ABSTRACTS OF PAPERS 58TH ANNUAL MEETING NEW ORLEANS, LOUISIANA

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ALPHA OLEFIN SULFONATE FORMULATIONS AND PROPERTIES

E. A. Knaggs, J. A. Yeager, M. Nussbaum and E. J. Buerk

The recent commercial availability of high-purity alpha olefins in the C_{10} to C_{20} range, combined with continuous SO_3 falling film processing technology, has made possible the commercialization of alpha olefin sulfonates.

The effect of olefin purity and chain length, and sulfonation processing conditions on derived product properties is reviewed, indicating that a variety of specialties can be tailored, depending on the raw material feedstock.

Alpha olefin sulfonates have been dried on a commercial scale, both in a spray tower and on a drum drier. The resultant beads and flakes are free-flowing and nonhygroscopic.

Emphasis is also placed on liquid detergent formulations both for household light-duty liquid applications, and for various cosmetic specialties including shampoos, bubble baths and skin cleansers. Results of Draize rabbit eye irritation studies, and rabbit skin irritation studies established the alpha olefin sulfonates to be significantly milder than conventional anionics used today.

The extreme mildness properties of these olefin sulfonates combined with their improved biodegradability, and formulation flexibility, underscore the importance of this "third generation" detergent in meeting our ever increasing detergent standards.

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NEW NONIONIC DETERGENTS DERIVED FROM EPOXIDIZED OILS. IV

K. L. Johnson

The results of an experiment designed to investigate optimal composition, catalyst level, molar ratio, and cosolvent level for the reaction of methylepoxystearate with polyoxyethylene glycol are presented. The results of this study show that methyl epoxystearate can be readily converted to nonionic wetting agents of technical importance in the most rudimentary processing equipment. The reaction takes place at ambient temperatures, atmospheric pressure, and without a cosolvent. Dry raw materials and adequate mixing are the only stringent requirements.

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THE EFFECT OF UNSATURATION ON DETERGENCY OF TALLOW ALCOHOL SULFATES

R. S. Klonowski, C. M. Josefson, F. Ozkan and T. W. Findley

Preparatory to a study of selective hydrogenolysis of animal fats, and to determine the effect of such selectivity on the function of detergents made from tallow alcohols. Soil removal and whiteness retention were measured for 5 concentrations (0.05% to 0.25%), 5 levels of unsaturation (0% to 50%) of tallow alcohol sulfates and ethoxylated (3 moles) tallow alcohol sulfates, with and without builder, at 3 levels of water hardness (0 ppm to 300 ppm), and at 3 temperatures (100F to 180F). Selective experimental design and statistical evaluation of results enabled conclusions to be reached with a minimum of experimental determinations.

Sulfates of tallow alcohol mixtures containing unsaturated alcohols have better soil removal properties than mixtures containing only saturated alcohols. This is especially true for built systems, but less evident with ethoxylated alcohol sulfates. It is most evident at low temperatures or high water hardness.

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ETHER ALCOHOL SULFATES FROM OLEYL ALCOHOL

J. K. Weil, A. J. Stirton and Eileen B. Leardi

A study on the effect of oxyethylation, oxypropylation and oxybutylation of tallow alcohols has now been extended to include the unsaturated component.

Distribution constants, the ratio of ether alcohol reactivity to parent alcohol reactivity, have been found to be about 2 and increasing for the ethylene oxide reaction, 0.5 and constant for the propylene oxide reaction, and 0.3 and constant for the butylene oxide reaction. Thus propylene oxide and butylene oxide reactions give higher yields of the important first derivative than the ethylene oxide reaction.

First and second derivatives from each epoxide were separated by distillation. The derivatives, which were 97–99% pure according to GLC, were characterized by freezing point and refractive index.

Sulfation of the unsaturated ether alcohols with dioxane—SO_3 led to high-purity ether alcohol sulfates which were evaluated as detergents. The products were soluble in water and toluene and were effective detergents and lime soap dispersing agents.

Sulfation of the unsaturated ether alcohol sulfates with 1.2 moles of chlorosulfonic acid gave a product with 60% double bond retention. Similar sulfation of oleyl alcohol gave only about 25% retention of the double bond. Alkyl substitution of the ether or additional groups did not change the degree of protection. Ether alcohols sulfated by this method likewise showed improved solubility and favorable properties in combination with saturated ether alcohol sulfates and soap.

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SPRAY DRYING OF HEAVY DUTY DETERGENT FORMULATIONS: ADVANTAGES OF TRIMETAPHOSPHATE IN PLACE OF TRIPOLYPHOSPHATE

S. J. Silvis and Mario Ballestra

Plant tests were made to evaluate the economic as well as processing advantages of using sodium trimetaphosphate in place of sodium tripolyphosphate. The Ballestra Spray Tower System containing the continuous feeding and crutching units was used. The sodium trimetaphosphate was converted to completely hydrated sodium tripolyphosphate continuously within the crutcher mixture. The spray tower operations and efficiencies as well as the finished product characteristics were compared when using the trimetaphosphate to produce the finished product and when using the low temperature-rise tripolyphosphate normally employed in this plant. Results showed that the use of trimetaphosphate gave the following advantages: 1) a fully hydrated slurry without the lumps and viscosity problems normally caused by tripolyphosphate; 2) a finished product with a higher percentage of the P_2O_5 in the desirable form of tripolyphosphate; 3) significantly increased tower capacity; 4) higher finished product moisture content; 5) steadier tower operation resulting in better control of finished product moisture and density.

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PENTANE FROM THERMAL DECOMPOSITION OF LIPOXIDASE-DERIVED PRODUCTS

C. D. Evans, G. R. List, Ami Dolev, D. G. McConnell and R. L. Hoffmann

Thermal decomposition of 13-hydroperoxyoctadeca-9,11-dienoic acid forms pentane predominantly to the exclusion of practically all other short-chain hydrocarbons. Oxidative polymers are simultaneously formed during lipoxidase oxidation of pure linoleic acid, and the polymer fraction also yields pentane to the exclusion of other hydrocarbons under similar thermal conditions. Chromatographic pure fractions of the hydroperoxide and the polymer were separated from the unoxidized linoleic acid in yields of 36 and 27%, respectively. Polymer formation simultaneously with hydroperoxides by lipoxidase is a new concept important to edible oil quality and oil processing. Thermal release of pentane is helpful in interpreting the structure of oxidative dimers and polymers and is in agreement with current findings on the properties of oxidative polymers.

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EDIBLE OIL QUALITY AS MEASURED BY THERMAL RELEASE OF PENTANE

C. D. Evans, G. R. List, R. L. Hoffmann and Helen A. Moser

Fatty hydroperoxides produce normal hydrocarbons as part of their thermal decomposition products. The extent of hydrocarbon formation can be measured and associated with the quality and potential stability of an oil. Edible oils, high in linoleic acid, develop 13-hydroperoxy-9,12-octadecadienoic acid as one product of autoxidation. Since this particular hydroperoxide isomer yields pentane on thermal decomposition, the amount of pentane released has been correlated with the flavor of soybean and cottonseed oils and with the peroxide values of the respective oils. The amount of pentane released has an inverse linear relationship to flavor and a direct linear relationship to the peroxide values.

Edible oils exposed to light have a different relationship between flavor score and thermally derived pentane than do the same, but autoxidized, oils. Curves showing the relationship between flavor and peroxide value to pentane for autoxidized and light-struck soybean and cottonseed oils have been plotted. Application and limitations of this analytical method for flavor and quality evaluation will be described.

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SIMPLE PILOT-PLANT BATCH PROCESSES FOR ELIMINATION OF THE HALPHEN-TEST RESPONSE OF COTTONSEED OILS IN CONJUNCTION WITH DEODORIZATION

P. H. Eaves, H. P. Dupuy, L. L. Holzenthal, E. T. Rayner and L. E. Brown

Cottonseed oils contain cyclopropenoid fatty acids, chiefly malvalic, in amounts ranging from about 0.3 to 0.7%. Even trace quantities of cyclopropenoids are qualitatively detected by the Halphen test, while they may be determined quantitatively by HBr titration. As unusual biological effects have been ascribed to the cyclopropenoids, research has been carried out to develop methods of treating cottonseed oils to render them Halphen-negative and thus eliminate the biological effects.

Based on a laboratory procedure, two simple but effective pilot plant processes utilizing a pilot-plant size conventional deodorizer were developed for the production of Halphen-negative cottonseed oils.

Refined and bleached winterized cottonseed oil containing about 0.53% cyclopropenoids (calculated as malvalic acid) was used as starting material. In one process from 0.5 to 1.5 equivalents (based on cyclopropenoid content in the oil) of distilled cottonseed acids were mixed with the oil; then the mixture was charged to the deodorizer and heated to 450F under about 17 in. Hg vacuum while being agitated vigorously with a small steam sparge. When tests showed the oil to be Halphen negative, the excess free fatty acid was removed and the oil deodorized at 29.9 in. Hg vacuum by sparging with 3% steam. The time required for rendering the oil Halphen-negative (after reaching 450F) ranged from 5 min for 1.5 equivalents of free fatty acid to 75 min for 0.5 equivalents.

In a second process the addition of free fatty acids was omitted, and the small amount of cottonseed acids required for reaction with the cyclopropenoids was generated in situ by heating the oil at 450–455F under about 17 in. Hg vacuum while sparging lightly with 1.5% steam. When the oil tested as Halphen-negative, it was deodorized under high vacuum in the conventional manner. About 1.5 hr of heating at 450–455F were required to render the oil Halphen-negative by this procedure.

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PILOT-PLANT SELECTIVE HYDROGENATION OF SOYBEAN OIL: ACTIVATION AND EVALUATION OF COPPER-CONTAINING CATALYSTS

K. J. Moulton, D. J. Moore and R. E. Beal

The linolenate content in soybean oil has been reduced to less than 1% under pilot-plant conditions without increasing the saturates by hydrogenation with an active copper-chromite catalyst.

When used for the selective hydrogenation of soybean oil, several commercial catalysts containing copper had less than optimum activity. Their activity was improved by heating the catalysts at an elevated temperature. During the correlation of hydrogenation data on catalyst activity with oil quality, differential thermal analysis proved a useful

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tool to predict 1) whether or not a catalyst was active, 2) if a heat treatment would increase activity and 3) what the approximate temperature should be for the most effective treatment. Six commercial catalysts were investigated for hydrogenating soybean oil at 170C and 30 psig. When at least two-thirds of the linolenate content was removed from the original oil, the linolenate selectivity ratio (K_{Le}/K_{Lo}) was in the magnitude of 9 to 12. When the hydrogenation was scaled up from 1 to 15 gal, oil quality, catalyst selectivity and operational ease were comparable.

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METHYL SILICONE IN FRYING FATS—ANTIOXIDANT OR PROOXIDANT?

Stanley Rock, Leonard Fischer and Howard Roth

Methyl silicone was shown to be both an antioxidant and a prooxidant, at a level of 2 ppm in frying fats, depending upon the method of heating. When thermostatically heated in a fryer, at 375F, with or without frying, fats containing methyl silicone deteriorated slower than controls without the additive. The reverse was true when these fats were heated and maintained at 375F in an oven.

The functional condition responsible for this difference in behavior has been shown to be the temperature of the air-fat interface, which is approximately 100F lower in the fryer.

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THE HYDROPHILIZATION PROCESS FOR THE SEPARATION OF FATTY MATERIAL IN SOLID AND LIQUID COMPONENTS WITH THE AID OF AQUEOUS SOLUTIONS OF SURFACE SUBSTANCES

Werner Stein

Fats and derivatives of fats consist of mixtures of components differing in chain length and saturation. Apart from fractional distillation several methods exist for the separation, based on the different melting points and solubility behavior of these components. The modern separation processes use recrystallization from organic solvents; others use press or filtration methods.

The hydrophil process uses an entirely different approach: the mixture of solid and liquid fatty material, for instance tallow fatty acid, is treated with an aqueous solution of surface-active substances. The liquid fatty acids are removed from the surface of the fatty acid crystals and are emulsified in the aqueous phase. By the surface activity of the solution the crystals are hydrophilized, so that they leave the oil phase and enter completely the aqueous phase, where they are suspended.

In a solid bowl centrifuge the aqueous solution containing the suspended crystals of stearic acid is separated from the oleic acid phase. The stearic acid is obtained by heating the aqueous suspension above the melting point.

The composition and concentration of the surfactants, the salt content and the volume of the aqueous phase, the crystallization performance of the fatty material and the operative design are very important for the efficiency of the separation. The composition of the components is based on the phase diagram which controls iodine number, melting or cloud point of the oleic and stearic acid in relation to the separation temperature. The stearic and oleic acid obtained is practically identical with the expected composition from the phase diagram readings.

This separation principle can be used for fatty acids, fatty alcohols or fats.

A plant with a capacity of ca. 6,000 lb/hr has been operating continuously for a number of years.

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THE EFFECTS OF MICROMOLAR CONCENTRATIONS OF IONS ON THE LIPID OXIDATION OF ISOLATED LYSOSOMES

Kiyoshi Hayase and D. B. Menzel

The oxidation of isolated rat kidney lysosomes was followed by measurement of the light-scattering properties and the release of lysosomal enzymes. Micromolar amounts of ferrous ion initiated the reaction. The reaction was autocatalytic resulting in the irreversible swelling of the lysosomal particles and release of the enzymes contained within. Swelling of the lysosomes by this reaction was all or none. The rate of swelling of the lysosomes depended upon the composition of the suspending medium being most rapid in KCl and NaCl solution and less in sucrose. The swelling in sucrose solution was most reproducible and was initiated by 1 to 25 μM Fe^{++} ion. The latent period was extended with increasing concentrations of Fe^{++} ion; 50–100 μM Fe^{++} inhibited lipid oxidation as measured by the TBA reaction and caused contraction, as opposed to swelling, of the lysosomes. Ten μM Mn^{++} ion inhibited the reaction as did 0.68 mM EDTA. The pattern of swelling and release of enzymes is interpreted as a result of the oxidation of the lipid of the lysosome.

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THE ISOLATION AND IDENTIFICATION OF LACTONES IN OXIDIZED VEGETABLE OILS

J. A. Fioriti and R. J. Sims

Both gamma and delta lactones up to C_{10} can be removed efficiently from oxidized vegetable oils by high vacuum distillation at 100C. The quantitative removal of higher lactones requires relatively long periods of time at much higher temperatures. Under these conditions additional lactones may be formed by the decomposition of peroxides. Furthermore, fatty acids and other interfering substances are also distilled along with the lactones.

Liquid-liquid extraction of the oxidized oils with methanol avoids this decomposition. The free fatty acids extracted can then be removed by washing with base. Unfortunately substantial amounts of the shorter chain lactones, particularly delta lactones, are also removed by this treatment. Column or thin-layer chromatography appears, thus far, to be the best way of separating fatty acids from the lactones. The interference of nonlactonizable hydroxy fatty acids has also been studied. In this case silylation followed by TLC and GLC gives the best results.

The above techniques have been used to confirm the presence of C_5–C_{12} lactones and to extend the search for long-chain and unsaturated lactones in heated vegetable oils.

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OXIDATION OF VARIOUS LIPID SUBSTRATES WITH UNFRACTIONATED SOYBEAN AND WHEAT LIPOXIDASE

P. L. Guss, T. Richardson and M. A. Stahmann

Quantitative determinations of lipoxidase in wheat were made on five milling fractions and the whole wheat from four varieties including two hard red winter and two hard red spring wheats. Lipoxidase content decreased with increasing refinement and was the lowest in the flour fraction. In general, the mill fractions from spring wheats exhibited slightly higher lipoxidase content than the corresponding mill fractions from winter wheats.

Aqueous extracts from soybeans (variety Hawkeye) and wheat (variety Selkirk, break shorts fraction) were adjusted in concentration so that the lipoxidase activity with respect to linoleic acid was approximately the same. The diluted extracts were then tested for their ability to oxidize other lipid substrates possessing the necessary pentadiene system. Soybean lipoxidase was far more reactive toward methyl linoleate and trilinolein than wheat lipoxidase. Mono- and dilinolein were relatively unreactive in both systems although dilinolein was a better substrate in the soybean system than in the wheat. Neither system oxidized highly unsaturated digalactosyl diglycerides alone. However, the soybean extract oxidized this substrate to a small extent in the presence of catalytic amounts of linoleic acid, while under identical conditions the wheat extract remained inactive.

Polyacrylamide gel electrophoresis of the aqueous extracts of soybeans and wheat milling fractions coupled with a staining procedure specific for lipoxidase indicated 3 to 4 isoenzyme bands in the soybeans, and 2 to 4 isoenzyme bands in the wheat milling fractions. Isoenzymes may be responsible for differences in enzymatic activity on various substrates.

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THE ISOLATION AND CHARACTERIZATION OF THE NON VOLATILE COMPOUNDS FROM THERMALLY OXIDIZED TRIOLEIN

E. G. Perkins and J. A. Anfinsen

Triolein was subjected to thermal oxidation at 200C with an air flow of 0.15 ml/min/g. The oxidized triglyceride was converted to its corresponding methyl esters and the oxidized components isolated with the aid of preparatory thin-layer and gas-liquid chromatography. Six compounds were isolated in this manner and their structures determined with the aid of nuclear magnetic resonance and mass spectroscopy, as well as by supplementary chemical methods and by comparison with known compounds. These compounds were 1) 2-cyclohexyldodec-3-enoic acid, 2) a disubstituted cyclohexyl compound, 3) a mixture of 2,8,9, and 10-mono hydroxystearic acids, 4) 9,10-dihydroxystearic acid, 5) di octyl phthalate, and 6) 1-decyl-2-(dec-6-enyl)-cyclohexane. The characterization and synthesis of these compounds and their isomers will be outlined.

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MECHANISM AND STEREOCHEMISTRY OF α-OXIDATION OF FATTY ACIDS IN PLANTS

L. J. Morris, C. Hitchcock and A. T. James

The existence of biological systems capable of degrading fatty acids by α-oxidation, or one carbon at a time, has been recognized for some years. The best known of such systems in animals is the brain wherein long-chain saturated and unsaturated acids are metabolized by this mechanism to odd-number acids and to α-hydroxy acids which are incorporated into cerebrosides. This system has been extensively studied by Mead and Radin and their respective co-workers.

In plants also, α-hydroxy acids have been shown to be constituents of the cerebroside fraction, which is, however, a relatively minor component of plant lipids. However, certain seed oil triglycerides contain relatively high proportions of acids whose structures imply an α-oxidation step during their biosynthesis, notably the cyclopropenoid acid, malvalic acid, in Malvaceous oils and the series of C_{17} acetylenic acids in *Acanthosyris spinescens* seed oil. In *Pachira* and *Bombacopsis* seed oils, we have characterized α-hydroxysterculic acid as a relatively major component along with sterculic and malvalic acids. No other α-hydroxy acids were detected implying that the α-oxidation is a specific terminal step in the biosynthesis of malvalic acid.

