy to account for the quantitative aspects.

BIOSYNTHESIS OF COPROSTANYL ESTERS. R. S. Rosenfeld (Montefiore Hosp. and Med. Center, Bronx). *Arch. Biochem. Biophys.* 112, 621–5 (1965). Portions of a fecal suspension were incubated in separate experiments with 7-α-H^3-cholesteryl palmitate and C^{14}-palmitic acid. The major route of formation of coprostanyl esters is from esterification of free coprostanol and not by reduction of cholesteryl esters. The data are in accord with the following sequence: (1) hydrolysis of cholesteryl palmitate; (2) reduction of cholesterol to coprostanol; and (3) esterification of coprostanol. There was no conclusive evidence for reduction of cholesteryl palmitate without prior hydrolysis. The author considers it probable that the reactions are carried out by fecal bacteria.

EFFECT OF VITAMIN B_6 DEFICIENCY ON THE COMPOSITION OF YEAST LIPIDS. Betty E. Haskell and E. E. Snell (Univ. of California, Berkeley). *Arch. Biochem. Biophys.* 112, 494–555 (1965). When the yeast *Hanseniaspora valbyensis* was grown in a vitamin B_6-deficient medium, the amount of palmitoleic acid, the principal monounsaturated fatty acid component, decreased dramatically. Saturated fatty acid levels remained relatively constant. Deficient cells contained only about 60% as much total lipid as control cells; the phytosphingosine content also was substantially lowered. Lipid phosphorus levels were unchanged. Nicotinic acid deficiency increased the total lipid content; pantothenic acid deficiency decreased it. Pantothenate deficiency affected both chain length and degree of saturation of the fatty acids, whereas vitamin B_6 deficiency affected both chain length and degree of saturation alone. These results support previous postulates of a role for B_6 in synthesis of unsaturated fatty acids and show that this role extends to synthesis of monounsaturated compounds.

BIOSYNTHESIS OF STEROLS IN THE SOYBEAN PLANT. J. A. Waters and D. F. Johnson (National Institute of Arthritis and Metabolic Diseases). *Arch. Biochem. Biophys.* 112, 387–91 (1965). Radiochemically pure stigmasterol and β-sitosterol were isolated as major products of mevalonic acid-2-C^{14} incubation with excised leaves of the soybean plant. Two minor biosynthetic products of this radioactive sterol precursor were partially characterized; evidence suggests that they are monohydroxy, saturated compounds. Incubation experiments with radioactive stigmasterol and β-sitosterol indicate that the two are not directly related biosynthetically via hydrogenase-dehydrogenase enzyme systems.

VITAMIN E DEFICIENCY IN THE MONKEY. VII. THE ROLE OF VITAMIN E AND THE VITAMIN-LIKE ACTIVITY OF COENZYME Q. C. D. Fitch (Univ. of Arkansas School of Med.), J. S. Dinning, F. S. Porter, K. Folkers, H. W. Moore and J. L. Smith. *Arch. Biochem. Biophys.* 112, 488–93 (1965). Young Rhesus monkeys were fed a vitamin E deficient purified diet containing, by weight, 11% fat and 18% isolated soybean protein. After 1–2 years they developed the characteristic deficiency syndrome of nutritional muscular dystrophy and anemia. The syndrome was not influenced by extra dietary methionine and cystine, by the presence or absence of choline, or by selenium. Complete remission of the muscular dystrophy and anemia was induced either by *d*-α-tocopherol or by its *l*-epimer, but the duration of the remission after treatment with the *l*-epimer was relatively short. Coenzyme Q_{10} treatment evoked a reticulocytosis in the anemic- vitamin E deficient monkey, and a complete hematologic remission followed treatment with a compound of lower molecular weight, hexahydrocoenzyme Q_4. Vitamin E either is involved in the maintenance of coenzyme Q activity or can substitute for coenzyme Q.

BIOSYNTHESIS OF CHOLESTEROL AND UBIQUINONE IN THE LESSER SPOTTED DOGFISH. A. T. Diplock and G. A. D. Haslewood (Vitamins Ltd., Tadworth, Surrey). *Biochem. J.* 97, 36P–37P (1965). *In vitro* a dogfish liver was homogenized with phosphate buffer and incubated with H^3-sodium acetate in the presence of ATP, NAD and NADP and the product was saponified after the addition of carrier ubiquinone. Between 15 and 30% of the total unsaponifiable fraction radioactivity was found in squalene. As purification of cholesterol and ubiquinone proceeded, a progressive drop in the specific activity, usually to zero, was noted. This was interpreted as a failure to biosynthesize either compound.

A MECHANISM FOR THE BIOSYNTHESIS OF α-CAROTENE. T. W. Goodwin and R. J. H. Williams (Univ. College of Wales, Aberystwyth). *Biochem. J.* 97, 28C–32C (1965). Carrot root slices were incubated with labeled mevalonic acid and the composition of the unsaponifiable matter was determined. Results suggested that α-carotene is not synthesized from β-carotene,

but that it arises separately. Thus although β-zeacarotene is a possible precursor of α- and β-carotene via γ-carotene, it is ruled out as a precursor of α-zeacarotene. β-Carotene is also ruled out as a precursor of α-carotene, but the possibility that α-carotene is a precurosor of β-carotene remains. It is more reasonable, however, to assume that two enzymes are concerned with cyclization of carotenoids; both cause the formation of the same transient carbonium ion, and the disposition of the substrate on the active sites of the two enzymes causes the ejection of a proton from C-6 (β-ionone formation) or C-4 (α-ionone formation). The probable pathways from neurosporene to the various cyclic carotenes are shown schematically.

EVIDENCE THAT THE OXIDATION OF THE SIDE CHAIN OF CHOLESTEROL BY LIVER MITOCHONDRIA IS STEREOSPECIFIC, AND THAT THE IMMEDIATE PRODUCT OF CLEAVAGE IS PROPIONATE. K. A. Mitropoulos and N. B. Myant (Hammersmith Hosp., London, W. 12). *Biochem. J.* **97**, 26C–28C (1965). Labeled cholesterol was incubated with mitochondria from rat liver. Results showed that the initial step in the enzymic oxidation of the isopropyl unit of the side chain of cholesterol is stereo-specific. The immediate product of cleavage is propionate.

EFFECT OF INTRAVENOUS INFUSIONS OF ACETATE, β-HYDROXYBUTYRATE, TRIGLYCERIDE AND OTHER METABOLITES ON THE COMPOSITION OF THE MILK FAT AND BLOOD IN COWS. J. E. Storry and J. A. F. Rook (Nat. Inst. Res. Dairying, Shinfield, Reading, Berks). *Biochem. J.* **97**, 879–86 (1965). The effects in the cow of intravenous infusions of sodium acetate, butyrate, propionate, β-hydroxybutyrate, malonate, citrate or succinate, of glucose or of an emulsion of cottonseed oil on the secretion of the component fatty acids of milk fat and on the composition of the blood plasma of the jugular vein have been studied. Glucose and cottonseed oil were the only metabolites consistently to affect the yield of milk fat. Glucose decreased the yield of milk fat through a diminished secretion of the C_{18} fatty acids and in 2 out of 3 cows also of the steam-volatile fatty acids (C_4–C_{10}). The cottonseed oil caused an increase in the yield of milk fat through an increased secretion of linoleic acid. In 3 of 4 cows, acetate caused an increase in the yield of milk fat through an increased secretion of mainly palmitic acid.

THE EFFECT OF DIET ON THE FATTY ACID COMPOSITIONS OF SERUM, BRAIN, BRAIN MITOCHONDRIA AND MYELIN IN THE RAT. L. Rathbone (Guy's Hosp. Med. School, London). *Biochem. J.* **97**, 620–8 (1965). Three groups of female rats were maintained on a linoleic acid rich diet (sunflower seed oil), a linoleic acid poor, predominately saturated fatty acid diet (coconut oil) and a normal diet. Of the serum fatty acids, linoleic acid showed the greatest change in the percentage of the total acids in response to diet; the change in the proportion of oleic acid was considerable. The percentages of arachidonic acid in serum fatty acids in the groups on the linoleic acid-rich and linoleic acid-poor diets were similar, but higher than those in the normal group. Changes in the proportions of linoleic, arachidonic and docosahexaenoic acids occurred in brain fatty acids that to some extent paralleled those occurring in serum. Changes in the proportions of most other acids in the serum fatty acids were not accompanied by corresponding changes in the brain fatty acids. Changes in the relative proportions of linoleic, arachidonic and possibly some docosapolyenoic acids were demonstrated to occur in brain mitochondria and myelin as a result of diet. The author suggests that similar changes may result in humans.

EXTENT OF TOCOPHEROL DEPLETION VERSUS ONSET OF CREATINURIA IN RATS FED SATURATED OR UNSATURATED FAT. L. A. Witting, E. M. Harmon and M. K. Horwitt (L. B. Mendel Res. Lab., Elgin State Hosp., Elgin, Ill.). *Proc. Soc. Exp. Biol. Med.* **120**, 718–21 (1965). Rats fed a diet containing either a saturated or an unsaturated fat were depleted of α-tocopherol (whole body) at comparable rates. Upon depletion to an average tissue α-tocopherol level of 2.4 μg/g wet weight of tissue, the group fed the unsaturated fat showed a significant creatinuria. Depletion of the saturated fat-fed group to an average tissue α-tocopherol level of 0.4 μg/g did not produce creatinuria.

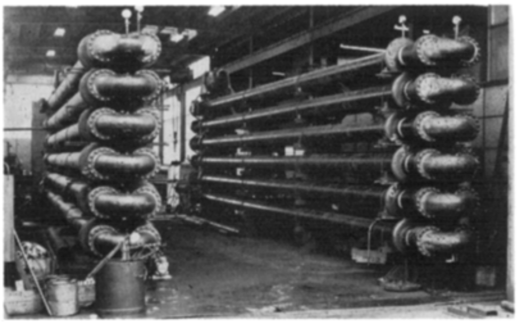
METABOLISM OF POLYUNSATURATED FATTY ACIDS AND SERUM CHOLESTEROL LEVELS IN THE RAT. H. C. Tidwell, J. L. Pipe and Patricia Gifford (Biochem. Dept., Univ. of Texas, Sw. Med. School, Dallas, Texas). *J. Nutr.* **88**, 111–14 (1966). Male albino rats were fasted for 4 days and then fed various fats in order to study the effect of the fat upon blood ketone and liver glycogen levels. The fats included coconut oil, lard, and linseed, safflower and tung oils. The ingestion of the more saturated fats, and tung oil with its conjugated double bonds, was associated with higher blood ketone levels than the other more unsaturated fats. The opposite was true for liver glycogen levels except for coconut oil which may have spared glycogen by its more rapid oxidation. The results obtained are in line with the speculation that the polyunsaturated fatty acids during their utilization might be so degraded that the 3-carbon fragments between the double bonds formed would be more glycogenic in nature than the ketolytic 2-carbon fragments produced when saturated fatty acids are oxidized. The speculation was advanced that such degradation, if true, might play some part in affecting blood cholesterol and lipid levels.

INFLUENCE OF VITAMIN A AND D FORTIFICATION ON THE FLAVOR OF INSTANT NONFAT DRY MILK. E. L. Thomas, S. T. Coulter and J. M. Kudale (Dept. of Dairy Industries, Univ. of Minn., St. Paul, Minn.). *J. Dairy Sci.* **48**, 1561–65 (1965). Twenty-one experimental batches of instant nonfat dry milk (NDM) were prepared in cooperation with four commercial manufacturers and three suppliers of vitamins. Vitamins A and D were added to yield levels of 10,000 and 2,000 IU/lb of NDM, respectively, in fortified batches. Flavor evaluations by a trained panel (triangle difference test) were made initially and periodically during storage at 100 and 70F up to 5 and 12 months, respectively. No correlation between vitamin retention and flavor was evident. It is concluded that although a flavor associated with vitamin A deterioration doubtless develops on storage of NDM containing vitamin A of the types and levels added in this study, other changes are of at least equal significance and, in general, overshadow it in importance.

CONTRIBUTION OF FREE FATTY ACIDS TO THE FLAVOR OF RANCID MILK. R. A. Scanlan, Lois A. Sather and E. A. Day (Dept. of Food Sci. and Tech., Oregon State Univ., Corvallis, Ore.). *J. Dairy Sci.* **48**, 1582–84 (1965). Fatty acids added to fresh milk in the quantities reportedly liberated by lipase, produced a flavor similar to the flavor of rancid milk. Samples of milk with groups of fatty acids and fatty acids singly omitted from the fatty acid mixture were evaluated for rancidity by a flavor panel. The flavor panel results indicated that the even-numbered fatty acids from butyric to lauric accounted for the fatty acid contribution to rancid flavor and that the fatty acids above lauric acid contributed little, if any, to the rancid flavor. No single fatty acid in the butyric to lauric acid series exerted a predominating influence in its contribution to the rancid flavor.

COMPARISON OF NEUTRAL FAT AND FREE FATTY ACIDS IN HIGH LIPID-LOW CARBOHYDRATE DIETS FOR THE GROWING CHICKEN. S. Brambila and F. W. Hill (Dept. of Poultry Husbandry, Univ. of Calif., Davis, Calif.). *J. Nutr.* **88**, 84–92 (1966). Three experiments were conducted with day-old chick a) to determine if they require an exogenous source of carbohydrate when fed diets high in lipids, and b) to study the comparative nutritional properties of soybean oil (SO) and soybean oil fatty acids (SOFA). Semipurified diets based on isolated soybean protein were used. The reference high carbohydrate diet supplied in percentage of metabolizable calories: glucose, 45; protein, 32; and SO, 23. For the high lipid diets, both glucose and SO were equicalorically replaced with the lipid under study; supplemental carbohydrate added to these diets replaced an equicaloric amount of lipid. Diets supplying the nonprotein calories as SO permitted growth nearly equal to the high carbohydrate controls; addition of 3% glycerol or up to 6% glucose calories did not improve it. In contrast, chicks fed the high SOFA diet grew slowly and developed foot dermatitis and beak deformities. This syndrome was not prevented by feeding the fatty acids as the methyl or ethyl esters. The supplementation of these diets with 6% glucose markedly improved growth but did not prevent the development of the foot and beak abnormalities.

PROCESS FOR PRODUCING PHYTOSTEROL MATERIAL FROM CUCURBITACEAE. G. J. Lammers (Chemetron Corp.). *U.S. 3,219,542.* The *Cucurbitaceae* is extracted with an organic solvent such as methyl or ethyl alcohol, ethyl oxide, chloroform, ether or petroleum ether. At least part of the solvent is removed to provide a nontoxic concentrate of the phytosterol material.



