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

Deterioration During Storage

The following reviews were published on the oxidative deterioration of oils and fats. (Prasad, *J. Proc. Oil Technologists' Assoc.* India, Kanpur *17*, 77; Notevarp, *Rappt. Nord. Fettharskningssymp. 3*, Sandefjord, Norway, *1961*, 18, (Pub. 1962)).

The changes caused by autoxidation in highly unsaturated fatty esters were characterized in a series of papers by Fukuzumi et al. The methyl esters of cuttle fish oil were oxidized at 35C in the air while exposed to scattered sunlight. The product was analyzed by IR and UV spectrophotometry before and after further decomposition in the dark under nitrogen with the addition of Fe or Cu soaps (*Yukagaku 12*, 392; 396). Furthermore, the methyl esters of eicosapentaenoic, docosahexaenoic and docosapolyenoic acids from the cuttle fish were oxidized in the dark at 0–2 or 33–5C and the resulting products characterized (*Yukagaku 12*, 89; 351; 348).

The stability of several vegetable oils was tested under various conditions. Aging of olive oil in the presence of oxygen caused the formation of conjugated dienes with max wavelength at 232 mμ, as well as conjugated trienes and unsaturated ketones with maximum wavelength at 260–280 mμ. On further aging (and also by bleaching or refining) the extinction at 270 mμ increased at the expense of that at 232 mμ with a parallel increase in acidity (Spiteri, *Deut. Lebensm.-Rundschau 58*, 155).

Small increases in the free fatty acid content occurred in crude, refined, or bleached samples of cottonseed oil stored in closed containers at 27–34C for six months. The color of the crude sample increased upon storage whereas that of the refined or bleached ones decreased (Ninan et al., *Indian Oilseeds J. 6*, No. 2, 89). Bleaching also caused color increase in sesame oil, probably due to the conversion of sesamoline to sesamol (de Castro, *Grasas y Aceites 14*, 1). The oxidized acids responsible for color reversion during the saponification of oils could be removed by salting out, using a suitable salt concn plus adequate washing (Lechartier, *Rev. Franc. Corps Gras 10*, 19).

Light had some influence on the preservation of olive oils. A considerable reduction in the formation of peroxides was noted when olive oils were processed under Na vapor lamps (de la Borbolla et al., *Grasas y Aceites 14*, 12). Oils containing linolenic acid developed bad taste in the presence of even extremely small amt of oxygen. Taste deterioration could be slowed by careful treatment of the oil before and after refining and deodorizing (*Rappt. Nord. Fettharsknings-symp. 3*, Sandefjord, Norway, *1961*, 94 (Publ. 1962)). Other studies were undertaken on the influence of different storage conditions on the keeping quality of sunflower and cottonseed oils (Kozlova and Ermakova, *Maslob.-Zhir. Prom. 28*, No. 10, 20), as well as castor seed oils (Paulose and Achaya, *Indian Oilseed J. 6*, 243).

The changes provoked by autolysis in the lipids of fish flesh were reviewed by Lovern (*Recent Advan. Food Sci., Papers*, Glasgow 1960, *1*, 194 (Pub. 1962)). Various fish oils were also tested for autoxidation changes. Samples of cod liver oils were exposed to sunlight for 80 days in white or tinted glass containers or kept in the dark in the presence of air at −3 to 0C or 18 to 20C. Samples exposed to light and/or air deteriorated rapidly (Byczkowski and Kiewlicz, *Poznan. Towarz. Przyjaciol. Nauk, Wydzial Lekar, Prace Komisji Farm 1*, No. 3, 67). Packing of salted herring in refrigerator drawers compared favorably with others packed in barrels containing brine. Hydrolysis was the main process involved in the destruction of muscle tissue fat during storage (Bukhryakova, *Sb. Rabot. po Biol. Tekhn. Rybolovstva i Tekhnol. Tikhookeansk. Nauchn.-Issled. Inst. Morsk. Rybn. Khoz i Okeanogr. 1960*, No. 1, 74). Storage of herring in cooled sea water was also tested (Kenopkaite et al., *Kholodiln. Tekhn. 39*, No. 5, 29). The susceptibility to rancidity of cold-stored herring and mackerel was higher than that of rainbow trout, possibly due to the thinner skin of the former (Bramsnaes and Hansen, *Rappt. Nord. Fettharskningssymp 3*, Sandefjord, Norway *1961*, 180 (Pub. 1962)). The carbonyl compounds developed in autoxidized salmon oils were qualitatively analyzed. Main components were C_1 to C_{12} alkanals, C_4 to C_{12} alk-2-enals and C_6 to C_{10} alk-2,4-dienals (Wyatt and Day, *J. Food Sci. 28*, 305). The polyunsaturated fatty acids of raw tuna underwent little change upon processing (Roubal, *JAOCS 40*, 215). A patent was issued for the treatment of fish with absolute alcohol before smoking in order to prevent falling away of the skin and to retard the oxidation of the oil (Fujii et al., *Japan 1662*, 1962).

Oxidized lipids such as thermally oxidized corn oil or autoxidized linoleic acid were able to form complexes with protein compounds such as egg-albumin, lactalbumin, fresh egg white, or casein (Narayan and Kummerow, *JAOCS 40*, 339). *In vitro* experiments demonstrated that the peroxides in oxidized unsaturated fatty acids caused damage to the protein with which they were incubated (Desai and Tappel, *J. Lipid Res. 4*, 204). Sulfhydryl groups in protein and amino acids proved to be specially sensitive to oxidized fatty acids, the rate of their destruction being proportional to the peroxide value of the lipid compound (Lewis and Wills, *Biochem. Pharmacol. 11*, 901). Autoxidized menhaden oil oxidized cysteine to cystine (Wademeyer and Dollar, *J. Food Sci. 28*, 537). Loss of SH groups from flour doughs mixed in oxygen or air was attributed to the reaction of oxidized lipids with flour SH groups. When oxidized flour lipids, oxidized methyl linoleate, *tert*-butyl-hydroperoxides, methyl ethyl ketone peroxides and acetone peroxides were mixed with flour and incorporated into doughs under nitrogen, the content of the SH groups decreased (Tsen and Hlynka, *Cereal Chem. 40*, 145).

The aerobic mixing of flour and water caused loss of free fatty acids, probably due to oxidation of linoleic and linolenic acids by lipoxidase and enzymic oxidation of all free fatty acids (Morrison, *J. Sci. Food Agri. 14*, 245). The variation in free acidity of sterilized moistened samples of palm oil that occurred during storage or transportation could be explained on the basis of a chemical autocatalytic or a biochemical process, depending on the conditions (Coursey and Simmons, *Oléagineaux 18*, 161).

Treatment of doughs with KIO_3 produced the appearance of peaks in the fatty acid gas-liquid chromatograms which were absent in the chromatograms of fatty acids from flour or bread (Coppock and Daniels, *Brot Gebaeck 16*, No. 6, 117). That fatty acids were affected by artificial drying, molds and heat was evident from chromatograms of corn and wheat fatty acids in which the area between caproic and capric methyl esters showed the presence of a complex mixture of fatty acids, most probably coming from breakdown of longer chain fatty acids (Baker, *Cereal Chem. 39*, 393). Changes in the fatty acid composition were also found in potatoes stored at 4C. Pontiac potatoes showed a marked decrease in linoleic

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acid and an increase in palmitic acid, whereas Ontario potatoes showed a decrease in both palmitic and linolenic acids. The fatty acids with more than 18 carbon atoms increased during storage (Mondy et al., *J. Agr. Food Chem. 11,* 328). Also the content of volatile carbonyl compounds of potato chips changed upon storage (Dornseifer and Powers, *Food Technol. 17,* 118). Peanut cake, grits obtained by crushing it and flour were kept for 6, 12, and 18 weeks in different containers. The differences in organoleptic qualities were not very great when the products were kept at 27C and 60–70% relative humidity but differences arose at 38C and 90% relative humidity. The development of free fatty acids was less at the lower temp (Urs et al., *Food Sci.* (Mysore) *11,* No. 10, 273). Treatment with insecticides, as well as exposure to air at 38–57C and 100% relative humidity for one hour prevented the development of free fatty acids in stored peanuts (Naudet and Biasini-Bonjour, *Rev. Franc. Corps Gras 10,* 127).

Raw freeze-dried pork chops containing 37% fat were equilibrated at 0–32% relative humidity; hydrolysis proceeded more rapidly at higher relative humidities but oxidation was faster at lower values (Salwin, Freeze-Drying Foods, *Proc. Conf. Chicago 1961,* 58 (Publ. 1962)). No chemical changes could be detected in the lecithin of eggs held at 21C and 50% relative humidity for 400 days (Stute, *Arch. Gefluegelk 26,* 441).

A wide variety of carbonyl compounds was recognized in autoxidized oils and esters. The method most used consisted in the formation of 2,4-dinitrophenylhydrazones followed by adsorption (Suzuki and Maruta, *Yukagaku 11,* 282; Suzuki and Takeuchi, *Ibid. 12,* 354) or paper chromatography (Taüfel and Heder, *Fette Seifen Anstrichmittel 65,* 85). Steam distillation plus GLC was also used (Kadar, *Rappt. Nord, Fettharskningssymp. 3, Sandefjord, Norway,* 1961, 49 Pub. 1962). Rapeseed oil heated under dried air at 90C for 12–24 hr showed the presence of volatile and non-volatile compounds. More than 70% of the volatile compounds were C_4 and C_6 aldehydes and more than half of the C_8 and C_{10} compounds were unsaturated. The non-volatile carbonyl compounds contained more saturated components than the volatile carbonyl (Suzuki, OP. CIT.). Autoxidized safflower oil presented as volatile components hexanal, octenal, nonanal and nonenal. The autoxidized methyl esters yielded caproaldehyde, the C_{5-9} satd, the C_8 and C_9 monounsatd aldehydes and decadienal. Autoxidized cod liver oil yielded propanal, pentanal, pentenal, hexenal, nonenal, nonodienal whereas the Me esters yielded butanal, hexenal, heptanal, heptenal, and octanal (Kadar, OP. CIT.). In reverted but not oxidized soybean oil, six saturated methyl ketones, ten saturated aldehydes, five 2-enols, and a small amt of 2,3-dienols were identified by similar procedures (Mookherjee and Chang, *JAOCS 40,* 232).

The specificity and nature of the lipolytic enzymes in rice bran were characterized. The lipolysis reaction rate increased with the concn of the bran and the time of contact with the oil (El Hinnawy, *Oil Soap* (*Egypt*) *8,* No. 1, 23). Storage under CO_2 increased considerably the stability of refined and crude sunflower, cottonseed and whale oils at room temp (Kozin and Sitnikova, *Sb. Nauchn. Rabot. Mosk. Inst. Nar. Khoz. 1962,* No. 17, 114). A CO_2 atmosphere was superior in providing stability than air, nitrogen or SO_2 atmospheres (Masson, *Oleagineux 18,* 89). Another way of protecting unsaturated free fatty acids and ethyl esters was their conversion into urea adducts. However, glycerides, which did not form adducts were more rapidly oxidized in the presence of urea (Pokorny and Danickova, *Rev. Univ. Ind. Santander 4,* 221). Corn with high carotene content was dried at 38C to 11.0 or 3.0% H_2O and stored at 7 or 25C. The drying process reduced the amt of carotenoids by 25%. Losses were most rapid early in storage and greater at the higher temp and water content (Quackenbush, *Cereal Chem. 40,* 266). A new oxidation product of γ-tocopherol was isolated and partially characterized (Shone, *Chem. Ind.,* (London) *1963,* 335).

The effect of the presence of several substances on the alteration of fat during processing or storage was studied in various different ways. When added to an autoxidizing polyunsaturated fat in the form of soaps, several metals exhibit approx the same catalytic activity, but when chelated compounds were used, chelates of Fe^{++} were most catalytic, whereas those of Ni^{++}, Cu^{++} and Zn^{++} all had a typical induction period and that of Co^{++} had a decided negative (antioxidant) effect (Fedeli et al., *Riv. Ital. Sostanze Grasse 40,* 300). It was found that the removal of phospholipids and associated impurities from oxidizing linseed and soybean oils led to a considerable reduction in the induction period. Comparatively small additions of the ordinary drier metals (Pb, Mn or Co) reduced the induction period to zero (Anonymous, Tech. Notes *45,* 1 p (British Oil and Cake Mills)). The effect of several ions and acids on the stability of the hydroperoxides present in autoxidized methyl oleate was surveyed. Cu^{++} ion was stronger than Fe^{++} ion in destroying hydroperoxides. Mineral acids (sulfuric, hydrochloric and phosphoric acids tested) and organic acids (oxalic, monochloroacetic, citric and benzoic acids tested) led to decomposition of hydroperoxides in mixtures of esters and to a deepening of color. Decrease in peroxide value, decrease in the extinction coefficient at 232 mμ and color deepening were parallel to the strength and concn of the mineral acids (Täufel et al., *Fette Seifen Anstrichmittel 65,* 6). The methyl esters of sunflower oil fatty acids served as model substrate for a systematic evaluation of its susceptibility to oxygen in the presence of heavy metals, bleaching earth and/or metal chelator. In the presence of 0.1–1.0γ Cu or Fe/g sunflower oil, autoxidation was more significantly retarded by a mixture of bleaching earth and citric acid plus 0.1% metal chelator than with bleaching earth and Na polyphosphate plus 0.2% metal chelator; the latter combination enhanced autoxidation in the presence of Fe (Täufel and Linow, Nahrung *7,* 399). The catalytic activity of Cu added to lard emulsions was nearly the same whether the metal existed in a hydrophilic or in a lipophilic form prior to the preparation of the emulsion. The Cu ions acted at the interphase between water and fat (Finholt et al., *Medd. Norsk Farm. Selskap 24,* 61). Cu and hemin had a strong catalytic effect on the oxidation of fractions of pure phospholipids. Phosphatidylethanolamine and phosphatidylserine were oxidized faster than lecithin and sphingomyelin. These and previous evidences pointed out that the rancidity of milk, butter or dry milk was caused or at least initiated by oxidation of phospholipids (Swratling and Mattsson, *Rappt. Nord. Fettharskingssymp 3, Sandefjord, Norway 1961,* 25 (pub. 1962)). Cu contributed also to the rancidity of margarine samples containing 81% fat and oil, and 16% water. After 16 weeks' storage the samples with more than 1.0 ppm Cu were rancid (Nakazawa et al., *Yukagaku 11,* 477).

Pure lecithin in aqueous dispersion was treated with different substances in order to check their effect as accelerators or inhibitors of browning. Ascorbic acid and $FeSO_4$ produced rapid browning, whereas $CaCl_2$ had no effect. Glycine sped color development and phenylglycine produced a dark solid which eventually became black tar. Browning of phospholipids appeared to be due to formation of chromophores by a carbonyl-amino reaction (Burton and McWeany, Nature *197,* 1086). The oxidation of pure lipids like methyl linolenate and methyl arachidonate in the presence of glutathion required the addition of both the oxidized and the reduced forms of it. Both phosphate and arsenate prevented glutathione-induced peroxidation (Weinstein et al., *Biochem. Biophys. Res. Comm. 11,* 452). Protoporphyrin derivatives such as methemoglobin, metmyoglobin, imidazolehemoglobin hemichrome, carbon monoxide hemoglobin, oxyhemoglobin and nitric oxide hemoglobin were active catalysts for the oxidation of unsaturated lipids. The existence of ferrous forms provoked the appearance of induction periods but they were in the ferric state once the oxidation had started (Brown et al., *Arch. Biochem. Biophys. 101,* 14). However, relatively high concn of the above compounds had an inhibitory effect on linoleic acid oxidation (Lewis and Wills, *Biochim. Biophys. Acta 70,* 336). In an experiment with model compounds, cholesteryl linoleate and hemoglobin were made to react in an aqueous solution. Apparently, dihydroxy fatty acids were among the reaction products (Kaufmann et al., *Fette Seifen Anstrichmittel 64,* 1104). The autoxidation behavior of unsaturated wax-esters was studied (Kaufmann and Zollersberg, *Fette Seifen Anstrichmittel 64,* 908).