The α-oxidation systems of such seeds have not yet been studied biochemically but degradation of fatty acids by this route in plant leaves has been studied extensively in our laboratory. The basic pathway has already been shown to be, for example: palmitic acid → α-hydroxypalmitic acid → pentadecanal → pentadecanoic acid, and so on. We have now synthesized pure D- and L-α-hydroxypalmitic acids and D- and L-α-tritiopalmitic acids and using these compounds with leaf systems in vitro we have determined the absolute configuration of the α-hydroxy acids produced, the stereochemistry of the hydroxylation reaction and the fact that further oxidation of these hydroxy intermediates is stereospecific for the L-enantiomer.

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THE INTERACTION AND STRUCTURE OF BIOLOGICALLY IMPORTANT LIPIDS IN AQUEOUS SYSTEMS

D. M. Small

Polar lipids may be classified into three major groups depending on their bulk and surface properties. 1) *Insoluble amphipaths* form stable monolayers at aqueous-air interfaces, but are insoluble in water in the bulk (e.g., triglycerides, diglycerides, cholesterol, cholesterol esters and protonated fatty acids). 2) *Swelling amphipaths* also form stable monolayers at the aqueous-air interface, but swell in water to form liquid crystals, the molecules of which remain associated and do not disperse (e.g., lecithin, cephalin, monoglycerides and lipid extracts from myelin and erythrocytes). 3) *Soluble amphipaths* have a measurable finite solubility in water (critical micelle concentration) and form unstable films. At higher concentrations soluble amphipaths form micelles and with rare exceptions, e.g., bile salts, form liquid crystals (e.g., soaps, detergents, lysolecithin, phosphatidic acid). To study the interactions of various lipids in water, ternary and quaternary phase diagrams were constructed and the individual phases found studied by a variety of techniques including x-ray diffraction, light scattering, ultracentrifugation, viscosity and surface balance. Among

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the systems studied are: lecithin-cholesterol-water; lecithin-lysolecithin-water; cephalin-cholesterol-water; bile salt-cholesterol-water; bile salt-lecithin-water; bile acid-lecithin-water; bile salt-lecithin-cholesterol-water; bile salt-soap-water and soap-fatty-acid water. A variety of crystal, liquid crystal (lamellar, hexagonal, cubic, etc.) and isotropic aqueous phases have been found. Certain insoluble amphipaths are solubilized in liquid crystal phases of swelling amphipaths and in turn these liquid crystals can be dispersed as mixed micelles by soluble amphipaths. The phase equilibria of mixtures of biologically important lipids as well as the structure and some physical characteristics of the individual phases will be discussed and correlated with certain biological phenomena.

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ROLE OF LIQUID CRYSTALLINE STRUCTURES IN LIPIDS

J. L. Ferguson and G. H. Brown

Most lipids do not pass on heating directly from a crystalline structure to an isotropic structure. They are often characterized by a number of intermediate phases ranging from the plastic crystal where the center of gravity of the molecule may rotate about one or more axes while the three-dimensional order of the crystal remains to nematic liquid crystals which have birefringent properties of crystals and yet are characterized by completely random ordering of the molecular centers. The smectic and cholesteric liquid crystalline structures are most commonly encountered in lipids. The structural characteristics of these systems will be discussed.

Of particular interest in living systems are liquid crystals which are formed by cholesteric esters and many protein materials and the two-dimensional crystals (smectic structure) formed by fatty acid derivatives. The mechanisms of energy transfer and the mechanical alignment in these liquid crystalline systems are unique and require different considerations than one finds adaptable to liquids or solids. The properties of liquid crystals which might best be associated with living systems will be discussed. These will include surface properties for catalytic processes and diffusivity.

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VOLATILE PRODUCTS FROM OXIDATION OF CIS,CIS-6,9-OCTADECADIENE

G. Fuller, R. J. Horvat, W. H. McFadden and T. H. Applewhite

The hydrocarbon analog of linoleic acid, *cis,cis*-6,9-octadecadiene, was prepared from methyl linoleate by a series of reactions. The hydrocarbon was then used as a model oxidation system and subjected to oxidation both at ambient temperature and at 185C. The volatile oxidation products were separated and analyzed by a tandem gas chromatographic-mass spectral technique. Major products from the hydrocarbon were similar at high and low temperatures, but some of the minor products were quite different, reflecting differences in mechanism and in techniques of product isolation. Volatiles included hydrocarbons, aldehydes, ketones, esters and cyclic compounds. Some mechanistic interpretation of product formation and the relationship to oxidation processes with linoleate derivatives will be discussed.

Calendar of Ladies' Program
Spring Meeting—New Orleans

Monday, May 8, 1967

Assembly and Continental Breakfast—9:00–9:30 AM.
Rendezvous Room—Roosevelt Hotel

Boat trip through canals and bayous to the Barataria area aboard cruise boat "Voyageur"—Departure 10:00 AM. Bus transportation to dock at foot of Canal Street will be provided. Picnic lunch aboard "Voyageur"—return to hotel about 2:30 PM.

Tuesday, May 9, 1967

Assembly and Continental Breakfast—9:00–9:30 AM.
Rendezvous Room—Roosevelt Hotel

Bus tour of city through the French Quarter and near uptown area, followed by brunch in the Grand Court—Pontchartrain Hotel, St. Charles Avenue near Lee Circle. Menu will feature typical Creole breakfast cuisine. Return to Roosevelt Hotel by street car. Schedule will leave afternoon free.

Wednesday, May 10, 1967

Assembly 9:00–9:30 AM. Rendezvous Room—Roosevelt Hotel, followed by short walk to Kolbs Restaurant on St. Charles Avenue near Canal. Coffee party in Kolbs' Tyrolean Room. Exhibit of research products of the Southern Regional Research Laboratory, featuring newest in cotton fabrics and wearing apparel. Return to Roosevelt Hotel will be timed to permit those who wish to attend the Awards Luncheon, 12:30 PM.

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THE KINETICS OF EPOXIDATION OF METHYL-12,13-EPOXYOLEATE WITH PERACETIC ACID

M. E. Snook, Jr., W. E. Scott and H. L. Rothbart

The kinetics of epoxidation of methyl-12,13-epoxyoleate (methyl vernolate) with peracetic acid was studied in benzene and chloroform at a variety of temperatures from 25C to 40C. The rate of disappearance of the ester and formation of the *syn* and *anti* diepoxyesters was followed with the aid of gas-liquid chromatography. Rate constants, and energies and entropies of activation were determined for the second-order disappearance of monoepoxy ester and the formation of the diepoxides. Error analysis indicates that knowledge of the initial concentrations and temperature control are extremely important for the accurate determination of the rate constants for this relatively slow reaction. Although the entropies of activation for the formation of the two isomeric diepoxides, on the order of 30 eu., are known to only one significant figure the differences in these two entropies of activation, about 1 eu., may be determined. These small differences lead to large differences in the yields of the diepoxides.

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SPECIFICITY OF A LIPASE FROM GEOTRICHUM CANDIDUM FOR CIS-9-UNSATURATION

T. A. Marks, J. G. Quinn, J. Sampugna and R. G. Jensen

A lipase from *Geotrichum candidum* did not differentiate between oleate and palmitoleate when glyceryl 1-palmitoleate 2,3-dioleate was the substrate. The enzyme released equimolar quantities of oleate and linoleate from glyceryl 1-oleate 2,3-dilinoleate and from equimolar mixtures of triolein and trilinolein. When glyceryl 1-*cis*-vaccenate 2,3-dioleate was the substrate oleate comprised 92.8 M% of the FFA. When glyceryl 1-palmitoleate 2,3-dipetroselinate was the substrate the FFA contained 94.6 M% palmitoleate. Since earlier, it was found that the lipase would not hydrolyze elaidate, the enzyme apparently has a specificity at least for *cis*-9 unsaturation and probably also for *cis*-9, *cis*-12 unsaturation.

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RATE OF REACTION OF HEXAHALOCYCLOPENTADIENES WITH LONG CHAIN MONOENES

C. K. Lyon, G. Fuller and T. H. Applewhite

Second-order rate constants were calculated for the Diels-Alder type reaction of hexachlorocyclopentadiene and hexabromocyclopentadiene with various monounsaturated fatty esters and a model alkene. Diene concentrations during reaction were determined by measurement of ultraviolet absorption at 322 mμ (hexachloro diene) or 346 mμ (hexabromo diene). The following structural effects were noted on the rate of reaction of hexachlorocyclopentadiene at 150C: 1) Terminal double bonds reacted about 12 times as fast as mid-chain double bonds; 2) The presence of a homoallylic hydroxyl group (ricinoleate esters) reduced the reaction rate about 35%; 3) *cis*-double bonds reacted 5 to 6 times as fast as *trans*-double bonds. At 130C, hexabromocyclopentadiene reacted about one half as fast with ricinoleate esters as did hexachlorocyclopentadiene. The bromo-compounds were considerably less stable to heat than the chloro-compounds. We conclude that steric factors have a pronounced effect on the rate of reaction and stability of these heavily substituted dienes.

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SOME REACTIONS OF THE STEAROYLATED ENOLIC FORM OF ACETONE

E. S. Rothman

After observing the ease of addition of acetyl hypobromite to the olefin, oleic acid, we considered that similar addition of stearoyl hypobromite to isopropenyl acetate would give rise to the highly unusual geminal diester structure. During a short-cut procedural try using commercially available isopropenyl acetate and stearic acid with a trace of acid catalysis we found that acid-ester interchange occurred instead to give the new enol ester, isopropenyl stearate. Isopropenyl stearate is a powerful stearoylating agent at elevated temperatures under conditions of acid-catalysis and performs such unusual stearoylations as that of N-butylstearamide and maleiimide. Extension of these principles to dioic acids gave diisopropenyl esters which are potential polymer-formers. Thus diisopropenyl sebacate reacts with N,N'-dimethylazelaiamide to form a linear polyimide, and with sucrose to form a three-dimensional network polymer. There is evidence to believe that the effective causative, intermediary agent is hexadecylketene. We have prepared and characterized the cyclobutanedione and four-membered-ring-lactone dimers supporting this conclusion. In situ preparation of hexadecylketene in the presence of hydroxylamine has enabled the preparation of the previously unknown di- and trihydroxamic acids. We have also observed a rearrangement of isopropenyl stearate to heneicosane-2,4-dione.

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THE ROLE OF DETERGENT IN AUTOMATIC DISHWASHING PERFORMANCE

F. W. Gray, V. J. Richter and R. C. Odioso

The ability of automatic dishwashing products to perform satisfactorily in mechanical machines is dependent upon a number of factors, including machine design and composition of product. Although most leading dishwashers are highly satisfactory for food soil removal, the detergent composition and its usage can affect performance characteristics.

In this paper, the objectionable tendency of automatic dishwashing detergent compositions to cause glassware spotting or filming, fading of fine china decoration, and silverware tarnishing is considered. The means to minimize these deficiencies are presented.

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THE EFFECT OF WATER HARDNESS ON DETERGENCY

Sadao Kakegawa, Michio Aoki and Haruhiko Argai

The effect of ionic or nonionic detergents, and builders on oil removal efficiency in hard water has been studied. As ionic detergent (sodium dodecyl benzene sulfonate), maximum oil removal efficiency

at same water hardness for given oil soil was found. But, in case of nonionic detergent (polyoxyethylene (10) nonyl phenyl ether), it was not influenced on oil removal efficiency by water hardness.

The action of sodium tripolyphosphate (STPP) on oil removal efficiency in hard water was that of softening the water. On the other hand it is interesting to note that the action of STPP on oil removal efficiency for some oil soil was increased. As sodium sulfate, it was not remarkable effect on oil removal efficiency.

The relationship between the oil removal efficiency and the dispersion of oil was discussed.

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A DETERGENCY TEST BASED ON RAPID AGING OF UNREMOVED SEBUM

W. G. Spangler, R. C. Roga and H. D. Cross, III

A laboratory screening test has been developed for screening detergent compositions with respect to the removal of sebum soil in the absence of particulate soil. The fabrics are uniformly soiled with an aqueous emulsion of synthetic sebum and then laundered under controlled conditions in a Tergotometer. The unremoved soil is rapidly aged and the resulting yellowness is measured instrumentally.

This test can be run in a minimum amount of time and with a minimum amount of equipment. It enables one to check many variables, (such as sequestering capacity, temperature effects, brightener buildup under soiled conditions, etc.) on various fabrics, with or without special treatments. The results are in terms of yellowness which is recognized by the housewife and are not a measurement of total soil removal. These values, when combined with grayness values derived from the sebum airborne test are good prognosticators of practical performance.

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THE DISTINCTION BETWEEN DEPOSITION AND REDEPOSITION OF SOIL

A. R. Martin and W. H. Smith

A method for testing dry-cleaning detergents for their ability to inhibit soil redeposition is described. It involves the measurement of soil transfer from a dirty to a clean fabric. The usual practice of using suspensions of a model soil in a detergent solution is a soil *deposition* test and does not give comparable results to the soil *redeposition* test described here.

Hensley has already attacked the validity of deposition tests to measure redeposition in aqueous detergent solutions, and our results confirm Hensley's conclusion. The major argument against redeposition tests in the past has been that they do not permit two detergents to be compared at the same soil concentration in the suspension and therefore they "stack the cards" against the better detergent because soil must be removed before it can be redeposited. Our results refute this argument and show that detergents exhibiting low soil removal invariably show high graying and vice versa. These two qualities either go hand-in-hand or they are merely two aspects of the same quality.

A possible explanation for the difference between the two test procedures is that the degree of dispersion of soil is much greater in the redeposition test.

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EFFECTIVE BLEACHING WITH SODIUM PERBORATE

A. H. Gilbert and F. K. Rubin

Sodium perborate has been known for a long time as a mild, but fairly effective, bleach at temperatures exceeding 60C. These washing conditions are prevalent in many European countries, and the United Kingdom, and consequently sodium perborate is found in a great many of their detergent powders. Much lower washing temperatures, concentrations and times are common in the United States than are used in Europe, and this negates the use of sodium perborate as an oxidizing agent unless it is activated in some way.

Activation of sodium perborate falls into two distinct classes: a) metal activation and b) potential peracid activation. Both methods will be discussed. Each works via a different mechanism.

Heavy metal catalysis is difficult to control but under ideal conditions can be remarkably effective.

Peracid precusors are much easier to control in solution, but tend to be somewhat unstable in the product. The structure, mechanism, safety, use properties and stability of representative materials will be discussed.

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THERMAL ANALYSIS OF ODD AND EVEN SODIUM SOAPS AND THEIR BINARY MIXTURES

H. L. Spier

In recent years a lot of information has been assembled about the structural behavior of anhydrous even-chained sodium soaps. Progress in the development of thermal analyzers permitted a rapid determination of enthalpy and transition points of small quantities of very pure substances. Also the construction of phase diagram of binary mixtures was simplified.

With the advent of synthetic fatty acids, which differ from the components of natural fats in that apart from the even chains, odd straight and branched fatty acid chains are present, it was thought necessary to apply this technique to odd and even straight chain sodium soaps, which were purified for this investigation in the range from C_{11}–C_{19}.

Measurement of the transition points, from room temperature up to the melting point as a function of the chain length, disclosed some transitions hitherto not mentioned in the literature.

The enthalpy of the transitions subwaxy/waxy and subneat/neat clearly alternated with odd and even chains; whereas, for the other transitions this kind of alternation was either absent or difficult to detect. The consequences of this behavior will be discussed.

Finally we were able to show that for a number of binary systems in this range of carbon chains almost complete miscibility exists from room temperature up to the melting point.

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SURFACE ACTIVITY OF SODIUM SALTS OF ALPHA-SULFO FATTY ESTERS: THE AIR/WATER INTERFACE

E. A. Boucher, T. M. Grinchuk and A. C. Zettlemoyer

Adsorption at the air/water interface of the four esters sodium hexyl α-sulfopelorgonate ($C_7H_{15}CH(SO_3Na)COOC_6H_{13}$), sodium heptyl α-sulfopelorgonate ($C_7H_{15}CH(SO_3Na)COOC_7H_{15}$), sodium methyl α-sulfomyristate ($C_{12}H_{25}CH(SO_3Na)COOCH_3$) and sodium methyl α-sulfopalmitate ($C_{14}H_{29}CH(SO_3Na)COOCH_3$) has been investigated.

The amount of surfactant adsorbed Γ at the surface of an aqueous solution of surfactant is given by the Gibbs equation in the form

$$d\gamma = -RT\Gamma d \ln c, \qquad [1]$$

where γ is the surface tension and c is the molarity of surfactant ions in solution of constant counterion (Na^+) concentration. Surface tensions were determined using the drop volume method.

Surfactant concentrations were used with counterion concentrations of 0.01 and 0.04 N. Plots of γ against ln c are in all cases linear at surfactant concentrations below the critical micelle concentrations indicating that the amount of surfactant adsorbed is constant. The reciprocal of Γ is the area occupied at the surface by a surfactant ion. Values observed are in the range 44 to 59 Å/surfactant anion and are in each case consistent with close-packed adsorption with the carbon chains oriented normal to the surface. There is no appreciable dependence of cross-sectional areas upon counterion concentration.

Increase in counterion ion concentration from 0.01 N to 0.04 N results in a decrease in surface tension, for a given surfactant concentration, of about 7.5 dyne/cm. The effect of change in counterion concentration upon γ can be successfully predicted by using bulk activity coefficients obtained from the Debye-Hückel theory.

For a solution of given concentration, the surface tension for $C_7H_{15}CH(SO_3Na)COOC_8H_{13}$ is about 10 dyne/cm greater than that for $C_7H_{15}CH(SO_3Na)COOC_7H_{15}$, i.e., an increase of one $-CH_2$ group. The increase of two $-CH_2$ groups in going from $C_{12}H_{25}CH(SO_3Na)COOCH_3$ to $C_{14}H_{29}CH(SO_3Na)COOCH_3$ results in a surface tension decrease of about 18 dyne/cm, or almost double that for the addition of one methylene group. This is a good indication that the carbon chains of all four compounds play similar roles at the water/air interface.

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DETERMINING BOTH ORGANIC AND SULFURIC ACID CONTENT BY A SINGLE DIFFERENTIAL TITRATION

William Carasik, Marvin Mausner and Gerald Spiegelman

The need for determining the individual content levels of organic and sulfuric acids in sulfonic acids and sulfate esters is recognized because of the marked effect these components have on end properties. Present methods require separate analysis to determine the amount of each acid.