• Detergents

THE EFFECT OF ELECTROLYTES ON THE STABILITY OF OIL IN WATER EMULSIONS. H. Sonntag and H. Klare, Jr. (Inst. Physic. Chem., Berlin, Germany). *Tenside* 2, 33–5 (1965). A special apparatus is described for studying the effect of electrolytes of different types on the coalescing process of water in oil emulsions, using a model droplet. A stabilizing effect is found at low salt concentrations which is attributed to the high concentration near the phase boundary. At higher concentrations there is a demulsifying effect caused by dehydration of the surface active materials.

TWO IMPORTANT STAGES IN THE HISTORY OF SURFACTANTS. G. Schwen (Ludwigshafen/Rhein, Germany). *Tenside* 2, 65–8 (1965). The discovery of alkyl-naphthalene sulfonates and of ethylene oxide adducts is reviewed.

THE EFFECT OF SURFACTANTS ON THE BIOLOGICAL EFFECTIVENESS OF PHENOLS AND PHENOL DERIVATIVES. H. Bellinger (Henkel & Cie. Gmbh, Düsseldorf, Germany). *Tenside* 2, 295–305 (1965). While the reaction mechanism between phenolic materials and bacterial cells is still poorly understood, more is known about the effect of phenol substitution on bacteriological effectiveness. Surfactants also act on bacterial cells, but because the reaction mechanism between phenolic substance and bacterial cells is not yet elucidated, it is not possible to obtain a clear picture on the chemistry of the effect of surfactants on the biological effectiveness of phenolic substances. In a few cases it is possible to explain the effect of surfactants on phenolic disinfectants and preserving agents on the basis of chemical or physico-chemical data. In complicated systems such as cosmetic emulsions and creams, however, it is practically impossible to predict the mutual influence. It is therefore necessary to study problems of preservation of such products experimentally.

EVALUATION OF SOME PROPERTIES OF SOAP BARS BY X-RAYS. V. A. Rossetti, F. Fulignoli and G. Schippa (Univ. of Rome, Italy). *Riv. Ital. Sostanze Grasse* 42, 260–4 (1965). Samples of milled toilet soap from different points in the bar making process were found by X-rays to have approximately the same β and ω phase content. An interesting observation was made using the ratio (σ_D) between the minimum and the maximum density of blackening measured on one of the rings of the X-ray spectrum obtained in a Weissemberg chamber, since samples with lower values of σ_D can be assumed to have a higher degree of crystalline order and orientation. The finished bar was found to have lower σ_D (0.7) than simply milled soap (0.8), as expected. A definite correlation exists between σ_D and sudsing behavior, with the lower σ_D values (higher crystalline order) yielding lower suds values.

BIOLOGICAL BEHAVIOR OF SURFACE-ACTIVE AGENTS. A. de Jong (Unilever Res. Lab., Vlaardingen, Holland). *Informaciones sobre Grasas y Aceites (Buenos Aires)* 3 (3–4), 4–7 (1965). The detergent contamination of some European rivers is reported and its influence in microorganisms, fish and plants discussed. A number of beavers were fed for 300 days a diet containing 30,000 ppm of surface-active agents plus a carcinogenic substance. No specific effect could be attributed to the detergent. Experiments on persons taking up to 100 ppm of different detergents failed to shown any chronic effect.

INFRARED ANALYSIS OF DETERGENTS. E. R. Wright and A. L. Glass (Naval Air Engineering Center, Philadelphia). *Soap Chem. Specialties* 41 (11), 59–62, 83, 85 (1965). A procedure is presented for the removal of the sulfonate groups from alkyl aryl sulfonates used as detergent raw materials. This removed permits the use of infrared techniques to distinguish biodegradable straight chain from non-biodegradable branched chain structures.

WETTING AGENTS AND SURFACE ACTIVE COMPOSITIONS THEREFROM. A. J. Stirton, R. G. Bistline, Jr., J. K. Weil and W. C. Ault (Secty. of Agr., U.S.A.). *U.S. 3,219,584*. A wetting agent composition effective in distilled and hard water consists of 20–80% by weight of a member selected from the group consisting of a sodium alkyl α-sulfopelargonate, a sodium alkyl α-sulfolaurate, a sodium alkyl α-sulfomyristate, each alkyl containing from 2–12 carbon atoms, the sodium salts of the 2-ethylhexyl esters of the α-sulfonated coconut oil fatty acids; and 80–20% of a member selected from the group consisting of the sodium salts of the methyl esters of the isopropyl esters of α-sulfonated hydrogenated tallow acids, sodium methyl α-sulfostearate, sodium isopropyl α-sulfostearate, sodium methyl α-sulfopalmitate, and sodium isopropyl α-sulfopalmitate.

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assayed for activity and compared to total activity from Triton x-100 data. In 0.15 m sucrose no significant differences in enzyme release were observed after 1 and 30 min, but significantly less enzyme was released from EFA deficient lysosomes after 60 min. Significantly more enzyme was released from EFA deficient lysosomes after 1 min in 0.45 m sucrose, and after 1 and 30 min in 0.70 m sucrose.

Similar kinetic studies of acid phosphatase (β-glycerophosphate) and aryl sulfatase (p-nitrocatechol sulfate) release from kidney lysosomes were initiated.

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SERUM NEUTRAL LIPID ANALYSIS BY TLC AND DENSITOMETRY

Virginia S. Whitner, O. T. Grier and R. F. Witter

Further investigation has been made of the quantitative technique, previously reported from this laboratory (JAOCS *42,* 1154), for the determination of neutral lipids by TLC in which serum is applied directly to the plate. The densitometric technique described by Privett et al. (JAOCS *42,* 380) has been used for the quantitative determination of the separated lipids. In these experiments 5 serum pools were analyzed on 4 occasions. These pools ranged in triglyceride content from 68–300 mg% as determined by a modified Carlson procedure. The cholesterol values ranged from 47–97 mg% free cholesterol and 163–331 mg% total cholesterol as determined by a modified Sperry-Webb method. The resulting free and total cholesterol values were 15% lower than the corresponding values found with the Sperry-Webb procedure. The triglyceride values were 20% lower than those found with the Carlson method. However, when conventional lipid extracts were used, the values obtained by densitometry for total cholesterol and triglycerides agreed with those found by the previously mentioned reference procedures. Investigations of these differences are being carried out in an attempt to increase the apparent recovery of lipids from serum applied directly to thin-layer plates.

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COMPARATIVE EVALUATION OF ANTIBACTERIAL SOAPS

Eric Jungermann, James Brown, Jr., Frank Yackovich and David Taber

Antibacterial soaps containing (1) a mixture of hexachlorophene and 3,4,4′-trichlorocarbanilide, and (2) a mixture of 3,4,4′-trichlorocarbanilide, 4,4′-dichloro-3-(trifluoromethyl) carbanilide, and 3,4′, 5-tribromosalicylanilide were compared with a nonmedicated soap and a detergent-based formula containing hexachlorophene for in vitro antibacterial activity against twelve pathogenic staphylococci and streptococci. The mixture of hexachlorophene and 3,4,4′-trichlorocarbanilide was superior in every case to unmedicated soap and to the ternary system. The first-mentioned system also exhibited broader spectrum activity than the product containing hexachlorophene alone. In vitro comparison of both medicated soaps against three microorganisms of the types responsible for human body odor, showed the hexachlorophene-3,4,4′-trichlorocarbanilide system to be superior to the three-component system.

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AN AUTOANALYZER METHOD FOR DETERMINING HEXACHLOROPHENE IN SOAP

W. E. Hoover, M. E. Ginn and Eric Jungermann

An AutoAnalyzer method has been developed to determine hexachlorophene in soap by condensing the free phenol group with 4 amino-antipyrine, oxidizing with alkaline potassium ferricyanide and colorimetrically measuring the resulting quinone-type dye at 480 μ. Internal standards are used consisting of soaps containing all of the same ingredients as the samples, including known amounts of hexachlorophene. The method is applicable to the range that would be expected in a germicide soap, i.e. from 0–2%.

The overall precision was found to be ± 0.022% (95% confidence limits) at the 1.00% level of hexachlorophene in soap.

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ALPHA OLEFIN SULFONATES

D. M. Marquis, S. H. Sharman and Ralph House

Alpha olefin sulfonate (AOS) can be made by SO_3-air sulfonation of straight chain alpha olefins followed by saponification of the neutralized product. The sulfonation step forms unsaturated sulfonic acids, sultones, and sultone sulfonic acids. Hydrolysis of the various sultones yields a mixture of unsaturated and hydroxy sulfonates. Sulfonation of commercial mixtures of straight chain alpho olefins in a large scale SO_3 falling film unit has given AOS of 1.5–2.0% oil based on active content and tristimulus color of about 40% saturation (2% solution) which is readily bleachable with 1–3% NaOC1 to about 10–15% saturation.

Performance of AOS made from C_{15}-C_{18} alpha olefin is comparable to that of the high-foaming $C_{12.5}$ LAS in both detergency and dishwashing foam. The product shows a low order of toxicity and biodegradability better than that of LAS. The sulfonate from alpha olefins is generally superior in properties to similar products made from internal straight chain olefins.

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PREPARATION AND PROPERTIES OF DIALKYLINDAN SULFONATE

R. C. Taylor, B. J. Meehan, W. J. DeWitt and J. C. Reid

Work concerning the alkylation of benzene with chloroparaffins has brought to light compounds having the empirical formula $CnH_2n—_8$. These compounds have been identified as being predominately dialkylindans, probably the 1,3 isomers. These dialkylindans have been prepared in high concentration by alkylating benzene with dichlorododecane (mixed isomers, predominately secondary chlorides) using aluminum chloride catalyst under conditions used for making monoalkylbenzenes from monochlorododecanes. Under sulfonation conditions which are normally used for dodecylbenzene, the dialkylindans give good yields of the monosulfonate.

The dialkylindan sodium sulfonates have properties and detergent performance similar to those of dodecylbenzene sodium sulfonate. They are biodegradable as determined in the river water die-away test.

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EFFECTS OF TOTAL DISSOLVED SOLIDS IN WATER ON DETERGENCY

W. C. Powe

A synthetic laundry effluent formulated to simulate wash water with soils and surfactants removed was used to study concentration effects of dissolved solids on detergency. This work was to determine what quantities of dissolved solids could be present in reclaimed wash water and still be reuseable water for laundering.

The synthetic laundry effluent contained the sodium salts of tripolyphosphate, orthophosphate, silicate, carbonate, sulfate, chloride and also urea.

Soil removal and deposition data with clay as the model soil for several typical laundry detergents in the presence of varying concentrations of the synthetic laundry effluent are discussed. Effects of initial water hardness on cleaning are also noted. Some carbon test data are given for comparison.

In general, the presence of more than 100 ppm dissolved solids produced a drop in detergency and an increase in deposition. The individual anions causing the greatest loss in detergency at 5000 ppm level were chloride and sulfate.

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A MINIATURE DISHWASHING EVALUATION METHOD

E. J. Schuck and R. M. Anstett

A new procedure for evaluating dishwashing products has been developed. Good precision and favorable correlation with older methods are obtained with minimum requirements of manpower, time and materials. The relationships between dishwashing performance and such factors as detergent concentration, water hardness, and fatty soils are discussed.

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LIQUID SCINTILLATION DETERMINATION OF DETERGENTS ON COTTON FABRIC

B. E. Gordon, W. T. Shebs, D. H. Lee and R. V. Bonnar

A method for the determination of anionic surfactants on cotton fabric has been developed using S^{35} and H^3 labelled compounds. The method is simple, involving immersion of the white fabric in liquid scintillation counting solution and counting. No interference by quenching or self-absorption is encountered.

Combination of the pulse height shift method with a automation data handling has permitted the analysis of several hundred samples per week with little increase in operator time.

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PROGRESS IN THE MEASUREMENT OF FILM ELASTICITY

R. I. Razouk and K. J. Mysels

The elasticity of a soap film, i.e., the increase of its tension when it is stretched, must play an important role in foam stability. It has been defined already by Gibbs, who also explained its origin and calculated its value for a two-component system. A method for measuring film elasticity and first results for complex systems have been reported by Mysels, Cox and Skewis [J. Phys. Chem. *65,* 1107 (1961)]. We have now attempted to obtain significant results for a two-component system by purifying solutions of high quality sodium dodecyl sulfate and sulfonate samples by foaming in situ. Reproducible results of greatly improved precision have been obtained and the large effect of the purification procedure demonstrated. Independent tests of the surface purity, especially the presence of Marangoni effects, i.e., the increase in surface tension of bulk solution when the surface is expanded, and the independence of film elasticity from film thickness make it doubtful, however, that sufficient purity to satisfy Gibbs' premises has been attained. The significance of this problem and possible further approaches to its solution will be discussed.

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INFRARED DETERMINATION OF PARTS PER MILLION OIL AND DEMULSIFIER CHEMICAL CONTENT IN WATER

B. B. Stafford

Antipollution regulations are being proposed prohibiting discharge of water containing too much residual oil. Water containing oil is usually treated with an efficient biodegradable chemical demulsifier to lower the concentration of residual oil. In order to comply with proposed water pollution regulations, a useful method is needed to differentiate between oil and treating chemical in water and determine the parts per million concentration of each of these components. A rapid, precise analytical procedure would also provide a means for controlling efficiency of chemical treatment.

A number of water samples containing oil and chemical have been analyzed by infrared spectroscopy. New infrared data are presented for fresh and salt water samples containing 0-100 ppm of crude oil and an oxide adduct detergent in a heavy aromatic solvent. The concentration by weight of each of the components is determined from infrared spectra using an absorption compensation method. Standardizations are done through the use of suitable model compounds. Extractions of the water samples are necessary for the standardizations and determinations.

The method has been used to monitor oil tanker waste water discharge, and to evaluate chemical treatment efficiency of these and other waste water effluent sources.

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GENETICS AND THE LIPIDOSES

A. G. Knudson, Jr.

Formal genetic analyses of family data in cases of errors of lipid metabolism are able to distinguish monogenic vs. multigenic and nongenetic disorders. These data, together with population data, provide criteria for homogeneity of cases which can be useful in the interpretation of biochemical findings. The peculiarly elevated incidence of three main genotypes of sphingolipidoses among Ashkenasic Jews has no reasonable explanation except for heterozygote selection. This inference has strong implications for the biochemist in that it suggests (1) the existence of some biochemical abnormality in common among the heterozygotes for these conditions, and (2) the utility of such a heterozygote abnormality in the defense of the body against some adverse environmental stress, such as some specific infectious disease. The detection of heterozygous carries by biochemical means could be important not only for this reason but also as a means for anticipating marriages which may produce a lethal lipidosis. Finally, as long as preventive measures are either imperfect or not practiced, major attention is directed toward therapy, which in turn requires not only more knowledge of the basic defect but awareness of the fact that such knowledge may not be helpful without the development of more advanced devices than those employed in, for example, phenylketonuria or galactosemia. The deep-seated nature of the presumed lesions in the lipidoses seems to require a different type of corrective measure, such as gene replacement or transformation.

