# Report of the Literature Review Committee

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

**V. MAHADEVAN, University of Minnesota, The Hormel Institute, Austin, Minnesota**

### DETERIORATION OF FATTY MATERIALS

Studies on the deterioration of fatty materials and on the changes in physical and chemical properties occurring during storage continued to make progress.

**Deterioration by Heat**

The chemistry, manufacture, and uses of dimeric fatty acids were reviewed by Byrne (*Offic. Dig. Federation Socs. Paint Technol., 34,* 229), and by Cowan (*JAOCS, 39,* 534).

Heat treatment of linoleate esters for short periods and spectrophotometric analysis of the resultant conjugated products showed that Diels-Alder's type addition is not the sole dimerization mechanism during thermal polymerization of unsaturated fats, Norton et al., *Chem. Ind., 1961,* 1452). When four different isomers of methyl linoleate were polymerized in an inert gas atmosphere, it was evident that isomerization of the double bond resulted in the formation of conjugated *trans-trans* and nonconjugated *trans* isomers and that these two isomers underwent Diels-Alder's type addition (Nagano and Tanaka, *J. Oil Chem. Soc. Japan, 2* (3), 119). Heating of fatty acid esters of more than four nonconjugated double bonds caused their structure to change to the conjugated form. The compounds produced at 200C were linear and had the *cis-cis-trans* conjugated triene form. Treatment at 215C caused the formation of both linear and cyclic compounds in approximately equal proportions. In both cases, the conjugated triene compounds were monomeric but not dimeric (Miyakawa and Nomigu, *J. Oil Chem. Soc. Japan, 10* (12), 724).

Heating of pure triglycerides provided evidences indicating that triglycerides decompose at high temperatures in different ways, according to the presence or absence of oxygen. In the absence of air even a fully saturated triglyceride had only limited stability and the first important degradation product is the component fatty acid (at 240–260C). Unsaturated triglycerides were more readily degraded (Crossley et al., *JAOCS, 39,* 9). The attack of oxygen at high temperatures caused dehydrogenation of saturated triglycerides. Subsequently, hydroperoxides were produced, as well as intermediates containing hydroxyl, carbonyl, and carboxyl groups. Hydrolysis of the ester linkage between glycerol and fatty acid also occurred and it was found to occur whether the acid was a long chain saturated one such as palmitic, a short chain saturated, such as lauric, or a long chain unsaturated such as oleic acid (Endres et al., *Ibid., 39,* 118). Of the wide variety of compounds formed during thermal oxidation of these pure triglycerides, long chain carbonyls were produced during the initial stages. Under longer oxidation periods such as 24 hours, volatile saturated aldehydes from $C_1$ to $C_{16}$, and ketones, developed. Also fatty acids from $C_4$ to $C_{16}$ were identified, as well as dicarboxylic acids. The main tool for identification of these products was gas-liquid chromatography (GLC) (Endres et al., *Ibid., 39,* 159).

When natural oils were treated similarly, linseed oil led to the formation of distillable esters which contained a nonurea-adducting fraction. This fraction was separable into three subfractions by GLC. All three had the 1-propyl-2-alkencarboxycyclohexane skeleton, but differed in the position of the double bond in the side chain (McInnes et al., *Canadian J. Chem., 39,* 1906). When herring oil was heated at 100C, the volatiles entrapped in a stream of nitrogen and passed through a solution of 2,4-dinitrophenylhydrazine, the 2,4-dinitrophenylhydrazones of the following main compounds were identified: acetaldehyde, propionaldehyde, isobutylaldehyde, 2-methyl-butylaldehyde, and acetone (Hughes and Herring, *J. Sci. Food Agr., 12,* 822). Bodying of soybean oil in an inert atmosphere at around 300C increased the concentration of monounsaturated acids and reduced that of the polyunsaturated ones (Capella et al., *Chim. e Ind.,* (Milan), *43,* 1004).

Thermal oxidation of oils rich in unsaturated fatty acids was characterized by passing 2–3 ml of $O_2$ per g of oil per min at 80–200C. The oxidation process occurred in four distinct stages: a) saturation of one double bond with formation of one-peroxy- and one epoxy-group; b) increase of the peroxide and epoxide contents to a maximum; c) decrease of the content of double bonds, peroxides, and epoxides, accompanied with an increase in the level of carbonylic and carboxylic derivatives to a maximum, thus causing high viscosity and gelatinization (Porkorny, *Sbornik Vysoke Skoly Chem. Technol., Oddil Fak., Potravin Technol., 1958,* 123). Whereas no significant changes were detected in the physical properties of the oil in the first stage of oxidation, in the second there was an almost linear relationship between the increase in refractive index and viscosity and the simultaneous decrease in specific rotation and iodine value (Pokorny, *Sbornyk Vysoke Skoly Chem. Technol., Oddil Fak., Potravin. Technol., 1957,* 237; *Ibid., 1958,* 123; Pokorny and Mares, *Ibid.,* 221). The overall process was equally accompanied with increases in specific gravity, acidity, peroxide number, and acrolein content, and with a decrease in the linoleic acid content (Simko et al., *Cesk Gastroenterol. Vyzivy, 15,* 429). The change of color accompanying the autoxidation of blown linseed oil was correlated to the temperature (Pokorny, *Sbornyk Vysoke Skoly Chem. Technol., Oddil Fak., Potravin. Technol., 1960,* 205). It was also observed that the peroxide content of the decomposed oils was an important commanding factor of the rate of autoxidation. When the peroxide concentration was low, the reaction proceeded as an autocatalytic second order reaction, but when significant amount of peroxides accumulated, the reaction order changed to the first order (Pokorny, *Sbornyk Vysoke Skoly Chem. Technol., Oddil Fak, Potravin. Technol., 3,* 261).

The following volatile compounds were identified in air-heated (240C) soybean oil chiefly by GLC: normal saturated and monounsaturated hydrocarbons from $C_2$ to $C_8$; methyl cyclopentane, cyclohexane, benzene, aldehydes from $C_2$ to $C_8$, acrolein, crotonaldehyde, and ketones such as acetone and methyl ethyl ketone (Tor et al., *J. Oil Chem. Soc., Japan, 10* (9), 536). Vinyl ketones were recognized in oxidized safflower oil and butter fat as responsible for metallic flavor (Forss et al., *JAOCS, 39,* 308). In cooked pork, agents causing sex odor were found in the unsaponifiable fraction (Craig et al., *J. Food Sci., 27,* 29).

Several papers concerned with the alterations produced in oils by frying appeared. Products like potato chips, wheat flour doughs, and fillets of pork and veal were treated under commercial frying conditions. Deterioration of the oil accelerated at higher temperatures. However, regarding the oxidation stability of the fried oil as indicated by the Active

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Oxygen Method (AOM) test, an oil fried at 200C proved to be more stable than another fried at 170C (Kumazawa, *J. Oil Chem. Soc. (Japan), 10* (9), 531). Addition of fresh fat during frying had little effect whereas the addition of natural antioxidants delayed oxidation (Janicek et al., *Vopr. Pitaniya, 20* (6), 12; Pokorny and Supova, *Sbornyk Vysoke Skoly Chem. Technol., Oddil Fak., Potravin Technol., 1958,* 249). In general, the type and extent of the changes in the oil during frying depended on the starting quality and the length of the treatment (Mroczkowski and Ziombski, *Roczniki Panstwowego Zakladu Hig., 13,* 175; *Riv. Ital. Sostanze Grasse, 39,* 81).

Reviews on the biological effects of heat-altered fats (Frazer, *Chem & Ind., 1961,* 417; Rinetti and Giovetti, *Minerva Dietol., 1,* 91), and on their carcinogenic properties (*Nutrition Revs., 20,* 346) appeared.

Toxic monomeric nonurea-adduct forming derivatives were isolated from thermally polymerized linoleate (Bottino, *JAOCS, 39,* 25) and linolenate esters (Matsuo, *Bull. Chem. Soc. Japan, 35,* 105). However, experiments with natural oils indicated that a treatment more severe than those commercially used was necessary to produce detectable damage and the subsequent harmful effects. Furthermore, no harmful substances were found in fat heated in the presence of food (Poling et al., *JAOCS, 39,* 315; Warner et al., *J. Am. Dietet. Assoc., 40,* 422). Peanut oil was isomerized to contain 60% *trans*-acids and fed to rats in admixtures with fresh oil. Although there was some evidence of the isomerized fats being less readily digested, the differences were small. However, the coefficient of lipid efficiency (defined as the weight gain/g of fat ingested) was 10–15% higher for isomerized fats (Raulin and Loriette, *Compt. Rend., 254,* 1154). Indeed, abusive heating of highly unsaturated oil (herring) resulted in products causing slow growth and high mortality when fed to rats (Raulin and Petit, *Arch. Sci. Physiol., 16,* 77; Raulin and Terroine, *Ibid.,* 89).

The flavor of French fried potatoes was found to improve as the acidity of the frying pork fat increased with repeated use up to a value of approximately 5.5; thereafter, this relation reversed as the acidity continued to rise (Hall et al., *J. Agr. Food Chem., 10,* 96).

The observation that the free fatty acid content of the diet affected the severity of atherosclerosis in cholesterol fed rabbits (Kritchevsky et al., *J. Atheroscler. Res., 2,* 115) stimulated studies on the influence of short term heating on the degree of hydrolysis of edible fats (Kritchevsky et al., *J. Nutrition, 77,* 127).

Co-carcinogenic effects were encountered in lipase-undigestible or nonurea adduct forming fractions isolated from heated oils when fed to rats. The effect could be best demonstrated when the fractions were fed in admixture with fresh oil (Sugai et al., *Cancer Res., 22,* 510).

Methods were developed for the determination of the polymerized portion of heated oils (Sedlacek, *Prumysl Potravin, 12,* 607; Rost, *Fette, Seifen, Anstrichmittel, 64,* 427). The sedimentation velocity procedure was applied to the estimation of the molecular weights of linseed oil polymers (Lück et al., *Fette, Seifen, Anstrichmittel, 64,* 239). The Tortelli-Jaffe reaction was used for the characterization of the cyclic compounds formed by heating highly unsaturated acids (Murase and Toyama, *Nagoya Sangyo Kagaku Kenkyusho Kenkyu Hokoku,* (12), 42 (1960); Ono, *Ibid.,* (12), 47 (1960). A significant correlation was found between the smoke-point and the free fatty acid content of thermally treated fats (Zabik, *Food Technol., 16,* 111).

Patents were issued for the preparation of polymeric acids (Unilever, *Ger., 1,110,642; Council of Scientific and Industrial Research, India, 67,636; Council of Scientific Research, India, 70,174; Emery Industries, Canada, 595,595 and 595,598;* Wolff and Rowe, *Brit., 878,985;* Glidden Company, *U.S., 2,978,468*) of oxidized, polymerized oil (Mozell and Gleason, *U.S., 3,026,279*) and of cyclic derivatives from linolenate (Scholfield et al., *U.S., 3,041,360;* Beal, *U.S., 3,005,840*). A continuous fryer was devised which prevented polymer formation during the manufacture of potato chips (Goodin and Melnick, *U.S., 2,973,268*).

### Deterioration During Storage

Reviews on the changes in the physical and chemical properties of fats and oils brought about by oxidation (Borodina, *Sbornik Nauch. Rabot, Leningrad Inst. Sovet. Torgovliim F. Engel'sa, 1959,* (13), 114), on the principles of the autoxidation process as related to the development of off-flavors (Badens, *Neth. Milk & Dairy J., 14,* 215) and on the biological aspects of fat deterioration (Lea, *Food Technol., 15* (7), 33, 37, 40) were published. Less general topics such as oxidation of fish lipids (Olcott, *Conf. Fish. Nutr.,* Washington, D.C., *1,* 1.9.1), the changes occurring in them during processing and storage (Lovern, *Conf. Fish. Nutr.* Washington, D.C., *1,* 1.8.1), soybean oil flavor reversion (Gutierrez, *Grasas y Aceites, 12,* 190), and the physiological and cooking value as well as the storage stability of compound-cooking fats (Polladina et al., *Tr., Vses. Nauchn.-Issled. Inst. Zhirov, 1960* (20), 223), were also discussed. Czok (*Deut. Lebensm.-Rundschau, 57,* 297) discussed the influence of light on the autoxidation of fat.

Alkali refined sunflower seed oil was stored in underground iron tanks and the changes in its physical and chemical characteristics, flavor, and stability were followed over 1.5–2.75 year periods at temperatures ranging from 3–15C. Whereas the free acidity remained constant, the peroxide value augmented. However, oils from good seeds stored well for more than 1.5-year periods (Stanculescu et al., *Lucrarile Inst. Cercetari Aliment., 5,* 99). Excessive increase in the moisture content of the seed contributed to increase the free acidity thus lowering the oil quality and making its refining more difficult (Aleshin and Ryazantseva, *Soobschen. i Referaty Vsesoyuz. Nauch. Issledovatel, Inst. Zerna i Produktov Ego Pererabotki, 1960* (3), 14). Also the level of acidity in the fat of stored raw coffee beans was higher in beans prepared by wet treatment (Baiao Esteves, *Rev. Cafe Port., 7* (28), 74). Oxidative deterioration of the lipids in crude leaf proteins was equally enhanced by moisture, having antioxidants little protective effect (Lea and Parr, *J. Sci. Food Agr., 12,* 785). That the phospholipids present in the oil hampered its storage qualities was demonstrated by keeping sunflower seed oil samples containing 0.25, 05, and 0.75% added phospholipids at relative humidities of 60, 75, and 90% and at 18–20C and 0–3C. Hydrated phospholipids formed during storage and precipitated, thereby enriching the lower layers of the oil with water, phospholipids, and free acids. The free acidity of the upper layers of the sunflower oil with phospholipids was the same at all humidities and was lower than that of the oil without phospholipids. The upper layers were more oxidized than the lower layers (Kozin and Sitnikova, *Sb. Nauchn. Rabot. Mosk. Inst Narod. Khoz., 1961* (17), 103). The hydration of the phospholipids themselves was studied under laboratory conditions by putting phospholipids in solution in soybean oil, in the presence of water. The solubility of the water in hydrated oil augmented with its content of nonhydratable phospholipids (Jacubowski, *Oléagineux, 17,* 201).

Lipolytic alteration of frozen substrates by microorganisms was shown to be related to the degree of unsaturation of the fat in the substrate. Considerable lipase activity was found within 2–4 day storage at −7C and within a week at −18C (Alford and Pierce, *J. Food Sci., 26,* 518). Gas-liquid chromatographic characterization of the liberated fatty acids revealed that the rate of fatty acid liberation fell off sharply after the first few hours at low temperatures, but that the ratios of the types of fatty acids were not affected until after 24 hours (Alford et al., *J. Food Sci., 26,* 234). Also hydrolysis, and the degradation of nonfatty, nonnitrogen-containing substances were the causes of the undesirable properties of the sulfur olive oil obtained from olive presscake (Gracián et al., *Grasas y Aceites, 12,* 174; Gracián and Martel, *Ibid., 13,* 128). There was a relationship among the free fatty acid content of the residual oil, the dryness of the olive cake, and the number of microorganisms present. However, the total fat content of the olive cake was not affected by the presence of moulds and yeasts (Gracián et al., *Grasas y Aceites, 13,* 17). The microorganisms responsible for the oxidation of palm oil during storage were also characterized and their participation in lipolytic processes was studied (Coursey and Eggins, *Oléagineux, 16,* 227). When rice-bran oil was kept under deteriorating conditions, the rate of hydrolysis rose immediately while oxidation occurred only after an induction period, paralleling the development of off-flavors (Ivanov, *Compt. Rend. Acad. Bulgare Sci., 14,* 799). The upper limit of the acid value reached by the same oil after 1–2 year storage was discussed statistically (Takeshita, *J. Oil Chem. Soc. Japan, 11* (3), 105). Damaged rapeseed grains possessed higher water content and absorbed more atmospheric moisture causing a faster mold growth and an intensified lipase activity (Rutkowski et al., *Oléagineux, 17,* 91). The lipids of fermented cabbage and Brussels sprouts equally showed considerable increases in the amount of free fatty acids (Vorbeck, Univ. Microfilms, Order No. 62–2512, Dissertation Abstr., *23,* 33). Lecithin added lecithin-barley walls mixtures were kept at various relative humidities at 30C. Constant values for phospholipase B and D were detected after 48 days at a relative humidity above 55% (Acker, *Vortraege Originalfassung Intern. Kongr. Grenzflaechenaktive Stoffe, 3, Cologne, 4,* 578).

Some work was devoted to the hydrolytic alterations of fish lipids during cold storage. There seemed to be a relationship between lipid hydrolysis and denaturation of frozen fish muscle proteins (Bligh, *J. Fish Res. Board Canada, 18,* 143; Jonas and Tomlinson, *Ibid., 19,* 733). Nevertheless, a thorough

study of the two phenomena indicated that there was not simple cause-and-effect relationship. Freezing and thawing was found to lead to free fatty acid production and in many instances phospholipase was found to be as important as lipase in producing free fatty acids (Olley et al., *J. Sci. Food Agr., 13,* 501). In cellophane-wrapped lingcod fillets, lipid hydrolysis proceeded at a roughly uniform rate. On the contrary, the rate of alteration of gray cod lipids varied with time. At the end of 15 weeks, the respective degree of hydrolysis was 25 and 40% (Wood and Haqq, *J. Fisheries Res. Board Can., 19,* 169). The fishy odors and flavors in deteriorated fish oils resulted from the formation of many types of substances some of which were characteristic of the individual species of fish, others being produced by oxidative alteration of fish oils and phospholipids and by bacterial and/or enzymatic hydrolysis (Stansby, *Food Technol., 16* (4), 28). The volatile carbonyl compounds developed in haddock flesh upon storage at 2C could be grouped according to their rate of formation. The relatively low molecular weight compounds augmented linearly with time. The relatively high weight ones rose to a maximum which occurred between 8 and 11 days of storage (Mendelsohn and Steiwberg, *Food Technol., 16,* 113). Other studies dealt with the deterioration of medicinal fish liver oils (Parteshko et al., *Aptech. Delo S.S.S.R., 10,* 65).

Various alterations in the properties of fats and oils had their origin in the processing operations. Refining, bleaching, and deodorization of soybean oil generally caused the development of objectionable flavors and changes in color. Deodorization temperatures around 295C were found to decrease both the tocopherol content and the stability of the oil while increasing the resistance to changes in color (Yashuda et al., *J. Oil Chem. Soc. Japan, 11* (1), 2). The absorbents present were also important with respect to oxidation stability (Harada et al., *J. Agr. Shem. Soc. Japan, 35,* 212). A fishy-odor producing compound was isolated from refined, bleached, and deodorized soybean oil which had been subsequently autoxidized and hydrogenated (Chang et al., *Chem. Ind. (London), 1962,* 1023). Refining produced, in olive oil, unidentified compounds which gave brown color on $HNO_3$ addition (Hauchecorne reaction) (Martinenghi, *Olearia, 15,* 308). Commercial methods of compression and extraction accelerated the oxidation process in sunflower seeds and peanuts as in the case of pulverized seeds heated at the same temperature in the laboratory (Rzhekhin et al., *Tr. Vses. Nauchn. Issled Inst. Khirov, 1959* (19), 276). Changes induced upon drying sunflower seeds and their influence on the storage stability were also characterized (Plyushkina, *Maslob.-Zhir. Prom., 27* (10), 23). Bleaching of peanut oil caused the development of a substance which absorbed light in the 260–280 mμ region. It did not appear in the tissue fat of rats fed the oil (Raulin et al., *Rev. Franc. Corp. Gras., 9,* 75). The degree of processing affected the nutritional properties of soya-bean oil meals for chicks (Bornstein et al., *J. Sci. Food Agr. 12,* 80).

The methyl esters of fatty acids from linseed, sesame, camellia, and sardine oils were oxidized up to peroxide numbers of 50 and 100. The higher peroxide number decreased rapidly upon storage at 30C. The decomposition of peroxides was accelerated by UV-rays, metallic soaps, and antioxidants (Bito, *Yukagaku, 7,* 428). Unsaturated fatty acid peroxides inhibited many enzymes of various types, the inhibition depending on the peroxide value, and on the time of contact (Wills, *Biochem. Pharmacol., 7,* 7).

Exposure to air and sunlight increased the rancidity and peroxide number of edible oils. The oxidizing effect of air progressed more rapidly than the deleterious effect of sunlight. Diffused light and darkness reduced, whereas UV light enhanced oxidation by air (Romani and Valentinis, *Boll. Lab. Chim. Provincialis (Bologna), 13,* 190). In oils stored in glass containers, the best protection against flavor reversion was obtained with glass which absorbed light wavelengths below 450 mμ (de la Borbolla and Vazquez, *Grasas y Aceites, 13,* 75). Pork fat was equally exposed to sunlight. A total deterioration, accompanied with a change in taste, occurred in the first 24 hours. The peroxide value rose sharply during the same period, the increase continuing for the next 4 days and diminishing afterwards. The acid number increased steadily meanwhile (Starikova, *Sb. Nauchn. Rabot. Zaochn. Inst. Sov. Torgovli, 1959* (4), 63).

Physical and chemical modifications produced in pork fat by long term freezing storage were discussed (Valdecantos Jimenez et al., *Rev. Frio, 5* (3), 137; *Ibid., 5* (4), 191). Fatty tissue from pigs on 4 kinds of feed was stored at −25C and tested for rancidity. The thiobarbituric acid test (TBA) high in the samples which had fishy flavor at slaughter, was also high in samples without flavor defects. Pigs fed fish oil or cooked garbage tended to give fat with rancid flavor (Fredholm, *Acta Agr. Scand., 11,* 335). Analytical data including peroxide number, aldehyde number, acid number, and fatty acid composition, and unsaponifiable material were collected for stored bone fat. Fresh bone fats proved unsuitable for feeding (Niesar, *Sonderh. Z. Landwirtsch. Forsch., 15,* 151).

The nature of wheat lipids and their role in flour deterioration were studied (Nelson, *Univ. Microfilms, Order N° 62–1808. Dissertation Abstr. 22,* 3842). Lipid extracts from doughs mixed in air or oxygen presented higher TBA values than those from doughs mixed under nitrogen. Addition of lipoxidase increased, and addition of antioxidants diminished peroxidation. That lipid peroxides also act as flour improvers was suggested by the increase in the structural relaxation constant produced by the addition of tert-butylhydroperoxide, succinic acid peroxide, $H_2O_2$, acetyl peroxide, and methylethyl ketone peroxide. Lipid peroxidation was enhanced by adding SH reagents (p-chloromercuric benzoate, iodoacetic acid, N-ethyl maleinimide, and $HgCl_2$) and improving agents (iodate and bromate) (Tsen and Hlynka, *Cereal Chem., 39,* 209).

Slow, moderate, and rapid wilting at 32, 50, and 70F respectively, in kale, collards, turnip green, and rape, were tested as cause of losses in carotent content. Conditions favorable to wilting resulted in a rapid loss of carotene, but high temperatures provoked a much more rapid destruction (Ezell and Wilcox, *J. Agr. Food. Chem., 10,* 124).

Krukovsky (*J. Agr. Food Chem., 9,* 439) discussed the influence of natural varietal factors on the biochemical properties of milk as referred to lipid deterioration. Milk from cows giving higher yields contained more lactoglobulins and its fat was more susceptible to autoxidation than the milk of cows of lower yields. This suggested that a relation existed between high lactoglobulin content of milk and increased oxidizability (Pijanowski and Benedykcinska, *Roczniki Technol. i Chem. Zywnosci, 5,* 17). Milk lipase and not bacterial lipases was causal agent of fat hydrolysis in high-temperature short-time pasteurized milk. Pasteurization for 15 sec at 72C was insufficient in the prevention of fat hydrolysis and the subsequent rancid flavor, whereas 74C was found to be sufficient. Higher homogenization pressures promoted fat hydrolysis. Homogenized milk presented more marked deterioration tendencies than the nonhomogenized one (Stadhouders, *Neth. Milk Dairy J., 15,* 10; Sjostrom, *Svenka Mejeritidn, 50,* 655). The presence of a lipid phase during pasteurization adversely affected heat and storage stabilities of milk at high temperatures for a short period of time (Leviton and Pallansch, *J. Dairy Sci., 44,* 633). In another experiment, milk was steam heated and then subjected to vacuum treatments, high-temperature short-time pasteurization, and finally it was homogenized. The high retention of ascorbic acid and the resistance to oxidized flavor development noted, were attributed chiefly to the removal of oxygen by the vacuum treatment and the activation of sulfhydryl group by the heat treatment (Kleyn and Shipe, *J. Dairy Sci., 44,* 1603). Actually, copper was demonstrated to be an essential metal catalyst for the development of oxidized flavor in milk, while the relation of xanthine activity to the development of oxidized flavor was not consistent. The destruction of ascorbic acid by ascorbic acid oxidase prevented peroxidation, but when the original level of ascorbic acid was restored, oxidized flavor developed (Smith and Dunkley, *J. Dairy Sci., 45,* 170; Goded, *Anales Lactol. Quim. Agr. Zaragoza, 1962* (4), 1). However, the theories about the role of ascorbic acid in the initiation of oxidized flavor in milk were questioned on the basis of experiments showing that the correlation between lipid peroxidation and ascorbic acid oxidation rate was highly significant in milk from alfalfa-fed but not from pasture-fed cows (Smith and Dunkley, *J. Food Sci., 27* (2), 127).

In purified milk fat containing carotenoids, autoxidation was delayed until the carotenoids had been oxidized (Drozdov, *Dokl. Akad. Nauk. SSSR., 137,* 349).

The changes produced in the volatile flavor components of sterile concentrated milk upon storage were investigated (Patel, *Univ. Microfilms, Order N° 61–3146, Dissertation Abstr., 22,* 726).

The fat from autoxidized butter was distilled under high vacuum and the flavor concentrates obtained were fractionated into three main fractions by GLC. Recombination of the fractions reproduced the tallowy flavor of the concentrate. The first of these components had a retention time close to that of octanal and gave an oxidized flavor to milk. The second component migrated close to undecanal and provoked a grassy flavor. The third component was 2,4-decadienal, and gave milk an oily flavor. The first and third components could be formed from methyl linoleate and the second one from methyl linolenate. The view was advanced that these compounds could be responsible for the major off-flavor characteristics of other autoxidized fats (El-Negoumy et al., *J. Dairy Sci., 45,* 311). Packaged butter fat deteriorated and color and flavor altered as a consequence of losses of water from the surface layers

(Pont, *Australian J. Dairy Technol., 16,* 146). Wrapping of household margarine influenced moisture loss but not development of rancidity. In the absence of mold no increase in the acid value was detected in both wrapped and unwrapped margarine. The inner part of the pieces was darker than the surface (Nakazawa et al., *J. Oil Chem. Soc. Japan, 10* (12), 761). Testing of bakery margarine demonstrated that oxidation took place at almost the same rate at the inside as in the outside, and that bakery margarine discolored more than household margarine (Nakazawa et al., *J. Oil Chem. Soc. Japan, 11* (4), 195).

Nutritional experiments were performed which showed once more the adverse alimentary characteristics of autoxidized fats and oils. Pure fatty acid hydroperoxides were lethal to mice when fed at a 1.5% level (Khan, *Pakistan J. Biol. Agr. Sci., 3* (1), 3), and exhibited some toxicity when injected parenterally to chicks and rats (Glavind et al., *Acta Pharmacol. Toxicol., 18,* 267). Ethyl linoleate hydroperoxides inhibited respiration as well as aerobic and anaerobic glycolysis in Ehrlich-ascites tumor cells and yeasts (Schauenstein et al., *Monastsh., 92,* 442). Rats fed autoxidized tallow grew less than fresh fat-fed controls, the fat peroxides in the ration not being deposited in the body lipids nor excreted in the feces (Kreier et al., *Am. J. Vet. Research, 22,* 795). On the contrary, 6% dietary rancid lard had no effect on chick growth (Scolari, *Atti. Soc. Ital. Sci. Vet., 13,* 144).

Several analytical procedures for predicting the oxidative stability of lipids were applied to combinations of animal and vegetable fats and oils, with and without added monoglycerides, and were compared (Pohle et al., *JAOCS, 39,* 226). They included the Eckey Oxygen Absorption, a modification of the ASTM Oxygen Bomb, and the Active Oxygen Method.

A thorough investigation was made of the thiobarbituric reaction as applied to the *in vitro* autoxidation of polyunsaturated fatty esters. The TBA values of esters with more than two double bonds bore a linear relationship with the peroxide value and diene conjugation at the early stages of oxidation and only with the diene conjugation throughout the whole oxidation period. Other results strongly suggested that TBA color would vary with the polyunsaturated fatty acid composition of the oxidizing lipids. Thus, at equal levels of autoxidation, a tissue rich in highly unsaturated fatty acids would develop more TBA color than one rich in less unsaturated fatty acids. Therefore, in crude mixtures the TBA reaction could not be used as a direct measure of peroxides but as only an empirical test for the presence of autoxidation products (Dahle et al., *Arch. Biochem. Biophys., 98,* 253; Dahle, *Univ. Microfilms., Order No. 62-1171, Dissertation Abstr. 22,* 3839). Other studies on the TBA test covered the chemistry of some of its side reactions (Tarladgis et al., *JAOCS, 39,* 34); the factors affecting its use in homogenates (Singh and Pritchard, *Can. J. Biochem, Physiol., 40,* 317); the removal of substances interfering with it (Yu and Sinnhuber, *Food Technol., 16,* 115); and its application in testing chicken fat quality (Grabowski et al., *Roczniki Technol. Chem. Zywnosci, 6,* 43) as well as for the determination of malonaldehyde in cured meats (Zipser and Watts, *Food Technol., 16,* 102).