Application of a single differential nonaqueous titration to the analysis of sulfonic acids and sulfate esters containing sulfuric acid is presented.

A titration of these materials in water measures their total acidity. Analysis of individual acids is difficult to make because of the leveling effect of water. In solvent, however, which diminishes the leveling effect, a titration can measure the hydrogen sulfate ion, which is equivalent in content to the sulfuric acid, at a second end point. The organic acid portion is then determined by subtraction.

Analysis by a single differential, nonaqueous titration, using tetrabutylammonium hydroxide (TBAH) as the titrant, is discussed step by step. It is suggested as a faster means of determining individual acid contents in sulfonic acids and sulfate esters.

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A DETERMINATION OF THE MOLAR ABSORPTION COEFFICIENTS OF THE COBALT THIOCYANATE COMPLEXES OF ETHYLENE OXIDE ADDUCTS OF NONYLPHENOL

N. T. Crabb and H. E. Persinger

Cobalt thiocyanate is used as a reagent to determine traces of polyoxyethylene nonionic surfactants. The minimum number of detectable ethylene oxide units attached to a hydrophobe has been in conjecture for some time due to the unavailability of samples of the pure individual ethylene oxide adducts.

The individual ethylene oxide adducts of high purity were obtained by liquid column chromatography on silica gel. The mixed solvent was similar to a system developed for thin-layer chromatography. The composition of the separated isomers has been determined by infrared, ultraviolet, nuclear magnetic resonance, and mass spectroscopy.

Molar absorption coefficients have been obtained for the individual cobalt thiocyanate complexes both in benzene and chloroform. For the low molecular weight adducts studied, the efficiency of color development and extraction into the organic phase has been found to be dependent on the concentration of the cobalt thiocyanate reagent. A saturated aqueous solution of cobalt thiocyanate was found to be preferable and benzene was found to be a more reliable extractant than chloroform.

Molar absorption coefficients do not vary linearly with chain length at lower molecular weights and the minimum number of ethylene oxide units that will form a reliable color was found to be three.

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A SURVEY OF THE CONJUGATED FATTY ACIDS OF SEED OILS

C. Y. Hopkins and Mary J. Chisholm

Rapid progress has been made in the last few years in the isolation and identification of fatty acids with conjugated unsaturation, most of them found in seed oils. This interesting group of about 30 individual acids is reviewed with emphasis on their detection, isolation, and structure determination. The pattern of occurrence in the seed oils of ten or more plant families is shown. There are instances of closely-related plant species producing different positional and geometric isomers of conjugated acids of the same chain length. The formation of these isomeric acids, particularly the hydroxydiene acids discovered by the Peoria research group and others, provides a basis for theories of the general route of biosynthesis of unsaturated acids. The theories are discussed briefly.

The natural conjugated acids contain such groupings as conjugated diene, triene, tetraene, various combinations of olefinic and acetylenic groups, and oxygenated derivatives of most of them. Some reactions and derivatives of the acids are described. The newer techniques of fat chemistry are extremely useful in these studies.

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OCCURRENCE, ISOLATION AND DETERMINATION OF THE CYCLOPROPENE FATTY ACIDS

H. W. Kircher

The 1,2 disubstituted cyclopropene ring occurs in two fatty acids that are found in the seed oils of numerous plants of the order Malvales. The development of a red color when such oils are heated with carbon disulfide and sulfur (Halphen test) and the thermal instability of *Sterculia foetida* oil and the acids derived from it first suggested an unusual structure for these acids. The discovery of their biological activity subsequently stimulated many investigations.

The isolation of the C_{19} acid, sterculic acid, from *S. foetida* oil by urea clathration and low temperature crystallizations was facilitated by its relatively high mp (18C) and concentration in the oil (~50%). Later work showed that the more stable ester, methyl sterculate, could be isolated in good yield in the same fashion.

The isolation of the C_{18} acid, malvalic acid, is considerably more difficult. Its lower mp (10C), lower concentration in oils and the presence of larger quantities of oleate and linoleate in the same oils require extensive chromatography and recrystallization. Sterculic acid and its derivatives, therefore, have been used almost exclusively for chemical and biochemical studies. The relative reactivities of sterculic and malvalic in chemical (Halphen test) and biochemical systems (physiological effects in hens) are still an open question.

A number of analytical methods have been developed for the cyclopropene acids, of which three have been most widely used; the Halphen test, HBr titration, and gas chromatography. The Halphen test is the most sensitive and is preferred when low concentrations of the acids are encountered. It is an empirical method and requires carefully standardized conditions and a primary standard. The HBr titration is a stoichiometric method that has undergone extensive modification to remove interfering compounds. It is the source of a current active controversy concerning the purity of "pure" methyl sterculate. Gas chromatography of the methyl esters of malvalic and sterculic acids leads to their destruction and incomplete elution from columns. The degradation products of the two esters overlap the peaks of linoleate and linolenate, respectively. Modifications of the gas chromatographic method to eliminate these problems have included hydrogenation of the esters, hydrogenation after pyrolysis and after column chromatography on silver nitrate-silica gel, and treatment with methyl mercaptan and methanolic silver nitrate.

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NATURALLY OCCURRING EPOXY OILS

C. F. Krewson

Fatty acids which contain oxirane functional groups are widely distributed in nature and have usually been found in the seeds of plants from different families growing in moderate climates. Some of the plants which have been found to produce such acids are *Vernonia anthelmintica, V. colorata, Tragopogon porrifolius, Chrysanthemum coronarium* (Compositae); *Euphorbia lagascae, Cephalocroton cordofanus, C. peuschelii* (Euphorbiaceae); *Clarkia elegans* (Onagraceae); *Hibiscus esculentus, H. cannabinus, Malope trifida* (Malvaceae); *Camelina sativa* (Cruciferae); Oroju oil (Oleaceae) and others to be included. The amount of epoxy derivatives in seed oils varies widely and ranges from traces up to 70–75%. In some cases dihydroxy compounds are also present in the oils in varying quantities depending upon how the oils are prepared. These dihydroxy acids are believed to be precursors of the epoxy derivatives in maturing seeds. Enzymes present in some seeds have the ability to reconvert epoxides to dihydroxy compounds as soon as the seeds are crushed.

A number of methods are used to determine the oxirane content of seed oils which include thin-layer and gas-liquid chromatographic techniques. The HBr method of Durbetaki, or some variation thereof such as the Jay Method, is a useful procedure in common practice although some naturally occurring substances such as dimorphecholic acid and cyclopropenoid acids do cause interference in this technique.

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PLANT GLYCOLIPIDS

H. E. Carter, Alemka Kisic and Judith Koob

Some years ago we devised a procedure for obtaining an "inositol lipid" fraction from commercial plant phosphatide preparations. Methods have now been developed for the separation of the inositol lipid mixture into its major components. They key to this development was the recognition that the magnesium and calcium counter-ions were binding phosphatidyl inositol (and possibly other materials) to the phytoglycolipids. Replacement of the divalent cations of the inositol lipid fraction by sodium or potassium by use of a chelating resin made possible for the first time a clean separation of glycerophosphatides from the phytoglycolipids. In a hexane-butanol-methanol-water system phosphatidyl inositol (Na^+) distributes into the alcohol phase and the phytoglycolipids into the hexane phase. The phytoglycolipid fraction from flax has now been found to contain two types of ceramide phosphate-inositol oligosaccharides. The previously unrecognized material yields a higher oligosaccharide containing inositol, glucuronic acid, galactose, arabinose, fucose and mannose but devoid of glucosamine which is present in the previously characterized phytoglycolipid. These two types of phytoglycolipids can be separated by CCD in a butanolacetic acid-water system. The hexosamine-containing species distribute into the alcohol phase; the higher oligosaccharide (hexosamine-free) phytoglycolipid distributes into the aqueous phase. Further purification of the latter was achieved by Sephadex gel filtration. Recent studies disclosing the presence of phytoglycolipids in plant leaves will be presented.

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STUDIES ON THE STRUCTURES OF THE TRIGLYCERIDES OF BOVINE MILK SERUM: SHORT CHAIN TRIGLYCERIDES

L. J. Nutter and O. S. Privett

The structures of the triglycerides containing short chain fatty acids of bovine milk serum were determined by a combination of argentation-TLC and liquid-liquid partition chromatography. Some 168 different molecular species of triglycerides containing short chain acids were detected on the basis of a difference in degree of unsaturation or carbon number exclusive of positional isomers. All species present in amounts greater than the order of 0.01% were determined. The short chain fatty acids were found to be widely distributed among the triglycerides but significant amounts of dimolecular species containing short chain acids were also detected. Although previous evidences for an interrelationship in the biosynthesis of triglycerides and lecithin were observed, comparison of the fatty acid and molecular species composition did not reveal a direct relationship between the synthesis of these compounds.

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LIPOGENESIS IN CELL-FREE PREPARATIONS OF LACTATING MAMMARY GLAND OF THE MONGOLIAN GERBIL

J. G. Coniglio

Lipogenesis is being investigated in cell-free preparations of lactating mammary gland of the Mongolian gerbil for eventual comparison with other species. Incubation of such preparations with factors necessary for lipogenesis in rat mammary gland preparations [Lipids *1*, 76 (1966)] resulted in incorporation of radioactivity into various fatty acids depending on the labeled substrate used. Use of ^{14}C-malonyl CoA with whole homogenates resulted in predominant labeling in 14:0 and 16:0. A small percentage of the incorporated label was in low molecular weight compounds. These results are similar to those obtained with whole homogenates of rat lactating mammary gland. Incubation with ^{14}C-acetate resulted in predominant incorporation into low molecular weight compounds. About half of the counts incorporated into longer chain fatty acids (>10:0) was in materials having retention times greater than 18:1. One of these had a retention time identical with that of 18:2 and another with 20:2. This is in contrast to rat mammary gland in which only a small proportion of the ^{14}C incorporated into longer chain fatty acids was in compounds of retention time greater than 18:1. Similar findings were indicated with use of ^{14}C-acetyl CoA as substrate. Chemical identification of these materials is in progress.

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AVIDIN SENSITIVITY AND ELONGATION OF FATTY ACID BY RAT LIVER MITOCHONDRIA

J. P. Jordan, E. B. Harris and Carolyn W. Musser

Using an acetone powder preparation from rat liver mitochondria, a study was made of the fatty acids formed in the presence and absence of avidin. Comparing synthesis in the presence of avidin with that occurring in the absence of any nonenzyme protein, inhibition greater than 50% is seldom demonstrable. In the presence of high levels of avidin, there appears to be stimulation of mitochondrial fatty acid synthesis. Such stimulation can be mimicked with comparable levels of bovine serum albumin. Comparing synthesis of fatty acids in the presence of avidin with that occurring in the presence of equivalent amounts of bovine serum albumin, inhibition is almost complete. Several other lines of evidence suggest the role of malonyl-CoA in the elongation of fatty acids in this system. The types of products formed in the presence and absence of avidin are essentially the same; the ratio of C^{14} in the carboxyl carbon compared to that in all the carbon atoms in the fatty acid products (CC:TC) is approximately the same in the presence or absence of avidin (this applies to the saturated, monounsaturated and polyunsaturated acids); acetyl-1-C^{14}-CoA and malonyl-1,3-C^{14}-CoA are incorporated into the same products with the

same CC:TC ratio but with malonyl-CoA incorporation being slightly greater; malonyl-1,3-C^{14}-CoA is incorporated into the same products with the same CC:TC ratio even in the presence of acetyl-CoA. In all of these instances, the CC:TC ratio ranges from 1:1.8 to 1:3.0.

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DETERMINATION AND IMPORTANCE OF NON-ESTERIFIED FATTY ACIDS IN BLOOD

H. L. Davis

The "free fatty acids" constitute only a small fraction of the blood lipids, but they are the most labile. Common marked fluctuations in plasma FFA (NEFA, UFA, IFA) are associated with significant alterations in the colloidal stability of fibrinogen, and in the coagulability of circulating blood. At pH 7.4 the major portions of the NEFA are present as *soaps*. All soaps are procoagulant, and especially effective in lowering clot times and tending to produce thromboembolic episodes are the soaps of long-chain saturated NEFA [JAOCS *43*, 114A (1966)].

Determination of NEFA concentrations in serum has been facilitated by modifications of the Dole-Gordon-*Trout* procedure [J. Lipid Res. *1*, 199 (1960)]. Use of isopropanol reduces the fading of end-points observed with ethanol, and thymolphthalein (colorless 9.3–10.5 blue) permits titrations of the acids to pH 9.6–9.8, a more appropriate end-point. The procedure has been validated by tests with fatty acids or with soaps, alone or added to serum samples. Potassium acid phthalate is a convenient standard for alkali, and KOH appears preferable to NaOH. Automation of such titrations has been described.

In stress episodes mobilization of NEFA from adipose stores is the usual source of emergency energy. As these soaps pass through plasma, they produce a transient hypercoagulability, and may be conducive to impaired blood flow in heart, brain, lungs, kidneys, etc. Such effects have been studied in women in childbirth, in surgical patients, and in many dogs under a variety of stresses. Many other instances will be shown as reliable and convenient procedures facilitate determinations of plasma NEFA. Various research projects demonstrate important effects of these fluctuations in NEFA levels on other phenomena.

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BIOSYNTHESIS OF OLEIC ACID IN SOYBEANS

P. J. Thomas, Ami Dolev, F. L. Little and H. J. Dutton

Fatty acid synthesis in green soybeans was studied by incubating slices of freshly picked beans with acetate-1-C-14 and then measuring the isotopic carbon incorporated into the fatty acids as a function of time. The specific activity of the polar lipid fractions leveled off after 2 hr, at which time it was equal to 20 times the specific activity of the neutral lipid fraction; the neutral lipids showed some additional increase up to 3 hr. In the fatty acids of the polar lipids, the relative specific activity in oleic acid went up sharply, reaching its highest level within ¼ hr and then decreasing toward unity over the 3-hr period. At the same time, the relative specific activity of linoleic acid gradually increased toward unity. The specific activity of stearic acid was always less than the specific activity of the total lipid. High activity was consistently observed in fractions having the chromatographic properties of lauric, myristic and palmitoleic acids, although corresponding mass peaks were not always observed. Since a high specific activity was not recorded for palmitic acid, we believe that initial desaturation occurs on a 12- or 14-carbon chain, followed by chain elongation to hexadecenoic and oleic acids. These data are consistent with the hypothesis that stearic acid is not a precursor of oleic acid.

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THE EFFECT OF TEMPERATURE AND SUBSTRATE ON THE PRODUCTION OF AFLATOXIN

R. Y. Mayne, A. O. Franz, Jr. and L. A. Goldblatt

Five strains of *Aspergillus flavus*, selected for known variation in ability to produce aflatoxins, and one of *A. parasiticus* were grown on three substrates (autoclaved, moistened shredded wheat, undelintered cottonseed, and split peanut kernels with skins removed) at four different temperatures for 7 days. The amount of growth of mold was observed and aflatoxin assays were made of chloroform extracts of the whole cultures. As expected from the choice of strains, they varied greatly in the quantity and kinds of aflatoxins produced even when on the same kind of substrate and at the same temperature. Some of the strains elaborated very little aflatoxin G, but all produced some of this aflatoxin under at least one set of conditions. On shredded wheat and peanut kernels, elaboration of aflatoxin (when it was produced) was less at 37C than at the three lower temperatures (20, 24.5, and 30C) although mold growth was as good or better at this temperature. On cottonseed, although one strain produced no aflatoxin, more aflatoxin was produced at the two higher temperatures, and this was accompanied by a corresponding increase in mold growth. For the three strains of *A. flavus* which consistently produced aflatoxin G, the ratio of aflatoxin G to B produced was generally highest at 24.5C. The effect of temperature on production of aflatoxins was dependent more on the substrate than on the strain of *A. flavus*.

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THE LIPIDS OF NEUROSPORA CRASSA

J. G. Hamilton and Ernesto Barbosa

The cytoplasmic lipids of wild type and the osmotic mutant of *Neurospora crassa* were compared. The lipids were separated into classes by silicic acid and DEAE cellulose column chromatography. The lipids classes were assayed by silica gel glass paper chromatography (GPC) for purity and further purifications were accomplished with GPC. The fatty acids of each class were assayed by gas chromatography (GLC). The total amount of lipids for 3-day old cultures was 9% of the dry weight of the cytoplasm in wild type and somewhat higher in the osmotic mutant. The total fatty acid containing lipids consisted of approximately 3% ergosterol esters, 45% triglycerides, 10% diglycerides, 12% phosphatidyl choline, 9% of an unknown polar lipid. Linoleic acid was the dominant fatty acid representing about 40% of the total fatty acids. Linolenic, oleic and palmitic acid represented from 10 to 20% of the total lipids. Stearic, palmitoleic and myristic were present in small amounts. All of the fatty acids were found in all lipid classes. Lecithin contained about 85% unsaturated acids, whereas phosphatidyl ethanolamine, triglycerides and diglycerides contained relatively large amounts of palmitic acid. Ergosterol esters contained more unsaturated fatty acids than phosphatidyl ethanolamine but less than lecithin. In general, a relatively larger percentage of linoleic acid and a smaller percentage of linolenic acid was found in the osmotic mutant of *Neurospora crassa* than in

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the wild type. Ergosterol, lanosterol and ergosterol peroxide were present in small amounts.

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THE EFFECT OF ULTRA-HIGH FREQUENCY SOUND WAVES ON LIPASE ACTIVITY

L. P. Goodman and L. R. Dugan, Jr.

Crude pork pancreatic lipase was subjected to ultra-high frequency sound waves from 1–30 min using a Branson model S-75 "Sonifier" at a frequency of 20,000 cps and a setting of 8 amperes. Sonication was carried out at temperatures of 10, 30, 40, and 50C. The sonicated enzyme was used for the subsequent hydrolysis of an olive oil emulsion at 38C. The rate of hydrolysis was determined by continuous titration with 0.1 N NaOH to pH 9.0. Sonication affected the original activity of the lipase very little even after treatment for 30 min at 10 and 20C. However, at 40 and 50C, there was a progressive decrease in lipase activity as sonication time was increased.

The lipase preparation and olive oil-substrate were sonicated at 38C for 4.5 min and the lipolysis compared to that in another sample which was stirred at 600 rpm for 4.5 min. The degree of hydrolysis was determined, after stopping the reaction, by titration with 0.1 N NaOH of the fatty acids liberated. Sonication increased the rate of hydrolysis by a factor of 2.7.