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NEUROPATHOLOGICAL AIDS TO THE LIPID BIOCHEMISTRY OF THE CENTRAL NERVOUS SYSTEM

A. L. Prensky

The need for histological control of lipid work involving the central nervous system is stressed. Information is presented indicating that the central nervous system (CNS) is an anatomically heterogeneous mass in which adjacent areas may differ radically in both cell structure and degree of myelination. The pertinent literature concerning developmental changes within the CNS are reviewed with particular reference to the normal time sequence of myelination. Examples of the complex manner in which disease affects the nervous system are presented with emphasis on the restricted changes that may be produced by relatively diffuse insults.

Because of these variables, the selection of samples for biochemical study becomes a critical problem. Methods of sectioning fixation and storage of pathological tissues are reviewed. Since many of these techniques alter both histological and biochemical preparations, it is pointed out that collaboration between the neuropathologist and biochemist should begin at biopsy or autopsy and a method of approach utilizing fresh brain is suggested.

Subdivision of the brain into meaningful pathological and biochemical areas is approached by two methods. The first outlines a plan for the survey of various representative areas of the brain which will delineate most disease processes. The second combines histological and biochemical studies to fit a known disease process. It is reasoned that diffuse diseases of white or gray matter will require large samples to yield a statistical summation of variability, while focal lesions are best appreciated by serial sections comparing the lesion with surrounding normal tissue. Micro samples can be used for either diffuse or focal disease. The development of biochemical techniques for lipid analysis at the micro level makes possible the use of very small samples allowing close correlation of histological appearance and chemical composition.

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GENERAL PATHOLOGY AND LIPID RESEARCH

G. P. Vennart

The *raison d'etre* of a pathologist is to elucidate the natural history of disease processes. Once this natural history has been defined, he hopes to be in a position to know where best to interrupt or modify the course of the disease in order to shed new light on the basic mechanisms involved and/or to benefit a specific afflicted patient. General pathology embraces those areas of disease mechanisms which are common to all organ systems, and as such its techniques have wide applicability.

The techniques of the biochemist have been more or less completely absorbed by the pathologist. Unfortunately, techniques involving microscopy, the time-tested method of the pathologist, have not found equal utilization by the biochemist. This presentation will consider the techniques of microscopy, particularly light microscopy, which are directly applicable to the areas of interest of lipid biochemists. Many of these techniques are quite simple to perform even with minimal experience and can yield valuable information in many areas, for example: 1) in studying the localization and zonal distribution of individual chemical components within tissues and cells; 2) in the screening of tissue changes as a "general unknown"; and 3) in the control of experimental design especially of synthetic, so-called "complete" diets.

In addition to examples from the various areas, general principles of tissue handling, equipment needed, staining, and results to be expected will be discussed.

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HISTOCHEMICAL AND ELECTRON MICROSCOPIC STUDIES OF SOME LIPIDOSES

B. W. Volk and Barbara J. Wallace

The present study describes the fine structural and some enzyme histochemical changes in biopsies obtained of brain, cerebellum, liver, rectal wall, and lymph nodes from eight patients afflicted with various lipidoses. In the central nervous system of Tay-Sachs disease (TSD), late infantile lipidosis (LIL) and acid mucopolysaccharidosis (AMP) concentrically lamellated lipid bodies, as well as granular pleomorphic bodies with irregularly arranged membranes were seen. Most cytosomes showed a single limiting membrane. In Purkinje cells of Niemann-Pick disease (NPD) the cytosomes consisted of vacuoles with a few loosely arranged lamellae. These vacuoles in NPD were confluent with ER while the ER of TSD showed markedly dilated cisternae, some of which contained dense bodies and small vesicles. There were marked variations of the morphology of the lipid bodies in cerebral and cerebellar neurons on one hand, and liver and rectal mucosa on the other hand in NPD. In AMP a marked difference occurred also between cytosomes of cerebellar neurons and those in viscera. In TSD many large granular bodies were seen in neurons of Meissner's plexus, which were different from those observed in cerebral neurons. In Gaucher's disease the typical Gaucher bodies were present. In most organs of all cases AcPase and organo-phosphorus resistent esterase activities were localized in these abnormal lipid structures. The presence of a limiting membrane and of hydrolytic enzyme activity in these cytosomes as well as their proximity to ER and Golgi complex suggests that they are lysosomal in nature. These observations would be in accord with the concept that many disorders of inborn error of metabolism are classified as "lysosomal diseases," presumably precipitated by lack of some of the lysosomal hydrolytic enzymes.

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PATHOLOGY AND LIPID METABOLISM OF THE EYE

G. L. Feldman

The accumulation of lipoidal material is frequently associated with diseases of the eye. This lipoidal accumulation sometimes manifests itself as a localized deposit(s) such as that which precedes the retinal degeneration that occasionally results from diabetes. The lipoidal deposit can also involve not only a specific tissue, but a specific lipid as well. In arcus senilis, a disease of the cornea, cholesterol accumulates in an opaque ring at the corneal periphery. These, and other observations, clearly establish the importance of lipids to the metabolism of the eye.

In the normal eye, each specific ocular tissue has a characteristic pattern of lipid composition. For example, the lens contains an abundance of cholesterol and is also very rich in sphingolipids. Not only do individual tissues differ from each other, but similar tissues from other species contain a different distribution pattern of qualitatively similar lipids.

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INTERACTIONS IN THE METABOLISM OF POLYUNSATURATED ACIDS

R. T. Holman

The conversion of oleate to higher unsaturated acids in animal tissues, when unsuppressed by dietary polyunsaturated acids, lends to the incorporation of large amounts of $20:3\ \omega\ 9$ in tissue lipids. Normal dietary levels of linoleate or other polyunsaturated acids hinder the incorporation of this and other oleate metabolites into tissue lipids. The metabolism of linoleate to $20:4\ \omega\ 6$ and $22:5\ \omega\ 6$ is suppressed by the presence of linolenate in the diet. The metabolism of linolenate to $20:5\ \omega\ 3$, $22:5\ \omega\ 3$, and $22:6\ \omega\ 3$ is inhibited by dietary linoleate. The interactions are such that $18:3 > 18:2 > 18:1$ when tendencies to metabolize to higher polyunsaturated acids are compared. The influences of dietary monoenoic and saturated acids upon linoleate metabolism are of a much lower order. Dietary odd chain fatty acids tend to suppress the appearance of $20:3\ \omega\ 9$ in tissue lipids. The interactions will be demonstrated in total tissue lipids, in individual lipid classes, and in the fatty acids in the two positions in lecithin.

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POLYENOIC ODD-NUMBERED ACIDS

Hermann Schlenk, D. M. Sand and Joanne Gellerman

Odd-numbered straight-chain fatty acids are present in virtually all natural lipids but normally represent only a very minor portion of all acids. Mullet oil with 15–30% odd acids is a rather unique exception which made possible structural identification of naturally occurring polyenoic odd acids.

Compositional data from mullet show pair-wise resemblance of chain lengths C_{15} and C_{16}, C_{17} and C_{18}, C_{19} and C_{20}, in regard to relative amounts of saturated, mono-, di- and higher unsaturated components. The structures are, in reference to the carboxyl group, identical when considering appropriate pairs: e.g., 9,12–17:2 and —18.2, or 5,8,11,-14–19:4 and —20:4. This suggests biological correlations for unsaturated odd acids similar to those which are established for the common unsaturated even acids.

Origin and conversions of odd acids in mullet are not known. However, odd polyenoic acids from mullet were administered to rats and to the protozoon *Ochromonas danica*. The conversion products were investigated and bear out the surmised correlations. The acids, 9,12–17:2 and 6,9,12–17:3, converted readily to 5,8,11,14–19:4 and related acids like linoleic and γ-linolenic into arachidonic. When synthetic unsaturated acids of various double bond structures were applied, it became apparent that with chain lengths C_{17}, C_{18} and C_{19}, the distance between carboxyl and first double bond is of deciding influence on introduction of further double bonds and on chain elongation.

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INFLUENCE OF SEX AND GONADAL HORMONES ON LIPID METABOLISM IN EFA-DEFICIENT RATS

Rosemarie Ostwald and R. L. Lyman

This paper will review work which has grown out of the observation first made in Burr's laboratory that female rats made deficient in EFA stored more fat than did comparable males. Subsequent work from a number of laboratories suggested that female rats had a more efficient system for utilizing EFA and that therefore there may be sex differences in their intermediary metabolism. In the course of studies in this laboratory concerning the effects of dietary cholesterol, Dr. Okey and her co-workers observed sex differences in the levels and in the composition of both cholesterol ester and phospholipids in plasma and liver of rats which were indicative of sex differences in the metabolism of polyunsaturated fatty acids. Further work in which testosterone and estrogen were injected in castrated male rats showed the influence of estradiol on the sex differences. In an attempt to further characterize the sex differences in the metabolism of EFA, the lipid composition of carcass, liver and serum were determined in intact male and female rats and in castrated males treated with testosterone and estrogen at intervals during the development of an EFA deficiency. The results indicate that some of the observed differences could be attributed to the sex hormones while others are more likely due to differences in organ size and growth rate. The results of a study using C^{14}-linoleic acid to study its metabolism in these EFA-deficient male and female rats as well as in the hormone-treated castrates will be included in the discussion.

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POLYUNSATURATED FATTY ACID-ANTIOXIDANT RELATIONSHIPS IN METABOLISM

J. G. Bieri

The work of numerous investigators which bears on this subject will be reviewed. It has been recognized for many years that antioxidants derived from the diet must be incorporated into tissue lipids in order to stabilize the polyunsaturated fatty acids (PUFA) contained primarily in phospholipids. These antioxidants represent diverse organic and inorganic compounds. The dietary requirement for antioxidants can range from almost zero to relatively high levels. The varying susceptibility of different PUFA to peroxidation has led to attempts to relate antioxidant requirement to the total "estimated peroxidizability" of dietary PUFA. Evidence indicates that various tissues have specific qualitative and quantitative antioxidant requirements. In vitro biochemical studies have correlated loss of enzyme activity with peroxidation of specific phospholipids. Certain mitochondrial changes of previously unexplained origin are now recognized to involve the antioxidant-PUFA ratio in the structural membrane. A possible involvement of the principal naturally occurring antioxidant, α-tocopherol, in interconversions of polyunsaturated fatty acids has been suggested.

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AUTOMATED TECHNIQUES IN SERUM LIPID ANALYSIS

A. Antonis

Recent research in lipid metabolism has created the need for rapid and reproducible methods for the routine investigation of serum lipid levels. In this paper automated or semiautomated methods developed by the author and other investigators will be reviewed and evaluated against manual methods previously described. Techniques will be discussed for the colorimetric and fluorimetric estimation of various serum lipid components such as cholesterol, total esters, free fatty acids, triglycerides and phospholipids.

An enzymatic fluorimetric method will also be described for the estimation of glycerol in hydrolysates from triglycerides, as well as for the estimation in plasma of various intermediary metabolites such as pyruvic, lactic, aceto-acetic and β-hydroxy-butyric acids.

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RECENT ADVANCES IN AUTOMATED LIPID ANALYSIS

Nathan Gochman

This paper will review some of the recent developments in automated instrumentation which apply to the determination of clinically significant blood lipids such as cholesterol, phospholipids and triglycerides. The continuous-flow analytical system, described by Skeggs in 1957 for the determination of blood urea nitrogen, has been adapted to a wide variety of chemical determinations which can be accomplished using the principles of proportional pumping of sample and reagents, with subsequent colorimetry or fluorometry and automatic recording.

The introduction of new equipment to perform specific analytical functions such as extraction, digestion, hydrolysis has gradually expanded the capabilities of this system.

The combination of simple preliminary manual purification, followed by automated processing for the remainder of the analytical procedure, permits application to complex lipid determinations.

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AUTOMATED DETERMINATION OF SERUM CHOLESTEROL AND TRIGLYCERIDE

R. F. Witter, Virginia S. Whitner, G. R. Cooper and A. Mather

Two methods have been studied for the determination of serum cholesterol. One procedure, based on the Abell-Kendall reaction, has been used for the routine duplicate analyses of as many as 16,000 samples per year with a standard deviation between duplicates, randomized over a 6-day period, of from 2.7–3.0 mg%. The method was made possible by the development of a Liebermann reagent stable for 8 hr. Mechanized pipettes are used to measure all reagents and the 0.1 ml samples of serum. Extraction, evaporation, and color reactions are carried out in tubes which can be washed mechanically. One analyst can analyze 60 samples per day in duplicate. Conditions necessary to obtain accurate and reproducible cholesterol analyses with the AutoAnalyzer N-24 method have been investigated. Unlike other ferric chloride methods which give high results, it is possible to obtain close agreement between the results of the reference Abell-Kendall method and those of the modified AutoAnalyzer N-24 method. The time of the color reaction at 95C is kept between 95 and 120 sec. The rate of flow of extract is raised to 0.9 ml/min, and the ratio of extract to color reagent is maintained within specified limits. With 120 samples ranging in concentration from 142 to 338 mg%, a standard deviation of 2.6 mg% between duplicates randomized over a 6-day period was obtained with this modified procedure. A method for the determination of triglycerides also was investigated. Triglycerides are extracted from serum with chloroform in the presence of silicic acid which adsorbs the phospholipids. After saponification, the liberated glycerol is determined on the AutoAnalyzer at the rate of 40 determinations per hour with the periodate-chromatropic acid reaction. Preliminary experiments with 53 samples show that the standard deviation between duplicates is 4.8 mg% in the range from 40 to 180 mg%.

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SEMIAUTOMATIC ANALYSIS OF SERUM TRIGLYCERIDES AND CHOLESTERYL ESTERS BY INFRARED ABSORPTION

N. K. Freeman, E. Lampo and A. A. Windsor

An instrument has been developed for the semiautomatic analysis of mixtures of triglycerides and cholesteryl esters. The method is based on high-resolution infrared spectrophotometry, and has previously been shown to be applicable to the determination of these components in the nonionic fraction of human serum lipids. A simple nonrecording grating spectrophotometer has been suitably modified to carry out this analysis; and appropriate computing circuitry has been coupled with it for performing the two-component calculation. The supplementary electronics consist of operational amplifiers, a logarithmic conversion circuit, a digital voltmeter, and a printer. Automatic operation is accomplished by a control mechanism, which programs the measurements, the steps in the calculations, and print-out of the results.