The methods used to accelerate fat oxidation were compared and from the results the "Swift-AOM" method recommended (Alemani Verdaguer and del Pozo, *Galenica Acta, 15,* 433). The relationship between the stability of coned milk and protein-lipid interactions was discussed by Brunner (*J. Dairy Sci. 45,* 943). Removal of colloidal $Ca_3(PO_4)_2$ from milk by acidification and analysis increased its heat stability. Once the phosphate approached complete dissolution, the normal heat stability-pH relation was not recovered upon reprecipitation of the phosphate. There was a correlation between heat stability and β-lactoglobulin content, but not between stability and content of inorganic components (Rose, *J. Dairy Sci. 45,* 1305). Various milk flavors were listed and classified from the organoleptic point of view (Smith, *Australian J. Dairy Technol. 18,* 34). The following investigations were intended to clarify the relation between undesirable flavors and chemical alterations in the lipids of milk and related products. The rate of development of oxidized flavor in milk was related to the initial concn of ascorbic acid. At constant fat globule membrane material concn, the rate of

lipid oxidation increased with the initial concn of ascorbic acid up to 20/40 mg/l. Further increases inhibited oxidation. At constant initial ascorbic acid concn, the rate of lipid oxidation increased directly with the fat globule membrane material concn. The rate of ascorbic acid oxidation was proportional to the rate of lipid oxidation. Addition of Cu in concn lower than 0.1 ppm inhibited both the rates of lipid and ascorbic acid oxidation. Cu addition to more than 0.1 ppm enhanced ascorbic acid oxidation but lipid oxidation was further inhibited. The results suggested that the lipid oxidation system was activated by association with ascorbic acid and inhibited by products of ascorbic acid oxidation (King, *J. Dairy Sci. 46,* 267) see also (Budlowski and Zakrzyewska, *Sbornyk Vyoska Skoly Chem. Technol., Praze, Oddil. Fak. Potravin Technol. 6,* No. 2, 27). The addition of ascorbic acid also affected the acid and peroxide values of milk, and provided a means of improving its quality (Cerutti, *Intern. Dairy Congr. Proc. 16th, Copenhagen 1962,* Sect. A, 663).

Milk with augmented linoleic acid content showed a diminished stability toward oxidation and an increased susceptibility to Cu-induced oxidized flavor. The latter effect seemed to be dependent on the augmented linoleic acid content of the phospholipids rather than of the milk fat (Smith et al., *J. Dairy Sci. 46,* 7). Intravenous injection of emulsified tocopherol to cows increased markedly milk tocopherol content and oxidative stability. For individual cows, correlation between milk tocopherol and oxidative stability was highly significant. This did not happen when milks from different cows were compared (Erickson et al., *J. Dairy Sci. 46,* 911). The carbonyl compounds present in oxidized milk and commonly associated with its off-flavor were thoroughly investigated. Tentative identification was made of C_5 to C_{16} saturated aldehydes, C_6 to C_{11} alk-2-enals and C_8 to C_{12} alk-2,4-dienals. Flavor studies and quantitative carbonyl analyses indicated that alk-2,4-dienals, especially 2,4-decadienal, played a significant role in the off-flavor of milk (Parks et al., *J. Dairy Sci. 46,* 295; Day et al., *Ibid.* 291). Petroleum ether-soluble fractions from aged evaporated milk included C_5 to C_{13} odd chain ket-2-ones, δ-decalactone, γ- and δ-dodecalactone, C_6 to C_{16} even chain fatty acids, and probably acetone and 2-butanone (Muck et al., *J. Dairy Sci. 46,* 774; see also Univ. Microfilms Order No. 63-3306; *Dissertation Abstr. 23,* 4303). When milk fat was heated in the absence of oxygen and moisture, C_3 to C_{11} odd-chain ket-2-ones were most abundant. Capillary GLC revealed the presence of many other components in relatively small quantities (Nawar et al., *J. Dairy Sci. 45,* 1172). Similar compounds were detected in butter oil, cream and cheddar cheese of varying ages or after being steam distilled (Lawrence, *J. Dairy Res. 30,* 161) and in fresh and decomposed cream (Wong, *J. Dairy Sci. 46,* 571). A relation was established between fat acidity and short chain fatty acid values, and the intensity of rancid flavor in milk. Most of the acids freed by lypolysis probably contributed to flavor deterioration (Kolar and Mickle, *J. Dairy Sci. 46,* 569). Reviews on lipase in milk (Stickler, *Oesterr. Milchwirtsch 15,* 97) and on the effect of fat splitting by lipase on the quality of milk products (Sjoestrom, *Rappt. Nord. Fettharskningssymp 3, Sandefjord, Norway 1961,* 172 (Pub. 1962)) were published. The oxidation flavor in milk induced by light was attributed mainly to the oxidation products of whey proteins. Increased fat content of milk resulted in less oxidation (Storgards and Ljungren, *Milchwissenschaft 17,* 406; Storgards and Lindquist, *Ibid.* 483).

The color changes that occurred in butter fats upon oxidation were related to the formation of oxidized acids (Otake, *Nippon Chikusangaku Kaiho 32,* 190) and, in general, to the development of carbonyl compounds (*Ibid. 32,* 196). These compounds were isolated (*Ibid. 32,* 221) and spectrophotometrically studied (*Ibid. 32,* 65). The keeping quality of packaged cheese was also related to its initial acidity (Rohse and Mair-Waldburg, *Intern. Dairy Congr. Proc. 16th, Copenhagen 1962,* Sect. B, 861). The consequences of the presence of metals in cold stored butter were studied. More than five ppm Cu in the parchment wrapper caused surface defects (Parodi, *Australian J. Dairy Technol. 18,* 25; see also *Ibid. 17,* 179). Butter with more than 100 γ Cu/kg did not develop defects on storage. However, the addition of 25γ/kg caused early development of defects. Fe and Mn had no adverse effect, either alone or in combination (Menger, *Mededel Landbouwhogeschool Wageningen 61,* No. 10, 79 pp). Other investigators reported, however, that both natural and added Cu had catalytic activity. Differences in pH and in the amt of protein-bound and total Cu could explain the discrepancies (Koops, *Verslag. Nad. Inst. Zuivelonderzoek* No. *80,* 193). Phospholipid fractions from soybean oil contained different proportions of Cu. When added to margarine, they showed oxidative effects, but no relation was found between the effects and the Cu content of the fractions (Tolboe and Krog, *Rappt. Nord. Fettharskningssymp. 3, Sandefjord, Norway 1961,* 153 (Pub. 1962)).

The action of several additives on margarine stability was tested (Rutkowski and Dunaj, *Tluszcze Srodki Piorace 4,* 197). Very little change was observed in cocoa beans during roasting under normal commercial practices (Pokorny et al., *Sbornyk Vysoke Skoly Chem. Technol. Praze Oddil Fak. Potravin Technol. 6,* No. 2, 285). Storage for two years reduced the liposoluble-vitamin content of milk (Davidov et al., *Molochnaya Prom. 23,* 12, 19).

A review on tests for stability of anhydrous fats was reported (Mroczkowski, *Tluszcze i Srodki Piorace 4,* 146). Standard iodometric procedures for the determination of the peroxide value were compared (Amer et al., *J. Pharm. Sci. United Arab Rep. 2,* No. 1, 1; Froelich, *Pharmazie 18,* 125) and modifications were suggested (Amer et al., *Pharm. Bull. 42,* No. 4, 245; *Ibid.* 253; Driver et al., *JAOCS 40,* 504; Hautfenne and Jacqumain, *Rev. Ferment. Ind. Aliment. 17,* No. 4, 107). New iodometric methods were developed (Amer et al., *Egypt. Pharm. Bull. 42,* No. 4, 271; Oette et al., *J. Lipid Res. 4,* 212; Silles and Montes, *Pharm. Acta Helv. 38,* 746) as well as modifications for previous colorimetric methods (Pokorny, et al., *Sbornyk Vysoke Skoly Chem. Technol., Praze, Oddil Fak. Potravin Technol. 4,* 197; *Ibid.* 211; *Ibid.* 297; Janicek and Pokorny, *Tluszcze i Srodki Piorace 4,* 293), some of them allowing the determination in micro amt of samples (Mario and Istrara, *Riv. Ital. Sostanze Grasse 37,* 441); Vioque and Vioque, *Grasas y Aceites, 13,* 203). A qualitative micro-determination by paper chromatography was also reported (*Ibid. 13,* 211). Peroxidation, and indirectly the action of antioxidants, was evaluated polarographically (Hamilton and Tappel, *JAOCS 40,* 52; Maach and Lück, *Experientia 19,* 466; see review by Hayano, *Yukagaku 12,* 605). The determination of the peroxide value in oxidized fats seemed to be less useful than that of the carbonyl value, since the latter was less dependent on various chemical and physical factors (Birden et al., *Rev. Franc. Corps Gras 10,* 79). The peroxide value was compared also with the TBA method (Vinagre and Mella, *Nutr. Bromatol. Toxicol. 2,* No. *111,* 11). The TBA reaction was tested on model compounds in an attempt to evaluate to what extent compounds such as formic acid, glyoxal monohydrate, 1,1,3,3-tetraethoxypropane, heptanal and acrolein diethyl acetal interfered when the absorbance of the reaction products was measured at different wavelengths. Optimal reaction conditions were found for each compound (Kaerkkaeinen and Antila, *Finnish J. Dairy Sci. 29,* 69). Modifications in the procedure were reported (Khomutov et al., *Sb. Nauchn. Rabot. Mosk. Inst. Nar. Khoz. 1961,* No. 17, 69; Drozdov and Krylov, *Zavodsk. Lab. 29,* 1308). Applications of the TBA reaction in testing for oxidation in the following materials were described: heated and oxidized edible oils (Yamada, *Seikagaku 35,* 309; Budzynska, *Roczniki Panstwowego Zakladu Hig. 13*(6), 525; Szilas-Kelemen and Bereczky, *Budapesti Muszaki Egyet. Elelm.-kem Tansz Kozlemeny 1962,* 1); milk products (Karkkaeinen and Antila, *Milchwissenschaft 17,* 479; King, *J. Dairy Sci.* 45, 1165; Aristova and Makar'ina, *Molochn. Prom. 24,* No. 4, 11); roasted nuts (Winterton, *Queensland J. Agr. Sci. 19,* 291); fish oils (de Koning and Silk, *JAOCS 40,* 165). The theory and practice of the carbonyl-number determination was surveyed (Moeller, *Rappt. Nord. Fettharskningssymp. 3, Sandefjord, Norway, 1961,* 60 (Pub. 1962). Procedures were devised for the evaluation of aldehydes and ketones as their 2,4-dinitrophenylhydrazones by spectrophotometry (Suzuki and Maruta, *Yukagaku 12,* 44). Food vapors could be injected in a GLC and their volatile components determined directly (Buttery and Teranishi, *J. Agr. Food Chem. 11,* 504).

The absorption of oxygen by unsaturated fats could be measured in several different ways (Taüfel and Linow, *Nahrung 7,* 41; Flanzy et al., *Ann. Zootech. 11,* No. 4, 263; Heide-Jensen and Gravenhorst, *Rappt. Nord Fettharskningssymp 3,* Sandefjord, Norway *1961,* 62 (Pub. 1962); Pickering and Jayne-Williams, *J. Soc. Dairy Technol. 16,* 28; Pokorny et al., *Sbornyk Vysoke Skoly Chem.-Technol., Praze, Oddil Fak Potravin Technol. 4,* 319). The Warburg apparatus seemed to be one of the best for the above purpose. The interrelationships between oxygen uptake and changes in iodine, conjugated diene, peroxide and carbonyl values were evaluated in autoxidizing model compounds of different unsaturation. Linoleic acid isolated from safflower oil and a mixture of hexaenoic and pentaenoic acids from cod liver oil were aerated at 25C and analyzed every 24 or 36 hr for a period of 300 hr. The results indicated that probably conjugation occurred before peroxide formation, that most of the carbonyl oxidation products were not conjugated, and

that the carbonyl value best expressed the total oxidation occurring in a sample Notevarp and Sellaeg, *Rappt. Nord. Fettharskningssmyp. 3, Sandefjord, Norway 1961,* 55 (Pub. 1962)).

Other methods tested, compared and/or developed were: organoleptic methods (Aime, *Riv. Ital. Sostanze Grasse 39,* 80; Hvolby, *Rappt. Nord. Fettharskningssymp. 5, Sandefjord, Norway 1961,* 100 (pub. 1962)) and the achromatic scale procedure for rancidity determinations (Valentinis, *Boll. Lab. Chim. Provinciali 13,* 336); evaluation of both the hydrolyzed and oxidized acid numbers, for a better characterization of deterioration in edible oils (Ivanov, *Nauchni Tr. Vissh. Inst. Khranitelna Vkusova Prom., Plovdiv 8,* Pt. 2, 155); colorimetric micromethod for the hydroxyl value (Vioque and Maza, *Grasas y Aceites 13,* 210); column chromatography procedures for the evaluation of total oxidation products in vegetable oils (Pogonkina and Rzhekhin, *Maslob.-Zhir. Prom. 29,* No. 8, 7), and of very small amt of oxidized acids in peanut oil (Naudet and Lachamp, *Rev. Franc. Corps Gras 10,* 546); a colorimetric procedure for measuring color reversion upon saponification (Lauchard and Loury, *Rev. Franc. Corps Gras 9,* 525); a method for determining oxirane (epoxide) oxygen (Lyubavima, *Maslob.-Zhir. Prom. 21,* 38); a paper test for the detection of esterases in chocolate products (Purr, *Rev. Intern. Chocolat. 17,* 567); and a procedure using UV fluorescence to detect early stages of rancidity (Scheunemann, *Arch. Levensmittelhyg. 14,* 41). Changes in the relative absorbances of butterfat at different wavelengths were due to oxidation of β-carotene, and could serve as criteria of evaluation of the keeping quality of butterfat (Wurziger, *Milchwissenschaft 14,* 565).

The following procedures were patented: oxidation and esterification of fatty acids (Piekarski, *U.S. 3,031,480*), preparation of peroxy-acids (Silbert and Swern, *U.S. 3,079,411*); partial and total epoxidation (Viñals, *Span. 285,433,* 1963); Henkel, *Brit. 865,271*; Chemische Fabrik Hoesch K. G., *Ger. 1,150,063*; Murata and Higo, *U.S. 3,112,325*); decomposition of the peroxides formed during autoxidation (Engelhard Industries, *French 1,334,673*); treatment for fish and fish offal (Ehlert, *U.S. 3,041,174*). Also the use of additives for milk products (Leviton and Pallansch, *U.S. 3,065,086*; Rahm, *Belg. 618,696*) and for carotenoids (Reiners and Morgan, *U.S. 3,081,171*) were patented.

Deterioration by Heat

Problems related to the deterioration of fats due to heat treatment were reviewed (Custot, *Symp. Substances Etrangéres Aliments, 5e, Budapest 1959,* 201); (Ota, *Yukagaku 12,* 431). The relation between fat alteration and nutrition was discussed (Timms, *Australian J. Dairy Technol. 18,* 106; Lang, *Dechema. Monograph. 46,* 761).