A tripalmitin emulsion, containing methyl myristate as a carrier, was sonicated with lipase for 9.5 min at 45C. The degree of hydrolysis was compared to a duplicate sample stirred at 600 rpm. No hydrolysis of tripalmitin occurred during the stirring experiment while 14 μequivalents of palmitic acid were liberated during sonication.

The increased hydrolysis was attributed to the fact that a better emulsion was obtained during the lipolysis of the sonicated samples and to rapid renewal of surfaces for new complex formation.

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TRITIUM AND C^{14} COUNTING IN TISSUE SAMPLES USING LIQUID SCINTILLATION METHOD

C. K. Parekh and E. Eigen

While studying the incorporation of vitamin D_3–H^3 in duodenal tissue, the method of Kelly [Anal. Biochem. *2*, 267 (1961)] gave low recoveries.

The method was improved upon in the following manner. After combustion, the bottom of the flask was cooled in a dry ice-acetone bath while the top was irradiated with an infrared lamp. This led to a more efficient condensation of the water produced during combustion. The liquid scintillation counting data indicated that the use of this procedure resulted in at least 95% recovery of the tritiated water. These values were highly reproducible.

In instances where glycine-C^{14}-u.l. was used to study protein synthesis in skin, liver and muscles of the rat, the tissue samples were dissolved in NCR at 55C for 24 hr. Following addition of the scintillation fluid, the samples were counted in a liquid scintillation counter. NCR base was superior to hyamine in solubilizing tissue samples and it also gave higher counting efficiencies.

The described methods yielded reproducible H^3 and C^{14} data in tissue samples with high recovery of both isotopes.

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PROTEIN INTERACTION WITH PROTEIN MONOLAYERS

J. D. Arnold

The direction and strength of intermolecular forces at an air water or oil water interface is such that many proteins in the interface are distorted in structure. This involves substantial changes in solubility and cross sectional area. Many of the changes can be accounted for by rupture of the secondary and tertiary bonds and are often irreversible. The hydrophilic groups of the protein will be concentrated in the aqueous phase and participate in interactions with normal proteins in the supporting solution. It can be shown that certain types of interaction between these hydrophilic groups of a protein monofilm and a soluble protein are dependent on the interfacial pressure, that they are sensitive to a small (one or more amino acid) change in structure of the protein. Evidence will be given that they are related to certain antigen-antibody type reactions between molecules in three-dimensional systems. Since many proteins in vivo are exposed to oil-water and air-water interfaces, this laboratory model may have physiologic as well as chemical significance.

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SOAP FILMS AND SOME FUNDAMENTALS OF THIN LIQUID FILMS

K. J. Mysels

Factors influencing the formation and behavior of a stable thin liquid film will be examined. Some of these are common to water-in-air, water-in-oil, oil-in-air, and oil-in-water films, whereas others are different for each system. The common ones are often most easily studied in the water-in-air system, i.e., the ordinary soap films. Some of the experimental methods used, theoretical problems encountered, and results obtained in the study of these latter films will be reviewed with emphasis on those that seem to be also applicable to the oil-in-water films. Among topics covered will be the problem of optical measurement of thickness, the effect of surface viscosity and rigidity, motions due to gravity convection and to marginal regeneration, the attractive and repulsive forces between surfaces, the free energy-thickness diagram, and the determination of the free energy stabilizing an equilibrium film by contact angle measurements.

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THE DOMESTIC TUNG INDUSTRY TODAY

G. F. Potter

The tung tree (*Aleurites fordii*), which was introduced to the United States from China in 1904, requires a moderately acid soil, an annual rainfall of 45 to 70 in., and a long hot summer, yet must have a period of cold weather in winter. These factors limit its culture in North America to a narrow belt along the Gulf of Mexico from Florida to Texas. Many early plantings failed because of lack of information, but production research has determined soil adaptations and cultural and fertilizer requirements. Terrace systems adaptable to orchards have been worked out, and new varieties that have high productivity and bear fruit of high oil content have been originated.

The majority of the orchards presently consist of miscellaneous seedlings that are about 30 years of age. By replacing these with orchards of new varieties, on suitable soil, and by following recommended practices, growers can produce oil at lower costs than previously. However, crop loss from frost is still a serious problem. Labor costs, especially those of harvesting, are expected to rise in the near future, but machine harvesting is now a reality.

During World War II, the government requisitioned the entire domestic production of tung oil for military purposes, and regular customers had to turn to other products. This market has not yet been fully won back, and growers look to utilization research to widen and improve the market.

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TUNG OIL RESEARCH AND DEVELOPMENT

B. M. Kopacz

This paper reviews utilization research on tung oil conducted at the Southern Regional Research Laboratory in New Orleans. The tung oil program began with the inception of the Laboratory in 1940 and was treminated in July 1966. Utilization research on tung oil has been concerned with exploratory chemical reactions to develop new or improved industrial products, and the development of improved paint formulations. The emphasis of the domestic and foreign extramural program supervised by this Laboratory has been aimed at the practical applications of tung oil in plastics and the effects of heat on tung oil. Accomplishments over the past few years in these areas are described and some projections for future research needs proposed.

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DERIVATIVES OF ELEOSTEARIC ACID

Shelby F. Thames, J. S. Long, Oliver Smith, S. J. Jen and J. M. Evans

The chemistry of eleostearic acid, the principal component of tung oil, has received relatively little attention in the past. Concern has centered on the use of tung oil in coatings' applications—rightfully so since tung oil offers advantages not obtainable with other oils. However, our keen interest in eleostearic acid has prompted us to explore not only new polymeric resins containing eleostearic acid but also the potential of its derivatives. Our present research efforts are centered on reactions at (1) the terminal carboxyl group and (2) the conjugated triene system of eleostearic acid. During our investigation we have studied such reaction parameters as temperature, atmospheric conditions and pH and their effects on product isolation and purification. The spectrum of derivatives to be described includes the synthesis and evaluation of novel sulfonylisocyanate-based resins, copolymers whose composition can be varied to alter water solubility, derivatives of dimeric eleostearic acid, and monomeric products containing various moieties generally concluded as necessary for physiological activity.

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TUNG OIL AND ELECTRODEPOSITION A NEW CONCEPT

R. O. Austin, Herschel Bullock and Thomas McCraney

A polymer has been developed which is almost completely soluble in water; it contains all of the components necessary to deposit satisfactorily, and it leaves no undesirable residues in the bath.

The polymer carries a negative charge on the outside mass. When deposited, a negative charge is given up and a chemical reaction takes place within the polymer molecule probably simultaneously.

This polymer contains no soap and does not require coupling and emulsion agents or defoamers to produce a satisfactory electrodeposited film on current carrying surfaces such as steel, phosphatized steel, aluminum, and copper.

Wrinkle film patterns can be produced on steel and phosphatized steel by increasing the potential about 25 volts over good plating practices.

Baking temperatures at 275F for 30 min produce a Sward film hardness of 24. Clear baked films on copper are impervious to 95% denatured alcohol and toluene for 31 days and six months in distilled water, the length of exposure when partly submerged. At a baking temperature of 390F for 20 min, the film is also impervious to 1% potassium hydroxide solution for 31 days, the length of the test.

Films containing tribasic lead chromosilicate on phosphatized steel will remain intact for up to 500 hr in the salt fog cabinet.

Reaction mechanisms are discussed.

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RESINS CONTAINING ELEOSTEARIC ACID AND PROTECTIVE COATING FILMS FROM THEM THAT DO NOT BLISTER AND PEEL

Shelby Thames, J. S. Long and Oliver Smith

Long oil alkyd resins containing eleostearic acid up to 20% of the fatty acid content, the balance being nonconjugated fatty acids such as tall, soya or other semidrying or drying fatty acids, have been synthesized by solvent process direct esterification yielding resins of viscosity less than Z Gardner.

These have then been blown with air at 50–70C to a viscosity of Z-6 to promote polymerization of the eleostearic acid chains and partial oxidation of the nonconjugated ones. This oxidation causes some "preshrinking" of the films and confers other advantages in color, drying time, adhesion, and per cent elongation.

After 18 weeks' exposure on the roof at 45°South, the percentage of oxygen in films containing 20% eleostearic acid was 4.5% less than the corresponding films in which all of the fatty acids were tall oil. This indicates that the eleostearic acid was *polymerized* by the catalytic effect of the peroxides formed in the oxidation process on the nonconjugated fatty acids. These peroxides were identified. The lower dergee of oxidation indicates a much higher life expectancy of tung oil.

When pigmented with only nonalkaline pigments and no pigments that furnish appreciable concentrations of water soluble ions, the resultant coating films absorb less than 2.5% of water by weight during immersion and do not blister and peel on the Eagle Picher Blister Box—even on long periods of test.

Uralkyd hybrids containing up to 50% of eleostearic acid have been similarly synthesized but viscosity is higher. Partial oxidation can be achieved in xylene using Pt/C catalyst. Films from these uralkyds also show low water absorption and no blistering on the blister box. This successful solving of a major problem promises a great resurgence in use of tung oil.

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AN ION EXCHANGE-BASED PROCEDURE FOR THE ANALYSIS OF COMMERCIAL HOUSEHOLD DETERGENTS

A. E. O'Donnell

A procedure for the determination of organic components in light and heavy-duty household detergents is described. Principal separations of components are made according to ionic type by use of ion exchange resins. The main advantage of such separations compared to those made by the usual solvent partitioning procedures is that of completeness. Cations initially present, together with an urea present, are removed wtih an H^+ form cation exchanger and subsequently are displaced from the exchanger. Portions of the solution of organic acids from the exchanger are analyzed for anionic surfactant content and strong and weak acidity in conventional ways. The main portion is treated with an OH^- form anion exchanger to remove anions and yield the nonionics. The anions are displaced from the resin with hydrocholric acid and further separated and determined using conventional procedures. Spectroscopic, gas-chromatographic, and chemical techniques are used to characterize the various separated components. Determination of anionic surfactants by the bromocresol green or methylene blue indicator titration techniques before and after ion exchange or acid-catalyzed hydrolysis provides measurements of cationic actives or distinguishes between sulfonate and sulfate ester type anionic actives. Results of the analyses of a number of commercial household detergents are given.

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COLUMN CHROMATOGRAPHY OF SURFACTANT MIXTURES USING THE EVAPORATIVE ANALYZER

Edmund C. Steinle, Jr.

Initial results are reported on the application of a column chromatography-universal detector system to the analysis of surfactant mixtures containing components not amenable to gas chromatography because of nonvolatility or instability. The goal has been to analyze such mixtures with the convenience and speed of gas chromatographic procedures. The first system studied separates mixtures into classes of compounds according to differences in polarity. A solvent gradient is used to elute a sample of 5 to 25 mg total solids from a small column of alumina. Total analysis time is about 2 hr.

The use of gradient elution demands a detector not affected by the changing solvent background. One of the few such detectors available is the "evaporative analyzer" invented by D. L. Ford and W. Kenard of Union Carbide Australia Limited, for which patent protection is being sought. The analyzer works on the principle of atomizing the column effluent, removing solvent as the spray passes through a heater, and detecting nonvolatile solutes with a light-scattering technique. The main drawback is the loss of volatile solutes, but the end result parallels the much-used technique of evaporating solvent and weighing residues.

Examples presented show resolution of surfactant mixtures into two or three peaks such as "free oil," monofunctional sulfate or sulfonate, and perhaps difunctional components.

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A NEAR INFRARED DIFFERENTIAL SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF OXYPROPYLENE GROUPS IN POLYOXYALKYLENE GLYCOLS

R. D. Ring and William V. Floutz

The nonionic surface active agents, PLURONIC polyols, are polyoxyalkylene glycol ethers that contain blocks of oxyethylene and oxypropylene groups. The properties of these products depend on the number and ratio of oxypropylene and oxyethylene units in the polymer. A near infrared differential spectrophotometric method is described for the determination of the oxypropylene units in polyoxyalkylene glycol ethers.

The methyl, methylene and methine groups of polyoxyalkylene glycol ethers exhibit first overtone hydrogenic stretching absorption bands of moderate intensity in the region of 1.7 microns. These bands normally overlap and detection of absorption assigned to methyl and methine groups is difficult at low concentrations. It is possible to determine the amount of oxypropylene groups using the differential technique and employing a polyethylene glycol ether as the differentiating substance. Under these conditions, the hydrogenic stretching band of the methyl and methine groups of the oxypropylene unit can be detected and measured quantitatively in the range from 1 to 100 per cent oxypropylene units.

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ATOMIC ABSORPTION DETERMINATION OF IRON IN SODIUM BENZENE SULFONATE

W. F. McClune and G. O. Nardi

A procedure is described for the determination of trace iron in sodium benzene sulfonate or sulfonic acid by atomic absorption spectrophotometry, as follows: Samples are dissolved in 50% acetic acid and compared with standard solutions on the spectrophometer. Effects of solvent, sodium concentration, burner types and instrument parameters are investigated.

Also to be presented are comparisons with results obtained by other sample preparation techniques—wet oxidation and sulfuric acid ashing—with subsequent bathophenanthroline determination and a statistical study of the problem of sampling particularly in regard to slurries.

The method is rapid, and accuracy and precision are good.

Other metals can be determined using the same procedure.

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THE DETERMINATION OF DOUBLE LABELED SEBUM IN DETERGENCY STUDIES: A COMPUTER ASSISTED-EXTERNAL STANDARD METHOD

B. E. Gordon, W. T. Shebs and R. U. Bonnar

A liquid scintillation method for the determination of carbon-14 and tritium labeled sebum on white fabric has been developed. The method involves the use of an external standard to determine the counting efficiency of each isotope. The requirement that these determinations be made on swatches of fabric complicates the analysis because of their anti-quenching effect.

Comparison of the results obtained by the external standard method with those by the conventional internal standard method shows satisfactory agreement.

A computer program has been written which accepts the raw counting data and performs all necessary calculations printing out the final data in a form required by these studies.

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AN AUTOMATED METHOD FOR DETERMINING FREE ALKALINITY OR ACIDITY IN SOAP

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

An AutoAnalyzer method has been developed to determine free alkalinity or acidity in soap by use of a mixed cresol red-phenolphthalein indicator solution and measuring the color developed at 550 mμ. An internal standard or reference solution is used consisting of a soap solution which has been adjusted to represent neutrality. The method was applied for 0 to 0.10% free alkalinity, calculated as sodium hydroxide, and for 0 to 0.50% free acidity, as oleic acid. Attainable precision of the method (95% confidence limits) in the ranges explored was found to ±0.0025% for free alkali and ±0.05% for free acidity.

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POTENTIOMETRIC TITRATION OF SULFATE IN SOME TYPICAL ANIONIC SULFONATE AND SULFATE SURFACTANTS

N. T. Crabb and H. F. Persinger

The inorganic sulfate content of several typical anionic surface active agents has been determined by a potentiometric titration with lead nitrate using the potassium ferri-ferro cyanide couple to detect the end point. The recovery of added sodium sulfate varied from −6% to +5% of the contained amount at the 0.5% sulfate level.

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THE USE OF POLYSACCHARIDE GELS IN THE ANALYSIS OF SURFACTANT FORMULATIONS

H. O. Locke

Polysaccharide gels have been used with water as solvent in the analysis of formulations containing surface-active materials. Formulations containing either nonionic or anionic surfactants, or a mixture of both, have been analyzed. The technique has been found to be especially helpful in the separation of organic sulfates and sulfonates from other ionic materials.

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RAPESEED AND RAPESEED OIL PRODUCTION IN CANADA

B. M. Craig

Commercial production of rapeseed in Canada started in 1941 as a wartime emergency. The acreage declined in the immediate postwar years but an interest in a domestic edible oil coupled with an increased demand on the export market has resulted in steadily increasing production to a level of 1.5 million acres for the past two years.

Spring types of two species *Brassica napus* and *Brassica campestris* are grown in Western Canada. Initial studies on the variation of fatty acid composition with variety and environment showed an inverse relation between oleic and erucic acids for both species. Single

(Continued on page 133A)

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plant selections from a German variety, Liho, resulted in rapeseed producing an oil that contained no erucic acid. Further research showed that the erucic content of rapeseed oils was under genetic control and opened the way for developing varieties of rapeseed in both species which would give vegetable oils with low saturated acid contnet and an absence of eicosenoic and erucic acids. Research work on biosynthetic pathways revealed that eicosenoic and erucic acids were synthesized from an oleic precursor by a chain elongation system. Since this is coupled with the *novo de* synthetic system, the oil content of the zero erucic seed would be similar to the erucic-containing types.

The zero erucic rapeseed (Canbra) oil has been evaluated on laboratory and commercial scales for uses as an edible oil. The oil has the following fatty acid composition, palmitic 4, stearic 1, oleic 66, linoleic 20, and linolenic 9, with minor proportions (<1%) of palmitoleic, arachidic, eicosenoic and behenic acids. The yield of liquid salad oil produced by partial hydrogenation to reduce linolenic acid to 1% followed by winterization is about 90%, compared to soybean at 75%.

Investigations are also underway to reduce the linolenic acid content of rapeseed oils and to reduce or eliminate the content of "mustard-oils" in the rapeseed.

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COMPOSITIONAL AND STEREOCHEMICAL APPROACHES TO FATTY ACID BIOSYNTHESIS IN PLANTS

L. J. Morris and A. T. James

The pathways of biosynthesis of the more common fatty acids in plants are now fairly well understood and the normal series of unsaturated acids (oleic, linoleic and linolenic acids) are known to be formed, in the order given, by sequential desaturations. These pathways have been established in a wide variety of vegetable tissues by biochemical studies using ^{14}C-labelled substrates. Earlier compositional approaches to fatty acid biosynthesis gave rise to widely divergent hypotheses, most of which have now proved to be wrong.

More recent chemical work, particularly on seed oils, has revealed the existence of many "unusual" fatty acids; acids containing hydroxy, epoxy, keto or cyclopropene groups, or containing acetylenic bonds or conjugated systems of unsaturation. The structures of many of these compounds and the coexistence of numbers of them readily suggest pathways by which they may have been formed biologically. Evidence in favor of some of these pathways has been obtained by the characterization of postulated or probable intermediates for their formation. Conversely, compositional studies have on occasion given rise to hypothetical pathways which are wrong, or at least, improbable.

