# Report of the Literature Review Committee

## Annual Review of the Literature on Fats, Oils and Detergents Part V.

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## COMPOSITION AND CHARACTERISTICS

### OFFICIAL METHODS AND REVIEWS

The Commercial Fats and Oils Analysis Committee of the AOCS reported on several subjects (Stillman et al., *JAOCS 41*, 454). The FAC Color Standards Subcommittee reported that it had agreed upon a new set of permanent glass color standards to replace the liquid standards in current use. A new subcommittee on Fish and Marine Oils in Vegetable Oils was formed. The Hydrocarbons Subcommittee reported that they had developed a column chromatography procedure for determining hydrocarbons in fats and oils. The Oxygen Absorption Subcommittee has been carrying out comparisons of the AOM and bomb methods for determining oxygen uptake. The Refining Subcommittee reported that it has been working on determination of the refining loss in sesame oil. A new procedure for neutral oil (Ca 9F-57) was approved. The Epoxidized Oils Subcommittee reported comparative tests of three methods for oxirane oxygen and suggested some modifications of the AOCS Method Cd 9-57 (Barlow et al., *Ibid.* 86). The Gas Chromatography Subcommittee of the Instrumental Techniques Committee reported the comparative analyses of some samples of menhaden, castor, dehydrated castor, and tall oil (O'Connor et al., *Ibid.* 158). Additional work needs to be done on identifying the components of menhaden oil. The methods for tall oil and castor oil are satisfactory. The Special Task Group for Preparation of Methyl Esters reported that the use of $BF_3$ or sulfuric acid as a catalyst had little effect on the determination of *trans* unsaturation.

A review of the 1962–3 literature on composition and characteristics was compiled by the AOCS Literature Review Committee (Mahadevan and Hammond, *Ibid.* 630). Reviews were published on gas chromatography (Van Reysselberge, *Ind. Chim. Belge 6*, 575; Horning and Vandenheuvel, *JAOCS 41*, 707; and Jaforte, *Riv. Ital. Sostanze Grasse 40*, 678); thin-layer chromatography (Mangold, *JAOCS 41*, 762; Loury, *Rev. Franç. Corps Gras 11*, 259; and Hiroshi and Yasuhiro, *Yukagaku 12*, 597); neuclear magnetic resonance (Melera, *Riv. Ital. Sostanze Grasse 41*, 70); rheology (Tsuchiya, *Yukagaku 13*, 100 and Date and Fukada *Ibid.* 52); the physico-chemical properties of fatty oil solutions (Kusane, *Ibid.* 185); the separation of unsaturated fatty acids. (Takagi, *Ibid.* 247); the evaluation of unfamiliar vegetable oils (Mills, *J. Oil Colour Chemists' Assoc. 47*, 187); the composition of oiticica oil (Anon., *Rev. Afg. Grasas Aceites 5*, 62); the composition of isano oil (von Mikusch (*Farbe Lack 70*, 101); the Haucherorne reaction for adulteration in olive oil (*Olearia 17*, 106); detection of re-esterified olive oil (Adam, *Lipidos 22*, 117); sesamin, sesamolin, and related compounds (Budowski, *JAOCS 41*, 280); composition of margarine (Montefredine, *Riv. Ital. Sostanze Grasse, 1962 Symposium Issue*, 97); the structure of marine fatty acids (Ackman, *J. Fisheries Res. Board Can. 21*, 247); cutaneous lipids (Wheatley, *Drug Cosmetic Ind. 93*, 161); and gangliosides (Svennerholm, *J. Lipid Res. 5*, 145).

### ANALYSIS OF FAT SOURCES

The AlfaLaval method for determining the fat content of milk was advocated as having precision equal to the Babcock-Gerber test and possessing several operating advantages (Randolph and Gould, *Ind. Aliment 2*, 60). The fatty acid composition of various fats and oils determined by gas chromatography was found to be unaffected by a number of solvent and extraction procedures commonly used (Hivon et al., *JAOCS 41*, 362). A study of the extraction of cholesterol from serum lipoproteins with heptane led to the conclusion that the degree of extraction depends on the disruption of the lipoprotein structure at interfaces and that other proteins may protect the lipoprotein from disruption (Zilversmit, *J. Lipid Res. 5*, 300). Lipid extracts in chloroform-water solution could be purified by passing them through a Sephadex column (Wells and Dittmer, *Biochemistry 2*, 1259). This procedure removed amino acids, carbohydrates, nucleotides, and inorganic contaminants completely and gave quantitative recoveries of lipid.

The possibility of predicting the percentage of protein and solids-not-fat in milk from fat determinations was investigated (Horwood and Williams, *Spectrochim. Acta 19*, 1351). The lipid content of oats was found to be inversely proportional to its protein content (Pokorny et al., *J. Inst. Chem. Tech. Prague 7*, 223). The iodine value and oil content of various locations on the endosperm and embryo of several seeds were found to vary (Kartha, *J. Sci. Food Agr. 14*, 515).

Refining losses in sunflower seed oil were unrelated to the moisture content but approximately equal to the free fatty acid plus a constant factor (Nosti, *Grasas Aceites [Seville, Spain] 14*, 210). The moisture of butter was determined by passing the butter through a capacitor cell and measuring the dielectric constant (Fexa and Burianec, *Papers Inst. Chem. Tech. Prague 6*, 131). The standard error was 0.2%. The residual solvent in extracted meal was determined by letting the meal sample stand in a closed bottle at room temperature for one hour and reading the pressure on a water manometer (Lewis and Nelakantan, *JAOCS 41*, 211). The method would detect as little as 0.04% hexane.

## ANALYSIS FOR FUNCTIONAL GROUPS

The problem of completely characterizing an unfamiliar vegetable oil was discussed and illustrated (Mills, *J. Oil Colour Chemists' Assn. 47*, 187).

The free fatty acid content of blood serum could be determined by automatic titration of a single phase system obtained by adding isopropanol to nonpolar lipid extracts (Schnatz, *J. Lipid Res. 5*, 483). The free fatty acids in vegetable oils were determined colorimetrically as copper salts in benzene solution. The results agreed well with the titration method (Baker, *JAOCS 41*, 21). A similar procedure was used to measure the free fatty acids in peanuts and peanut products (Bains et al., *Ibid.* 831).

Glycerol and fatty acids of glycerides were estimated by a simplified hydrogenolysis-acetylation procedure by gas-liquid chromatography (Holla et al., *J. Lipid Res. 5*, 263). In cases where the fatty acids interfered, the glycerol could be estimated by a saponification-acetylation procedure. Preliminary acetolysis was necessary when this method was applied to phosphatidyl ethanolamine. A similar procedure for glycerol and fatty acids was reported (Mason et al., *Anal. Chem. 36*, 587). Free glycerol in blood serum was determined by acetylation and gas chromatography with butane-1,4-diol as an internal standard. As little as 0.01 mg of glycerol/100 ml of serum could be detected (Jellum and Bjornstad, *J. Lipid Res. 5*, 314). The estimation of glycerol by conversion to formaldehyde and colorimetric analysis was disturbed by the presence of proteins that absorbed the formaldehyde in lipoprotein lipase experiments (Levy and McGee, *Ibid.* 265).

The analysis of α-unsaturated ether and aldehydogenic lipids in rat liver was disturbed by the vitamin A. Vitamin A interferes with both the spectrophotometric determination of α-unsaturated ether with iodine and the estimation of aldehydogenic lipids as nitrophenylhydrazones (Camejo et al., *Ibid.* 75). Nuclear magnetic resonance was used to determine the total unsaturation, 15,16-double bonds, 1,4-pentadiences, and allylic methylene groups in hydrogenated methyl linolenate (Johnston et al., *JAOCS 41*, 788). The absorption maximum for pure methyl elaidate obtained by gas chromatography was found to be at 967.7 $cm^{-1}$ rather than 965.0 $cm^{-1}$. The apparent peak at 965.0 $cm^{-1}$ is caused by the presence of *cis*-methyl esters which have an absorption at 963.1 $cm^{-1}$ in some cases (DeFrancesco, *Riv. Ital. Sostanze Grasse 41*, 20). Conjugated double bonds were determined by a combination of the Hanus and bromine vapor methods in linseed oil samples which could not be investigated by the spectrophotometric technique. The conjugated double bonds do not react quantitatively by the Hanus method but do by the bromine vapor method (Pokorny, *J. Inst. Chem. Tech. Prague 5*, 51). Correlations were determined between the iodine value and refractive index of linseed and safflower oil so that the iodine value might be estimated by the refractive index (Price, *Australian Paint J. 9*, (4) 17).

Comparative determinations of oxirane oxygen with hydrogen chloride in ether, dioxane, and acetic acid and hydrogen bromide in acetic acid were made. Water in the sample decreased the accuracy. Hydrogen bromide–acetic acid was the most accurate reagent, and an improved procedure for preparing and standardizing the reagent was suggested (Suhara, *Yukagaku 13*, 378). Silicone rubber (SE-30) was suggested as the best stationary phase for the gas chromatographic determination of epoxyoleate. Ethylene glycol succinate, Carbowax, and Apiezon phases all led to decomposition of the oxirane group (Herb et al., *JAOCS 41*, 222). Cyclopropenoid fatty acids were determined in cottonseed oil by stepwise titration with hydrogen bromide at 3 and 55C after removal of interfering materials with alumina (Harris et al., *Ibid.* 309). Highly oxidized samples required conversion to methyl esters.

1-Monoglycerides were determined by oxidation with periodic acid and conversion of the resulting glycol aldehyde ester to the 2,4-dinitrophenylhydrazone which was determined colorimetrically. Glycerol does not interfere (Szonyi and Sparrow, *Ibid.* 535). To obtain 99% acetylation of monoglycerides in 30 min reflux, it was necessary to use 450% excess acetic anhydride (Tomankova and Pokorny, *J. Inst. Chem. Tech. Prague, 6*, 243). The reaction rate was highly dependent on temperature, and the time could be decreased to 10 or 15 min by reacting at 160C under slight pressure.

A polarographic method to determine cobalt and copper as their respective soaps was developed. Pyridine complexes of the soaps could be used with any of three electrolyte-solvent combinations (Malik and Rizwanul, *JAOCS 41*, 411).

## LIPID CLASS DETERMINATIONS AND STRUCTURAL ANALYSIS

GENERAL. The lipids of serum and serum lipoproteins were separated into hydrocarbons, cholesterol esters, triglycerides, cholesterol, free fatty acids, and phosphatides by chromatography on columns of Silica gel G. The recovery was quantitative except for phosphatides where the recovery was 60–80% (Crider et al., *J. Lipid Res. 5*, 479). A method for fractionating the lipid-pigment mixture of lucerene by chromatography on silicic acid columns was described. Thin-layer chromatography (TLC) revealed that the fractions were impure (Thirkell and Tristram, *J. Sci. Food Agric. 14*, 488).

The separation of various types of lipids by TLC was reviewed (Mangold, *JAOCS 41*, 762 and Loury, *Rev. Franç. Corps Gras. 11*, 259). A quantitative procedure for the fractionation of complex lipid mixtures was described which is based on chromatography on diethylaminoethyl cellulose followed by TLC. This method was compared with other methods based on column chromatography and TLC (Rouser et al., *JAOCS 41*, 836). Methods were devised for the separation of serum and tissue lipids into classes by TLC and recovery of each class from the plate for fatty acid analysis by gas chromatography. In the case of serum lipids, this method gave results comparable to column method for the neutral lipids, but the agreement was not as good for phosphatides, especially sphingomyelin (Bowyer et al., *Biochim. Biophys. Acta 70*, 423 and Dobiasova, *J. Lipid Res. 4*, 481). Techniques for quantitative recovery of lipids from thin-layer plates were described (Dobiasova, *Ibid.* and Goldrick and Hirsch, *Ibid.* 482). Neutral and phospholipids were determined quantitatively on thin-layer plates by charring and densiotometry of the charred spots (Blank et al., *JAOCS 41*, 371). Radioactive lipids were analyzed by autoradiography and densiotometry of the radiograms. A similar method for the determination of lipids on thin-layer plates by oxidation with acid dichromate and reading the absorbance at 350 μ was reported (Amenta, *J. Lipid Res. 5*, 270). A preparative TLC procedure for lipids was described that uses a thicker layer of adsorbent, a longer plate, and development with two consecutive solvents (Komarek et al., *Ibid.* 268). A simple method for the fractionation of blood lipids by TLC was described (Sachs and Wolfman, *Proc. Soc. Exptl. Biol. Med. 115*, 1138). TLC on silicic acid with solvents of carefully controlled water content permitted the separation of the lipids of lettuce and cabbage into a number of components. The chloroplast fraction contained a new unidentified lipid (Nichols, *Biochim. Biophys. Acta 70*, 417). Iodine vapors used as a detection agent were found to reduce the amount of polyunsaturated fatty acids that could be recovered from thin-layer plates. The more double bonds there were in the acid the greater the loss from iodine vapors (Nichman et al., *J. Lipid Res. 4*, 484). When Rhodamine 6G was used as a detection agent, the fatty acid analysis of fractions from thin-layer plates agreed with the results of column chromatography, indicating that little autoxidation occurred on the plates. Saran wrap (a commercially available film) can be used to protect portions of thin-layer plates when they are to be exposed to iodine vapors for detection of spots (Negishi et al., *Ibid. 5*, 486).