Investigations were undertaken on the effects of thermal treatment on pure lipids. Stearic acid was heated at 200,250,300 and 350C under different pressure conditions. Decomposition of the acid started at 350C at atmospheric pressure and at 250C under reduced pressure. At the same temp, decomposition was faster under reduced pressure (Levin and Elchaninova, *Tr. Vses. Nauchn.-Issled. Inst. Neftkhim Protsessov 1962,* No. 5, 139). Volatile decomposition products from methyl oleate heated in air at 200C included water, C_{8-9} aldehydes, semialdehyde methyl esters, C_{7-8} hydrocarbons, methyl esters of fatty acids, several fatty acids, mono-methyl esters of dibasic fatty acids and some alcohols (Toi et al., *Yukagaku 11,* 508). Soybean oil was heated at 200–240C for 2–10 hr. The tendency to foam increased with time, being closely related to the amt of polar fractions present. Those fractions were composed mainly by polyhydroxyacids (Ota et al., *Yukagaku 12,* 409). The volatile decomposition products formed upon further heating at 240C for one hr were gas chromatographically shown to be normal C_5,C_6 and C_8 hydrocarbons, 1-heptene, 1-octene, other octenes, C_2 to C_8 aldehydes, acrolein, crotonaldehyde and methyl ethyl ketone. During heating there was a general tendency for loss in wt of oil, iodine number, smoke point, monomer content and dienoic and trienoic acids and an increase in viscosity, acid number, carbonyl number and conjugated dienoic acid. Oils heated longer than four hr lost their edible value (Ota et al., *Yukagaku 12,* 403). In the same series of experiments, soybean oils having peroxide values of 10 or 290 were heated at 250C under a stream of nitrogen. The nature of the decomposition products depended on the peroxide value of the original oil and the length of treatment. Very few of these products arose from soybean oil having a peroxide value of 10, they being mainly hydrocarbons (more olefins than saturated hydrocarbons) and small amt of carbonyl compounds. Similar products developed at late stages of heating in the oils with a peroxide value of 290. At early stages a higher amt of decomposition products was produced, they being mostly *n*-pentane and propionic and caproic aldehyde. These and similar studies on safflower and olive oils suggested that the first step in the thermal decomposition of vegetable oils was the formation of hydroperoxides, followed by cleavage on either side of the carbon atom containing the hydroperoxide group (Toi et al., *Yukagaku 11,* 504). Other work consisted of heating previously oxidized cod-liver oils (peroxide numbers 13,29 and 54, respectively) at 125–300C and detecting the changes in peroxide and carbonyl values. Below 125–50C the carbonyl value rose to levels which depended only on the peroxide value. Above 150C, the max carbonyl value depended upon the peroxide value, time and temp. Conjugation of the double bonds of the aldehydes, enhanced by peroxides, and polymerization probably occurred simultaneously (Aure et al., *Rappt. Nord. Fettharskningssymp. 3, Sandefjord, Norway 1961,* 119, (Pub. 1962)). Epoxy fatty acids and derivatives were heated under nitrogen at 70,95,120 and 150C. The oxirane oxygen in the *cis*-epoxy fatty acids was destroyed more readily than the carbonyl group. The opposite happened to the *trans*-epoxyacids. At the temp tested, the *trans*-compounds were more stable than the *cis*-ones. Ester and amide derivatives proved to be much more stable than the original epoxy acids (Suhara, *Yukagaku 11,* 473).

When linseed oil was heated in the presence of air, the peroxides which were formed helped in reducing the drying time of the oil. Furthermore, other oxidation products besides peroxides also affected the drying rates (Pokorny, *Sbornyk Vysoke Skoly Chem.-Technol. Praze. Oddil. Fak. Potravin Technol. 4,* 223). Changes in the physical and chemical constants of fish oils produced by high-temp treatment with air blowing were measured and plotted against time and temp (Jangaard and Ackman, *J. Fisheries Res. Board Can. 19,* 839). Also modifications in the fatty acid composition in cod liver oil were studied. After air blowing at 130C for six hr, the saturated fatty acids did not change but the monounsaturated ones decreased 1–3%. There was also a marked decrease in the content of eicosapentaenoic and docosahexaenoic acids (Jangaard et al., *J. Fisheries Res. Board Can. 20,* 89). Identification was made of the carbonyl compounds developed in heat-processed herring (Hughes, *J. Sci. Food Agr. 14,* 893). Cholesterol and several oils and fats were oxidized at 105,130 and 180C for 72 hr, saponified, and the sterol precipitated with digitonin. The filtrate was analyzed by the color reaction with alcoholic KOH and UV absorption in order to detect changes in the sterol-free unsaponifiable fraction produced by heat. The conversion of cholesterol, when heated alone, to the non-precipitable compounds at the three above temp was 12,56 and 100%, respectively. The absorption max of the unsaponifiable at 260–70 mμ increased with temp. Similar changes were noted in the fats tested. Max formation of peroxides occurred at 130C with cholesterol and at 105C with the fats (Wurziger and Junker, *Deut. Lebensm.-Rundschau 59,* No. 5, 133).

The modifications induced by thermal treatment on oat flake lipids (Janicek and Pokorny, *Sbornyk Vysoke Skoly Chem.-Technol., Praze, Oddil Fak. Potravin Technol. 4,* 327), and coffee bean lipids (Kaufmann and Hamsagar, *Fette Seifen Anstrichmittel 64,* 734) and on the frying medium for potatoes (Rinetti and Giovetti, *Minerva Dietol. 2,* No. 3, 131) and for doughnuts (Downs, *Baker's Digest 2,* 66) were also investigated. The adjustment of the temp within fine limits by means of high frequency heating of sunflower seeds produced better quality oil from them (Gondar, *Fette Seifen Anstrichmittel 65,* 196). Some work was devoted to study the action of added siccatives on the autoxidation of vegetable oils (Juhasz, *Periodica Polytech. 6,* No. 4, 203). Studies on the thermal treatment of lipid-soluble vitamins showed that boiling, frying or roasting of fish did not reduce the content of vitamin A of their liver oil (Norcos and Saleh, *J. Pharm. Sci.* (United Arab. Rep.) *2,* No. 1, 129), and that a dimeric product was formed when γ-tocopherol containing products were thermally oxidized (McHale and Green, *Chem. Ind.* (London) *1963,* 982).

Several groups worked on the behavior of fats and oils when heated in the absence of oxygen. Glyceryl-1-monoeleostearate was treated in sealed tubes at 200–70C for 1–6 hr. Dimers were the most abundant product as revealed by mol wt and UV spectrum determinations (Kuck et al., *Rev. Agr. Grasas y Aceites 4,* 3). Heat-bodied linseed oil methyl esters were fractionated in urea adduct-forming and nonforming fractions. The nonadducting fraction was then molecularly distilled. Analysis of the products by GLC revealed the presence of at least 5–7 components in the distillate, they being cyclic or branched fatty esters. The residue was composed mainly of polymeric esters (Gast et al., *JAOCS 40,* 287; see also Eisenhauer et al., *JAOCS 40,* 129). The formation of polymers seemed to take place through dimerization, the degree of polymerization depending on the temp and times used. Thus, linseed oil heated for eight hr at 240C contained appreciable amt of dimeric glycerides. After 16 hr at 260C tetramers first appeared,

whereas higher polymers were formed at more prolonged periods of heating or higher temp. The linolenic acid in the oil was the only acid involved in the polymerization process, at least at the lower temp (Fedeli et al., *Fette Seifen Anstrichmittel 65*, 402; Fedeli et al., *Riv. Ital. Sostanze Grasse 40*, 321). One of the cyclic fatty acids developed in heated linseed oil was isolated in pure form and shown to be ethyl-11-(2-methylcyclohex-2-en-1-yl) undec-*trans*-9-enoate by means of chemical degradation and synthesis and by physical methods (Hutchinson and Alexander, *J. Org. Chem. 28*, 2522). The methyl esters of the highly unsaturated cuttle-fish oil once heated at 200–50C resulted in the formation, first, of *cis-cis-trans* and then of *trans-cis-trans* triene compounds. These materials then underwent partly inter- and intra-molecular cyclization (Miyakawa and Nomizu, *Fette Seifen Anstrichmittel 64*, 593). Up to 8% conjugated components was found in safflower oil bodied at various temp (250–310C). The absorption spectra also showed isolated *trans* bonds and conjugated *cis-trans* bonds (Sivaramaiah and Kulkarni, *Paintindia 12*, No. 1, 117). The fluorescence of corn and soybean oils increased remarkably upon heating (Ogawa et al., *Eiyo To Shokuryo 12*, 83). To identify the fluorescent material developed in edible fats by heat, cholesterol, phytosterol and ergosterol were heated at 400C under a current of nitrogen. Several aromatic compounds were isolated in crystalline form from the resulting material. Neither cholesterol nor phytosterol gave UV fluorescence after thermal treatment at 200C for several hr, but ergosterol did after two hr (Schmid, *Mitt. Gebiete Lebensm. Hyg. 53*, 507). Ionene was found among the thermal degradation products of β-carotene (Day and Erdman, *Science 141*, 808).

Methods were developed for the determination of the polymeric and cyclic compounds in heat treated oils and fats (Firestone, *JAOCS 40*, 247; Rost, *Fette Seifen Anstrichmittel 65*, 463; Sahasrabudhe and Bhalerao, *JAOCS 40*, 711) (Black and Eisenhauer, *Ibid. 40*, 272). Processes for the dimerization of fatty acids were also described (Byrne, *Chem. Prods. 25*, 496). The following patents were issued for various procedures for the polymerization of unsaturated fatty products (Barrett and Goebel, *U.S. 3,097,220*; Goebel, *U.S. 3,100,784*; Myers et al., *U.S. 3,076,003*; Zenkoku, *Japan 10,165/62*; Nitto Chem. Ind. Co., *Japan 5,130/63*) and for obtaining heat-stable emulsions (Unilever, *Neth. 102,037*, 1962).

Chemical alterations in fats and oils produced changes in their nutritional properties. In a series of experiments, different synthetic and natural glycerides were heated at different temp and fed to animals. Pure trilinolein and soybean and linseed oils were heated for two hr at 200C: no effects on growth were detected when fed to mice at a 15% level (Akiya et al., *Eiyo To Shokuryo 14*, 71). Commercial soybean, rice bran and rapeseed oils were similarly heated for 12 hr and fed to rats. Only the treated rapeseed oil retarded growth (Akiya, et al., *Ibid. 14*, 397). Fractions from linssed oil heated at 300–20C for three hr also retarded growth when given at a 20% level. Finally, synthetic trilinolein heated at 280C for two hr was injected intraperitoneally to mice. All the animals died within 48 hr. Specially toxic was a fraction that distilled at less than 280C (Akiya, *Ibid. 15*, 226). Lard and cottonseed oil were oxidized at 95–100C for 210 hr and then separated into fractions by molecular distillation, urea complex formation and alembic distillation. The fractions were administered *per os* to rats for three weeks at an 8% level. Several parameters like growth, thirst, organ weights and liver lipids served for the evaluation of the nutritional effects. Rather specific actions were associated with some of the fractions. A number of them provoked toxicity symptoms but others were associated with pharmacological effects such as depression of neutral fat deposition or enhancement of urine excretion (Kaunitz et al., *Symp. Substances Etrangéres Aliments, 5e*, Budapest *1959*, 189 (Pub. 1962); Kaunitz and Johnson, *Metab. Clin. Exptl. 11*, 1187). Other experiments on the subject led to the conclusion that the extent of the oxidative treatment was relevant for nutrition purposes. The effects were different for oils containing certain peroxide levels as compared to others in which further treatment had led to breakdown of the peroxides and to consequent polymerization. Peroxides were bearers of toxicity as determined from growth criteria, skin changes and mortality. They induced changes of a functional type. Instead, polymerized oils had effects of a more biological type, depressing growth (Benes and Sedlacek, *J. Hyg. Epidemiol., Microbiol., Immunol.* (Prague) *3*, 96). Other experiments, however, showed that dimers isolated from autoxidized sunflower oil caused, when fed to rats (100 mg/day for 12–14 weeks) not only growth depression but also development of catarrhs, desquamation and necrosis of the epithelium. Fatty livers and dystrophic disintegration of the liver cells were also observed (Parteshko, *Cesk. Gastroenterol. Vyziva 16*, 305; Parteshko, *Gigiena i Sanit. 28*, No. 4, 42). Oral administration to rats of air-heated soybean oil (180C, 2–7.6 hr) led to the deposition of lipids in the renal medulla. Also Cu and Fe were detected (Griem, *Arch. Pathol. Anat. Physiol. 336*, 592). In other cases, similarly treated soybean oil was lethal to rats after two weeks feeding but no histological changes could be detected (Kieckebusch et al., *Klin. Wochschr. 40*, 1076).

Pigs were shown to be rather sensitive to the *per os* administration of deteriorated fats. Corn oil with a peroxide value of approximately 222 provoked the appearance of acute tissue necrosis, severe skeletal and muscle degeneration, leading to death after 16–37 days (Thafvelin, *Rappt. Nord. Fettharskings-symp. 3, Sandefjord, Norway 1961*, 189 (Pub. 1962?)). Chicks given commercial and semisynthetic diets supplemented with oxidized safflower oil showed a 100% incidence of encephalomalacia (Mokadi and Budowski, *British J. Nutrition 17*, 347). Feeding partly autoxidized soybean oil to rats on diets containing vitamin E caused a reduction in the dienoic acid levels mainly in organ (heart, liver, kidneys) and subcutaneous fat and a reduction in the trienoic acid levels in most fractions. In vitamin E depleted animals the same oil led to no change in the proportions of the fatty acids in organs. To the other body fat fractions a higher decrease in the dienoic acids but a very slight one in the trienoic acids occurred. No change in the proportions of unsaturated acids were found upon feeding of a strongly autoxidized soybean oil plus vitamin E. The different effect was attributed to an incomplete intestinal absorption of the strongly autoxidized oil due to its high content of polymers (Degkwitz and Lang, *Fette Seifen Anstrichmittel 64*, 893). Studies by others proved that thermally oxidized fats were approximately 10% less well absorbed than the corresponding fresh fats. The higher the content of unsaturated fatty acids of the original fat, the larger the difference in the degree of absorption (Bhalerao et al., *J. Dairy Sci. 46*, 176).

Deteriorated fish oils were also tested as nutrients. Pure triglycerides from menhaden oil were oxidized at room temp to different levels. When given to rats at a 10% level, the oils with high peroxide values caused steatitis, enlarged livers and high TBA values in blood and excreta. Feeding of menhaden oil previously autoxidized for 24 hr provoked anorexia, steatitis, lowered hemoglobin levels and death, the severity of these symptoms increasing with the time of autoxidation. Ethoxyquin or α-tocopherol caused the remission of most of these symptoms, being more effective when added to the fresh oil than when given as dietary supplements (Rasheed et al., *J. Nutrition 79*, 323). Analogous results were obtained in swine (Oldfield et al., *JAOCS 40*, 357). Deodorized (180–200C, 8 hr) herring oil had impaired digestibility as compared to that of the fresh oil, and caused slower rate of growth as well as variations in the excretion of neutral fat (Raulin et al., *Symp. Substances Etrangéres Aliments 5e Budapest, 1959*, 227 (Pub. 1962)). In pregnant rats, the oral administration of oxidized cod-liver oil decreased the tissue levels of linoleic acid (Kaunitz et al., *Nature 197*, 600).