The fact that the biological desaturations leading to oleic, linoleic and linolenic acids are all oxygen-requiring has, of course, raised the possibility that oxygenated acids, such as ricinoleic or vernolic acids, for example, may be intermediates of such desaturations. Despite considerable work, it has never been demonstrated that such oxygenated acids are intermediates. However, they are now, indirectly, providing the key with which we can elucidate the mechanism and stereochemistry of double bond formation and, incidentally, of their own biogenesis. Most of the natural hydroxy and epoxy acids are optically active and the absolute optical configurations of many of them have been determined. It is thus possible to prepare from them, by chemically defined routes, fatty acids which are stereospecifically labelled with tritium. We have prepared a number of palmitic, stearic and oleic acids stereospecifically tritiated and, in some cases, deuterated and are using these as substrates for the study of a wide range of biological desaturations and hydroxylations in plant systems.

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THE MICROREACTOR APPARATUS AND TECHNIQUE FOR GAS CHROMATOGRAPHIC ANALYSIS

H. J. Dutton, V. L. Davison and E. D. Bitner

Versatility and ease of analysis are provided gas chromatography by the development of the microreactor apparatus (MRA). This accessory consists of a copper-clad, stainless steel U-tube fitted with a septum and gas inlet at one end and a needle for sample injection at the other. Since the tube is fastened between the poles of a soldering gun, it may be heated rapidly to any temperature desired up to and exceeding 500C.

Among the applications of the MRA are chromatographic procedures for determining positional unsaturation in natural and hydrogenated fat products and petroselinic acid content in vegetable oils by ozonization-pyrolysis and for measuring fatty acid composition by microalcoholysis, esterification and hydrogenation reactions.

The MRA has now been combined into a compact, portable unit easily adaptable to any chromatograph.

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AQUATIC ANIMAL LIPIDS AND THEIR COMPONENTS

E. H. Gruger, Jr.

The types of lipids found in aquatic animals have been shown to differ in their abundance and relative proportions, depending on the species and anatomical sources. This is exemplified by lipids of certain marine sources that are predominantly wax esters and lipids of certain fish liver oils that are largely alkoxydiglycerides. Common teleost fish generally possess triglycerides as the major portion of their combined lipids. Fatty acid compositions are shown to vary among triglycerides of fishes of freshwater and marine origin. Analyses of limited samples of crustacea and mollusks indicate some possible characteristic lipid patterns.

Fatty acids of the several moiety positions of triglycerides and phospholipids are shown to possess different average degrees of unsaturation, of which the patterns are by no means generally defined. Some species are sources of certain fatty acids which appear solely unique in their natural abundance, e.g. isovaleric acid of dolphin jawbone oil and C_{15}–C_{17} acids from mullet and others.

Fatty acid composition of combined fish lipids are shown to vary as to geographical locations and seasons of catch. These variations may be related to either maturity differences, or dietary differences, or combinations of both variables among fish of the same species.

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TRACE COMPONENTS OF BUTTERFAT

Mark Keeney

Butterfat contains a variety of interesting trace components. Some are characteristic of ruminant fats and originate from metabolic activity of the rumen microorganisms. Examples of these compounds are the branched-chain fatty acids, the *cis* and *trans*-isomers of oleic and linoleic acids and the bound aldehydes of butterfat. Other compounds appear to result from the forced metabolic activity of the mammary gland. These are mono- and diglycerides and hydroxy- and ketoacids. The steroid, Δ^7-cholesten-3-one has recently been discovered in butterfat. Discussion will be concerned with analytical methods needed for the trace compounds. the roles of many of the compounds in the unique flavor properties of butterfat, and the prospect of butterfat being a useful source material for the isolation of intermediates in fat metabolism.

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THE STUDY OF LIPID BILAYERS AT A WATER-WATER INTERFACE: APPARATUS DESIGN PARAMETERS

R. E. Howard

Lamellar lipid-protein membranes separating aqueous media provide a unique type of interfacial film closely analogous to biological membranes. Following the initial report of Mueller et al. [Nature *194*, 979 (1962)], our laboratory has developed apparatus and methods for the formation and study of these membranes as models of biological membranes. In particular, our systems have been designed to facilitate permeability studies in electrically and optically characterized lamellar membranes.

A number of thoeretical criteria (e.g., chemical and dimensional analogy, aqueous phase boundaries) relating biological membranes and experimental models such as the aqueous-bounded lipid bilayer will be discussed. Rigorous physiochemical characterization of the experimental membrane requires control, measurement, or nullification of a number of theoretical variables such as membrane area, thickness, continuity. These variables will be considered with the design factors they impose.

Methods of measuring membrane properties lead to practical considerations in apparatus design, which will be traced through the historical development of the apparatus. Various methods of formation of such model membranes will be reviewed.

Our current apparatus design and methods of study will be described and various parameters illustrated with experimental examples. Ovolecithin-cholesterol-hydrocarbon membranes thin to a black (bilayer) state characterized electrically by 10^3–10^5 ohm-cm^2 specific resistance and 0.2 μfd/cm^2 specific capacitance. Preliminary experiments on the permeability of such lamellar lipid membranes to polar and nonpolar compounds will be described. The versatility and potential uses of the aqueous-bound lipid bilayer system will be presented.

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PROPERTIES OF LIPID BILAYERS AT A WATER-WATER INTERFACE

D. A. Haydon

This paper is essentially a review of the work on black hydrocarbon films in aqueous media which has been carried out by the author and his colleagues during the last few years.

The theory of the formation and stability of the films is discussed in terms of the structure and physical properties of the constituent molecules. Particular consideration is given to the adsorbtion of the stabilizing molecule and the metastable equilibrium of the resultant thin film. The various systems which have been examined experimentally are then described. The interrelation of the film capacitances, thicknesses and compositions is discussed in the light of the theoretical expectations.

The films are permeable to water, although the measurement of their permeability is complicated by the difficulty of stirring the boundary layers of the aqueous solutions. A discussion of the progress in this problem and the interpretation of the permeability measurements in terms of the structure and composition of the films is given.

Finally, some conditions under which films become strongly conducting are described.

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MEASUREMENT OF VAN DER WAALS INTERACTIONS IN MONOLAYERS

M. D. Rosenberg

Measurement of the interfacial tensions of phospholipids and of fatty acids have been made simultaneously at gas-liquid and liquid-liquid interfaces. The experimental apparatus consists of an electrobalance with a Wilhelmy plate sensitive to the differences in tension. The measurements can be made with a tenfold increase in sensitivity and the data obtained provide an estimate of the van der Waals interactions as functions of intermolecular distances and chain length. Deviations from the expected dependency can be interpreted as resulting from the formation of molecular clusters in equilibrium with free molecules. The formation of these clusters is sensitive to the ionic content of the soline phase. The observations can be interpreted in terms of chain length, steric packing, and possible formation of lattice-type structures.

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THE DETERMINATION OF THE SOLIDS CONTENT OF FATS AND OILS BY NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

W. A. Bosin and R. A. Marmor

A nuclear magnetic resonance (NMR) method is presented for the determination of the solids content of fats and oils at various tem-

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peratures. This method is more rapid, accurate and universally applicable than the generally accepted solids fat index (SFI) procedure. A standard deviation of 1.0% solids, unaffected by parameter interactions and attainable by inexperienced operators, indicates good reliability and reproducibility. The utilization of an NMR variable temperature accessory and a more precise mode of NMR tuning has optimized the precision and accuracy. The direct calculation of percent solids from the NMR signals eliminates the need for standards and compensates for the temperature variable. Results are given which indicate that two separate laboratories using two different methods and modes of NMR operation agree reasonably well.

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EVALUATION OF THE DIFFERENTIAL SCANNING CALORIMETRIC METHOD FOR FAT SOLIDS

A. P. Bentz, B. G. Breidenbach and L. A. Wheelan

The use of the DSC for fat solids has been studied extensively using the method of Denison and Justin (AOCS Fall Meeting, 1966, Philadelphia, Paper 112). For Smalley Committee Samples EF-1,2, and 3, the method is excellent. It was found that interlaboratory correlations were exceptionally good.

A statistical comparison of dilation, NMR, and DSC for solids fat index with six random fat samples shows the DSC equivalent to, or superior ot the other methods in 14 of 15 pairs.

For hard butters, the DSC method loses precision and reproducibility, if the scanning rate and pretempering cycles are kept constant, i.e. equilibration is slower. Slower scanning rates improve the reproducibility. Presumably, the tempering cycle could be adjusted to improve it still further.

One important fact which emerged from numerous statistical studies was that there is a marked dependence of results on sample size. This would affect heats of fusion, heat capacities and other thermal measurements on certain types of fats.

Basically, this method appears sound and could be adapted for quality control purposes.

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PHASE EQUILIBRIA AND COUNTERCURRENT DISTRIBUTION. I

R. A. Barford, R. J. Bertsch and H. L. Rothbart

Ternary diagrams for methyl oleate/*n*-hexane/acetonitrile and methyl palmitate/n-hexane/acetonitrile systems were constructed from equilibria data obtained at three temperatures (20C, 25C, and 30C). The diagrams varied significantly with temperature, both systems showing expanding regions of immiscibility as temperature decreased. Both the partition coefficient of the methyl ester and the mutual solubility of hexane and acetonitrile, at each temperature, varied with ester concentration in the system. Although these effects were most pronounced at concentrations of 0.35 M and above, some variation of partition coefficient was observed in much more dilute solutions. These observations are related to phenomena observed in a separation by countercurrent ditsribution. Therefore, phase equilibria studies are indicated to be of great value when use of this powerful fractionating technique is being contemplated.

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THE USE OF THE TENSIOLAMINOMETRIC TECHNIQUE FOR THE STUDY OF THE FOAMABILITY OF SOLUTIONS

Andre Eydt and H. L. Rosano

The factors responsible for foam formation and foam stability are reviewed and a dynamic method which considers most of these factors is presented. The experimental set-up is a modification of a method proposed by R. Matalon. It involves the measurement of the work of formation of a liquid lamella inside a vertical, rectangular, rigid, wettable frame which is withdrawn from a foamable solution. The frame is connected to a transducer-recorder and the solution is placed on a platform which can be raised or lowered at various controlled speeds. The force versus displacement curves obtained allow the direct determination of the free energy of formation of a liquid lamella. Moreover, the dynamic as well as the static surface tension of the solution can be measured. Upon pushing the film back into the solution the work opposing surface reduction—due to hindered desorption or chemical changes of the surface-active material—is also determined. The interpretation of the extension and contraction curves provides a direct measurement of the liquid lamella behavior as to foam stability. The tensiolaminometric technique can be applied to determine the rate of adsorption of surfactants. The efficiency of suds-boosters and the aging processes of protein solutions have been investigated with this technique. The application to the study of emulsification processes has also been considered.

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USE OF THE DIFFERENTIAL SCANNING CALORIMETRIC METHOD IN THE STUDY OF LIPID PHASE TRANSITIONS

J. W. Hampson and H. L. Rothbart

The use of the differential scanning calorimetric method for studying phase transitions of several representative lipids will be discussed. Some of the lipids studied include methyl oleate, methyl palmitate and their mixtures, tristearin, tripalmitin and their mixtures. Instrumental parameters such as heating rate, cooling rate, sample size and emissivity will be discussed as to their effects on the characteristic thermal profiles of the samples.

Data such as temperatures and heats of phase transformation will be compared with literature values for some of the afore-mentioned and other materials. The use of this method as an approach for the construction and study of phase diagrams of binary mixtures offers certain advantages over classical techniques such as ordinary heating or cooling curves. The technique is rapid, simple and allows the determination of heat capacity and enthalpies of transition.

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GAS CHROMATOGRAPHIC-MASS SPECTROMETRIC ANALYSIS OF TRIMETHYL SILYL ETHERS OF GLYCERO-LACTO-ESTERS OF FATTY ACIDS

Jens Birk Lauridsen, P. E. Brandt, Niels Krog and Ole Tolboe

Mass spectrometric data for trimethyl silyl (TMS) ethers of synthetically prepared glycerol-lactic acid-esters and glycero-lacto-palmitates were used for the analysis of the commercial emulsifying agent, glycero-lacto-esters of fatty acids. The commercial product was fractionated on a silicic acid column and separated in a small fraction containing triglycerides and three fractions with 1, 2, and 3 hydroxyl groups, respectively. The latter were transformed to TMS ethers and separated on a Perkin-Elmer model 880 gas chromatograph equipped with dual 2 m × ⅛ in. columns packed wtih 10% SE 30 on Gas-Chrom Z 80/100 mesh and programmed from 100 to 300C. The three fractions showed up to 45 peaks. By combination of the gas chromatograph with a Hitachi-Perkin Elmer RMU-6D mass spectrometer mass spectra from the individual peaks in the gas chromatogram were recorded. Mass spectra of compounds with molecular weights as high as 750 were recorded. By comparison of these mass spectra with the mass spectra of the synthetically prepared compounds the structure of the main portion of the commercial product was elucidated.

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THE MASS SPECTRA OF LONG-CHAIN ALDEHYDES, DIMETHYL ACETALS AND ALK-1-ENYL ETHERS

Kirsten Christiansen, V. Mahadevan, C. V. Viswanathan and R. T. Holman

Aldehydogenic lipids are often present in biological systems; and it is desirable to determine the structures of the aldehydes formed by their hydrolysis, either as the free aldehydes or, preferably as the dimethyl acetals formed by methanolysis of complex lipids. Application of mass spectrometry to identification of these compounds requires correlations between the mass spectra and the structures of series of authentic compounds.

Our study presents the mass spectra of long-chain saturated and unsaturated aldehydes, dimethyl acetals and alk-l-enyl methyl ethers. Each series of compounds studied included saturated members with 12–18 carbon atoms, and the unsaturated members 18:1, 18:2 and 18:3.

The mass spectra of the saturated aldehydes were in agreement with previous knowledge of short-chain aldehydes, whereas the unsaturated aldehydes gave only a few fragments of analytical importance. In comparison with fragmentation of short-chain dimethyl acetals, the saturated and the unsaturated long-chain dimethyl acetals gave fewer correlations. Loss of a fragment of 32 mass units, resulting in the formation of an ion of the structure of an alk-l-enyl methyl ether, is characteristic for the long-chain dimethyl acetals. Other fragments derived from the dimethyl acetals may arise directly or indirectly from an alk-l-enyl methyl ether. This was confirmed by the study of the mass spectra of the corresponding alk-l-enyl methyl ethers.

A series of even-numbered mass peaks $68 + n \cdot 14$ ($n = 0, 1, 2, \ldots$) is common to all three types of compounds. The structures of these fragments which seem to be characteristic for these three classes of long-chain compounds are tentatively assigned the structure of furan and alkyl derivatives of furan. Mass spectra of all three types of compounds will be discussed in detail.

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A CONVENIENT SEPARATION OF THE AFLATOXINS

R. D. Stubblefield, Odette L. Shotwell and Gail M. Shannon

The four aflatoxins B_1, B_2, G_1 and G_2 have been separated on a series of columns. The crude product (2 g) containing the four (670 mg B_1 and 530 mg G_1) was made by precipitation in hexane from a concentrated chloroform extract of wheat molded with *Aspergillus flavus* NRRL 3145. Chromatography of the crude product on silicic acid with 1% ethanol in chloroform gave 330 mg of free and relatively pure B_1. Aflatoxin G_2 was separated from other aflatoxins, but in an impure state. Thin-layer chromatography was used to follow the column development. The remaining column fractions containing quantities of G_1 and sizable amounts of B_1 and B_2 were pooled and rechromatographed on Silica Gel G. The mobile phase was 2% acetone in chloroform. Quantities of free G_1 (340 mg) were eluted from this column. Isolation of more B_1 (145 mg) and of moderate amounts of B_2 was also achieved. Each of the four aflatoxins could be further purified by repetition of the Silica Gel G or silicic acid columns. Products from these columns crystallized readily.

These procedures have been successfully applied to other products from culturing *A. flavus* NRRL 2999 on rice. However, this organism produces little G_1 and G_2.

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THE INFLUENCE OF DIETARY FATTY ACIDS ON THE METABOLISM OF POLYUNSATURATED FATTY ACIDS IN RAT LIVER

J. W. Andrews, Jr., W. O. Caster, Hans Mohrhauer and R. T. Holman

The effects in rats of individual dietary fatty acids were studied by feeding mixtures of fatty acids made up in such a way that the correlations between the individual acids were low. Dietary and liver fatty acids were analyzed by GLC. Questionable GLC peaks were collected and identified by hydrogenation, ozonization and mass spectra. Major emphasis in this experiment was placed on the behavior of polyunsaturated fatty acids (PUFA). Feeding of most fatty acids resulted in a corresponding increase in those same fatty acids in liver. Between the $\omega 9$, $\omega 6$ and $\omega 3$ families of PUFA, metabolic competition was demonstrated. Linoleate was metabolized preferentially to 22:5ω6. But arachidonate and other high members of the $\omega 6$ family were metabolized preferentially to 22:4ω6 rather than to 22:5ω6. Linolenate in the diets studied tended to be metabolized preferentially to 22:5ω3. Higher members of the $\omega 3$ family were metabolized to 22:6ω3 in a greater degree than linolenate. These observations suggest that the $\omega 6$ and $\omega 3$ families of fatty acids conversion pathways are more complex than previously recognized.

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OBSERVATIONS ON THE EFFECT OF HYPOPHYSECTOMY ON THE INTERRELATIONSHIPS OF THE METABOLISM OF LIPIDS IN RAT TESTES

Benny Jensen, Masami Nakamura and O. S. Privett

Male rats in an advanced stage of EFA-deficiency were hypophysectomized, maintained on the fat-free diet for 6 days and transferred to the basic diet supplemented with 9% methyl linoleate. One group of control animals was sham-operated and given the linoleate diet, another group was transferred directly to the linoleate diet. After 23 days on this diet all animals were sacrificed by exsanguination.

The testicular lipids were extracted by homogenization in 2:1, 2:1, and finally 1:2 chloroform:methanol, cleaned by Sephadex chromatography, and separated by TLC into polar lipids and the neutral lipid classes. Through methylation and GLC the fatty acid composition was determined of the cholesteryl esters, glyceryl ether diesters, triglycerides, and total polar lipids. The fatty acid distribution at the β-position of the triglycerides was determined via pancreatic lipase

hydrolysis. The total polar lipids were subjected to a prolonged treatment with phospholipase A and the liberated acids isolated, esterified and chromatographed.

One of the main effects of hypophysectomy appeared to be on the distribution of the fatty acids among the triglycerides. In contrast to the control animals there was an accumulation of triglycerides with saturated fatty acids in the β-position. No major differences between experimental groups were observed in the composition or distribution of the fatty acids between the α- and β-positions of the phospholipids. Also, there were no major differences in the fatty acid composition of the glycerol ether diesters. However, there was an elevation of the latter compounds and an increase in the polyunsaturated cholesteryl esters in the hypophysectomized animals as compared to the control groups, indicating that the enzymes involved in the interrelationships in the metabolism of these compounds are hormone-sensitive.