Colorimetric, iodometric and polarographic methods for the determination of the peroxide number were compared (Janicek and Pokorny, *Sbornik Vysoke Skoly Chem. Technol., Prague, Oddil Fak. Potravin. Technol., 3,* 233; *Nahrung, 5,* 399; Pokorny, *Revista Ital. Sostanze Grasse, 38,* 482). A detailed study was made of the adequacy of the diphenylcarbazide spectrophotometric method for the estimation of the degree of rancidity of lipids. Butter, lard, fresh and highly oxidized sunflower oil, and peanut, cottonseed, rapeseed, and soybean oils were tested, together with the interfering action of several organic and inorganic substances (Sedlacek, *Nahrung, 5,* 637). Spectrophotometry was also applied to the assay of olive oil before and after oxygen absorption (Montefredine et al., *Riv. Ital. Sostanze Grasse, 38,* 131). A combination of infrared spectrum and gas liquid chromatographic behavior was useful in the analysis of several oxygenated fatty acid derivatives (Kitagawa et al., *JAOCS, 39,* 217). These derivatives could also be separated by thin layer chromatography, using mixtures of ether and petroleum ether in varying proportions as solvent systems. Feasible separations included: a) compounds with the same chain length but different number of OH groups; b) compounds differing in chain length by about 4 or more C atoms; c) *cis* isomers from *trans* isomers (Subbarao et al., *J. Chromatog., 9,* 295). Paper chromatography on paraffin impregnated paper was applied to the separation of fatty acid peroxides and found useful in the identification of monohydroperoxides, cyclic diperoxides, and polymerized peroxide compounds (Pokorny, *Riv. Ital. Sostanze Grasse, 38,* 484).

Early changes in a substrate undergoing oxidation could be monitored by means of a self-indicating-autoxidation-catalyst system using Fe (ortho) 1, 10-phenantroline in methanol benzene. It served to study the effect of moisture upon development of peroxide values in beef fat (Ferren, *Univ. Microfilms, Order No. 62-2595. Dissertation Abstr., 23,* 197.

Tertiary butyl alcohol was proposed as a relatively inert solvent suitable for carbonyl analysis in autoxidized fats by the Girard T reagent and 2,4-dinitrophenylhydrazine (Gaddis et al., *Nature, 19,* 1391). Glacial acetic acid, on the other hand, was the solvent of choice for the estimation of carbonyl groups by the method of Noguchi and Barnstein (Pokorny et al., *Sbornyk Vysoke Skoly Chem. Technol., Oddil. Fak., Potravin. Technol., 1960,* 187). The mono- and dinitrophenylhydrazones of the dicarbonyl and other polar compounds formed in oxidized milk could be separated by liquid-liquid partition chromatography in 3 different columns, each suited to handle a different range of compound polarities (Corbin, *Anal. Chem., 34,* 1244). Vacuum (Lea and Swoboda, *J. Sci. Food Agr., 13,* 148) and steam (Ivanov, *Compt. Rend. Acad. Bulgare Sci., 14,* 683) distillation procedures were devised for the determination of similar volatile compounds. GLC, also, proved useful in this respect (Miyahara, *Nippon Suisan Gakkaishi, 27,* 42; Vorbeck, et al., *J. Food Sci., 26,* 569).

The stability of lipids against oxidation was checked by measuring refractive index changes (Janicek and Pokorny, *Sbornyk Vysoke Skoly Chem. Technol., Oddil. Fak., Potravin. Technol., 1960,* 157), oxygen absorption (Martin, *Chem. Ind., 1961* (12), 364), and by determining free radicals formation (Zhuravlev, *Zh. Prikl. Khim., 35,* 1153). Two rapid tests used to assess oil quality gave little or no information on the initial deterioration in taste which occurred in stored vegetable oils (Paul and Roylance, *JAOCS, 39,* 163). No useful correlation was found between peroxide value and flavor scores in foam-dried milk (Klimän, *J. Agr. Food Chem. 10,* 496). The Kreis and oxidation (Issoglio) tests provided good correlation with the organoleptic properties of stored olive oil but the Stamm test did not (Aime, *Riv. Ital. Sostanze Grasse, 39,* 80).

A method for the analysis of hydroxy fatty acids and esters was reported (Frankel et al., *JAOCS, 39,* 297). Another for the preparation of *trans-trans* methyl linoleate hydroperoxide was also given (Banks et al., *J. Sci. Food Agr., 12,* 724), and factors that disturbed the determination of lipoperoxides were examined (Glavind and Hartmann, *Acta Chem. Scand., 15,* 927). A procedure for the extraction of lipids from oxidized fish tissue was developed (Zipser et al., *J. Food Sci., 27,* 135).

Patents were issued for procedures for the separation of fatty acids from oxidized fish oil (Bulloff, *U.S. 2,972,624*), for improving the storage properties of edible oils (Unilever, *Brit. 865,807*), and for preventing flavor reversion (Copenhaver, *U.S., 3,004,048*).

### Deterioration by Irradiation

"Influence of Irradiation Preservation on the Nutritive Value of Fish and Fishery Products" was the title of a review presented by Shewan (*Conf. Fish. Nutr.,* Washington, D.C., *1,* 1.17.1).

The free radicals formed by irradiating tristearin with $Co^{60}$ were detected by electron paramagnetic resonance (EPR). Samples irradiated *in vacuo* and stored under oxygen or air changed their original EPR spectra into a single asymmetric peak, which was associated with oxygen uptake. At about −25C, the free radical decay rate for tristearin was optimum for the onset of peroxide formation. Irradiations at −196C and −78C resulted in nonequivalent free radical systems, as demonstrated by their differing responses to identical storage conditions. (Bradshaw and Truby, *U.S. Dept. Com. Office Tech. Serv., BP Rept. 154, 306,* (1961)).

When oleic acid was exposed to $5 \times 10^5 - 10^6$ rads of gamma-rays in the presence of amino acids, a yellow discoloration was noted which was enhanced by incubation at 37C. This effect was attributed to the reaction between the amino acid and carbonyl compound derived from the fatty acid during irradiation (Okada et al., *J. Tokyo Univ. Fisheries, 47,* 289).

Vegetable oils showed to be stable upon treatment with a dose of $10^6$ rads, but similar doses resulted in marked increase in the carbonyl derivatives and peroxide content of lard (LeClerc et al., *Ann. Nutrition Aliment., 15,* 65). Higher doses up to 15,000 rads provoked no observable effects on sunflower oil. At sterilization doses an increase in the degree of peroxidation and a lowering in organoleptic value were observed (Zhuravlev, *Voprr. Pitaniya, 20,* 65). Similarly, a change in taste was noticeable in margarine and tallow stored after treatment with ionizating radiations. Peroxides formed and increased throughout standing, and the amount of water in the original fat was to be important in this case. Irradiation under a nitrogen atmosphere did not prevent the formation of peroxides and the liberation of fatty acids, and in fact, after a month storage the peroxide number was greater

in samples treated under nitrogen than in those under air. Antioxidants had no influence on the amount of peroxides formed but prevented their subsequent accumulation (Fomin, *Gigiena i Sanit., 27* (3), 34). The effect of gamma-rays on antioxidant-added lard were rather similar to those of heat-treatment, both causing accumulation of peroxides and breakdown of antioxidants (Piul'skaya, *Vopr. Pitaniya, 21,* (1), 65). The level of intramuscular fat affected organoleptic, physical and chemical properties of pre-cooked gamma-irradiated pork loin muscle (Whitehair, *Univ. Microfilms, Order N° 62-4757. Dissertation Abstr., 23,* 1320).

Pure solutions of fat-soluble vitamins were exposed to gamma rays both aerobically and anaerobically. Vitamin E proved the most sensitive and the only one affected adversely by the presence of oxygen during irradiation (Knapp and Tappel, *J. Agr. Food Chem., 9,* 430).

Significant formation of fatty peroxides was detected in the scutellum of growing gamma-irradiated (40,000 rad) corn seeds. The maximum amount was found at the 6–7th day; at the 9–19th day the quantity of peroxide diminished and the plant died (Kuzin et al., *Rol. Perekisei i Kosloroda v Nachal'nykh Stadiyakh Radiobiol. Effekta, Akad. Nauk. S.S.S.R., Inst. Biol. Fiz., 1960,* 33). On the other hand, pre-planting exposition to lower levels (2,000–15,000 rad) improved the oil content of cotton seeds, without modifying their chemical constitution and food quality (Usmanov et al., *Uzbeksk. Khim. Zh., 1961* (3), 45). Gamma-irradiation of corn grain with $2.5 \times 10^5$ rads lowered the lipase activity. Higher levels had no effect on the acid number of the seed fat. No changes were detected in the fat content of dry grains irradiated and stored for 360 days (Chmyr, *Trudy Vsesoyuz. Nauch.-Issledovatel. Inst. Zerna i Produktov ego Pererabotki, 1960* (38), 97).

The formation of toxic products was demonstrated in both irradiated and oxidized sunflower oil (Zhuravlev et al., *Med. Radiol., 6* (2), 46)), and fish fat (Burkalova et al., *Nauch. Doklady Vysshei Shkoly, Biol. Nauki, S.S.S.R., 1,* 145). Moreover, irradiation of oleic acid in the presence of oxygen produced substances that were found to be toxic for yeasts (Kudryashov et al., *Doklady Akad. Nauk, S.S.S.R., 144,* 443). However, irradiation alone of uncured bacon did not produce carcinogens or growth-altering substances (Dixon et al., *J. Food Sci., 26,* 611). Experiment performed on *Endomyces magnusii* demonstrated that, in general, fat-rich cells were 30% more radio-stable than fat-poor ones. The irradiated cells metabolized their internal reserves less rapidly if rich in fat (Alekseeva, *Biofizica, 3,* 94).

Rats were treated with 700 rad of $Co^{60}$ gamma-rays and the concentration of peroxides and antioxidants in their fat was determined. The exposed animals presented up to a 60-fold increase in peroxide concentration and up to a 3-fold decrease in antioxidizing ability over the nonirradiated ones (Zhuravlev, *Rol Perekisei i Kisloroda v Nachl'nykh Stadiyakh Radiobiol Effekta, Akad. Nauk. S.S.S.R., Inst. Biol. Fiz, 1960,* 55; Kolomiitseva and Kuzin, *Ibid., 1960,* 26).

The lipid peroxides in X-irradiated leaves of kidney bean seedlings originated in an enzymic process with the participation of lipoxidase. The enzyme was activated by irradiation, with the subsequent increase in peroxides (Budnitskaya and Borisova, *Tr. Tashkentsk. Konf. po Mirnomu Izpol'z. At. Energii, Akad. Nauk. Uz. S.S.S.R., 3,* 200).

Several changes were noted as a result of electron-irradiation of natural fats. They included increases in viscosity, density, dielectric constant, refractive index, and degree of polymerization, as well as a depression of the melting point. The dielectric loss factor also increased with large doses of radiation due to the formation of oxidation products and the splitting of fats (Lück and Kohn, *Fette, Seifen, Anstrichmittel, 63,* 812).

The changes in viscosity and acid number upon UV-irradiation of vegetable oils was found to depend on the content of polyunsaturated fatty acids. Animal fats (sheep, hog, horse, and milk fat) were less sensitive, with the exception of cod liver oil in which a sharp increase in viscosity and acid number occurred at various radiation levels. This effect was associated with the presence of clupanodonic acid (Ryspaev, *Trudy (Pervoi) Nauch. Sessii po Voprosy:Problemy Zhira v. Pitaniz, Leningrad, 1959,* 100). The oxidative alterations produced by UV-light treatment of roasted coffee beans could be prevented by treating the beans with a hydrogen-chlorine gas mixture (Inagaki and Uchio, *J. Utilization Agr. Products, 6,* 280).

The modifications provoked by ultrasonic irradiation in some phospholipids sols were also investigated (Saunders et al., *J. Pharm. Pharmacol. 14,* 567).

### Lipoxidase Oxidation

The reactions catalyzed by lipoxidase in a linoleate ester system were reviewed by Khan (*Pakistan J. Sci., 13,* 178).

Trilinolein and linoleic acid substrates were used for the characterization of the lipoxidase activity in several legumes by tracing the pH curves. The triglyceride activity presented two distinct peaks. Whereas a lag or induction period was observed with linoleic acid, there was no lag with trilinolein (Dillard et al., *Food Research, 25,* 544). Lipoxidase activity was demonstrated in all parts of wheat seedlings at different growth stages. In fact, the activity of the enzyme rose during the early stages of growth, and diminishing afterwards (Pshenova and Kolesnikov, *Biokhimiya, 26,* 1008).

Treatment with hydrogen-chlorine gas mixture apparently inactivated the lipoxidase in coffee beans (Inagaki and Uchio, *J. Utilization Agr. Products, 6,* 280).

Intraperitoneal injection with lipoxidase plus X-ray irradiation was lethal to mice, which only survived 4–30 days, depending on the radiation level (Cheymol et al., *Bull. Acad. Natl. Med., 145,* 515). When mice received a solution of lipoxidase in olive oil, then were exposed to lethal doses of radiation, no influence on the survival rate was noted (Cheymol et al., *Bull. Acad. Natl. Med., 145,* 518).

### Deterioration Mechanisms

Reviews on the subject covered the properties of lipid peroxides, the reactions that take place during lipid autoxidation, the reaction mechanisms, and the reaction products (Loury, *Rev. Franc. Corps Gras, 8,* 688). The synthesis, analysis and reactions of fatty peroxides were further discussed by Silbert, *JAOCS, 39,* 480).

Highly purified preparations of fatty acid esters were utilized in studies on the autoxidation reaction. Contrary to previous theories (see Heaton and Uri in the 1961 Literature Review) nonhydroperoxidic substances exhibiting UV-absorption were found to be formed during the induction period in autoxidizing linoleate. Thus, autoxidation of polyunsaturated lipids probably started with a reaction previous to the formation of stable hydroperoxides. The peroxygenic substance resulting from this reaction affected the stability of preparations of polyunsaturated fatty esters (Privett and Blank, *Ibid., 39,* 465). The increase with time of the peroxide content of pure methyl esters in the presence of oxygen was followed and the effect of the temperature was testd (Paquot and Mercier, *Rev. Franc. Corps Gras, 9,* 275). In other series of experiments low-molecular weight unsaturated compounds were shaken with oxygen and the rate of formation of peroxides was determined. Autoxidation of esters of the type $CH_2{=}CHCH_2COOR$ (I) and $CH_3CH{=}CHCOOR$ (II) led to the formation of $H_2O_2$, 3 different hydroperoxides, and dimerized products. The autoxidation of alpha,beta- and beta,gamma-unsaturated enol esters resulted in the formation of hydroperoxides and polymeric compounds. The introduction of an alpha–OH group in I and II led to increases in the oxidation rates of both esters, thus showing that the alcohols formed during the autoxidation of fatty esters and glycerides are oxidized at a faster rate than the parent compounds. The oxidation of $HOCH_2CH{=}CHCOOR$ gave $OHCCH{=}CHCOOR$ but only polymeric compounds were obtained when $H_2C{=}CHCHOHCOOR$ was oxidized. The oxidation of divinyl carbinol yielded only divinyl ketone and water. At least 3 peroxides formed during the oxidation of divinyl methane and 5 isomeric hydroperoxides resulted from methyl 3,6-heptadienoate. The results showed that the position of free-radical formation depended on the activation of the CH groups and on the factors which affected it, i.e., double bonds, ester and OH groups. Hydroperoxides were formed at this position only if there was no possibility of intermolecular rearrangement leading to a conjugated structure (Rieche et al., *Fette, Seifen, Anstrichmittel, 64,* 198).

The presence of formic acid among the volatile compounds evolved in the oxidation of oleic acid prompted to a theory for its mechanism in which a transitory diperoxide probably formed. It resulted from the fixation of a second oxygen molecule on a primary monoperoxide by double bonding. The diperoxide would then degrade with the liberation of formic acid (Loury, *Oléagineux, 17,* 197; Loury and Lechartier, *Rev. Franc. Corps Gras, 9,* 133). The presence of 1-octen-3-ol and 2-*trans*-heptenal in oxidized esters was explained by a reaction exchange which assumed the formation of an intermediary acetal (Hoffman, *JAOCS, 39,* 439; Treibs, *Fette, Seifen, Anstrichmittel, 63,* 1151). Oleic and linoleic thick layer oxidation experiments led to an equation for the calculation of the peroxide content in those systems. According to these experiments, the energy of activation of peroxide formation from free radicals is lower for linoleic acid than oleic acid, whereas the activation energies of peroxide formation in chain reactions and peroxide decomposition are lower in the case of oleic acid (Pietrzyk, *Roczniki Technol. i Chem. Zywnosci, 6,* 19).

During bulk oxidation of linseed oil in the presence of Co, Mn, and Pb linoleates the structure of the oil changed from homogeneous to microheterogeneous. The addition of

antioxidants in the presence of the metallic catalysts did not modify the character of the curves of catalyst concentration versus oxygen absorbed. It only shifted the maximum and minimum points to the side of greater catalyst concentration (Ivancheva, *Nauchn. Ezhegodnik za 1957, Chernovitsk. Univ., Chernovtsy, 508*).

## ANTIOXIDANTS

Reviews on antioxidants discussed their use in foods (Dalfo, *Lipidos, 20,* 274; Daghetta, *Aliment. Animale, 5,* 713; Kotake, *Shohuhin Eiseigaku Zasshi, 3,* 114; Anonymous, *Oil, Paint and Drug Reporter,* April 24, *39,* (1961)) their mechanism of action and physiological role (Brun, *Rev. Franc. Corps Gras., 9,* 192), their toxicity and metabolism (Aoki, *Shokuhin Eiseigaku Zasshi, 3,* 120) and their relationship with vitamin E nutrition in chicks (Scott, *Nutrition Abstr. and Revs., 32* (1) 1).

Regulations were proposed covering the identity and labeling of antioxidants which might enter into food use (*Federal Register, 26,* 847), and for the use of butylated hydroxyanisole (BHA) (*Federal Register, 27,* 8423), butylated hydroxytoluene (BHT), BHA and stearyl monoglyceridyl citrate (*Federal Register, 27,* 2568), monoglyceridyl citrate (*Federal Register, 26,* 1949), BHT (*Federal Register, 26,* 1984), BHA (*Federal Register, 26,* 1984), 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline (ethoxyquin) (*Federal Register, 26,* 644), as well as BHA and BHT (*Federal Register, 27,* 3883).

The protection of fats by antioxidants was explained on the grounds of the fatty acids forming a protective complex with the antioxidant. Thus, fatty acids near the antioxidant might be well protected whereas the protection of those further away would diminish with distance. The "effective complex area" varied with different antioxidants, heat and metals probably disturbing the complex formation (Khan, *Pakistan J. Sci. Res., 13,* 133). The temperature level was an important factor in determining whether antioxidants acted as such or as pro-oxidants (Heimann and Pezold, *Z. Lebensm. Untersuch. u. Forsch., 112,* 463).

Using linoleate esters as substrates, it was demonstrated that the antioxidative activity of tocopherol depended on factors like type of substrate, temperature, and concentration (Kaufmann et al., *Fette, Seifen, Anstrichmittel., 64,* 309). In order to study the relationship between tocopherol content and peroxide development during storage, various types of oils were kept at 10, 22, 37, and 43C for 4–18 months. Most of the oils showed at 22, 37, and 42C a rapid initial decrease in the tocopherol level accompanied with a slight increase in peroxide number. Among the temperatures tried, storage at 10C caused the slowest oxidation rate (Nazir and Magar, *Indian J. Appl. Chem., 24,* 18). The stability of beta-carotene in the presence of various antioxidants was tested by dispersing beta-carotene in agar containing methyl linoleate. The protecting effect of the antioxidants was found to depend chiefly upon the presence and nature of pro-oxidative factors (Blain and Shearer, *Chem. Ind. (London), 1962,* 217).

Several antioxidants were tried in widely different substrates. Thus, alpha-tocopherol, cetyl gallate, octyl gallate, and ascorbyl palmitate were shown to be of value as preservatives for edible fats (Somogyi and Kuendig-Hegedues, *Mitt. Gebiete Lebensm u. Hyg., 52,* 104). BHT, nordihydroguaiaretic acid (NDGA), NYOX 52, G-15 and G-16 (Gutierrez, *Grasas y Aceites, 12,* 234) and BHA and Tenox II as well (Gutierrez, *Grasas y Aceites, 13,* 69) were found to act as antioxidants in refined olive oil. However, they were not able to retard the development of some atypical flavors. NDGA (0.01%) with ascorbic acid (0.005%) or BHT (0.02%) lengthened the preservation of lard but 0.01% NDGA or BHT were ineffective in salted and frozen bacon (Ciobanu and Ionita, *Ind. Aliment., Produse Animale, 9,* 365). The supplementation with BHT could reduce the effect of oxygen that took place during heating of lard at 130C. The presence of casein in the system substantially contributed to inhibit oxidation (Janicek and Pokorny, *Nahrung, 5,* 387).

Propyl gallate proved to be a useful antioxidant in spray-dried milk at a concentration of 0.01%. However, concentrations of 0.02% produced a bitter taste (Akhmedova, *Trudy Nauch.-Issledovatel. Inst. Zhivotnovodstva, Uzbek. Akad. Sel'skokhoz. Nauk. 1960* (5) 215). Frozen whole milk was also effectively preserved for at least 6 months by the addition of sodium gentisate, gentisic acid or ethyl hydrocaffeate (Gelpi et al., *J. Agr. Food Chem., 10,* 89). Both gentisic acid and sodium gentisate were equally active antioxidants in lard, cottonseed oil, yellow grease, fish and other oils (Wishnetsky and Stuckey, *Food Technol. 15,* 503). The fat in confections was well preserved by the addition of NDGA (Gryuner and Soboleva, *Sb. Nauchn. Rabot. Mosk. Inst. Nar. Khoz., 1961,* (17) 3) especially in admixture with BHT and citric acid (Gryuner et al., *Ibid., 1961,* 64). Rancidity in potato chips was considerably delayed by adding either a 0.02 solution of Saschim 3-F or 0.01% propyl gallate in propylene glycol (Kihara and Inoue, *Kaseigaku Zasshi, 13,* 5). Of several antioxidants tested, BHT showed to be the best in protecting the fat in fish products (Egorova and Treshcheva, *Rybn. Khoz., 37,* N°11, 76; Thomson, *Commercial Fisheries Rev. 24,* (4), 5). In salted marine fish, BHA presented good antioxidative qualities (Toyama et al., *J. Tokyo Univ. Fisheries, 46,* 107), while ascorbic acid, sodium glutamate, propyl gallate, and hydroquinone retarded the formation of peroxides (Natenzon, *Nauch. Tekhnol. Byull. Polyar. Nauch. Issledovatel. Inst. Morsk. Rybn. Khoz. i Okeanogr., 1958,* (3), 60). Oils for pharmacological use were found to be better preserved by AMIF 72, which showed the synergistic properties of BHA, propyl gallate and citric acid (Jonas, *J. Pharm. Belg., 43,* 187).

Other compounds tried as antioxidants and the substrates in which they were tested are gossypol and related compounds in methyl oleate (Hafez et al., *Grasas y aceites, 12,* 227; Chahine et al., *Grasas y aceites, 13,* 10; Nedoma, *Prum. potravin., 12,* 633); the smoking liquid in wet smoking of fish (Khomutov et al., *Sb. Nauchn. Rabot. Mosk. Inst. Nar. Khoz., 1961,* N°18, 99); marjoram in lard (Zalewski, *Przemysl Spozywczy, 16,* 159; Zalewski, *Gospodarka Miesna, 1960,* (5), 11); cocoa bean shell extracts in palm oil (Deschreider, *Inds. Aliments. et Agr., 75,* 759); sorbic acid in pressed olive oil residues (Foresti and Giuffrida, *Boll. Inform. Ind. Olearia e Saponica, 7,* (2), 15); egg yolk cephalins in olive oil (Urakami et al., *Osaka Shiritsu Daigaku Kaseigaku bu Kiyo, 9,* 1); oat extracts in lard (Supova et al., *Sb. Vyseke Skoly Chem. Technol. Praze, Oddil Fak. Potravinauske Technol. 3,* 525); malonic acid and its esters in sunflower seed oil, and in beef and pork fat (Asnina, *Trudy Ukrain. Nauch.-Issledovatel. Inst. Pishch. Prom., 1959,* (2), 113); hydroquercetin in dairy products (Rajan, *Univ. Microfilms, Order* N°62–2196, *Dissertation Abstr. 22,* 4319); ubiquinones and related compounds in sunflower and linseed oil methyl esters (Lea and Kwietny, *Chem. Ind. (London), 1962,* 1245); hydropolyporic acids in linoleic acid (Bennet and Uri, *J. Chem. Soc., 1962,* 2753); synthetic 2,3-dicaffeoyl glycerol and 1,2,3-tricaffeoyl glycerol in oat oil (King, *Chem. Ind., 1962,* 1468); sorbitol and citric acid in soybean oil and lard (Cowan et al., *JAOCS, 39,* 6); 3,5-di-tert-butyl-4-hydroxybenzyl derivatives in stripped corn oil (Kim and Kummerow, *JAOCS, 39,* 150); a phenolic compound with antioxidant properties was isolated from leaves of the olive tree (Mazuelos and Vasquez, *Grasas y Aceites, 13,* 124).

Nutrition experiments demonstrated that antioxidants like BHA, BHT, and propyl gallate reduced the assimilation of lard when mixtures containing them were given to rats (Budagyan and Smirnova, *Vopr. Pitaniya, 21* (2), 47). Feeding 0.02% propyl gallate also caused lesser weight gain, and diminished resistance to the effects of acute underfeeding, and a reduced survival under these conditions (Buhman, *Vopr. Pitaniya, 21* (1), 68). Similar harmful effects on growth rate were demonstrated for BHT and BHA (Johnson and Hegwill, *Australian J. Exptl. Biol. Med. Sci., 39,* 253). In rats, the $LD_{50}$ of BHT and NDGA was more than 1500 mg/kg of body weight (Schobesch, *Igiena, (Bucarest), 9,* 243). Other studies on BHT-treated herring meal showed that the utilization by chicks was 91% for freshly prepared meal and no less than 82% for a meal stored for 11 months (March and Biely, *Poultry Sci., 41,* 873). Experiments were undertaken to investigate the rate of excretion of some of the most common antioxidants. The rat excreted about 90% BHA within 4 days (Golder et al., *J. Pharm. Pharmacol., 14,* 268); the dog excreted 60% in the feces and the remainder in the urine; in man, urinary excretion within 24 hours was mostly as BHA glucuronide (Astill et al., *J. Agr. Food Chem., 10,* 315).

Feeding of cottonseed oil previously supplemented with lauryl peroxide (0.25–2.0%) and then heated, caused encephalomalacia within 20 days in chicks. This effect was prevented by the addition of ethoxyquin to the treated oil (Machlin, *Poultry Sci., 40,* 1631).

In hens, supplementation of antioxidant-depleted diets with vitamin E or ethoxyquin ameliorated egg production, fertility, and hatching of fertile eggs. However, there was no need of antioxidants when the birds were fed low linoleic acid diets (Machlin et al., *J. Nutrition, 76,* 284). In the rat, it was possible to maintain fertility of animals receiving vitamin E-deficient diets by means of the addition of N,N′-diphenyl-p-phenylenediamine (DPPD) or ethoxyquin to the diet (Crider et al., *J. Nutrition, 73,* 64; Crider, *Univ. Microfilms, Order N° 62–579, Dissertation Abstr., 22,* 3369). When chicks were reared on a ration containing torula yeast without or with supplements of vitamin E, sodium selenite, ethoxyquin, or DPPD, it was evident that the lowest dietary concentrations of vitamin E, Se, and ethoxyquin needed to protect almost

completely against exudative diathesis reduced lipid peroxidation in the liver by 33, 35, and 85%, respectively. This pointed to a fundamental dissimilarity between the nutritional effects of vitamin E and Se and their effect on lipid peroxidation. Probably, they did not function *in vivo* solely as lipid antioxidants (Bunyan et al., *Brit. J. Nutrition 16,* 519).

The action of fat pro-oxidants was also investigated. The effects of the presence of $Cu^{++}$, $Co^{++}$, and $Fe^{+++}$, singularly and collectively, as well as those of an increase in temperature on the reactive surface were examined. The time of the oil-stability test was reduced one-half by the addition of traces of $Cu^{++}$ as well as by an increase in the temperature from 60–98C (Mroczkowski and Ossowska, *Prace Inst. Lab. Budawczych Przmyslu Spozywczego, 11* (3), 1). Ferrous iron was more active than ferric iron in initiating peroxidation of heterogeneous linoleate at 30C. Ferric iron produced an autocatalytic reaction whereas ferrous iron produced a linear one, except for the more dilute solutions in which the reaction was first linear and then autocatalytic. The hypothesis was advanced that the unstable reduced form of the metal could produce a free radical upon oxidation to the higher state (Smith and Dunkley, *Arch. Biochem. Biophys., 98,* 46). The oxidation susceptibility of rapeseed oil during adsorptive bleaching was tested in the presence of 4 fuller's earth preparations containing varying amounts of Fe and Cu. The oxidation susceptibility increased with the amount of fuller's earth added. This effect was attributed to the decomposition of secondary products formed during autoxidation (Taüfel et al., *Nahrung, 5,* 646). Actually, the addition of a keto acid like 12-oxo-*cis*-9-octadecenoic acid at a 0–10% level accelerated the formation of peroxides in autoxidizing stripped corn oil. The keto acid blocked the action of alpha-tocopherol acetate as antioxidant but BHA, BHT, propyl gallate, NDGA, ethoxyquin, and DPPD lengthened the induction period (Bhalerao et al., *JAOCS, 39,* 28).

The effects of the presence of some amino acids, alone or with other substances like citric acid, Cu, or both was examined (Kwapniewski et al., *Riv. Ital. Sostanze Grasse, 39,* 190). Most amino acids, and especially histidine and tryptophane, showed antioxidant properties in linoleate and linolenate esters. High amino acid concentrations or low pH tended to provoke pro-oxidative effects, and phosphate or emulsifiers caused the opposite (Marcuse, *JAOCS, 39,* 97). Instead, the addition of histidine plus ferrous or ferric ions augmented markedly the peroxide content of autoxidizing linoleate (Saunders, *Ibid., 39,* 434). Also chlorophyll contributed to increase the oxygen consumption by linoleic acid, thus presenting a lipoxidase-like activity (Imura, *Toho Igakkai Zasshi, 8,* 875).

Methods for the qualitative detection of antioxidants in fats and fat-containing foods were described. Dry materials were extracted with cyclohexane, while the liquid ones were diluted with hot water and extracted. Gallates, BHA, and NDGA were extracted with 75% alcohol. BHT was either steam distilled or chromatographed on silica gel. Once separated, oxidizable substances were recognized by a spot test with phosphomolybdic acid. Gallates and BHA were identified by TLC (Meyer, *Deut. Lebensm. Rundschau, 57,* 170). Rapid qualitative screening methods were also described for propyl gallate, NDGA, BHA, and BHT (Heidrick and Conroy, *J. Assoc. Offic. Agr. Chemists, 45,* 244).