The glycerides, cholesterol esters, and free cholesterol of the lipids from 0.1 ml of serum can be determined by partitioning the lipids between 87% ethanol and petroleum ether. Three partitions with 3 ml of upper phase and 1 ml of lower phase yields a upper phase containing all the glyceride and no phosphatide. Three partitions with 1 ml of upper phase and 5 ml of lower phase leaves all the cholesterol ester in the upper phase and all the free cholesterol in the lower phase (Galanos et al., *Ibid.* 242). Direct fractionation of mixed neutral lipids into sterol, sterol ester, and triglyceride was achieved by gas chromatography (Kuksis, *Can. J. Biochem. Physiol. 42*, 419). The separation is by carbon number. The cholesterol ester and triglyceride content of lipid mixtures may be obtained by differences in their ester carbonyl absorption frequency in the infrared (Freeman, *J. Lipid Res. 5*, 236). A high resolution instrument must be used, and the phosphatides must be removed by a preliminary adsorption on silicic acid. The overall accuracy is ±5%.

GLYCERIDES. The triglycerides of serum or plasma were determined by separation in thin-layer plates of silicic acid 1 mm thick, elution of the glycerides and measurement by infrared spectrophotometry. The relation between absorptivity and concentration is not linear. The precision is 2% and the accuracy 4% (Krell and Hashim, *J. Lipid Res. 4*, 407). The seed oils of the Chinese tallow tree, *Sapium semiferum* and *Sebastiana lingustrina* were shown to be optically active. The optical activity was associated with the glyceride fraction and

was separated into several components by countercurrent distribution. The optically active fractions were rich in unsaturated carboxyl-conjugated fatty acids (Maier and Holman, *Biochemistry 3,* 270).

Triglycerides were separated on columns of silver nitrate-impregnated silica gel. The triglycerides were separated on the basis of the number of double bonds and whether they were *cis* or *trans*. When applied to palm oil six fractions were obtained (deVries, *JAOCS 41,* 403). The triglycerides of coconut oil and linseed oil and mixtures of mono-, di-, and trilaurin were separated on columns of rubber powder with methanol-acetone as the mobile phase (Trowbridge et al., *Ibid.* 306). The possibilities of separating glycerides by crystallization and co- and countercurrent distribution were discussed (Sambuc, *Rev. Franç. Corps Gras 11,* 319 and Naudet, *Ibid.* 326). Castor oil glycerides were successfully fractionated in a 100-tube countercurrent distribution apparatus according to the number of molecules of ricinoleic acid they contained (Achaya et al., *JAOCS 41,* 783).

Mono-, di-, and triglycerides were separated by thin-layer chromatography on silicic acid, and triglyceride with different degrees of unsaturation were separated on silver nitrate-impregnated silica gel (Jurriens et al., *J. Lipid Res. 5,* 267). The accuracy of the analysis was found to be quite good. The separation of triglycerides on silver-nitrate-impregnated silica gel was reported (Jurriens, *Riv. Ital. Sostanze Grasse 41,* 4). The triglycerides of a number of vegetable oils were separated by reverse phase TLC with liquid paraffin as a stationary phase and aqueous actic acid as the mobile phase (Anker and Sonanini, *Pharm. Acta Helv. 37,* 360). A reverse phase TLC method was applied to the separation of seed oil triglycerides and the fatty acid composition of each fraction was determined. In some cases a preliminary fractional crystallization was carried out (Gunstone et al., *Chem. Ind. [London] 1964,* 483). Partial separation of triglycerides was obtained by chromatography on uncoated glass paper with pyridine-water as a mobile phase. The glycerides could be saponified on the paper and the fatty acids separated by development in the second dimension (Swartwout and Gross, *JAOCS 41,* 378).

The specificity of lipases was reviewed (Desnuelle and Savary, *J. Lipid Res. 4,* 369). A procedure for rapid pancreatic hydrolysis of triglycerides, isolation of the products by TLC, and analysis of the fatty acid methyl esters from the fractions by gas chromatography was reported (Luddy et al., *JAOCS 41,* 693). Pancreatic lipase was found to release butyrate and palmitate at the same rate from 1-palmito-2,3-dibutyrin, but when this glyceride was mixed with 1-myristo-2,3-diolein, the former glyceride was hydrolyzed more rapidly (Jensen et al., *J. Dairy Sci. 47,* 727). Shorter chain fatty acids located on the 2-position of triglycerides were quickly hydrolyzed by pancreatic lipase (Entressangles et al., *Biochim. Biophys. Acta 84,* 140). This was due to the rapid migration of the shorter chains to the alpha positions of the partially hydrolyzed glycerides. Trilaurin, 1,3-dilaurin, and 1- and 3-monolaurins were hydrolyzed by pancreatic lipase at rates which decreased in the order listed. Acyl migration was noted when 2-monolaurin was the substrate (Jensen et al., *J. Dairy Sci. 46,* 907). Incubation of the seed oil of *Vernonia anthelmintica* with pancreatic lipase yielded mostly 2-monoglyceride, showing that the formation of 1,3-divernolin by the natural lipase of this plant is not caused by the structure of the triglyceride (Sampugna et al., *JAOCS 41,* 132). Microbial lipases were found to exhibit specificity for triglycerides at either the 1-position, the 1- and 2-positions, or for olcic acid regardless of its position (Alford et al., *J. Lipid Res. 5,* 390).

A chemical technique for determining the fatty acid distribution on disaturated triglycerides was reported which depends on the preferential hydrolysis of the azelaic acid from azelaoglycerides obtained by permanganate oxidation. The hydrolysis is carried out by refluxing an acetone solution of the glycerides over potassium carbonate. The hydrolyzed product is further oxidized with permanganate, upon which the 1,2-diglycerides give an acid product which may be separated from the neutral product of the 1,3-diglycerides (Kartha, *JAOCS 41,* 456). A method for analyzing the glyceride structure of fats was worked out which consists of the oxidation of the triglycerides with permanganate-periodate, esterification of the oxidized glycerides, and gas chromatographic analysis of the esterification product (Youngs and Subbaram, *Ibid.* 218). The method gives the distribution of the individual saturated fatty acids in each glyceride class.

The possibility of using gas-liquid, liquid-liquid, and thin-layer chromatography in sequence to separate various natural triglyceride mixtures was discussed (McCarthy and Kuksis, *Ibid.* 527). Triglycerides were separated by chromatography on columns of silver nitrate-impregnated silica gel and then each fraction was analyzed by a previously described oxidation-gas chromatographic method (Subbaram and Youngs, *Ibid.* 445). Preparative silver ion TLC was used to separate triglycerides and each fraction was then analyzed by gas chromatography directly (Litchfield et al., *Ibid.* 588).

The triglyceride structure of palm oil obtained from silver ion-thin-layer chromatography agreed with Vander Wal's theory (Jurriens et al., *J. Lipid Res. 5,* 366). Good agreement with Vander Wal's theory was found for most of 14 fats whose glyceride structure was determined by gas chromatography of the oxidized glycerides (Subbaram and Youngs, *JAOCS 41,* 595). The exceptions were human fat, palm oil and cocoa butter. The glyceride structure of the seed fat of the bitter gourd, *Momordica Charantia,* did not agree with Vander Wal's theory (Subbaram et al., *Ibid.* 691). The glyceride structure of castor oil obtained by countercurrent distribution agreed with Vander Wal's theory (Achaya et al., *Ibid.* 783). The results of pancreatic lipase hydrolysis of lard support Vander Wal's theory, but they are subject to other interpretations (Coleman, *Ibid.* 247). The triglycerides from a number of even-toed animals *(Artiodactyla)* were examined by pancreatic lipase hydrolysis. There was a tendency for the palmitic acid to be found at the 2-position in domestic pigs, European and American wild boars, and two species of peccary. In other cases the palmitic was distributed randomly or preferentially at the 1- and 3-positions. In all species only a small amount of stearic acid was found at the 2-position. Myristic acid tended to esterify at the 2-position, and no pattern was discernible for the unsaturated fatty acids (Mattson et al., *J. Lipid Res. 5,* 363). The triglycerides of 28 plants were examined by pancreatic lipase and palmitic and stearic acid were found to be predominantly at the 1- and 3-positions. An earlier study had indicated that fatty acids longer than 18 carbons accumulated at the 1- and 3-positions. Oleic, linoleic, and linolenic acid were distributed at random among the positions left after the preferential distribution of palmitic, stearic, and acids with chains longer than 18 (Mattson and Volpenhein, *Ibid.* 392). Triglyceride analysis on a number of natural and rearranged butter and coconut oils by gas chromatography showed that the natural oils were not randomly distributed (Kuksis et al., *JAOCS 41,* 201). The high-melting triglyceride isolated from milk fat-globule membranes was 71.2% trisaturated and the individual glycerides were not randomly distributed (Wolf and Dugan, *Ibid.* 139).

2-Palmito-1-$C^{14}$-1,3-diolein was fed to lobster, cod, trout, and rat and the retention of the glyceride distribution during digestion was determined. The glyceride structure was retained 50–80% in the fish and rat depot fat and was still retained after 4 weeks. In rat liver there was no retention of the structure after 1 day. The glyceride structure was not retained in the lecithins (Brockerhoff et al., *J. Biol. Chem. 239,* 735). A number of fats were fed to rats and the rat fat was examined for the influence of the dietary fat on its structure. The rats showed a tendency to place the unsaturated fatty acids in the 2-position, and the distribution of saturated and unsaturated fatty acids in the rat is not greatly influenced by the dietary fat. Hydroxy-fatty acids were metabolized like other acids and did not disturb the glyceride structure (Perkins, *JAOCS 41,* 285).

A paper chromatographic method was described for separating the α-glyceryl ethers from one another (Todd and Rizzi, *Proc. Soc. Exptl. Biol. Med. 115,* 218).

UNSAPONIFIABLES. A liquid-liquid partition chromatography method for analyzing the nonpolar unsaponifiable fraction obtained from soybean oil deodorizers was reported (Hoffman et al., *JAOCS 41,* 116).

Procedures for the gas chromatography of sterols were discussed (Horning and Vandenheuvel, *Ibid.* 707). Phenyl silicone and neopentyl glycol succinate were suggested as stationary phases for the gas chromatographic separation of cholesterol and desmosterol (Fumagalli and Vandenheuvel, *Riv. Ital. Sostanze Grasse 40,* 470). Gas chromatography was used for the analysis of urinary estrogens after preliminary purification by TLC (Wotiz and Chattoraj, *Anal. Chem. 36,* 1466).

A micromodification of the Sperry and Webb procedure was adapted to the determination of cholesterol in red and white blood cells from 0.4 ml of blood (Hawthorne et al., *J. Nutr. 81,* 241). Eggs from hens fed large amounts of polyunsaturated fatty acid cannot be analyzed accurately colorimetrically for cholesterol with Zlatkis reagent. The polyunsaturated fatty acids in the eggs react to give a high determination (Weiss et al., *Arch. Biochem. Biophys. 105,* 521).