The nutritional significance of the alterations induced in proteins by oxidized lipids was tested by giving to rats and chicks test meals with different combinations and levels of the protein and lipid fractions of fresh and oxidized herring meal. The results provided no evidence of toxicity in the oxidized fish lipids. The protein fractions that had been in contact with deteriorated fat caused a somewhat lesser degree of growth but this could be attributed to an appetite depressant effect (Carpenter et al., *Brit. J. Nutrition 17*, 151). Epoxides which might occur in superheated fats were fed to rats and mice in a pure form. No definite evidences of carcinogenic effects were obtained (Seelkopf and Salfelder, *Z. Krebsforsch. 64*, 459). On the other hand, two of the hydroperoxides formed in ethyl linoleate dispersed in water at 40C showed antitumor activity (Schauenstein and Esterbauer, *Monatsh. 94*, 164). The poisoning symptoms provoked by certain foods could be due to the presence of phospholipase C-producing bacteria. Phosphorylcholine, which is formed from lecithin by the enzyme, was found to be chemically related to certain substances which increase intestinal contractions (Nygren, *Acta Pathol. Microbiol. Scand. Suppl. 160*, 88 pp.). β-carotene was neither toxic nor active as vitamin A precursor after being treated with N_2O_4 and fed to rats under varied dietary conditions (Emerick and Lievan, *J. Nutr. 79*, 168).

Deterioration by Irradiation

An international symposium held on the "Implications of Organic Peroxides in Radiobiology" was published (*Radiation Research 18*, Suppl. 3 (1963)). Topics like chemistry of organic peroxides (Walling, *Ibid.* 3); biochemical implications of pro-oxidants (Bernheim, *Ibid.* 17); mechanisms of lipid peroxide formation in rat tissue homogenates (Barber, *Ibid.* 33) and techniques for the detection, estimation, identification, separation, etc., of organic peroxides (Philpot and Milas, *Ibid.*

55, 71) were discussed. The effects of ionizing radiations on alimentary fatty substances were reviewed (Mercier, *Ann. Nutr. Aliment. 16*, 59). The chemical and physical changes induced by Co^{60} γ-irradiation from a 16,000-c source on unsaturated fatty acids were determined by GLC and IR absorption spectra. No change was observed in unsaturated esters of C_{18} when irradiated with a dose of ca. 10^6r in an oxygen-free state (Kitahara and Tanaka, *Yukagaku Zasshi 83*, 203). In cottonseed oils, γ-rays provoked hydrolysis of the oil, reflected in a higher free fatty acid content. Changes in the absorption max from 220–280 mμ indicated that conjunction of unsaturated linkages had taken place (Ibragimov and Mukhamedov, *Vopr. Sovrem. Fiz. i Mat., Akad. Nauk. Uz. SSR 1962*, 95). Mixtures of ethyl linoleate and linolenate, as well as samples of lard, were treated with 300,000-rad of γ-rays with or without BHA, BHT or a fraction of phenol separated from smokehouse smoke. Irradiation of the ethyl esters increased their peroxide contents, but the presence of an antioxidant inhibited peroxide accumulation. The phenol fraction was less effective as an antioxidant than BHA or BHT. In lard the three antioxidants were equally effective (Piul'skaya, *Tr. Vses. Nauch.-Issled. Inst. Myasn. Prom. 1962*, (3) 86). Radiation with $1.5\text{-}2.0 \times 10^6$ rad of Co^{60} γ-rays could not stop enzymic activity in fresh carp, sheat fish, pickerel or herring, the storage life being limited to two months. A lower iodine number and a higher acid value were found after irradiation while the pH of irradiated herrings increased (Kardashev and Korzhova, *Tr. Vses. Nauch. Issled. Inst. Morsk Rybn. Khoz i Okeanogr 45*, 15). Changes in plasticity of hydrogenated rape seed oil caused by low and medium doses (10^6 to 5×10^7 rad) of γ-radiation appeared before other observable changes in the physical or chemical properties (Kaplan and Pelcik, *Jaderna Energie 9*, 20). No loss was found in the vitamin K content of six vegetable foods which had been frozen, canned by conventional heat treatment and irradiated with 2.79 and 5.58 megarads of γ-rays (Richardson, *U.S. At. Energy Comm. NP-9589*, 19 pp. (1960). A method based on paper chromatographic separation was developed for the analysis of γ-irradiated fatty acids. Results indicated that hydrolysis and some decarboxylation had occurred (Ibragimov and Arifzhanov, *Vopr. Sovrem. Fiz. i Mat., Akad. Uz. SSR 1962*, 105). In leaves irradiated with 1,000–10,000 rads of X-rays, the free lipid fraction contained 2–6 times as much peroxides as that in nonirradiated leaves, whereas the bound lipid fraction had only negligible amt. Exposure to 20,000 rads provoked considerable changes in the unsaturated fatty acid content of the free lipid fraction. Since there was no increase in peroxide content upon irradiation subsequent to inactivation of lipoxidase with steam, the conclusion was drawn that ionizing radiation activates lipoxidase (Budnitskaya, *Proc. Intern. Congr. Photobiol., 3rd, Copenhagen 1960*, 367 (pub. 1961) (See also Farkas and Goldblith, *J. Food Sci. 27*, 262). The effect of X-rays on liver mitochondria phospholipids was also studied (Schwarz et al., *Arch. Biochem. Biophys. 101*, 103). The GLC analysis of fats subjected to a 100-megarad electron beam showed the presence of more than 20 C_4 to C_{12} compounds. The splitting of the C-O-C ester bond was often accompanied by a simultaneous breaking of the acid chain. Carbonyl compounds of C_6 to C_{12} were mostly produced, those compounds up to C_9 being largely aldehydes, while ketones were formed in the heavier compounds. Irradiated palmitic acid and tripalmitin produce large amt of unsaturated C_9 ketones and a few C_{13} ketones. Unsaturated fatty acids did not produce any of these compounds, but gave mostly C_{11} and C_{12} ketones. (Lück and Kohn, *Experientia 18*, 62). Exposure of arachis, linseed, olive, soybean and chinese wood oils and of lard, partially hydrogenated margarine and beef and horse suet, to UV and IR radiations resulted in the conversion of *cis* to *trans* fatty acids, and vice versa, but the balance of displacement was favored in the direction of the form richest in energy and most stable (i.e., the *trans* form). *Cis-trans* isomerization was independent of any simultaneous shift in double bonds (Lück and Kohn, *Nahrung 7*, 199). IR treatment of groats lengthened their storage life as compared to heat treatment (Luchkin, *Sb. Nauchn. Rabot Mosk. Inst. Nar. Khoz, 1959* (16) 121). Gas plasma irradiation of rice slowed the liberation of free fatty acids when stored at different temp, relative humidities and lengths of time. The mol wt of the extractable lipids was higher for the irradiated sample as compared to an untreated one. Polymerization and increase in saturation resulted from irradiation rather than from the heat and/or vacuum treatment accompanying irradiation (Roseman et al., *Cereal Chem. 40*, 568). Studies on the nutritional quality of irradiated fats showed that when soybean oil irradiated with 2.5, 10.0 and 50.0 megarads was fed to rats at a 20% level for three weeks, the following changes were detected: Serum cholesterol in the ten megarad treated animals decreased while total lipids and total phospholipids increased. No change in the serum lipid content occurred in the rats that had received 50 megarad, probably because of lowered absorption (Degwitz and Lang, *Z. Ernaehrungswiss 3*, 164). No changes could be detected in rats fed previously irradiated canned pork (Sporn et al., *Igiena* (Bucharest) *11*, 31).

Lipoxidase Oxidation

Fresh methyl linoleate was oxidized in the presence of partially purified lipoxidase and the product was shown to be methyl monohydroxylinoleate by elemental analysis, H-absorption, and IR analysis. The methyl monohydroxylinoleate was then hydrogenated to monohydroxystearate which was resolved in two fractions by column chromatography. Analysis of the fractions proved them to be 9- and 13-hydroxystearate (Khan, *Pakistan J. Biol. Agr. Sci. 4*, No. 1, 72). The lipoxidase oxidation products of methyl linoleate which had an adsorption max at 277 mμ were isolated by a combination of column and TLC. The chromophore was demonstrated to be a mixture of 9-keto-11, 13-octadecadienoate and 13-keto-9,11-octadecadienoate. Reduction followed by dehydrogenation yielded octadecatrienoate (Vioque and Holman, *Arch. Biochem. Biophys. 99*, 522). Lipoxidase action on buffered solutions was very sensitive to heat and ionizing radiations. Heating before irradiation produced inactivation proportional to the sum of the separate treatments, whereas the reverse order produced inactivation greater than the sum of the effects of each treatment. Addition of 20% pea solids to a buffered solution of the enzymes afforded a 6–10 fold protection with respect to inactivation by heat and a 70 fold protection against inactivation by ionizing radiation. Combined heat and irradiation treatment of soybean lipoxidase, in the presence of 20% pea solids, showed that, contrary to the results in buffer, heating before irradiation produced more inactivation than irradiation before heating (Farkas and Goldblith, *J. Food Sci. 27*, 262 see also Budnitskaya, *Proc. Intern. Cong. Photobiol., 3rd, Copenhagen 1960*, 367 (pub. 1961)). The storage temp of germ flakes and whole germs of different varieties of Ukranian wheat affected lipoxidase activity, being max when germ flakes were stored during six months at 16–21C. Storage in an open atmosphere at 100% relative humidity resulted in spoilage (Yakovenko, *Tr. Odessk. Tekhnol. Inst. 11*, (2) 31). The addition of lipoxidase to wheat flour increased the time for max on the farinograph curve, but the addition of lipoxidase plus K linoleate had no additional effect (Dahle and Sullivan, *Cereal Chem. 40*, 372). A lipoxidase inhibitor was found in peanut testa. (Narayanan et al., *Chem. Ind.* (London) *1963*, 1588).

Deterioration Mechanisms

Recent theories on the process of autoxidation of monounsaturated fatty acids were reviewed (Skellon and Wharry, *Chem. Ind.* (London) *1962*, 929). Studies with model systems contributed to the clarification of the complicated problem of fat oxidation. Methyl oleate hydroperoxide was prepared by three different methods. Thermal degradation studies showed it to be an α-methylenic hydroperoxide; the double bond of the original methyl oleate was displaced during the autoxidation and finally occupied the positions 9,10; 8,9; and 10,11. This hydroperoxide remained stable at 0C for a month; at room temp it lost 13% active oxygen in 30 days, 30% in 60 days; at 150C, it completely decomposed in 15 min. Methyl linoleate autoxidized about 12 times faster than oleate. The first attack was on the α-methylenic carbon between the two double bonds; a hydroperoxide first formed at position 11, then shifted to form two hydroperoxides at 9 and 13, in dienic conjugation. A peroxide was then formed from these. Methyl linolenate hydroperoxides containing 70% conjugated isomers formed at a speed that was twice that of the formation of methyl linoleate hydroperoxide, the initial attack for its formation taking place at the 2 α-methylenic carbon located between the two double bonds. Two isomeric peroxides were isolated from methyl eleostearate and they lost no oxygen at 100C after seven days. This remarkable stability led to the assumption that peroxide formation was competitive with polymerization and did not constitute an intermediary stage as in the case of non-conjugated esters (Karnojitzky, *Peintures, Pigments, Vernis 39*, 144). The incorporation of oxygen into methyl linoleate was measured in a Barcroft apparatus in the darkness at 40C. The addition of water or a pH 6.5 buffer decreased the rate of oxidation at the beginning. The pH of the water had no effect on the rate of autoxidation between pH 5 and 9. Most antioxidants were affected in their action by the presence

of water, the pH of the medium, the presence of proteins, carbohydrates and emulsifying agents as well as heavy metals (Spetsig, *Rappt. Nord. Fettharskningssymp 3, Sandefjord*, Norway *1961*, 146 (Pub. 1962)). In other series of experiments, methyl ester of linoleic acid was oxidized at 50C for ten hr. Differently timed samples showed only hydroperoxide formation at the initiation of the reaction. The hydroperoxides gradually decreased due to autoxidation of conjugated double bonds to unstable diallyl peroxides, which split into carbonyl compounds. The mol wt of the oxidized esters was double that of the original esters. This was not due to acid association or bonding of peroxides (Dulog and Burg, *Deut. Farben-Z. 17*, 21). When oleic acid was oxidized, saponification or autolysis of the products yielded C_7 to C_{16} fatty acids and 9,10-dihydroxystearic acid. This was in agreement with the autoxidation mechanism postulated by Deatherage and Matill (Loury, *Rev. Franc. Corps Gras 9*, 481). These and other experiments supported the view that in autoxidative rancidification of fats, the peracid formed cleaved into the next lower aldehyde, and formic acid; the latter was further oxidized to CO_2 and H_2O, while the newly formed aldehyde oxidized then to peracid, which underwent the same cycle (Loury, *Compt. Rend. 256*, 2870; Loury and Lechartier, *Rev. Franc. Corps Gras 10*, 21, 273).

Antioxidants

Several reviews were published on antioxidants and their action on the oxidation of fats and oils (Froelich, *Pharmazie 18*, 20; Hik, *Suara Pharm. Madjalah. 5*, No. 6, 159; Mattil, *Chem. Biol. Hazards Food, Proc. Intern. Symp. Food Protect., Ames, Iowa 1962*, 60; O'Neil, *Rept. Progr. Appl. Chem. 46*, 184; Scott, *Chem. Ind.*, (London) *1963*, 271). The antioxidants used in milk and milk products were also surveyed (Adda, *Lait 42*, 378).