Reverse-phase chromatography was utilized for the separation and identification of gallates, BHA, and ethyl protocatechuate (Kawashiro et al., *Eisei Shikensko Hokoku, 77,* 175), and GLC proved to be suitable for the determination of BHA and BHT in potato granules (Buttery and Stuckey, *J. Agr. Food Chem., 9,* 283). In milk powder, dodecyl gallate was estimated by a method using ferrous tartrate, and BHA by the 2,6-dichloroquinone chlorimide procedure (Raadsveld and Kooy, *Neth. Milk Dairy J., 15,* 282).

Different aspects of the UV-spectrophotometric determination of antioxidants were examined. Spectral constants were determined for 2-*tert*-BHA, 3-*tert*-butyl-4-hydroxyanisole, NDGA, propyl gallate, trimethyl hydroquinone, naphthoquinone, N-palmitoylphenetidine, ascorbyl palmitate, alpha-tocopherol acetate, and alpha-tocopherol (Khomutov and Kulakovskaya, *Maslob.-Zhir. Prom. 28* (5), 13) for gallic acid and its methyl, ethyl, propyl, octyl, and dodecyl esters, BHA, BHT, ascorbic acid, and ascorbyl palmitate as well. Satisfactory solvents for the above purpose included chloroform and toluene (Sedlacek, *Fette, Seifen, Anstrichmittel, 64,* 683).

A previously described complexometric method was extended to the assay of BHT, ascorbyl palmitate and gallates in lard (Sedlacek, *Fette, Seifen, Anstrichmittel, 63,* 1053). Traces of BHA and BHT in foods (Sloman, *J. Assoc. Offic. Agr. Chemists, 45,* 76) and of BHT in fats and oils (Heidrick and Conroy, *J. Assoc. Offic. Agr. Chemists, 45,* 244) were distilled and subsequently estimated colorimetrically. BHT could also be estimated by refluxing it in ethanol and HCl and then making it to react with p-nitrobenzenediazonium salt with the production of a stable azo dye (Nakamura et al., *Bunseki Kagaku, 11,* 978).

The diene conjugation in an autoxidizing system was used to evaluate antioxidants. The results obtained agreed with those of peroxide value and viscosity measurements (Tsukamoto, *Congr. Sci. Pharm., 1959,* 405). A rapid method for evaluating the effectiveness of antioxidants was also developed (Placer and Sedlacek, *Nahrung, 6* (3), 227).

Patents were issued for the following antioxidants: BHT or BHA or their mixture with the addition of 3–20% of its weight $MgCO_3$ or its mixture with powdered $SiO_2$ to prevent rancidity development in fish (*Nippon Volatile Oil Co., Japan, 2876*), chromans (Folkers and Wolf, *U.S. 3,026,330*), glycerol palmitate plus citric and tartaric acids (Pottier, *Fr. 1,284,683*), bis-(hydroxynaphthylmethyl) alkylphenols (U.S. Rubber Co., *Brit. 874,869*), hydroxyflavone derivatives (National Research Development Corp., *Brit. 875,164*), mixtures of ascorbic and citric acids with fatty acids or their esters to make the formers more soluble in lipids (Pottier, *Fr. 1,239,940*), 4,4'-alkylene dioxy bis-(alkylated phenols) (Bell et al., *U.S. 2,967,774*) were used for the protection of many different fats and oils. Stabilizers for edible fats (Williams et al., *U.S. 3,001,878*), fat and milk products (Borden Co., *Brit., 886,519*) and fish and animal meals (Olivier and Nguyen, *Fr. 1,253,279*) were also described.

## COMPOSITION AND CHARACTERISTICS

### OFFICIAL METHODS AND REVIEWS

The Spectroscopy Committee of the AOCS announced the adoption of a tentative method for isolated *trans* isomer (O'Connor et al., *JAOCS, 39,* 32). Samples containing less than 15% *trans* isomer had to be converted to methyl esters, and a special committee was appointed to study this conversion. The Spectroscopy Committee is now studying an infrared determination of the hydroxyl number. The Fat Analysis Committee of the AOCS announced a revision of the AOCS Tables (Stillman, *Ibid.,* 431). Progress was also reported on methods for determining the viscosity of liquids, a saponification value which avoids the use of ethanol, the gas chromatographic determination of common commercial fatty acids, the determination of hydrocarbons in fats, and the determination of moisture in lecithin. The AOCS committee on the analysis of fatty nitrogen compounds reached agreement with the corresponding A.S.T.M. committee on a number of analyses.

A review of the 1960-61 literature on composition and characteristics was compiled by the AOCS Literature Review Committee (Mahadevan and Hammond, *Ibid.,* 389). Reviews were published on the chromatographic separation, identification, and analysis of phosphatides (Marinetti, *J. Lipid Res., 3,* 1), the properties of phosphatides (Loew, *Rev. Centro Quim. Ind., 1961,* 34), and the isolation and analysis of glycerides (Mattson and Volpenhein, *J. Lipid Res., 3,* 281).

### ANALYSIS OF FAT SOURCES

A method for the rapid and reproducible extraction of total lipids from adipose tissue and muscle was given (Ostrander et al., *JAOCS, 39,* 178). This method depended on a methanol-chloroform type extraction. A method for the fat content of edible meats such a hamburger, pork trimmings, meat loaf, and sausage, based on the detergent test for fat in milk, was proposed (Anderson et al., *J. Assoc. Offic. Agr. Chemists, 45,* 13). The method was said to be simple and accurate and required only 30 minutes to complete. The total lipids, lipid phosphorus, and total polyenoates extracted from mullet tissues with chloroform-methanol decreased progressively during autoxidation of the tissue (Zipser et al., *J. Food Science, 27,* 135). The modified A.O.A.C. Babcock method (18.013) for crude fat in canned fish was studied with frozen boneless salmon, swordfish, and tuna and found to give results 0.2–0.4% lower than the present official method (Risley, *J. Assoc. Offic. Agr. Chemists, 45,* 259). The method was recommended for further study and application to raw fish. A simple ethyl alcohol-ethyl ether-petroleum ether extraction procedure was described for the estimation of total lipid in egg white (Cotterill, *Poultry Sci., 40,* 1514). The procedure recovered 90% of the lipids from egg white contaminated with 0.05 to 0.4% yolk. The fat content of olives was estimated by petroleum ether extraction of olive paste which had been dried with sodium sulfate (Gracian et al., *Grasas Aceites [Seville, Spain], 12,* 73). It was suggested that the seed density of flax could be used for the screening of flax lines of high oil content (Zimmerman, *JAOCS, 39,* 77). Seed densities were determined with a Beckman air comparison pycnometer. A modified Folch procedure was used for extraction of the lipids from

wheat products (Tsen et al., *Cereal Chem., 39,* 195). The method gave higher values than petroleum ether extractions. A method was devised for determining the rate of extraction of oil from unextracted soybean products (Carew et al., *Poultry Sci., 41,* 188). The oil was extracted rapidly from pelleted, flaked, or expanded soybeans, 90% being removed in 40–60 min. In ground soybeans, four hours were required for the same degree of extraction. The rate of extraction was correlated with the availability of the oil in chicken feeds for digestion by the chicks. The degree of extraction of cholesterol and esterified fatty acids from human serum by various alcohol-ether systems was found to be significantly different in normal and diseased states (Amatuzio et al., *Circulation , 25,* 540). The degree of extraction was not related to the total level of cholesterol or esterified fatty acids. A simple chromatographic procedure was reported for removing the nonlipid material from a lipid extract (Biezenski, *J. Lipid Res., 3,* 120). The lipid extract was applied in a line to a silica impregnated paper, and the chromatogram was developed 16–24 hr with 20% methanol in chloroform at 30C. The lipids from about 1 gram of tissue could be handled on one paper, and the recovery of all classes of lipids was close to 100%. The method removed all ninhydrin and ammoniacal silver nitrate-staining compounds and inorganic phosphate. Metal salts persisted in the purified lipids in small amounts. The amount and purity of the lipids extracted from serums by the Bloor, Delsal, and two Folch procedures was compared (deJongh and van Pelt, *Ibid.,* 385). There was little difference in the amounts of lipid extracted by the three methods.

A laboratory method for determining the refining loss of sulfur olive oil was described (de Castro, *Grasas Aceites, [Seville, Spain], 12,* 221). The refining loss was usually slightly more than twice the free fatty acid percentage. Laboratory tests were applied to 20 samples of Spanish cottonseed oils to determine the relation between free fatty acid content of the crude oil, refining conditions, refining loss, and bleached color (*Ibid.,* 259). The refining losses and bleached color were highest with crude oils of high fatty acid content. Increasing the concentration or amount of lye during refining increased refining losses and decreased bleached color.

## ANALYSIS FOR FUNCTIONAL GROUPS

A gas chromatographic method was proposed for classifying a compound as a member of the homologous series of normal alkanes, alkyl benzenes, alcohols, aldehydes, ketones, ethers, esters, thioalkanes, or thiols (Merritt and Walsh, *Anal. Chem. 34,* 903). When two different columns containing appropriate liquid phases were used, the ratio of retention volumes for homologous series on the two columns was constant. The ratio was different for different homologous series. The method was applicable on isothermal or programmed apparatus and packed or capillary columns. An appartus called an "osmophile" was invented which was used to measure the presence of fatty acids, aldehydes, and ketones in oxidized oils (Berton, *Rev. Franc. Corps Gras, 9,* 187). The apparatus consisted of a metallic grid simultaneously in contact with the vapor to be analyzed and an electrolyte. The electrolyte was chosen so that it become polarized when exposed to the analyzable component of the vapor. The polarization was detected by a galvanometer.

A coulometric method for determining the organic acids in a non-aqueous medium was reported (Crisler and Conlon, *JAOCS, 39,* 470). The samples were dissolved in benzene-methanol 1:1 which contained lithium chloride as an electrolyte. The acid was titrated by reduction of the hydrogen ion at a platinum cathode. At the anode silver chloride was formed from silver. The endpoint was detected by an antimony-glass electrode pair, and the derivative of the output from these electrodes was used to shut off the current automatically. The method required no standard solutions, little attention from the analyst, and was rapid. Samples in the range of 0.01 meq. were used. A method for determining long-chain fatty acids in the 0.05–0.5 $\mu$ mole range was reported (Duncombe, *Biochem J., 83,* 6P). A chloroform solution of the fatty acids was shaken with a cupric nitrate-triethanolamine reagent, and the copper extracted into the chloroform phase was detected spectrophotometrically with sodium diethyldithiocarbamate. The formation of sterol ester during sample isolation and preparation may cause errors in the acid and ester values of fats (Blank, *JAOCS, 39,* 122). The effect of ester value variation on the results obtained in soap analysis was described. Micromethods for determination of the acid and saponification values of fats and oils were described (Fedeli, *Riv. Ital. Sostanze Grasse, 9,* 401). An improved hydroxamic method for determining the ester values of lipids was described which gives equivalent colors for all types of lipids (Skidmore and Entenman, *J. Lipid Res. 3,* 356). The main factor in improving the method was controlling the amount of water present during the formation of the hydroxamates. A quantitative hydroxamic color determination of the ester in various fractions of lipids separated by thin-layer chromatography was suggested (Vioque and Holman, *JAOCS, 39,* 63). The zones from the thin-layer chromatogram were scraped from the plates and freed of silicic acid before running the color reaction.

The average carbon chain length of saturated fatty acid esters was determined by comparing absorption intensities in the 3.3 and 5.75 $\mu$ infrared regions (Keeney, *Ibid.,* 304). The method was used on triglycerides, monoglycerides and methyl esters, and is particularly applicable for small samples which contain both long and short chain saturated fatty acids. The proton nuclear magnetic resonance spectra of triglycerides were used to determine their average molecular weight and degree of unsaturation (Johnson and Shoolery, *Anal. Chem., 34,* 1136). The NMR spectra gave four sets of signals from: the olefinic protons, the four glyceride methylene protons, protons of methylene groups between two double bonds, and the remainder of the protons on saturated carbon atoms. The glyceride methylene protons were used for an internal standard to determine the number of molecules, and the total number of protons and the olefinic protons yielded the average molecular weight and degree of unsaturation. The method agreed well with the Wijs iodine number except for tung oil. A micro iodine number was described (Fedeli, *Riv. Ital. Sostanze Grasse, 9,* 401). A rapid and accurate infrared method for determination of the *trans* unsaturation of fats was described (Szonyi et al., *JAOCS, 39,* 276). The method utilized the absorption at 965 $cm^{-1}$ and is directly applicable to triglycerides with low amounts of *trans* unsaturation and variable fatty acid chain length. The Spectroscopy Committee of the American Oil Chemists' Society reported on an infrared method for the determination of *trans* unsaturation in fats (O'Connor et al., *Ibid.,* 32). Infrared spectroscopy permitted the determination of *trans* unsaturation in olive oils and other fats or their methyl esters (Pallotta, *Riv. Ital. Sostanze Grasse, 9,* 387). *Trans* isomer contents as low as 1% could be detected but the results were reproducible only above 5%. *Trans* isomer was found in olive oil only after heating. A method for determining the enol ether type of linkage found in plasmalogens by reaction with iodine was presented (Williams et al., *J. Lipids Res., 3,* 378). The enol ether in lipid extracts could be determined in concentrations between 0.02 and 0.125 $\mu$moles. The reproducibility was $\pm 3.6\%$. The determination of conjugated diene fatty acids in human serum lipids was improved by conversion of the lipid to free fatty acid or methyl ester (Benedikt and Ichaisenstein, *Fette, Seifen, Anstrichmittel, 63,* 706).

A modification of the Kaufmann and Funke method for determining hydroxyl value in free fatty acids was reported (Kaufmann and Schmülling, *Ibid., 64,* 319). An acetyl chloride-pyridine reagent was used. A method for the determination of the hydroxyl value of mono- and diglycerides was reported which depended on esterification of the hydroxyl groups with 3,5-dinitrobenzoyl chloride (Jensen and Sampugna, *JAOCS, 39,* 309). The acid released was titrated with tetrabutylammonium hydroxide. The method gave results similar to the AOCS pyridine-acetic anhydride method.

A fluorometric method for the micro determination of alpha-keto acids was described (Spikner and Towne, *Anal. Chem., 34,* 1468). The keto acids were converted to the corresponding substituted quinoxalines with o-phenylenediamine, and the fluorescence of the latter was measured. The acids could be determined in the range of 0.05–0.5 $\mu$g/ml.

## LIPID CLASS DETERMINATIONS AND STRUCTURE ANALYSES

GENERAL. A rapid separation of the neutral lipids and phospholipids of serum was achieved by thin-layer chromatography on silicic acid plates (Vogel et al., *J. Lipid Res., 3,* 138). The plates were developed with chloroform-methanol-water 80:25:3 by volume. The application of thin-layer chromatography to analytical separations of fats and oils was surveyed (Peereboom, *Chem. Weekblad, 57,* 625). The method was used to separate different kinds of glycerides, soybean lecithin, styrene modified oils, and emulsifiable oils. The unsaponifiable components were separated with *iso*-octane-ethyl acetate. Iodine vapor was advocated as a general detection agent in thin-layer chromatography (Sims and Larose, *JAOCS, 39,* 232). A method for the ultramicro detection of lipids was proposed (Lands and Dean, *J. Lipids Res., 3,* 129). A liquid fraction containing the lipid was dried on a photographic ferrotype plate. Amounts of lipid from 1–100 $\mu$g could be detected by the visible change in reflectivity of the shiny surface.

GLYCERIDES. The isolation, analysis, stability, and synthesis

of glycerides was reviewed (Mattson and Volpenhein, *J. Lipid Res., 3,* 281).

Thin-layer chromatography was used to separate mono-, di-, and triglycerides and pentaerythritol esters of linseed oil fatty acids (Rybica, *Chem. Ind.* (London), 1962, 308). On silica gel plates developed with petroleum ether-diethyl ether mixtures, the separation was predominantly on the basis of the hydroxyl content of the molecule. Conditions were reported for the quantitative analysis of mono-, di-, and triglycerides on thin-layer plates by charring (Privett and Blank, *JAOCS, 39,* 520). The difficulties encountered in estimating the total monoglyceride in commercial products were surveyed (Hartman, *JAOCS, 39,* 126). Certain modifications were suggested for previous methods, and a tentative method for determining total monoglyceride based on simultaneous isomerization and periodate oxidation was described. A method for the simultaneous determination of glycerol and fatty acids in glycerides was described (Horrocks and Cornwell, *J. Lipid Res., 3,* 165). The glycerides were converted to glycerol and fatty alcohols with lithium aluminum hydride, and then the glycerol and fatty alcohols were converted to their acetate esters with acetic anhydride. The acetate esters were then fractionated by gas phase chromatography. Quantitative results with mono-, di-, and triglycerides were reported.

A theory of glyceride distribution was proposed which assumes that the secondary hydroxyl of glycerol is preferentially acylated by unsaturated eighteen carbon acids such as oleic, linoleic, and linolenic (Gunstone, *Chem. Ind. (London),* 1962, 1214). The two primary hydroxyls are acylated with the remaining acids. The equations suggested by Hammond and Jones for calculating restricted random glyceride distribution were criticized (Kartha, *JAOCS, 39,* 272).

Methods for separating the four azelaoglyceride types obtained by the permanganate oxidation of glycerides were reported (Haigton et al., *Riv. Ital. Sostanze Grasse, 3,* 130). One method was based on countercurrent distribution between *iso*-octane and methanol which separated the trisaturated and mono-azelaoglycerides from the di- and tri-azelaoglycerides. A second method was based on partition chromatography on rubber columns with aqueous acetone. The latter gave somewhat more complete separation, especially of the trisaturated and mono-azelaoglycerides. The errors involved in the permanganate oxidation of triglycerides and the subsequent analyses of products by Kartha's procedure were discussed (Kartha, *JAOCS, 39,* 478).

Triglycerides were separated by thin-layer chromatography on plates impregnated with a petroleum fraction boiling 240–250C (Kaufmann et al., *Fette, Seifen, Anstrichmittel, 63,* 689). The plates were developed with acetone-acetonitrile 8:2. Detection of the spots on the plates was facilitated by exposing the plates to the vapors of dichlorodimethyl silane which made it possible to dip the plates in aqueous solutions without destroying them. Methods for carrying out hydrogenation and bromination of double bonds in lipid molecules on thin-layer plates were reported (*Ibid., 64,* 1). The hydrogenation was accomplished by a palladium catalyst deposited on the plate; the bromination was carried out by adding bromine to the mobile phase. Bromination led to improved separation of triglycerides. The use of pure plaster of paris to make plates for thin-layer chromatography of lipids was suggested (Kaufmann and Khoe, *Ibid.,* 81). These plates, which may be washed without destruction, were used for the separation of triglycerides. Thin-layer chromatography was found to separate some triglyceride "critical partners" (Kaufmann and Das, *Ibid.,* 214). The silica gel plates were coated with a high boiling petroleum fraction and developed with acetone-acetonitrile 8:2.

The diglycerides of lipolyzed milk were isolated by chromatography on silicic acid columns and thin-layer plates in about 93% yield, and their fatty acid composition was determined (Jensen et al., *J. Dairy Sci., 44,* 1983). The fatty acid composition of the diglycerides was different in some respects from that of the triglycerides in the intact milk. Similar data were given for the monoglycerides which were recovered in 70% yield. The butyl and palmityl esters of 1,2- and 1,3-diethoxypropanol, 1,2- and 1,3-dichloropropanol, and 1- and 2-propanol were subjected to hydrolysis by milk lipase (Gander et al., *Ibid.,* 1980). Significant hydrolysis occurred only when the fatty acids were esterified with primary alcohols. The fatty acid specificity of milk β-esterase was tested with 1-oleo-dicaproin and 1-palmito-dibutyrin (Jensen et al., *Ibid., 45,* 842). The fatty acid composition of the isolated free fatty acids, mono-, and diglycerides indicated no specificity for short chain fatty acids. On long incubation there was an apparent specificity for butyric acid which was thought to be caused by the preferential hydrolysis of dibutyrin.

VITAMINS, STEROIDS, AND PIGMENTS. An acetone extract of the lipids of green leaves was chromatographed in a two dimensional paper system (Booth, *Biochem. J., 84,* 444). About 40 components were observed including chloroplast pigments, quinones, and tocopherols. A new chlorophyll, three new carotenoids, and 20 other unknown compounds were observed. Thin-layer chromatography on alumina plates was used to separate the fat-soluble vitamins and other lipids (Davidek and Blattna, *J. Chromatog., 1,* 204). The spots were detected with a perchloric-sulfuric acid reagent. Steroids, fat-soluble vitamins, provitamins, and triterpenoid alcohols were fractionated by chromatography on paraffin impregnated paper with 84% acetic acid as the mobile phase (Peerboom et al., *Ibid., 5,* 500). A linear relation was found between the number of carbon atoms and the $R_m$, and rules were developed for predicting the effect of the introduction of a double bond on the $R_f$ of the molecules. The separation of the pairs cholesterol-stigmasterol, β-cholestanol-coprostanol, cholesterol-epicholesterol, lanosterol-dihydroxylanosterol, and ergosterol-lumisterol were achieved.

A circular paper chromatographic procedure for the separation of the fatty acid esters of vitamin A on silicone impregnated paper developed with methanol-butanol-water-85:10:5 was reported (Mahadevan and Ganguly, *Biochem. J., 81,* 53). The color reaction between vitamin $A_2$ aldehyde and antimony trichloride was studied (Plack, *Ibid.,* 556). With 14% antimony trichloride in chloroform containing 2% acetic anhydride, a blue-green color was observed which increased with the concentration of aldehyde and decreased with time. In the absence of acetic anhydride the maximum absorption was at 741 mμ. Some observations on antimony pentachloride and 1,3-dichloropropan-2-ol as a reagent for vitamin $A_2$ aldehyde were presented. A method for the determination of vitamin $A_1$ and $A_2$ aldehydes in solutions containing a mixture of the two was described (Black and Kon, *Ibid.,* 561).

An infrared spectrophotometric technique was proposed to distinguish vitamin $D_2$ and $D_3$ (Morris et al., *Anal. Chem., 34,* 381). This can be done either by visual inspection of the spectra between 10 and 11 μ or by the spectrophotometric neutralization technique. The latter technique can also be used to estimate the amounts of the two vitamins. A reference curve was made relating the ratio of the absorbance differences at 10.34 and 10.5 μ to the percentage composition of the mixture. The amount of each form could then be calculated from the total vitamin D content with an accuracy of ± 15%.

A procedure for tocopherol was reported which depended on the concentration of tocopherols from the unsaponificable fraction of vegetable oils by adsorption chromatography (Morris and Haenni, *J. Assoc. Offic. Agr. Chem., 45,* 92). The tocopherols were detected by a spot test with phosphomolybdic acid. Infrared spectrophotometry was used to identify the form of tocopherol present, and the α-tocopherol content was estimated by the spectrophotometric neutralization technique in samples with a high non-uniform background. The different forms of α-tocopherol were utilized to the same relative degree in both a long chick liver storage bioassay and a short bioassay (Dicks et al., *J. Nutr., 75,* 165). The same authors modified the saponification and color determination procedure of the Bro-Rasmussen and Hjarde method for tocopherol, and found it a possible substitute for the molecular distillation-Florex chromatography analysis of liver α-tocopherol.

A number of steroids were separated by gas chromatography on QF-1-0065, a fluorinated silicone (Knights and Thomas, *Anal. Chem., 34,* 1046). The logarithm of the retention times relative to cholestane were calculated, and these values were found to be reducible to additive contributions of the individual groups in the molecule and of the steroid nucleus. The contribution of a group to the retention time of a steroid depended upon its chemical nature, its position in the molecule, and its stereochemical configuration. Conditions for the gas phase chromatography of the methyl esters of hydroxy-, acetoxy-, and ketocholanic acids were given (Sjörall et al., *J. Lipid Res., 2,* 317). The effect of these functional groups on the retention times was studied using two types of silicone gum rubbers as stationary phases. The sterols of butter and margarine were analyzed by gas chromatography after isolation by saponification and chromatography of the unsaponifiable matter on Florisil columns (Eisner et al., *J. Assoc., Offic., Agr. Chem., 45,* 337). Only cholesterol was found in butter but margarine contained β-sitosterol, γ-sitosterol, and stigmasterol. The unsaponifiables from refined soybean oil were separated into three groups by liquid-liquid column chromatography (Hoffmann et al., *JAOCS, 39,* 323). The least polar fraction contained hydrocarbons and made up 15–30% of the unsaponifiables, the most polar fraction contained steroids and made up 35–45% of the unsaponifiables, and the fraction of intermediate polarity varied in composition. Further analyses were performed by thin-layer and gas chromatography and by chemical tests for functional groups. The effect of activation on three silica gels of known structure in the chro-

matography of cholesterol acetate was evaluated (Klein, *Anal. Chem., 34,* 733). The separation of steroids by paper chromatography was extended to liquid-liquid column chromatography (Kabasakalian and Talmage, *Ibid.,* 273). A series of Zaffaroni-type solvents were employed. A paper partition chromatography method for the fractionation of 17-ketosteroids was proposed which used heptane as a mobile phase and dimethylsulfoxide as a stationary phase (Katy and Broich, *J. Chromatog., 6,* 514). It gave excellent resolution in 2.5–24 hr and the solvents did not interfere with characterization tests. The use of tomatine instead of digitonin in the analysis of cholesterol in serum was investigated (Rinehart et al., *J. Lipid Res., 3,* 383). The iron reagent gave satisfactory results with tomatine if more time was allowed for precipitation of samples with small amounts of cholesterol and the washing and handling of the precipitates was done carefully. An acetone extraction procedure was worked out for the isolation of free and combined sterols in phosphatides (Aylward and Nichols, *J. Sci. Food Agr., 13,* 86).

PHOSPHATIDES. The use of chromatography for the separation, identification, and analysis of intact phosphatides and analytical procedures for the component parts of the phosphatides were reviewed (Marinetti et al., *J. Lipid Res., 3,* 1). The polar lipids of egg yolk and milk were isolated from the triglyceride portion by countercurrent distribution between petroleum ether and 87% ethanol (Galanos and Kapoulas, *Ibid.,* 134). A technique was described for the resolution and quantitative determination of the nitrogenous constituents of dog tissue lipids (McKibben, *Ibid., 2,* 328). These were choline, ethanolamine, serine, amino acids, ammonia, sphingosine, and hexosamine. Phosphatides containing unsaturated fatty acids were eluted from silicic acid columns more rapidly than their saturated analogues (Nelson, *Ibid., 3,* 71). Clean separations of lecithin and sphingomyelin could not be obtained. A method was proposed for the detection of phospholipid in chromatography column eluates which was based on the color obtained when Rhodamine 3GO was added to phospholipid (Coulson and Somerville, *Biochem. J., 80,* 45P). The color was linear with the amount of phospholipid over a wide range and in various solvents. As little as 10 $\mu$g of phosphorus could be detected. The reagent was also useful in detecting phospholipids on paper chromatograms. Techniques for the uniform impregnation of filter paper with silicic acid for the chromatography of phosphatides were reported (Hack, *J. Chromatog., 5,* 531). A method for the thin-layer chromatography of lecithin and phosphatidyl ethanolamine on silica gel plates was described (Wagner, *Fette, Seifen, Anstrichmittel, 63,* 1119). The plates were developed with chloroform-methanol-water-65:25:4, and the spots were detected by ninhydrin and ammonium molybdate-perchloric acid.

Hydrolysis of lecithin was found to occur on aluminum oxide columns under the conditions normally used in the chromatography of phosphatides (Renkonen, *J. Lipid Res., 3,* 181). About 1% of the lecithin was destroyed per hour at 22C, but this was reduced by working at 2C. A considerable part of the lecithin was recovered as lysolecithin. The alpha-beta distribution of fatty acids in lecithin was determined by hydrolyzing the beta position of the lecithin with snake venom (Hawke, *Chem. Ind. (London), 1962,* 1761). Most of the molecules had unsaturated fatty acids in the beta position. The lecithin was obtained from egg yolk by acetone precipitation and column chromatography on cellulose, alumina, and silicic acid. An improved separation of phosphatides on silicic acid columns revealed that rat plasma contains significant amounts of lysolecithin (Newman et al., *J. Lipid Res., 2,* 403). The lysolecithin did not seem to be an artifact. Silicic acid chromatography was also used to separate the mono- and dimethylethanolamine containing phosphatides of choline-requiring *Neurospora crassa* mutants (Hall and Rye, *Ibid.,* 321). The phosphatides were shown to be phosphatidyl mono- and dimethylethanolamine.

A serine containing phosphatide of unknown structure with properties similar to that of phosphatidic acid was isolated from cereal lipid (Aylward and Showler, *J. Sci. Food Agr., 13,* 92).

Gradient elution chromatography of rat brain lipids on alumina columns gave a fraction which contained all the ethanolamine containing lipids (Long and Staples, *Biochem. J., 80,* 557). The main components were phosphatidyl ethanolamine and phosphatidal ethanolamine with small amounts of sulphatide, lysophosphadityl ethanolamine and methyl esters (the latter resulting from transesterification between the phosphatidyl ethanolamine and methanol). Further chromatography on silicic acid resolved the mixture into methyl esters, phosphatidyl + phosphatidal ethanolamine, and sulphatide + lysophosphatidyl ethanolamine. The lipids of human brains were also separated on alumina columns, and the fractions were further analyzed by chemical procedures and paper chromatography (Davison and Wajda, *Ibid., 82,* 113).

A method for the preparation of pure plasmalogen (phosphatidal choline) was based on the selective hydrolysis of phosphatidyl choline by *Crotalus atrox* venom (Gottfried and Rapport, *J. Biol. Chem., 237,* 329). Cobra venom would not work. The aldehydogenic chains of human erythrocyte plasmalogens were isolated as a group and converted to alcohols, acetylated alcohols, methyl esters, and dimethylacetals, and analyzed by gas phase chromatography (Farquhar, *J. Lipid Res., 3,* 21).