A reverse phase TLC procedure was described for the separation of desmosterol and cholesterol. Undecane is the

stationary phase and acetic acid-acetonitrile the mobile. The undecane can be evaporated from the plates to facilitate spot detection with phosphomolybdic acid (Wolfman and Sachs, *J. Lipid Res. 5,* 127). The development time of thin-layer plates for the separation of steroids can be shortened by incorporating 50% Celite 545 into the silica gel used on the plates. $R_f$ values increased but the degree of separation was not affected adversely (Vaedtke et al., *J. Chromatog. 12,* 208). Thin-layer plates of silicic acid impregnated with silver nitrate were used to analyze mixtures of sterols and triterpenic alcohols (Capella et al., *Riv. Ital. Sostanze Grasse 40,* 645). Cholesterol esters of varying degrees of unsaturation were separated on paper impregnated with silicic acid (Gabbay and Waterhouse, *J. Chromatog. 11,* 241).

A silicic acid column chromatographic method was reported for the fractionation of the lipid-pigment mixture of lucerne. Subsequent TLC of the fractions showed they were not pure (Thirkell and Tristram, *J. Sci. Food Agr. 14,* 488). Anhydroretinol, methyl retinyl ether, retinol, and methyl retinoate could be separated by gas chromatography at 150C with SE-30 as a stationary phase (Dunagin and Olson, *Anal. Chem. 36,* 756). Retinol and retinyl acetate were largely converted to anhydroretinol under gas chromatographic conditions; however after the columns were treated with β-carotene, these compounds could be separated without much distruction. The carotenoids of vegetable oils can be separated by TLC and the xanthophyll and β-carotene can be extracted from the plates for quantitative spectrophotometry. About 90% of the β-carotene is recovered (Capella et al., *Riv. Ital. Sostanze Grasse 40,* 666). All-*trans*-β-carotene, all-*trans* retinol 13-*cis*-retinol, all-*trans* retinyl acetate, all-*trans* retinal, and vitamins $D_2$ and $D_3$ were reduced at a dropping mercury electrode (Kuta, *Science 144,* 1130). Three double bonds were required before reduction took place, and as the number of conjugated bonds increased, the reduction took place at a lower potential. The waves were proportional to the concentration of the vitamins. A carotenoid having an absorption maximum at 460 mμ and having the properties of 3′-hydroxy-3,4-dehydro-β-carotene was isolated by saponification and chromatography of alfalfa lipids. It was named anhydrolutein. Chicks converted this compound to vitamin $A_2$ (Budowski et al., *Ibid. 142,* 969).

Gas-liquid chromatography was used to analyze tocopherols and vitamin $K_1$ (Carroll and Herting, *JAOCS 41,* 473). Vitamin A preparations were altered by the gas chromatographic conditions and ubiquinones gave no peaks. Ascorbic acid was used to stabilize the tocopherols of milk fat during saponification procedures (Krukovsky, *J. Agr. Food Chem. 12,* 289). The tocopherol content of milk and dairy products was determined by a new procedure. The sample was extracted with ethanol and hexane, the tocopherols were purified by silicic acid chromatography, and the color developed with 4,7-diphenyl-1,10-phenanthroline was measured spectrophotometrically. A correction was made for the interference of carotenoids (Erickson and Dunkley, *Anal. Chem. 36,* 1055). A metabolite of α-tocopherol isolated from mammalian liver which could also be synthesized from the vitamin by treatment with potassium ferricyanide was shown to be two tocopherol moieties joined by carbon-carbon double bond at the 7,7′-positions. It was named di-α-tocopherone (Csallany and Draper, *J. Biol. Chem. 238,* 2912). The cis and trans-isomers of di-α-tocopherone were separated (*Ibid. 239,* 574).

The literature on sesamin, sesamolin, and related compounds since 1951 was reviewed (Budowski, *JAOCS 41,* 280). The triterpenes were separated from oil of petitgrain by chromatography on columns of powdered rubber with methanol acetone as a mobile phase (Trowbridge et al., *Ibid.* 306). A TLC method was described for fractionating the triterpenic alcohols and sterols of vegetable oils (Capella, *Riv. Ital. Sostanze Grasse 40,* 660).

PHOSPHOLIPIDS. The phosphoinositides of beef brain were converted to their sodium salt by treatment with disodium ethylenediaminetetraacetate and with a chelating resin, and the sodium salts were separated by chromatography on a diethylaminoethyl cellulose column. The inositides were eluted with a gradient of ammonium acetate (Hendrickson and Ballou, *J. Biol. Chem. 239,* 1369). Phospholipids containing dimethylaminoethanol and dimethylaminoisopropanol as bases were separated by chromatography on columns of silicic acid and diethylaminoethyl cellulose (Bieber and Newburgh, *J. Lipid Res. 4,* 397). Column and paper chromatographic methods were used to identify the bases in the phosphatide hydrolyzate of pilchard oil (Silk and DeKoning, *JAOCS 41,* 619). A method for the estimation of glycerol in phosphatidyl ethanolamine was described which involved acetolysis and determination by gas chromatography (Holla et al., *J. Lipid Res. 5,* 263).

The TLC of phosphatides was reviewed (Hiroshi and Yasuhiro, *Yukagaku 12,* 597). A method for the quantitative determination of animal phosphatides by two-dimensional paper chromatography was described which uses formalin-treated paper and development with butanol-acetic acid-water and tetrahydrofuran-diisobutylketone-water. The spots from the paper are ashed and analyzed for phosphorous (Wagner et al., *Biochem. Z. 339,* 34). A modification of the methods of Fiske-Subbarow and Bartlett for the microdetermination of phosphate compounds on paper chromatograms was described (Gerlach and Deuticke, *Ibid. 337,* 477). As little as 0.005 mole of phosphatidyl ethanolamine or phosphatidyl choline could be detected on thin-layer plates sprayed with a modified Zinzadze molybdenum blue reagent (Dittmer and Lester, *J. Lipid Res. 5,* 126). A wide range of phosphatides could be detected.

A paper chromatographic procedure was described for the separation of the various α-glyceryl ethers from the phosphatide fraction of mammalian tissues and starfish (Todd and Rizzi, *Proc. Soc. Exptl. Biol. Med. 115,* 218). The phosphatidyl ethanolamine fraction of bovine erythrocytes was found to contain a monoacyl, monoalkyl analogue. The glyceryl ether group was shown to have the L-1 configuration, and evidence was obtained that the acyl group was on the 2-position. The glyceryl ethers obtained by hydrolysis of the phosphatides were converted to isopropylidine derivatives and analyzed by gas chromatography. The glyceryl ethers could also be separated according to their degree of unsaturation by chromatography on alumina as the mercury derivatives (Hanahan et al., *Biochemistry 2,* 630).

The cardiolipin of rat liver was separated into five subfractions by gradient elution on silicic acid columns (Rose, *Biochim. Biophys. Acta 84,* 109). Structural and compositional studies did not reveal the basis for the fractionation. The presence of a free hydroxyl group could not be confirmed. Cardiolipin from beef heart and from *Mycobacterium phlei* was shown to have a 1,3-diphosphatidylglycerol structure by deacylation and periodate oxidation (LeCoeq and Ballou, *Biochemistry 3,* 976). Synthetic 1,3-di-O-(L-glycerol-3′-phosphoryl)-glycerol was found to have the same optical activity as the deacylated cardiolipin.

Hydrolysis of the phosphoinosidites of ox brain indicated that mono-, di-, and triphosphoinositics were present (Ellis et al., *Biochem. J. 88,* 125). The phosphatidyl-myoinositol dimannosides of *Mycobacterium tuberculosis* and *Mycobacterium phlei* were shown to be 1-phosphatidyl-L-myo-inositol-2,6-di-α-D-mannopyranoside (Lee and Ballou, *J. Biol. Chem. 239,* 1316). The structural proof was based on methylation-periodate oxidation and on nuclear magnetic resonance studies.

The sphingomyelin of human plasma lipids was fractionated into two components by TLC (Wood and Holton, *Proc. Soc. Exptl. Biol. Med. 115,* 990). The less polar material contained more fatty acids above $C_{20}$, but both fractions yielded a number of different bases on hydrolysis.

GLYCOLIPIDS. Methods of isolation and analysis of gangliosides were reviewed (Svennerholm, *J. Lipid Res. 5,* 145). The glycolipids of blood serum were isolated quantitatively by TLC on silica gel and four such compounds were characterized. They were ceramide-monohexoside, -dihexoside, -trihexoside, and -trihexoside-N-acetylgalactosamine. The monohexoside was a glucocerebroside. All the hexosides except the one containing N-acetylgalactosamine contained both normal and hydroxy fatty acids (Svennerholm and Svennerholm, *Biochim. Biophys. Acta 70,* 432). The long chain bases of gangliosides from several sources were analyzed by gas chromatography and two $C_{20}$ bases were discovered. The names gangliosphingosine and dihydrogangliosphingosine were proposed for them (Sambasivarao and McCluer, *J. Lipid Res. 5,* 103). The phytoglycolipid fraction of corn was hydrolysed and the oligosaccharide fraction was separated by chromatography on ion exchange resin. A trisaccharide, glucosaminido-glucuranido-inositol, was found which can be obtained from all the higher oligosaccharides by mild hydrolysis and which accounts for their content of these three sugars. The tetrasaccharide contained also mannose. The higher oligosaccharides were not individual components and contained also galactose, arabinose and fucose (Carter et al., *Biochemistry 3,* 1103). The sulfolipid and glycolipids of *Chlorella pyrenoidosa* and alfalfa were separated by chromatography on Florisil, diethylaminoethyl cellulose and silicic acid columns (O'Brien and Benson, *J. Lipid Res. 5,* 432). A sterol-containing glycolipid was discovered in wheat (McKillican, *JAOCS 41,* 554). The mycoside B of *Mycobacterium tuberculosis* was found to be a mixture containing different fatty acid homologues. It contains one molecule of 2-O-methyl-D-rhamnose bound in a β-linkage to the phenolic hydroxyl group of a phenolic methoxylated glycol whose composition was $C_{32}H_{58}O_4$. The hydroxyl groups of the glycol are esterified with palmitic and mycocerosic acid (De-

marteau-Binsburg and Lederer, *Biochim. Biophys. Acta 70,* 442).

LIPOPROTEINS. The lipid content of egg yolk granules obtained by water washing was 35% while that of the yolk plasma was 81%. Two fractions were obtained by ultracentrifugation of the plasma lipoproteins (Saari et al., *J. Food Sci. 29,* 307). Subcellular lipoprotein particles were obtained from cottonseed by tanning the cell contents and then centrifuging (Yatsu and Altschul, *Science 142,* 1062). A simplified method for the analysis of the $S_f$ 20–$10^5$ and $S_f$ 0–20 low-density lipoproteins of blood serum was proposed. The lipoproteins are separated by preparative ultracentrifugation and analyzed by refractometry. The results are comparable to those with more difficult analytical ultracentrifuge techniques (Lindgren et al., *J. Lipid Res. 5,* 68). Long chain fatty acids were found to be firmly bound to plasma lipoproteins even after extensive extraction with solvents. Proteolysis revealed that the fatty acids were bound to peptides containing organic phosphate (Fisher and Gurin, *Science 143,* 362).

ALCOHOLS. The even-numbered n-aliphatic alcohols from $C_{12}$ to $C_{18}$ were separated by reverse-phase paper chromatography (Mukai et al., *Yukagaku 12,* 505). The stationary phase was liquid paraffin and the mobile phase was aqueous acetic acid. The separation of aliphatic alcohols from hydrocarbons and unsaponifiable components was accomplished by TLC on silica gel with various solvent mixtures (Hashimoto and Mukai, *Ibid.* 613).

FATTY ACIDS. Reviews were written on the separation of unsaturated fatty acids (Takagi, *Yukagaku 13,* 247) and oxidized fatty acids (Naudet, *Oleagineux 19,* 449). The recovery of fatty acids from aqueous solutions of surface active agents by centrifugation was studied, and the possibility of using this to separate saturated and unsaturated fatty acids was discussed (Fuchsova and Zalud, *J. Inst. Chem. Technol. Prague 6,* 251).