Sample preparation consists of an extraction of lipids from serum in such a way as to exclude phospholipids. This may be done in a single step, although a two-step procedure—total lipid extraction followed by adsorption separation of the phospholipids—appears to be more reliable. Measurements are made on a solution of the neutral lipid fraction in carbon tetrachloride.

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THE USE OF THE COMPUTER IN SERUM LIPID AND LIPOPROTEIN ANALYSIS

J. C. Jensen, A. M. Ewing, R. D. Will and F. T. Lindgren

The usefulness of computers in data evaluation is generally recognized, however, the problem of utilizing a computer in the most intelligent manner deserves careful consideration.

True programs requiring a minimum of manual measurements have been developed to analyse gas-liquid chromatograms. These programs perform many operations including corrections for baseline, linearity, Gaussian resolution, and variation in column conditions.

Finally a general purpose statistical analysis program has been developed which greatly aids in summarizing and correlating data from these programs, as well as other sources, such as ultracentrifugal data.

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THE STRUCTURE AND HYDRATION OF METHYL DIEPOXYSTEARATES

G. Maerker, E. T. Haeberer and W. C. Ault

Epoxidation of methyl linoleate results in two forms of methyl 9,10,12,13-diepoxystearate, each of which is a racemic mixture and therefore optically inactive. Epoxidation of methyl vernolate (methyl 12,13-epoxyoleate) also leads to two forms of methyl 9,10,12,13-diepoxystearate, a solid and a liquid, which have significant but different optical activity. These facts were combined to arrive at a structure assignment for the geometrical isomers involved.

Several previous workers have attempted to prepare 9,10,12,13-tetrahydroxystearates from epoxidized linoleates but have obtained other products instead. Hydration of methyl 9,10,12,13-diepoxystearate with aqueous fluoboric acid in dioxane gave a dihydroxy ester which was oxidized to a diketo ester. Infrared and NMR analyses of the two products have been used to demonstrate that the dihydroxy ester is a derivative of tetrahydrofuran, and that one of the two hydroxy functions is a substituent on the ring while the other is in a side chain. The cyclic product results from the acidcatalyzed interaction of the two oxirane functions.

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HOMOGENEOUS HYDROGENATION OF METHYL LINOLENATE CATALYZED BY PLATINUM-TIN COMPLEXES

E. N. Frankel, E. A. Emken, Hiroshi Itatani and J. C. Bailar, Jr.

Our studies of homogeneous catalysis are aimed, practically, at finding selective catalysts for the hydrogenation of linolenate in soybean oil and, theoretically, at obtaining a better understanding of the mechanism of fat hydrogenation.

The homogeneous hydrogenation of pure methyl linolenate was catalyzed by mixtures of chloroplatinic acid and stannous chloride in methanol-benzene solution at 220–500 psi hydrogen. Other effective soluble catalysts investigated include dichloro-bistriphenylphosphine-platinum (II), *cis* and *trans*-hydrido-chloro-bis-triphenylphosphine-platinum (II), and dichloro-bis-triphenylarsine-platinum (II), each in mixture with stannous chloride. At 65C dienes and conjugated diene-trienes were formed as major products, and monoenes, conjugated dienes and conjugated trienes as minor products. No stearate was formed. At 100C monoenes were the major products.

The expected platinum-tin complex involved in the various reductions was synthesized as the (trichloro-tin)-hydrido-bis-triphenylphosphine-platinum (II). With this bimetallic complex, linolenate underwent more isomerization and conjugation than reduction to dienes.

The products from hydrogenated linolenate were fractionated by countercurrent distribution into monoenes, dienes and trienes. Further separation of the monoenes by silver cation-exchange column chromatography yielded 28% *cis* and 72% *trans* isomers in which the double bond was distributed (oxidative cleavage) predominantly between the 4 and 15 positions. A diene fraction was fractionated by preparative gas chromatography into components with *trans* double bonds separated by 2 to 4 methylene groups. Apparently conjugation is an important initial step before reduction.

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STRUCTURE OF UNSATURATED GLYCERIDES: A DIRECT METHOD OF CALCULATION

C. D. Evans, D. G. McConnell and C. R. Scholfield

Composition and structure of unsaturated glycerides of vegetable oils can be calculated directly from the fatty acid composition of the oil. Fatty acid distribution on the 2-position, as normally determined by lipase hydrolysis, is calculated from the composition of the whole oil by applying the following three rules in their respective order: 1) Saturated fatty acids and those with chain length greater than 18 carbons are first distributed equally and randomly on the 1- and 3-positions of the glycerol moiety. 2) Oleic and linolenic acids are treated equally, or as a unit, and distributed equally and randomly on all three glyceride positions with any excess from the 1- and 3-positions being added to the 2-position. 3) All remaining positions are filled by linoleic acid.

Remarkably good agreement between the calculated and experimentally determined fatty acid distributions is shown for soybean, linseed, safflower and many other vegetable oils whose compositions are reported in the literature. This evidence of a biosynthetic association between oleic and linolenic acid in vegetable oils is presented and discussed.

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CONJUGATION OF SOYBEAN OIL VIA THE IRON TRICARBONYL COMPLEX AND CARBON MONOXIDE DECOMPOSITION

E. N. Frankel and S. Metlin

Studies with iron carbonyl complexes of fatty esters have led to the development of a procedure to conjugate vegetable oils. We previously described a method based on complexing the polyunsaturated fatty esters with iron pentacarbonyl and then decomposing the complexes with ferric chloride. Although 95–97% of the polyunsaturates were conjugated by this procedure, the iron tricarbonyl moiety of the complex was completely decomposed and lost. This procedure has now been improved by decomposing the complexes with carbon monoxide under pressure and by permitting the regeneration of free $Fe(CO)_5$, which is recovered for reuse.

When iron carbonyl-complexed soybean oil was heated at 180 to 200C at CO pressures of 1100 to 3800 psi, 90–97% of the complex was decomposed. At 180C and 3600 psi CO, the $Fe(CO)_5$ formed was 84% of theory, and 82% of the polyunsaturates was conjugated. At 190C and 1100 psi CO, 98% $Fe(CO)_5$ was recovered, but the oil was less conjugated (75%). No evidence of carbonylation of the oil was indicated. It may now be possible to develop an economical continuous process to conjugate vegetable oils efficiently by consecutive reactions with $Fe(CO)_5$ and CO.

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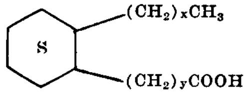
C_{18}-SATURATED CYCLIC ACIDS FORM LINSEED OIL: A STRUCTURAL STUDY

J. P. Friedrich

The previously described preparation of C_{18}-saturated cyclic acids (HCAL) from linseed oil by heat treatment in the presence of alkali gives rise to a series of at least 12 isomers as evidenced by GLC. These isomers have now been shown to be comprised of geometric pairs with the following general formula:



$x + y = 10$

An unequivocal synthesis of isomers (x = 1, 2 and 3) indicates that the principal positional isomer of HCAL is 9-(2'-*n*-propylcyclohexyl)-nonanoic acid. This isomer would result from cyclization of the 10,12,14-octadecatrienoic acid. The predominance of this isomer is further substantiated by mass spectral analysis. The three positional isomers prepared constitute most of the HCAL. The exact percentage cannot be calculated from the GLC curves because of insufficient resolution. In the synthesis of the three positional isomers the parent aromatic compounds show a single peak on the GLC, whereas their hydrogenated counterparts show two peaks. Thus, each of these positional isomers is composed of two geometric isomers.

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DETERMINATION OF THE STRUCTURE OF LECITHINS VIA THE DERIVED ACETO-1,2-DIGLYCERIDES

O. S. Privett, L. J. Nutter and E. C. Nickell

The conversion of lecithins to aceto-1,2-diglycerides permits the determination of the structure of these compounds by techniques used for the structural analysis of triglycerides.

Acetolysis and phospholipase C-acetylation methods for the preparation of aceto-1,2-diglycerides from lecithins are compared.

Multichromatographic procedures employing combinations of liquid-liquid, gas-liquid column and thin-layer chromatography in various ramifications are described for the fractionation and quantitative analysis of aceto-diglycerides.

Steps in the procedures are demonstrated with standard mixtures of aceto-diglycerides as well as with mixtures of other triglycerides and the method is demonstrated on a number of natural lecithins.

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POTENTIAL SYNTHETIC LUBRICANTS: DIOL ESTERS OF C_{18}-SATURATED CYCLIC ACIDS

J. P. Friedrich and L. E. Gast

Monoalkyl esters of C_{18}-saturated cyclic acids (HCAL) described by Friedrich, Bell and Gast [JAOCS *42:* 643 (1965)] showed promise as lubricants for military specification MIL-L-7808 but lacked the increased oxidative stability and viscosity required by the later MIL-L-23699.

Six diol esters of HCAL (IV < 1) containing < 1% saturated straight-chain acids and no aromatic contaminants have now been prepared. The dihydric alcohols used, both hindered and unhindered, included ethylene glycol, diethylene glycol, 1,4-cyclohexanedimethanol, 1,4-benzenedimethanol, 2,2-dimethyl-1,3-propanediol and 2,2–4,4-tetramethyl-1,3-cyclobutanediol (I). The viscosities of these esters at 210F ranged from 10.1 to 19.6 centistokes and the pour point, from −35 to −62F. The oxidative stabilities at 400F were determined by using 1% each of phenyl-α-naphthylamine and *p,p'*-dioctyldiphenylamine as inhibitors. The esters of the unhindered diols showed poor stability. However, esters of the hindered diols, in particular I, showed excellent resistance to the formation of acidic decomposition products. Although I by itself is too viscous to meet specification MIL-L-23699, its ASTM slope (0.650) is excellent. Blends of this material with a less viscous ester commercially available show promise for application in the field of low-temperature lubricants.

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CYCLIC FATTY ACIDS: SEPARATION FROM STRAIGHT-CHAIN FATTY ACIDS BY UREA ADDUCTING

R. A. Eisenhauer and R. E. Beal

Unsaturated cyclic fatty acids made by high-temperature alkali treatment of linseed fatty acids were separated by the urea adduct method in over 95% purity and 90–95% recovery from monomer mixture containing 37% cyclic and 63% straight-chain fatty acids. Previous reports from this Laboratory described a process for separating cyclic fatty acids from stearic acid by crystallization at −40C following hydrogenation. The urea adduct method avoids the hydrogenation step and has the further advantage that unsaturated cyclic and unsaturated straight-chain products are obtained as separate fractions. By further urea adduct treatment of the crude unsaturated straight-chain fatty acid fraction, the small amounts of palmitic and stearic were removed leaving an unsaturated fraction containing oleic, linoleic and conjugated linoleic acids. The possible separation of the unsaturated C_{18} straight-chain fatty acids into individual components is being investigated.

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THE ENERGETICS OF SURFACTANT ADSORPTION AT THE AIR-WATER INTERFACE

A. M. Mankowich

The standard free energy, enthalpy, and entropy changes (ΔG, ΔH, and ΔS, respectively) for the adsorption at the air-water interface of a commercial ethylene oxide (EO) adduct of straight chain nonylphenol from monomer solution at the critical micelle concentration (CMC) have been calculated from surface tension-concentration data at 21C-45C using the Gibbs equation, the standard free energy change equation $\Delta G = RT \ln$ (interfacial monomer concentration/CMC), and the Gibbs-Helmholtz equation which gave ΔH directly from the slope of the ($\Delta G/T$) vs (I/T) function. The CMC and surface tension at the CMC (γ CMC) decreased, and $-\Delta G$ and molecular area increased slightly, with increasing temperature. The ΔH and ΔS values were positive, and appear explainable by the postulations applied to micellization. At ambient temperature (28C) an increase in the (EO) mol ratio of straight chain nonylphenol and C_{13} secondary alcohol ethoxylates resulted in increases of CMC, γ CMC, and molecular area, and slight decreases in $-\Delta G$. A comparison of the 9(EO) mol ratio adducts of C_{13} straight chain primary and secondary alcohols showed that the CMC and molecular area of the secondary alcohol ethoxylate were larger, and the γ CMC and $-\Delta G$ smaller, than the corresponding values of the primary alcohol ethoxylate.

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FATTY ACIDS IN SEDIMENTS: A REVIEW

K. A. Kvenvolden

Fatty acids have been found by several investigators in a number of sediments ranging in age from Precambrian to Recent. A variety of molecular distributions of fatty acids has been observed. In most sediments even-carbon-numbered fatty acids are much more abundant than odd-carbon-numbered fatty acids; in some sediments, however, concentrations of even- and odd-carbon-numbered fatty acids are about equal.

Molecular distributions of normal fatty acids have recently been compared with molecular distributions of normal paraffin hydrocarbons in the same samples. In many ancient sediments molecular distributions of normal fatty acids, especially those larger than about C_{20}, seem to parallel distributions of normal paraffins having one less carbon atom than the acids. This parallelism indicates that these acids and paraffins may be related. In modern sediments, however, distributions of fatty acids and normal paraffins are not parallel suggesting that acids and paraffins in modern sediments are not related, at least not in the same manner as in ancient sediments.

Fatty acids have been postulated as possible precursors for paraffin hydrocarbons in petroleum because of (1) structural similarities between the two kinds of molecules, (2) ubiquity of fatty acids in biological materials, (3) acid-paraffin relationship in sediments, and (4) distribution of paraffins in some crude oils. Evidence suggests that normal fatty acids serve as source for many normal paraffins of intermediate and high molecular weights found in petroleum. Detailed mechanisms of chemical reactions by which normal paraffins can be derived from fatty acids in sediments under geologically reasonable conditions have yet to be defined.

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FATTY ACIDS IN RECENT SEDIMENTS

P. L. Parker

Fatty acids were quantitatively and qualitatively measured in a variety of recent sediments. All types of acids were accounted for by considering the sediment as a receiver of biochemical debris from the ecosystem. The low concentrations of fatty acids, especially the polyunsaturates, were explained by assuming that they react quickly with other biochemicals and become part of the "kerogen" fraction.

In recent sediments vertical variations (down a core) of the fatty acid patterns were much less than environmental variations. Carefully measured fatty acid ratios may be used to empirically distinguish different environments.

The even carbon number, saturated, straight chain fatty acids are the most abundant acids in recent sediment. However, the odd carbon number ones are more abundant in some environments than previous work indicates. The homologous series based on the methyl iso and anteiso branched chain acids were found in sediment for the first time.