Triphosphoinositide was isolated from ox brain (Dittmer and Dawson, *Biochem. J., 81,* 535). It was tightly bound to brain protein and pre-treatment of the tissue with acetone partially degraded the complex to diphosphoinositide. The triphosphoinositide contained inositol, phosphate, glycerol, and fatty acid in the ratio 1:3:1:2. No nitrogen was found. Monophosphoinositide was also found in the brain tissue and from hydrolytic studies found to be diacylglycerylphosphorylinositol. The structure of brain triphosphoinositide was studied (Dawson and Dittmer, *Ibid., 81,* 540). Mild acid hydrolysis produced inositol triphosphate and some inositol diphosphate, glycerophosphate, diglyceride, and monoglyceride. Alkaline hydrolysis produced glycerylphosphorylinositol diphosphate with some inositol triphosphate, inositol diphosphate, and some glycerophosphate. Acid hydrolysis of the glycerylphosphorylinositol diphosphate yielded inositol triphosphate, inositol diphosphate, and glycerophosphate. These results were interpreted as showing the original phosphatide to be diacylglycerylphosphorylinositol diphosphate. The inositol-containing lipids of flaxseed phosphatides were isolated by solvent fractionation (Carter et al., *JAOCS, 39,* 107). Countercurrent extraction separated the inositol fraction into phosphatidyl inositol and a fraction containing inositol and a long chain nitrogenous base. The phosphatidyl inositol existed as a mixed magnesium-calcium salt and it could have been attached to phospitidyl ethanolamine through such salt linkages. The long-chain base fraction contained phosphatidyl inositol and two phytoglycolipids one of which was similar to that previously isolated from corn and soybeans. The other phytoglycolipid contained galactose, arabinose, and fucose. The long chain base was dehydrophytosphigosine.

A method for the determination of sphingosine based on its reaction with methyl orange was described (Lauter and Trams, *J. Lipid Res., 3,* 136). From 0.01–0.1 $\mu$moles of sphingosine can be determined in this way.

Lipids extracted from human carcinomas of the stomach, small intestine, and colon were fractionated on silicic acid columns and the fractions tested for serological activity (Graf et al., *Cancer Res., 21,* 1532). One antiserum contained anticytolipin H and the other was reactive to the crude lipids of the gastrointestinal system, both normal and neoplastic. Two different haptens could be demonstrated, cytolipin H and a new hapten designated cytolipin G.

MISCELLANEOUS LIPIDS. Glycolipids possessing blood group activity were extracted from human blood type A+ and B+ (Hakomori and Jeanloz, *J. Biol. Chem., 236,* 2827). These were purified by chromatography on alumina, silica gel, and cellulose. Partial hydrolysis of the glycolipid showed a resistant core composed of fatty acid, sphingosine, glucose, and an additional sugar. In A type blood the sugar was galactose, in B it was galactosamine. In both glycolipids part of the galactose and galactosamine was located at, or near, the end of the carbohydrate chain.

The occurrence of methyl esters of fatty acids in blood and body lipids was reported (Dhopeshwarkar and Mead, *Proc. Soc. Exp. Biol. Med., 109,* 425). It was demonstrated that the methyl esters were not formed during homogenization or extraction of the tissue.

The extraction of peat wax with various solvents was studied (Howard and Hamer, *JAOCS, 39,* 250). Benzene appeared to be the best solvent. The crude wax was further fractionated by chromatography into nine components. Hydrocarbons, carboxylic ester, carbonyl, and hydroxyl groups were detected.

Several new glyceryl ethers were demonstrated in the liver oils of the grey dogfish, Greenland shark, and ratfish (Hallgren and Larsson, *J. Lipid Res., 3,* 31). The ethers were isolated by chromatography on alumina and converted to the corresponding dimethyoxy derivatives. The latter were rechromatographed on alumina and fractionated by gas phase chromatography. Besides the usual chimyl, batyl, and selachyl alcohols, compounds with saturated $C_{14}$, monounsaturated $C_{18}$, $C_{20}$, and $C_{22}$, and diunsaturated $C_{18}$ ether chains were found. The hexadecenyl chain was unsaturated at the 9,10 position, and the eicosenyl and docosenyl chains were unsaturated at the 13,14 position, according to the results of chromic acid oxidation. The molecular weights of the dimethoxy derivatives were confirmed by mass spectrometry. An analysis of the

glyceryl esters of various human and bovine tissues also demonstrated the occurrence of several new glyceryl ethers (*Ibid.*, 39).

*Lipoprotein:* A study of the β-lipoprotein of human serum indicates that three forms exist (Lawrence and Shean, *Science, 137,* 227). The three forms are not present in all individuals, and the amounts present are variable. They are not separable by sedimentation, and they cross-react immunologically. They can be demonstrated on starch gel electrophoresis and immunoelectrophoresis. The β-lipoprotein of human serum was isolated by chromatography on hydroxyapatite (Cramer and Brattsten, *J. Atherosclerosis Res., 1,* 335). It was shown to be immunologically homogeneous, and was further purified by electrophoresis. The dextran sulfate-precipitable lipoprotein was compared with lipoprotein fractions obtained by ultracentrifugation at densities of 1.019, 1.063, and 1.21 and with paper electrophoretograms (Sakagami and Zilversmit, *J. Lipid Res., 3,* 111). Low molecular weight dextran sulfate and calcium chloride quantitatively precipitated the low-density lipoproteins from dog serum. The precipitate was demonstrated to contain both $a_2$ and β-lipoprotein by electrophoresis.

## FATTY ACID DETERMINATIONS AND STRUCTURE ANALYSIS

GENERAL. A method for the separation of free fatty acids from other lipids was reported (McCarthy and Duthie, *J. Lipid Res., 3,* 117). The separation was achieved with a column of silicic acid treated with potassium hydroxide in *iso*-propanol. The neutral lipids were eluted from the column with ethyl ether and the fatty acids with 2% formic acid in ethyl ether. Phosphatides were retained on the column. The fatty acid recovery averaged 98.3%.

The analysis of polyunsaturated fatty acids by gas phase chromatography and alkali isomerization were compared. Comparative analyses on 12 varieties of pecan oils, 11 peanut oils, 2 avocado oils, and one analysis on each of citrus seed oil, corn oil, Wesson oil, and lard showed good agreement for the two methods except on the latter three samples (French, *JAOCS, 39,* 176). The iodine values calculated from the gas phase results agreed closely with the values obtained by the Wijs method. In another study the results obtained by gas phase chromatography, alkali isomerization, and the lipoxidase methods were compared (Guillaumin and Drouhin, *Rev. Franc. Corps Gras, 9,* 415). Gas chromatography was felt to be the easiest technique, but in certain cases it was less sensitive than alkali isomerization. Lipoxidase was useful in cases where determination of *cis-trans* isomerism was important, but did not distinguish linoleate from linolenate.

GAS CHROMATOGRAPHY. The idea that "eddy diffusion" adds a significant contribution to the height of a theoretical plate in gas chromatography was examined critically (Giddings and Robison, *Anal. Chem., 34,* 885). An alternative to the classical eddy diffusion concept, the coupling theory of eddy diffusion, was proposed. The van Deemter-Jones plate height equation was used to evaluate the performance of small packed gas chromatography columns (Nogare and Chiu, *Ibid.*, 890). The relation between β, the ratio of free gas space to liquid phase volume in the packing, and resolution was derived. A criterion was established for the β which gave the best resolution and efficiency. The relation between plate height in ordinary gas chromatograph and programmed temperature gas chromatography was discussed (Giddings, *Ibid.*, 722). The zone compression caused by the continual heating and the thermal lag in heating from the wall of the columns to the center were treated theoretically. The use of programmed temperature in capillary gas chromatography was considered (Habgood and Harris, *Ibid.*, 882). The large dead volume of capillary columns restricts much of the effectiveness of temperature programming to very low ratios of heating rate/flow rate. Even so the theory predicts that significant decreases in analysis time without loss in resolution can be expected with programmed temperature capillaries.

The effect of operating parameters on the sensitivity of electron capture detectors for gas chromatography were studied (Landowne and Lipsky, *Ibid.*, 726). With a constant direct current voltage as the source of applied potential, relatively high detector temperatures and low gas flows yielded optimum results. Electron affinity depended on the hydrocarbon structure in addition to the electronegativity of the heteroatom. A gas chromatography apparatus was described which employed two columns having different liquid phases and which displayed simultaneously the chromatograms obtained from each column on separate channels of a dual channel recorder (Merritt and Walsh, *Ibid.*, 908). Carbon deposits in the preheater section of gas chromatography apparatuses were indicated as a potential source of error with compounds such as fatty acids and amines (Smith and Gesnell, *Ibid.*, 646). The deposits caused low detector response and false peaks. The deposits were formed by thermal decomposition of the injected samples. Solvents and filter paper were pointed out as possible sources of contamination in the microanalytical determination of fatty acids by gas liquid chromatography (Lindgren et al., *J. Lipid Res., 3,* 390). A device for the quantitative collection of the effluents from gas chromatography columns was tested (Hajra and Radin, *Ibid.*, 131). It consisted of a Millipore filter held in a Swinny hypodermic adapter. A semilogarithmic plot of elution volumes aided in identifying components which were members of the same homologous series (Evans et al., *JAOCS, 39,* 210). This technique was especially helpful in flavor studies for identifying small peaks.

The assay of tritium in the effluent from gas chromatography columns was discussed (Lee et al., *Anal. Chem., 34,* 741). It was important to maintain constant efficiency for tritium detection. Variation in detection efficiency may occur at high count rates and from composition changes in the operating counter gas during the passage of large peaks. Long chain fatty acids were recovered from the effluent of a gas chromatograph and assayed for radioactivity (Meinertz and Dole, *J. Lipid Res., 3,* 140). The vapors were trapped in a glass tube connected to the exit of the gas chromatograph. The tube was plugged with a wad of cotton dampened with toluene to insure complete recovery. The tube contents were washed into a scintillator for counting. A method for the radioassay of carbon-14 labeled substances in gas chromatography effluents was reported (*Ibid.*, 44). The effluent was passed through a cartridge filled with anthracene crystals coated with silicone oil. The radioactivity in the cartridge was assayed directly by scintillation counting. A series of such cartridges could be mounted on an automatic fraction collector for routine work.

Methods for the quantitative preparation of methyl esters for gas chromatography were investigated (deFrancesco and Maglitto, *Riv. Ital. Sostanze Grasse, 5,* 245). When volatile fatty acids were present, a transesterification with methanol and sodium methoxide in a sealed vial prevented the loss of short chain components. If more than 0.5% acidity was present, sodium methoxide could not be used unless the fat were pretreated with an alkaline ion exchange resin. Concentrated sulfuric acid or zinc and zinc chloride made good transesterification catalysts with acid samples. When fatty acid methyl esters were hydrogenated in ethanol in preparation for gas chromatography, the final product contained both the methyl and ethyl esters (Poukka et al., *J. Lipid Res., 3,* 128). This could be avoided if the hydrogenation were done in methanol.

The type of column packing and the proportion of stationary phase were found to have an appreciable effect on the retention times of linolenic and arachidonic acids but not of oleic, stearic, and linoleic acids (Poy, *Riv. Ital. Sostanze Grasse, 3,* 137). Celite C22 packing at low liquid concentrations may actually reverse the order of appearance of arachidonic and linolenic acids, but such effects were not observed with Chromosorb W. Improvements in the techniques for preconditioning polyester columns were reported (Gerson, *J. Chromatog., 6,* 178). The introduction of samples within a capsule onto a gas chromatography column, ionization detector overloading, and methods of predicting the retention volumes of methyl esters of fatty acids were also discussed.

The simultaneous determination of glycerol and fatty acids in glycerides was accomplished by reduction with lithium aluminum hydride, acetylation of the resulting alcohols, and analysis by gas chromatography (Horrocks and Cornwell, *J. Lipid Res., 3,* 165). The four geometric isomers of methyl linoleate were determined by gas chromatography on Apiezon L and diethyleneglycol succinate capillary columns (Litchfield et al., *JAOCS, 39,* 330). The Apiezon L column gave three peaks containing the *cis-cis,* the *cis-trans,* and *trans-cis* + *trans-trans* isomers, respectively. The polyester column gave three peaks containing the *trans-trans,* the *cis-cis,* and the *trans-cis* + *cis-trans* isomers, respectively. The content of the four isomers could be calculated from the results on the two columns, and the final values agreed well with *trans* isomer determinations by infrared spectrophotometry. Methods for the separation of chlorinated fatty acids on a variety of column packings were reported (Smith and Gosnell, *Anal. Chem., 34,* 438). The gas chromatographic analysis of marine oils was discussed (Pfeifer et al., *JAOCS, 39,* 292). A gas chromatographic method for the identification of the stock from which a sample of soap originated was developed (Beck et al., *Ibid.*, 53). At the same time a novel automatic attenuator was described. The methyl esters were prepared from the soaps with methanolic boron trifluoride. A gas chromatographic procedure for the quantitative determination of the unsaturated fatty acids in tall oil was reported (Iden and Kahler, *Ibid.*, 171). Since

some of the tall oil components were not volatile enough to be chromatographed, an internal standard of margaric acid was used. The accuracy obtained with thermal conductivity and beta-ray ionization detectors was compared.

A gas chromatographic method for the analysis of milk fatty acids was reported (Gander et al., *J. Dairy Sci., 45,* 323). The methyl esters were separated on diethyleneglycol succinate; butyl esters were separated on Apiezon L with temperature programming to estimate the short-chain acids. Even with butyl esters the recovery of the four, six, and eight carbon acids was not quantitative and correction factors had to be applied. Another technique for the gas chromatographic determination of the fatty acids of butter and margarine was described (Anselmi et al., *Riv. Ital. Sostanze Grasse, 38,* 436). Two chromatograms were made, one for the esters up to $C_{10}$ and the other for the total sample. They also found appreciable losses with the short-chain methyl esters, but reported that ethyl ester would give satisfactory results. An extensive examination of the fatty acids of milk fat using gas chromatography and other techniques was reported (Magidman et al., *JAOCS, 39,* 137; Herb et al., *Ibid.,* 142). The esters were first fractionated by distillation and silica gel chromatography to reduce the complexity of the mixture. The fractions were then separated by gas chromatography on several types of columns, and the components separated in this way were analyzed by iodine value, alkali isomerization, infrared and ultraviolet spectrophotometry, and rechromatography after hydrogenation. At least 60 fatty acids were found. Water and short chain fatty acids were estimated directly by gas chromatography on a column of stearic or 12-hydroxy-stearic acid on a Teflon support at 120° (Prevat and Cabeza, *Rev. Franc. Corps Gras, 8,* 632).

A gas chromatographic method for calssifying a compound as a member of the homologous series of normal alkanes, alkyl benzenes, alcohols, aldehydes, ketones, ethers, esters, thioalkanes, or thiols was proposed (Merritt and Walsh, *Anal. Chem., 34,* 903). When two different columns containing appropriate liquid phases were used, the ratio of retention volumes for homologous series on the two columns was constant. The ratio was different for different homologous series. The method was applicable to isothermal or programmed apparatuses and packed or capillary columns. A gas chromatographic method was developed for the separation of the aldehydes and aldehyde esters resulting for the ozonolysis of unsaturated fatty acids esters (Privett and Nickell, *JAOCS, 39,* 414). The method was useful in studying the structure of unsaturated fatty acids and in the quantitative analysis of mixtures of unsaturated fatty acids which would not be completely separated by gas chromatography. A gas chromatographic method was described for the identification of the di- and monocarboxylic acids present in alkyd and polyester coating resins (Esposito and Swann, *Anal. Chem., 34,* 1048). The resins were transesterified with lithium methoxide and separated by programmed temperature gas chromatography on both polar and nonpolar columns.

PAPER AND THIN-LAYER CHROMATOGRAPHY. Losses by volatilization of short chain fatty acid sodium salts from paper chromatograms were studied by means of $C^{14}$-tagged fatty acids (Molloy and Kowkabany, *Ibid.,* 491). The losses were attributed to hydrolysis of the sodium salts on the paper, and the humidity and temperature were important factors in the process. Fatty acid critical pairs (lauric-linolenic, myristic-linoleic) were separated by paper chromatography on undecane impregnated paper using acetic acid-water, methanol-water, and dioxane-water as mobile phases (Aparicio, *Grasas Aceites [Seville, Spain], 12,* 109). Palmitic and oleic acids could not be separated by these solvents but could with acetonitrile-water. A paper chromatographic method was reported for the detection of small amounts of polymers in fats and oils (Rost, *Fette Seifen Anstrichmittel, 64,* 427). The dimeric fatty acids were concentrated from the total fatty acids with urea and the concentrate was further fractionated on paper impregnated with a petroleum fraction (b.p. 190C) developed with 90% acetic acid. The spots were visualized with copper acetate-sodium diethyldithiocarbamate. Qualitative and quantitative analyses of long-chain hydroxy- and keto-fatty acids on undecane impregnated paper developed with aqueous acetic acid were reported (Kaufmann and Ko, *Ibid.,* 434). The spots were detected with copper acetate-rubeanic acid and the color intensity was measured photometrically. A circular paper chromatographic method was reported for the identification, estimation, and preparation of fatty acids (Viswanathan and Bai, *J. Chromatog., 6,* 264).

Silica gel thin-layer chromatographic plates were treated with dichlorodimethylsilane to make them water repellent (Kaufmann et al., *Fette, Seifen, Anstrichmittel, 63,* 689). This made it possible to dip the plates into aqueous solutions to detect the spots on the plates. The copper acetate-dithiooxamide solutions used for detection in fatty acid paper chromatography could then be used for thin-layer plates. A colorimetric method for the quantitative determination of esters on thin-layer plates was described (Vioque and Holman, *JAOCS, 39,* 63). The spots or zones were scraped from the plates and the ester determined by the hydroxamic acid method. Techniques for carrying out hydrogenation and bromination of fatty materials on thin-layer plates were described (Kaufmann et al., *Fette, Seifen, Anstrichmittel, 64,* 1). These techniques were useful in the separation of critical pairs. The hydrogenation was performed by depositing a palladium catalyst on the plate. The bromination was accomplished by adding bromine to the developing solvent. Silicic acid thin-layer plates impregnated with silver nitrate were used to separate the *cis* and *trans*-isomers and vinylogues of fatty acids (Morris, *Chem. Ind.* (London), *1962,* 1238). Also, plates impregnated with boric acid were used to separate the *threo* and *erythro*-isomers of vicinal dihydroxy-fatty acids. Gypsum was advocated as an adsorbant for thin-layer plates (Kaufmann and Khoe, *Fette Seifen Anstrichmittel, 64,* 81). Such plates were less fragile than silicic acid plates and could be dipped into aqueous reagents without destruction.

MISCELLANEOUS FATTY ACID ANALYSES METHODS. The fatty ester-mercuric acetate adducts of unsaturated fatty acids were separated from saturated fatty acids and from each other by chromatography on alumina columns (White and Quackenbush, *JAOCS, 39,* 511). Oleate and linoleate were obtained in a pure form but the linoleate could not be separated completely from the linoleate. The original fatty esters could be regenerated from the adducts in high yield. A similar chromatographic separation of the mercuric acetate adducts of unsaturated fatty acid esters on alumina columns was described (Kuemmel, *Anal. Chem., 34,* 1003). This gave fractionation into saturated, monounsaturated, and polyunsaturated components. The geometrical isomers of the various unsaturated fractions could be determined by infrared spectrophotometry of the regenerated esters. The method was useful with partially hydrogenated oils. The mercuric acetate addition products of oleic, linoleic, and linolenic acids were prepared and completely separated from each other by adsorption chromatography (Jantzen et al., *Fette, Seifen, Anstrichmittel, 63,* 685). The original esters were recovered completely and without isomerization.

Geometrical isomers and isologous fatty acid esters were separated by countercurrent distribution between *iso*-octane and 0.2 molar silver nitrate in 90% methanol (Dutton et al., *Chem. Ind. (London), 1961,* 1874). Methyl oleate and methyl elaidate were separated. Selenium isomerized methyl linoleate was resolved into *trans-trans, trans-cis* + *cis-trans,* and *cis-cis* isomers. Alkali conjugated methyl linoleate was resolved into *cis-cis*-unconjugated, *trans-cis* + *cis-trans*-conjugated, and *trans-trans*-conjugated isomers. Lesquerolic acid, a new hydroxy acid from Lesquerella seed, was isolated by countercurrent distribution (Smith, *J. Org. Chem., 26,* 2903).

An improved micromethod for the location of double bonds in naturally occurring polyenoic fatty acids was reported (Chang and Sweeley, *J. Lipid Res., 3,* 170). The acids were cleaved by permanganate-periodate oxidation and the fragments were analyzed by gas chromatography. Another method for locating the double bonds in unsaturated fatty esters was based on the gas chromatographic separation of the aldehydes and aldehyde esters obtained by the reduction of the fatty ester ozonides (Privett and Nickell, *JAOCS, 39,* 414). The ozonolysis was carried out at −60 to −70C by adding the sample to a pentane solution of ozone. The reduction was affected by a Lindlar catalyst at 0C. The method was careful for structural analyses or for the quantitative determination of mixtures of isomers which are difficult to separate.

## MEASUREMENTS OF PHYSICAL PROPERTIES

A film tensiometer was described which was capable of continuous measurement of the surface tension of a solution undergoing constant changes in its surface properties (Peterson, *Kolloid Z., 183,* 141). The surface tension was measured as a function of the pull exerted on a pair of vertical rods by a thin moving liquid film supported by these rods. A variation in surface tension caused a change in the force applied to the rods. This resulted in the movement of one of the rods which was spring loaded. The movement was converted by a transducer to a continuously recorded electric signal. The instrument could be calibrated to an accuracy of 0.5 dynes/cm and detected a difference as small as 0.3 dynes/cm.

An apparatus was described which was capable of measuring the color of the diffuse reflected light from solid objects such as fats (Naudet et al., *Rev. Franc. Corps Gras, 9,* 202). Light is projected onto the solid object at a 45° angle and the light reflected by the solid is picked up by a photocell at a 90° angle with respect to the solid. The AOAC method

for determining egg yolk color agreed well with readings taken on a Heiman-Carver color rotor except when the latter gave scores over 17 (Sunde, *Poultry Sci., 41,* 532).

## COMPOSITION AND CHARACTERISTICS: ANALYTICAL DATA

Many analyses of the composition and physical properties of fats and lipids were published in 1962. 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 of fats, oils, and unfractionated lipid mixtures. An asterisk appearing after the reference indicates that fatty acid composition data are given. The next division of the list includes reports in which there was at least partial fractionation into lipid groups, or in which some particular lipid groups were analyzed for. An asterisk after the reference indicates that fatty acid composition data are given. The third division of the list gives measurements of physical properties. Many of the references in the previous sections on methodology contain information on the composition and physical properties of certain materials. These references have not been completely cross-listed in the present section. Reports that deal primarily with the effect of environmental, dietary, and genetic factors on composition and physical properties are given in the succeeding section.

UNFRACTIONATED LIPIDS. The oils from the seed of 37 plant species representing 18 families were analyzed by alkali isomerization and other techniques in a search for new industrial oils (Earle et al., *JAOCS, 39,* 381)*. Species examined were: *Nolina texana* S. Wats, *Yucca peninsularis* McKelvey, *Humulus scandens* (Lour.) Merr, *Deutzia scabra* Thunb, *Brongniartia alamosana* Rydb, *Coursetia glandulosa* A. Gray, *Willardia mexicana* (S. Wats.) Rose, *Melia azedarach* L, *Rhus radicans* L, *Abutilon incanum* (Link) Sweet, *Malva* cf. *parviflora* L, *Daphne mezereum* L, *Godetia amoena* (Lehm.) G. Don, *Acanthopanax spinosum* Miq, *Hedera helix* L, *Trachymene caerulea* (DC.) R. Grah, *Pastinaca sativa* L, *Thevetia thevetioides* (H.B.K.) K. Schum, *Ipomoea* sp, *Phlox paniculata* L, *Datura metel* L, *Solanum nodiflorum* Jacq, *Tabebuia palmeri* Rose, *Proboscidea altheaefolia* (Benth.) Decne, *Actinomeris alternifolia* (L.) DC, *Anthemis tinctoria* var. *Kelwayi, Bidens frondosa* L, *Calendula officinalis* L, *Coreopsis lanceolata* L, *Dimorphotheca pluvialis* (L) Moench, *Doronicum caucasicum* Bieb, *Matricaria copensis* L, *Osteospermum ecklonis* (DC.) T. Norl, *Osteospermum spinescens* Thunb, *Rudbeckia hirta* L, *Tagetes erecta* L, *Zinnia elegans* Jacq. The seed oils from 14 species of the genus *Lesquerella* were analyzed by gas chromatography, and all but two contained hydroxyeicosenoic acid, and the remaining two contained hydroxyoctenoic acids (Mikolajczak et al., *Ibid.,* 78)*. The species examined were: L. *augustifolia,* L. *argyraca,* L. *densipila,* L. *engelmannii,* L. *fendleri,* L. *globosa,* L. *gordonii,* L. *gracilis,* L. *grandiflora,* L. *lasiocarpa,* L. *lescurii,* L. *lindheimeri,* L. *ovalifolia,* L. *pinetorum. Lesquerella lasiocarpa* seed oil was shown to contain 40–45% (+) 14-hydroxy-*cis*-11-eicosenoic acid (lesquerolic acid) (Smith et al., *J. Org. Chem., 26,* 2903)*. The fatty acid composition of a number of fats and oils was examined by gas chromatography (Prevot and Cabeza, *Rev. Franc. Corps Gras, 9,* 149)*. The substances investigated were: corn gluten, apricot kernel, sweet almond, voantamenaka, *Lallementia imberica,* calaphyllum, grape endosperm, olive seed, and *Parinarium macrophyllum* kernel*. The seed oil of *Thalictrum polycarpum* was shown to contain 35% *trans*-5-*cis*-9-*cis*-12-octadecatrienoic acid, *trans*-5-octadecenoic acid and two unknown $C_{18}$ acids (Bagby et al., *Biochemistry, 1,* 632)*. *Limanthes douglasii* seed oil was found to contain 10% *cis*-5-*cis*-13-docosadienoic acid (Bagby et al., *J. Org. Chem., 26,* 1261). The seed oil of *Lunaria biennis* was reported to contain 21% *cis*-15-tetracosenoic acid (Wilson et al., *JAOCS, 39,* 104). The fatty acid composition of *Argemone Mexicana* seed oil was determined by liquid-liquid column chromatography (Badami and Gunstone, *J. Sci. Food Agr., 13,* 255)*. Alkali isomerization and gas chromatography data were reported on the oils from 12 varieties of pecans and 11 of peanuts as well as citrus seed, Wesson oil, corn oil, and lard (French, *JAOCS, 39,* 176)*. The presence of positional isomers of linoleic acid in corn, cottonseed, and safflower oils was detected (Sreenivasan and Brown, *Ibid.,* 255)*. *Vernonia anthelmintica, Artemisia absinthium,* and castor oils were analyzed by gas and thin-layer chromatography (Vioque and Holman, *Ibid.,* 63)*. A number of castor oil samples were analyzed by gas chromatography (Binder et al., *Ibid.,* 513)*. An analysis was reported for *Vernonia anthelmintica* seed oil (Krewson et al., *Ibid.,* 334)*.

The unsaturated fatty acids of the total lipids of various microorganisms were analyzed by oxidative degradation and gas chromatography (Scheuerbrant and Bloch, *J. Biol. Chem., 237,* 2064)*. Fatty acid analyses were also reported on *Rhodopseudomonas* particulate fractions (Hands and Bartley, *Biochem. J., 84,* 238)*, *Stemphylium dendriticum, Pithomyces chartarum, Cylindrocarpon radicicola,* and *Etemphylium dendriticum* (Harman et al., *Ibid., 82,* 76)*, and *Cooanephora cucurbitarum* (White et al., *JAOCS, 39,* 123)*.

Sweet potato mitochondria were analyzed (Richardson et al., *J. Lipid Res., 3,* 344)*. The glyceride structure of Erythrina Indica seed fat was examined (Kartha and Narayanan, *J. Sci. Food Agr., 13,* 411).

Polyunsaturated fatty acids were analyzed in the adipose and muscle lipids of the loin or rib roasts of pork, beef, lamb, and veal (Ostrander and Dugan, *JAOCS, 39,* 178)*. Meat fats were examined by gas chromatography (Beare, *J. Agr. Food Chem., 10,* 120)*. Mitochondrial lipids from the hearts and livers of marine birds were examined by gas chromatography (Richardson et al., *J. Lipid Res., 3,* 344)*. The fatty acids of human brain tissue were analyzed by gas chromatography (Baker, *Biochem. J., 79,* 642)*. Canine adrenal lipids were found to contain 8,11,14-eicosatrienoic, 5,8,11,14-eicosatetraenoic, and 7,10,13,16-docosatetraenoic acids (Chang and Sweeley, *J. Lipid Res., 3,* 170)*. The lipid and mineral content of men's aortas were determined (Whitehead et al., *J. Atherosclerosis Res., 2,* 199 and 210). The glyceride structure of the fat from the lymph of rats fed known diets was determined with lipase (Mattson and Volpenhein, *J. Biol. Chem., 237,* 53)*.

The fatty acids and alcohols of sperm whale were analyzed by paper chromatography (Kaufmann and Schoeb, *Fette Seifen Anstrichmittel, 63,* 609)*. The mitochondrial lipid of the hearts and livers of several fresh water and marine fish were analyzed for polyunsaturated fatty acids (Richardson et al., *J. Lipid Res., 3,* 344)*. An analysis for fatty acid structural isomers in dogfish liver oils *(Squalus acanthias)* revealed that there were several monounsaturated fatty acids of various chain lengths (Mallins and Houle, *Proc. Soc. Exp. Biol. Med., 108,* 126)*. The polyunsaturated acids of cuttlefish oil (Toyama and Takagi, *Fette, Seifen, Anstrichmittel, 64,* 134)*, and finback whale oil (*Ibid.,* 137)* were examined.

The fatty acid composition of 106 raw milk samples taken over a year's time was determined by gas chromatography (Jensen et al., *J. Dairy Sci., 45,* 329)*. A detailed analysis of the polyunsaturated fatty acids in a milk sample was obtained (Herb et al., *JAOCS, 39,* 142)*. A detailed analysis of the $C_{18}$ polyunsaturated fatty acids of milk fat was made (Sambasivarao and Brown, *Ibid.,* 340)*. *Trans*-octadec-16-enoic acid (Hansen and Cooke, *Biochem. J., 81,* 233), and 3,7,11,15-tetramethylhexadecanoic acid (Sonneveld et al., *J. Lipid Res., 3,* 351) were reported in milk fat.