The oxidation of model compounds in the presence or absence of inhibitors was studied. On the basis of the different mechanisms involved, inhibitors could be graded as: peroxide decomposers, metal deactivators, light absorbers, inhibitor regenerators and chain stoppers (Shelton, *Off. Dig. 34*, No. 449, 590). Experiments in which the oxidation of purified *cis*-1,4-polyisoprene was inhibited with secondary aromatic amines and a hindered phenol indicated that the direct oxidation of the inhibitor was a significant initiation process. Two discrete stages of retarded oxidation were evident. The amt of oxygen absorbed at a well defined break between an initial stage and a faster second stage was independent of temp and inhibitor concn. This was probably due to the onset of bimolecular hydroperoxide decomposition as a major initiation process (Shelton and Vincent, *J. Am. Chem. Soc. 85*, 2433). Many common antioxidants were tested in different substrates and conditions. A number of them were added to autoxidizing ethyl esters of linseed oil and their inhibitory effects were compared. Of the 38 compounds tested, lecithin was the least, whereas *p*-hydroxyphenylamine was the most effective. Also the relative ability to increase the induction period was surveyed: lecithin was the poorest, while pyrogalol was the best (Paquot and Mercier, *Rev. Franc. Corps Gras 10*, 337). Other commercial antioxidants were tested in olive oil (Gutierrez, *Grasas y Aceites 14*, 49), dough frying oils (Narayanan, and Hlynka, *Cereal Chem. 39*, No. 5, 351), and wool fat (Nitschke, *Fette Seifen Anstrichmittel 65* 51). NDGA, BHA, propyl gallate, 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline (EMQ), BHT and α-tocopherol in order of decreasing effectiveness were used to preserve shark liver methyl esters. Shark liver oil was best preserved by EMQ, followed by BHA, NDGA, propylgallate, α-tocopherol, BHT and α-tocopherol. Antioxidants were less effective when oleic acid was added or the phospholipids removed from the oil (Chahine and El-Shobaki, *Grasas y Aceites 14*, 61, 116; Toyama, *J. Tokyo Univ. Fisheries 48*, 111, 119; Yudina, *Tr. Vses. Nauchn.-Issled. Inst. Morsk. Rybn. Khoz i Okeanogr 45*, 32). Other animal fats such as hen, horse, heifer and ewe fats were protected by BHT, dodecyl gallate and octyl gallate, in this order (Lopez et al., *Anales Bromatol. (Madrid) 14*, No. 3, 197). BHA was the most effective single antioxidant for lanolin and castor oil among the others tested, namely, NDGA, the gallates, BHT, citric acid and commercial "Antracine" and "Tenox" (Rutkowski et al., *Tluszcze i Srodki Piorace, 4*, 220). Sorbic acid was tested alone or in combination with dodecyl gallate and NDGA as oxidation inhibitor in lard, butter and butterfat. The enhancement of oxidation provided by sorbic acid was almost completely inhibited by the simultaneous use of antioxidants. The mixture was satisfactory for storage, inhibiting both microbial and chemical spoilage (Steinbach and Franzke, *Nahrung 6*, 713). NDGA, hydroquinone, pyrogallol or BHA at the 0.01% level were acceptable in keeping tallow from color changes (Houdouin and Loury, *Rev. Franc. Corps Gras 10*, 552). The relative effectiveness of antioxidants in reducing flavor deterioration in foam-dried whole milk decreased in the following order: lauryl gallate, propyl gallate, NDGA, ascorbyl palmitate, BHA, ascorbic acid, dihydro quercetin, Na diethyl dithiocarbamate, thiodipropionic acid, quercetin, dilauryl thiodipropionate (Tamsma et al., *J. Dairy Sci. 46*, 114). Several flavone compounds were also useful for that purpose, 6-dodecyl-2′,3,5′,7-tetrahydroflavone having keeping properties comparable to those of propyl gallate and NDGA (Abbot and Waite, *J. Dairy Res. 29*, 55). No retarded development of oxidized flavor in cold-stored butter was noted when kept at −18 or 38C with added NDGA, BHT, and BHT plus iso-propyl citrate (El-Negoumy and Hammond, *J. Dairy Sci. 45*, 848). A series of compounds or groups of compounds were tested in different products as antioxidants. A mixture of BHA, $K_2S_2O_5$ and hydrogenated peanut oil was tested in dehydrated potato and carrot powder (Bhatia et al., *Current Sci.* (India) *32*, 311); a solution containing HCHO and 1% nitrite, in fish and fish offal (Carpenter and Olley, *Dept. Sci. Ind. Res., Torry Tech.* Paper No. *2*, 31 pp); 2,6-di-*tert*-butyl-p-cresol or 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline, in grass meal (Astrup, *Acta Agr. Scand. 12*, 199); substituted phenols in different substrates (Hedenburg, *I E C Fundamentals 2*, 265); hydroquinone in lard (Sarsunova and Selecky, *Gyogyszereszet 3*, 181); cephalins and derivatives in methyl oleate (Urakami et al., *Osaka Shiritsu Daigaku Kaseigakubu Kiyo 10*, 1); lecithin and cephalin with added ethoxyquin in menhaden oil (Olcott and Van der Veen, *J. Food Sci. 28*, 313); emulsifiers like Tween 20, Tween 40 and gum arabic in soybean oil emulsions (Nerlo et al., *Ann. Univ. Mariae Curie-Sklodowska, Lublin-Polonia, Sect.* D *17*, 33 (1962; Pub. 1963)); hot water extracts of vegetables and several concn of tripolyphosphate in cooked beef (Ramsey and Watts, *Food Technol. 17*, 102); ionol as compared to gentisic acid and Na gentisate in whole frozen milk (Piñeiro, *Ind. Lechera 44*, No. 519, 302); nitrogen- and sulfur-chelating compounds like the Na_2Ca salt of ethylenediaminotetraacetic acid, carboxymethylmercaptosuccinic acid, and its monolauryl ester, in milk (Pierpont et al., *J. Dairy Sci. 46*, 1044); extracts from rosemary (*Rosemarinus officinalis*), in lard and meat (Ostric-Matyasevic, *Rev. Franc. Corps Gras 10*, 443); 17 spice powders in lard (Kihara and Inoue, *Nippon Shokuhin Kogyo Gakkaishi 9*, (7) 290); NaCl in cotton phosphatide conc (Kats and Popova, *Maslob. Zhir. Prom. 29*, No. 2, 37). Other compounds with antioxidant properties were: flavonoids (Aft, *Plant Phenolica Ind. Significance, Proc. Symp. 2nd. Corvallis 1962*, 93 (Pub. 1963)); esters of phenolic acids extracted from oats (Daniels et al., *J. Sci. Food. Agr. 14*, 385); analogs of 3,5-di-*tert*-butyl-4-hydroxy toluene (Rachinskii et al., *Plasticheskie Massy 1963*, No. 7, 48); amino acids (Marcuse, *Rappt. Nord. Fettharsknungssymp. 3, Sandefjord, Norway 1961*, 75 (pub. 1962)); sunflower oil (Lapshev, *Myasn. Industr. SSSR 33*, No. 3, 53); nucleic acids and derivatives (Matsushita, *Arch. Biochem. Biophys. 102*, 446). The synergistic action of liquid smoke on BHA activity was characterized (Kajimoto and Endo, *Eiyogaku Zasshi 20*, 125).

The nutritional effects of several dietary antioxidants were examined. Dodecyl gallate (Karplyuk, *Gigiene i Sanit. 27*, No. 12, 34), BHA and BHT caused no pathological symptoms in rats and mice but propyl gallate did (Karplyuk, *Tr. 2-oi (Vtoroi) Nauchn. Konf. po Vopr. Probl. Zhira v Pitanii, Leningrad 1962*, 318). The addition of antioxidants like tocopherol or ethonyquin to a vitamin E deficient diet with low content of polyunsaturated fatty acids protected the tissues of young swine from increased TBA values and from increased hemolysis. There was a relation between dietary polyunsaturated fatty acids and the needs of α-tocopherol. For each 1% of peroxidized corn oil added to the diet above 4%, roughly 100 mg *d*-α-tocopherol acetate was necessary to protect pigs from erythrocyte hemolysis (Hill, *JAOCS 40*, 360). Analogous relations were estimated in monkeys for dietary fat and vitamin E requirements (Fitch and Dinning, *J. Nutrition 79*, 69). The antioxidant effect promoted by both α-tocopherol and selenium in the tissues of chickens, rats and sheep seemed to follow largely independent paths. Selenium-promoted antioxidant activity was mainly associated with tissue selenoproteins (Hamilton and Tappel, *J. Nutri. 79*, 493). The storage life of loin, roasts, sausage and lard from pigs fed standard rations was not improved by supplementing the diets with ethanolamine hydrochloride as an antioxidant (Harrison et al., *Food Technol. 16*, 127). The life of the antioxidants themselves was affected by the peroxide content of the fat to be preserved. In breakfast cereals, the amounts of BHA and BHT remaining at any time during storage were inversely proportional to the amt of peroxides

present (Anderson et al., *JAOCS 40*, 349). Na triphosphate was the best of several compounds tested in preserving the stability of BHA in conditions resembling those in stored marine products (Toyama and Suzuki, *J. Tokyo Univ. Fisheries 48*, 127). Regulations were given for the amount of BHT and BHA (*Federal Register 27*, 11639) and of 2,4,5-trihydrobutyrophenone (*Federal Register 28*, 1141) to be added to food as an additive.

The methods of analysis of antioxidants were reviewed (Kawashiro and Hosogai, *Shokuhin Eiseigaku Zasshi 3*, 297). Newly developed procedures consisted of solvent extraction followed by a color reaction (Biefer, *Mitt. Levensm. Hyg. 53*, 243); by UV spectrophotometry (Choy et al., *J. Agr. Food Chem. 11*, 340) (Sedlacek, *Fette Seifen Anstrichmittel 64*, 962), or by column chromatography plus UV spectrophotometry (Alicino et al., *J. Agr. Food Chem. 11*, 496; Khomutov and Kulakovskaya, *Maslob.-Zhir. Prom. 28*, No. 8, 19).

Patents issued for the use of various compounds for protecting fats and/or oils from oxidation included: sesame as an antioxidant in edible fats (Marcus, *U.S. 3,058,995*; mixtures of BHA, BHT, and pyrogallol in raw fish (Matsuda and Ueno, *Japan 23,467*, 1961); hydroxy naphthyl-aminomethyl bis (propionic acid) derivatives in lard, castor oil and corn oil (Miles Laboratories, Inc., *Fr. 1,339,334*; 5-arylaminoacenaphthenes in fatty acids and their esters (Geigy, *Brit. 904,068* (1962); compounds with the general formula 2,4,5-HO(Me)(RNH)-$C_6H_2CHMe_2$, where R was a methyl, ethyl, propyl, butyl, acetyl, acetonyl or benzyl group in cottonseed, castor, rapeseed, soybean, linseed, coconut, olive, sesame and peanut oils, lard, beef tallow, fish oil and hardened oils (Tanabe Seiyaku Co., Ltd., *Jap. 13,917* 1961). Other patents claimed were for *bis*(3,5-dihydrocarbyl-4-hydroxybenzyl) sulfides (U.S. Rubber Co., *Brit. 917,370* 1963); mixtures of dialkylphosphite and methylene biphenol (Ethyl Corp., *Brit. 923,190*); mixtures of stearic acid, hardened whale oil, BHA, BHT, and dodecyl gallate plus lecithin, citric acid, and propylene glycol (N.V. Chemische Fabrik "Naarden," *Belg. 621,645*); coenzyme Q^{10} derivatives (Folkers and Wolf, *U.S. 3,064,012*, 1962) and ascorbic acid (Arles, *Fr. 1,330,601*). A procedure for stabilizing vitamins A and D and related compounds was also patented (de Wilde and Rappoldt, *U.S. 3,100,783*).

COMPOSITION AND CHARACTERISTICS

OFFICIAL METHODS AND REVIEWS

The Spectroscopy Committee of the AOCS published a preliminary report of the Subcommittee on Methods of Preparing Long Chain Fatty Acid Methyl Esters. A comparison of the methanol-sulfuric acid, diazomethane, boron trifluoride and dimethoxypropane techniques indicated that all gave satisfactory results. The boron trifluoride and methanol-sulfuric acid methods gave the lowest yield of free acids and were favored by the subcommittee. Standards were also made available for the determination of isolated *trans* isomer, and progress was reported on the determination of the hydroxyl value with near infrared spectroscopy (O'Connor and Chipault, *JAOCS 40* (*3*), 14). The Commercial Fats and Oils Analysis Committee issued a report on the standardization of a new lot of bleaching earth (Stillman, *Ibid.* 596). A revision of Method Ca 5a-40 for the determination of free fatty acids was submitted which involved a change of solvent and an alternative electrometric method for dark colored oils. The Industrial Oils and Derivatives Committee submitted minor revisions of Method Cd 9-57 for oxirane oxygen, Method Ka 3-58 for color, and Ka 6-59 for viscosity (Holt, *Ibid.*, 605). The subcommittee on polymerized fatty acids found the following AOCS Official Methods applicable for polymerized oils: L 2b-57 for moisture, L 3a-57 for acid value, L 4a-57 for unsaponifiable matter, L 7a-57 for saponification value and Ka 3-58 for color. The fatty nitrogen subcommittee found the methods for color, moisture, total amine value and iodine value in section N of the Official Methods of the AOCS were applicable to amino compounds.

A review of the 1961–2 literature on composition and characteristics was compiled by the AOCS Literature Review Committee (Mahadevan and Hammond, *Ibid.* 478). Reviews were published on methods for glyceride structural analysis (Vander Wal, *Ibid.*, 242); methods for determining essential fatty acids (Williams and Reiser, *Ibid.* 237); methods for the analysis of thermal and oxidative polymers in fats and fatty acids (Firestone, *Ibid.*, 247); methods for the determination of short chain fatty acids (Churacek, *Fette Seifen Anstrichmittel 64*, 679); the analysis of food fats by gas chromatography (de Saint-Rat and Bertrand, *J. Gas Chromatog. 1*, 31); methods for the analysis of *cis-trans* isomers of fatty acids by gas chromatography (Litchfield et al., *JAOCS 40*, 302); the equivalent chain length method for predicting the structure of fatty acids detected by gas chromatography (Miwa, *Ibid.*, 309); gas chromatographic methods for the analysis of partially hydrogenated and polymerized oils (Rybicka, *Chem. Ind. (London) 1963*, 390); melting phenomena in fatty materials (Meskens, *Lab. Tech. 6*, 287); methods for determining the rheological properties of foods (Janer, *Grasas Aceites (Seville, Spain) 13*, 216); the composition of soybean oil (Ramos, *Ibid. 14*, 122); the polyunsaturated fatty acid content of a number of fats and oils (Blattna and Manouskova, *Nahrung 1962*, 332); nutritional factors affecting milk fat production (Van Soest, *J. Dairy Sci. 46*, 204); and the composition and biosynthesis of milk lipids (Garton, *J. Lipid Res. 4*, 237).

ANALYSIS OF FAT SOURCES

A method for determining the oil content of seed based on nuclear magnetic resonance was devised (Conway et al., *J. Lipid Res. 4*, 265). The method was applicable to oils with a number of different structures. It agreed closely with results obtained by petroleum ether extraction. A method was given for determining the oil content of oil bearing materials which depends on the extraction of the oil with α-monobromonaphthalene and reading the refractive index of the oil solution (accuracy was ±5%). (Ovcharenko and Borisova, *Maslob.-Zhir. Prom. 12*, 6). A gravimetric method for determining the oil content of sunflower seed was based on extraction of the seed with hexane in a Twisselmann extractor and weighing the lipid in the extract. The procedure took 2.5 hr and gave results equivalent to standard methods. (Costanzo and Sameh, *Inst. Arg. Grasas Aceites 3*, 57). A rapid method based on density was published for the determination of oil in sunflower seeds (Lazarov and Atanasova, *Compt. Rend. Acad. Bulgare Sci. 14* (*4*), 401). A 10-g sample is ground with 70 ml *o*-dichlorobenzene, the solution is filtered and the specific gravity of the solution is determined with a hydrometer. Precise temp control is required. There was poor correlation between the oil content and percentage of kernel in sunflower seed (Costanzo and Maymo, *Rev. Arg. Grasas Aceites 3*, 109). The yield and composition of the lipid extracted from liver by Bloor's procedure and Soxhlet extraction with diethyl ether were compared. The extracts prepared by Bloor's procedure were more stable in storage, contained nearly 100% more total lipid and phospholipid, and produced more reproducible fatty acid patterns in gas chromatography (Sheppard, *JAOCS 40*, 545). A reagent for the determination of fat in blood serum was patented. It consisted of a nonionic and an anionic surface active agent and ethoxy triglycol to make the anionic surface active agent miscible with the serum (Schain, *U.S. 3,062,623*). A simple one-step operation to remove and extract fat from mouse carcasses and tissues was based on the properties of toluene (Wolff and Baky, *Proc. Soc. Exptl. Biol. Med. 112*, 524). The tissue is heated in toluene and the water is distilled out as the fat is extracted.

The neutral oil analysis method proposed by the National Soybean Processors Association Technical Committee was compared with the cup loss method (Potts, *JAOCS 40*, 535). The premiums paid under the two methods were the same, but better quality oil would have received a higher premium and poorer oils would have been penalized under the new procedure. The cup refining losses of sesame oil by the AOCS Method Ca-9c-52 were found to follow the relation: % loss = 1.8 × % free fatty acid + 1.4 (de Castro, *Grasas Aceites 14*, 1). Certain bleaching earths were found to increase the oil color. This was attributed to the conversion of sesamoline to sesamol by acid earths.