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LIPIDS IN RECENT SEDIMENTS: CAROTINOIDS AND STEROLS

R. B. Schwendinger

For over fifty years the soil scientist has been interested in the identification and characterization of the organic fraction of recent terrestrial sediments, specifically of arable soils. More recently the organic geochemist has extended such interests to the study of aquatic sediments, fresh water and marine. Both disciplines have contributed to knowledge of the occurrence and distribution of carotinoids and sterols in the upper few feet of the earth's mantle.

The composition of the organic matter in a recent sediment reflects not only the flora and fauna native to the environment above it, but also the microflora and microfauna that is usually so abundant within it. Impressed upon the biogenetic origin of the multitudinous organic compounds synthesized and released from these various forms of life are the diagenetic transformations that can take place when these compounds are exposed to each other as well as to air, water and the possible catalytic activities of the inorganic fraction. Such things considered, it is surprising that relatively reactive molecules as the carotenoids and sterols not only exist in recent sediments in the 10–1000 ppm range but seem to be ubiquitous. It is questionable whether sampling or analysis of such complicated and nonhomogeneous systems presents the greater problem.

Such investigations extend our knowledge of the function of soil organic matter as well as provide basic insight into the first steps of petroleum genesis.

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ALKANES, FATTY ACIDS, AND ALCOHOLS IN GREEN RIVER OIL SHALE

D. L. Lawlor and W. E. Robinson

Normal alkanes, isoprenoid paraffins, fatty acids, and alcohols were shown to be present in the organic material of Green River Formation oil shale, providing evidence for the biological origin of the organic material. Fractionation of extracts from mined oil shale samples and from cored samples of different stratigraphic depths was accomplished by elution chromatography, GLC, and TLC. Identification was done by mass, infrared and nuclear magnetic resonance analysis. The data indicated metamorphic conversion of even-carbon-numbered fatty acids to odd-carbon-numbered fatty acids by carbon loss. It was evident also that decarboxylation of fatty acids to n-alkanes had occurred. The ratio of odd to even n-alkanes present in extracts from the core samples decreased progressively with change in stratigraphic depth. The presence of the isoprenoid compounds provides conclusive evidence of the biological origin of oil shale because the compounds identified are related to the phytol group of chlorophyll. The presence of fatty acids and alcohols also provides evidence of biological origin. This study represents a part of a continuous effort to evaluate the chemical nature of the organic material present in oil shale and to show the relationship of this organic material to petroleum and other carbonaceous materials.

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THE HYDROCARBON COMPOSITION OF PETROLEUM AND SOME POSSIBLE LIPID PRECURSORS

H. M. Smith

During the last decade organic geochemists in general have come to believe that the hydrocarbons in petroleum are derived by chemical processes from the lipids found in organic debris. It is therefore useful to consider the hydrocarbons that have been identified in petroleum and any quantitative data available.

The normal and isoalkanes, the monocyclic and dicyclic alkanes, the benzenes, naphthalenes, phenanthrenes, and other polycyclic aromatics, and a few cycloalkano aromatic hydrocarbons that have been identified in petroleum will be presented by means of an imaginative circular map called "The Petroleum World." On this map the various hydrocarbons are placed according to boiling point on temperature isotherms, and arranged so that each series is depicted as a river originating near the center of the map at low temperatures and proceeding to the Petroleum Ocean through increasing temperatures. Quantitative information on certain hydrocarbons will be pointed out, and where applicable possible lipid precursors of petroleum hydrocarbons will be mentioned, but not discussed in detail.

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DECARBOXYLATION: MECHANISMS AND GEOCHEMISTRY

I. A. Breger

Decarboxylation of organic compounds can be achieved by procedures commonly used in organic synthesis, by thermal degradation, or by methods involving biochemical processes or radiation chemistry. The various techniques and mechanisms are described and compared in a review primarily directed toward a better understanding of the diagenesis or organic substances under geological conditions. Factors such as temperature, pressure, time, pH, and Eh, and the role of catalysis, are considered and evaluated.

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ESSENTIAL FATTY ACIDS IN INFANT NUTRITION

Hilda F. Wiese

In early studies on effects of feeding low-fat diets to infants, impaired growth, possible susceptibility to infection and occasional skin eruptions were noted. Specific clinical evidences of a requirement for dietary linoleate were not observed. However, in a study of 426 young infants fed milk mixtures varying in linoleic acid content from < 0.1 to 7.3% of the calories, clinical manifestations of linoleic acid deficiency were evident in a high percentage of infants fed either a milk mixture low in fat or one in which the fat was very low in linoleic acid. These babies showed dryness and thickening of the skin with desquamation. Histologic changes in the skin showed thickening of the epidermal layer, dense keratin and often parakeratosis. Before cereals were added to the diet at 3 months of age, blood serum levels for linoleic and arachidonic acids were significantly lower in infants fed milk mixtures deficient in linoleic acid than in infants fed diets that provided 1% or more of the calories as linoleic acid. In serum of babies in the former group, 5,8,11-eicosatrienoic acid was relatively high. Differences in mean caloric efficiency (kcal consumed/g wt gain) also were demonstrated between groups of infants fed milk mixtures containing different amounts of linoleic acid. Clinical, histologic, blood serum and caloric efficiency evidences of linoleic acid deficiency disappeared when dietary linoleate was fed as the ester or triglyceride or in a milk mixture having fat which provided 1% or more of the calories as linoleic acid.

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POLYUNSATURATED FATTY ACIDS IN RELATION TO STEROL AND BILE ACID EXCRETION IN HUMAN SUBJECTS

Peter Wood and L. W. Kinsell

There is abundant documentation that in human subjects replacement of saturated fat in the diet by predominantly unsaturated fat results in decreased levels of plasma lipids, with particular reference to cholesterol and phospholipids. There has not been agreement as to the mechanism(s) of this effect.

Studies dealing with the effects of unsaturated fat on sterol and bile acid excretion have been unsatisfactory in many instances for methodological reasons and some of the results are contradictory.

Studies in this laboratory carried out in human subjects maintained under quantitatively constant conditions on the metabolic ward and using an isotope dilution technique have been pursued during the past few years. Without exception all of these subjects studied have had increased levels of cholesterol and bile acid excretion during the intake of polyunsaturated as compared to saturated fat. There is good reason to believe that the techniques used are accurate and reproduceable. (Details of procedures used will be presented.) It therefore seems probable that a major factor responsible for the effects of polyunsaturated fats upon plasma lipid levels is by way of acceleration of sterol and bile acid excretion in the stool.

Current studies upon *bile* composition are designed to investigate effects of unsaturated fat upon formation and excretion of specific bile acids.

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PROSTAGLANDINS AND ESSENTIAL FATTY ACIDS

J. E. Pike

Prostaglandins comprise a hormone-like group of oxygenated lipid acids with activities in a physiological range and which affect smooth muscle, cardiovascular phenomena and lipid mobilization. These humoral agents which occur widely distributed in animal species including man are biosynthesized efficiently from essential fatty acids. Recent findings relating to the biological activities of the prostanoic acids will be discussed with special emphasis on their possible role in essential fatty acid deficiency.

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THE BIOSYNTHESIS OF UNSATURATED FATTY ACIDS

S. J. Wakil

The biosynthesis of unsaturated fatty acids is accomplished by two independent major pathways; the aerobic and anaerobic pathways. The oxygen dependent pathway is the most frequently found route in biological systems and involves the direct desaturation of saturated fatty acids to olefinic acids. This type of mechanism appears to be responsible for the synthesis of not only monounsaturated but also polyunsaturated fatty acids, though different enzymes may be used in each system. The enzymes catalyze the desaturation of the thioester derivatives of the fatty acid (CoA or ACP derivatives) in the presence of O_2 and reduced pyridine nucleotides (DPNH or TPNH). They appear to be associated with internal-membrane structures and in the case of stearyl CoA desaturase system a requirement for lipid micelles was recently demonstrated. In the latter enzyme a combination of three phospholipids: lecithin, phosphatidyl, ethanolamine and phosphatidyl serine were needed for regeneration of the desaturase activity. The exact role of lipids in this enzyme remains to be determined.

The synthesis of the polyunsaturated fatty acids may be accomplished by further desaturation of oleic acid. Most animal systems convert Δ^{9}-C_{18} to $\Delta^{6,9}$-C_{18} acids whereas the plant systems convert Δ^{9}-C_{18} to $\Delta^{9,12}$-C_{18} and $\Delta^{9,12,15}$-C_{18} acids. The cofactor requirements for these conversions are essentially the same as those for the stearyl desaturase enzyme.

The anaerobic pathway, found in most bacteria, yields only monounsaturated fatty acids, and its mechanism is closely related to that of the de novo synthesis of saturated fatty acids, β-hydroxydecanoyl ACP being the common intermediate in the two pathways. Two dehydrases have been isolated from extracts of *E. coli* which dehydrate the β-hydroxydecanoyl ACP to *trans*-α-β and *cis*-β-γ-decenoyl ACP, respectively. The α-β-decenoyl ACP is then reduced to decanoyl ACP and elongated to saturated fatty acids (palmitic and stearic). The *cis*-β-γ-decenoyl ACP, on the other hand, is elongated without reduction to unsaturated fatty acids (*cis*-vaccenic).

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THE USE OF A COMPUTER TO CALCULATE AND TABULATE DATA IN METABOLIC EXPERIMENTS USING P^{32} AND INVOLVING QUANTITATIVE PHOSPHOLIPID ANALYSIS

A. I. Holtz

The sample of cells previously incubated with P^{32} as the phosphate was washed five times with Dulbecco's phosphate buffer to remove the incubating media. An aliquot of the suspended cells were counted, the rest extracted on a high-speed homogenizer with chloroform-methanol (Folch method) and the phospholipids are separated by TLC on Silica Gel G.

The individual phospholipids were analyzed quantitatively by a modification of the Bartlett phosphorus procedure. An aliquot of the final mixture of the phospholipid analysis was plated on a planchet and analyzed in a model D-47 Nuclear-Chicago gas-flow radiation detector with a model C-110B automatic sample changer.

In this work dealing with several experiments and involving numerous time-consuming chemical manipulations, it was found that a computer program was of value in helping to calculate and tabulate the final data in terms of counts per minute correcting for the decay of the isotope between the time of injection of the isotope into the experiments and the final assay and analysis of the radioactive compounds. An additional benefit was the substraction of the background from each sample.

The computer program was set up as follows:

$$\text{CPM} = \frac{\text{CPS} \times (\text{TB-Ti}) \times 2^{\frac{\left\{\text{T2} - \text{T1} + (\text{i} - 1)\ \Delta\text{T} + \sum\limits_{\text{K}=1}^{1} \text{T}_{\text{k}}\right\}}{1440 \times \text{HALF}}}}{\text{TB} \times \text{Ti}}$$

where (for the i^{th} sample)

CPM = Number of counts per minute
CPS = Number of counts per sample
Ti = Time required for i^{th} sample i 2,3 . . .
TB = Time required for background sample TB = T1
T1 = Time when experiment ended
T2 = Time when experiment began
T = Time between sample counts
Half = Half-life of radioactive substance

All times are in minutes.

Input to the program is as follows:
Date (day, month, year) and time when experiment ended.
Date and time when counting began.
Number of counts per sample.
Time between samples (in minutes).

This method is recommended for the analysis of relatively short-lived isotopes especially in the biochemical field where time-consuming chemical manipulations of many samples must be made between the initial metabolic experiment and the final chemical analysis of the metabolites.

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USES OF DIGITAL COMPUTERS IN THEORETICAL ANALYTICAL CHEMISTRY. I. SEPARATION OF A COMPOSITE INFRARED BAND INTO TWO COMPONENT PEAKS. II. SIMULATION OF THE PASSAGE OF A COMPOUND THROUGH A COUNTERCURRENT DISTRIBUTION APPARATUS

C. R. Eddy and J. S. Showell

A procedure has been developed for representing an infrared absorption peak by a general mathematical form, not restricted to Gaussian or Lerentzian shape. A computer program using this procedure will be described for obtaining the individual components from an infrared band consisting of two overlapping peaks. The method is also applicable to other overlapping peaked curves, such as are obtained in chromatography, countercurrent distribution, X-ray diffraction, and other branches of spectroscopy.

The progress of a compound through a countercurrent distribution apparatus has been followed theoretically by use of a digital computer. The computer avoids the usual assumption that all the tubes in the apparatus are alike. Instead, it deals with individual volumes and cut-off levels and allows time variation of solvent input rate. This gives a more realistic theoretical model and also makes it possible to test the properties of new designs of equipment before construction.

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AUTOMATED GRADIENT ELUTION COLUMN CHROMATOGRAPHY OF LIPIDS

Gary Nelson

Gradient elution in liquid-solid or liquid-liquid column chromatography lends itself to complete automation because it is a continuous process. The system to be described in this paper was specifically developed for the separation of complex mixtures of naturally occurring lipids obtained from various tissues and uses silica gel columns with concave gradients of increasing polarity. Concave gradients offer advantages over the convex type due to the absorptive properties of most lipid classes on silica gel.

The entire system is operated automatically once the charge is placed on the column. The flow rate is constant throughout the run and the shape of the gradient is predetermined by selecting known values for three parameters. The selection of the gradient is arbitrary but previous knowledge of the chromatographic behavior of the substances to be separated is useful in determining the shape of the gradient. Nongradient elution can also be performed by this apparatus. The entire system is controlled by timers, pumps, and solenoid valves. Details of the construction of the apparatus will be discussed and criteria for the selection of acceptable pumps for organic solvents will be mentioned. Several examples of the separations obtained with the system will be shown and the use of automatic column monitoring devices in conjunction with the gradient column system described.

While the chromatographic run described in this work used silica gel as the absorbent, there is no reason to believe that other adsorbers could not be substituted for other application. In addition, column size and elution times can be changed to provide better resolution for specific problems. The addition of continuous effluent monitoring to the apparatus will allow the system to be used in a manner similar to present gas-chromatographic systems, greatly broadening the application of liquid column chromatography particularly in the area of routine quantitative analysis of nonvolatile substances.

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MICROCOLUMN LIQUID ANALYSIS OF LIPIDS WITH FLAME IONIZATION DETECTION

J. E. Stouffer and P. L. Oakes

A system for the rapid analysis of compounds which may be either too large or too sensitive to be subjected intact to gas chromatographic or other methods is described. Capillary glass columns of 0.5 mm and 1 mm I.D. and various lengths are packed with very fine particle size specially prepared silica gel and other media. A simple pumping arrangement is employed for either single solvent or gradient elution and the solute in the effluent is detected by means of a highly sensitive flame ionization detector. The application of this system is illustrated by the separation of microgram and submicrogram amounts of triglycerides, steroids and other neutral lipids as well as some phospholipids.