The gas chromatographic determination of fatty acids was reviewed with special attention to quantitative measurement of the peak (Jaforte, *Riv. Ital. Sostanze Grasse 40,* 678). Results from the analyses of the standard fatty ester mixture obtainable from the National Heart Institute were reported, and recommendations about their use were given (Goldwater, *J. Lipid Res. 5,* 20). The relative response for flame ionization detectors in the analysis of long chain fatty esters was shown to be related to the weight percentage of "active" carbon (Aekman and Sipos, *JAOCS 41,* 377). Acid-treated kieselguhr and hexamethyl disilane-treated firebrick were recommended as stationary phase supports in the gas chromatography of fatty acid methyl esters (Choy et al., *J. Chromatog. 12,* 171). The amounts of unsaturated eighteen carbon fatty acids determined by gas chromatography with hydrogen as a carrier gas were lower than when helium was used (Tiscornia and Boniforti, *Riv. Ital. Sostanze Grasse 41,* 140). This phenomenon was evidently related to impurities in the hydrogen. Contaminants which might be erroneously identified as fatty acid methyl esters during gas chromatography were found to arise from the anhydrous methanolic hydrogen chloride used to prepare the methyl esters (Johnson and Roots, *J. Lipid Res. 5,* 477).

The procedure of Stoffel et al. for preparing methyl esters and dimethylacetals from lipid extracts was found to give low recoveries of 2-hydroxy methyl esters and contamination by cholesterol derivatives (Eng et al., *Ibid.* 128). A TLC method is given for the separation of the hydroxy esters, nonoxygenated esters, and cholesterol and its derivatives. Fatty acid methyl esters were prepared for gas chromatographic analysis without elevated temperatures by using 2,2-dimethoxypropane to drive the transesterification to completion (Mason and Waller, *Anal. Chem. 36,* 583). No extraction of the reaction mixture is required. The dimethoxypropane ties up the glycerol as isopropylidine glycerol. A rapid method for preparing fatty acid methyl esters from triglycerides with chloroform-methanol-sulfuric acid was reported (Peisker, *JAOCS 41,* 87). Methods for preparing methyl esters from triglycerides for gas chromatography that could be carried out at room temperature were favored over those requiring a higher temperature (Hivon et al., *Ibid.* 362). Methyl esters and triglycerides were reduced to alcohols with lithium aluminum hydride and the alcohols were identified by gas chromatography of the p-phenylazobenzoate derivatives. The alcohol derivatives could also be separated by thin-layer liquid column chromatography and determined quantitatively by their absorption at 322 mμ (Katz and Keeney, *Anal. Chem. 36,* 231). Procedures were described which allow the simultaneous quantitative determination of glycerol as well as fatty acids of fats and oils by gas chromatography (Mason et al., *Anal. Chem. 36,* 587 Holla et al., *J. Lipid Res. 5,* 263). Methyl esters may be hydrogenated with a platinum catalyst in 95% ethanol without fear of transesterification, but the loss of esters during the evaporation of the solvent may be different for different chain lengths (Ackman and Burgher, *Ibid.* 130). Free fatty acids were analyzed by gas chromatography using a strongly acidic stationary phase in a capillary type column or by using a inert stationary phase support such as Teflon (Kabot et al., *Riv. Ital. Sostanze Grasse 41,* 131).

A stationary phase of β-cyanoethylmethylsiloxane was recommended for the separation of the geometric isomers of unsaturated fatty acid methyl esters by gas chromatography (Litchfield et al., *JAOCS 41,* 52). On bromination, octadecenoic acids give derivatives that can be resolved into *cis* and *trans* isomers by gas chromatography more readily than the original acids (Cartoni, *Riv. Ital. Sostanze Grasse 40,* 653). By this technique as little as 1% elaidic acid can be detected. The positional isomers of linoleic acid formed during alkali isomerization were separated by gas chromatography (Cartoni et al., *Ibid.* 482). The isomers of oleic acid such as elaidic and petroselenic were measured by gas chromatography on capillary columns (Riva et al., *Ibid. Sympos. Issue 1962,* 66). Gas chromatography was used to separate the geometric isomers of methyl oct-2-enoate, (Mercuri et al., *JAOCS 41* 89). There was very little interaction of the double bond with the stationary phase so that separation was a function of volatility. The separation factors among various unsaturated fatty acid methyl esters were dependent on the distance from the carboxyl and methyl ends of the chain to the nearest double bond (Ackman and Burgher, *J. Chromatog. 11,* 185). Large errors in the determination of polyunsaturated fatty acids by gas chromatography were reported which seem to be caused by polar stationary phase supports (Gerson et al., *Biochem. J. 91,* 11 C). Gas chromatography was shown to cause both elaidinization and positional shifts of the conjugated double bonds of elaeostearic (Mikolajszak and Bagby, *JAOCS 41,* 391).

Isothermal gas chromatography can be applied to butter fatty acids with accurate results if the peak areas are calculated from the retention time rather than the peak width (Daghetta and Jaforte, *Riv. Ital. Sostanze Grasse 40,* 597). Volatile fatty acid may be isolated by steam distillation for analysis by gas chromatography (Vandenheuvel, *Anal. Chem. 36,* 1930). The distillate is neutralized, dried, and mixed with carbon disulfide containing formic acid just before injection.

Silicone rubber (SE-30) was recommended as a stationary phase for the gas chromatography of epoxyoleic acid. Other stationary phases caused decomposition (Herb et al., *JAOCS 41,* 222). Gas chromatographic carbon numbers were determined for all 17 isomers of hydroxy and acetoxy methyl stearates and for 15 of the 16 isomers of methyl oxostearate on SE-30, QF-1, and ethylene glycol succinate stationary phases (Tulloch, *Ibid.* 833). The carbon number increases as the distance between the carboxyl group and point of substitution increase. Isomers with substituents at 9 to 13 were not distinguishable on any of the stationary phases.

Nuclear magnetic resonance spectra of the protons on hydrogenated linolenic acid was used to measure the amount of unsaturation, 15,16-double bond, 1,4-pentadiene, and allylic methylene groups present (Johnston et al., *Ibid.* 788). The percentage of linoleic acid in cottonseed oil was predictable from the iodine value by a regression equation (Driessche, *Ibid.* 248). Bioassays of essential fatty acids in unhydrogenated samples agreed well with the results by the alkali isomerization method (Alfin-Slater and Melnick, *Ibid.* 145). In hydrogenated fats, the linoleic acid could be estimated by a combination of the alkali isomerization and thiocyanogen methods. The isomers formed during the alkali isomerization of linoleic acid were followed by gas chromatographic analysis (Cartoni et al., *Riv Ital. Sostanze Grasse 40,* 482). The theoretical prediction of a mixture of 50% 9-*cis*-11-*trans* and 50% 10-*trans*-12-*cis* was partially confirmed, but there was a tendency to form *trans-trans* isomer as the reaction time increased.

The use of TLC in separating fatty acids was reviewed (Loury, *Rev. Franç. Corps Gras 11,* 259). Lipids could be recovered quantitatively from thin-layer plates by loosening the adsorbent from the plate and sucking it up into a tube closed at one end with a sintered glass filter (Goldrick and Hirsch, *J. Lipid Res. 4,* 482). The lipid could then be eluted from the adsorbent by pouring solvent through the tube. The presence of as little as 1% methyl elaidate in a mixture could be detected by TLC using silica gel-silver nitrate as an adsorbent (Pallotta and Mararese, *Riv. Ital. Sostanze Grasse 40,* 579). A thin-layer method for elaidic acid which would detect as little as 0.25% was later reported (*Ibid. 41,* 210). Tall oil fatty acids were fractionated by reverse phase paper chromatography (Berger and Muller, *Plaste Kautschuk 10,* No. 9,

566 and No. 10, 631). Preliminary hydrogenation of the mixed acids on the paper improved the separation. A TLC procedure for the separation of hydroxy methyl esters from nonoxygenated esters was reported (Eng. et al., *J. Lipid Res. 5,* 128). A reverse phase TLC system for the fatty acids of common vegetable oils was described (Anker and Sonanini, *Pharm. Acta Helv. 37,* 360). Two experimental dyes, Brilliant Green and Lauth Violet, were found to be excellent for detecting fatty acid spots on paper chromatograms (Sliwiok and Kwapniewski, *Riv. Ital. Sostanze Grasse 41,* 288). A technique for reverse phase paper chromatography of fatty acids is also described using dioctylphthlate as a stationary phase. Critical pairs are separated by adding bromine to the developing solvent. Critical pairs of fatty acids were separated by reverse phase paper chromatography of fatty acids which had been bromomethoxylated with N-bromsuccinimide (Jobtscheff et al., *Fette Seifen Anstrichmittel 65,* 913).

Chromatography on silicic acid-silver nitrate columns was used to fractionate fatty acids for structural determinations (Bhatty and Craig, *JAOCS 41,* 508). A silver nitrate-saturated ion exchange resin was used to separate *cis* and *trans* isomers of unsaturated fatty acid methyl esters (Emken et al., *Ibid.* 388). Methyl elaidate and methyl oleate and the *cis-trans, cis-cis,* and *trans-trans*-9,12-octadecadienoates were completely separated. Chromatography on columns of powdered rubber was used to resolve the methyl ester of coconut oil (Trowbridge et al., *Ibid.* 306).

Various solvents were screened for the most effective separation of solid and liquid fatty acids by crystallization (Martinenghi, *Olearia 17,* 99). 1,2-Dichloroethane-acetonitrile (6:4) is recommended. Mixtures of solid and liquid fatty acids from olive oil were separated almost quantitatively with 90% yield by two crystallizations from dichloroethane at −35C (*Ibid.* 187). The effect of the molar excess of urea and other factors in the separation of oleic and stearic acid by adduct formation were discussed, and a 10–20 molar excess of urea was recommended (Zajic and Capova, *J. Inst. Chem. Tech. Prague 6,* 275). Oils of low or medium acidity can be partially deacidified by treatment with urea (Zajic, *Ibid. 7,* 181). Optimum results were obtained at 40C with 30% water and agitation for 2 hr.

Fatty acid methyl esters were fractionated by countercurrent distribution between hexane and 0.2*N* silver nitrate in 90% methanol. *Cis* and *trans*-monoenoates were completely separated and polyunsaturated fatty esters were separated on the basis of the number and configuration of the double bonds (Scholfield et al., *Anal. Chem. 35,* 1588). Countercurrent distributions between hexane-acetonitrile and hexane-methanolic silver nitrate were used to fractionate the isomers produced by the hydrogenation of linolenic acid with hydrazine (Butterfield et al., *JAOCS 41,* 397). The *cis,cis*-9,15-isomer could be isolated from the *cis,cis*-9,12- and 12,15-isomer. Countercurrent distribution between heptane and methanol-acetic acid-formamide (1:1:1) was used to separate tall oil fatty acids (Aho et al., *Teknillisen Kemian Aikokusilehti 19,* 390).

The monobasic and dibasic acids produced by periodate-permanganate oxidation of unsaturated fatty acids were determined quantitatively by gas chromatography to determine the position of the double bonds (Tulloch and Craig, *JAOCS 41,* 322). A comparative study of the permanganate and peracetic acid techniques for determining the position of double bonds in unsaturated fatty acids indicated that the permanganate method gave considerable degradation while the hydroxylation gave quantitative results (Fedeli et al., *Riv. Ital. Sostanze Grasse 40,* 313). Hydrazine was shown to reduce the double bonds of elaeostearic acid without change in geometric configuration, and it was suggested that a combination of hydrazine reduction with permanganate-periodate oxidation could be used to determine the structure of conjugated fatty acids (Takagi and Craig, *JAOCS 41,* 660). The mechanism of the ozone reaction with unsaturated fatty acids was studied (Privett and Nickell, *Ibid.* 72). Chromatography on silver nitrate-silicic acid columns was recommended as a method of separating unsaturated fatty acids in preparation for oxidative structural studies (Bhatty and Craig, *Ibid.* 508). A scheme was worked out for the identification of unsaturated fatty esters by comparison of their retention times during gas chromatography on different polyester stationary phases (Ackman and Burgher, *J. Chromatog. 11,* 185).