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LIPID COMPOSITION OF SERUM AND SERUM LIPOPROTEINS FROM MINIATURE SWINE FED A CORN OIL-CHOLESTEROL DIET FOR SIX MONTHS

D. P. J. Goldsmith, H. P. Jacobi, D. M. Findlay and M. Kelly

In a study of the relationship between serum lipid components and atherosclerosis in swine, miniature boars were fed for six months a standard hog ration modified so as to contain 20% corn oil and 2% cholesterol. This diet resulted in a 56% increase in total serum lipids, a 130% increase in serum cholesterol, a 150% increase in serum phospholipids and a 72% increase in free fatty acids over control values. There was no change in serum triglycerides. The distribution of fatty acids in each of the lipid fractions was determined. The test diet caused increases in linoleic acid in all lipid components of the serum, while a decrease in the greater-than-18 carbon fatty acids and in linolenic acid was observed in most fractions.

Each of the lipoprotein fraction (S_f 20+, S_f 0–20, high-density fraction and sediment button) was assayed for total lipids, cholesterol esters, triglycerides, phospholipids and free fatty acids. In addition, the distribution of fatty acids in each of the lipid components in each lipoprotein fraction was determined. Changes were produced by the high lipid diet in the lipid composition and fatty acid distribution of most lipoprotein fractions. In contrast to the human, for this strain of hog it was observed that high-density lipoproteins are the most abundant lipoproteins in serum and most subject to change by lipid feeding. Total lipids in this lipoprotein fraction increased 98%; cholesterol esters, 153%; triglycerides, 52%; phospholipids, 150%; and free fatty acids, 38%.

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DIETARY FAT AND LECITHIN AND CEPHALIN FATTY ACIDS IN LIVERS OF MATERNAL AND FETAL PIGS

J. P. Feaster, F. C. Neal and H. D. Wallace

Feeding pregnant pigs a highly unsaturated fat, corn oil, at a high level of 15% of the diet for 85 days caused a significant increase in maternal liver total cholesterol. Feeding a highly saturated fat, coconut oil, increased liver cholesterol in both maternal and fetal pigs and liver total lipid in maternal pigs. Total lecithin and cephalin content of livers was not altered by feeding either fat at the 15% level, but concentrations of several of the constituent fatty acids were significantly affected. The diet of 15% corn oil, very high in linoleate, increased linoleate in lecithin and cephalin, but oleate, also in high concentration in corn oil, was reduced by feeding this diet. Palmitate was decreased when the high corn oil diet was fed. Fetal liver myristate and palmitoleate values were 5 to 10 times as high as maternal in both lecithin and cephalin. Linoleic, an essential fatty acid, was far lower in fetal than in maternal livers, indicating that while placental transfer did take place, it was at a limtied rate. Palmitoleate was significantly reduced with the 15% corn oil diet, indicating a suppressive effect of the high fat intake on its synthesis; this effect was not observed, however, with the high-level coconut oil diet.

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EFFECT OF A POLYUNSATURATED DIET UPON ADIPOSE TISSUE FATTY ACIDS IN YOUNG CORONARY MALES: A FIVE YEAR COHORT STUDY

A. I. Fleischman, Thomas Hayton and M. L. Bierenbaum

Thirty-four electrocardiographically confirmed coronary male outpatients, aged 20 to 50 years, were placed on a 30% of calories fat diet for periods up to five years. The diets contained 14.1% of calories as polyenoic fats and 5.5% as saturated fats. Dietary adherence was monitored subjectively by a 24-hr dietary rceall at 10-week intervals and annually by a one-week detailed food diary. Objective biochemical monitoring was accomplished by gas-liquid chromatographic assay of constituent fatty acids in the triglycerides and serum free fatty acids. Adherence was good to excellent. Adipose tissue aspirations were done annually. The men were at ideal weight at the start and maintained ideal weight within 5% during the study. The means of observation, grouped in 6-month intervals, could be fitted reasonably well by a sigmoid curve. For approximately the first 9 months, the mean linoleate remained constant at 10.9%, after which it approxi-

$$\frac{x-9}{6}$$

mated the expression: $y = 19.5 - 8.7\ (0.4\ )$ where "y" is the adipose tissue linoleate as mole % of total fatty acids, and "x" is the time in months. The major increase, approximately 50%, in adipose tissue linoleate occurred between 12 and 18 months, with small increases occurring for the remaining 42 months. The turnover time is of the order of 18 months. Fatty acid determinations of adipose tissue are valuable in epidemiological studies and as a monitor of very long-term dietary adherence, if weight is stable.

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ALTERATION OF SALMON FATTY ACIDS BY DIETS OF ENCHYTRAEID AND TUBIFICID WORMS

J. B. Saddler, H. M. Krueger, I. J. Tinsley and R. R. Lowry

Coho salmon were taken from the river and maintained for 21 days and fed diets of enchytraeid or tubificid worms. The total lipid content of the enchytraeid worms was 4.2%, of the tubificid worms 3.2%, of the river salmon 2.8%, of the salmon fed enchytraeids 6.0%, and of the salmon fed tubificids 3.4%. An alteration of the fatty acid composition accompanied the increase in total lipid content. The ratio of saturated to unsaturated fatty acids in the enchytraeids was 2:3 and in salmon fed enchytraeids also was 2:3; however, in tubificids the ratio was 1:4 and in salmon fed tubificids the ratio increased to 1:3. In salmon fed enchytraeids the level of 14:0 was 107 mg per 10 g while the level in salmon fed tubificids was 11 mg; the enchytraeids contained 7 mg of 14:0 and the tubificids contained 14 mg per 10 g.

In enchytraeids 8% of the total fatty acids were ω6 and in salmon fed enchytraeids they averaged 25%. In the tubificids 36% of the total fatty acids were ω6 and in salmon fed tubificids they averaged 25%. Thus ω6 fatty acids were selectively accumulated in the salmon fed enchytraeids and were selectively metabolized in the salmon fed tubificids. The amount of 22:6 per 10 g averaged 19–21 mg in river salmon, starved salmon, and salmon fed tubificids; but in salmon fed enchytraeids was only 9 mg. Apparently diet influences the incorporation of selected fatty acids in salmon while the salmon selectively metabolizes certain acids to meet its requirements.

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LIVER AND PLASMA PHOSPHOLIPID TURNOVER DURING HEPATIC TRIGLYCERIDE ACCUMULATION

C. A. Olmsted

Total liver lipids increase in female rats from 6–24 hr after administration of *dl*-ethionine [Olmsted, C.A. 1955 Fed. Proc. 14, 1212.] while liver phospholipids (PL) show little or no change, and serum PL decrease. Rees and Schotlander [Proc. Roy. Soc. *157*, 517 (1963)] confirm these findings and show additionally that serum NEFA increases in both male and female rats at 6, 13, and 24 hr. The data of the present study show that ethionine decreases synthesis of plasma and liver PL as measured by the incorporation of intravenous $NaH_2P^{32}O_4$. The turnover of plasma PL in male and female rats, and the disappearance of native, labeled plasma PL from the plasma of normal, and depancreatectomized dogs are both markedly decreased by ethionine. However, as indicated by relative specific activity of plasma and liver PL, an increased exchange of phospholipids between liver and plasma occurs under these conditions. Therefore, the data suggest an active role of phospholipid transport during hepatic triglyceride accumulation. The possible relationship of these findings to the triglyceride cycle involving serum NEFA turnover, and the role of phospholipids in low-density lipoprotein formation and release by the liver is to be discussed.

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THE PHOSPHOLIPIDS OF PLASMA FROM VARIOUS MAMMALIAN SPECIES

G. J. Nelson

The plasma phospholipids in several common mammalian species, including rat, rabbit, pig, dog, horse, sheep, cow, goat, cat, and guinea pig, were analyzed using chromatographic and spectrophotometric procedures. Lipids were extracted from the plasma with chloroform-methanol, 2 to 1, v/v, and freed of nonlipid material by passage through a Sephadex column. The phospholipids were separated by 2-dimensional thin-layer chromatography (TLC) using Silica Gel HR to which was added 10% $MgSiO_2$. Spots were identified by various spray reagents (charring, I_2 vapor, ninhydrin, Dragendorff's) and also infrared spectroscopy of the lipids after collecting the spot from the TLC plate and freeing the lipid from the absorbent. Phosphorus analyses were performed directly on spots, visualized by charring (sulfuric acid-dichromate spray reagent), which were scraped off the TLC plate directly into the color development tubes without removing the absorbent.

The phospholipid distribution in all species was similar. Lecithin, lysolecithin, and sphingomyelin were detected in the plasma of all species, and accounted for more than 95% of the phospholipid content in most species' plasma. Lecithin, without exception, was the major phospholipid in the plasma of these animals (60 to 80% of the total phospholipid) and this is particularly interesting in the case of ruminants because these animals have little or no lecithin in their erythrocytes. The lysolecithin and sphingomyelin content of the plasma phospholipid in these species ranged between 5 and 20% for both compounds.

Phosphatidyl ethanolamine and its plasmalogen analogue and phosphatidyl inositol were the only acidic phospholipids commonly found in the plasma; combined, they usually were less than 5% of total plasma phospholipids. Guinea pig plasma was the only exception to this observation; almost 20% of its total phospholipids were acidic forms, mainly phosphatidyl ethanolamine. Phosphatidyl serine was detected in only trace amounts and in only a few species.

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MUSCLE LIPIDS OF VERTEBRATES AND INVERTEBRATES

Gerald Simon, George Rouser and Gene Kritchevsky

Because the lipids of muscle have not been studied as extensively as the lipids of other organs, a systematic investigation of lipids of this important organ in different species was begun. Initial chromatographic studies revealed several interesting features: 1) Human, rat, and mouse muscles have a very high neutral lipid content. 2) Ethanolamine and choline phosphoglycerides are the most abundant phospholipids of both vertebrates and invertebrates. 3) Vertebrates and some invertebrates contain sphingomyelin but in some invertebrates this lipid is absent. 5) New lipid classes were found in human, rat, abalone, scallop and sea urchin muscles. The quantitative distribution of phospholipids and some characteristics of several new lipids will be presented.

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PRECISION AND ACCURACY OF GLC ANALYSIS OF FATTY ACIDS

S. F. Herb

The results of gas-liquid chromatographic analyses from approximately forty collaborators of the AOCS Smalley and Instrumental Techniques Gas Chromatography Subcommittees are evaluated. Precision

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between the collaborators and the accuracy of the analyses of four methyl ester mixtures and two vegetable oils will be discussed.

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THE GAS CHROMATOGRAPHY OF FATTY ACIDS AND DERIVATIVES USING NEW LIQUID PHASES

L. D. Metcalfe, R. J. Martin and A. A. Schmitz

The gas chromatography of fatty acids and derivatives has become stabilized in recent years. However, the search for liquid phases that can give better or specialized separations has continued. A number of new liquid phases have been tested for separating fatty acids, esters, fatty amines, nitriles, and other fatty derivatives. Among these have been cyanoethylsucrose, cyanoethyl starch and cationic surfactant-treated polyesters.

A new concept of column technology we call "permanent liquid phases" has been explored. This latter idea consists of chemically binding the organic phase to inorganic support. Polyesters, acrylonitrile, and other organic materials have been reacted with Celite and other inorganic compounds. The resulting column packings have been used to separate fatty compounds. These "permanent liquid phase" columns exhibit unique properties.

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GAS-LIQUID CHROMATOGRAPHY OF TRIGLYCERIDE MIXTURES CONTAINING BOTH ODD AND EVEN CARBON NUMBER FATTY ACIDS

Carter Litchfield, R. D. Harlow and Raymond Reiser

By critically selecting optimum operating conditions, quantitative gas-liquid chromatography (GLC) of triglycerides has been extended to natural fats containing both odd and even carbon number fatty acdis. GLC analysis of triglycerides on a 1.83 m glass column containing 3.0% JXR silicone on 100/120 mesh Gas-Chrom Q resolved triglyceride molecules differing by only one carbon number. The column temperature was programmed from 210 to 375C at 4.0C/min with 100 ml/min helium carrier gas. Operating temperatures to 375C required a special Kovar glass-to-metal seal at each end of the column. Peak resolution was significantly improved by hydrogenating each triglyceride sample prior to GLC analysis.

The triglycerides of four fish oils (mullet, tuna, menhaden, and pilchard) and one seed fat (*Acanthosyris spinescens*) containing various amounts of odd carbon number fatty acids were analyzed by this technique. The method was also useful for defining the triglyceride composition of the cyclopentene fatty acid oil from *Hydnocarpus wightiana* seeds. The carbon number distribution of triglycerides in each fat was quantitatively determined.

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ANALYSIS BY GAS-LIQUID CHROMATOGRAPHY OF FATTY ALCOHOLS, HYDROCARBONS AND METHYL ESTERS FROM SELECTIVE HYDROGENOLYSIS OF TALLOW ESTERS

G. D. Lee, R. S. Klonowski, G. A. Ayer and T. W. Findley

Mixtures of alcohols, hydrocarbons and methyl esters predominantly in the C14:0, C16:0, C18:0 and C18:1 range were anticipated as reaction products from selective hydrogenolysis of animal fats to produce fatty alcohols retaining unsaturation.

The use of gas-liquid chromatography techniques to analyze the resulting mixtures was found to be feasible. Preliminary investigations for establishing this involved the use of weighed known mixtures of C14:0, C16:0 and C18:0 compounds of each class. This was paralleled by correlating retention times of hydrocarbons, esters and alcohols having the same carbon chain length. The most satisfactory column was found to be a cyanoethylene succinate polyester on an acid-washed Chromosorb. Multistage columns composed of mixed packings also were investigated. Three-stage temperature programming of the column was found to be required for a complete resolution of the desired components. The resulting baseline shift on temperature programming was minimized to prevent quantitative interference by the design and fabrication of a precision-matched column where required. Hydrogen-flame detection of the dual type was selected. An additional requirement was the resolution of mixtures also containing methyl oleate and oleyl alcohol.

Accomplishment of acceptable resolution of the basic components of the reaction mixtures revealed improper quantitative effects on the part of the alcohols. Corrections for the alcohols from the raw quantitative data were calculated. These correction factors ranged from a minor nature for the C14:0 alcohol, to major for the C18:0. These nonquantitative phenomena were shown to be not a function of the hydrogen-flame detector. Use of internal standards showed the presence of nonchromatographable material in some reaction mixtures.

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PREPARATION AND ANALYSIS OF SILYL ETHER DERIVATIVES

W. R. Supina and Nicholas Pelick

Several types of silylating reagents were evaluated for the preparation of silyl ether derivatives of polar biochemicals. Fatty acids, partial glycerides, sterols, other important lipids and sugars will be discussed. This paper will present information on the chemical reactions of the various reagents and their use in analysis in chromatography. Both gas- and thin-layer chromatography were used to study the effectiveness of the reagents in preparing the silyl derivatives. Retention data for the various derivatives including dimethylsilyl and trimethylsilyl ethers have been tabulated. The silyl ether derivatives in some cases are eluted later than the free compounds, but peak symmetry is better.

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THE DIRECT CONVERSION OF 2,4-DINITROPHENYLHYDRAZONE DERIVATIVES OF CARBONYL COMPOUNDS TO DITHIOACETALS AND DITHIOKETALS FOR ANALYSIS BY GAS LIQUID CHROMATOGRAPHY

M. M. E. Metwally, C. H. Amundson and T. Richardson

A method has been developed for the conversion of 2,4 dinitrophenylhydrazone (2,4 DNP) derivatives of aldehydes and ketones to dithioacetals and dithioketals for subsequent analysis by gas-liquid chromatography (GLC). The conversion was accomplished by using 1,2 ethanedithiol and boron trifluoride etherate (BF_3).

The 2,4 DNP derivative of the aldehyde or ketone was dissolved in a minimum amount of chloroform, and 1,2 ethanedithiol and BF_3 were added. The mixture was heated to 125C for 2 hr, or for shorter times at higher temperatures. After cooling, the reaction mixture was washed with alkali and extracted with pentane. Yields were determined by GLC using a 10% FFAP column. The dithioacetals and the dithioketals chromatographed without any observed decomposition.

The whole spectrum of monocarbonyl derivatives from acetone to 2-nonadecanone and from acetaldehyde to palmitaldehyde can be chromatographed using temperature programed GLC. Yields of over 90% were obtained from the 2,4 DNP derivatives of 2-octanone and palmitaldehyde. Slightly lower yields were obtained from the 2,4 DNP derivatives of acetaldehyde and acetone. Some extraneous peaks were observed in the chromatograms, but they did not interfere with yield determinations.

Additional investigations are being conducted on obtaining quantitative yields from short chain 2,4 DNP derivatives, eliminating extraneous GLC peaks, and characterizing the derivatives formed from the 2,4 DNP derivatives of dicarbonyls and unsaturated carbonyls.

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ANALYSIS FOR GEOMETRICAL AND POSITIONAL ISOMERS OF FATTY ACIDS IN PARTIALLY HYDROGENATED FATS

C. R. Scholfield, V. L. Davison and H. J. Dutton

A liquid chromatographic procedure for fractionation and analysis of methyl esters from fats was developed. First, esters are separated by liquid chromatography on a partially vulcanized rubber column into trienoate, dienoate, monoenoate-palmitate and stearate fractions. The monoenoate-palmitate fraction is separated by liquid chromatography on a silver-saturated cation exchange resin into palmitate, *trans* monoenoate and *cis* monoenoate fractions. Double bond positions in *trans* and *cis* monoenoates are located by ozonization and gas chromatography of fragments. This separation procedure requires less time and uses simpler apparatus with smaller samples than a previously described method based on countercurrent distribution. Data from analyses of two liquid oils and four shortenings are presented. *cis*-Monoenoates from liquid oils had about 80% of the double bonds in the 9-position. Those from shortening had 73 to 77% in the 9-position. In all-*trans* monoenoates double bonds were widely scattered with maxima at the 10- and 11-positions.

Infrared analyses indicated 5–12% *trans* in liquid oils and 17–29% in shortenings. Monoenoate fractions from liquid oils contained 7–20% *trans* and dienoate fractions, 6–11%. These same fractions from shortenings contained 26–35% *trans* and 10–34%, respectively.

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DETERMINATION OF PETROSELINIC ACID BY MICROREACTOR CHROMATOGRAPHY

R. Kleiman, V. L. Davison, F. R. Earle and H. J. Dutton

The microreactor-ozonolysis technique, developed to locate double bonds in fatty acids, was applied to the quantitative determination of the relative amounts of petroselinic and oleic acids in vegetable oils. When the technique was tested on ester mixtures of known composition, results were excellent. Comparison of data on esters from seven Umbelliferae oils analyzed by another newly devised procedure, which utilizes thin-layer chromatography followed by gas-liquid chromatography or ultraviolet spectrophotometry, shows good agreement between the two methods. The speed and accuracy of the microreactor technique permitted analysis of numerous oils of the Umbelliferae, five oils of the Araliaceae, and a few from other families.