The polyunsaturated, oleic, saturated, and *trans* fatty acids of 26 brands of margarine were determined (Rice et al., *J. Am. Dietet. Assoc., 41,* 319)*. The fatty acid composition of various margarines, spreads, and shortenings were analyzed by gas chromatography (Beare, *J. Agr. Food Chem., 10,* 120)*. A gas chromatographic analysis of tall oil fatty acids was performed (Iden and Kahler, *JAOCS, 39,* 171)*. The glyceride structure of soybean oil before and after various degrees of hydrogenation was determined by the lipase method (Mattson and Volpenhein, *Ibid.,* 307)*.

The copper and iron content of ice cream and mellorine samples were determined (Vanderzant and Miah, *Food Technol., 15,* 515).

FRACTIONATED LIPIDS. The lipid of coffee seeds was fractionated into glycerides, diterpenes, phytosterols, and phosphatides by chromatography (Kaufmann and Hamsago, *Fette, Seifen, Anstrichmittel, 64,* 206)*. The lipids of *Azobacter agilis, Agrobacterium tumefaciens,* and *Escherichia coli* were fractionated into phosphatides (mostly phosphatidyl ethanolamine) and esters of coenzyme Q (Kaneshiro and Marr, *J. Lipid Res., 3,* 184)*. Barley, oat, and rye lipids were fractionated into phosphatidyl inositol, phosphatidic acid, and a serine containing phosphatide (Aylward and Showler, *J. Sci. Food Agr., 13,* 92)*. The acetone-soluble lipids of red clover were reported to contain minor amounts of sterol esters, triglycerides, diglycerides, free sterols, and hydrocarbons, and the nature of the sterols and hydrocarbons was examined (Weenik, *Biochem. J., 82,* 523)*.

The free and combined sterols of commercial phosphatides from groundnut, rapeseed, and linseed were analyzed (Aylward and Nichols, *J. Sci. Food Agr., 13,* 86). The unsaponifiable matter of tung oil *(Aleurites montana)* from Nyasaland was found to contain sterols, carotenoids, and tocopherols (Shone, *Ibid.,* 315). The unsaponifiables of soybean oil were fractionated by partition chromatography into hydrocarbons, sterols, and a fraction of intermediate polarity, and the amounts and compositions of these fractions were investigated (Hoffmann et al., *JAOCS, 39,* 323).

The constitution of peat wax was investigated (Howard and Hamer, *Ibid.,* 250).

The following animal lipids were analyzed by chromatographic resolution into various lipid fractions: Pig spleen, lung, and kidney, and pigeon, trout, and rabbit muscle (Gray and MacFarlane, *Biochem. J., 81,* 480)*; the subcellular particles from rat liver (Getz et al., *Ibid., 83,* 181)*; beef heart mitochondria and various mitochondrial enzyme subunits (Fleischer et al., *J. Biol. Chem., 236,* 2936)*; phosphorylating "digitonin particles" and water- and saline-washed mitochondria from rat liver (Bartley et al., *Biochem. J., 82,* 540)*; beef brain and spine (Carroll, *J. Lipid Res., 3,* 263)*; human brain (Radin and Akahori, *Ibid., 2,* 335)*; human brain (Davison and Wajda, *Biochem. J., 82,* 113); inter- and intramuscular tissue of pork, beef, lamb, and veal (Ostrander and Dugan, *JAOCS, 39,* 178)*; beef and pork muscles (Crowe and Heimberg, *J. Food Sci., 26,* 581)*; egg yolk and developing chick embryos (Bieber et al., *Biochemistry, 1,* 532); the cell sap and floating fatty layer from centrifuged rat liver homogenates (Getz et al., *Biochem. J., 81,* 214); Swiss laboratory mice liver (Nelson, *J. Lipid Res., 3,* 256)*; mouse dermis and epidermis (Carruthers, *Cancer Res., 22,* 294)*; human bone marrow (Lund et al., *J. Lipid Res., 3,* 95)*; ox plasma (Dunean and Garton, *Ibid.,* 53)*; chick erythrocytes and plasma (Leveille et al., *Proc. Soc. Exp. Biol. Med., 109,* 345); human maternal and umbilical cord blood and amniotic fluid (Helmy and Hack, *Ibid., 110,* 91); and human serum lipoprotein (Nelson, *J. Lipid Res., 3,* 71)*.

A total of 19 aldehydogenic compounds was identified in erythrocyte plasmalogens (Farquhar, *Ibid.,* 21). Iso- and anteiso branched chain aldehydes were isolated from animal tissue (Gray, *J. Chromatog., 6,* 236). The fatty acid distribution on the alpha and beta positions of egg lecithin was analyzed (Hawke, *Chem. Ind. (London), 1962,* 1761)*.

β-Lipoprotein was isolated from human serum and analyzed for cholesterol and phosphorus (Cramer, *J. Atherselerosis Res., 1,* 317). The lipoprotein level of chicken plasma was determined by dextran sulfate precipitation (Homma and Kato, *Poultry Sci., 41,* 608). The composition of the lipoprotein fractions of dog serum was determined (Sakagami et al., *J. Lipid Res., 3,* 111).

The phosphatides of the sponges *Lissodendoryx* and *Speciosphngia* were analyzed (Landowne and Bergman, *J. Org. Chem., 26,* 1257). The former contained a choline and sugar-free phosphosphingolipid.

Analyses were reported on the diglycerides from lipolyzed milk (Jensen et al., *J. Dairy Sci., 44,* 1983)*; and the lipid fractions of milk (Smith and Lowry, *Ibid., 45,* 581)*.

The carotenoid content of human serum was measured under various dietary conditions (Cornwell et al., *J. Lipid Res., 3,* 65). Analyses for various forms of vitamin A and carotenoid were presented for the eggs of lampreys, marine elasmobranchs, marine teleosts, freshwater teleosts, salmon, amphibians, reptiles, and hens (Plack and Kon, *Biochem. J., 81,* 561). The fatty acid esterified with vitamin A in sheep liver was shown to be palmitic (Rao et al., *Arch. Biochem. Biophys., 95,* 285). The amounts of the various tocopherols in wheat germ and bran were determined (Bacharach and Green, *Chem. Ind.* (London), *1961,* 2135).

*Physical Properties:* Cooling curves and micropenetration tests were reported on a cocoa butter-like fat obtained from palm kernel oil (Tateishi et al., *J. Japan Oil Chemists' Soc., 10,* 662). The melting point, optical rotation, and infrared spectrum of cholesterol arachidonate were reported (Mahadevan and Lundberg, *J. Lipid Res., 3,* 106). The physical properties of *Limanthes douglasii* seed oil were reported (Miwa and Wolff, *JAOCS, 39,* 320). Phase diagrams were prepared for 9- and 10- oxooctadecanoic acids and 9- and 10-hydroxyoctadecanoic acids (Cocchrane and Harwood, *J. Org. Chem., 26,* 1278). The solidification points of the binary mixtures dodecanol-tetradecanol, tetradecanol-hexadecanol, hexadecanol-octadecanol, and dodecanol-octadecanol were tabulated (Paquot et al., *Oleagineux, 6,* 555). A freezing point diagram was worked out for the ternary reciprocal salt pair system morpholine, 2,2'-dipyridylamine, stearic acid, palmitic acid (Mod et al., *JAOCS, 39,* 444). The crystal form of lard and interesterified lard was examined (Lutton et al., *Ibid.,* 233). Differential thermal analysis data were presented for binary mixtures of 1-mono-, 1,3-di-, and tristearin (Perron et al., *Rev. Franc. Corps Gras, 8,* 559) and for tertiary mixtures of the same (*Ibid., 9,* 341). The solubility of soaps of wool wax acids formed from 10 cations was determined in seven solvents at 25° (Noble et al., *JAOCS, 39,* 31). A phase diagram of methanol-cottonseed oil-cottonseed oil fatty acids was prepared (Harris, *Ibid.,* 314). The interaction of oleic acid with various aqueous buffer solutions was noted (Saunders, *Biochem. J., 82,* 52P). Infrared spectra were given for hydroxy-, acetoxy-, oxo-, and unsaturated oxo-stearic acid (Kitagawa et al., *JAOCS, 39,* 217). The rheological properties of the emulsifier films in ice cream were studied (Sherman, *Food Technol., 15,* 394). A series of studies of the physical properties of ground olive oil pastes was reported (Martinez et al., *Grasas Aceites [Seville, Spain], 12,* 118, 166, and 213).

## 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.

The fatty acid composition of 106 milk samples taken during the period June 1960–June 1961 and analyzed by gas chromatography was reported (Jensen et al., *J. Dairy Sci., 45,* 329). The oleic and stearic acids were higher in the summer. The carotenoid content of spinach and lima bean leaf segments was found to change on exposure to light in a nitrogen atmosphere (Yamamoto et al., *Arch. Biochem. Biophys., 97,* 168). Violaxanthin decreased and this was accompanied by a nearly stoichiometric increase in zeaxanthin. The light reaction was accompanied by a transient increase and decrease in antheraxanthin concentration. In spinach leaf exposure to strong light followed by dark incubation in oxygen caused a decrease in zeaxanthin, an increase in antheraxanthin, and a small increase in violaxanthin. In bean leaf, the decrease in zeaxanthin was accompanied by a nearly stoichiometric increase in antheraxanthin. The level of dry matter, crude fat, neutral fat, and fatty acid composition of various parts of soybean plants undergoing germination was recorded (Brown et al., *JAOCS, 39,* 327). There was a continuous decrease in the total dry matter and crude fat during germination, but the fat composition was almost constant. There was a small but significant difference in the mobilization of the oleic acid compared to the other fatty acids in the seeds. Samples of subcutaneous body fat were taken from hens maintained at three environmental temperatures and the fatty acid composition was determined (Fisher et al., *Proc. Soc. Exp. Biol. Med., 110,* 832). The fat from hens maintained at 0C was significantly more unsaturated than that from hens at 21 or 32C. There were no differences in the body temperature of the hens at the site of the biopsy. Liver vitamin A levels were much higher in cold-acclimated rats than in rats maintained at 25C (Porter and Masoro, *Ibid., 108,* 609). A greater need for vitamin A for rats in a cold environment could not be shown, and the increase did not stem from a sparing action induced by cold acclimation. The increase was apparently due to the increased food consumption of the rats maintained at low temperatures. Female mosquitos, in contrast to male mosquitos or to male and female houseflies, synthesized triglycerides from glucose, but polyunsaturated fatty acids were absent from the newly synthesized fat (Van Handel and Lum, *Science, 134,* 1979). The fatty acid composition of the oils from 12 varieties of pecans, 11 of peanuts, and other oils were measured by gas chromatography and alkali isomerization (French, *JAOCS, 39,* 176). The deposition of lipid material in the fat organs of developing chick embryo was followed (Feldman et al., *Poultry Sci., 41,* 1232). The results indicated that yolk lipids may be transported to the fat organs by serum lipoproteins. The fatty acid compositions of the ovaries, mesenteric fat, whole blood, and planktonic eggs of Pacific sardines were compared by gas chromatographic analysis (Lasker and Theilacker, *J. Lipid Research, 3,* 60). Whole blood, ovaries, and planktonic egg lipids had similar fatty acid composition and contained more linoleic acid than mesenteric fat. Changes in the fatty acid composition occurred when the sardines were fed an artificial diet rich in oleic and linoleic acids.

The serum cholesterol, lipid phosphorus, total fatty acids, and serum triglycerides were determined for 12 young men receiving different dietary fats (Morse et al., *J. Am. Dietet. Assoc., 41,* 323). All received 37% of their total calories from fat; one group ate butter, another an all corn oil margarine, and the third a liquid corn oil margarine. The butter group had a higher serum cholesterol level; the margarine group had a higher serum triglyceride level. Fats high in various kinds of polyunsaturated fatty acids were used to replace the fat in a control diet of human volunteers (Kingsbury et al., *Biochem. J., 84,* 124). The dietary changes were quickly reflected by the plasma lipids but not by the depot fats. Individual changes occurred in the plasma and depot fat which could not be related to the dietary fat. The tallow from sheep fed purified diets contained less stearate and more oleate than tallow from either sheep fed hay-grain diets or grazing animals (Tove and Matrone, *J. Nutr., 76,* 271). In some instances the tallow was liquid at room temperature. The results were explained in terms of rumen hydrogenation. *Trans* acids in the diet seemed to resist further hydrogenation in the rumen. Holstein cows were fed three types of concentrates, a control, 6% added tallow, and 6% added cottonseed oil; along with either high or low roughage diets (Brown et al.,

*J. Dairy Sci., 45,* 191). Volatile fatty acids in the rumen were affected by the roughage in the diet. Cottonseed oil depressed the percentage of fat and total fat production on the low roughage diets. The fatty acids of the milk fat from $C_6$ to $C_{14}$ were depressed by cottonseed oil and the $C_{18}$ acids were increased. Tallow had less effect than the cottonseed oil.

Dietary linoleic acid profoundly affected the fatty acid composition of the depot, heart, liver, testes, and cerebrum lipids of chickens (Machlin and Gordon, *J. Nutr., 75,* 157). Linoleic acid added to the diets of chickens which had been maintained on a fat-free diet decreased the percentage of $C_{20}$ trienoate and increased the linoleic and arachidonic acid in all tissues. The levels of linoleic and linolenic acids incorporated into the egg lipids were found to increase with the amounts of these acids present in the diet (Marth and Reiser, *Ibid.,* 287). The levels of these acids in the eggs reached a plateau when they comprised 5% of the diets. Linoleic reached a higher level than linolenic acid in the eggs. The level of incorporation of each was decreased when tallow was included in the ration. Linoleic acid was the precursor of arachidonic and docosapentaenoic acids; linolenic acid of eicosapentaenoic and docosahexaenoic acids. The influence of changes in dietary protein level, calorie/protein ratio, and the type of fat on the cholesterol and iodine number of egg fat was studied (Edwards et al., *Poultry Sci., 41,* 713). Only the type of fat appeared to exert a definite effect on the egg fatty acid composition. These factors had little influence on the cholesterol level. Hens were fed purified diets containing either 15% hydrogenated coconut oil or 15% safflower oil as the sole fat source (Machlin and Gordon, *Ibid.,* 1340). The feeding of coconut oil increased the quantities of lauric, myristic, and myristoleic acids and decreased the arachidonic acid of the eggs. Lauric and myristic acids were also increased in the depot, liver and heart lipids, and linoleic acid was decreased in hens fed coconut oil rather than safflower. The composition and alpha-beta distribution of the fatty acids in egg phosphatidyl choline were determined (Hawke, *Chem. Ind. (London), 1962,* 1761). The preparation contained about 30% palmitic acid with about 60% of the molecules having palmitic acid in the alpha position. Most of the beta positions were unsaturated. The proportion of palmitic acid in the molecules was not influenced much by the diet, but the monounsaturated fatty acids were replaced by polyunsaturated fatty acids when cod liver oil was fed. The effect on egg fat composition of feeding diets to hens with corn oil and beef tallow supplements was studied (Jordan et al., *Food Technol., 16,* 118). Corn oil increased the iodine value of the egg lipid. Eggs from the corn oil fed chickens gave better cake volumes and lighter yolk colors. Hens fed cottonseed oil or *Sterculia foetida* seeds had higher concentrations of stearic and lower concentrations of oleic acid in liver, blood plasma, and ovaries than hens fed a control diet (Evans et al., *J. Nutr., 76,* 314). Heart and depot lipids were little changed. Feeding as little as 200 mg/day of *Sterculia foetida* oil to sexually developing pullets resulted in retardation of comb development, enlargement of gall bladder and liver, retarded ovary and oviduct development, inhibition of egg production, and a decrease in the iodine number of the depot fat from 71–41 (Schneider et al., *Ibid., 77,* 403). Addition of as little as 25 mg of *Sterculia foetida* oil to the diets of laying hens increased the mortality of the embryos to over 80% (Schneider et al., *Poultry Sci., 40,* 1644). The active factor may be the same as that found in crude cottonseed oil. Eggs from hens fed 0.15 g of *Sterculia foedita* oil daily showed a pink discoloration after two weeks storage, and the pH of the yolks increased to essentially the same values as the whites after one month storage (Dobernz et al., *Ibid., 41,* 700). The yolks were more viscous than those from the eggs of chickens fed corn oil, and the yolks became semi-solid and putty-like at refrigerator temperatures. The methoxy derivative of sterculyl alcohol, the corresponding hydrocarbon, methyl sterculate, and polymers of sterculic acid were fed to laying hens. (Norby et al., *JAOCS, 39,* 183). The compounds all caused pink egg formation except the polymers. The simultaneous feeding of crude cottonseed oil with crystalline gossypol to laying hens intensified the discoloration caused by gossypol in eggs stored at 35C for three months (Kemmerer et al., *Poultry Sci., 41,* 1101). The cottonseed oil caused a pink discoloration in the eggs.

The effect of the feeding program of the hens on the yolk color of their eggs was investigated (Sunde, *Ibid.,* 532). Six to seven percent corn approximately equalled 1% of alfalfa in ability to pigment the yolks, even though alfalfa contained 10 times as much xanthophyll and 60 times as much vitamin A as corn. High levels of vitamin A reduced the yolk color in diets containing 55% corn and 5% alfalfa. The light colored yolks contained about as much carotenoid + vitamin A as the dark yolks. As the amount of vitamin A in the yolk increased the amount of xanthophyll decreased. Rats receiving diets both with and without 3% sodium nitrate or 0.5% sodium nitrate were fed vitamin A palmitate in oil or in water dispersion both orally and by injection, or they were given β-carotene in oil by stomach tube (Emerick and Olson, *J. Nutr., 78,* 73). In all instances the aqueous source of vitamin A contributed to liver storage to a much greater extent than the oil solution; the oral sources contributed more than the injected ones. The feeding of nitrite but not nitrate lowered liver storage of vitamin A from oral sources but not from injected sources. The type of vitamin A ester found in the intestinal contents, mucosae, small intestinal muscle, blood, and liver of rats fed vitamin A in water dispersions or in oil solutions was studied (Mahadevan and Ganguly, *Biochem. J., 81,* 53). When water dispersions were fed, vitamin A palmitate was found in all fractions. The types of esters in the intestinal contents and mucosae were governed by the fatty acids of the carrier oils. No such relation was found for the ester composition of intestinal muscle and blood which was almost entirely palmitate ester. The liver, under all conditions, stored exclusively palmitate ester.

## DETECTION OF ADULTERATION

The presence of 1% margarine in butter could be detected by gas chromatographic examination of the sterols (Eisner et al., *J. Assoc. Offic. Agr. Chem., 45,* 337). The sterols were isolated by saponification of the fat and chromatographic separation of the unsaponifiable matter on a Florisil column. Butter contained only cholesterol while margarine samples contained stigmasterol and β- and γ-sitosterol. A method for the detection of other fats in milk fat was proposed which was based upon molecular distillation of the fat (Sander and Bird, *J. Dairy Sci., 45,* 317). The distillate yield from mixtures of milk fat and domestic food fats and oils indicated that the presence of food fats and oils other than coconut oil could be detected. If the season of the production of the milk fat were unknown 18–20% adulteration could be detected, but the detectable amount could be less than 5% at certain seasons. The presence of coconut oil could be detected by measuring the refractive index of the distillate. If the production season were unknown 30% adulteration with coconut oil could be detected, but at certain seasons less than 5% could be detected.

Removal of pigments from fat and fat extracts prior to analyses for DDT, DDE, and Rhothane was accomplished by precipitation from acetone and chromatography on Florisil (McKinley et al., *J. Agr. Food Chem., 10,* 226). Data on the recovery of the three pesticides from several types of animal fats were given. Dieldrin was concentrated from a 50 gram sample of butter oil by partition between acetonitrile and n-hexane followed by elution from a Darco G60-Solka Floc column with acetone (McKinley and Savary, *Ibid.,* 229). The dieldrin was then free enough of fat to be identified by paper chromatography. Silicic acid and silicic acid-sulfuric acid mixtures were used in the chromatographic concentration of chlorinated hydrocarbon pesticide residues from butterfat (Moats, *J. Assoc. Offic. Agr. Chem., 45,* 355). Both columns gave excellent separations of DDT and other chlorinated hydrocarbons from large amounts of fat. A rapid colorimetric method for the detection of DDT in milk and butter was devised which required 1.5 hours and detected 0.2 ppm of DDT in 100–150 grams of milk and less than 3 ppm in 6 g of butter (Gunther et al., *Ibid.,* 359). The method could be scaled up to increase the sensitivity. The DDT was separated by inert solvent partition followed by reaction-solvent partition. The DDT was then nitrated and determined colorimetrically. An analytical method was reported for the determination of perthane [1,1-dichloro-2,2-bis (p-ethylphenyl)-ethane] in rat fat and milk (Gordon et al., *J. Agr. Food Chem., 10,* 380). A study was made of the DDT and DDE content of the diet and body fat of native Alaskans who lived in isolated, primitive areas (Durham et al., *Science, 134,* 1880). These Eskimos had considerably less DDT and DDE in their body fat than the U.S. population. Very little was found in their food supply. Dairy cows were placed on pasture at various intervals after application of 0.25 lb/acre of heptachlor (Rusoff et al., *J. Agr. Food Chem., 10,* 377). The butterfat from these animals showed continuous low levels of heptachlor epoxide when the animals were allowed to graze the treated pasture continuously 1,8,15,29, and 43 days after application of the heptachlor. Traces of residue were found in animals allowed to graze 57 days after application. Animals continued to excrete the residue 40 days after removal from the treated pasture.

Olive oils produced by re-esterifying olive oils high in free fatty acid or distilled olive oil fatty acids with glycerol were analyzed by determining the hydroxyl number of the unsaponifiable fraction (Gracian and Martel, *Grasas Aceites [Seville, Spain], 12,* 123). Re-esterified oils made from distilled fatty acids had a low unsaponifiable hydroxyl number

due to the loss of unsaponifiables during distillation. Vizern's test for detecting the presence of solvent-extracted olive oil in virgin olive oil was found to give more reliable results when the unsaponifiable fraction of the oil was used instead of the oil itself (Espejo, *Ibid.*, 115).

A method based on the determination of conjugated dienoate, *trans* isomer, and *cis* monoenoate was proposed for the determination of mutton tallow in mixtures of beef and mutton tallow (Szonyi, *JAOCS, 39,* 185). The analytical findings were combined in an empirical equation and gave results accurate to ± 14%. The adulteration of lard by tallow could be determined by analysis of the ratio of $C_{14}$ to $C_{16}$ acyl groups by gas chromatography (Wolff, *Rev. Franc. Corps Gras, 8,* 677). The ratio is normally 0.054 in lard, but 0.114 in tallow. About 10% tallow can be detected. The adulteration of fats with pig fat could be detected by the Boemer value (Tollenaar et al., *Fette, Seifen, Anstrichmittel, 64,* 303). Three methods for determining the Boemer value, the D.F.G.-ether method, the R.Z.S.-ether method, and the AOCS-acetone method, were compared. All the methods gave comparable results. The removal of ether insoluble glycerides was found to have a great influence on the melting point of the glycerides of tallow and hardened fats (Roos, *Ibid.*, 6). This could be used for the detection of these fats in lard. In this way the Boemer number gives a sensitive index for the detection of tallow in lard.

A reliable rapid chick assay procedure for the hydropericardium factor was reported (Alexander et al., *Poultry Sci., 41,* 22). Assays of a number of fats showed that all the fats and fatty acid components being used in the formulation of hydrolyzed animal and vegetable fats were free of the factor. The nature of the unknown factor was studied. Chicks fed fractions of an epoxy-resin paint developed hydropericardium and ascites similar to the symptoms observed in the toxic-fat syndrome (McCune et al., *Ibid.*, 295). The toxicity was caused by a chlorinated biphenyl used as a plasticizer in the paint. It was toxic at the level of 0.02% of the diet. The pathological symptoms were described. Fat containing the toxic factor was found to have a marked effect on testicular development without appreciably affecting the development of secondary sex characteristics (Allen and Lalich, *Proc. Soc. Exp. Biol. Med., 109,* 48). Apparently in immature chickens testicular hypoplasia is a more sensitive index of toxic fat than hydropericardium or ascites.

## NUTRITION, PHYSIOLOGY, BIOCHEMISTRY

### NUTRITION

MAN. The effect of dietary changes on the fatty acid composition of normal human depot fat was studied using diet supplements or substitutions of ethyl arachidonate, a cod-liver oil fraction, and corn and cod-liver oils (Kingsbury et al., *Biochem. J., 84,* 124). Selectively hydrogenated oils exchanged for the corresponding natural oils in diets of physically healthy hospitalized men produced relatively small mean increases of serum cholesterol in several experiments, and phospholipid and triglyceride mean increases as well in one experiment (Anderson et al., *J. Nutrition, 75,* 388). A palatable diet high in unsaturated fat (40% of calories) was taken by institutionalized men for as long as two years. Serum cholesterol depressions were maintained for as long as 20 months (Hiscock et al., *J. Am. Dietet. Assoc., 40,* 427). Effects of dietary butter, "100% corn oil margarine," and "a margarine containing liquid corn oil" on blood lipids of young men were studied. Margarine eating groups had lower cholesterol levels than the butter eating group (Morse et al., *J. Am. Dietet. Assoc., 41,* 323). Findings of another study suggest that the level of linoleic acid in the diet, age, and possibly other factors may influence the serum cholesterol level and fatty acid composition of the serum lipid fractions (Swell et al., *Proc. Soc. Exp. Biol. and Med., 109,* 682).

ANIMALS. A few studies dealt with nutritive properties of several different fats. A comparison of lard, tallow, butter, and hydrogenated cottonseed oil in starters and of pelleted vs. nonpelleted coastal Bermudagrass hay was made in calves. The control starter was decidedly more palatable than those containing added fat (Miller, *J. Dairy Sci., 45,* 759). The nutritive properties of several fats indigenous to the Pakistani dietary were studied in weanling rats. Nitrogen retention was compared under the extreme dietary conditions provided by feeding a fat-free diet and one rich in sunflowerseed oil. It was found that, as a source of energy in the diet, fat may be completely replaced with carbohydrate without adversely affecting the utilization of protein. The observations were discussed in relation to the caloric deficiency of the Pakistani diet (Naismith and Quresh, *J. Nutrition, 77,* 373). In a similar study dietary cholesterol, per se, had no influence on protein metabolism. Growth was markedly reduced with an EFA-free diet, and characteristic signs of EFA deficiency developed within five weeks (Naismith, *Ibid., 77,* 381). The influence of varying levels of dietary protein, carbohydrate, and fats in the nutrition of the rat was reported (Siedler et al., *J. Nutrition, 77,* 149).

Relatively few nutritional studies of the essential fatty acids (EFA) were reported. A bioassay of EFA in rats is described in which amplitude of the response (weight gain) was magnified by equalizing water intake for all animals. From bioassay of a large number of oils and fats, a straight line relationship between biological activity and diunsaturated acids content was found to exist (Thomasson, *Rivista Ital. delle Sostanze Grasse, 38,* 541). Determination of linoleate requirement of swine by a new method is reported. There are four distinct metabolic processes related to linoleate nutrition. Calculations for two of the metabolic processes were based on decrease in tissue diene vs. increase in tissue triene or on tetraene metabolism in tissues vs. total body weight gain (Caster et al., *J. Nutrition, 78,* 147). *cis*-2-Octenoate administered to EFA deficient rats was not incorporated *in vivo* into linoleate (Brenner et al., *J. Nutrition, 77,* 203). In puppies the rate of development of fat deficiency signs and symptoms was related directly to the rate of growth which in turn was dependent on the caloric intake of the low-fat diet (Wiese et al., *J. Nutrition, 76,* 73). In similar studies in puppies the distribution of fatty acids in erythrocytes and plasma were studied and findings on trienoic acid levels were reported (Patil and Hansen, *J. Nutrition, 78,* 167).

Numerous studies were made in the area of diet, blood and liver lipids, and lipid transport. Adult male rhesus monkeys were maintained for six months on purified diets containing saturated and unsaturated fats and their mixtures. Levels of different plasma lipid classes were measured and lowerings of plasma lipids were correlated with the amounts of the unsaturated fat component of the diet (Emerson et al., *J. Nutrition, 76, 6*). The effects of feeding cottonseed oil blended with hydrogenated peanut oil so as to provide different intakes of linoleic acid was studied in monkeys and in cholesterol fed rats. Supplements providing linoleic acid at the 7.5% level prevented the hypercholesterolemic and elevated liver cholesterol effects of the diets (Jagannathan, *J. Nutrition, 77,* 317). The same study was extended with feedings of butter fat, coconut oil, and hydrogenated peanut oil admixed with cottonseed oil (Jagannathan, *J. Nutrition, 77,* 323). Rhesus monkeys were fed cholesterol along with mustard-seed oil, or sesame-seed oil, or coconut oil. Fecal cholesterol and plasma lipid and lipoprotein classes were estimated at intervals up to six months. The depressent effect of the different oils on plasma cholesterol level did not seem to be due to saturation, unsaturation or fatty acid composition of the oil (Banerjee et al., *Proc. Soc. Exp. Biol. Med., 109,* 313).

Dog serum lipid responses to dietary fats differing in the chain length of the saturated fatty acids was studied. The glycerides of saturated fatty acids of 12- and 14-carbon atoms produced significantly higher serum cholesterol and phospholipid values than either the glycerides of 16- and 18-carbon saturated fatty acids or of 8- and 10-carbon saturated fatty acids (Grande, *J. Nutrition, 76,* 255).

Hypercholesterolemic rats were used to investigate the lipid depressant activities of lyophilized whole fish prepared from menhaden, silver salmon, mullet, and ocean perch. Ingestion of the whole fish supplements promoted a significant reduction in levels of plasma cholesterol and phospholipids, and in TC/TP ratios (Peifer et al., *JAOCS, 39,* 292). A ration, in which whole-ground, drained, canned sardines replaced casein and lard, prevented or caused regression in rats of hypercholesterolemia produced with cholesterolcholic acid diet supplements. Sudanophilia of cardiovascular tissues was mitigated under the experimental conditions by the sardine supplements (Miller et al., *J. Nutrition, 77,* 397). Lead and cadmium in drinking water were reported to lower serum cholesterol of the rat; chromium was without demonstrable effect (Schroeder et al., *Proc. Soc. Exp. Biol. Med., 109,* 859). The effects of dietary bile acids: lithocholic, hyodeoxycholic, and chenodeoxycholic acids on the plasma cholesterol of rats were studied and compared (Howe and Hutchison, *J. Nutrition, 77,* 237). The effects of dietary butter or vegetable fat, fed at different levels, on the plasma cholesterol level of rats were studied. The butter diets produced higher serum cholesterol levels and higher liver lipid levels (Roehm and Mayfield, *J. Am. Dietet. Assoc., 40,* 417). The effect of fat-free diets and several degrees of dietary lipid unsaturation on rat plasma and tissue cholesterol was reported (Diller et al., *Proc. Soc. Exp. Biol. Med., 108,* 637). An increased lipotropic requirement (choline) was shown in rats with renal necrosis induced by high-fat diets. A lipotropic effect of sodium cholate was observed (O'Neal et al., *J. Nutrition, 75,* 309).