## MEASUREMENTS OF PHYSICAL PROPERTIES

Methods of rheological measurements (Fukada, *Yukagaku 13,* 52), the rheology of edible fats (Sone, *Ibid.* 93), and the rheology of condensed milk and ice cream (Tsuchiya, *Ibid.* 100) were reviewed. The consistencies of fats were predicted by differential thermal analysis. It was assumed that temperature differences between the sample and reference substance are proportional to the rate of melting of solid glycerides. Abrupt changes in slope of the differential thermal analysis curve indicate the beginning and termination of melting, and the curve maximum indicates the maximum melting rate. It is proposed that the ratio between the maximum melting rate and the melting range be defined as the coefficient of hardness. This coefficient correlates well with a corresponding coefficient which can be obtained from dialometric data (Pokorny et al., *J. Inst. Chem. Tech. Prague 5,* 141). Nuclear magnetic resonance was used to determine the percentage of solid fat in samples. This method was more reliable than dialometric measurements and was applicable over a wider range (Taylor et al., *JAOCS 41,* 177). It was proposed that the different polymorphic forms of long chain n-saturated compounds correspond to certain preferred angles of tilt in the crystal latice. This hypothesis provides a consistent pattern for the long spacings observed for acids, salts, alcohols, esters, monoglycerides, and diglycerides. Formula were derived for predicting the long spacing of simple and mixed triglycerides, but the calculation could not be extended to unsaturated compounds (Gunstone, *Chem. Ind.* [*London*] *1964,* 40).

The hydrophile-lipophile balance of surface active compounds can be predicted from retention volumes during gas chromatography on polar and nonpolar stationary phases (Becher and Birkmeier, *JAOCS 41,* 169). Methods of estimating the physico-chemical properties of solutions were reviewed (Kusano, *Yukagaku 13,* 185).

## COMPOSITION AND CHARACTERISTICS: ANALYTICAL DATA

Many analyses of the composition and physical properties of fats and lipids were published in 1964. A detailed report of these results is beyond the scope of this review, and only a listing of the lipids analyzed and the type of information obtained can be given. This listing has been subdivided. The first division includes chemical analyses on fats, oils and unfractionated lipid mixtures. An asterisk (*) appearing after the reference indicates that fatty acid composition data are given. A dagger (†) indicates information on the fatty acid combinations that occur is given. The second division contains data on certain lipid classes. The third division of the list gives measurements of physical properties. Reports which deal primarily with the effect of environmental, dietary, and genetic factors on composition and physical properties are given in the succeeding section.

UNFRACTIONATED LIPIDS. Analyses were reported on *Corynebacterium diptheriae, Micrococcus lysodeikticus,* and *Bacillus megaterium* KM (Fulco et al., *J. Biol. Chem. 239,* 998)*, the aldehydes of rumen bacteria (Katz and Keeney, *Biochim. Biophys. Acta 84,* 128), the slime mold, *Dictyostelium discoideum* (Davidoff and Korn, *J. Biol. Chem. 238,* 3210)*, and *Euglena gracilis* (Korn, *J. Lipid Res. 5,* 352).

*Cis*-11-octadecenoic acid and other minor unsaturated fatty acids were found in olive, soybean, cottonseed, corn, peanut, rapeseed, and safflower oil (Kuemmel, *JAOCS 41,* 667)*. Evidence for the presence of 11-octadecenoic acid was also reported in olive, sunflower, linseed, rapeseed, and coriander oils (Tulloch and Craig, *Ibid.* 322)*. Odd-chain saturated and monoenoic acids were reported in olive, rapeseed, and sunflower seed oils (Bhatty and Craig, *Ibid.* 508)*. Analyses were reported on several commercially available food oils and shortenings (Hivon et al., *Ibid.* 362)*, peanut oil (Martinenghi, *Olearia 18,* 47)*, and (pimiento pepper, *Capsicum annum L.,* (Marion and Dempsey, *JAOCS 41,* 548)*. Thirty-six different species of oil seeds were investigated (Grieco and Piepoli, *Riv. Ital. Sostanze Grasse 41,* 283)*. An annotated list of 13 minor Indian oil seeds was published (Lakshminarayana et al., *Indian Oilseeds J. 7,* 233). Analyses were also reported on mandarin orange seed oil (Shalika et al., *Indian Soap J. 29,* 71)*; peach seed, *Polygonum japonicum,* and cowpea oils (Hamada and Ueno, *Yukagaku 13,* 195)*; and rice (Lugay and Juliana, *JAOCS 41,* 273)*. Analytical data were collected on tall oil (*Teknillisen Kemian Aikakausilehti 19,* 390)*; oiticica oil (Anon., *Rev. Arg. Grasas Aceites 5,* 62); isano oil (von Mikusch, *Farbe Lack 70,* 101), isano oil (Badami and Gunstone, *J. Sci. Food Agr. 14,* 863)*. In a search for new industrial oils most of the known varieties of *Limnanthes alba, L. bakeri, L. douglasii, L. floccosa, L. gracilis, L. montana,* and *L. striata* were examined (Miller et al., *JAOCS 41,* 167)*. *Cuphea hookeriana, C. painteri, C. agnea, C. llavea,* and *C. carthagensis* oils were examined and found to be rich in capric acid (Miller et al., *Ibid.* 279)*. Seed oil from 29 species in

five genera of the tribe *Calenduleae*, family *Compositae* were examined with special attention to dimorphecolic acid (Earle et al., *Ibid.* 345)*. The species examined were *Calendula arvensis L, C. officinalis L, Castalis nudicalaulis DC, C. nudicaulis var. graminifolia (L.) T. Norl, Chrysanthemoides incana (Burm. f.) T. Norl, C. monilifera (L.) T. Norl, Dimorphotheca chrysanthemifolia DC, D. cuneata Less, D. pluvialis (L.) Much, D. sinuata DC, D. zeyheri Sond, Osteospermum amplectans (Harv) T. Norl., O. asperulum (DC) T. Norl, O. calendulaceum L. f., O. caulescens Harv., O. clandestinum (Less) T. Norl, O. corymbosum L., O. dregei (DC) T. Norl, O. ecklonis (DC) T. Norl, O. fruticosum (L) T. Norl, O. hyoseroides (DC) T. Norl, O. imbricatum L, O. imbricatum ssp. nervatum (DC) T. Norl, O. jucundum (E. P. Phill.) T. Norl, O. junceum Berg, O. microphyllum DC, O. Muricatum E. Mey. ex DC, O. scariosum DC, O. Sinuatum (DC) T. Norl, O. spinescens Thunb*, and *O. spinosum L.* Seed oils from 29 species of the family Boraginaceae were examined (Kleiman et al., *Ibid.* 459)*. The species were: *Cordia oblique Willd., Ehretia acuminate R. Br., E. aspera Roxb., Heliotropium europaeum L., Cynoglossum amabile Stapf.* and *Drumm., C. officinale L., C. pictum Soland, Paracaryum angustifolium Boiss, P. caelestinum Benth. & Hook, Cryptantha bradburiana Payson, C. angustifolia (Torr) Greene, Lappula redowskii (Hornem.) Greene, Anchusa capensis Thunb, A. hybrida Ten., Borago officinalis L., Symphytum officinale L., Cerinthe major L., C. minor L., Lithospermum apulum Vahl., L. officinale L, L. tenuiflorum L.f., Moltkia aurea Boiss, M. coerulea Lehm., Myosotis sylvatica Hoffm., Onosma sericeum Willd., O. stellulatum Waldst & Kit, Onosmodium molle Michx., Echium italicum L.*, and *E. plantagineum L.* Analyses were reported on *Gynandropsis pentaphylla* and *Capparis aphylla* seed oil (Gupta and Chakrabarty, *J. Sci. Food Agr. 15*, 69)*; *Pachira aquatic* kernels (deBruin et al., *Ibid. 14*, 758)*; and *Citrullus colocynthis* seed (Gupta and Chakrabarty, *Ibid. 15*, 74)*. 9-Keto-*trans,trans*-10-12-octadecadienoic acids was found in *Cimorphotheca sinuata* seed oil (Binder et al., *JAOCS 41*, 108)*. All *cis*-5,11,14-eicosatrienoic acid was found in *Podocarpus nagi* seed oil along with *cis,cis*-11,14-eicosadienoic acid (Takagi, *Ibid.* 516)*. All-*cis*-6,9,12,15-octadecatetraenoic acid was found in *Onosmodium occidentale* seed oil (Craig, and Bhatty, *Ibid.* 209)*, and *Echium plantagineum* seed oil (Smith et al., *Ibid.* 290)*. *Trans,trans*-9,12-octadecadienoic acid was found in *Chilopsis linearis* seed oil (Chisholm and Hopkins, *Can. J. Chem. 41*, 1888)*. The same seed oil was found to contain *trans,trans*-10-12 octadecadienoic acid and *trans*-9-*trans*-11-*cis*-13-octadecatrienoic acid (Hopkins and Chisholm, *JAOCS 41*, 42)*. 11-Hexadecenoic acid was isolated from *Gevuina avellana* seed oil (de Tomas et al., *Hev. Arg. Grasas Aceites 5*, 53)*.

Analyses were reported on Acanthamoeba (Korn, *J. Biol. Chem. 238, 3584*, and *Ibid. 239*, 396)* and *Leishmania Enrietti* (Korn, *Science 142*, 1301)*. The latter was the first instance of the biosynthesis of α-linolenic acid by a nonphotosynthetic organism. *Cis*-vaccenic acid was shown to be an important constituent in rat liver cells (Holloway and Wakil, *J. Biol. Chem. 239*, 2489)*. The fatty acid composition of gerbils was compared to that of rats (Gordon and Mead, *Proc. Soc. Exptl. Biol. Med.* 116, 730)*. Phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) was isolated from ox plasma (Lough, *Biochem. J. 91*, 584)*. The branched chain fatty acids of various tissues from newly born lambs was assayed (Downing, *J. Lipid Res. 5*, 210)*. The positional isomers formed during hydrogenation of linolenic acid in an artificial rumen were characterized (Ward et al., *Biochem. J. 92*, 60)*. Analyses were reported on adipose tissues from various locations in Somali sheep (Read and Awdeh, *J. Sci. Food Agr. 14*, 770)*; and weasels, stoats and ferrets (Hartman and Johnson, *Ibid. 15*, 127)*. Twenty-five new mono- and dienoic fatty acids were discovered in pig brain (Kishimoto and Radin, *J. Lipid Res. 5*, 98)*. Analyses were reported on human depot fat (Kingsbury et al., *Biochem. J. 90*, 140)*; human skin surface fat (Wooley, *Arch. Biochem. Biophys. 105*, 634)*; and tallow from calf, mutton, horse and bones, and lard (Wolff and Audiau, *Rev. Franç. Corps Gras 11*, 77)*.

The polyunsaturated fatty acids of marine oil are believed to be entirely *cis* methylene interrupted structures (Ackman, *J. Fisheries Res. Board Can. 21* 247). The fatty acid composition of the oils from 21 species of aquatic animals was reported (Gruger et al., *JAOCS 41*, 662)*. The species were: Atlantic cod *(Gadus morrhua)*, Atlantic cod liver *(Gadus morrhua)*, mackerel *(Scomber scrombrus)*, menhaden *(Brevoortia tyrannus)*, ocean perch *(Sebastes marinus)*, striped mullet *(Mugil cephalus)*, spiny dogfish *(Squalus acanthias)*, spiny dogfish liver *(Squalus acanthias)*, Pacific halibut *(Hippoglosus-sus stenolepsis)*, Pacific herring *(Clupea harengus pallasi)*,

rockfish *(Sebastodes pinniger)*, sablefish *(Anoplopoma fimbria)*, chinook salmon *(Oncorhynchus tshawytscha-)*, chum salmon *(Oncorhynchus keta)*, coho salmon *(Oncorhynchus kisutch)*, pink salmon *(Oncorhynchus Gorbuscha)*, pink salmon egg *(Oncorhynchus gorbuscha)*, lake herring *(Coregonus artedii)*, rainbow trout *(Salmo gaironeri)*, lake whitefish *(Coregonus clupeaformis)*, blue crab *(Callinectes sapidus)*, littleneck clam *(Protothaca staminea)*, Pacific oyster *(Crassostrea gigas)*, and sea scallop *(Placopecten magellanicus)*. Structural analyses were reported on the polyenoic acids of mullet *(Mugil cephalus)* (Sen and Schlenk, *Ibid.* 241)*. Analyses were also reported on cod liver oil (Ackman and Burgher, *J. Fisheries Res. Board Can. 21,* 319)*; cod roe (*Ibid.* 469)*; seal blubber (*Phoca vitulina concolor* DeKay) (Jangaard et al., *Can. J. Biochem. 41,* 2543)*; and *Euphausia superba* recovered from the stomach contents of a finback whale (Tsuyuki et al., *Yukagaku 13,* 203)*.