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GAS LIQUID CHROMATOGRAPHY OF THE TRIMETHYLSILYL DERIVATIVES OF SUGAR PHOSPHATES

M. W. Hurst, C. G. Huggins and J. G. Hamilton

Silylation of several hexose phosphates was accomplished by the use of Bis (tri-methylsilyl) acetamide (BSA). The compounds were converted to free acids by addition of 1.0 N HCl and evaporated to dryness. After addition of BSA the samples were heated to 60C for 1 hr in sealed tubes and the excess BSA was evaporated with a stream of N_2 at room temperature. The samples were dissolved in acetone and analysis was performed on an Aerograph 200 gas-liquid chromatograph using a 5 ft × ¼ in. glass column packed with 3% OV-1 on Chromosorb G. The effluent was split 1:1 and detected with a flame ionization detector and a thermionic detector (phosphorus). Malathion was used to evaluate the performance of the phosphorus detector. Methyl esters of fatty acids gave no response in the phosphorus detector while the silyl derivative of inositol PO_4 and glucose-6-PO_4 gave a response in both detectors. At a column temperature of 220C inositol PO_4 gave two peaks at retention times of 11.0 and 13.3 min and glucose-6-PO_4 gave a major peak at 15.0 min retention time. The symmetry of the peaks suggest that other biologically important phosphates may be amenable to the formation of trimethylsilyl esters by this technique.

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THE METABOLIC CONSEQUENCES OF THE CONTROL OF LONG-CHAIN FATTY ACID OXIDATION BY CARNITINE

I. B. Fritz

Carnitine is involved in translocation of fatty acyl groups across mitochondrial barriers which are impermeable to acyl CoA but not to acylcarnitine derivatives. The site of long-chain fatty acid activation is often functionally separated from the site of fatty acid oxidation, and the rate-limiting step for fatty acid oxidation under certain conditions may be the rate of fatty acyl group translocation mediated by carnitine palmityltransferase. Part of the evidence supporting this hypothesis has been previously reviewed [Advan. Lipid Res., *1*, 286 (1963)]. More recent evidence will be presented which strengthens this viewpoint.

Since carnitine and the long-chain acyltransferase system appear to have the potential for controlling the rate of long-chain fatty acid oxidation, it becomes of interest to examine effects of carnitine on other metabolic parameters known to be influenced by altered rates of fatty acid degradation. It is gentrally recognized that increased fatty acid oxidation is associated with increased rates of ketogenesis and gluconeogenesis, but with decreased rate of fatty acid synthesis [Physiol. Rev. *41*, 52 (1961); Proc. Roy. Soc. Series B, *159*, 545 (1964); J. Biol. Chem. *241*, 2523 (1966)]. Evidence will be presented that carnitine increases not only fatty acid oxidation but also ketogenesis and gluconeogenesis. In addition, data will be reviewed demonstrating that long-chain acylcarnitine derivatives are capable of enhancing both fatty acid synthesis activity in complex systems and acetyl CoA carboxylase activity in partially purified systems. The possible physiological significance of long-chain acylcarnitine interactions with enzymes which are inhibited by acyl CoA derivatives will be discussed. An hypothesis concerning the

possible regulatory role of the carnitine palmityltransferase system in different physiological states will be presented. A functional role for carnitine acetyltransferase in fatty acid metabolism will also be discussed in relation to the possbile existence of two metabolically distinct intramitochondrial acetyl CoA pools.

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INHIBITION OF THE LONG CHAIN ACYL CoA-CARNITINE ACYLTRANSFERASE BY HYPOGLYCIN (L-α-AMINO-β-METHYLENECYCLOPROPANEPROPIONIC ACID)

R. Bressler and M. Entman

"Vomiting Sickness" is a disease which occurs in Jamaica and has been attributed to the ingestion of the unripe fruit of the tropical plant *Blighia sapida* (ackee fruit). The toxic illness is characterized by hypoglycemic convulsions and coma. The toxic agent was found to be a plant amino acid, hypoglycin (L-α-amino-β-methylenecyclopropanepropionic acid) which was only active in vivo. The compound undergoes transamination and oxidative decarboxylation in the liver to yield methylenecyclopropaneacetic acid, which is active in vivo or in vitro. The degradation compound was found by von Holt to decrease the oxidation of palmitate, but not of octanoate, and cause hypoglycemia. Because of these effects on long- but not short-chain fatty acid oxidation we undertook a study of the effect of hypoglycin on the long-chain acyl CoA-carnitine acyltransferase. This enzyme has been shown to be the rate-limiting step in long-chain fatty acid oxidation.

Intravenous administration of hypoglycin to mice resulted in marked hypoglycemia, which was preceded by a decrease in palmitate oxidation in myocardial homogenates from the treated animals. The homogenates from the toxin-treated animals showed normal rates of glucose and hexanoate oxidation. Palmityl CoA-carnitine acyltransferase activity was markedly depressed. The addition of carnitine to myocardial homogenates of toxin-treated animals restored both the depressed palmitate oxidation and the palmityl CoA-carnitine acyltransferase activity to normal levels. The administration of carnitine to hypoglycin-treated mice prevented the decreases in myocardial palmitate oxidation, palmityl CoA-carnitine acyltransferase activity, and the hypoglycemia. The data suggest that hypoglycin administration results in an inhibition of the rate-limiting enzymatic step in long-chain fatty acid oxdation, which can be reversed by carnitine.

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OXIDATION OF LONG-CHAIN FATTY ACIDS BY RAT LIVER MITOCHONDRIA

D. M. Gibson

The initial step in the oxidation of a long-chain fatty acid by mitochondria is the formation of an acyl CoA ester. In recent studies of Rossi, Galzigna, and Gibson three separate acyl CoA synthetase sites have been identified in rat liver mitochondria. If the ATP which is initially present in isolated mitochondria is depleted by pretreatment with dinitrophenol (DNP), external ATP will initiate fatty acid oxidation provided that either carnitine or oligomycin is added concurrently. The acyl CoA synthetase which is the starting point for the carnitine-dependent route of fatty acid oxidation is not inhibited by atractylate. However the oligomycin-dependent pathway is blocked by atractylate. The observation that no carnitine is required for the fatty acid oxidation initiated by ATP and oligomycin suggests that a second acyl CoA synthetase site exists within the outer mitochondrial membrane. Oligomycin blocks DNP-induced ATP-ase thereby permitting ATP (and fatty acids) to pass inside.

In phosphate-free media fatty acids are oxidized by DNP-treated mitochondria in the absence of added ATP, carnitine, or oligomycin. This DNP-insensitive route probably relies on the GTP-specific fatty acyl CoA synthetase which has recently been purified from mitochondria. Long-chain acyl CoA formation by the isolated enzyme is inhibited by orthophosphate.

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CONTROL OF FATTY ACID BIOSYNTHESIS

P. R. Vagelos, A. W. Alberts, John Elovson, A. R. Larrabee and D. F. Silbert

Acyl carrier protein (ACP) plays a central role in all enzymatic systems that synthesize fatty acids *de novo*. This conjugated protein contains a prosthetic group, 4′-phosphopantetheine, to which all the biosynthetic intermediates are linked through a thioester bond. Although the peptide structure of ACP is important in determining the activity of the acyl-ACP derivatives with various enzymes, presence of the prosthetic group ultimately determines whether ACP will be active or inactive within a cell.

The biological control of the concentration of holoACP has been studied in *Escherichia coli*. The concentration of the holoACP is rigidly controlled in these cells. Two enzymes have been discovered which might govern the concentration of ACP in the cell. One of these, ACP hydrolase (ACPase), catalyzes the specific hydrolysis of ACP to yield apoACP and the prosthetic group, 4′-phosphopantetheine. A second enzyme, ACP synthetase, catalyzes the transfer of 4′-phosphopantetheine from CoA to apoACP to yield biologically active holoACP.

Another approach to the study of the control of fatty acid biosynthesis is through classical biochemical genetics. Mutants of *E. coli* are being selected which have a specific requirement for long-chain fatty acids for growth. One such fatty acid biosynthetic mutant requires unsaturated fatty acids. *In vivo* and *in vitro* experiments have indicated that this mutant is unable to synthesize unsaturated fatty acids. Such mutants can be utilized to study the factors determining characteristic cellular fatty acid composition and perhaps to gain some insight into the physiological function of fatty acids.

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FATTY ACID SYNTHETASES FROM AVIAN AND MAMMALIAN LIVER

J. W. Porter, D. N. Burton, P. H. W. Butterworth, A. G. Haavik, E. J. Jacob and J. E. Nixon

The isolation of the pigeon liver fatty acid synthetase system as a stable multienzyme complex (mol wt 450,000) is well documented [Hsu, Wasson and Porter, J. Biol. Chem. *240*, 3746 (1965)]. A second soluble animal fatty acid synthetase (obtained from rat liver) has now been isolated as a homogeneous multienzyme complex and the latter has been stabilized in a buffer of high ionic strength containing 0.01 M reduced thiol (dithiothreitol). Preliminary studies on the properties of the rat liver system indicate that they are similar to those of the pigeon liver fatty acid synthetase. The molecular weight and the fatty acid synthesizing activity are of the same order and neither system requires flavin. Further studies with the pigeon liver fatty acid synthetase have demonstrated its dissociation into at least seven major peptide subunits. This result is obtained by treatment of the complex with a mixture of phenol, acetic acid and urea, and subsequent electrophoresis on polyacrylamide gel. N-terminal amino acid analysis yields similar results. Previous studies demonstrated that the substrates (acetate and malonate) for fatty acid synthesis are covalently bound to the protein as thioesters. In current studies enzyme containing covalently bound acetate or malonate has been subjected to proteolytic digestion and the resulting peptides have been fractionated by cation exchange chromatography, molecular filtration and high-voltage electrophoresis. One substrate-containing peptide which has been extensively purified has been found to contain phosphate and equimolar amounts of substrate, β-alanine and taurine (after oxidation). Further studies on the characterization of this prosthetic group are in progress. When the pigeon liver fatty acid synthetase is incubated with acetyl-CoA and 2-^{14}C-malonyl-CoA in the absence of NADPH, β-hydroxym thylglutaryl CoA (HMG CoA), triacetic acid lactone (TAL) (and a small amount of free triacetic acid) and acetoacetic acid are formed. About twice as much TAL as HMG CoA is formed. The activities for the formation of the latter compounds purify with the activity for fatty acid synthesis.

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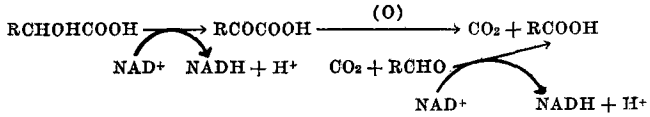
THE ALPHA OXIDATION SYSTEM OF BRAIN MICROSOMES

J. F. Mead

Stumpf and James and their co-workers have demonstrated a fatty acid degradation system in higher plants that appears to involve a peroxidative attack on the alpha carbon with the possible formation of the hydroxy acid and the aldehyde as intermediates and with the one-carbon shorter fatty acid as the product. Mead and Levis found evidence for an alpha oxidation system in rat brain *in vivo* and a system that led to decarboxylation of alpha-hydroxy long-chain fatty acids in a brain microsomal fraction. This enzyme system required the microsomal fraction and the 110,000 × *g* supernatant and was markedly stimulated by ATP and NAD. However, although the alpha keto acid was also rapidly decarboxylated under the same conditions and could be shown to be formed during decarboxylation of the alpha hydroxy acids under special conditions, neither the keto acids nor the long-chain aldehydes could be shown to be intermediates. More recently, a requirement for NAD has been shown by the use of NADase and the keto acid can be detected in the reaction medium during decarboxylation of the hydroxy acid. This and other evidence now makes it seem likely that the reaction proceeds as follows:



Whether the decarboxylation of the keto acid also involves an NAD-requiring dehydrogenation of the aldehyde or whether it may be an oxidase mediated by Fe^{++} and ascorbate has not been determined.

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FACTORS CONCERNED IN THE REGULATION OF LIPID SYNTHESIS IN DEVELOPING TISSUES OF HIGHER PLANTS

P. K. Stumpf

Some of the stiking features of lipid synthesis in developing tissue of higher plants are the controls imposed on the tissue at a given period of its development with regard to rate of synthesis as well as types of fatty acids synthesized during that period. Discussion will revolve around the path of the initial product of photosynthetate namely, sucrose, to the final product which accumulates in the endosperm tissue, triricinolein, the relationship between the micostructures of endosperm tissue and the function of these structures to lipid synthesis, and a careful survey of lipid synthesis in developing endosperm tissues of *Ricinus communis L.* in terms of the properties of the mixed function oxygenase which specifically hydroxylates oleyl CoA to ricinolyl CoA, the major fatty acid of endosperm tissue.

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BIOSYNTHESIS OF CYCLOPROPANE AND BRANCHED FATTY ACIDS

J. H. Law

Fatty acids which contain a cyclopropane ring occur widely in bacteria, ciliated protozoans and plants of the *Malvaceae* and *Sterculiaceae* families. Studies with isotopically labeled precursors indicate that these acids are formed from the corresponding olefins by addition of a methylene group arising from the methyl group of methionine. In this process the olefinic protons and two of the methionine methyl protons are retained.

A soluble enzyme has been purified from extracts of the bacterium, *Clostridium butyricum*, which catalyzes the following reaction:

unsaturated phospholipid + S-adenosylmethionine
→ cyclopropane phospholipid + S-adenosylhomocysteine

Various phospholipids serve as substrates, depending upon the conditions of incubation. The enzyme shows strict stereospecificity for natural 3-phosphoglycerol derivatives. It shows a positional preference for olefinic acids in fatty acids esterified at the 1-position of the 3-phosphoglycerol derivatives.

The enzymatic synthesis of 10-methylstearic acid is an analogous process catalyzed by extracts of *Mycobacterium phlei*. Incubation of these extracts with labeled adenosylmethionine leads to the formation of a labeled unsaturated fatty acid which may be 10-methylene stearic acid.

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ELECTRON MICROSCOPIC STUDIES ON LYTIC MECHANISMS

S. C. Kinsky and Sarah A. Luse

Previous studies with lipid monolayers and bilayers have suggested that polyene antibiotics, such as nystatin, amphotericin B, and Filipin, interfere with the ability of the cell membrane to function as a selective restraining barrier by interaction with the sterol localized in the membrane of sensitive organisms [Kinsky et al., Federation. Proc. *25*, 1503 (1966)]. In the present investigation, the effect of Filipin on the morphology of erythrocyte ghosts and lipid dispersions was examined by negative staining, and compared with the effects produced by other lytic agents.

Filipin produces "pits" in lecithin:cholesterol dispersions (i.e., dark areas, approximately 140 Å in diameter, surrounded by a light band). Similar pits are present in ghosts obtained by lysis of either rat or human erythrocytes with the antibiotic. Spherical substructures can be detected in the light bands which may be indicative of a lamellar to micellar phase transition. Lipid dispersions, devoid of cholesterol, retain their characteristic lamellar appearance when treated with Filipin suggesting that pit formation may represent the terminal stage in the mechanism by which the antibiotic affects cell membrane structure. This contention is also supported by the observation that Filipin, whose biological activity has been completely destroyed by brief illumination with visible light in the presence of flavinmononucleotide, has no visible effect on lecithin:cholesterol dispersions. Furthermore, perhydrofilipin, which has 1/100 the hemolytic potency of Filipin, also does not produce pits but results in a pattern which is similar to that obtained with lysolecithin [cf. Bagham and Horne, J. Mol. Biol. *8*, 660 (1964)]. The pits produced by Filipin are clearly distinguishable from those induced by saponin (also a hemolytic agent which complexes with sterols). Whereas the former are nearly circular and distributed randomly, the pits produced by saponin are not isolated but occur in regular array and are hexagonal in shape [cf. Lucy and Glauert, J. Mol. Biol. *8*, 727 (1964)]. The pits produced by Filipin are remarkably similar to those found in erythrocyte ghosts, or bacterial protoplasts, after immune lysis in the presence of complement [cf. Rosse et al., J. Exptl. Med. *123*, 969 (1966)].

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CELLULAR ORGANELLES AND LIPIDS

J. J. Wolken

The living cell is a complex system of macromolecular structures enclosed within a semipermeable membrane. Cells vary in shape, size and internal organization depending upon their environment and function. The cell is a dynamic system concerned with the processes of energy conversion and transfer, processes so necessary for maintenance, growth and reproduction. To do so the cell has evolved subcellular organelles, for example, nucleus, mitochondria, endoplasmic reticulum, chromatophores, and more highly specialized organelles such as the flagella for cell movement, nerve membranes to initiate excitatory phenomena, photoreceptors such as chloroplasts for photosynthesis, retinal rods and cones for vision, and other receptor organelles that respond to the environmental stimuli. The cell membrane and these organelles are highly ordered structures, double layers, of the order of 100 Å in thickness, of lipid and protein. Lipids make up a considerable part of these lamellar structures. Phospholipids in these membranes, for example, tend to form molecular layers (lamellae) observed as myelin structures. Such a highly ordered molecular structure greatly increases the surface area and minimizes the volume, thus providing space for pigments, reacting cites for enzymes and close association with other necessary molecules. Besides the phospholipids, lipids also include the phytols, the carotenes, and vitamin A, molecules necessary for light capture and to the function of all photoreceptors. Therefore we are investigating the properties and molecular organization of the pigment-lipid-protein and associated enzymes in these organelle structures in attempts to elucidate how they function as physical-chemical systems. From these studies experimental systems of pigments-lipids-proteins have been devised for clues as to how such model systems may function in the living state.

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RED BLOOD CELL LIPIDS AND THE PLASMA MEMBRANE

D. G. Cornwell, R. E. Heikkila and R. S. Bar

The red blood cell occupies a unique position both in the historical development of membrane theory and current experimentation on membrane properties. The cell is readily accessible and has been studied in many animal species and disease conditions. Differences in composition, structure, and properties have been described and a number of highly specific cell types are now available for investigation.