The effects of roughage and dietary fat on the fatty acid

composition of milk were studied in Holstein cows. The amounts of shorter chain acids of milk were diminished by feeding long chain acid fats regardless of the roughage of the diet. Volatile fatty acids in the rumen were affected by the amount of roughage and dietary fat (Stull and Stott, *J. Dairy Sci., 45,* 191).

Changes in tissue lipids of rats in response to diet were reported. Fatty acid compositions of subcutaneous, mesenteric, and interscapular fat as well as of liver and plasma lipids were determined. Semisynthetic diets contained safflower or coconut oil. The data support the hypothesis that the rat can store dietary linoleic acid in its adipose tissue for slow release when the dietary supply is low. Characteristic sex differences were observed both in the concentration of the different liver and plasma lipids, and in their fatty acid patterns (Okey et al., *J. Nutrition, 76,* 341, 353). Effects of corn oil on the amounts of cholesterol and the excretion of sterol in the rat were reported. Some indication of changes of body cholesterol could be gained by measuring the rate of sterol excretion. The fatty acid composition of the tissue and serum lipids showed an inverse relationship between the concentrations of cholesterol in the tissues and that of arachidonic acid in the lipids (Gerson and Shorland, *Biochem. J., 81,* 584). Studies of the influence of dietary fat on the fatty acid composition of monkey erythrocytes emphasize that the fatty acid composition of structural lipids may be significantly influenced by the fatty acid content of the diet (Fitch et al., *J. Nutrition, 75,* 409). In rats the fatty acid compositions of erythrocyte and liver mitochondrial lipids were drastically and easily altered by varying the dietary fat. Brain mitochondrial lipids were similarly altered by dietary fat (Witting et al., *J. Lipid Research 2,* 412). Effects of the fat-deficiency syndrome, aggravated by dietary cholesterol, on the liver lipid constituents of male and female rats were studied. Liver-lipid measurements involved: vitamin A stores, cholesteryl esters, triglycerides, phospholipids, triene/tetraene ratio and diene/tetraene ratio (Morton and Horner, *Biochem. J., 79,* 636). Dietary asparagine was reported to prevent alcohol-induced rat liver triglyceride elevations (Lansford et al., *J. Nutrition, 78,* 219).

Tissue lipid fatty acid changes following the feeding of high-cholesterol, essential fatty acid-supplemented diets were studied in rabbits. Aortas were graded visually. There did not appear to be any difference with respect to the effect of the various diets on atherogenesis with the possible exception of the group receiving linoleic acid and pyridoxine (Swell et al., *J. Nutrition, 75,* 181). A subsequent report indicated the effect of dietary fat on serum and tissue cholesteryl ester and triglyceride fatty acid compositions. Aortal lipid compositions also showed dependency on dietary fat. The data also suggest that derangements of cholesteryl oleate metabolism may be an important factor in the deposition of cholesteryl esters in liver and aorta of rabbits fed cholesterol (Swell et al., *J. Nutrition, 76,* 429).

Effects of chronic food restriction were studied in swine. Serum lipid levels, vascular pathology, and the relation of fatness to other measured characteristics were reported (Calloway et al., *J. Nutrition, 76,* 365).

In sheep fed purified diets a high content of *trans* acids in the dietary fat resulted in high levels of *trans* acids in the sheep tallow irrespective of the remainder of the diet, indicating the resistance of the *trans* isomers to hydrogenation by the rumen flora (Tove and Matrone, *J. Nutrition, 76,* 271).

The fatty acid composition of the lipids of some Pacific sardine tissues in relation to ovarian maturation and diet were reported. Studies of planktonic eggs and a number of tissues are presented (Lasker and Theilacker, *J. Lipid Research, 3,* 60).

The effect of vitamin A intake on some biochemical and physiological changes which included cerebrospinal fluid pressure and plasma vitamin A concentrations in swine was reported (Nelson et al., *J. Nutrition, 76,* 325). Vitamin K deficiency was produced in baby pigs with a "synthetic" liquid diet. Animals died in 4–5 weeks (Schendel and Johnson, *J. Nutrition, 76,* 124).

The vitamin K requirement for puppies in the active growth stage was found to be 10 μg or greater /kg of body weight and less than 5 μg/kg body weight as the animals matured (Quick et al., *J. Nutrition, 77,* 28).

Vitamin E deficiency with steatitis was produced in cats by feeding purified diets (Gershoff and Norkin, *J. Nutrition, 77,* 303).

Evidence is presented to indicate that the level of dietary vitamin A is closely related to development of symptoms of vitamin K deficiency in the rat (Matschiner and Daisy, *Proc. Soc. Exp. Biol. Med., 109,* 139). The feeding of nitrite, but not nitrate, significantly lowered liver storage of vitamin A from orally administered sources of preformed vitamin A, but not from injected sources in rats (Emerick and Olson, *J. Nutrition, 78,* 73). The effect of vitamin $B_{12}$ deficiency on serum total cholesterol levels and incorporation of labeled acetate into liver cholesterol was studied in rats (Icayan and Chow, *J. Nutrition, 78,* 109). Liver necrosis in adult and young rats fed a protein-free diet deficient in vitamin E and the effects of certain supplements and of inanition are reported (Goettsch, *J. Nutrition, 76,* 30).

The relation of selenium, vitamin E, and other factors to muscular dystrophy was studied in the rabbit (Proctor et al., *Proc. Soc. Exp. Biol. Med., 108,* 77).

Studies with non-vitamin minor components or unusual lipids included one showing the mechanism of gossypol detoxification in ruminants to binding to soluble proteins with a bond that is permanent during the protein digestion (Reiser and Fu, *J. Nutrition, 76,* 215), and another in which dietary epoxyoleic acid was deposited as such in rat tissues and did not produce toxic symptoms under the experimental conditions (Chalvardjian et al., *J. Nutrition, 76,* 52).

BIRDS. A number of reports dealt with energy and nutritive values of diets and performance. Metabolizable energy, absorbability and effect on growth and feed conversion have been measured on a number of fat samples including different grades of tallows and greases, hydrolyzed animal and vegetable fat, and methyl esters of fatty acids when fed to chicks (Cullen et al., *Poultry Sci., 41,* 360). A number of fats, fatty materials and mixtures thereof were evaluated in terms of metabolizable energy, chick weight gains, and gain:feed ratios (Sibbald et al., *Poultry Sci., 41,* 46). Feed grade tallow and acidulated soapstock were compared in practical chick starter rations (Sibbald et al., *Poultry Sci., 41,* 120). The energy values of a number of fats and fatty acids for chicks were evaluated by a controlled feed intake method (Young and Artman, *Poultry Sci., 40,* 1653). An *in vitro* method for determining the availability of soybean oil in unextracted soybean products for the chick was studied. A high degree of correlation was found between the rate at which the oil in soybean products was extracted by ether and the biological availability to the chick (Carew et al., *Poultry Sci., 41,* 188). The comparative value of dietary rapeseed oil, sunflower seed oil, soybean oil, and animal tallow was studied in chickens. Fatty acid composition of adipose tissue reflected that of dietary fat (Sell and Hodgson, *J. Nutrition, 76,* 113). Protein-energy relationships in the diet of the chick were reported (O'Neil et al., *Poultry Sci., 41,* 739). The effect of protein source on the growth promoting action of soybean oil, and the effect of glycerine in a low fat diet were studied (Campbell and Hill, *Poultry Sci., 41,* 881). It was shown that coconut oil meal (copra meal) can be used at relatively high levels in poultry diets (Thomas and Scott, *Poultry Sci., 41,* 477). In studies of effects of dietary antioxidants and unsaturated fatty acids it was concluded that apparently the hen responds to the same dietary stresses that produce encephalomalacia in chicks by exhibiting a reduction of egg production, fertility, and hatchability (Machlin et al., *J. Nutrition, 76,* 284). The effect of dietary fat and cellulose on the apparent calcium digestibility in growing chickens was reported (Griffith et al., *Poultry Sci., 40,* 1492). Mortality from liver derangement in caged layers was approximately doubled when either 7.5 or 12.5% of fat was fed but no other untoward effects on mortality rate were noted (March and Biely, *Poultry Sci., 41,* 9).

Reports on blood and liver lipids and lipid transport follow. Amino acid imbalance and serum cholesterol levels were studied in chicks. Among the amino acids observed to be essential for optimal growth in chicks, 6 have been tested for their effect on the serum cholesterol levels in diets which were either deficient or more than adequate in one of the amino acids. Cholesterol level changes were observed (Kokatnur and Kummerow, *J. Nutrition, 75,* 319). Another group also reported hypocholesterolemic effects of certain amino acids and protein in chicks (Leveille et al., *J. Nutrition, 76,* 321). The influence of changes in dietary protein level, calorie protein ratio, and the type of fat on serum and egg cholesterol levels in the mature hen was reported (Edwards et al., *Poultry Sci., 41,* 713). The effects of lard, vegetable oil, or fish oil, with or without cholesterol supplementation, on plasma and liver cholesterol concentration in female chicks were studied (Miller et al., *J. Nutrition, 75,* 367). The same group reported the effect of high levels of different types of dietary fat on tissue fat and plasma cholesterol in eight-week-old broilers (Miller et al., *Poultry Sci., 41,* 970). Nine diets, approximately equal in energy and protein content, were fed to chicks to study the effect of feeding 8 per cent of animal fat and 8 per cent of soybean oil with three different levels of choline on weight gains, feed efficiency, liver lipids, and liver and serum cholesterol (Daghir and Balloun, *Poultry Sci., 40,* 1712). The effect of bile acids on egg production, serum cholesterol, and egg cholesterol in hens was reported (Edwards et al., *J. Nutrition, 77,* 253).

Triparanol seemed to have no effect on either serum

cholesterol or egg yolk cholesterol concentrations (Nichols et al., *Poultry Sci., 41,* 1494), while others reported that MER-29 caused accumulation of desmosterol in the egg followed by cessation of egg production. After withdrawal of the drug, egg production returned to normal only after a lag phase of 12 days (Burgess and Wilson, *Proc. Soc. Exp. Biol. Med., 109,* 218). Laying hens administered triparanol for 10 days exhibited no significant variation in serum calcium levels from those of normal laying hens. Triparanol did raise serum cholesterol levels about 90% by the 10th day of administration of the drug. Decreased yolk and egg size and rate of egg production were observed (Nelson et al., *Poultry Sci., 41,* 664).

Interactions among dietary fat, protein, and cholesterol in atherosclerosis-susceptible pigeons were reported. Limited evidence suggests that the coronary arteries and aortae in non-cholesterol-fed birds are independent of each other in their susceptibility to atherosclerosis (Clarkson et al., *Circulation Res., 11,* 400). Effects of levels of saturated and unsaturated dietary lipids and vitamins A and E on plasma cholesterol and atherosclerosis in the chick were reported (Beeler et al., *J. Nutrition, 78,* 184).

A number of studies in birds dealt with effects of diet and dietary fat on body fat as follows: Effect of dietary fatty acids and cholesterol on growth and fatty acid composition of the chicken (Machlin and Gordon, *J. Nutrition, 75,* 157), Influence of graded levels of dietary linoleic and linolenic acids on the fatty acid composition of hen's eggs (Murty and Reiser, *J. Nutrition, 75,* 287), Effect of dietary fat on the fatty acid composition of eggs and tissues of the hen (Machlin and Gordon, *J. Poultry Sci., 41,* 1340), Influence of diet and age upon liver lipid changes in the chick (Marion and Edwards, *J. Nutrition, 77,* 23), Depletion and synthesis of fatty acids in chickens fed a diet low in unsaturated fatty acids (Machlin, *Proc. Soc. Exp. Biol. and Med., 108,* 819), Studies on eggs from hens on diets differing in fat content (Jordan et al., *Food Technol., 16,* 118). Effects of different levels of vitamin A, carotenal, and alfalfa on yolk color (Sunde, *Poultry Sci., 41,* 532).

Studies of the effects of dietary sterculic acid or derivatives and cottonseed oil on chickens and on coloration of eggs were: Sterculic derivatives and pink egg formation (Nordby et al., *JAOCS, 39,* 183), Physical changes in eggs produced by hens receiving *Sterculia foetida* oil supplements (Dobernz et al., *Poultry Sci., 41,* 700), Effect of cottonseed oil on discoloration of cold storage eggs (Kemmerer et al., *Poultry Sci., 41,* 1101), Fatty acid distribution in tissues from hens fed cottonseed oil or *Sterculia foetida* seeds (Evans et al., *J. Nutrition, 76,* 314), Effect of *Sterculia foetida* oil on mortality of the chick embryo (Schneider et al., *Poultry Sci., 40,* 1644), Delay of sexual maturity in chickens by *Sterculia foetida* oil (Schneider, *J. Nutrition, 77,* 403).

Vitamin A utilization by chicks, as judged by liver storage and survival, is best when the vitamin is given as a gelatin-coated preparation (Ascarelli and Senger, *J. Sci. Food Agr., 13,* 332). The cholesterol content of the chicken egg during incubation or of the developing chick embryo did not differ from that of its vitamin $B_{12}$-deficient counter-part (Daniel et al., *Proc. Soc. Exp. Biol. Med., 108,* 119). Studies in chicks indicate that the variation among antioxidants in their ability to substitute for vitamin E is due primarily to differences in their availability to the body and subsequent deposition in the tissues (Krishnamurthy and Bieri, *J. Nutrition, 77,* 245). The results of both a long chick liver-storage bioassay and a short bioassay indicated that the different forms of *alpha*-tocopherol were utilized to the same relative degree under the two sets of conditions (Dicks and Matterson, *J. Nutrition, 75,* 165). A specific effect of cystine in the prevention of nutritional muscular deficiency in vitamin E-deficient chicks is reported (Scott and Calvert, *J. Nutrition, 77,* 105). Results are presented that both vitamin E and selenium are concerned in the prevention of nutritional muscular dystrophy in the chick (Calvert et al., *Proc. Soc. Exp. Biol. Med., 109,* 16).

Encephalomalacia and hydropericardium in birds received some research study. Feeding of cottonseed oil in which vitamin E was destroyed by a pro-oxidant and heat caused encephalomalacia in birds. An ethoxyquin supplement prevented this encephalomalacia (Machlin, *Poultry Sci., 40,* 1631). Effects of ubinquinones and phytyl-ubichromenol upon encephalomalacia and muscular dystrophy were studied in chicks (Sondergaard et al., *J. Nutrition, 78,* 15). The etiology of exudative diathesis, encephalomalacia, and muscular degeneration in the chicken is reviewed (Machlin and Gordon, *Poultry Sci., 41,* 473). The effects of diet and encephalomalacia on the fatty acid composition of chicken brain is reported (Machlin et al., *JAOCS, 39,* 229). Hydropericardium and ascites in chicks fed a chlorinated hydrocarbon are described (McCune et al., *Poultry Sci., 41,* 295). A hydropericardium assay and the safety of a number of fats and fatty acid products were studied (Alexander, *Poultry Sci., 41,* 22). The response of chickens to prolonged feeding of crude "toxic fat" was studied (Allen and Lalich, *Proc. Soc. Exp. Biol. Med., 109,* 48).

### Diets, Diet Components, and Supplements

A few reports considered the effects of processing and particularly heat on the nutritive value of fats. The nutritive value of methyl linoleate and its thermal decomposition products were studied in rats (Bottino, *JAOCS, 39,* 25). Studies in rats of the influence of temperature, heating time, and aeration upon the nutritive value of fats indicated that treatments more severe than those usually encountered in processing or cooking are necessary to produce detectable damage (Poling et al., *Ibid., 39,* 315). The influence of short-term heating on the development of increased titratable free fatty acids in edible fats was studied (Kritchevsky et al., *J. Nutrition, 77,* 127). Fats were extracted from a number of samples of meat cuts cooked well-done. The biologically available energy of these fats to rats was not changed during cooking (Warner et al., *J. Am. Dietet. Assoc., 40,* 422).

It was shown that volatile carbonyls develop in haddock flesh during storage at 2C (Mendelsohn and Steinberg, *Food Technol., 16,* 113). Nutritive values for the chick and chemical changes in the lipid fraction of herring meals with and without antioxidant treatment were studied (March et al., *Poultry Sci., 41,* 873). Results obtained on lipids extracted from flour provide direct evidence that peroxides are formed in dough during mixing in air or oxygen (Tsen and Hlynka, *Cereal Chem., 39* (3), 209).

The effect of free fatty acid content on the flavor of fat is described (Hall et al., *J. Agr. Food Chem., 10,* 96).

Miscellaneous studies in the area of foods and food components are presented next. Evidence was obtained for the presence of a thermolabile growth inhibitor in raw wheat germ which seems specifically to block utilization of fat (Creek et al., *Poultry Sci., 41,* 901). Data are presented to show that loss of thiamine alleged to be due to "thiamine destroying factor" in soybeans is based on an unreliable thiochrome assay procedure (Weakley et al., *J. Agr. Feed Chem., 9,* 435). The amino acid composition of *Lesquerella* seed meals is reported (Miller et al., *JAOCS, 39,* 115). Glyceryl lactate-$C^{14}$ palmitate was studied in rat experiments to prove the safety of the non-labeled analogue in baking (Treon et al., *J. Agr. Food Chem., 10,* 111). Effects of extraction of lipids on the nutritional value of fish flour were reported (Morrison et al., *J. Nutrition, 77,* 97).

Patented preparations of interest to the nutritionist were: Synthetic cocoa butter substitute, Dutton and Scholfield (Sec'y of Agriculture, U.S.A.) *U.S. 3,012,890.* Cocoa butter substitute, Best et al., (Lever Bros. Co.) *U.S. 3,012,891.* All-purpose culinary oils, Baur (Procter and Gamble Co.) *U.S. 3,047,401.* Fluid shortening, Schmidt (Lever Bros. Co.) *U.S. 3,047,402.* Yogurt containing an unsaturated vegetable fat, Metzger (Beatrice Foods Co.) *U.S. 3,025,165.* Method of enhancing the xanthophyll content of poultry feeds, Kruse (Central Soya Co.) *U.S. 3,020,159.* Method for preparing a granular oil-free phosphatide product, Davis and Fello (Central Soya Co.) *U.S. 3,012,888.* Fatty food composition, Berndt and Krett (National Dairy Products Co.) *U.S. 3,010,830.* Manufacture of a hard, dry fat containing feed pellet, Guidarelli (Cargill, Inc.) *U.S. 3,014,800.* Soap in animal feed, Patterson et al., (Swift and Co.) *U.S. 3,010,828.* Plasticizer for fat, Farbak et al., (Swift and Co.) *U.S. 3,021,221.* Preparation of stable, aqueous, isomeric vitamin A compositions, Ames (Eastman Kodak Co.) *U.S. 3,026,249.* Vitamin A ester compositions, Stieg and Nielson (Chas. Pfizer and Co.) *U.S. 3,047,598.* Stabilized carotene compositions, Borenstein (Nopco Chemical Co.) *U.S. 3,039,877.* Process for the production of the reduction products of the vitamin $D_2$, vitamin $D_3$, and the corresponding irradiated provitamins, Schenck (Farbenfabriken Bayer Aktiengesellschaft) *U.S. 3,049,553.* Process for manufacturing powdered preparations containing fat soluble vitamins, essential oils, and mixtures thereof, Ohtaki *U.S. 3,056,728.* Water-soluble compositions of lipid-soluble vitamins, Sevigne (Collett—Week Corp.) *U.S. 3,035,981.* Chewable, palatable vitamin B preparations, Stoyle et al., (Merck and Co.) *U.S. 3,037,911.*

## PHYSIOLOGY

### Digestion, Intestinal Absorption, and Excretion

The effects of different dietary fats on the body and the important properties of a food fat from the health point of view are reviewed. Digestion, absorption, transport,

body fats, biosynthesis and interconversions, utilization, and excretion of fat are discussed (Frazer, *Chem. & Ind.*, (London), *1962*, 1438). Rearrangement of glyceride fatty acids during digestion and absorption was studied in rats using $C^{14}$-labeled substrates (Mattson and Volpenheim, *J. Biol. Chem., 237*, 53). A series of experiments indicated that dietary raw soybean meal depressed fat absorption in chicks of two breeds at two weeks of age but not at four weeks of age (Nesheim et al., *J. Nutrition, 78*, 89). Studies on the absorption of *beta*-carotene and the distribution of total carotenoid in human serum lipoproteins after oral administration are reported (Cornwell et al., *J. Lipid Research, 3*, 65). Studies of absorption of vitamin A alcohol in rats indicated that the palmitate ester was an important transport and storage form of the vitamin (Mahadevan and Ganguly, *Biochem. J., 81*, 53). Coprostanol excretion by rats was found to be accelerated by dietary linoleic acid and depressed by oleic and palmitic acids (Wilson, *J. Lipid Research, 2*, 350). Evidences for fat secretion in the intestine of the fish are presented (Bottino and Brenner, *JAOCS, 39*, 519).

### Lipid Transport and Body Fats

Without change in diet or exercise it has been shown in humans that striking alterations in the serum concentration of cholesterol and triglycerides correlate with the occurrence of emotionally stressful situations (Wolf et al., *Circulation 26*, 379). The lipids in maternal and cord blood and of human amniotic fluid have been compared (Helmy and Hack, *Proc. Soc. Exp. Biol. Med., 110*, 91). The role of liver and of extrahepatic tissues in the transport and metabolism of fatty acids and triglycerides was studied in the dog (Havel and Goldfien, *J. Lipid Research, 2*, 389). A technique is described to determine changes in plasma free fatty acids (FFA) on passage through adipose tissue *in vivo*. Mobilization of FFA was studied in dogs (Spitzer and Hohenleiter, *J. Lipid Research, 2*, 396). The effect of 2,4-dinitrophenol on free fatty acid uptake by skeletal muscle was studied in anesthetized dogs (Spitzer et al., *Proc. Soc. Exp. Biol. Med., 108*, 89). Reserpine treatment diminished but did not abolish the mobilization of FFA which occurs during fasting in rats. The effect of reserpine on mobilization of FFA by hormonal substances was also studied (Edmonson and Goodman, *Proc. Soc. Exp. Biol. Med., 110*, 761). Findings on formation and fate of endogenous triglycerides in blood plasma of rabbits are discussed in relation to the roles of lipoprotein lipase activity and esterifying capacity of tissue in the fate of circulating FFA and triglyceride fatty acids (Havel et al., *J. Lipid Research, 3*, 297). An emulsifier system for experimental intravenous fat emulsions appeared to give satisfactory physiologic results when infused in dogs (Singleton et al., *JAOCS, 39*, 260). Thyroid-active substances were effective agents in lowering plasma cholesterol of weanling mice receiving hydrogenated coconut oil as the main source of fat (Howe and Bosshardt, *J. Nutrition, 77*, 161). Thyroactive compounds lowered serum and liver cholesterol levels in rats (Kritchevsky et al., *Proc. Soc. Exp. Biol. Med., 108*, 254). Studies of the effect of fasting on serum and liver lipid levels in the rat are reported (Mayfield and Roehm, *J. Nutrition, 75*, 265). Strain and sex differences in serum cholesterol levels of mice were reported (Bruell et al., *Science, 3*, 1071). Levels of lipoprotein in rooster and hen plasma were studied. In hens, lipoprotein levels were correlated with ovarian activity (Homma and Kato, *Poultry Sci., 41*, 608). The lipid content of the subcutaneous fat organs of the chick embryo was studied. Results were discussed in relation to lipid transport by serum lipoproteins (Feldman et al., *Poultry Sci., 41*, 1232).

### Lipid Metabolism in the Intact Animal

Additive plasma cholesterol-lowering effects were noted in cockerels and dogs when treated with a bile acid binding polymer and cholesterol synthesis inhibitors (Tennent et al., *Proc. Soc. Exp. Biol. Med., 108*, 214). 24-Dehydrocholesterol was reported to accumulate in human blood vessel walls following MER-29 therapy (Blankenhorn, *Proc. Soc. Exp. Biol. Med., 108*, 43). A lipotropic effect and an increased liver lecithin concentration in oophorectomized rats receiving a vitamin $B_{12}$ and estradiol supplemented diet were reported (Bowser et al., *J. Lipid Research, 2*, 278).

The influence of sex and hair growth cycle on lipid composition of mouse epidermis was studied (Carruthers, *Proc. Soc. Exp. Biol. Med., 109*, 390). A number of surfactants, when introduced into the eye mucosa, are capable of producing an anaesthesia or an increase in the threshold of pain (*Drug Cosmetic Ind., 91* (1), 30).

Several studies utilized drugs or pharmacologic agents. The hypocholesterolemic effect of methyl testosterone was reported to occur in rats. Results were interpreted as indicating that the effect was not produced by redistribution of cholesterol between the serum and the tissues examined (Abell and Mosbach, *J. Lipid Research, 3*, 88). Findings in rats were interpreted as indicating that sodium polyethylene sulfonate and heparin increase the rate of oxidation of triglyceride fatty acids indirectly by increasing the rate of hydrolysis to fatty acids, which in turn are rapidly oxidized (Miller and Krake, *Proc. Soc. Exp. Biol. Med., 110*, 309). The effect of diphenylhydantoin administration upon levels of liver, aortic, and dermal lipids was studied in rats (Chung et al., *Proc. Soc. Exp. Biol. Med., 109*, 454, *Ibid., 110*, 788).

Studies are reported in which reticuloendothelial stimulation significantly inhibited dietary-induced hepatic cholesterosis in rats fed cholesterol-cholate diet (Riggi and DiLuzio, *J. Lipid Research, 3*, 339).

Hepatic vitamin A levels which were much higher in cold-acclimated rats than in rats maintained at 25C are interpreted as being a corollary to increased food consumption that accompanies cold-exposure (Porter and Masoro, *Proc. Soc. Exp. Biol. Med., 108*, 609). Samples of subcutaneous body fat were analyzed for fatty acids and iodine values in hens maintained at 3 environmental temperatures. It was found that fat from hens maintained at 0C was significantly more unsaturated and contained more dienoic and hexaenoic acid than fat from birds kept at either 21C or 32C (Fisher et al., *Proc. Soc. Exp. Biol. Med., 110*, 832).

The fate of the antioxidant, butylated hydroxyanisole, was studied in man and dogs. Although excretion was not the same in man and dogs the compound was excreted primarily unchanged in the feces or as sulfate and glycuronide conjugates in the urine (Astill et al., *J. Agr. Food Chem., 10*, 315).

Described is a neomycin fatty acid salt, Dale (Upjohn Co.) *U.S. 3,013,007* and fatty acid salts of physiologically acceptable organic bases, Ginger and Kartinos, (Baxter Labs., Inc.) *U.S. 3,055,923*. A non-oral antibiotic composition which exhibits a protracted therapeutic effect is described, Jacobsen (Novo Terapeutisk Laboratorium A/S, Copenhagen) *U.S. 3,016,330*. An ophthalmic composition, Anderson, *U.S. 3,035,971* and a chewable fatty coating of iron particles Stoyle et al., (Merck and Co., Inc.), *U.S. 3,035,985* are described.

## BIOCHEMISTRY

### Analytical and Methodology

This section contains a bibliography of methods of interest to the biochemist and experimental biologist. Reports dealing with fatty acids or their derivatives will be listed first. "Separation of unsaturated fatty acids with the aid of mercury adducts" (Jantzen et al., *Fette, Seifen, Anstrichmittel, 63*, 685). "A mercury derivative-chromatographic method for the separation of unsaturated fatty acid esters" (Kuemmel, *Anal. Chem., 34*, 1003). "Separation of fatty ester-mercuric acetate adducts on alumina" (White and Quackenbush, *JAOCS, 39*, 511). "Isolation of pure linolenate as its mercuric acetate adduct" (White and Quackenbush, *JAOCS, 39*, 517). "Separation of geometric isomers and isologues of fatty acid esters by counter-current distribution" (Dutton et al., *Chem. and Ind. 1961*, 1874). "Determination of the hydroxyl value of free fatty acids" (Kaufmann and Schmülling, *Fette, Seifen, Anstrichmittel, 64*, 319). "Fluorometric microdetermination of *alpha*-keto acids" (Spikner and Towne, *Anal. Chem., 34*, 1468). "The colorimetric determination of long-chain fatty acids in the 0.05–0.5 micromole range" (Duncombe, *Biochem. J., 83*, 6P). "The determination of esterified fatty acids in glycerides, cholesterol esters, and phosphatides" (Skidmore and Entenman, *J. Lipid Research, 3*, 356). "Concentration of polyunsaturates in fats: study of different methods" (Guillaumin and Drouhin, *Rev. Franc. Corps., 9*, 415). "Adsorption chromatography of sucrose palmitates" (Mima and Kitamori, *JAOCS, 39*, 546). "A rapid quantitative method for the separation of free fatty acids from other lipids" (McCarthy and Duthie, *J. Lipid Research, 3*, 117). "Purification of $C^{14}$-labeled fatty acids by chromatography on acid-treated Fluorosil" (Carroll, *J. Lipid Research, 3*, 388).