Butter samples collected periodically over a year were analyzed (Chioffi and Magon, *Riv. Ital. Sostanze Grasse 41,* 243)*. The free fatty acids of cheddar cheese were analyzed (Bills and Day, *J. Dairy Sci. 47,* 733)*. The milk lipids of humans (Pokorny and Zeman, *J. Inst. Chem. Tech. Prague 6,* 85)* and the grey (Atlantic) seal (Ackman and Burgher, *Can. J. Biochem. 41,* 2501)* were examined.

Analytical data were given on margarine oils and hydrogenated fats (Pokorny et al., *J. Inst. Chem. Tech. Prague 7,* 223)*; blended and hydrogenated margarines (Alfin-Slater and Melnick, *JAOCS 41,* 145)*; margarines (Montefredine, *Riv. Ital. Sostanze Grasse, Sympos. Issue 1962,* 97)*; wheat flour (Muntoni et al., *Ibid. 41,* 154)*; the free fatty acids of wheat flour (Morrison, *J. Sci. Food Agri. 14,* 870)*; corn meal lipids (Kaderavek and Gay, *Olearia 17,* 145)* and bread (Fleischman et al., *J. Ah. Dietet. Assoc. 43,* 537)*. The percentage of fat in deep-fat fried chicken pieces was determined (Smith and Vail, *Ibid.* 541). The change in fatty acid composition of fats used for frying were determined (Kilgore, *JAOCS 41,* 496)*. The glycerol and free fatty acids formed during the storage of tallow were related to the amount of water originally present (Crespo et al., *Rev. Arg. Grasas Aceites 5,* 48). 11-(2-Methylcyclohex-2-en-1-yl) undec-*trans*-9-enoic acid was isolated from linseed oil (Hutchison and Alexander, *J. Org. Chem. 38,* 2522).

FRACTIONATED LIPIDS. The following plant materials were analyzed for the substances indicated: *Escherichia coli* strain $O_{111}B_4$ for lipopolysaccharides (Burton and Carter, *Biochemistry 3,* 411); *E. coli* for phosphatides (Kanfer and Kennedy, *J. Biol. Chem. 238,* 2919); *Streptococcus cremoris* for glycerides, waxes, and phosphatides (Brown and MacLeod, *J. Dairy Sci. 47,* 831)*; *Neurospora crassa* for lipid bases related to choline (Crocken and Nye, *J. Biol. Chem. 239,* 1727); the slime mold, *Dictyostelium discoideum,* for phosphatides (Davidoff and Korn, *Ibid. 238,* 3199)*; *Euglena gracilis* for glycerides, phosphatides, and glycolipids (Hulanicka et al., *Ibid. 239,* 2778)*; *Chlorella pyrenoidosa* and alfalfa for galactolipids and sulfolipids (O'Brien and Benson, *J. Lipid Res. 5,* 432)*; lettuce and cabbage tissues for sterols, sterol esters, glycerides, phosphatides, and glycolipids (Nichols, *Biochim. Biophys. Acta 70,* 417); the sterols and glycerides of lima, blackeye, pinto, kidney, and Californiia small white beans (Korytnyk and Metzler, *J. Sci. Food Agr. 14,* 841)*; green beans (*Phaseolus vulgaris* var Slender-green) (Wagenknecht, *J. Food Sci. 28,* 489); yam bean *(Pachyrrhizuz frosus)* seed for sterols and glycerides (Broadbent and Shone *J. Sci. Food Agr. 14,* 524)*; wheat endosperm for phosphatides, glycolipids, and a new sterol-containing glycolipid (McKillican, *JAOCS 41,* 554)*; and three varieties of Canadian wheat (McKillican and Sims, *Ibid.* 340)*.

Information on the glyceride structure of the following glycerides was reported: palm, linseed, corn, olive, cottonseed, and soybean oils and cocoa butter (Subbaram and Youngs, *Ibid.* 595)*†; 28 species of plants (Mattson and Volpenhein, *J. Lipid Res. 4,* 392)*†; palm oil (de Vries, *JAOCS 41,* 403)*†; coconut oil (Kuksis, et al. *Ibid.* 201)*†; palm oil (Jurriens et al., *J. Lipid Res. 5,* 366)*†; cocoa butter (Chacko and Perkins, *JAOCS 41,* 843)*†; cocoa butter (Subbaram and Youngs, *Ibid.* 445)*†; cocoa butter, olive, cottonseed, and soybean oils (Youngs and Subbaram, *Ibid.* 218)*†; *Garcinia indica* and *Vateriain indica* seed fats (Kartha, *Ibid.* 456)*†; coconut and linseed oils (Trowbridge et al., *Ibid.* 306)*†; olive, peanut, almond, linseed, sesame, castor, rape, soybean, and coconut oils, and cocoa butter (Anker and Sonanini, *Pharm. Acta Helv. 37,* 360)*†; castor oil (Achaya and Craig, *JAOCS 41,* 783)*†; *Cuphea llavia var. miniata* seed (Litchfield et al., *Ibid.* 588)*†; *Jatropha curcas* seed Gunstoen et al., *Chem. Ind.* [*London*] *1964,* 483)*†; and bitter gourd *(Momordica charantia)* seed (Subbaram et al., *JAOCS 41,* 691)*†.

The following lipids were analyzed for the indicated unsaponifiable constituents: yeast mutants for ubiquinone, tocopherol, vitamin A, ergosterol and total sterol (Mackler, *Biochemistry 3,* 893); *Clostridium sticklandii, Mycoplasm gallisepticum, Ochromonas malnamensis, Polyphorous schweinitzii* for coenzyme Q (Gale et al., *Arch. Biochem. Biophys. 104,* 169); Italian olive oil for unsaponifiables (Montefredine and Iacone, *Riv. Ital. Sostanze Grasse, Sympos. Issue 1962,* 49); rice bran oil for the ferulate ester of β-sitosterol (Tanaka et al., *Yukagaku 13,* 260); lucerne, white clover, cocksfoot and other grasses for α-tocopherol (Booth, *J. Sci. Food Agr. 15,* 342); castor, linseed, wheat germ, coconut, cottonseed, corn; safflower, soybean and other oils for vitamin E (Herting and Drury, *J. Nutr. 81,* 335); spinach for plastoquinones (Henninger and Crane, *Biochemistry 2,* 1168); soybean oil deodorizer scum for hydrocarbons (Yamada, *Yukagaku 13,* 321 and Evans et al., *JAOCS 41,* 406); corn, wheat germ, cottonseed, olive, safflower, soybean, and sunflower seed oils for hydrocarbons (Kuksis, *Biochemistry 3,* 1086); olive, linseed, and several other oils for hydrocarbons (Capella, *Riv. Ital. Sostanze Grasse 40,* 603); and cottonseed oil for gossypol (Rzehin et al., *Trudy Vniiz 23,* 70).

The variability of lipids from various Czech oat varieties was examined statistically (Pokorny et al., *J. Inst. Chem. Tech. Prague 6,* 199). Oat hull lipids were partially fractionated (Zeman et al., *Ibid.* 205)*. The composition of Japan wax was reported (Sano et al., *Yukagaku 13,* 324)*.

The following lipids of animal origin were analyzed for the indicated constituents: embryonic liver lipid in chicks for cholesterol and other fractions (Feldman and Grantham, *Poultry Sci. 43,* 150)*; bison serum lipoproteins, liver, rumen contents and abomasal fluid for free fatty acids, sterol esters, glycerides, and phosphatides (Evans, *J. Dairy Sci. 47,* 46)*; bovine semen lipids (Komarek et al., *J. Lipid Res. 5,* 268); bovine spermatozoa and seminal plasma for phosphatides, cholesterol, glycerides and waxes (Komarek et al., *J. Dairy Sci. 47,* 531); bovine blood plasma lipids during parturition and lactation for sterol esters, triglycerides, and phosphatides (Duncan and Garton, *Biochem. J. 89,* 414)*; mice plasma for sterol, sterol ester, and phosphatides (Yammamoto et al., *J. Lipid Res. 4,* 413); brown and yellow adipose tissue from rats for triglycerides and phosphatides (Chalvardjian, *Biochem. J., 90,* 518)*; cutaneous lipids (Wheatley, *Drug Cosmetic Ind. 93,* 161); human epidermis for triglyceride phosphatide, and cholesterol (Carruthers, *Proc. Soc. Exptl. Biol. Med. 115,* 215)*; chylomicrons from humans fed butter fat for triglycerides and phosphatides (Wood et al., *J. Lipid Res. 5,* 225)*; human red blood cells for cholesterol ester, glyceride and phosphatide (Hill et al., *JAOCS 41,* 393)*; human red blood cells for cholesterol and phosphatide (Ways and Hanahan, *J. Lipid Res. 5,* 318); human plasma lipoproteins for cholesterol esters, triglycerides, and phosphatides (Goodman and Shiratori, *Ibid.* 307)*; and human fibroblasts cultivated in vitro for phosphatides, cholesterol, and glycerides (Bole and Castor, *Proc. Soc. Exptl. Biol. Med. 115,* 174).

Glyceride structure information was obtained for the following fats: rats (Brockerhoff et al., *J. Biol. Chem. 239,* 735 and Perkins, *JAOCS 41,* 285)*†; Indian goat tallow (Rajagopal and Achaya, *J. Sci. Food Agr. 15,* 497)*†; Artiodactyla (domestic pig, European and American wild boar, and two species of peccary) (Mattson et al., *J. Lipid Res. 5,* 363)*†; human, dog, ground squirrel, chicken, pig, rabbit, and Guinea pig (Subbaram and Youngs, *JAOCS 41,* 595)*†; and lard (*Ibid. 445*)*†.

The following materials were analyzed for the indicated unsaponifiable components: baboons for blood serum cholesterol (Stron et al., *Circulation Res. 14,* 367); human blood serum, erythrocytes and white cells, platelets for free and total cholesterol (Hawthorne et al., *J. Nutri. 81,* 241); native Ethiopians for blood cholesterol (Loginov, *Federation Proc. 23,* T145); humans feces for sterols (Eneroth et al., *J. Lipid Res. 5,* 245); beef heart for cytochromes (King et al., *J. Biol. Chem. 239,* 1989); monkey heart, frog nerve, mice tumors, shark liver, *Cecropia* moth thorax, and for coenzyme Q (Gale et al., *Arch. Biochem. Biophys. 104,* 169); charcoal-broiled steaks for benzo (A) pyrene and other polynuclear hydrocarbons (Lijinsky and Shubik, *Science 145,* 53); and wool wax for hydrocarbons (Mold et al., *Biochemistry 3,* 1293).

Human tissues were found to contain methyl and ethyl esters of long chain fatty acids (Kaufmann and Viswanathan, *Fette Seifen Anstrichmittel 65,* 925). The alcohols, acids, and hydrocarbons of several coccid waxes were determined (Faurot-Bouchet and Michel, *JAOCS 41,* 418).