Several techniques are used to study the membrane of the intact cell. The membrane surface may be labeled in specific areas by the selective exchange of lipids such as cholesterol. Exchange studies provide information about lipid-lipid and lipid-protein interactions in the intact structure. Membrane lipids are hydrolyzed by phospholipases and enzymatic activity is modified by the action of penetrating hemolytic agents. Permeability is readily measured by hemolysis and correlated with chemical structure, partition coefficient, and biological activity of different metabolites and drugs. The red cells of vitamin E deficient animals are particularly susceptible to hyperoxia. Hemolysis in these cells is correlated with alterations in membrane structure through the formation of lipid peroxides.

Gorter and Grendel first used monolayers prepared from red cell lipids to show that sufficient lipid was present in the membrane to form a bimolecular layer. Lipid extracts from the red cells of different animal species and disease states have been used in recent monolayer studies. The surface properties of these lipids and their purified neutral lipid and phospholipid fractions yield additional information about lipid-lipid interactions which may exist in the membrane. Enzyme hydrolysis and penetration studies indicate that lipids in monolayers and membranes have similar properties.

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FATTY ACID ESTERS OF MONOMERIC AND POLYMERIC LACTIC ACID

A. E. Thomas, III, and J. A. Kelers

Fatty acid esters of monomeric and polymeric lactic acid have been analyzed. These esters, which are used as dough conditioners in bread and pie shortenings, as aerating agents in cake shortenings and cake mixes, and as starch complexing agents in dehydrated potatoes are converted into their methyl ester derivatives with diazomethane. The derivative can be analyzed by temperature-programmed gas-liquid chromatography. This procedure was applied to the above esters composed of fatty acids ranging from 12–20 carbon atoms and of lactic acid or its polymers ranging from the monomer to the hexomer. Free fatty acids were also determined as their methyl esters.

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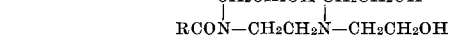
IDENTIFICATION OF AN AMIDO-AMINE COMPONENT IN DIETHANOLAMIDES

J. H. Benedict and Mary Ellen Wittekind

Diethanolamide products made by the condensation of diethanolamine and fatty acid are known to contain, in addition to the amide, excess diethanolamine, fatty acid soap of diethanolamine and water. In a recent examination of some amide products, a new amido-amine compound was isolated and identified. This compound is formed from two moles of diethanolamine and one mole of fatty acid and is present at levels as high as 10% of amide product.

The amido-amine was isolated by extraction with isobutanol from an acidic solution of the amide. The structure of this new compound, which was verified by its chemical reactivity and by instrumental analyses, is as follows:



Synthesis studies and methods of analyses will also be discussed. The presence of other compounds, such as substituted piperazines, were not detected.

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ANALYSIS OF TRACE PEROXY COMPONENTS AND IMPURITIES IN INTRAVENOUS FAT EMULSIONS

Judith M. Schultz and Arthur Rose

Procedures are described for determining the extent of the impurities and minor components found in intravenous fat emulsions. New data and information are presented on peroxides and spray reagents for peroxy-type lipids, on pH and chemical analysis, on storage problems with frozen emulsions, and on an experiment in densitometry as applied to analysis of intravenous fat emulsion systems. Advances in thin-layer chromatography separation procedures and colorimetric tests have made it possible to examine the emulsions and their components for peroxide content and chemical variability, whether due to impurities or changes during storage and handling. The results of the investigations on peroxides are compared with data obtained from published procedures in an attempt to establish some guidelines in studies involving peroxy and other polymeric materials present in fat emulsions. Use of the simple procedures described can give valuable information on the extent of impurities in intravenous fat emulsions and can thus serve as a guide in animal and clinical testing of emulsions.

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AROMATIC ACIDS OF CARNAUBA WAX

L. E. Vandenburg and E. A. Wilder

Cinnamic acid with *para*-hydroxy and *para*-methoxy substitution has been isolated from carnauba wax in a yield of about 5%. The *para*-hydroxy cinnamic acid accounts for approximately 75% of the total aromatic acids.

These hitherto unreported aromatic acids occur predominantly as part of a polymerizable diester of approximate average molecular weight 1025. Certain properties attributed to carnauba wax are believed to be due to the presence of about 30% of these diesters. Except for trace amounts, no significant quantities of these aromatic acids could be isolated in the free state from the whole wax.

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A SENSITIVE METHOD FOR THE DETECTION OF CHEMICAL CHANGES IN THE LIPIDS OF EGG YOLK TREATED WITH HYDROGEN PEROXIDE

Lee-Shin Tsai and L. M. Smith

Hydrogen peroxide is a useful bactericide and is being employed commercially in pasteurization treatments to improve the quality of liquid egg white and whole egg. The addition of H_2O_2 makes it possible to reduce the temperatures and times employed below the point of heat damage to the functional properties of the egg product. However, very little is known about the basic effects of H_2O_2 treatments on white and yolk constituents. We have developed a spectrophotometric method to detect the slight chemical changes in egg lipids produced by dilute H_2O_2.

Lipids extracted from raw egg yolk have an absorption peak at 267 mμ and the absorptivity at this wavelength increases linearly when the lipids are held in air. Our method is based on the observation that lipids extracted from H_2O_2-treated yolk and held in air at 80C, show faster rates of increase in absorptivity than the control samples.

To test the method, both whole egg yolk and yolk lipids were subjected to mild treatment with H_2O_2 and then examined. Lipid samples were extracted from yolk treated with 0.3%, 0.1%, or no H_2O_2 at 37C for 60 min. The treated samples showed faster increases in absorptivity with holding time at 80C than the control samples, even though differences were not detected by thin-layer chromatography, infrared spectroscopy, or induction period determinations. The sensitivity of the method was further confirmed in tests on egg yolk treated with 0.150%, 0.125%, 0.100%, 0.075%, 0.050%, or no H_2O_2 at 57.4C (135.5F) for 3.5 min. All the H_2O_2-treated samples had higher rates of increase in absorptivity at 267 mμ than the control samples. Lipids from yolk treated with 0.150% H_2O_2 gave the highest rate.

Total lipids were extracted from yolk and separated into phospholipid and phospholipid-free fractions. Aliquots from each fraction were treated with 1.0% H_2O_2 for 10 min at 20C, then the H_2O_2 was removed and the lipid fraction held in air at 20C. Both treated and control phospholipid-free samples showed no increase in absorptivity at 267 mμ up to 8 days. However, both treated and control phospholipid samples rapidly increased in absorptivity and the rate for the treated sample was higher.

It is concluded that the spectrophometric method may be useful to indicate slight changes in the lipids of yolk or whole liquid egg treated with dilute H_2O_2.

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SEARCH FOR NEW INDUSTRIAL OILS. XV. SEED OILS OF BORAGINACEAE

R. W. Miller, F. R. Earle and I. A. Wolff

The fatty acid composition of seed oils has been determined for 33 species of *Boraginaceae*. Iodine values of the oils range from 72 to 216. All-*cis* 6,9,12-octadecatrienoic acid, a source material for the synthesis of prostaglandins, occurs in every member (27 species) of the subfamily Boraginoideae in amounts from 0.2 to 18%. It is also in one species of another subfamily to the extent of 0.8%. All-*cis* 6,9,12,15-octadecatetraenoic acid occurs in most of the same oils in amounts up to 15%. The predominant common unsaturated acid varies among the oils, with the maxima represented by the 45% linolenic in *Arnebia griffithii*, the 69% linoleic in *Heliotropium strigosum* and the 63% oleic acid in *Cynoglossum creticum*. Oils of the tribe Cynoglosseae of the subfamily Boraginoideae contain smaller amounts of linoleic acid than other oils of the family which have equal iodine values. Many Boraginaceae oils contain traces of components more

volatile in gas chromatography than the usual triglycerides; the seed oil of *Cordia verbenacea* has a significant amount, 23%. It also differs from the other samples in its large content, 43%, of C_{20} acids.

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SOME HEATED FAT COMPONENTS IDENTIFIED

N. R. Artman and J. C. Alexander

Soybean oil, hydrogenated to I.V. 78, was heated for 84 hr in a commercial frying kettle at 182C. The oil was not used for frying. The oil was converted to ethyl esters which were distilled and treated with urea. The distillable, non-urea-adducting fraction (DNUA) was subjected to repeated column chromatography on silicic acid and on silica gel-silver nitrate, and to gas chromatography. Several of the materials obtained appeared to be either single substances or mixtures of closely related positional isomers. Several of these were characterized by physical methods, and more or less specific structures were assigned to them. Among them were hydrocarbons, aromatic esters, saturated and unsaturated cyclic esters, hydroxy- and keto-containing esters.

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SPECIFICITY OF HYDROLYSIS IN HEATED TRIGLYCERIDES

C. Buziassy and W. W. Nawar

The purpose of this study was to determine whether there is a fatty acid or positional specificity in thermal hydrolysis of triglycerides. Samples were heated in the presence of water under controlled conditions and the released fatty acids quantitatively analyzed by gas chromatography. Experiments with both a mixture of monoacyltriglycerides and glycerides with equimolar amounts of randomly distributed fatty acids showed a preference for the hydrolysis of the shorter chain and the unsaturated fatty acids. The C_4, C_8, C_{12}, C_{16} and $C_{18:1}$ fatty acids were used in the above mixtures.

A trilaurin in which the fatty acid in the 2-position is labelled with C^{14} was synthesized. When the free acids released by heat were analyzed by a combination gas chromatographic-radioactivity detector system, no evidence for a positional specificity was apparent.

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THE FATTY ACID AND FATTY ALDEHYDE COMPOSITION OF THE MAJOR PHOSPHOLIPIDS OF MOUSE BRAIN

G. Y. Sun and L. A. Horrocks

Individual phospholipids were separated from mouse brain lipid extracts by preparative thin-layer chromatography. Methyl esters were prepared from the intact phospholipids by direct transmethylation in the presence of silica gel using 0.5 M NaOH—methanol at room temperature in order to prevent interference by aldehydes or derivatives. Dimethyl acetal derivatives of aldehydes were prepared using 5% concentrated hydrochloric acid in methanol, followed by preparative thin-layer chromatography for isolation. The major phospholipids present were ethanolamine phosphoglycerides, 39.1%, choline phosphoglycerides, 40.9%, serine phosphoglycerides, 15.2%, and sphingomyelin, 4.9%. One-fifth of the total phospholipids were in the form of plasmalogens, of which more than 90% was found in the ethanolamine phosphoglycerides. Choline and serine plasmalogens were present in trace quantities. The major aldehyde components of the ethanolamine plasmalogens were 16:0, 18:0, and 18:1. The ethanolamine phosphoglycerides were rich in long-chain polyunsaturated fatty acids, including 28.3% of 22:6, and 16.8% of 20:4, but contained only 7.1% of 16:0. In contrast, the choline phosphoglycerides contained 39.2% of 16:0, and 30.9% of 18:1 with a very small content of polyunsaturated fatty acids. The serine phosphoglycerides exhibited a still different pattern containing 37.4% of 18:0, 22.7% of 18:1, 23.7% of 22:6, 2.8% of 16:0, and 3.9% of 20:4. No fatty acids with a chain length of more than 22 carbon atoms were observed in sphingomyelin under these conditions.

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MECHANICAL DESIGN REQUIREMENTS OF PRESSURE LEAF FILTERS FOR THE FATTY OIL INDUSTRY

T. J. Warning

Although the filtration applications from one fatty oil plant to another are generally the same, there are certain specific conditions which do vary. Because of these varying conditions, the mechanical design requirements of the filtration equipment will depend on the specifics of the plant in question. A great deal has been accomplished in recent years in the development of design improvements to the point where the list of tried and proven filter designs and features is quite extensive. This paper is an attempt to make you aware that this relatively wide selection of equipment exists and to discuss the selection of design that most ideally fills the needs and conditions of a specific plant.

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PROCESSING OF OILSEEDS ABROAD COMPARED TO PROCESSING IN THE UNITED STATES

L. R. Watkins

Most oil mills in the US normally confine their crushes to cottonseed or soybeans, while their counterparts abroad are many times called on to crush a variety of oilseeds. This variety along with many unusual economic factors cause some processing practices used abroad to seem unrealistic.

This paper will attempt to touch on some of the economic factors and natural conditions encountered abroad as they affect oil mill design and operations in the various departments of the oil mill.

Cottonseed oil mills in the US may be forced to use some of the unorthodox practices as other seed crops slowly move into their areas.

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AQUEOUS ACETONE EXTRACTION OF COTTONSEED

W. A. Pons, Jr. and P. H. Eaves

Extraction of cottonseed flakes with acetone containing 25–30% water removes essentially all of the gossypol, most of the free fatty acids, half the raffinose and negligible quantities of neutral oil and protein. After drying and reflaking of the aqueous acetone extracted marc, the oil may be removed either by hexane extraction or mechanical pressing to produce meals essentially free of gossypol pigments and exceptionally high in protein and available lysine content. Crude oils are light-colored, contain negligible gossypol, high neutral oil content and refine and bleach to a prime color value. The process is effective for the removal of toxic mold metabolites such as aflatoxins from mold-damaged seed.

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THE PRODUCTION OF PROTEIN CONCENTRATES BY AIR CLASSIFICATION OF DEFATTED COTTONSEED FLOUR

Wilda H. Martinez, Leah C. Berardi, V. F. Pfeifer and A. J. Crovetto

Appropriate grinding of solvent extracted cottonseed meals provided flours which were air-classified to produce protein concentrates. Fractions from a four-cut profile of glandless and glanded cottonseed flours were analyzed for protein, fat, total and reducing sugars, fiber, gossypol, ash, phosphorus and calcium.

Microscopic examination indicated that the grinding of defatted cottonseed meal in a pin mill ruptured the cell wall without disrupting the integrity of the aleurone grains or protein bodies of the cell and that the finest air-classified fraction consisted predominantly of aleurone grains. A minimum of cell wall fragments and pigment glands (in the glanded materials) could also be observed in this fraction. As the mass median diameter of the fraction increased, the number of hull particles, pigment glands (in the glanded materials) and cell fragments which consisted of clusters of aleurone grains embedded in the cytoplasmic matrix increased.

Protein, ash and phosphorus contents increased as the mass median diameter of the fraction decreased. The concurrent increase in protein and phosphorus is in agreement with the cytology of the seed. All other chemical constituents investigated decreased. In the three flours studied, the two finest fractions which represented 50% of the weight of the flour averaged 68 to 73% protein on a dry weight basis. Differences in the molecular weight and amino acid profiles of the whole meal and the finest fraction indicated that fractionation of the proteins of the seed also occurred.

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OPTIMIZATION OF NEW PROCESS FOR PRODUCTION OF ALDEHYDIC ESTERS FROM SOYBEAN OIL

P. E. Throckmorton, L. I. Hansen, R. Christenson, J. N. Kellen and R. M. Gilbert

Reaction of ozone with unsaturated fatty compounds and reduction of the ozonide provides fatty aldehydes. The process had previously been carried out in the laboratory and small lots using methyl oleate as a starting material. The reductive ozonolysis products from methyl oleate are pelargonaldehyde and the aldehydic-ester, methyl azelaaldehydate (MAZ). Such material is conveniently isolated as the dimethyl acetal (MAZDA). Manufacture of MAZDA from soybean oil has now been accomplished on a pilot plant scale in high yield by this process. The process was developed through a factorially designed laboratory experiment to optimize the process variables. The process was then demonstrated by employing the optimum conditions to operate a continuous ozonolysis sieve-tray reactor. The predicted yield of MAZDA, which was based on regression analysis and optimization of the laboratory data, was achieved.

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ESTIMATION OF CRUDE FIBER IN DEHULLED SOYBEANS USING MICROSCOPY AND POLARIZED LIGHT

R. E. Anderson and K. E. Holt

The determination of crude fiber in soybeans is a lengthy and time-consuming procedure requiring extraction, digestion and incineration and is not suitable as a routine processing control tool. Described in this paper is a short control procedure employing photomicroscopy and is based on the characteristics of soybean hull cellulose to rotate plane polarized light. The sample is mounted on a microscope slide, placed in a polarizing projection microscope and the screen image compared with a series of standard photomicrographs. Estimation of crude fiber in dehulled soybeans can be made in 15 min compared to 8 to 12 hr using the official digestion procedure.

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STUDIES ON PHOSPHOLIPID MONO- AND BILAYERS

R. A. Demel

Lipids play a fundamental part in the lipid-protein network of biological membranes. Some of the variations in biological membranes may be brought about by differences in the chemical structure of the lipid constituents. A study was made by the interfacial force-area characteristics of saturated and highly unsaturated phospholipids and lyso phosphatidyl compounds. The action of some of these phospholipids on lipid bilayers has also been studied. Comparisons were made between the interfacial behavior of individual phospholipid species which were chemically synthesized and phospholipids from natural source. The influence of diets on the force-area characteristics of liver lecithins has been studied. From studies of mixed monolayers of cholesterol and phospholipids it was found that, the mean area per molecule in mixed films of cholesterol with (1,2-distearoyl)-3-lecithin and (1,2-didecanoyl)-3-lecithin at 22C followed practically the additivity rule. A condensing effect of cholesterol was evident with films of (1-stearoyl-2-lauroyl)-3-lecithin; (1,2-ditetradecanoyl)-3-lecithin, (1-stearoyl-2-oleoyl)-3-lecithin and the corresponding ethanolamine analogue, as well as with (1,2-dioleoyl)-3-lecithin at 22C. At 5C the condensation effect with (1,2-ditetradecanoyl)-3-lecithin was much reduced. The very expanded films of synthetic lecithins and phosphatidylethanolamines containing linoleic or linolenic acid showed no appreciable condensation effects with cholesterol. The behavior of the mixed-phospholipid films is governed by a number of factors, including van der Waals interactions, configurational entropy effects and alterations in the structure of water adjacent to the monolayers. These factors depend on chain length and degree of unsaturation.

Polyene antibiotics are found to lyse fungi, protozoa and erythrocytes while bacteria protoplasts and blue-green algae are not. Cholesterol as well as lecithin were found by some authors to reduce the effective polyene antibiotic concentration. It therefore seemed desirable to determine whether polyene antibiotics can interact with lipids other than sterols. Filipin, nystatin, amphotericin B, etruscomycin and pimaricin readily penetrate monolayers of cholesterol and ergosterol at initial surface pressures greater than the collapse pressure of the antibiotics. Under the same conditions there was essentially no interaction with a variety of pure synthetic phospholipids unless sterol was present. Filipin did not penetrate monolayers prepared from polyene-insensitive bacteria. The increase in surface pressure of mixed films of phospholipid and cholesterol after the injection of filipin was highly dependent on the relative quantity of sterol as well as on the molar ratio lipid-polyene antibiotic. From the results of mono- and bilayer experiments which are in very good agreement with the physiological experiments it is concluded that cholesterol is a necessary membrane constituent for the polyene antibiotic action.