"Gas-liquid chromatography: The introduction of samples, the preconditioning of polyester liquid phases, and the measurement of Rf values in the analysis of fatty esters" (Gerson, *J. Chromatog., 6*, 178). "Capillary programmed temperature gas chromatography; some theoretical aspects" (Habgood and Harris, *Anal. Chem., 34*, 882). "Theory of minimum time operation in gas chromatography" (Giddings, *Anal. Chem., 34*, 903). "Simultaneous dual column gas concept of gas chromatography" (Giddings and Robison, *Anal. Chem., 34*, 885). "Plate height theory of programmed temperature gas chromatography" (Giddings, *Anal. Chem., 34*, 722). "A study of the performance of packed gas

chromatography columns'' (Nogare and Chiu, *Anal. Chem., 34,* 890). ''Qualitative gas chromatographic analysis by means of retention volume constants'' (Merritt and Walsh, *Anal. Chem., 34,* 903). ''Simmultaneous dual column gas chromatography'' (*Ibid.,* 908). ''Graphic aid for interpreting gas chromatograms'' (Evans et al., *J. Lipid Research, 39,* 210). ''Effect of preheater contamination on gas chromatographic analysis of strongly absorbed substances'' (Smith and Gosnell, *Anal. Chem., 34,* 646). ''Potential contamination in the analysis of methyl esters of fatty acids by gas-liquid chromatography'' (Lindgren et al., *J. Lipid Research, 3,* 390). ''Collection of gas-liquid chromatographic effluents'' (Hajra and Radin, *J. Lipid Research, 3,* 131). ''Analysis of the geometric isomers of methyl linoleate by gas chromatography'' (Litchfield et al., *JAOCS, 39,* 330). ''Radioassay by gas-liquid chromatography of lipids labeled with carbon-14'' (Karmen et al., *J. Lipid Research, 3,* 44). ''Radioassay of low activity fractions encountered in gas-liquid chromatography of long chain fatty acids'' (Meinertz and Dole, *J. Lipid Research, 3,* 140). ''Proportional counter assay of tritium in gas chromatographic streams'' (Lee et al., *Anal. Chem., 34,* 741). ''Electron capture spectrometry, an adjunct to gas chromatography. Quantitative study of operating parameters and the qualitative and quantitative distinction between compounds containing the same heteroatom'' (Landowne and Lipsky, *Anal. Chem., 34,* 726). ''Catalytic hydrogenation of fatty acid methyl esters for gas-liquid chromatography'' (Poukka et al., *J. Lipid Research, 3,* 128). ''Direct measurement of water and short chain fatty acids by gas chromatography'' (Prevat and Cabeza, *Rev. Franc. Corps Gras, 8,* 632). ''Analyses of milk fatty acids by gas-liquid chromatography'' (Gander et al., *J. Dairy Sci., 45,* 323). ''Gas chromatographic analysis of fatty acids from dialyzed lipoproteins'' (Nichols et al., *J. Lipid Research, 2,* 203). ''Volatile flavor of sauerkraut. Gas chromatographic identification of a volatile acidic off-odor'' (Vorbeck et al., *J. Food Sci., 26,* 569).

''Separation of higher fatty acid isomers and vinylogues by thin-layer chromatography'' (Morris, *Chem. and Ind.* (London) *1962,* 1238). ''Quantitative estimation of esters by thin-layer chromatography'' (Vioque and Holman, *JAOCS, 39,* 63). ''Thin layer chromatography of fats. III. Visualization of the analyzing substances on the plate'' (Kaufmann et al., *Fette, Seifen, Anstrichmittel, 63,* 689). ''VII. Separation of fatty acids and triglycerides on gypsum plates'' (Kaufmann and Khoe, *Ibid., 64,* 81). ''VI. Hydrogenation and bromination on the plate'' (Kaufmann et al., *Ibid., 64,* 1).

''Paper chromatographic analysis of fatty acid esters of monohydroxy and polyhydric alcohols and some phenols. Test of a generalization of the PC number'' (Kaufmann and Grothues, *Ibid., 63,* 1021). ''Paper chromatography of fats L: qualitative and quantitative determination of hydroxy and keto fatty acids'' (Kaufmann and Young Su Ko, *Ibid., 64,* 434). ''Identification, estimation, and preparation of fatty acids by circular paper chromatography'' (Viswanathan and Bai, *J. Chromatog., 6,* 264). ''Separation of 'critical pairs' of fatty acids by paper chromatography'' (Aparicio, *Grasas y Aceites 12,* 109). ''Volatility effects in the paper chromatography of the lower fatty acids'' (Molloy and Kowkabany, *Anal. Chem., 34,* 491).

''Application of infrared spectroscopy to the analysis of primary fatty amide mixtures'' (Link and Buswell, *JAOCS, 39,* 39). ''Infrared spectra and gas chromatography of some oxygenated fatty acid derivatives'' (Kitagawa et al., *JAOCS, 39,* 217).

Reports dealing with glycerides and fats are presented next. ''Appraisal of methods of total monoester estimation in commercial monoglycerides'' (Hartman, *JAOCS, 39,* 126). ''Determination of hydroxyls in mono- and diglycerides'' (Jensen and Sampugna, *Ibid., 39,* 309). ''The simultaneous determination of glycerol and fatty acids in glycerides by gas-liquid chromatography'' (Horrocks and Cornwell, *J. Lipid Research, 3,* 165). ''Some alleged errors in the azelaoglyceride technique'' (Kartha, *JAOCS, 39,* 478). ''Stoichiometric transesterification of glycerides by methyl alcohol'' (de Francesca and Maglitto, *Rivista Ital. Sost. Grasse, 5,* 245). ''Chromatographic analysis of seed oils. Fatty acid composition of castor oil'' (Binder et al., *JAOCS, 39,* 513). ''Analysis of pecan, peanut, and other oils by gas-liquid chromatography and ultra-violet spectrophotometry'' (French, *Ibid., 39,* 176). ''Charring conditions for the quantitative analysis of mono-, di-, and triglycerides by thin-layer chromatography'' (Privett and Blank, *Ibid., 39,* 520). ''Thin-layer chromatography of fats. VIII. Triglycerides and their critical partners'' (Kaufmann and Das, *Fette, Seifen, Anstrichmittel, 64,* 214). ''The use of iodine vapor as a general detecting agent in the thin-layer chromatography of lipids'' (Sims and Larose, *Ibid., 39,* 232). ''Concentration gradient development in thin-layer chromatography'' (Rybicka, *Chem. and Ind.* (London) *1962,* 308). Application of thin-layer chromatography to the analysis of oils and fats'' (Peereboom, *Chem. Weekblad, 57,* 625). ''Application of differential thermal analysis to the study of fats and oils. I. Binary mixtures of 1-mono-, 1–3 di- and tristearin'' (Perron et al., *Rev. Franc. Corps Gras, 8,* 559). ''Applications of differential thermal analysis to the study of fats and oils II. Tertiary mixtures of mono-1, di-1-3, and tristearins'' (Perron et al., *Rev. Franc. Corps Gras, 9,* 341). ''Direct determination of *trans* unsaturation in triglycerides by infrared spectrophotometry'' (Szonyi et al., *JAOCS, 39,* 276). ''The analysis of mixtures of animal and vegetable fats. II. The paper chromatography of some sterols, provitamins, vitamins, and pentacyclic triterpenoid alcohols'' (Peereboom et al., *J. Chromatog., 5,* 500). ''The determination of mutton tallow in mutton/beef tallow mixtures'' (Szonyi et al., *JAOCS, 39,* 185). ''Statistical evaluation of the Boemer Number of lard'' (Roos, *Fette, Seifen, Anstrichmittel, 64,* 6). ''Investigations on the Boemer Value and a comparison of the ether and acetone methods'' (Tollenaar et al., *Ibid., 64,* 303). ''Correct determination of the fat content of olives'' (Gracian et al., *Grasas y Aceites, 12,* 73). ''The Banco Test: A rapid method for fat in meat and edible meat products'' (Anderson et al., *J. Assoc. Offic. Agr. Chem., 45,* 13).

Reports on phospholipid methods are presented next. ''Chromatographic separation, identification, and analysis of phosphatides'' (Marinetti, *J. Lipid Research, 3,* 1). ''The chromatography of phosphatides on silicic acid impregnated filter paper'' (Hack, *J. Chromatog., 5,* 531). ''Isolation of polar lipids from triglyceride mixtures'' (Galanos and Kapoulas, *J. Lipid Research, 3,* 134). ''Quantitative estimation of lecithin and colamcephalin in pharmaceutical preparations using thin-layer chromatography'' (Wagner, *Fette Seifen Anstrichmittel, 63,* 1119). ''Rapid thin-layer chromatographic separation of phospholipids and neutral lipids of serum (Vogel et al., *J. Lipid Research, 3,* 138). ''Breakdown of lecithin on aluminum oxide columns'' (Renkonen, *Ibid., 3,* 181). ''Identification and gas-liquid chromatographic behavior of plasmologen aldehydes and their acetal, alcohol, and acetylated alcohol derivatives'' (Farquhar, *Ibid., 3,* 21). ''A sensitive and specific method for plasmologens and other enol ethers'' (Williams et al., *Ibid., 3,* 378). A spectrophotometric determination of sphingosine'' (Lauter and Trams, *Ibid., 3,* 136). ''Purification of phosphatides'' Pardum (Lever Bros. Co.), *U.S. 3,047,597.* ''Process for the production of natural phospholipids and substances produced thereby'' Klenk et al., *U.S. 3,031,478.*

Reports on steroids follow. ''Separation of sterols by counter-current crystallization'' (Poulos et al., *Ind. Eng. Chem., 53,* 949). ''Liquid-liquid partition chromatography of steroids. Systematic approach relating column to paper chromatography using the $R_M$ function'' (Kabosakalian and Talmage, *Anal. Chem., 34,* 273). ''Effects of substituents on relative retention times in gas chromatography of steroids'' (Knights and Thomas, *Anal. Chem., 34,* 1046). ''Gas chromatography of unsaponifiable matter. I. Butter and margarine sterols'' (Eisner et al., *J. Assoc. Offic. Agr. Chem. 45,* 337). ''A study of the separation of substituted cholanic acids by gas-liquid chromatography'' (Sjovall et al., *J. Lipid Research, 2,* 317). ''A new paper chromatographic system for the resolution of 17-ketosteroids'' (Katy and Broich, *J. Chromatog., 6,* 514). ''Determination of cholesterol as the tomatidine using the iron reagent'' (Rinehart et al., *J. Lipid Research, 3,* 383).

Reports on vitamins and minor constituents are presented next. ''The colorimetric reaction between vitamin $A_2$ aldehyde and antimony trichloride'' (Plack, *Biochem. J., 81,* 556). ''Differentiation of vitamins $D_2$ and $D_3$ by infrared spectrophotometry'' (Morris et al., *Anal. Chem., 34,* ''Tocopherol determination: characterization of tocopherols in vegetable oils by infrared spectrophotometry'' (Morris and Haenni, *J. Assoc. Offic. Agr. Chem., 45,* 92). ''Chromatography of fat-soluble vitamins on thin layers of alumina'' (Davidek and Blattna, *J. Chromatog., 1,* 204). ''Antioxidants in oils, fats, and waxes'' (Heidrick and Conroy, *J. Assoc. Offic. Agr. Chem., 45,* 244). ''Complexometric estimation of antioxidants'' (Sedlacek, *Fette, Seifen, Anstrichmittel, 63,* 1053 and *Ibid., 62,* 669). ''Trace analysis of BHA and BHT in food products'' (Sloman et al., *J. Assoc. Agr. Offic. Chem., 45,* 76).

Reports of a general or miscellaneous nature follow. ''Report of the Fat Analysis Committee, 1962'' (Stillman, *JAOCS, 39,* 431). ''New methods of analyzing industrial aliphatic lipids'' (Mangold and Kammereck, *Ibid., 39,* 201). ''A simple procedure for the estimation of total lipids in liquid egg white'' (Cotterill, *Poultry Sci., 40,* 1514). ''A method

for separating lipid components of leaves'' (Booth, *Biochem. J., 84,* 444). ''A rapid method for the extraction of lipids from wheat'' (Tsen et al., *Cereal Chem., 39,* 195). ''Extraction of lipids from blood serum and liver tissue'' (de Iongh and van Pelt, *J. Lipid Research, 3,* 385). ''Analysis of lipids from fresh and preserved adult human brains'' (Davison and Wajda, *Biochem. J., 82,* 113). ''Total solids and ether extract in fish and other marine products'' (Risley, *J. Assoc. Offic. Agr. Chem. 45,* 259). ''A simple chromatographic technique for removal of non-lipid contaminants from lipid extracts'' (Biezenski, *J. Lipid Research, 3,* 120). ''Silica gel structure and chromatographic process. Surface energy and activation procedures'' (Klein, *Anal. Chem., 34,* 733). ''The ultramicro detection of lipids'' (Lands and Dean, *J. Lipid Research, 3,* 129). ''Determination of unsaturation and average molecular weight of natural fats by nuclear magnetic resonance'' (Johnson and Shoolery, *Anal. Chem. 34,* 1136). ''Method for the determination of dimeric acids in oils and fats'' (Rost, *Fette, Seifen, Anstrichmittel, 64,* 427). ''Identification of higher alcohols by paper chromatography'' (Capella et al., *Rivista Ital. Sost. Grasse, 1,* 2). ''Solidification point of binary mixtures of fatty alcohols'' (Paquot et al., *Oleagineux, 6,* 555). ''Dextran sulfate precipitation and ultracentrifugation of lipoproteins from hypercholesterolemic dog serum'' (Sakagami and Zilversmit, *J. Lipid Research, 3,* 111). ''Correlation of smoke point to free fatty acid content in measuring fat deterioration from consecutive heatings'' (Zabik, *Food Technol., 16,* 111). ''The adsorption and chromatography of alkyl benzene sulfonates on charcoal'' (Mysels et al., *JAOCS, 39,* 66). ''The film tensionmeter, an instrument for continuous measurement of surface tension of liquids and solutions'' (Peterson, *Kolloid-Z., 183,* 141). ''Determination of total and water-insoluble-combined lactic acid in lactic acid modified fatty glycerides'' (Stetzler and Andress, *JAOCS, 39,* 509). ''The chemistry of the 2-thiobarbituric acid test for the determination of oxidative rancidity in foods. I. Some important side reactions (Tarladgis et al., *Ibid., 39,* 34). ''Removal of interfering pigments in determining malonaldehyde by the 2-thiobarbituric acid reaction'' (Yu and Sinnhuber, *Food Technol., 16,* 115). ''A modified 2-thiobarbituric acid (TBA) method for the determination of malonaldehyde in cured meats'' (Zipser and Watts, *Food Technol., 16,* 102). ''Rapid colorimetric determination of DDT in milk and butter'' (Gunther, *J. Assoc. Offic. Agr. Chem., 45,* 359). ''Insecticide residues in butterfat, residues of heptachlor epoxide in butterfat of dairy cows grazing pastures treated with granular heptachlor'' (Rusoff et al., *J. Agr. Food Chem., 10,* 377). ''Insecticide residues, analytical method for determining 1, 1 dichloro-2, 2-bis (P-ethylphenyl) ethane in rat fat and cow's milk'' (Gordon et al., *J. Agr. Food Chem., 10,* 380). ''One-step chromatographic cleanup of chlorinated hydrocarbon pesticide residues in butterfat. I. Chromatography on silicic acid'' (Moats, *J. Assoc. Offic. Agr. Chem., 45,* 355). ''Analysis by a galvantic osmophile of the volatile components of fatty materials'' (Berton, *Rev. Franc. Corps Gras, 9,* 187). ''Rapid detection of traces of peroxide in ethers'' (Dugan, *Anal. Chem., 33,* 1630).

**Lipid Biosynthesis and Bio-oxidation**

The biosynthesis of linoleic acid from 1-$C^{14}$-cis-2-octenoic acid by the laying hen has been demonstrated (Reiser et al., *J. Lipid Research, 3,* 56). The site of stimulation of fatty acid synthesis by compounds of the tricarboxylic acid cycle has been localized to the acetyl coenzyme A (Acetyl-CoA) carboxylase reaction in a rat adipose tissue preparation in which this reaction is the rate-limiting step in fatty acid synthesis from acetyl-CoA (Martin and Vagelos, *J. Biol. Chem., 237,* 1787). Fatty acid synthesis by a highly purified preparation from pigeon liver is stimulated by sulfhydryl agents and inhibited by sulfhydryl agents and arsenite. A mechanism of fatty acid synthesis is proposed and discussed (Bressler and Wakil, *J. Biol. Chem., 237,* 1441). The pathways of biosynthesis of fatty acids from acetyl-CoA are discussed. Some work concerning the synthesis of fatty acids from acetate in the presence of ATP, $Mn^{++}$, and $CO_2$ by cellular cytoplasm is reported (Seubert, *Fette, Seifen, Anstrichmittel, 63,* 702). The *in vitro* incorporation of acetate-1-$C^{14}$ into six lipid fractions of normal and leukemic leukocytes was studied. Leukemic leucocytes incorporated the highest percentage of radioactivity into the phospholipid fractions whereas normal leukocytes incorporated the highest percentage into the glyceride fractions (Malamos et al., *J. Lipid Research, 3,* 227). The *in vitro* incorporation of acetate-1-$C^{14}$ into individual fatty acids of adipose tissue from young and old rats was studied. Diet related differences in incorporation of isotope into monoenoic acids of young and old rats were observed. Relationships of monoene synthesis, carbohydrate metabolism, TPNH generation, and insulin effects were discussed (Gellhorn et al., *J. Lipid Research, 3,* 314).

The incorporation of $C^{14}$-stearate into milk fat following its infusion into mammary tissue was interpreted as suggesting that the plasma lipids are hydrolyzed in the udder and thereby provide a ''pool'' of fatty acids for milk fat synthesis (Luick and Lucas, *Proc. Soc. Exp. Biol. Med., 110,* 275). The incorporation of palmitate-1-$C^{14}$ into mono-, di-, and triglycerides of excised rat diaphragms was studied. The data are consonant with the hypothesis that diglyceride is a precursor of triglyceride and glycerol phosphatides (Neptune et al., *J. Lipid Research, 3,* 229). In similar experiments the same group of workers studied incorporation of palmitate into plasmalogens and phosphatides of rat diaphragm. Estimated turnover times of lecithin, triglyceride, and cephalin are reported (Colodzin et al., *J. Lipid Research, 3,* 234). Biosynthesis of cerebroside odd-numbered fatty acids was studied in rats injected with radioactive acetate or propionate. Both of these precursors were incorporated (Hajra and Radin, *J. Lipid Research, 3,* 327).

The biosynthesis of cholesterol and fatty acids by the pregnant rat was studied (Schwenk and Joachim, *Proc. Soc. Exp. Biol. Med., 108,* 665). Allyl pyrophosphates (3, 3-dimethylallyl, geranyl, and farnesyl pyrophosphate), which are known intermediates in the biosynthesis of squalene from mevalonate, are also metabolized in the liver by an alternate pathway to prenoic acids as shown with liver enzyme systems. The formation of free prenols and prenoic acids from mevalonate was also observed *in vivo* (Christophe and Popjak, *J. Lipid Research, 2,* 244). Thyroxin, 3, 3′, 5-triodo-thyropropionic acid, *alpha*-diphenylbutyric acid, *alpha*-diphenylvalerie acid, nicotinic acid, and other compounds were tested with regard to their effect on the biosynthesis of liver cholesterol from labeled acetate and mevalonate *in vivo* and *in vitro* (Garratini et al., *Fette, Seifen, Anstrichmittel, 63,* 1027). Effects of cholic acid on the feedback control of biosynthesis of hepatic cholesterol and bile acids were studied in mice receiving single intraperitoneal injections of mevalonic acid-2-$C^{14}$ or cholesterol-4-$C^{14}$ (Beher et al., *Proc. Soc. Exp. Biol. Med., 109,* 863). A rapid conversion of desmosterol to cholesterol was demonstrated in washed rat liver mitochondria and microsomes. The reaction by the desmosterol reductase system requires TPNH. Sulfhydryl inhibitors inhibit the reduction, and inhibition is reversed with reduced glutathione (Avigan and Steinberg, *J. Biol. Chem., 236,* 2898). The conversion of $\Delta^7$-cholestenol-4-$C^{14}$ and 7-dehydrocholesterol-4-$C^{14}$, prepared by chemical synthesis, to cholesterol by cell-free homogenates of rat liver has been demonstrated. The latter reaction did not require the presence of molecular oxygen (Schriepfer and Frantz, *J. Biol. Chem., 236,* 3137). 7-Dehydrocholesterol and lathosterol are readily interconverted in the intestine of the rat *in vivo* and *in vitro,* the equilibrium of the enzyme system favoring lathosterol. Interconversions from 7-dehydrocholesterol to cholesterol and back were also studied. These results indicate that the cholest-7-enols are precursors of cholesterol rather than metabolites (Mercer and Glover, *Biochem. J., 80,* 552). *In vitro* synthesis of labeled cholesterol by aortic tissue of cockerels was found to fall off rapidly with maturation, with either acetate-1-$C^{14}$ or mevalonate-2-$C^{14}$ as precursor. Thoracic and abdominal aorta displayed similar rates of cholesterol synthesis (Dayton, *Proc. Soc. Exp. Biol. Med., 108,* 257). Localization and turnover of desmosterol was studied in chick embryos, ranging in age from 9 days' incubation to hatching, which were injected with acetate-1-$C^{14}$ (Fish et al., *J. Biol. Chem., 237,* 334). Following 12 hours of bile drainage in rats livers were excised and *in vitro* synthesis of cholesterol by the liver from acetate but not from mevalonate was increased compared with livers from control rats with normal enterohepatic circulation (Myant and Eder, *J. Lipid Research, 2,* 363). With rat liver homogenates $10^{-3}$ M arsenite inhibited the formation of labeled cholesterol, and labeled lanosterol accumulated. This inhibitory effect of arsenite provides a convenient method of preparing labeled lanosterol in high yield ($>10\%$) from commercially available $C^{14}$-mevalonic acid (Moller and Tchen, *Ibid., 2,* 342). Tracer amounts of cholesterol-4-$C^{14}$ added to hog adrenal homogenates and incubated under conditions favorable for steroid synthesis were rapidly and efficiently converted to labeled steroids. Addition of cholesterol-4-$C^{14}$ esters, prepared enzymatically and incubated under the same conditions did not give $C^{14}$-activity in the steroids produced (Dailey et al., *Proc. Soc. Exp. Biol. Med., 110,* 571).

Recent information which has significance to the problem of carotene biosynthesis is summarized, and a detailed scheme is presented. Evidence in support of this scheme of biosynthesis, including that from species other than the tomato, is given (Porter and Anderson, *Arch. Biochem. Biophys., 97,* 520).

The effects of fatty acids on respiratory activity and oxidative phosphorylation by rat-liver, -kidney, and -brain-cortex slices, were studied. Oxygen uptake was initially increased

followed by a gradual decrease. At lower concentrations, fatty acids increased the respiratory activity of rat-liver and -kidney slices. Fatty acids inhibited incorporation of $P^{32}$-phosphate into organic phosphate compounds. The effects produced were dependent on the concentration of the fatty acids added (Ahmed and Scholfield, *Biochem. J., 81,* 37, 45). The effects of medium fatty acid concentration, epinephrine, and glucose on palmitate-1-$C^{14}$ oxidation and incorporation into neutral lipids by skeletal muscle *in vitro* are reported (Eaton and Steinberg, *J. Lipid Research, 2,* 376). It has been observed that (a) blood plasma, even when diluted several hundred times, has an inhibitory action on palmitate oxidation by washed blood cells (rat); (b) plasma albumin is one of the substances contributing to this inhibitory action of blood plasma; (c) under certain experimental conditions involving very high dilutions of body blood plasma, its action is stimulating rather than inhibitory (Hrachovec, *Proc. Soc. Exp. Biol. Med., 110,* 239). Feeding to rats of high fat-high casein diet alone, or with thyroid, induces overproduction of acetoacetate, both endogenous and exogenous, by liver slices. Utilization of acetoacetate by the kidney slices obtained from such rats is not, however, increased. Consumption of oxygen by liver and kidney slices of rats fed high casein and high fat-high casein is less than that in normal tissues. Varying degrees of hyperglycemia, fatty liver, and hypertrophy of liver, kidney and adrenal tissue were observed in the rats fed such diets (Khanade and Nath, *Proc. Soc. Biol. Exp. Med., 110,* 7). The oxygen consumption, *in vitro,* of segments from different levels of the aorta taken from cockerels receiving either an ordinary or an atherogenic diet has been measured and compared. The thoracic area of the cockerel aorta on either type of diet had a lower oxygen consumption than the other segments of the vessel. Similar measurements were also made in rat aortas. The results suggested that atheromatous lesions occur in those areas of the vessel wall with higher oxygen uptakes (Munro et al., *J. Atherosclerosis Res., 1,* 296).

GENERAL LIPID BIOCHEMISTRY. Recent developments in lipid chemistry were reviewed (Paquot, *Rivista Ital. delle Sostanze Grasse, 38,* 532). Lectures of the American Oil Chemists' Society 1962 Short Course on Developments in Fat Chemistry are published (*JAOCS, 39,* 448, 480, 521). The heptane-soluble, organic acid pool in fat pads is composed of long-chain fatty acids produced by lipolysis of intracellular fat. This pool can discharge acids into an incubation medium, or contract by re-esterification, but it seems not to be entered by fatty acids taken up from the medium (Dole, *J. Biol. Chem., 236,* 3121). Metabolism of stearate-1-$C^{14}$ was studied in the isolated cow udder. Much of the added $C^{14}$ (in the perfusing blood) was recovered from the glyceride of the udder tissue. Stearic and oleic acids of the glycerides showed significant activities. Negligible activities were detected in the shorter chain fatty acids of the glycerides (Laurysens et al., *J. Lipid Research, 2,* 383). In the presence of glucose, propionate-1-$C^{14}$ was extensively incorporated into shorter chain length odd-carbon atom fatty acids by mammary gland slices of lactating rats (Katz and Kornblatt, *J. Biol. Chem., 237,* 2466). Methylmalonyl coenzyme A racemase has been purified approximately 180-fold from extracts of sheep liver. A requirement of this enzyme for the conversion of succinyl coenzyme A to propionyl coenzyme A and $CO_2$ has been shown. The results obtained are consistent with the view that methylmalonyl coenzyme A is racemized by a shift of its *alpha*-hydrogen atom (Mazumder et al., *J. Biol. Chem., 237,* 3065). The uptake and metabolism of $C^{14}$ labeled sodium palmitate by macrophages was studied *in vitro.* Of the labeled acid taken up most was converted to triglyceride and phospholipid, but a small amount was converted to cholesterol ester and to mono- and diglyceride (Day and Fidge, *J. Lipid Research, 3,* 333). The fatty acid compositions of the triglycerides and phospholipids of Hela cells and Strain L. fibroblasts grown *in vitro* were reported (Geyer et al., *Ibid., 3,* 80). Methyl elaidate-1-$C^{14}$ was fed to fat-deficient guinea pigs. Methyl ester seemed to have been absorbed without complete hydrolysis and deposited in body tissues as the methyl ester. Direct *in vivo* hydrogenation of elaidic acid to stearic acid was shown to occur (Dhopeshwarkar and Mead, *Ibid., 3,* 238). From metabolic studies in rats data were obtained indicating that fed elaeostearic acid was hydrogenated after being absorbed to a 2 double-bond acid (Moore and Sharman, *Biochem. J., 81,* 10P).

Evidence supporting the specificity of milk lipase for the primary ester position of glycerides instead of the 2 position was obtained in studies with model substituted compounds of glycerol (Jensen et al., *J. Dairy Sci., 44,* 1980). Differences in composition of free fatty acids and natural triglycerides of natural fats and oils were studied. The lower iodine value of the free acids (in some cases, e.g. whale oil, as much as 35 units lower than in triglyceride) is explained by the lipase's preferential attack on the triglycerides' *alpha* position, more often occupied by saturated acids (Duyvis, *Rivista Ital. Sost. Grasse, 4,* 188). Absence of specificity for short chain fatty acids compared with long chain fatty acids during lipolysis of some synthetic triglycerides by $\beta$-esterase preparations from milk was reported (Jensen et al., *J. Dairy Sci., 45,* 842). An enzyme present in homogenates of rat intestinal mucosa has been found to hydrolyze long chain saturated and unsaturated fatty acid monoglycerides, but little, if any, of either the di- or triglycerides. The *alpha*- and *beta*-forms of these monoglycerides appear to be hydrolyzed to a similar extent by this enzyme preparation (McPherson et al., *Proc. Soc. Exp. Biol. Med., 110,* 744). The effect of nucleic acid metabolites on lipolysis in adipose tissue is being studied and has been reported (Dole, *J. Biol. Chem., 236,* 3125). The metabolism of adipose tissue *in vitro* is reported. The enzymatic pathways of fatty acid metabolism, fatty acid synthesis, glyceride synthesis and hydrolysis are summarized. The metabolism of carbohydrates, and the effects of hormones on the metabolism of adipose tissue are discussed (Vaughan, *J. Lipid Research, 2,* 293).

The activities of eight selected enzymes were determined in cell-free extracts of the lateral thoracic fat organs of the developing chick embryo (Feldman et al., *Poultry Sci., 41,* 1423). A comparison was made of lipid metabolism of chicken embryo organs and cells in culture. Acetate incorporation by established cell lines (Hela and Chang liver) resembled more closely that of chick embryo organs *in vivo* than that of chick embryo tissues *in vitro* (Halevy and Geyer, *Proc. Soc. Exp. Biol. Med., 108,* 6).

A comparative study of distribution of vitamin A aldehyde in the eggs or ovaries of various cephalochordates, lampreys, marine elasmobranchs, marine teleosts, salmon, amphibians, reptiles, and the domestic hen is reported (Plack and Kon, *Biochem. J., 81,* 561). Levels of carotene and vitamin A in pregnant patients were shown to be significantly different from those found in non-pregnant and puerperal patients. The possible association of vitamin A and carotene changes with changes in lipids during pregnancy was considered (Pulliam et al., *Proc. Soc. Exp. Biol. Med., 109,* 913). The conversion of vitamin A, to retinene$_2$ was studied in a freshwater fish (Naito and Wilt, *J. Biol. Chem., 237,* 3060). Autoxidation, hematin-catalyzed oxidation, and lipoxidase-catalyzed oxidation of *beta*-carotene were studied in linoleate-agar gels. The effectiveness of several antioxidants in these systems was studied (Blain and Shearer, *Chem. and Ind.* (London), *1962,* 217).

The effect of vitamin E-deficiency on protein synthesis in skeletal muscle was studied in glycine-1-$C^{14}$ injected rabbits. In all subcellular fractions and at all time intervals the proteins of vitamin E-deficient animals had a much higher specific activity (Diehl and Sanders, *Proc. Soc. Exp. Biol. Med. 109,* 8). In leg muscle from vitamin E-deficient rabbits the free and total activities of four lysosomal indicator enzymes were increased. Increases of total activity were: ribonuclease, 11-fold; cathepsin, 15-fold; *beta*-galactosidase, 61-fold; and arylsulfatase, 00 (Zalkin et al., *J. Biol. Chem., 237,* 2678).