The following materials were analyzed for complex lipids

as indicated: housefly for phosphatides (Crone and Bridges, *Biochem. J. 89,* 11); human chylomicrons, egg yolk, sheep blood serum, and hog blood serum for phosphatides (Woods and Kinsell, *Proc. Soc. Exptl. Biol. Med. 114,* 225); hog serum phosphatides (Leat, *Biochem. J. 91,* 437)*; hog serum lecithin and lysolecithin (*Ibid.* 444)*†; blood serum glycolipids (Svennerholm and Svennerholm, *Biochim. Biophys. Acta 70,* 432); rat brain, heart, muscle, lung, liver, and kidney for phosphatides (Wagner et al., *Biochem. Z. 339,* 34); liver and pancrease for phosphoinositides (Kfoury and Kerr, *Biochim. Biophys. Acta 84,* 391); livers for phosphatide (Snyder and Lewis, *Proc. Soc. Exptl. Biol. Med. 116,* 459); rat liver for phosphatides (Skipski et al., *Biochem. J. 90,* 374); rat liver lecithin (Collins, *Ibid. 88,* 319)*; rat heart, lung, liver, spleen and skeletal muscle for plasmalogens (Gottfried and Rapport, *Biochemistry 2,* 646); rat liver mitochondria for phosphatidyl glycerol (Gray, *Biochim. Biophys. Acta 84,* 35)*; bovine bone marrow for α-glyceryl ether phosphatides (Thompson and Hanahan, *Biochemistry 2,* 641); cardiolipin (Marinetti, *Biochim. Biophys. Acta 84,* 55)*; Guinea pig forebrain subcellular particles for phosphatides and glycolipids (Eichberg et al., *Biochem. J. 92,* 91); human adult and infant brains for cerebroside sulfate (O'Brien et al., *J. Lipid Res. 5,* 109)*; human brain for phosphatides (*Ibid.* 329); beef brain phosphoinositide (Hendrickson and Ballou, *J. Biol. Chem. 239,* 1369); rat brain for α-glyceryl ether phosphatides (Ansell and Spanner, *Biochem. J. 88,* 56); human brain cerebrosides (Bernhard and Lesch, *Helv. Chim. Acta 46,* 1798)*; beef brain sphingolipids (O'Brien and Rouser, *J. Lipid Res. 5,* 339)*; egg yolk and rat and beef liver lecithins (Menzel and Olcott, *Biochim. Biophys. Acta 84,* 133)*†; gangliosides (Sambasivarao and McCluer, *J. Lipid Res. 5,* 103)*; human blood plasma for sphingomyelin, (Sweeley, *J. Lipid Res. 4,* 402)*; and pig brain for sphingolipids (Kishimoto and Radin, *Ibid.* 94)*.

The following marine and fresh water sources were analyzed for the indicated constituents: marine and fresh water sediments for carotenoid pigments (Schwendinger and Erdman, *Science 141,* 808); shark *(Carcharias ellioti Day)* liver, heart, and muscle for ubiquinone and tocopherol (Nazir and Magar, *Biochem. J. 90,* 268); tuna white muscle for phosphatides (Shuster et al., *JAOCS 41,* 36)*; South African pilchard *(Sardina ocellata* Jenyas) for phosphatides and glycolipids (Silk and De Koning, *JAOCS 41,* 619)*; tuna salmon, and menhedan muscle lecithin (Menzel and Olcott, *Biochim. Biophys. Acta 84,* 133)*†; and cod flesh phosphatides (Ackman and Burgher, *J. Fisheries Res. Board Can. 21,* 367)*.

The following analyses were performed on milk fat for the indicated constituents: β-keto fatty acid glycerol esters as precursors of methyl ketones (Parks et al., *J. Lipid Res. 5,* 232)*; triglycerides (Kuksis and McCarthy, *JAOCS 41,* 17 and Kuksis et al., *Ibid.* 201)*†; triglycerides from milk fat globule membranes (Folf and Dugan, *Ibid.* 139)*†; milk fractions for tocopherol (Eirckson et al., *J. Food Sci. 29,* 269); and phosphatides (Patton et al., *J. Dairy Sci. 47,* 489)*.

The lipids of dehydrated alfalfa were analyzed for glycolipids and unsaponifiables (Van der Veen and Olcott, *J. Agr. Food Chem. 12,* 287)*; butter for artificial fat soluble dyes (Janicek et al., *J. Inst. Chem. Tech. Prague 6,* 75); and margarine for tocopherol (Lambertsen et al., *J. Food Sci. 29,* 164).

Two lipoproteins were isolated from egg yolk (Saari et al., *Ibid.* 307).

PHYSICAL PROPERTIES. The crystal structure of long chain esters was reviewed (S. Aleby, *Acta Cryst. 16 Pt 13,* A 55). Infrared spectra gave evidence of new crystal forms of fatty acids (Holland and Nielsen, *Ibid. Pt 9,* 902). Information was obtained on the crystal structure of simple triglycerides, and 1- and 2-monoglycerides in which the terminal methyl group of the fatty acid chain was replaced with a bromine atom (Larsson, *Ibid. Pt. 13,* A 57). Differential thermal analysis demonstrated differences in the cooling curves of cocoa butter obtained by crushing and extraction (Mathieu et al., *Ind. Aliment. 2,* 57). Differential thermal analysis data was reported on milk fat, milk fat fractions, randomized milk fat, and butter obtained by conventional and continuous churning (Cantabrana and DeMan, *J. Dairy Sci. 47,* 32). Differential thermal analysis, melting point, and X-ray data was reported on six binary mixtures of the glycerides of palmitic and oleic acids (Moran, *Riv. Ital. Sostanze Grasse 40,* 412). The effect of mixtures of cocoa butter substitutes and cocoa butter in chocolate coatings was investigated (Janicek et al., *J. Inst. Chem. Tech. Prague 6,* 323 and Pokorny et al.,

*Ibid. 7*, 239). The melting point and dilation of partially acetylated mono- and diglycerides was investigated (Pokorny and Tomankova, *Ibid. 6*, 171). Melting point and differential thermal analysis data were given for some margarine oils and hydrogenated fats (Pokorny et al., *Ibid. 7*, 223). The thermal dilation of liquid glycerides was found to be linear and the effect of different glycerides structures was investigated (Hendrikse, *JAOCS 41*, 184).

Glycerides isolated from the Chinese tallow tree, *Sapium sebiferum* and from *Sebastiana lingustrina* were optically active (Maier and Holman, *Biochemistry 3*, 270). The refractive indices of saturated and unsaturated methyl esters were measured and correlated with theoretical equations (Gouw and Vlugter, *JAOCS 41*, 426). The same authors studied the dispersion and molar dispersion (*Ibid.* 514); dielectric constant (*Ibid.* 657); density and molar volume (*Ibid.* 142); and ultrasonic sound velocity (*Ibid.* 524).

The relative surface activity of 1- and 2-monoglycerides was tested (Hartman, *Ibid.* 519). The properties of aqueous dispersions of phosphatidyl serine were measured (Abramson et al., *J. Biol. Chem. 239*, 70). The effect of pH on the force-area relation and infrared spectra of calcium stearate monolayers was investigated (Bagg et al., *J. Am. Chem. Soc. 86*, 2759). The foam stability of thermally oxidized trilinolein was correlated with the degree of heat damage (Ota et al., *Yukagaku 13*, 264), and the relation of surface tension and viscosity to foaming was investigated (Ota, *Ibid.* 269). The solubilities of stigmasterol and sitosterol and their acetates were determined in 22 organic solvents at 20C (Yamada and Hosono, *Ibid. 13*, 200). The solubility of stigmasterol and nonstigmasterol sterols in butanol was investigated (Sato and Akashi, *Ibid. 12*, 617). α-Sitosterol was found to decrease the solubility of cholesterol in coconut oil (Wright and Presberg, *Proc. Soc. Exptl. Biol. Med. 115*, 497). The solubility of cholesterol in various fats and oils was measured and found to be the greatest in short chain glycerides (Kritchevsky and Tapper, *Ibid. 116*, 104). The solubility of 8-hydroxycaprylic acid in water and benzene was measured (Yasukawa and Abe, *Yukagaku 13*, 360).

The infrared spectra of pure methyl oleate and methyl elaidate were measured and discussed to measure the *trans* isomer dosage in the *cis* isomer (DeFrancesco, *Riv. Ital. Sostanze Grasse 41*, 20).

The role of phosphatides in the structure of mitochondria was discussed (Green and Fleischer, *Biochim. Biophys. Acta 70*, 554). The following lipid-protein interactions were studied: human serum albumin with phosphatidylserine (Theriault and Taylor, *JAOCS 41*, 490); various steroid harmones with human serum albumin, orosomucoid and transcortin (Westphal, *Ibid.* 481); cholesterol with human serum lipoprotein films (Zilversmit, *J. Lipid Res. 5*, 300); and phosphatides with cytochrome C (Das and Crane, *Biochemistry 3*, 696). The effect of vitamin A on lecithin-cholesterol monolayers was used to explain vitamin A toxicity (Bangham et al., *Biochem. J. 90*, 133).

## THE EFFECT OF ENVIRONMENT, DIET, AND GENETIC FACTORS ON COMPOSITION AND CHARACTERISTICS

Many reports in this area will be found in the section on nutrition. The present section is restricted to reports which are primarily of technological interest.

A study of 231 Italian olive oil samples revealed that northern oils have more oleic, less linoleic and a higher ratio of unsaturates to saturates than southern oil (Montefredine and Laporta, *Riv. Ital. Sostanze Grasse 40*, 382). The effect of temperature and rainfall on the oil content and fatty acid composition of linseed and rapeseed oils was discussed (Salans, *JAOCS 41*, 215). The iodine value of the fat from crustacean plankton in Lake Balaton was shown to have a regular annual cycle. The melting point of the lipid remained a little lower than the water temperature throughout the year, and the proportion of long chain polyunsaturated fatty acids increased with decreasing temperature (Farkas and Herodek, *J. Lipid Res. 5*, 369). Seasonal and environmental differences during the growth of wheat samples were correlated with their baking performance (Fisher et al., *J. Sci. Food Agr. 15*, 325).

Changes in the amounts and composition of the free fatty acids occurred during the growth cycle of lactic acid bacteria (Vorbeck et al., *J. Food Sci. 28*, 495). The composition and characteristics of almond *(Prunus amygdalus)* cotyledons remained constant during germination and the early stages of growth, but at a certain stage of development there was an increase in free acidity and iodine value, and the fatty acid composition changed (Lotti, *Riv. Ital. Sostanze Grasse 40*, 385). Changes in the fatty acid composition and oil content of ripening castor beans was recorded (Chandra, *JAOCS 41*, 251). The oil composition and characteristics of walnut (*Juglans regina* L.) cotyledons showed no change during the early stages of germination and growth, but at a certain stage the acid value increased, the iodine value decreased, and there was a change in fatty acid composition (Galoppini, *Riv. Ital. Sostanze Grasse 40*, 382). No acetyl value could be detected in the oil of ripening coconuts (Kartha, *J. Sci. Food Agr. 15*, 299). The degree of unsaturation and percentage of moisture in subcutaneous fatty tissue decreased as the fat content of young pigs increased (Kaufman et al., *J. Food Sci. 29*, 70). Tissues containing a greater amount of moisture and unsaturated fat were related to softer and leaner pork with less palatability.

The effect of cultivation media on the fat content of the yeast *Rhodotorula gracillis* grown on sulfite waste liquor was studied (Byr and Protive, *J. Inst. Chem. Tech. Prague 6*, 125). Lake crustaceans raised on algae containing fatty acids no longer than $C_{18}$ were rich in $C_{20}$–$C_{22}$ fatty acids, and fish fed on such crustaceans had a typical marine oil composition (Farkas and Herodek, *J. Lipid Res. 5*, 369).