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THE APPLICATION OF RAPID SCAN INFRARED SPECTROSCOPY TO GAS CHROMATOGRAPHIC FRACTIONS

K. E. Stine and H. J. Sloane

The recent introduction of an infrared spectrometer designed specifically for the analysis of gas chromatographic fractions has added new flexibility to both methods. Submilligram quantities of samples may now be examined directly as they elute from the column using heated, highly-efficient gas cells. Chromatographic fractions may be scanned "on-the-fly" as they elute from the column for immediate identification or may be trapped in the cell and scanned more leisurely.

In addition, the spectrometer can be used as a highly selective GC detector. This technique shows great promise for functional group monitoring. For example, it is possible to record chromatographic peaks for only those compounds in a mixture containing carbonyl or aliphatic CH absorptions.

Typical spectra obtained under both conditions will be shown and compared to standards. Application of this technique to typically-encountered mixtures containing carbonyl compounds, aromatics, and aliphatics, and its role in lipid chemistry will be discussed.

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MEASUREMENT OF RADIOACTIVITY IN THE EFFLUENT OF A GLC COLUMN

Arthur Karmen

If there is sufficient radioactivity in individual compounds to be measurable with acceptable precision in less than 15–20 sec, the effluent of a GLC column can be assayed for radioactivity during the course of an analysis. The effluent can be passed directly through a heated ionization chamber or proportional counter but both of these are somewhat sensitive to changes in the composition of the gas. The effluent can also be combusted to carbon dioxide and water, and the water then converted to HT for tritium assay. These gases can then be assayed in an ionization chamber, proportional counter or flow-through scintillation counter at ambient temperature. The detector volume and gas flow rate can then be made large to minimize the changes in gas composition that occur during the course of an analysis and adjusted to obtain the optimal speed of response. Convenient combustion trains have been developed that minimize memory effects in tritium assays that otherwise can cause difficulties. Convenient calibration methods are available for setting up the methods and for determining their efficiencies. When there is insufficient radioactivity in the sample to be measurable during this short time interval, the effluent can be fractionated, high boiling material in each fraction can be condensed out and assayed for radioactivity for longer periods. Automatic and quantitative fraction collecting devices have been developed for use with liquid scintillation counters that make this method of radioassay very accurate and sensitive.

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COMBINATION GAS CHROMATOGRAPHY-MASS SPECTROMETRY

J. A. McCloskey and F. A. J. M. Leemans

The direct combination of gas-liquid chromatography with mass spectrometry represents one of the most powerful techniques available for the analysis of complex mixtures of lipids. Principal advantages are the extremely small amounts of material required, the relative speed of analysis, and the wealth of molecular structural information available. The ability to rapidly record several mass spectra of one emerging chromatographic peak may also frequently be of use in detecting impurities, unresolved mixtures, or thermal decomposition of the sample. In the development of new gas chromatographic analytical procedures, the combination instrument may frequently prove highly useful for following chemical reactions in the preparation of new types of derivatives for GLC.

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SYNTHETIC FATTY ACIDS—A THREAT TO THE FATTY CHEMICAL INDUSTRY?

N. O. V. Sonntag

Panelists: K. T. Zilch, R. A. Peck, Morton Fefer and M. A. Baker

A round-table presentation of the chemistry, production and applications of synthetic branched-chain carboxylic acids. Included will be an up-to-date review of European synthetic fatty acids obtained by the oxidation of paraffinic hydrocarbons and an analysis of the prospects for the synthesis of straight-chain saturated and unsaturated fatty acids and derivatives from petrochemical base stocks. Dr. Fefer will discuss "Neo-Acids"; Dr. Zilch, "Synthetic Fatty Acids in Eastern Europe"; Dr. Peck, "New Technology"; and Dr. Sonntag, "Straight Chain Fatty Acids from Olfins and Ziegler Intermediates."

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THE USE OF GEL PERMEATION CHROMATOGRAPHY TO DETERMINE THE MOLECULAR SIZE DISTRIBUTION OF OIL-MODIFIED POLYESTER RESINS

D. A. Berry

Gel permeation chromatography (GPC) is a relatively new technique for quickly determining the molecular-size and molecular-weight distributions of a variety of polymer systems. Although it is better known as a tool for determining the distributions of addition polymers, with proper modification it can be used for relatively low molecular weight condensation products.

In this current study GPC was used to successfully obtain size distribution of oil-modified alkyd resins. This distribution is grossly affected by a number of variables including reaction conditions, type of dibasic acid and, of particular interest, the type of fatty acid. Light-scattering and vapor pressure osmometry were used to obtain absolute weight- and number-average molecular weights. From these data a "Q" value (molecular weight per Angstrom unit) can be derived, thus allowing conversion of the size distribution curves to weight distribution. Also, at critical points on the curve, samples were removed from the column and submitted to analysis by mass spectroscopy. Once again, the type and amounts of various fragments depend to a great extent on the variables listed above. It is believed that GPC could be an extremely useful tool in arriving at optimum conditions for the development of superior oil-modified resins.

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THE INFLUENCE OF GLYCERIDE STRUCTURE ON THE RATE OF AUTOXIDATION

E. G. Hammond and K. G. Raghuveer

Small amounts of trilinolein and trilinolenin were mixed with tridecanoin. Portions of these mixtures were randomized with a sodium methoxide catalyst, and all the preparations were deodorized. The rate of autoxidation of the randomized and unrandomized preparations at 37C was followed by determining the peroxide value. The unrandomized mixtures autoxidized much more rapidly than the randomized. In a second experiment, a mixture of trilinolein in tridecanoin was deodorized. A sample was withdrawn, and the rest of the mixture was randomized with a sodium methoxide catalyst. After allowing time for randomization, a sample was withdrawn and the remainder of the mixture was slowly cooled to direct the interesterification toward the formation of di- and trilinoleins. The randomized sample autoxidized much more slowly than the unrandomized sample, and the sample that had undergone directed interesterification autoxidized significantly more rapidly than the completely randomized one. Thus, the effects noted must be attributed to the glyceride structure and cannot be explained on the basis of contamination or the effect of the catalyst. A theory was derived to predict the effect of glyceride structure on the rate of autoxidation, and the theory accounted for the observed effects with reasonable accuracy. The theory indicates that the glyceride distribution frequently found in natural fats, in which the unsaturated fatty acids are concentration on the 2-position of the glycerol, should be more stable to autoxidation than a random distribution.

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LECITHINS AND LYSOLECITHINS OF WHEAT FLOUR

Mary E. McKillican

Lecithin and lysolecithin from the bound lipid of Thatcher Wheat endosperm were separated and purified by column and TLC. Lecithin was hydrolysed with phospholipase A (*Crotalus adamanteus*) and the fatty acids and lysolecithin separated chromatographically. The fatty acid composition of the original lecithin and lysolecithin and the products of hydrolysis was determined.

The fatty acids in the β-position of the lecithin were found to be almost entirely unsaturated, whereas those in the α-position were nearly equally saturated and unsaturated. There was little difference in fatty acid composition between the original lysolecithin and that obtained by hydrolysis with phospholipase A.

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OBJECTIVE FLUOROMETRIC MEASUREMENT OF AFLATOXINS ON TLC PLATES

W. A. Pons, Jr., J. A. Robertson and L. A. Goldblatt

Using a commercially available TLC densitometer, equipped for fluorescence measurements, the intensity of fluorescence of aflatoxins B_1, B_2, G_1, and G_2 in the dry state on silica gel coated TLC plates was measured after developing the plates with chloroform:acetone:2-propanol solvent. Using purified aflatoxins, essentially linear response was obtained between emitted fluorescence and concentration over a range of 2×10^{-4} μg to at least 100×10^{-4} μg of aflatoxin per spot. With a 465 mμ secondary filter, the response per unit weight was in the order B_2:G_2:B_1:G_1, with ratios of 4.3:3.0:1.7:1. The relative response of B_2:B_1 (2.5:1) and of G_2:G_1 (3.0:1) demonstrate that conventional measurements of aflatoxins B_2 and G_2 against B_1 and G_1 introduce significant errors.

Precision was determined by repetitive analysis of pure aflatoxins on TLC plates. Measurements made using a standard containing only aflatoxin B_1 and G_1 and spotting 60×10^{-4} μg of B_1, and 40×10^{-4} μg of G_1 on each of 9 plates, standard deviations of $\pm 1.69 \times 10^{-4}$ μg (B_1) and 1.40×10^{-4} μg (G_1) were obtained representing coefficients of variation of ±2.8% and ±3.5%, respectively. Similar analyses of a standard containing all four aflatoxins with aliquots ranging from 1 to 6 μl spotted on each of 9 plates yielded a precision of measurement, as represented by coefficients of variation, of ±7.6% (B_1), ±12.7% (B_2), ±7.8% ($B_1 + B_2$), ±8.4% (G_1), ±12.2% (G_2) and ±8.4% ($G_1 + G_2$). The technique has been applied successfully to the estimation of aflatoxins in purified extracts from cottonseed and peanut materials, mixed feeds and other agricultural products.

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DETECTION AND IDENTIFICATION OF PRODUCTS FROM THE AUTOXIDATION OF CHOLESTEROL

R. J. Sims and J. A. Fioriti

The autoxidation of cholesterol has been studied mainly by means of thin-layer chromatography and infrared spectrophotometry. In addition, chemical tests were used to follow the formation of hydroperoxides and epoxides. Spray reagents were used to distinguish hydroperoxides and epoxides from other oxidation products such as ketones, alcohols and acids. Epoxides were detected in the autoxidized mixtures. Several peroxide spots also appeared; an identification of these is being attempted.

Autoxidation in bulk was found to proceed very slowly at 80C. Higher temperatures or ultraviolet irradiation increased the rate markedly. The effect of temperature and storage conditions on the mechanism of the autoxidation reaction was investigated. A technique was developed for separating and identifying the several products from the reaction mixtures on TLC plates.

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AN IMPROVED 2-THIOBARBITURIC ACID (TBA) PROCEDURE FOR THE MEASUREMENT OF AUTOXIDATION IN FISH OILS

T. C. Yu and R. O. Sinnhuber

An improved 2-thiobarbituric acid (TBA) method suitable for routine testing of autoxidative changes of fish oil and other polyunsaturated lipids has been developed. Air oxidation of the lipid during the TBA reaction was found to produce misleading results. The air oxidation may be controlled by addition of antioxidants to the reaction system. Other factors causing inconsistent TBA results and methods of prevention are also described. A comparison of peroxide and TBA values in autoxidizing menhaden oil is presented.

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QUANTITATIVE DETERMINATION OF CYCLOPROPENOID FATTY ACIDS IN COTTONSEED MEAL

R. S. Levi, H. J. O'Neill, H. G. Reilich, E. L. Skau and A. F. Cucullu

A method has been developed for the quantitative analysis of the cyclopropenoid fatty acids (CPA) contained in cottonseed meal. Extraction of the meal was accomplished by a room temperature filtration with hexane-water-acetone azeotrope solvent followed by a benzene-methanol wash. The methyl esters were then formed by methanolysis with sodium methoxide. Extraction with petroleum ether followed by washing with aqueous acetone, resulted in a clean ester-containing fraction. This material was then analyzed for CPA by a spectrophotometric modification of the Halphen reaction.

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GAS CHROMATOGRAPHIC ANALYSIS OF POLYGLYCEROLS AND THEIR FATTY ACID ESTERS

M. R. Sahasrabudhe

Polyglycerols and polyglycerol fatty acid esters have been analyzed by GLC as silyl ethers, on a Perkin-Elmer model 800 Gas Chromatograph equipped with dual 3 ft S.S. columns packed with 3% JXR on Anakrom ABS and programmed from 120 to 325C at 10C/min. Commercial polyglycerols were found to be mixtures of tri- to decaglycerols with 20–70% of the specific polyglycerol. Application of the procedure to the analysis of polyglycerol esters in foods is discussed.

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GAS-SOLID CHROMATOGRAPHY OF HYDROCARBONS ON ACTIVATED ALUMINA. II. ALKANE, ALKENE AND ALKYNE SEPARATIONS WITH SUBTRACTIVE ADSORPTION

R. L. Hoffman, G. R. List and C. D. Evans

Various alkanes, -enes and -ynes of molecular weights below C_{10} have been rapidly separated by gas-solid adsorption chromatography on untreated activated alumina. Solid silver nitrate coated on alumina adsorb alkenes and alkynes irreversibly. Silver-nitrate concentration and column temperature markedly affect the extent of olefin removal. At temperatures near 300C some destruction of the C_8 and higher molecular weight saturated hydrocarbons has been observed. Conditions for this type of subtractive chromatography, the fate of unsaturated hydrocarbons and the application to the separation of hydrocarbons resulting from the oxidative deterioration of fats are discussed.

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SEPARATION OF FATTY ALDEHYDES AND THEIR DIMETHYL ACETALS BY TLC AND GLC

V. Mahadevan, C. V. Viswanathan, F. Phillips and W. O. Lundberg

Long-chain saturated aldehydes (C_{12}-C_{18}) and the C_{18} unsaturated—oleyl, linoleyl and linolenyl—aldehydes and their dimethyl acetals (DMA) were synthesized. The DMA were separated on the basis of their unsaturation by adsorption TLC using Silica Gel G impregnated with $AgNO_3$. The DMA of saturated aldehydes were separated from one another using reverse phase partition chromatoplates coated with Siliconized Kieselgur G. Separation of the various fatty aldehydes was effected similarly by a combination of $AgNO_3$ adsorption and reverse phase TLC.

The behavior of the aldehydes and the DMA on stationary liquid phases which are generally used in the GLC of fatty acid methyl esters was studied. Breakdown of the dimethyl acetals to the aldehydes was observed in some phases. The equivalent chain length values of the aldehydes and the DMA have been determined with respect to the corresponding fatty acid methyl esters to enable identification of DMA occurring in methyl ester mixtures prepared from natural samples. Besides, DMA standards are not as readily available as methyl ester standards.

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A NEW TECHNIQUE FOR COATING GLC CAPILLARY COLUMNS UTILIZING LONG CHAIN QUATERNARY AMMONIUM COMPOUNDS

L. D. Metcalfe and R. J. Martin

Open tube or capillary columns are finding increasing use in the gas chromatography of fatty acids and their derivatives. The great column efficiencies obtained with capillaries makes them an important adjunct in fat and oil research. Most metal capillaries are difficult to coat with the various liquid phases. This difficulty apparently is related to the geometry of the interior of the tube. Some columns are virtually impossible to coat with enough liquid to obtain useful chromatograms. It has long been known that quaternary ammonium compounds will plate out on metal or glass surfaces. It was decided to attempt to make use of this property to form an organic interface in the tube to which the liquid phase could adhere.