The relative antioxidative activity of tocopherols was shown to be dependent on several factors: substrate, temperature and concentration. Several studies were carried out using linoleate as substrate and studying the effects of cytochrome C, hemoglobin, and tritriplex III, on the absorption of oxygen with different levels of added tocopherols (Kaufmann et al., *Fette, Seifen, Anstrichmittel, 64,* 309). Antioxidants in fats and oils, their mechanism of action, and their eventual metabolic fate is discussed (Brun, *Rev. Franc. Corps Gras, 9,* 192). Conversion of D-*alpha*-tocopherol-$C^{11}$ to tocopheryl-p-quinone *in vivo* was studied in rats. Trace quantities of the tocopheryl quinone were also found in liver and muscle extracts of mice and pigs (Csallany et al., *Arch. Biochem. Biophys., 98,* 142). Lipid extraction with chloroform-methanol shows a progressive decrease of extractables in oxidizing mullet tissue. Thiobarbituric acid values from extracts of oxidized tissues are only 30–50% of the values obtained from unextracted tissue. It is believed that much of the TBA-reactive material becomes concentrated in the interfacial fluff during extraction (Zipser et al., *J. Food Sci., 27,* 135).

Analytical data are presented on the lipid composition of beef heart mitochondria and various submitochondrial particles which correspond to different segments of the electron transfer chain. The lipid content of the particles or subunits is 26 to 27% and is predominantly phospholipid of several types (Fleischer et al., *J. Biol. Chem., 236,* 2936). It has been shown that the electron transfer system in beef heart mitochondria may be reconstituted either totally or in any desired sequential segment by appropriate combinations of two or more of the four primary complexes that have been isolated in highly purified form. The four enzyme systems that col-

lectively comprise the complete machinery for transfer of electrons from DPNH and succinate to oxygen are indicated. Specific inhibitors of each complex have been studied (Hatefi et al., *J. Biol. Chem., 237,* 2661). Evidence is presented that phospholipids play a vital role in electron transport in the mitochondrion. Mitochondria that have been nearly depleted of phospholipids by extraction with aqueous acetone lose nearly all of their respiratory activity. Phospholipids recombine spontaneously when mixed with phospholipid-deficient mitochondria; the lost respiratory activity is thereby recovered. Complete reactivation is achieved when the amount of rebound phospholipid is equal to or somewhat less than that originally present (Fleisher et al., *J. Biol. Chem., 237,* 3264).

Isothiocyanates were shown to be present in enzymatic hydrolyzates from three species of *Lesquerella* seed meals (Daxenbichler et al., *JAOCS, 39,* 244).

Fractionation of the component(s) responsible for sex odor/flavor in pork is reported. The agents responsible for sex odor in pork are located in the unsaponifiable material. Cholesterol and squalene which were both found in this fraction, did not produce sex odor when heated (Craig et al., *J. Food Sci., 27,* 29).

The color of solid fatty materials was studied. The fundamental theory of diffuse reflection and the principle of measuring the color of solid objects is discussed. It was shown that the rapid spectrophotometric method for the measurement of color by transmission is applicable to the measurement of color by diffuse reflection (Naudet et al., *Rev. Franc. Corps Gras, 9,* 202).

*Phosphoglycerides, Phosphoinositides, Sphingolipids, and Other Complex Lipids.* Biosynthesis of phosphatidic acid from chemically synthesized *alpha-* and *beta*-lysophosphatidic acids and palmityl coenzyme A by an enzyme present in the cytoplasmic particulate fractions of either guinea pig brain or liver is described. *Alpha*-glycerphosphate was not implicated in this reaction (Pieringer and Hokin, *J. Biol. Chem., 237,* 659). A method for the preparation of a pure native plasmalogen, phosphatidal choline, has been developed, based on the selective hydrolysis of the phosphatidyl choline component of beef heart lecithin by rattlesnake *(Crotalus atrox)* venom (Gottfried and Rapport, *J. Biol. Chem., 237,* 329). Two proteins with phospholipase A activity have been isolated from the venom of *Crotalus adamanteus* (Eastern diamond back rattlesnake) by a procedure involving pH change, heat treatment, and subsequent chromatography on diethylaminoethyl-cellulose (Saito and Hanahan, *Biochemistry, 1,* 521). Utilizing separation of phospholipids on activated silicic acid columns rat plasma was shown to contain about 17.5% of its lipid phosphorus in the form of lysolecithin. Evidence was obtained which indicated that plasma lysolecithin is not an *in vitro* breakdown product (Newman et al., *J. Lipid Research, 2,* 403). The phospholipids of unincubated egg yolks and of chick embryos of eggs incubated 4,6,12,14, and 18 days have been separated by silicic acid chromatography and the phospholipid patterns studied (Bieber et al., *Biochemistry, 1,* 532).

Radioactive phosphate and sulfate were injected into neonatal rats and most of the persistent radioactivity was found in the lipids of the brain mitochondria. In other experiments differences between neonatal and adult rats were observed for distribution of lipid-incorporated radioactive phosphate in lipids of a myelin-enriched fraction and in lipids from the remaining brain (August et al., *Biochem. J., 81,* 8). The incorporation of $P^{32}$ into fractions of rat brain inositol phosphatides was studied and compared with the incorporation of the label into glycerophosphatides (Wagner et al., *J. Lipid Research, 3,* 177). Rabbit brain slices have been incubated separately with myoinositol-$H^3$ and glycerol-2-$C^{14}$, and the amount of incorporation of label into each of the three components of the phosphoinositide complex was determined. The evidence, taken together, offered conclusive support for the hypothesis that the glyceryl-phosphoryl-myoinositol structure remains intact during the metabolic interconversions of the three inositides (Brockerhoff and Ballou, *J. Biol. Chem., 237,* 1764). The stereochemistry of sphingosine formed in rat brain homogenates to which palmityl coenzyme A and labeled serine were added was studied. Under the experimental conditions *erythro*-dihydrosphingosine and, in lesser quantity, *erythro*-sphingosine were formed. The *threo* derivatives were not produced (Fujino and Zabin, *J. Biol. Chem., 237,* 2069).

A precipitable complex containing approximately 15% cytochrome *c* and 85% phosphatidylethanolamine has been studied and its properties reported (Reich and Wainio, *J. Biol. Chem., 236,* 3058). The enzymatic activity of a cytochrome *c* oxidase preparation, low in lipid, was restored, and that of an already active preparation increased by several hundred per cent, by the addition of purified phospholipids. In each instance, sodium deoxycholate acted as a competitive inhibitor with respect to the added lipid (*Ibid., 236,* 3062).

### Steroids

Evidence is presented that a high cholesterol diet to rats induces increased activity of the intestinal mucosa to esterify cholesterol (Murthy et al., *Arch. Biochem. Biophys., 95,* 176). The fatty acids of serum cholesterol esters in the gerbil and in the rat were studied and compared. Differences in arachidonic acid content were noted (Albers and Gordon, *Proc. Soc. Exp. Biol. Med., 109,* 860). The distribution of labeled cholesterol in animal tissues was studied in the rat and in the rabbit. The results indicate that the treatment of cholesterol in each tissue as a single homogeneous compartment in exchange with plasma cholesterol, with a provision of some cholesterol synthesis in the various organs, is adequate to explain the data in most cases examined (Avigan et al., *J. Lipid Research, 3,* 216). The turnover of cholesterol and fatty acids in liver, plasma, lung, and aorta of 36 control and 36 thyroidectomized rats was studied by means of acetate-1-$C^{14}$. The disappearance of radioactive cholesterol from tissues behaves almost exponentially while that of fatty acids is a non-exponential process involving multiple reaction rates. Other dynamic aspects are considered (Perin and Comolli, *J. Gerontology, 17,* 260). $C^4$- and $C^{26}$-labeled cholesterols were fed via stomach tube to normal and hypercholesterolemic rabbits. The serum, liver, and aortic cholesterol levels were measured, as were the liver and intestinal content and stool nonsaponifiable and saponifiable lipid fractions. The appearance of the isotopic cholesterol in these fractions and in the exhaled $CO_2$ was measured (Bortz, *Circulation Research, 11,* 343). The distribution of $C^{14}$ and the nature of the labeled metabolites were investigated after intraperitoneal administration of cholanic acid-25-$C^{14}$ to normal rats and rats with ligated or cannulated bile ducts. Several metabolites were detected and isolated. It is suggested that the metabolism of cholanic acid is initiated by hydroxylation at $C^7$ (Ray et al., *J. Biol. Chem., 236,* 3158).

Data are presented on the comparative tissue distribution of $C^{14}$-free and esterified sterols following injection of cholesterol-4-$C^{14}$ and $C^{14}$-phytosterols to normal and bile-fistula rats. Presence and esterification of both sources of label were studied in various tissues, intestinal contents, and feces. Conversion of the injected $C^{14}$-phytosterols to bile acid was observed (Swell and Treadwell, *Proc. Soc. Exp. Biol. Med., 108,* 810).

### Lipoproteins

The use of cholesterol-4-$C^{14}$ labeled lipoproteins as a tracer for plasma cholesterol in the dog is described. A method is presented for labeling lipoprotein cholesterol with cholesterol-4-$C^{14}$ (Porte and Hassel, *J. Lipid Research, 2,* 357). A method is described for measuring "cholesterol uptake" of serum incubated with solid cholesterol. The method was used to measure cholesterol uptake of serum from normal young men and from older men hospitalized with cardiovascular disease (Clark et al., *Proc. Soc. Exp. Biol. Med., 110,* 764). The exchange of such lipids as cholesterol, cholesteryl ester, free fatty acids, tripalmitin, and phospholipids between high- and low-density lipoproteins has been studied *in vitro* (Evans and Patton, *J. Dairy Sci., 45,* 589). A method of emulsifying liquefied fat in saline suspensions of meat or meat proteins was used in investigating the factors that influence the capacity of meat to emulsify fat, the characteristics of emulsions, and the relative efficiency of proteins as stabilizers (Swift et al., *Food Tech., 15,* 468).

The effect of ingested fat on the fatty acid composition of serum lipoproteins was studied in a human subject. The fatty acids of the chylomicrons, of the low-density lipoproteins ($d < 1.019$), and of the $d > 1.21$ fraction tended to resemble the fatty acids of the recently ingested fat (Bragdon and Karmen, *J. Lipid Research, 2,* 400). Chromatography on hydroxylapatite as described by Hjerten was used for the isolation of *beta*-lipoprotein from human serum. The isolated lipoprotein was characterized. The preparation obtained has been demonstrated to be immunologically homogeneous (Cramer and Brattsen, *J. Atherosclerosis Res., 1,* 335). Human serum *beta*-lipoprotein has previously been shown to form two lines on immunoelectrophoresis. In the study of a large number of individual sera it appears that both are not always present, that they are present in varying amounts in normal individuals, that a third form sometimes exists, that they are not separable by sedimentation techniques, and that they cross-react immunologically (Lawrence and Shean, *Science, 137,* 227). Cholesterol-27-$C^{14}$ in the form of chylomicron-containing lipoprotein fraction of chyle was injected intravenously into rats. About 75% of the injected sterol-$C^{14}$ was in the ester and 25% in the free form. After 10 minutes, the transfer of labeled sterol to the higher-density plasma lipoproteins in-

volved both esterified and free forms, but the proportion of free sterol transferred was greater than that in the injected preparation. The further disposition of the $C^{14}$-sterol in a number of rat tissues was also studied (Lossow et al., *J. Lipid Research, 3,* 207). Two kinds of fat particles in alimentary lipemia have been demonstrated with polyvinylpyrrolidone gradient columns. Evidence is presented that one group consists of particles from thoracic duct chyle, and that the other group consists of particles originating elsewhere, possibly the liver (Gordis, *Proc. Soc. Exp. Biol. Med., 110,* 657). In studies with perfused rat livers, the results indicate that addition of 5% cholesterol to the diet of organ-donor animals resulted in a 3-fold increase in amount of cholesterol in low density lipoprotein appearing in the perfusion fluid with no significant increase in amount of the protein moiety (Marsh and Sherry, *Proc. Soc. Exp. Biol. Med., 109,* 14). The stability or lability of serum low density lipoproteins appeared to be greatly affected by dietary fat and by the presence or absence of vitamin E. Unsaturated dietary fat was found to contribute to the oxidative denaturation of serum low density lipoproteins in chick serum (Nishida and Kummerow, *Proc. Soc. Exp. Biol. Med., 109,* 724).

Lipoprotein lipase activity was found in adipose tissue of all 18 samples from 9 human subjects (Nestel and Havel, *Proc. Soc. Exp. Biol. Med., 109,* 985). Lipoprotein lipase was demonstrated in subcutaneous adipose tissue obtained from 8 patients undergoing various surgical procedures (Stern et al., *Proc. Soc. Exp. Biol. Med., 110,* 366).

Quinones in lipoprotein electron transport systems are studied and discussed. Coenzyme Q, vitamin K, and plastoquinone are lipophilic quinones which participate in electron transport reactions associated with oxidative or photosynthetic phosphorylation (Crane, *Biochemistry, 1,* 510).

## LIPIDS IN DISEASED STATES

MAN. In an article entitled "Cholesterol Metabolism: A Guide Toward Its Understanding" several timely controversial questions are discussed. If there is reasonable assurance that the administration of large quantities of unsaturated fat will lower the plasma cholesterol (15–25%) there is almost no proof that the course of atherosclerosis is being influenced. Indeed, if enthusiasts advocate excessive ingestion of unsaturated fats, the strong possibility exists that an increase of lipemia-induced coronary thrombosis may occur (Friedman et al., *Prog. in Cardiovascular Diseases, 4,* 419). The question "Polyunsaturated Oils. Myth or Miracle?" is discussed (Anon., *Drug Cosmetic Ind., 90,* 508). Similar questions concerning blood cholesterol, dietary fat and calories, and atherosclerosis are discussed in the article "The Physician's Responsibility in the Age of Therapeutic Plenty" (Shelps and Shapiro, *Circulation, 25,* 399). The current status of knowledge of atherosclerosis with emphasis on blood cholesterol levels is discussed in "An Over-All Look at Atherosclerosis." The balance between saturated and unsaturated dietary fats is discussed (Sebrell, *J. Am. Dietet. Assoc., 40,* 403). A "Lecture on Hyperlipidemia and Atherosclerosis" is presented. The correlations between serum total lipid levels and age, body build, and clinical manifestation of atherosclerosis are discussed (Schrade and Böhle, *J. Atherosclerosis Res., 2,* 161).

Lipid and mineral matter in coronary arteries and aortas from a group of men dying from causes unrelated to coronary artery disease were studied. Mineral matter content is not greatly increased before the fifth decade but after this age there is a precipitous rise, particularly in the coronary arteries (Whitehead et al., *J. Atherosclerosis Res., 2,* 199). The above results and studies were compared with those in a group of men dying from coronary artery disease. The data indicated that patients dying of coronary artery disease have a higher degree of coronary atherosclerosis than those dying from other causes, whether they die with or without an occlusive thrombus (*Ibid., 2,* 210). Clinical studies of long-term estrogen therapy in men with myocardial infarction are reported (Marmorsten et al., *Proc. Soc. Exp. Biol. Med., 110,* 400).

Polyunsaturated fatty acids in relation to total fatty acids in male and female patients of different ages, with complaints of Angina Pectoris were studied. An inverse relationship between serum total fatty acid content and its relative amounts of polyunsaturated fatty acids was demonstrated in patients with anginal complaints (Pol et al., *J. Nutrition, 77,* 343). The fatty acid composition of cholesterol esters, phospholipids, and glycerides of sera from healthy and arteriosclerotic people were compared with one another and the changes in their composition during week long diets containing a linoleic acid rich seed oil were investigated (Shrade et al., *Fette, Seifen, Anstrichmittel, 63,* 1035). Cholesterol and phospholipid content of human *beta*-lipoprotein in different lipemic states and following myocardial infarction are reported. Effects of dietary linoleic acid and estrogen administration were also studied (Cramer, *J. Atherosclerosis Res., 1,* 317). Effects on serum cholesterol level of changes in dietary fat composition and of administration of vitamins, thyroid, and other substances were studied in women in a home for the aged (Turpeinen and Jokipii, *J. Atherosclerosis Res., 1,* 307). The extractability of serum lipids was studied in normal subjects, coronary disease, hyperlipemia, and hypercholesteremia (Amatuzio et al., *Circulation, 25,* 540). Observations on serum cholesterol levels in a twin population of Georgia are reported (McDonough et al., *Circulation, 25,* 862).

A study was made of the DDT and DDE content of the diet and body fat of native Alaskans who lived in isolated, primitive areas and had minimal contact with insecticides. Eskimos store considerably less DDT and DDE in their body fat than the general population in the United States (Durham, *Science, 134,* 1880).

The presence of a novel lipid hapten, Cytolipin G, in tumors and tissues of the gastrointestinal tract was demonstrated by immuno-chemical studies (Graf et al., *Cancer Research, 21,* 1532).

Decanoic hydroxamic acid was markedly inhibitory to growth of certain fungi, both mycelial and yeast-like. Toxicity studies were made. Topical application of 1% preparations on patients produced no observable sensitization or primary irritation (Gale et al., *Proc. Soc. Exp. Biol. Med., 109,* 188).

MAMMALS. A comparison of production of atherosclerosis in miniature male swine fed a human diet (military ration) or purified diets was made. Neither gross nor histopathological changes were related to serum lipids, to the ratio of one lipid component to another, or to diet (Calloway and Potts, *Circulation Research, 11,* 47). Renal hypertensive rats on an all-meat diet developed hypercholesterolemia and coronary atherosclerosis within 16 weeks. Thus, hypertension coupled with a high fat, high protein, but low cholesterol diet was related to the production of hypercholesterolemia and coronary atherosclerosis (Eades et al., *Proc. Soc. Exp. Biol. Med., 110,* 65). Production of atherosclerosis and hyperlipidemia in rats and rabbits and the influence of some alimentary fats are reported (Libert and Rogy-Effront, *J. Atherosclerosis Res., 2,* 186). Addition of either propylthiouracil or cholic acid to a chow diet, enriched in certain lipids, resulted in hypercholesterolemia and hyperlipemia of about the same amplitude in rats. However, only cholic acid could induce a high incidence of thrombosis, cardiac infarct, and serum lipoprotein changes associated with hyperlipemia or thrombosis (Renaud and Allard, *Circulation Res., 11,* 388). The cholesteryl ester fatty acid patterns of plasma, atheromata, and livers of cholesterol-fed rabbits were analyzed by GLC. The oleic-linoleic acid ratio varied with anatomic source and in treated vs. control animals (Evrard et al., *J. Nutrition, 76,* 219). Cholesterol-depressant effects by dietary means were studied in cholic-acid fed hypercholesterolemic mice (Howe and Bosshardt, *J. Nutrition, 76,* 242). Quantitative aspects of cholesterol flux in rabbit atheromatous lesions was studied. The accumulation of cholesterol in the atheromatous lesion is reported not to be a static process but to be subject to a continuous turnover (Newman and Zilversmit, *J. Biol. Chem., 237,* 2078).

The influence of fats and carbohydrates on cholesterol metabolism and gall-stone formation was studied in the golden hamster. Cholesterol gall stones are formed if the food contains an easily resorbable sugar as the carbohydrate component and no fat. Small quantities of fat protect against cholesterol stone formation; and this protective activity increases with unsaturation (Dam, *Fette, Seifen, Anstrichmittel, 64,* 193).

The phosphatides of some mouse ascites tumors and rat hepatomas were studied. Studies of the enzyme system for methylating phosphatidylethanolamine to phosphatidylcholine were included (Figard and Greenberg, *Cancer Res., 22,* 361). The influence of irradiated bacon lipids on body growth, incidence of cancer, and other pathologic changes was studied in mice. There were no major differences between the control and experimental groups (Dixon et al., *J. Food Sci., 26,* 611). A hereditary obesity in the rat associated with spectacularly high serum lipids and cholesterol is reported (Zucker and Zucker, *Proc. Soc. Exp. Biol. Med., 110,* 165). Fatty acid composition of dermal and epidermal triglycerides and phosphatides in mouse skin during normal growth and during malignant transformation as a result of application of methylcholanthrene was reported (Carruthers, *Cancer Res., 22,* 294).

Triglyceride level alterations in liver and plasma of normal and reticuloendothelial stimulated rats following carbon tetrachloride administration are reported (DiLuzio, *JAOCS, 39,* 194).

BIRDS. The effects of estrogen and estrogen-like compounds on coronary and aortic atherosclerosis were studied in cholesterol fed cockerels. The observations suggested that the

effects on atherosclerosis are mediated through molecular configurations different from those mediating feminization (Peck et al., *Proc. Soc. Exp. Biol. Med., 108,* 786). The influence of reserpine on plasma cholesterol, hemodynamics, and arteriosclerotic lesions was studied in turkeys (Spackman and Ringer, *Poultry Sci., 41,* 40). A study of the ataxias of vitamin A and vitamin E deficiencies in the chick is reported (Scott and Stoewsand, *Poultry Sci., 40,* 1517).

## LIPIDS IN MICROORGANISMS, PLANTS, AND INSECTS

MICROORGANISMS. Storage tests were conducted on stabilization of carotene produced intracellularly by mated cultures of the mold, *Blakeslea trispora* (Ciegler et al., *J. Agr. Food Chem., 9,* 447). The quantity and character of the microbial lipid isolated from rumen digesta are interpreted as indicating that significant quantities of milk fatty acids originate from rumen microbial synthesis of long chain acids from volatile fatty acids (Keeney et al., *JAOCS, 39,* 198). The biosynthesis of oleic and 10-methylstearic acids was studied in *Mycobacterium phlei.* In resting cells, molecular oxygen was required for conversion of stearic acid to oleic acid. Methionine serves as the source of the $C_1$ unit in the branched chain acid (Lennarz et al., *J. Biol. Chem., 237,* 664). Disproportionate labeling of oleic and linoleic acids during biosynthesis of uniformly labeled fatty acids by *Penicillium javanicum* varied in reverse manner with the source of the label: $C^{14}$-acetate or $C^{14}$-glucose (Coots, *J. Lipid Research, 3,* 84). Unsaturated fatty acids isolated from lipids of various microorganisms were studied and characterized. The correlation between the double bond structure of microbial fatty acids and the type of synthetic mechanism employed by the organism is discussed (Scheuerbrant and Bloch, *J. Biol. Chem., 237,* 2064). The lipids of the particulate fraction of *Rhodopseudomonas* contain largely (72–79%) octadecenoic acid under both aerobic and anaerobic conditions of growth. Of the octadecenoic acids 90–97% is vaccenic acid and the remainder is oleic acid (Hands and Bartley, *Biochem. J., 84,* 238). Microbial aspects of the alteration of olive cakes ("Orujo") during storage are reported. Free fatty acid content of the residual oil was related to the number of microorganisms present (Gracian et al., *Grasas y Aceites, 13,* 17).

Studies on lipids of *Pithomyces chartarum (Sporidesmium bakeri)* and related fungi are reported. Emphasis was on fatty acid composition (Hartman et al., *Biochem. J., 82,* 76). The phospholipids of *Azotobacter agilis, Agrobacterium tumefaciens,* and *Escherichia coli* were studied and characterized (Kaneshiro and Marr, *J. Lipid Research, 3,* 184). The isolation and characterization of phospholipids containing mono- and dimethylaminoethanolamine from a choline requiring mutant strain of *Neurospora crassa* is reported (Hall and Rye, *J. Lipid Research, 2,* 321).

The liberation and obtaining of ergosterol from yeast is described; Green et al. (Vitamins Ltd.) *U.S. 3,006,932.* The incorporation of 2-$C^{14}$-mevalonic acid and 2-$C^{14}$-acetic acid into lipids of mycobacteria was studied (Ramasarma and Ramakrishnan, *Biochem. J., 81,* 303). The hypocholesterolemic agents, triparanol and benzmalecene, inhibit the multiplication of several sterol-synthesizing phytoflagellates, and a non-sterol-synthesizing ciliate protozoa (Aaronson et al., *Proc. Soc. Exp. Biol. Med., 109,* 130). The ability of a variety of common intestinal microorganisms to grow in bile salt-containing media and to alter the structure of those bile salts was studied (Portman et al., *Ibid., 109,* 959).

Lipolytic activity of a number of microorganisms at low and intermediate temperatures is being studied. Activity of microbial lipases at temperatures below 0C was reported (Alford and Pierce, *J. Food Sci., 26,* 518).

HIGHER PLANTS. The two activities: acetyl coenzyme A carboxylase and acyl coenzyme A-malonyl coenzyme A transcarboxylase from wheat germ were found to be catalyzed by the same enzyme (Hatch and Stumpf, *J. Biol. Chem., 236,* 2879). Uptake of 2-$C^{14}$-acetate by isolated plant leaves through the stem of chopped leaves in phosphate buffer led to the synthesis of long chain fatty acids. Labeled octanoic, decanoic, and dodecanoic but not hexanoic or octanoic gave rise to oleic acid and other unsaturated acids. Labeled oleic acid produced labeled linoleic, the label position being preserved (James, *Biochem. J., 82,* 28P). Synthesis of oleic acid and palmitic acid from acetate by lettuce chloroplast preparations was studied. The authors suggest that the process of photosynthetic phosphorylation provides conditions for the synthesis of fatty acid, namely continuing formation of ATP, $O_2$, and TPNH (Stumpf and James, *Biochem. J., 82,* 28P).

The biosynthesis of gossypol has been investigated by the incubation of methyl- and carboxyl-labeled acetate with excised cotton roots. It is concluded that gossypol is metabolically formed by the isoprenoid pathway. Homogenates of cotton roots were capable of synthesizing gossypol from either acetate or labeled mevalonate (Heinstein et al., *J. Biol. Chem., 237,* 2643).

Studies on the light and dark interconversions of leaf xanthophylls are reported. Nearly stoichiometric intervonversions of some Carotenoids were observed (Yamamoto et al., *Arch. Biochem. Biophys., 97,* 168).

Oil content of flax seed varied linearly and inversely with seed density for 20 varieties of flax (Zimmerman, *JAOCS, 39,* 77). The effect of germination upon the fat of the soybean was studied. No loss of oleic acid occurred until after the second day of germination and its more rapid loss, compared to the other fatty acids, occurred during the period of most rapid fat loss. The significance of this observation and its relationship to oleic acid as the key intermediary in fat metabolism in plants is discussed (Brown et al., *JAOCS, 39,* 327).

The properties and substrate specificity of a castor bean lipase with a pH optimum of 4.3 are described (Ory et al., *J. Lipid Research, 3,* 99).

INSECTS. Sex was reported as the regulator of triglyceride metabolism in the mosquito; levels being markedly different in males and females under the experimental conditions. Polyunsaturated acids were absent from newly synthesized triglycerides (Van Handel and Lum, *Science, 134,* 1979).

## BOOK REVIEW

Several interesting reviews on various phases of the fats and oils field have appeared during the last year. Rapport and Norton reviewed "Chemistry of Lipids" (*Ann. Rev. Biochem., 31,* 103–108). Cowan presented a brief account of the availability and composition of oils rich in polyunsaturated fatty acids (*JAOCS, 39,* No. 9, p. 4). A review dealt with interpretation of various assays for vitamin A in margarine (Lehman, *Ibid., 39,* No. 10, p. 12). Wakil prepared a review on lipid metabolism (*Ann. Rev. Biochem., 31,* 369–406). Proceedings of the International Conference on diet, serum lipids, and atherosclerosis were published (Federation Proc., *21,* No. 4, Part II, pp. 101). Lectures of the 1962 short course on developments in fat chemistry, conducted by the American Oil Chemists' Society, were published (*JAOCS, 39,* 448–464, 480–508, 521–545). A review titled "The Alkyd Story" appeared (Jordan, *Chem. & Ind.* 2056).

New books of interest to chemists in lipid research are as follows:

Williams, P. N., Ed., "Refining of Oils and Fats for Edible Purposes." 2nd revised edition, Oxford, 236 pp., 1962.

Enselme, J., Ed., "Unsaturated Fatty Acids in Atherosclerosis." Pergamon Press, 148 pp., 1962.

Searcy, R. L. and L. M. Bergquist, "Lipoprotein Chemistry in Health and Disease." C. C. Thomas, 197 pp., 1962.

Kinsell, L. W., Ed., "Adipose Tissue as an Organ." Proceedings of Deual Conference on Lipids, C. C. Thomas, 278 pp., 1962.

Pezold, Fritz A., "Lipide und Lipoproteide in Blutplasma." Springer-Verlag, Berlin, 399 pp., 1961.

Schultz, H. W., Ed., "Symposium on Foods. Lipids and Their Oxidation." The Avi Publishing Company, 442 pp., 1962.

Lundberg, W. O., Ed., "Autoxidation and Antioxidants." Vol. II. Interscience Publishers, Inc., 1156 pp., 1962.

Gardner, H. A. and G. G. Sward, "Paint Testing Manual. Physical and Chemical Examination of Paints, Varnishes, Lacquers and Colors." Gardner Laboratory, P. O. Box 5728, Bethesda 14, Maryland, 550 pp., 1962.

Taylor, C. J. A. and Marks, S., Eds., "Paint Technology Manuals. Part 2. Solvents, Oils, Resins and Dries." Reinhold Publishing Corp., New York, 239 pp., 1962.

Hummel, D., "Analysis of Surface-Active Agents." Test volume, Carl Hauser, Munich, 323 pp., 1962.

Longman, G. F. and J. Hilton, "Methods for the Analysis of Nonsoapy Detergent Products." SAC Monograph No. 1, London: The Society of Analytical Chemistry, 30 pp., 1961.

Moilliet, J. L., B. Collie and W. Black, "Surface Activity. The Physical Chemistry, Technical Applications and Chemical Constitution of Synthetic Surface-Active Agents." Second Edition, D. Van Nostrand Co., 518 pp., 1961.

Randerath, K., "Dünnschicht-Chromatographie" Verlag-Chemie, G.M.B.H., Weinheim/Bergstr., 243 pp., 1962.

Stahl, E., "Dünnschicht-Chromatographie" Springer-Verlag, Berlin, 534 pp., 1962.

Smith, Ivor, Ed., "Chromatographic and Electrophoretic Techniques, Vol. I. Chromatography." Interscience Publishers, Inc., 617 pp., 1960.

Burchfield, H. P. and E. E. Storrs, "Biochemical Applications of Gas Chromatography." Academic Press, 680 pp., 1962.

Bayer, E., "Gas Chromatography." Elsevier Publishing Co., 238 pp., 1961.

Ambrose, D., and B. A. Ambrose, "Gas Chromatography." George Newness, Ltd., London, 220 pp., 1961.