Replacing carbohydrate in chick diets with fat increased the metabolic efficiency and the fat content of the tissues (Carew and Hill, *J. Nutr. 83*, 293). Chicks showed a marked growth response to dietary corn oil, and this response was not greatly influenced by the amount of unsaturated fatty acid residues found in the protein sources (Marion and Edwards, *Poultry Sci. 42*, 825). The efficiency of energy utilizations of chicks fed low fat diets or diets with corn oil, beef tallow, soybean oil, hydrogenated coconut oil or a lightly hydrogenated olive oil were compared (Carew et al., *J. Nutr. 83*, 300). The efficiency was higher in the diets containing fat except for hydrogenated coconut oil. A 10% level of corn oil was as good as higher levels. In evaluating acidulated cottonseed soap stock as a component of broiler rations, it was found that 0.01% dietary gossypol was a safe upper limit (Lipstein and Bornstein, *Poultry Sci. 43*, 686). Up to this limit cottonseed oil soap stock was as good as soybean oil soap stock. The gossypol content of cottonseed oil soap stock was influenced by extraction, storage, and production methods (*Ibid.* 694). A treatment with hot concentrated alkali just before acidulation reduced the gossypol to a safe level. The inclusion of 0.5% tung oil in chick rations caused slow growth and 2–5% caused death, but tung oil methyl esters and the unsaponifiables isolated from alkaline hydrolyzates were not toxic (Edwards, *J. Nutr. 83*, 365). Crude cottonseed oil included in the ration of laying hens caused the hens to lay eggs with larger proportions of stearic acid and smaller proportions of oleic acid. Inclusion of 10% corn oil in the diets increased the linoleic acid and slightly decreased the stearic acid, but corn oil did not reverse the effect of cottonseed oil when the two were fed together. Olive oil increased the oleic acid and decreased linoleic and stearic (Evans et al., *Poultry Sci. 42*, 875). Rate of egg production and hatchability was studied for two generations of hens on low fat diets. Hatchability was depressed by the low fat diet. Egg production was not improved in the first generation by addition of unsaturated fats in the diet, but this did help in the second generation. Eggs from second generation hen contained no essential fatty acids or trienoic acid (Jensen and Shutze, *Ibid.* 1014). Corn oil, tall oil, and safflower oil all improved egg weight of hens fed a semipurified diet. Methyl linoleate and linoleic acid in the diet had a similar effect (Shutze and Jensen, *Ibid.* 921). Feeding cholesterol to hens caused a temporary rise in serum and egg cholesterol at about 10 days, but by 20 days the cholesterol levels had returned to normal (Edwards and Jones, *Ibid. 43*, 877). High dietary levels of isolated soybean protein, purified isolated soybean protein, casein-gelatin, vitamin-free casein, and amino acid mixtures duplicating isolated soybean protein caused a decrease in liver vitamin A storage compared to moderate dietary protein levels (Stoewsand and Scott, *J. Nutr. 82*, 139). Treatment of male Broad-Breasted Bronze turkeys with diethylstilbesterol caused high mortality as a consequence of aortic rupture (Simpson and Harms, *Poultry Sci. 43*, 681).

Yields of milk and milk fat were depressed by 15% added safflower oil to diets of Guernsey and Jersey cows (Parry et al., *J. Dairy Sci. 47*, 37). The ratio of unsaturated to saturated fatty acids in the milk fat was increased. Susceptibility of milk to spontaneous and agitation-induced lipolysis was not affected by the diet or nutritional level of the cows but was a characteristic of the individual cows (Cannon and Rollins, *Ibid.* 41). The infusion of whale or linseed oil into the rumen of pasture-fed cows caused marked reduction in acetic and butyric acid in the rumen (Robertson and Hawke, *J. Sci. Food*

*Agr. 15*, 274). Propionate levels remained constant and ammonia levels increased. Mono- and diglycerides were found in the rumen after the infusion of linseed oil (*Ibid.* 283). After 6.5 hr the proportions of linoleic acid in the rumen was still high but linolenic had returned to prefeeding levels. The free fatty acids were more saturated than the unhydrolyzed and partially hydrolyzed glycerides. The level of short chain fatty acids in milk fell markedly from their normal values in cows with fasting ketosis (Luick and Smith, *J. Dairy Sci. 46*, 1251). Administration of growth hormone to dairy cows increased the blood plasma free fatty acid concentration 50% (Williams et al., *Ibid.* 1405).

The depot fat composition was compared for white, cape colored, and Bantu males and females in the third and fifth decades of life. Age had no effect on the depot fat composition. Females showed a higher ratio of monoenoates to saturates than males. Myristate was highest in the whites and lowest in the Bantu and was closely correlated with total fat intake of the three groups. Linoleate was significantly higher in the cape colored (Krut and Bronte-Stewart, *J. Lipid Res. 5*, 343).

Selection of rapeseed plants containing no erucic acid in their seed oil gave simultaneous selection for low eicosenoic acid; however, in populations whose genetic capacity to produce erucic acid was decreased only partially there was no decrease in the eicosenoic acid content (Downey, *JAOCS 41*, 475). The lipid content and composition of flour from different varieties of wheat were compared and correlated with baking properties (Fisher et al., *J. Sci. Food Agr. 15*, 325). Different varieties of wheat endosperm were found to have different fatty acid and lipid compositions (McKillican, *JAOCS 41*, 554). Eggs from five different strains of chickens from the same breed had significantly different fatty acid compositions (Edwards, *Poultry Sci. 43*, 751).

Various locations on the endosperm or embryo of several kinds of seeds differed in oil content and iodine value (Kartha, *J. Sci. Food Agr. 14*, 515). The fat content of pork was correlated with higher flavor, tenderness and juiciness (Kauffman et al., *J. Food Sci. 29*, 70). At higher fat content there was also less curing and cooking loss. Contrary to other cereals, the lipid content of oats was inversely rather than directly correlated with the protein content (Pokorny et al., *J. Inst. Chem. Tech. Prague 7*, 223).

## DETECTION OF ADULTERATION

Methods for the determination of re-esterified olive oil were reviewed (Adam, *Lipidos 22*, 117). Pancreatic lipase treatment of virgin and re-esterified olive oils followed by analysis of the fatty acids, and monoglyceride products allowed the detection of 25% re-esterified oil in virgin oil (Mazuelos et al., *Grasas y Aceites 15*, 12). The dark brown color induced by nitric acid in olive oils (Hauchecorne reaction) is not specific for esterified oils but rather detects autoxidation (Jacona, *Olearia 17*, 106 and Petruccioli, *Ibid.* 187). Adulteration of olive oil with 10–15% teaseed oil could be determined by the Fitelson reaction (Vitagliano and Vodret, *Riv. Ital. Sostanze Grasse, Sympos. Issue 1962*, 39). In some cases, genuine olive oil gives a positive reaction and this is correlated with late harvesting of the olives. Passage of olive oil through an alumina column makes it give a positive Fitelson reaction, but the reaction in teaseed oil is destroyed by alumina. The Fitelson reaction detected more than 20% adulteration of olive oil with teaseed oil, but autoxidation decreased the sensitivity of the reaction (Chindemi et al., *Ibid.* 296). As little as 5% sesame oil in olive, soybean, cottonseed, or corn oil can be detected by the yellow-green color of the acid layer when nitric acid is shaken with an oil (Synodinos and Konstas reaction) (Musurakis, *Ibid. Sympos. Issue 1962*, 183). It was possible to classify olive oils into grades of virgin-extra fine, virgin, and blends of virgin with treated oils by the free fatty acid content and the absorbance at 232 and 268 mμ (Casillo, *Olearia 18*, 9). These grades correlated with organoleptic evaluation. Alumina treatment of olive oils prior to ultraviolet absorption spectrophotometry was recommended to remove oxidation products (Corbi and Cicero, *Ibid. 17*, 148). Treatment of olive oil samples by passage through an alumina column to prepare them for spectrophotometric examination was poorly reproducible and inadequate to differentiate crude and rectified oils (Kaderavek, *Riv. Ital. Sostanze Grasse 40*, 420). The method was satisfactory when the free acidity of the oil was below 2%. Organic phthalates which are used to denature industrial olive oil were detected by a color reaction with quinizarine (Jaforte and Piepoli, *Ibid.* 425). Isopropyl alcohol residues left from the refining of olive oils were detected by oxidation to acetone (Garoglio and Boddi, *Olearia 18*, 5).

Adulteration of pumpkin seed oil was detected by paper chromatography of the fatty acids (Grobach and Weber, *Fette Seifen Anstrichmittel 65,* 989). Rapeseed oil could be detected by its erucic acid, soybean oil and linseed by their linolenic acid, and safflower by its lignoceric acid. The combination of zones from several papers for rechromatography increased the sensitivity.

Industrial-grade vegetable oils denatured with tricresylphosphate can be detected by a new rapid method (Armandola, *Ind. Aliment, 3,* 33).

Gas chromatography of the triglycerides could detect adulteration of milk fat with 5 to 10% lard or vegetable fats (Kuksis and McCarthy, *JAOCS 41,* 17). A certain mixture of coconut oil and lard escaped detection. Two branched chain fatty acids are present in tallow which are absent from lard (Grieco, *Riv. Ital. Sostanze Grasse, Sympos. Issue 1962,* 200). This allows 5–10% adulteration of lard with tallow to be detected by gas chromatography. By a combination of dilatometry, Bohmer index, and fatty acid composition, 10% tallow in lard was detectable (Jacini et al., *Ibid. 40,* 584). The ratio of saturated to linoleic acid determined by gas chromatography and the Bohmer index can be used to detect 10% tallow in lard (Pascucci and Paolini, *Ibid. Sympos. Issue 1962,* 194). The ratios of certain fatty acids determined by gas chromatography may be used to distinguish the following fats: tallow, calf, mutton, bone tallow, horse, and lard (Wolff and Audiau, *Rev. Franç. Corps Gras 11,* 77).

Evidence was presented that the two toxic compounds isolated from fats capable of producing hydropericardium in chicks were isomers of hexachlorohexahydrophenanthrenes (Wootton and Courchene, *J. Agr. Food Chem. 12,* 94). Endosulfan (Thiodan) in beef fat was detected by a color reaction between the residue and methanolic alkali and aqueous pyridine (Maitlen et al., *Ibid. 11,* 416). Captan, chlordan, and heptachlor interfered. A purely physical procedure was presented for the cleanup of butterfat for analysis for chlorinated insecticides (Ott and Gunther, *Ibid. 12,* 239). About 0.5 ppm of insecticide in 2 g of sample can be detected in an hour. Methoxychlor is detectable at 10 ppm. Heptachlor residues were found in milk fat from cows that had grazed 57 days on a pasture treated with 0.25 lb/acre of heptachlor (Rusoff et al., *Ibid. 10,* 377). The animals continued to excrete the residue for 40 days after removal from pasture. Dieldren was found in the body fat of the general population of the United States at a mean value of 0.15 to 0.02 ppm (Dale, *Science 142,* 593). This agrees with values from England. The mean concentration of benzene hexachloride was 0.20 to 0.04 ppm which is lower than comparable data from France. DDT determinations by colorimetric methods give incorrectly high values in human fat.

*The Annual Review of Literature Will Be Concluded in December*

## • *New Literature*

CELANESE CORPORATION OF AMERICA, New York, N.Y., announces the U. S. Food and Drug Administration regulation prescribing conditions for safe use of 1,3-butylene glycol (1,3-BG) in food flavorings. The four-carbon diol has significant potential use in food processing, artificial flavoring, extracts and food colors, as well as in pharmaceuticals and cosmetics.



## • *New Members*

**Active**

Graeme Levo Baker, Professor, Montana State University, Bozeman, Montana.

Robert Adam Blatz, Head Chemist, Swift Canadian Co., Ltd., St. Boniface, Manitoba, Canada.

William J. Carnes, Analytical Chemist, The Procter & Gamble Co., Cincinnati, Ohio.

John Gordon Erdman, Manager, Geochemical Branch Research & Development, Phillips Petroleum Co., Bartlesville, Oklahoma.

Albert James Fenton, Jr., Research Chemist, Procter & Gamble Co., Wyoming, Ohio.

Jerry Gagliardi, Production Superintendent, Cargill Inc., Kenosha, Wis.

Ignacio Garibay, Superintendent, Aceitera "La Gloria" S.A., Guadalajara, Mexico.

Cecil Barry Johnson, Research Fellow, The Hormel Institute, Austin, Minn.

Frederick C. Kast, Chemist, Eastern Industrial Oil Products, Stoughton, Mass.

Morris Kates, Senior Research Officer, National Research Council, Ottawa, Ontario, Canada.

Marius E. LePage, Research Scientist, Canada Department of Agriculture, Pte-Garineau, Canada.

Douglas John McIntosh, Laboratory Technician, University of California, Berkeley, California.

Raymond E. Meyer, Research Plant Physiologist, USDA, State College, Miss.

Martin John Moran, Chemist, Armour & Co., Oakbrook, Ill.

David William Newman, Assistant Professor, Miami University, Oxford, Ohio.

Rosemarie Ostwald, Assistant Professor, University of California, Berkeley, California.

Oscar Petersen, General Director, Aceitera "La Gloria" S.A., Guadalajara, Mexico.

William K. Rohwedder, Principal Chemist, Northern Regional Research Laboratory, Peoria, Illinois.

Donald N. Wright, Research Assistant, Naval Biological Laboratory, Richmond, California.

Robert L. Zipf, Plant Manager, Werner G. Smith, Inc., Cleveland, Ohio.

**Individual Associate**

Stanley T. Gibrowski, Eastern Division Manager, The DeLaval Separator Co., Westfield, N.J.