Approximately 1% of methyl trioctadecyl ammonium bromide was added to the solution used to coat the capillary column. A great increase in the amount of liquid phase that would adhere to the column was observed. A 100 ft stainless steel capillary which we had attempted to coat many times without success, was treated in this manner. The efficiency of this particular column increased from 1,250 to 13,100 theoretical plates with one treatment. Other columns which were easily coated had to be recoated at least once a month after normal use. With the quaternary treatment these same columns increased in efficiency and did not require recoating after many months of equivalent use. It was found that even the notorious glass capillary columns responded to the customary treatment.

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USE OF DIMETHYLSILYL ETHER DERIVATIVES IN GAS CHROMATOGRAPHY

W. R. Supina, R. F. Kruppa and R. S. Henly

In 1964 Wells, Sweeley, and Bentley ("Biomedical Applications of Gas Chromatography" H. A. Szymanski, ed., Plenum Press, N. Y., 1964, p. 199), described the gas chromatographic analysis of dimethylsilyl ether derivatives of glucose, mannose, and galactose. The dimethylsilyl ethers were found to have retention times approximately half that of the corresponding trimethylsilyl ether derivatives. The data is confirmed in this paper which includes relative retention times for both trimethylsilyl and dimethylsilyl ether derivatives of a number of carbohydrates. Also included are comparable data for other types of hydroxy and polyhydroxy compounds, including sterols, fatty alcohols, glycols, and phenols.

The effect of decreasing the retention time by using a dimethylsilyl derivative rather than a trimethylsilyl derivative becomes more pronounced as the number of hydroxy groups per molecule increases.

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CARBON ISOTOPIC EVIDENCE FOR THE ROLE OF LIPIDS IN PETROLEUM FORMATION

S. R. Silverman

The stable carbon isotope ratio (C^{13}/C^{12}) of modern marine organisms (plants and animals) is about 1% higher, on the average, than that of ancient organic matter in marine sediments. A similar relation exists between the ratios of terrestrial organisms and those of petroleums and other noncoaly organic materials genetically related to nonmarine sediments. In contrast, the C^{13}/C^{12} ratios of coals of all ranks fall into the same range as those noted for terrestrial plants.

Lipid fractions of organisms have consistently lower C^{13}/C^{12} ratios, by about 0.4–0.9%, than do the whole organisms. This suggests that petroleums and other noncoaly organic matter in ancient sediments are derived from lipids, or at least from certain components of the lipid fraction, whereas coal deposits are formed from the whole plants or from the cellulosic fraction, which is the major nonlipid constituent of plant tissues.

The implication that lipids play a major role in petroleum formation is supported by other geochemical information, including the distribution of C^{13}/C^{12} ratios and optical activity in petroleum fractions and the identification of specific organic compounds in petroleums that are structurally related to lipid components of modern organisms.

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OPTICAL ROTATION OF PETROLEUMS

W. D. Rosenfeld

The ability of crude oils and their derivatives to rotate plane polarized light indicates biochemical contributions to petroleum generation. Previous work implied that the optically active compounds are solely or predominantly naphthenic in structure and possibly derived from steroidal source materials. Later work showed the presence of optically active aromatic compounds and also extended widely the molecular weight range of active petroleum fractions, which suggests that the source of the rotatory compounds may include several classes of materials.

Distillation fractions of Arabian and Columbian crude oils show optical activity over a wide range of boiling points with a maximum in fractions whose molecular weight approximates 400. Similar maxima have been reported elsewhere for oils produced in France, the Soviet Union, and the United States.

Most optically active oil fractions are dextrorotatory, although levorotations are not uncommon. The latter are observed especially in the lower molecular weight range of petroleums. The existence of levorotatory compounds plus the wide molecular weight span of optically active oil fractions strongly reinforces the view that no single compound or class of compounds is likely to represent the source of rotatory materials in petroleum.

Polarimetry of dark, unfractionated crude oils was achieved with an instrument of high photometric sensitivity. Optical rotations range from a few thousandths to several degrees per decimeter of sample. All values are dextrorotatory and related apparently to the geochemical history of the petroleum.

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ALKANES IN NATURAL AQUEOUS SYSTEMS. II. THE EFFECT OF SODIUM CHLORIDE AND SODIUM BICARBONATE ON THE ACCOMMODATION OF C_{12}-C_{28} ALKANES

Eric Peake and G. W. Hodgson

The movement of hydrocarbons accommodated in ground and formation waters may be of importance in the migration of petroleum and the formation of oil fields. In order to more fully understand the petroleum migration and oil field formation processes a knowledge of the solubility or accommodation of n-alkanes in distilled water and saline solutions is desirable.

Using a simple solvent extraction procedure and gas-liquid chromatography the accommodation of n-alkanes C12 and C20, C22 to C24, and C28 was measured in solutions equilibrated with a liquid hydrocarbon mixture. To obtain equilibrium, distilled water or saline solution was shaken vigorously for 16 hr with 100 ppm of a mixture containing equal quantities of n-alkanes C7 to C20, C22 to C24, C28, and C32. After settling for periods of time up to one month, an aliquot was extracted several times by shaking with n-heptane and the extract was examined by program temperature gas chromatography for n-alkanes C12 to C28. The total n-alkane content of saline solutions, containing up to 50,000 ppm sodium chloride and sodium bicarbonate, ranged from a few parts per billion up to 25 ppm in distilled water.

Ultrafiltration of the hydrocarbon solutions indicated that micelles in saline solutions varied in size from those in distilled water. n-Alkanes accommodated in distilled water solutions readily passed through filters with pore sizes down to 0.8μ whereas the hydrocarbons of salt solutions were more easily filtered out.

These findings indicate that n-alkanes accommodated in fresh water may be forced out of solution by increased salinity.

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ABIOGENIC SYNTHESIS OF HYDROCARBONS

Cyril Ponnamperuma and Katherine Pering

In the study of chemical evolution we are interested in the path by which molecules of biological importance may have appeared on the primitive earth before the origin of life. In the present investigation, we have examined the formation of hydrocarbons by the action of a spark discharge on methane, a presumed constituent of the earth's primitive atmosphere.

Earlier studies revealed that the character of the C_6 to C_9 hydrocarbons formed varied with the type of discharge. A semicorona produced saturated compounds, while the arc discharge gave rise to unsaturated aromatic compounds. The higher hydrocarbons synthesized by the semicorona discharge have now been examined by gas chromatography and mass spectrometry. A comparative study showed that these hydrocarbons were different from those of the Posidonian shale, but similar to those from the Mountsorrel formation. Saturated normal alkanes and branched chain isoprenoid type compounds were found in the Posidonian, but not among the hydrocarbons from the spark discharge or Mountsorrel.

The results of our investigations may throw some light on the origin of hydrocarbons and enable us to establish reliable criteria for biogenicity.

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A NEW SILVER-IMPREGNATED GLASS PAPER FOR CHROMATOGRAPHIC SEPARATIONS BASED ON NUMBER OF DOUBLE BONDS

J. E. Muldrey, Jr.

The recent surge of interest in argentation separations in CCD, TLC and with silver-resins has indicated the need for a silver-impregnated glass paper. We reported in 1961 a silica-gel impregnated glass paper (SG) which is routinely used to separate cholesteryl esters into groups containing saturated, mono-, di-, tri-, tetraenoic (etc.) fatty acid esters. The separated "palmitate," "oleate," etc., are quantitated at the level of a microgram by densitometry of the charred spots produced by H_2SO_4 and heat. Mixtures of methyl esters are similarly separated on the SG paper, which also permits resolution of the acetates of desmosterol and cholesterol, of CoQ_9 from CoQ_{10}, and of tripalmitin from triolein. However, on the SG papers we have not been able to obtain the fine separations of triglycerides and lecithins into the many fractions separable by $AgNO_3$-TLC. Nor has the application of $AgNO_3$ to the SG papers proven satisfactory for this purpose. Indeed, $AgNO_3$-glass papers are extremely light-sensitive, and the HNO_3 released by the H_2SO_4 oxidizes away the carbon spots, lowering the sensitivity of the charring reaction.

When a reasonably good argentation medium was obtained by replacing NO_3 by PO_4, we were led to reinvestigate a zirconyl phosphate gel paper we had developed much earlier, which provided the answer to our present problem. Silver zirconyl phosphate impregnation of glass paper (Gelman microfiber or ITLC-Type SG) produces a stable, light-insensitive chromatographic medium capable of the same kinds of separations which are possible an $AgNo_3$-TLC, and on which as little as one-tenth microgram (100 nanograms) of material can be detailed by the H_2SO_4-char technique. The preparation and use of these media will be described.

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AUTOMATED GLC ANALYSIS OF FATTY ACIDS

Lars-Åke Appelqvist and Karl-Axel Melin

An apparatus capable of automatic analysis of up to 59 different samples of long chain fatty acid methyl esters has been constructed.

The automation involves a sample inlet system and a time-programmed print out of the detector signal after it has passed through a voltage-to-frequency converter. The sample inlet system consists of a storage section maintained at room temperature, and a vaporizer section maintained at about 275C. Chloroform solutions of the samples (usually about 20 μg) are applied with a microsyringe onto the inside wall of a short metal capillary open in both ends. After evaporation of the solvent, the tube is placed in the storage unit, from which it drops into the vaporizer by gravity when initiated by a timer. From the vaporizer the esters are carried onto the column by the argon carrier gas. The first-mentioned timer also initiates a second timer which controls a digital recorder printing out peak areas resulting from the separated components. The signal, produced by a standard PYE Sr^{90} ionization detector, is also fed to a 10 mV potentiometric recorder, which shows the normal separation pattern as well as the time intervals during which the integrated signals were counted.

In analysis of mixtures of C_{16}, C_{18}, C_{20}, and C_{22}-fatty acids with regard only to chain length a 40 cm stainless steel straight column packed with 10% silicon grease on Celite 60–80 mesh is being used. About 50 such samples can be analyzed overnight completely automatically. Mixtures containing linoleic and linolenic acid esters will not change chemically to a detectable extent during 24 hr, although stored without solvent, as an orgon atmosphere is maintained in the storage unit.

The potential use of the system for analysis of other high boiling compounds will be discussed.

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IDENTIFICATION AND ESTIMATION OF TOCOPHEROLS BY GLC

H. T. Slover, Lydia M. Shelley and T. L. Burks

Tocopherols are quickly and easily converted into their trimethysilyl ethers, using the method devised by Sweeley for carbohydrates. These derivatives may be chromatographed at lower temperatures, with less difficulty, than the free tocopherols. A method is described for the identification and quantitative estimation of alpha, beta, gamma, delta, epsilon, zeta-1 and zeta-2 tocopherols. Retention ratios, relative to octacosane, and Kováts Retention Indices have been determined on both Apiezon L and SE-30. All the forms investigated, with the exception of beta and gamma, may be completely resolved on both phases. The beta-gamma separation on Apiezon L is adequate for quantitative estimation, as shown by the results from the analysis of model mixtures. Qualitative data from the analysis of some natural tocopherol extracts are presented.

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THE RESOLUTION OF LONG-CHAIN ISOMERIC POLYHYDROXY ACIDS BY GLC

Randall Wood, E. L. Bever and Fred Snyder

Trifluoroacetate (TFA) derivatives of methyl 12-hydroxystearate, methyl ricinoleate, methyl *threo* and *erythro*-9,10-dihydroxystearate, methyl *threo* and *erythro*-9,10-12-trihydroxystearate, and methyl *erythro*-9,10- *erythro*-12,13- and *threo*-9,10-*threo*-12,13-tetrahydroxystearate were prepared and analyzed by GLC. Analyses of the TFA derivatives were carried out on polar and nonpolar packed columns containing 15% ethylene glycol succinate silicone polymer (EGSS-X) and 5% methyl silicone polymer (SE-30) liquid phases, respectively. The isomeric *threo* and *erythro* dihydroxystearates, which had not previously been resolved by GLC, were separated. The *threo* and *erythro* trihydroxystearates were separated into two components each. The *threo, threo* and *erythro, erythro* tetrahydroxystearates were also resolved into two components each. The order of elution of the various diastereoisomeric polyhydroxystearates can be predicted from structure and configurational considerations. The method is applicable for the determination of double bond configuration of unsaturated acids.

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AN OPTICAL INTEGRATOR FOR THE QUANTITATION OF TLC

W. O. Caster

An optical integrator was made with simple electronics and with optical parts available from a photographic enlarger. Thin-layer plates sprayed with sulfuric acid were heated until maximum contrast developed. The plates were photographed (Super Panchro-Press Type B cut film) and the resulting negatives were measured with the integrator. Essential portions of the integrator were a constant voltage, point light source provided by a "grain of wheat" bulb and battery, condensing lenses to produce a uniform collimated light beam, a combination mask and film holder constructed of sheet brass into which the film was inserted for reading, another set of condensing lenses to focus the light beam to a point (on a circle of opal glass), and a photoelectric microphotometer. The larger or the more dense the spot on the original thin-layer plate, the greater the amount of light that will reach, and be recorded by, the microphotometer. A series of known lipid standards and liver extracts were studied. For all components that migrate on the thin-layer plate, photometric results were substantially linear over a concentration range of 10-fold or more, and standard deviations were in the range of 5–10% relative error.

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SEPARATION OF BILE LIPIDS BY SEPHADEX COLUMN CHROMATOGRAPHY AND TLC

Akira Yamamoto and George Rouser

Sephadex column chromatography according to Siakotos and Rouser [JAOCS *42*, 913 (1965)] was used for preliminary separation of bile lipids. Phospholipids and unconjugated bile acids appeared in fraction 1, glycine conjugates in fraction 2, dihydroxy taurine conjugates in fraction 3, and trihydroxy taurine conjugates plus water soluble nonlipids in fraction 4. The various components of each fraction were then separated by two-dimensional TLC [JAOCS *42*, 215 (1965)] with 9 parts of silicic acid (no binder) mixed with one part of finely powdered magnesium silicate as adsorbent. Solvent pairs were (1) chloroform/methanol/water 65/25/4 followed by 1-butanol/acetic acid/water 60/20/20 and (2) chloroform/methanol/28% aqueous ammonia 65/35/5 followed by chloroform/acetone/methanol/acetic acid/water 5/2/1/1/0.5. Sephadex column chromatography provides a unique means for separation of bile salts since all unconjugated bile acids are separated from the conjugated forms, glycine and taurine conjugates are completely separated, and di- and trihydroxy taurine conjugates are separated.