ANALYSIS FOR FUNCTIONAL GROUPS

An iodometric determination of the acid value was proposed which is based on the liberation of iodine from a potassium iodide and iodate mixture. The procedure may be used on a micro scale and has an error of ± 1.2% (Vallant, *Mikrochim. Acta 1963*, 93). A colorimetric micromethod for the determination of long chain fatty acids was described which is suitable for fatty acids in the presence of cholesterol, cholesterol esters and triglycerides (Duncombe, *Biochem. J. 88*, 7). The reagent is a copper-containing chloroform solution, and the acids are ultimately determined as copper by means of diethyldithiocarbamate. Another micromethod for long chain fatty acids which is valid in the presence of material containing acetyl groups was reported (Duron and Nowotny, *Anal. Chem. 35*, 370). The fatty acids are transformed to methyl esters with boron trifluoride-methanol, and the methyl acetate is distilled from the long chain esters. Ultimately the fatty acids are determined by the hydroxamic

acid reaction. Chains of C_8 or higher may be determined quantitatively. Potentiometric titrations were described for acids ranging from mineral acids to phenols using commercial mixtures of N,N-dimethyl fatty amides as a solvent (Reynolds et al., *Ibid.*, 973). A glass electrode is used with a calomel cell in which the saturated potassium chloride has been replaced with saturated lithium chloride. A semi-micro determination of the neutralization equivalent of long chain fatty acids by titration was described (Tiwari et al., *Z. Anal. Chem. 187*, 161). The sample is titrated with potassium methoxide solution in methanol-benzene in the presence of a mixed indicator. The hydroxamic acid method of Snyder and Stephens (*Biochem. Biophys. Acta 34*, 244) for the determination of acyl esters was tested on highly purified glycerides and phosphatides (Renkonen, *Ibid.*, 54, 361). The color yield per equivalent of ester was independent of the kind of lipid. A direct method for determining methyl ester in fats based on paper chromatography was reported (Kaufman and Grothues, *Fette Seifen Anstrichmittel 64*, 805). Determinations of methyl ester as methanol by transesterification was not possible. A sensitive method for the determination of alkenyl ether linkages in plasmalogens in the presence of free aldehyde was described (Warner and Lands, *J. Lipid Res. 4*, 216).

An iodine value method which can be applied to paper chromatograms was reported. The method depends on reaction with I^{131}. A comparison of the results for 22 fats and oils with the Hubl method was given (Abdel-Wahab and El-Kinawi, *Z. Anal. Chem. 186*, 364). A modification of the hypochlorite method for the determination of I.V. was given. The results were in good agreement with other methods except on drying oils (Karvanek and Pokorny, *Papers Inst. Chem. Tech. Prague 3*, 413). The practical and theoretical difficulties in determining the *trans* unsaturation of fatty acids was discussed (Naudet, *Bull. soc. chim., France, 1962*, 664). The subcommittee of the AOCS for preparing methyl esters for use in estimation of *trans* unsaturation gave a preliminary report. They found the boron trifluoride and methanol-sulfuric acid methods suitable (O'Connor and Chipault, *JAOCS 40* (3), 14). The significance of the Diels-Alder reaction for analysis and structural research in fats was discussed (Kaufmann, *Fette Seifen Anstrichmittel 64*, 1115). The use of the diene and pan-diene values with maleic anhydride in structural investigations was illustrated (von Mikusch, *Double Liaison 1962 87*, 75). Bromination of the methyl esters of olive oil increased the difference in the retention time of *cis* and *trans* octadecenoate so that they could be separated by gas chromatography (Bigoni, *Riv. Ital. Sostanze Grasse 9*, 428). The separation of geometric isomers of monoenoate, ricinoleate, linoleate, conjugated, and epoxy fatty acids by gas chromatography was reviewed. New data was presented on the resolution of the geometric isomers of linolenate and of oleate and elaidate (Litchfield et al., *JAOCS 40*, 302). The analysis of mixtures of unsaturated compounds by the rate method was attempted. Bromination and hydrogenation reactions were plotted at second and pseudo first order reactions, respectively. The plot shows a linear portion for each component of the mixture (Hanna and Serencha, *Anal. Chem. 35*, 362).

A method for the determination of the hydroxyl value based on acetylation with acetic anhydride and estimation of the ester groups by the hydroxamic acid method was reported. The relative error was 0–4% (Vioque and Maza, *Grasas Aceites (Spain) 13*, 207). The near IR spectra of 1,2- and 1,3-diglycerides was studied, and the usefulness of this technique as an analytical tool was discussed (Susi et al., *JAOCS 40*, 329). The Spectroscopy Committee of the AOCS is testing a method for hydroxyl value based on near infrared spectroscopy (O'Connor and Chipault, *Ibid. 40* (3), 14). They find the scope of the method is quite restricted because only primary hydroxyls can be determined in the absence of secondary hydroxyls. A method was described for the quantitative isolation of 2-hydroxy fatty acids in brain. The lipid was saponified with potassium hydroxide-propylene glycol and the resulting saponification mixture was purified with a small silica gel column. The 2-hydroxy fatty acids were precipitated as a copper chelate, and the amt present was determined by a colorimetric copper determination. Further gas chromatographic identification of the isolated fatty acids could also be carried out (Kishimoto and Radin, *J. Lipid Res. 4*, 130).

Oxirane oxygen was determined with aqueous hydrochloric acid in acetic acid rather than the usual anhydrous reagents. The method gave satisfactory results on oxidized and non-oxidized herring fat (Lyubavina, *Maslob.-Zhir. Prom. 21* (1), 38). A technique for the determination of cyclopropenoid fatty acids was described which is based on reaction with concentrated hydrochloric acid. Epoxy and hydroperoxides interfere with the determination (Magne et al., *JAOCS 40*, 716). A rapid titrimetric method for the determination of cyclopropenoid and epoxy fatty acids was based on Durbetaki's hydrogen bromide reagent. At 3C, only the epoxy compounds reacted while at 55C the cyclopropenoid compounds reacted and gave a good end point (Harris et al., *Ibid.* 718).

LIPID CLASS DETERMINATIONS AND STRUCTURE ANALYSIS

GENERAL. Factice, a vegetable oil polymer, was used as a column packing for the chromatographic separation of glycerides, cholesterol, diglycerides, and monoglycerides (Hirsch, *J. Lipid Res. 4*, 1). Test tubes coated on the inner surface with a thin film of silicic acid were used for the TLC of various lipids (Lie and Nyc, *J. Chromatog. 8*, 75). Brain lipids were separated into lipid classes by TLC, and the lipid and silica gel from each zone was scraped off and put into a small column which was eluted with a suitable solvent to obtain the pure lipid. The separation of phosphatides and free fatty acids was poor (Davison and Graham-Wolfaard, *Biochem. J. 87*, 31P). The application of counter-current distribution to the fractionation of lipid mixtures was investigated (Therriault, *JAOCS 40*, 395).

GLYCERIDES. Methods for glyceride structural analysis were reviewed (Vander Wal, *JAOCS 40*, 242). Some implications of the 1,3 random, 2 random theory of glyceride structure were discussed. A mechanism was proposed to account for the incorporation of new fat appearing in a depot into the scheme (Vander Wal, *Ibid.*, 256). The "equivalence" or "non-equivalence" of the 1 and 3 positions of triglycerides could presumably be demonstrated by the absence or presence of optical activity. The data pertaining to optical activity in triglycerides was reviewed (Litchfield and Reiser, *Ibid.*, 750). A method was described for the separation of glyceride mixtures into classes according to their degree of unsaturation. The separation was accomplished by TLC on silica gel-silver nitrate. There was some separation of compounds having the same number of double bonds. The glyceride structure of a number of fats was determined by this method and compared to that obtained by pancreatic lipase hydrolysis (Barrett et al., *Ibid.*, 580). A procedure was given for the structural analysis of triglycerides based on ozonolysis and TLC. The ozonides and the "aldehyde cores" derived from the ozonides by reduction were separated and determined quantitatively. The constituent saturated faty acids in each group were then determined by gas chromatography. Several natural and synthetic triglycerides were analyzed (Privett and Blank, *Ibid.*, 70). The separation of substantial quantities (up to 40 mg) of triglycerides was achieved with a circular paper chromatographic system. The paper was impregnated with paraffin and the developing solvents were acetic acid, acetone-acetonitrile, or acetone-methanol (Kaufmann et al., *Fette Seifen Anstrichmittel 64*, 509). The triglycerides of milk fat were separated into fractions by gas chromatography according to the number of carbon atoms/molecule. Fractions containing 24–54 C were obtained. The results departed significantly from those predicted by a random distribution (Kuksis et al., *JAOCS 40*, 530). Liquid-liquid partition chromatography with a two-phase system of heptane-acetone-water was used to separate model triglycerides and cocoa butter. The effect of various operating variables on the separation was studied. Glycerides differing by four carbon atoms or two double bonds were completely separated. The results on cocoa butter were compared with various distribution theories (Black and Hammond, *Ibid.*, 575). A technique for the separation of triglycerides by chromatography on columns of factice, a polymerized vegetable oil, was described. The elution was followed by differential refractometry (Hirsch, *J. Lipid Res. 4*, 1).

Lard and illipe butter were hydrolyzed for various lengths of time with pancreatic lipase to check the validity of this technique. The monoglycerides of both fats showed a constant composition at various stages of hydrolysis. The composition of the tri- and diglycerides of illipe butter were independent of the stage of hydrolysis, but the tri- and diglycerides of lard became more saturated as hydrolysis proceeded. It was concluded that analysis of the monoglycerides provides the best estimate of the fatty acid distribution (Coleman, *JAOCS 40*, 568). The distribution of the fatty acids of lard were determined by crystallization and lipase hydrolysis techniques. None of the individual fatty acids were distributed randomly according to the crystallization technique. The saturated and unsaturated C_{14} and C_{16} fatty acids were concentrated on the 2 position, and the C_{18} fatty acids on the 1 and 3 positions (Arnold and Milloy, *Ibid.*, 296). The best conditions for the application of the pancreatic

hydrolysis technique to milk fat were explored. The majority of the fatty acids were found to be uniformly distributed on the glyceride except for butyric and caproic acids which tend to concentrate on the 1 and 3 positions, and palmitate which concentrates on the 2 position (Jack et al., *J. Dairy Sci. 46*, 284). The enzymatic characteristics of pure and homogeneous milk lipase were examined (Chandan and Shahani, *Ibid.*, 503). Errors in the technique for permanganate oxidation of fats and the separation of the azelaoglycerides was discussed by Lakshminarayana and Rebello (*JAOCS 40*, 300) in reply to statements by Kartha (*Ibid. 39*, 478). Hammond discussed the calculation of glyceride structure by restricted random distribution (*Ibid. 40*, 211) also in reply to statements by Kartha (*Ibid. 39*, 272).

The near IR spectra of 1,2- and 1,3-diglycerides was examined as a possible analytical tool (Susi et al., *Ibid. 40*, 329). The standard periodic acid oxidation technique for the determination of monoglyceride was not suitable for glyceryl-1-monoeleostearate (Crespo et al., *Rev. Arg. Grasas Aceites 3*, 76). After hydrogenation of the sample the method was satisfactory. The quantitative separation and analysis by TLC of the glyceride species of linseed oil during glycerolysis reactions was recommended for ascertaining when equilibrium had been reached (Rybicka, *Chem. Ind. (London) 1962*, 1947).

VITAMINS, STEROIDS, PIGMENTS. A new method for the determination of plasma or serum vitamin A based on trifluoroacetic acid as a chromogen was described. This reagent has advantages over the usual antimony trichloride reagent and gives results similar to the Carr-Price method on sera except in bovines where it gives high results. The method can be used on as little as 50 μl of serum (Neeld and Pearson, *J. Nutr. 79*, 454). A method for the detection and identification of lycopersene was developed which would detect 0.05 μg (Mercer et al., *Biochem. J. 87*, 317). A derivative of γ-tocopherol thought to be produced by oxidation by oxidized glycerides was isolated from tung oil. Some properties of the substance were described (Shone, *Chem. Ind. (London) 1963*, 335). A new form of coenzyme Q_{10} designated coenzyme Q_{10} (H-10) was isolated from *Gibberella fujckuroi*. It differs from coenzyme Q_{10} in that the terminal unit in the side chain is isopentanyl rather than isopentenyl (Gale et al., *Biochemistry 2*, 196). Vitamins E and K were separated, and α-tocopherolquninone and α-tocopherolhydroquninone were separated from α-tocopherol by gas chromatography on a mixed silicone rubber stationary phase (Nair and Turner, *JAOCS 40*, 353). An inverse relationship was found between vitamin K in the diets of chicks and their mortality from coccidiosis, but this was not satisfactory as an assay of vitamin K activity (Harms et al., *Poultry Sci 41*, 1836). A new napthoquinone was isolated from *Mycobacterium phlei* which differed from vitamin $K_{2(45)}$ in that one of the side chain isoprenoid units was reduced (Gale et al., *Biochemistry 2*, 200). It was designated vitamin K_9 (H). An unknown substance found in chromatograms of the unsaponifiable matter of several vegetable oils was found to be a mixture of cyclotriterpenic alcohols. Formerly it was thought to be a mixture of steroids (Jacini and Capella, *Riv. Ital. Sostanze Grasse 12*, 620).

Procedures were described for the quantitative gravimetric isolation of cholesterol and cholestan-3-β-ol as digitonides and the quantitative recovery of these sterols from their digitonides. It was suitable for samples of 0.5–4.0 mg (Sperry, *J. Lipid Res. 4*, 221). A revised digitonin-anthrone indirect determination of cholesterol was proposed and checked against the Sperry-Webb procedure on sera samples and for recovery of added cholesterol (Goodman et al., *Anal. Chem. 35*, 760). A number of investigations of the separation of sterols by gas chromatography were reported. The reader is referred to the section on fatty acid analysis for discussion of advances in gas chromatography theory and practice. An equation was derived to express the effect of substituent groups on the retention time of steroids (Knights and Thomas, *Chem. Ind. (London) 1963*, 43). Retention times relative to cholestane were given for 90 steroids on the silicone stationary phases SE 30 and QF-1 (Brooks and Hanaineh, *Biochem. J. 87*, 151). The argon ionization detector was not found to be linear enough to give quantitative analyses of sterols and methods for standardization were worked out (Bloomfield, *J. Chromatog. 9*, 411). Questions of instrument design, column packing preparation, temperature-programing and choice of detection systems were studied in the gas chromatography of steroids (Horning et al., *Anal. Chem. 35*, 526). A gas chromatographic method for the determination of fecal sterols was devised and compared with liquid column methods (Rosenfeld et al., *J. Chromatog. 7*, 293). The retention volumes of 12 sterol acetates were determined relative to cholesterol acetate on different kinds of silica gel (Klein and Szczpanik, *J. Lipid Res. 3*, 460). This allowed selection of the best gel for a given separation. A number of TLC methods for separating sterols were reported. Bird et al. developed a method for the quantitative assay of 6-chloro-17a-hydroxy-pregna-4,6-diene-3,20-dione acetate (*Anal. Chem. 35*, 346). The spots were quantitated by adsorption spectrophotometry at 283 mμ. Matthews et al. used a similar technique supplemented with a cholorometric reaction for non-ultraviolet absorbing steroids (*J. Chromatog. 9*, 331). Eight 3-B sterols differing in unsaturation in the B ring and in the side chain were separated by TLC (Bennett and Heftmann, *Ibid*, 359). A thin-layer method was also given for fractionating the metabolic precursors of cholesterol. It could be used for both analytical and preparative purposes (Avigan et al., *J. Lipid Res. 4*, 100). A method for the thin-layer separation of steroids used Celite as a support and Zaffaroni type solvents (Vaedtke and Gajewska, *J. Chromatog. 9*, 345). Another thin-layer method was applied to the separation of 18 corticosteroids and pregnane derivatives (Bennett and Heftmann, *Ibid.*, 348). Positional isomers, axial-equatorial hydroxy epimers, and A/B *cis-trans* isomers were successfully resolved. Thirty-eight 19-norsteroids were separated by TLC on silica gel (Golab and Layne, *Ibid.*, 321). A thin-layer method for steroids which used rice starch as a support was applied to the separation of 55 steroids in various solvents (Smith and Foell, *Ibid.*, 339). Solvents suitable for the TLC of 40 bile acids were examined (Eneroth, *J. Lipid Res. 4*, 11).

PHOSPHATIDES. A method was described for the purification of "lecithin" from crude soy "lecithin." The raw lecithin is treated with a ternary solvent of hydrocarbon, acetone and water, and a two phase system is formed. The phosphatides are coned in the lower layer (Pardun, *Fette Seifen Anstrichmittel 64*, 536). A gradient elution system for liquid column chromatography which gives both linear and non-linear gradients suitable for the elution of phosphatides on silicic acid columns was described (Wallach and Nordby, *Biochem. Biophys. Acta 70*, 188). Serine-containing phosphatides were freed of other lipids by chromatography on alumina columns. Partial decomposition of the phosphatidylserine occurred. Further purification was achieved on silicic acid columns (Long et al., *Biochem. J. 85*, 251). Acid-treated Florisil was found to give separations of phosphatides similar to those obtained on silicic acid, but the coarse mesh size allowed faster elution (Carroll, *JAOCS 40*, 413). Detailed instructions were given for the separation of complex lipids by two new schemes. The first scheme used DEAE cellulose columns for the initial separation and examination of each fraction by thin-layer and paper chromatography. The second scheme employed DEAE cellulose for the initial fractionation and further separation on silicic acid and silicic acid-silicate columns (Rouser et al., *Ibid.*, 425). A TLC method for the separation of tissue phospholipids and spingolipids was given (Horrocks, *Ibid.*, 235). A technique for the TLC of phosphatides and their hydrolysis products was given (Doizaki and Zieve, *Proc. Soc. Exptl. Biol. Med. 113*, 91). Neutral and basic thin-layer plates were made by including sodium acetate and sodium carbonate, respectively, in the silica gel matrix. The basic plates gave good separations of phosphatidyl serine, but the neutral plates gave better separation of cerebrosides (Skipski et al., *J. Lipid Res. 3*, 467). A two-dimensional thin-layer system was used to separate phosphatides and identify their hydrolysis products (Skidmore and Entenman, *Ibid.*, 471). The separation of phosphatides by countercurrent distribution was illustrated (Therriault, *JAOCS 40*, 395).

Deacylation of phosphatides by mild alkaline hydrolysis with little hydrolysis of the phosphate group was achieved by using polar solvents (Brockerhoff, *J. Lipid Res. 4*, 96). Lithium hydroxide in chloroform-methanol (2:8) gave only 0.1% hydrolysis of the phosphate in egg lecithin. The phosphatides of *Mycobacterium tuberculosis* were deacylated and separated to yield four products containing *myo*-inisitol. They were glycerol *myo*-inositol phosphate, glycerol *myo*-inisitol phosphate mannoside, glycerol *myo*-inisitol phosphate dimannoside, and glycerol *myo*-inositol phosphate pentamannoside. In all cases the glycerol phosphate moiety was attached to the L-1 position of the *myo*-inisotol ring (Ballou et al., *J. Biol. Chem. 238*, 69). IR spectra indicated that the α-unsaturated ether linkage in plasmalogens is *cis* (Nortaon et al., *J. Lipid Res. 3*, 456). Oxidative cleavage of the double bond of dehydrophytosphingosine from seeds indicated it to be in the 8,9 position. The phytosphingosine was shown to be D-ribo-1,3,4-trihydroxy-2-aminooctadecane, and by analogy, dehydrophotosphingosine is D-ribo-1,3,4-trihydroxy-2-amino-8-*trans*-octadecane (Carter and Hendrickson, *Biochemistry 2*, 389). The structure of lecithin was elucidated by ozonolysis and TLC. The ozonides as well as the "aldehyde cores" obtained by reduction were separated by TLC (Privett and Blank, JAOCS *40*, 70). Application of this method to egg lecithin revealed that the saturated fatty acids were mostly on the primary and the unsaturated on the

secondary hydroxyl group of the glycerol (Privett et al., *J. Fod Sci. 27*, 463). The selective β-esterase activity of *Crotalus adamanteus* and *Bothrops atrox* venoms was demonstrated on lecithins which contained a radioactive fatty acid specifically in the α- or β-position (Robertson and Lands, *Biochemistry 1*, 804). The lecithinase of heat-treated pancreatin was also specific for the β-position. Lecithinase activity was demonstrated in a number of tissues, but in none did lysolecithin accumulate, so lysolecithinase is evidently also present.

The glyceryl ethers of animal tissues were determined by deacylation and chromatography of the non-saponifiable portion on silicic acid. The α-glyceryl ethers can be oxidized with periodic acid and determined colorometrically as formaldehyde. They were detected in all tissues except bone marrow (Nakagawa and McKibbin, *Proc. Soc. Exptl. Biol. Med. 111*, 634). A liquid column chromatographic method was described for the isolation of the unsaponifiable portion of cod liver oil (Emmerie and Engel, *Fette Seifen Anstrichmittel 64*, 813). The bacteriostatic activity of various fractions was determined, and all the activity was found in the α-glyceryl ethers. The presence of glycerol alkenyl and alkyl ether diesters in the neutral lipids of starfish was demonstrated (Bilbertson and Karnovsky, *J. Biol. Chem. 238*, 893). These compounds were demonstrated in a number of mammalian tissues and glycerol alkenyl ether monoester was demonstrated in the epididymal fat of rats.

A method for the detection of the addition of peanut lipoprotein in comminuted meats at levels as low as 1% was proposed (Waldt et al., *Food Technol. 17*, 107).

FATTY ACIDS. Methods for determining the essential fatty acids were reviewed (Williams and Reiser, *JAOCS 40*, 237). Methods suitable for the analysis of thermal and oxidative polymers of fats and fatty acids were discussed (Firestone, *Ibid.*, 247). Methods for the chromatographic determination of short chain fatty acids were reviewed (Churacek, *Fette Seifen Anstrichmittel 64*, 679). Advances were made in the theory of gas chromatography and its applications to the analysis of fatty acids. A study was made of the contribution of longitudinal and eddy diffusion and mass transfer factors to the plate height in gas chromatography columns (Perrett and Purnell, *Anal. Chem. 35*, 430). Inhomogeneous distribution of the stationary phase across the column was believed to make an important contribution to the plate height. The theoretical basis of calculating plate height from column structure factors in chromatographic columns was reviewed, and new equations and proposals were suggested (Giddings, *Ibid.*, 439). The variation of plate height with gas velocity and outlet pressure was fitted with a three parameter equation, and the values of the parameters were determined for different loads of stationary phase (De Ford and Ayers, *Ibid.*, 426). The three parameters represent axial diffusion in the gas phase, non-equilibrium in the gas phase and non-equilibrium in the liquid phase. A study was made of the spreading of air peaks in capillary and packed gas chromatography columns (Knox and McLaren, *Ibid.*, 449). The contribution of various factors to the plate height was examined. The factors which detract from the efficiency of large diam gas chromatography columns were discussed, and suggestions were made for improving their performance (Giddings, *J. Gas Chromatog. 1*(1), 12). The effect of different methods of packing gas chromatography columns on efficiency was investigated (Hupe and Mack, *Anal. Chem. 35*, 492). The loss of efficiency by large diam columns was attributed to a porosity gradient across the column diameter. Large diam columns with high efficiencies were prepared by special techniques. Impregnating the support after filling the column is no different from packing an impregnated support. Open tube columns with a thin coating of support on the walls for holding the stationary phase were found to have advantages for some separations (Halasz and Horwath, *Ibid.*, 499). The permeability of the columns was similar to a conventional open tube column, but the sample size could be increased. Capillary adsorption columns were prepared by wetting the insides of capillary tubes with a suspension of an adsorbent in a volatile liquid and then evaporating the liquid (Schwartz et al., *Ibid.*, 496). Impregnating a column support with liquid phase after packing it into columns was advocated. This was a particularly useful technique for rejuvenating old columns (Averill, *J. Gas Chromatog. 1*(1), 34). Capillary gas chromatography columns could be used with a small volume thermal conductivity detector with samples of 1 to 10 μl (Schwartz et al., *Ibid. 1*(1), 32). Details were given for the construction of a simple radio frequency oscillator which could be used for the rapid vaporization of liquid samples, the pyrolysis of solid samples, and as an alternative to the normal radioactive ionizing source in an argon detector. It could also be used to study the discharge reactions of small quantities of vapors such as might be isolated from gas chromatograms (Andrew et al., *Ibid.*, 27). The sensitivity of an ionization cross-section detector was improved 100 fold by reducing the sensing volume (Lovelock et al., *Anal. Chem. 35*, 460). It was pointed out that electron absorption detectors are capable of giving false responses, and the cause of these anomalous responses were described (Lovelock, *Ibid.*, 474). An alternative method by the pulse sampling technique which avoided these anomalies was described. The determination of the carbon skeleton of organic compounds by hydrogenolytic gas chromatography has been extended to at least C_{20} compounds by improvement of the catalyst. The value of the technique was illustrated with several kinds of compounds (Beroza and Sarmiento, *Ibid.*, 1353). Techniques for measuring tritium in the effluent from gas chromatography columns were given. If there is sufficient tritium in the sample the effluent is decomposed in a combustion and reduction train to give tritium gas which is counted directly. If the tritium in the sample is low the effluent is trapped on *p*-terphenyl crystals coated with silicone oil. These are transferred to a vial containing diphenyl-oxazole-toluene and assayed by liquid scintillation counting (Karmen et al., *Ibid.*, 536).

Extraction of the lipids from liver tissue with Bloor's reagent gave preparations with reproducible fatty acid patterns in gas chromatography. Extraction of the tissues with diethyl ether in a Soxhlet apparatus did not give satisfactory results (Sheppard, *JAOCS 40*, 545). The preparation of esters of highly unsaturated fatty acids from fish oils was found to proceed rapidly to quantitative yields with virtually no loss in double bond structure (Gauglitz and Lehman, *Ibid.*, 197). The subcommittee of the AOCS on the preparation of methyl esters from long chain fatty acids issued a preliminary report that favored the methanol-sulfuric acid and boron trifluoride methods (O'Connor and Chipault, *Ibid. 40*(3), 14). The same compositions by gas chromatography were obtained on several oils when the methyl esters were prepared by transesterification in methanol or by esterification of the free acids by diazomethane, boron trifluoride, methanol-sulfuric acid or methanol-hydrochloric acid (Kaufmann and Mankel, *Fette Seifen Anstrichmittel 65*, 179). The methyl esters of fatty acids were isolated from non-saponifiable components for gas chromatography by TLC (Ruggieri, *Nature 193*, 1282).

Free fatty acids could be separated efficiently by gas chromatography on columns packed with 20% diethylene glycol succinate polyester and 3% phosphoric acid on 60–80 mesh Celite (Metcalfe, *J. Gas Chromatog. 1*(1), 7). For quantitative results a series of calibration factors are derived for each new column. When free fatty acids are analyzed on Golay columns with Trimer Acid as a liquid phase, tailing of the peaks occurs. This can be avoided by adding small portions of dinonylnaphthalene-disulfonic acid to the Trimer Acid (Averill, *Ibid. 1*(1), 22). The formation of ghost peaks from previous injections when aqueous solutions of volatile fatty acids were analyzed by gas chromatography could be avoided by adding formic acid to the carrier gas. This allows quantitative analysis of these acids with a flame ionization detector (Adkmen and Burgher, *Anal. Chem. 35*, 647). The acetate, propionate, butyrate and valerate esters of β-cyclodextrin were advocated as a stationary phase for the gas chromatography of fatty acid esters and related compounds. The change of retention volume on the various cyclodextrin phases helps identify the compounds (Schlenk et al., *Ibid 34*, 1529). Polyester columns at ten different temp were used to analyze complex fatty ester mixtures. None of the conditions gave completely satisfactory resolution (Ackman, *J. Gas Chromatog. 1*(6), 11). The retention time of unsaturated fatty acid methyl esters on polyester columns was found to depend on the chain length, the number of double bonds, and the number of carbons from the last double bond to the methyl end of the chain. This served to form a basis for a modified equivalent chain length system which is independent of temp effects and is based on common monoenoates rather than saturated acids (Ackman, *JAOCS 40*, 558). Gas chromatography separation factors between methyl esters of unsaturated faty acids was shown to be a feasible means of tentative identification (Ackman, *Ibid.*, 564). The separation factors can be between positional isomers of one chain length and the same number of double bonds or between acids of one chain length and differing number of double bonds. The separation factors derived from methyl ester of fatty acids longer than C_{16} on organosilicone and polyester stationary phases were applied to the identification of the unsaturated C_{16} esters (Ackman and Jangaard, *Ibid.*, 744). The principles and application of the equivalent chain length method for identification of complex molecules and prediction of the structure of unknowns were reviewed (Miwa, *Ibid.*, 309). A straight line relation was found between the logarithm of the retention time and the number of carbon atoms in linolenic, arachidonic and eicosenoic acid methyl esters at different temperatures. This relation proved helpful in identifying fatty

acids in gas chromatographic data (Buoncristiani et al., *Olearia 3*, 99). The fatty acids of chaulmoogra oil were investigated by gas chromatography. The cyclopentyl fatty acids were identified on the basis of their retention time on two stationary phases of different polarities and present knowledge of their structure and occurrence (Zeman and Pokorny, *J. Chromatog. 10*, 15).

A method was given for the determination of cyclic monomers in fatty acid ester mixture by gas chromatography. The accuracy of the analysis was established by comparison with a crystallization method (Black and Eisenhauer, *JAOCS 40*, 272). Modification of gas chromatography methods for use in the analysis of partially hydrogenated and polymerized oils was reviewed (Rybicka, *Chem. Ind. (London) 1963*, 390). The separation of geometric isomers of unsaturated, hydroxy and epoxy fatty ester by gas chromatography was reviewed and new data was presented on the resolution of linolenate geometric isomer on polyester and Apiezon columns (Litchfield et al., *JAOCS 40*, 302). The separation of methyl oleate and elaidate was reported and methods were given for the preparation of high resolution columns. The identification and analysis of *cis* and *trans* isomers of oleic and palmitoleic acid by gas chromatography on capillary columns with a hydrogen flame and argon detector was discussed (Liberti, *Boll. Lab. Chim. Provincial (Bologna) 12*, 411). Dienes with one *trans* double bond were separated into groups of *cis,trans* and *trans,cis* isomers by gas chromatography (Sreenivasan et al., *JAOCS 40*, 45). The loss of short chain methyl esters during preparation for gas chromatography was eliminated by the presence of long chain fatty acids or docosane in the petroleum ether extraction solvent (Kaufmann and Mankel, *Fette Seifen Anstrichmittel 65*, 179). The presence of docosane interfered with the analysis of the higher fatty acid methyl esters, so a duplicate extraction without docosane was required. The short chain fatty acids from propionic to pelargonic were analyzed by gas chromatography as their butyl, phenacyl, and decyl esters to overcome losses due to volatility. The decyl ester procedure was favored (Craig et al., *JAOCS 40*, 61). Temp programmed gas chromatography was recommended for clean separation of the volatile fatty esters of butter fat (Morgantini, *Riv. Ital. Sostanze Grasse 40*, 49). Programming the column gas flow rate was also advocated for the separation short chain fatty acids in butter fat (Valussi and Cofleri, *Ibid. 12*, 617). The use of peak area for the quantitative evaluation of gas chromatograms was discussed, and correction factors were determined for use with fatty acid methyl esters (Kaufmann et al., *Fette Seifen Anstrichmittel 64*, 501). The peak area determination was considered to be the main source of error in quantitative determinations. Correction factors for the gas chromagraphic analysis of fatty acid methyl esters were derived from retention time data (Edwards and Marion, *JAOCS 40*, 299). A curvilinear relation was found between retention time and the size of the correction factor. This was assumed to be logarithmic and a curve was fit by regression analysis. A new curve is needed for each new column. The effect of instrument design, column preparation, temp programming and detection system on the quantitative analysis of long chain fatty acid methyl esters was considered (Horning et al., *Anal. Chem. 35*, 526). A method was given for the quantitative analysis of fatty acids in blood (Glaser et al., *Biochem. Z. 336*, 274). It depended on a synchronous double recording of the elution pattern to allow an exact determination of minor constituents.