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STRUCTURAL DETERMINATION OF CERAMIDE AMINOETHYLPHOSPHONATE FROM THE SEA ANEMONE

Gerald Simon and George Rouser

The structure of ceramide aminoethylphosphonate (CAEP) proposed by Rouser et al. [JAOCS *40*, 425 (1963)] was established by degradative procedures. The long chain base obtained by acid hydrolysis of CAEP was shown to be sphingosine by gas chromatography (GC) of the trimethylsilyl derivative and TLC. Confirmation of the structure of sphingosine and demonstration of the point of attachment of the 2-aminoethylphosphonate grouping as the primary hydroxyl of sphingosine were obtained by a series of chemical reactions [J. Am. Chem. Soc. *75*, 313 (1953)]. CAEP was treated with performic acid and the product submitted to alkaline hydrolysis to yield CAEP hydroxylated at carbons 4 and 5. Hydroxylated CAEP was oxidized with sodium metaperiodate, the products separated and oxidized with chromic acid. Myristic acid was identified by GC of its methyl ester as the product obtained from the hydrocarbon portion of the long chain base thus demonstrating that the base is sphingosine. After acid hydrolysis of the product obtained from the other end of the molecule, serine was identified by TLC as the only amino acid released thus demonstrating that the 2-aminoethylphosphonic acid group is attached to the primary hydroxyl group (carbon 1) of sphingosine.

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SIMULTANEOUS QUANTITATION AND DETERMINATION OF SPECIFIC ACTIVITIES OF FATTY ACIDS BY DOUBLE-LABEL LIQUID SCINTILLATION COUNTING

G. A. Fischer and J. J. Kabara

Quantitation of fatty acids by esterification with methyl labeled methanol (H^3 or C^{14}) has proven to be rapid, accurate, convenient, and economical. This method of analyzing fatty acids is adaptible to a wide variety of problems in lipid chemistry. It is compatible with GLC and other methods of separation. Both nonlabeled and singly-labeled fatty acids can be measured. The wide range of samples size from molar quantities to 0.0001 μmole, enables accurate quantitative analysis beyond the range of existing methodology.

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TLC OF LIPIDS OF DESERT SOILS

A. J. Bauman and R. E. Cameron

Two dimensional TLC has been used to survey the chemical nature and number of lipid components of aridisol soils from widely different locations which varied in organic matter content, microfloral count, texture and salinity. Many substances were detected but few were identified. TLC is useful in determining the general approach to additional fractionation procedures such as column chromatography which may be required for soil organic analysis.

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TRIGLYCERIDE BIOSYNTHESIS VIA THE α-GLYCEROPHOSPHATE PATHWAY

J. M. Johnston, G. Ananda Rao and Patricia A. Lowe

Two pathways for the enzymatic synthesis of triglycerides exist in the intestinal mucosa. One pathway employs 2-monoglyceride as the acyl acceptor and is located in the microsomal fraction. On the other hand, the second pathway utilizes α-glycerophosphate and requires both the microsomal and supernatant fractions. The synthesis of triglycerides occurs when α-glycerophosphate, fatty acids, ATP and CoA are present. However, when the fatty acid activation system is replaced by fatty acid-CoA, only a trace synthesis occurs. Furthermore, the fatty acid-CoA derivatives inhibit the utilization of free fatty acids. The required supernatant is thermolabile, -SH sensitive, and not retained by Sephadex G-100. Parallel to the synthesis of triglycerides via the α-glycerophosphate pathway a polar lipid is formed as judged by TLC. Both labeled α-glycerophosphate and fatty acids give rise to triglyceride. In addition to the intestinal mucosa similar findings have been obtained with liver preparations. The relationship of these findings to the synthesis of triglycerides will be discussed.

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MINOR GANGLIOSIDES OF BRAIN: THEIR STRUCTURES AND INVOLVEMENT IN NEUROLOGICAL DISEASES

R. Ledeen, K. Salsman and M. Cabrera

Four minor gangliosides have been isolated from normal human brain by combinations of column and preparative thin-layer chromatographies. Carbohydrate and fatty acid compositions have been determined, and in some instances periodate/borohydride treatment has elucidated the oligosaccharide bonding arrangement. Two of the species lacked hexosamine and contained glucose, galactose, and NANA in molar ratios of 1:1:1 and 1:1:2, respectively. The other two had these compositions plus one unit of N-acetylgalactosamine. Neuraminidase removed sialic acid in all but one of the compounds. A number of neurological diseases are characterized by elevated brain levels of one or more of these gangliosides. Such pattern alterations are becoming increasingly useful in diagnosis.

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AN UNUSUAL LIPID PRESENT IN EHRLICH ASCITES CELLS AND OTHER TUMORS

Fred Snyder, Shu-Sing Cheng and Claude Piantadosi

Earlier work by DiPaolo and co-workers [Proc. Soc. Exp. Biol. Med. *113* 68–70 (1963)] demonstrated by phase contrast microscopy that Ehrlich ascites cells (EAC) possess refractile lipoid granules. These workers noted that the number of granules in each cell increased as the ascites tumor cells grew in the peritoneum of the mouse. Our data confirm their observation and further show that Ehrlich ascites cells grown in tissue culture also develop increased numbers of lipoid granules as the incubation period extends.

Using TLC, we have investigated the lipid composition of EAC and solid rat tumors. The lipid compositions of ascites cells grown in vivo and invitro were similar. Triglycerides and sterol esters were found to be present in approximately equal quantities within the cells; phospholipids, cholesterol, and free fatty acids were also found in the cells, but to a lesser extent. The most significant observation was the occurrence of an unknown spot (R_fX) on TLC directly above the triglycerides from all EAC but not in the extracellular fluid. The R_f of this unknown class of lipid is identical to that of an unidentified compound we have found in other tumors [Walker-256 (rat), Fisher (rat), spontaneous (rat), and human]. The polarity of this compound (R_fX) on TLC is similar to that of a glyceryl ether diester, but no definitive proof of structure is available at this time. Saponification of R_fX does not yield the free monoether of glycerol. Although palmitic-1-^{14}C acid and acetate-1-^{14}C were incorporated in vivo into triglycerides and phospholipids, none of the label was found in the R_fX component either in rat tumors or EAC.

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THE METABOLISM OF α-ALKOXY GLYCERYL MONOETHERS IN RAT LIVER

R. C. Pfleger, Claude Piantadosi and Fred Snyder

Data from our laboratories have shown that the liver is the primary site for the metabolism of alkoxy glyceryl monoethers [JAOCS *42*, 462A (1965)]. Continuation of this work led us to determine the quantity of intact alkoxy ethers incorporated into alkoxy ether phosphatides of the liver. Doubly labeled glyceryl ethers were administered to permit the calculation of the ratio of ether chain to glycerol. In addition, acetylation of the phospholipids with acetic acid:acetic anhydride (3:2 v/v) and subsequent saponification with 1 N KOH (ethanolic) was used to determine the original alkoxy ethers present.

Born in vivo and in vitro experiments were performed. ^{14}C-and ^{3}H-labeled chimyl and batyl alcohols were administered intravenously to female rats as synthetic fat emulsions. Six hours later the animals were etherized and the livers were perfused with saline before lipid extraction by a modified Folch procedure. In the in vitro experiments, 20 μg of the glyceryl ethers were incubated with liver slices (100 mg) in 1 cc Tyrode solution ($pH = 7.3$). After various time periods (0.5–5 hr) the lipids were extracted and thin-layer chromatograms and zonal scans of these extracts were used to determine the extent and type of metabolic products. The in vivo experiments confirmed earlier data indicating that only a small percentage ($<4\%$) of the injected labeled glyceryl ethers remain unreacted in the liver. Most of the activity (52% from the chimyl alcohol and 80% from the batyl alcohol) was found in the phospholipids, primarily in the phosphatidyl choline fraction. Radioactivity was also found in the triglycerides (37% from the chimyl alcohol and 9% from the batyl alcohol). Long-chain alcohols, fatty acids, diglycerides, and glyceryl ether diesters contained less than 10% of the total liver radioactivity. Similar metabolic transformations were observed in vitro. Acetolysis and saponification revealed that less than 0.7% of the activity in the phosphatides in all experiments was present as alkoxy ethers.

Since the ^{14}C from the chimyl alcohol (yielding palmitic acid) is greater in the triglycerides than that from the batyl alcohol, and since the ^{14}C from the batyl alcohol (yielding stearic acid) is greater in the phospholipids than that from the chimyl alcohol, it is apparent that the rat liver rapidly cleaves the ether linkage and that the fatty acid product enters the free fatty acid pool.

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STRAIN DIFFERENCES IN LIPID METABOLISM OF RATS

A. I. Fleischman, H. Yacowitz and M. L. Bierenbaum

Conflicting results have been reported on the effects of various fats on rat lipid metabolism. In studying possible mechanisms for the hypolipemic action of elevated levels of dietary calcium, different rat strains were employed. These experiments yielded strain dependent results.

Male Wistar SPF and male Holtzman albino rats, 400 days old, were housed in cages and fed a corn soya ration containing 18% added fat, cocoa butter or corn oil, and 2% added cholesterol for 21 days. Calcium levels were 0.08% and 1.2%. All diets were isocaloric and isonitrogenous.

Weight gains were comparable in both strains. Fecal excretion was 23% lower in the Wistar SPF than in the Holtzman under comparable conditions, $P < 0.01$. A similar effect was noted in fecal bile acids, $P < 0.01$. Serum total lipids were 45% higher in the Wistar SPF rat than in the Holtzman, $P < 0.01$, except with cocoa butter and 0.08% calcium. Serum cholesterol was similarly 24% higher in the Wistar SPF rat under all conditions, $P < 0.01$. Serum phospholipids were 23% lower on cocoa butter but 68% higher with corn oil in the Wistar SPF rat than in the Holtzman rat, $P < 0.01$. Liver total lipids were 31% higher in the Wistar SPF than in the Holtzman, $P < 0.01$.

Significant interactions were noted with diet, strain and calcium levels. Increased dietary calcium significantly lowered serum phospholipid in the Holtzman strain but not in the Wistar strain on cocoa butter. Serum triglycerides were lowered by increasing dietary calcium with both fats in the Holtzman rat, $P < 0.01$, but not in the Wistar SPF rat with corn oil. Calcium exhibited a significant hypocholesterolemic response in both rat strains.

These results emphasize the importance of specifying strains in reporting lipid data in rats.

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NUTRITIONAL EFFECTS OF SOME FRESH AND MILDLY OXIDIZED ANIMAL AND VEGETABLE FATS

Hans Kaunitz, Ruth Ellen Johnson and Lewis Pegus

Weanling male albino rats were fed purified, iodine-low diets containing 30% casein and 20% of butter, lard, corn oil, or soybean oil. These fats were fed fresh and after 40 hours of aeration (1 liter/min/gal) at 60C. Parameters studied included: weight; food intake; survival rate; fatty acid compositions of triglycerides, cholesterol esters, and phospholipids of liver, heart, kidney; total cholesterol levels of serum and tissues; and histology of tissues from all rats sacrificed or dying spontaneously. Iodine deficiency was associated with the occurrence of large, histologically abnormal thyroids; the animals fed vegetable oils had significantly heavier thyroids than did those fed animal fats. Otherwise, iodine deficiency seemed to exert only mild effects. The fatty acid composition of the dietary fats influenced those of the various lipid classes. The life span of the rats fed fresh animal fats was significantly longer than that of the rats fed fresh vegetable oils. Fibrosis of the heart muscle, kidney disease, and purulent lung disease were the main causes of death, and there were no apparent differences among the groups. Neoplasms (except for pituitary tumors) occurred in 11 out of 35 rats fed the butter diets and only 9 times in 65 animals fed the other diets. The results of this study will be compared with those of our earlier study involving cottonseed oil, olive oil, beef fat, and chicken fat.

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DIETARY INFLUENCE ON THE MOBILIZATION OF FAT FROM ADIPOSE TISSUE

D. G. Therriault, M. A. Mehlman and P. Killian

The previous dietary history of male rats of the Sprague-Dawley strain, was found to affect the mobilization of fat from adipose tissue during periods of starvation.

Rats were maintained on a highly purified synthetic diet or on the commercial purina chow rat diet. Measurement of the lipid content of the epididymal fat pads before and after a four-day period of starvation revealed that those animals on a purified synthetic diet lost significantly less lipid from adipose tissue than did the rats on purina chow. There was also a corresponding decreased body weight loss during starvation of rats fed the synthetic diet.

Varying the composition of the synthetic diet with respect to protein, carbohydrate or fat had no effect on the decreased mobilization of lipid adipose tissue during starvation.

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EFFECTS OF DIETARY SATURATED FATTY ACIDS UPON FATTY ACID COMPOSITION OF RAT LIVER LIPIDS

Hans Mohrhauer and R. T. Holman

Groups of weanling rats were fed a fat-free diet and supplements of a constant level of 0.5% of cal of linoleate. Additional supplements of individual saturated fatty acids C-4 to C-16 were fed in the form of their triglycerides at 10% of cal. The fatty acid composition of liver lipids was determined by GLC. The conversion of linoleate to arachidonate is not hindered by the dietary saturated fatty acids. The amount of 20:3 ω9 is slightly lowered by even-numbered saturated fatty acids shorter than C-16, whereas it is significantly diminished by C-5 and C-11 dietary acids. The administration of odd chain fatty acids gives rise to 15:0, 17:0, 17:1 and 19:3 in liver lipids as identified by GLC and mass spectrometry. These acids seem to substitute for 20:3 ω9.

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STUDIES ON THE METABOLISM OF TRANS ACIDS IN THE RAT. INTERCONVERSION OF TRANS-9, CIS-12, TRANS-9, TRANS-12, CIS-9, TRANS-12-, AND CIS-9, CIS-12-OCTADECADIENOIC ACIDS

E. M. Stearns and O. S. Privett

Studies on the structures of fatty acids obtained from the livers of male rats of the Sprague-Dawley strain fed *cis*-9, *cis*-12, *cis*-9, *trans*-12-, *trans*-9, *cis*-12-, and *trans*-9, *trans*-12-isomers of methyl octadecadienoate, as the sole source of lipid, are reported.

Animals were fed a fat-free diet supplemented with the test substances in a number of experiments which were varied with regard to both the duration and amount of the octadecadienoate isomers added to the diet.

The fatty acid composition of the livers of the individual animals were determined by gas-liquid chromatography of the methyl esters. The structures of the fatty acids were determined on pooled samples by a combination of silver nitrate chromatography, infrared spectral analysis and reductive ozonolysis, by a method which gives the specific positions of the *trans* double bonds.

The results indicate that for interconversion to higher polyunsaturated fatty acids, a *cis* double bond is required in the 9 position. None of the acids containing *trans* double bonds alleviated essential fatty acid deficiency symptoms.

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