A reverse phase circular paper chromatographic system with liquid paraffin as a stationary phase and aqueous methanol as the mobile phase was described (Viswanathan and Bai, *J. Chromatog. 7*, 507). Pyrogallol red, cresol red and cresolphthalein were suggested as suitable indicators for revealing the presence of fatty acids on paper chromatograms (Kwapniewski and Sliviok, *Riv. Ital. Sostanze Grasse 40*, 181). Resin acids were separated by paper chromatography in a silver ion system. The resin acid methyl esters are applied to glass fiber paper impregnated with hexadecane and the papers are developed with silver fluoroborate in aqueous methanol (Daniels and Enzell, *Acta. Chem Scand. 16*, 1530). Bromo and hydroxy derivatives of unsaturated fatty acids could be separated by TLC on silicic acid, and the *threo* and *erythro* isomers of the hydroxy acids could be separated (Sgoutas and Kummerow, *JAOCS 40*, 138). TLC was applied successfully to the separation of compounds with the same chain length but different hydroxyl number, compounds differing by four carbon atoms, compounds differing by a hydroxy vs. epoxy group, and *cis* and *trans* isomers (Subbarao et al., *J. Chromatog. 9*, 295). TLC was used to separate the sterioisomers of the ozonides of monounsaturated methyl esters (Privett and Nickell, *J. Lipid Res. 4*, 208).

A liquid column partition chromatography method was described for the quantitative determination of ricinoleic and dihydroxystearic acids in mixtures of normal saturated and unsaturated acids. The system consisted of a mixture of methanol-hexane-acetone on a silica gel column (Chobanov et al., *Compt. Rend. Acad. Bulgare Sci. 14*, 463). The use of liquid column partition chromatography in the preparation of unsaturated fatty acid methyl esters was illustrated (Privett and Nickell, *JAOCS 40*, 189). The methyl esters of oleic, linoleic and linolenic acids were separated cleanly by chromatography on silver ion columns. The silver ion was supported on ion exchange resin (Wurster et al., *Ibid.*, 513). The quantitative separation of *cis* and *trans*-monoenoic and polyenoic fatty acids in 30–100 mg quantities on silica gel impregnated with silver ion was repoted (DeVries, *Ibid.*, 184).

The analytical separations of "solid" fatty acids from "liquid" fatty acids by crystallization from dichloroethane and acetonitrile were compared with the Twitchell method using lead salts (Martinenghi, *Olearia 17*, 47). Isomeric dienes and monoenes produced by partial hydrogenation of linolenic acid were separated by a combination of low-temperature crystallization and countercurrent distribution (Sreenivasan et al., *JAOCS 40*, 45). *Cis,trans*-dienes were separated from *cis,cis*, and the conjugatable *cis,cis*-dienoates were partially separated from the *cis,cis* nonconjugatable ones. *Cis*-9-monoenoate was separated from *cis*-12, *cis*-15, and *trans*-monoenoate by crystallization. Countercurrent distribution partially separated the *cis*-12, *cis*-15, and *trans*-monoenoates. The mono-, di- and trienoate produced by the hydrogenation of methyl linolenate were separated by countercurrent distribution (CCD) and each of these fractions was further separated by CCD between petroleum ether and silver nitrate in methanol. The *cis* and *trans*-monoenoates were completely separated but only partial separation of the dienoates was achieved (Scholfield et al., *Anal. Chem. 35*, 386). The application of CCD to the fractionation of fatty acids and and fatty acid methyl esters was discussed (Therriault, *JAOCS 40*, 395). Plots of the rate of bromination (second order) and hydrogenation (pseudo first order) were used to find the number of components in an unknown mixture of unsaturated compounds. A linear section of the plot is obtained for each component (Siggia et al., *Anal. Chem. 35*, 362). The presence of β-keto fatty acid in butterfat was detected by formation of the pyrazolones with Girard's reagent T (Van der Ven et al., *J. Lipid Res. 4*, 91).

The carbon skeleton and other structural features of organic compounds were revealed by hydrogenolytic gas chromatography. By improving the catalyst, compounds up to C_{20} have been analyzed successfully (Beroza and Sarmiento, *Anal. Chem. 35*, 1353). The double bonds of unsaturated fatty acids were located by separating them into classes according to the number of double bonds by silver ion chromatography on thin-layer plates, separation of each class into pure chain lengths by gas chromatography, and reductive ozolysis of the fractions. The aldehydes obtained by reduction are analyzed by gas chromatography. The method can be used on less than 1 mg of lipid (Privett et al., *J. Lipid Res.* 4, 260). Partial oxidation of unsaturated fatty acids with peracids was investigated as a means of locating double bonds and as a means of distinguishing mono- and polyunsaturated fatty acids. The method is useful in locating the position of double bonds (Gunstone and Sykes, *Riv. Ital. Sostanze Grasse 11*, 561). X-ray diffraction patterns of single crystals of urea and thiourea adducts of long chain fatty acids were used to obtain structural information. The degree of branching, *cis trans* isomerism, and mol wt can be determined. The position of a methyl, hydroxy, keto or other side groups can be determined (Nicolaides and Laves, *JAOCS 40*, 400). The methyl esters of fish oil fatty acids were converted into urea adducts and examined by nuclear magnetic resonance. The double bonds were shown to be methylene interrupted and no ethylene interruption could be demonstrated. No terminal unsaturation was found (Hashimoto et al., *Ibid.*, 124). The usefulness of nuclear magnetic resonance in detecting branched chain and hydroxy fatty acid and mono- and diglycerides was investigated (Hopkins and Chisholm, *Rev. Natl. Res. Council (Canada)* No. 6816, 102).

MEASUREMENTS OF PHYSICAL PROPERTIES

Stereomodels were used to form hypotheses about the structure of lipoproteins. The rules determining the configuration of these models are discussed. Application of these rules to lipids of the myelin sheath showed that the two main classes, sphingolipids and phosphoglyceryl lipids, had similar configurations. This information was used to elaborate a structure for myelin (Vandenheuvel, *JAOCS 40*, 455).

The phenomena of melting in fatty materials was reviewed (Meskens, *Lab. Tech. 6*, 287). The proton magnetic resonance of ethyl stearate in the α-form and β-form was determined. The results suggest the α-form is composed partly of molecules in

a liquid-like state and partly of molecules constrained to rotate about their longitudinal axes (Grant and Williams, *Can. J. Chem. 41*, 378). The nuclear magnetic resonance (NMR) spectra of the α- and β-forms of tristearin and tripalmitin were obtained at various temp. The data support a hexagonal structure of the α-form (Nakajima, *J. Phys. Soc., Japan 16*, 1778). The NMR spectra and IR absorption spectra for the even numbered fatty acids from C_{10} to C_{18} near their melting points indicate that there is a breakdown of crystalline character and onset of liquid-like motion at scattered points in the solid structure several degrees below the melting point. This phenomena becomes more extensive as the melting point is approached (Barr et al., *Can. J. Chem. 41*, 1188). A simple method for obtaining a reproducible cooling curve was described and a microscopic technique was given for determining the melting point and transformation time of unstable polymorphic forms (Wilton and Wode, *JAOCS 40*, 707). For determining the melting point of cocoa butter the following tempering procedure was advocated: melt at 50–60C, filter, transfer to a capillary tube (inner diameter 1.0 ± 0.1 mm) at 50–60C, keep one hr at 16–18C, keep 24 hr at 23–4C, cool for one hour at 16–18C (Shimatani and Iwasaki, *Yukagaku 11*, 357). The solid/liquid ratio of soybean oil at various levels of hydrogenation was determined by wide-line NMR. The results were comparable to the absolute liquid/solid ratio and were slightly higher than values obtained by dilatometry (Ferren and Morse, *Food Technol. 17*, 112). Methods for measuring the consistency of butter, margarine, cheese, chocolate and other foods were reviewed (Janer, *Grasas Aceites (Seville, Spain) 13*, 216). The damping and deformation characteristics of suspension of tristearin in oil were measured at frequencies of 50 cps (Nederveen, *J. Colloid Sci. 18*, 276).

A modification of the trichromic technique for measuring oil colors was proposed that includes a transmittance measurement at 660 mμ to characterize the green color of some olive oils (Bigoni, *Riv. Ital. Sostanze Grasse 40*, 116). The determination of the mol wt of triglycerides by ultracentrifugation was carried out on peanut oil (Luck and Rickerl, *Fette Seifen Anstrichmittel 64*, 825). Mol wt in polar and non-polar solvents show no differences, but some association of molecules is observed at oil concentrations greater than 5–10%. A device was made for determining the density of solutions that could be used to measure the concn of oil in miscella (Yusupbekov, *Maslob.-Zhir. Prom. 12*, 33).

COMPOSITION AND CHARACTERISTICS: ANALYTICAL DATA

Many analyses of the composition and physical properties of fats and lipids were published in 1963. 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 unfractioned lipid mixtures. An asterisk (*) appearing after the reference indicates that fatty acid composition data are given. The second division contains data on certain lipid classes. The third division of the list gives measurements of physical properties. Many of the references in the previous section on methods contain information on the composition and physical properties of certain materials. These references have not been completely cross-listed in the present section. Reports which deal primarily with the effect of environmental, dietary and genetic factors on composition and physical properties are given in the succeeding section.

UNFRACTIONATED LIPIDS. Fatty acid analyses were given on the oils from commercial corn and wheat samples which had been damaged in various ways (Baker, *Cereal Chem. 39*, 393)*. The composition of the lipid extracted from oat hulls was examined (Pokorny et al., *Papers Inst. Chem. Tech. Prague 4-II*, 343)*. Oils obtained from the seed of flax grown for linen and for oil were quite similar (Alexa and Caraculacu, *Iassy 12*, 137). Changes of the lipid content and composition of potatoes during storage were reported (Mondy et al., *J. Ag. Food Chem. 11*, 328)*. The compositions of isano oils from various sources were compared (Pouliquen, *Peintures Pigments Vernis 38*(2), 69). A comparison of olive oils of different origins indicated that the amt of minor constituents vary considerably (Grieco, *Riv. Ital. Sostanze Grasse 9*, 432)*. Analyses on 518 samples of Greek olive oil showed that short-sized kernel olives gave oil of lower iodine value than large sized, but variations in climatic conditions had no effect (Anon. *Olive Oil Crop, 1961–1962 Ministry of Commerce Kingdom of Greece*). No lauric or myristic acids were found in pure olive oil and their presence in industrial oil was believed to be a result of autoxidation (Synodinois et al., *Rev. Franc. Corps Gras 10*(5) 285)*. The docosenoic acid of *Crambe abyssinica* was shown to be erucic acid (Miwa and Wolff, *JAOCS 40*, 742). The epoxyoleic acid and other fatty acids were measured in maturing *Vernonia anthelmintica (L.) willd.* (Miwa et al., *Ibid.*, 225)*. (+)-Threo-12, 13-dihydroxyoleic acid was isolated from the seed of *Vernonia anthelmintica (L.) willd.*, and the (−) isomer was obtained by acetolysis of the oil (Scott et al., *Ibid.*, 587). The oils of a number of *Vernonia* species were examined for oxygenated acids by reverse phase chromatography and the fatty acid composition of *Torresea cearensis* oil was reported (Badami and Gunstone, *J. Sci. Food Agr. 14*, 479). *Lesquerella densipila* oil was found to contain 12-hydroxy-*cis*-9, *cis*-15-octadecadienoic acid (Smith et al., *J. Org. Chem. 27*, 3112). *Spinacia oleracae* chloroplasts contained 70% polyenoic acids, and the presence of hexadecatrienoate and 3-*trans*-hexadecenoate was demonstrated (Debuch, *Experientia 18*, 61)*. Analyses were reported on tomato seed oil (Cescon and Giovetti, *Riv. Ital. Sostanze Grasse 7*, 349)*; coffee bean lipid (Calzolari and Cerma, *Ibid. 40*, 176)*; chaulmoogra oil (Gupta et al., *J. Sci. Food Agric.* 14, 457)*; and rubber seed oil (Azeimoddin and Rao, *Rubber Board Bull. 6*(2). *Chilopsis linearis* was found to contain octadeca-*trans*-9, *trans*-11, *cis*-13-trienoic acid and octadeca-10, 12-dienoic acid. (Hopkins and Chisholm, *Chem. Ind. (London) 1962*, 2064). *Catalpa ovata* was found to contain the same trienoate. The oils of a number of the *Bignoniaceae, Cucurbitaceae, Valerianaceae* and *Rosaceae* were examined (Hopkins and Chisholm, *Rev. Natl. Res. Council. Canada No. 6816*, 102). Punicic acid was discovered in *Momordica balsamina* and *M. charantia* contained α-elaeostearic acid. *Jacaranda mimosifolia D. don* was shown to contain *cis*-8, *trans*-10, *cis*-12-octadecatrienoate. (Chisholm and Hopkins, *J. Am. Chem. Soc. 27*, 3137). *Marshallia caespitosa nutt., Alyssum maritimum (L) lam.* and *Selemia grandis Martin* Seed Oils were rich in *cis*-11-eicosenoic acid (Mikolajczak et al., *JAOCS 40, 294*)*. Proximate analyses were carried out on the seed fat of *Acacia arabica, Citrullus vulgaris* var. *fistulosus, Sida cordifolia, Seasmum undicum,* and *Ricinus communis* (*Indian Oil Soap J. 28*(4), 83). Passion fruit seed yielded an oil suitable as soapstock and drying oil (Pruthi, *Ibid., 28*(3), 55). *Brassica pekinesis (hiroshimana)* and *Astragalus sinicus* oils were analyzed (Ueno and Hamada, *Yukagaku 12*, 358)*. *Comandra pallida A. DC.* and *Osyris alba L.* were found to contain ximenynic acid (Mikolajczak et al., *JAOCS 40*, 342)*. Analyses were reported on *Gevuina avellana mol., Lomatin hirsuta (Lam.) diels,* and *Embothrium coccieum forst* (Cattaneo et al., *Anales Asoc. Quim. Arg. 50*, 31)*; five species of *Cordyline,* two species of *Phormium,* and one of *Agavaceae* (Morice, *J. Sci. Food Agr. 13*, 666)*; *Bassia butyracea* seed (Agarwal et al., *J. Proc. Oil Technologists' Assoc. (Kanpur, India) 18*, 810)*; *Melia (Azadirachta) indica* or neem oil (Skellon et al., *J. Sci. Food Agr. 13*, 639)*; and the pollen of three *Pseudotsuga* and two *Pinus* species (Ching and Ching, *Science 138*, 890)*.

Three hydroxy fatty acids were reported among the lipids of *Serratia marcescens* (Bishop and Still, *J. Lipid Res. 4*, 81)*. Lactobacillic acid was found to be a major fatty acid of *Streptococcus lactis* (Macleod et al., *J. Bact. 83*, 806)*. Tubercle bacillus was found to contain 10–hexadecenoic acid, 10-methyl-9-hexadecenoic acid and 8- and 10-methyl hexadecanoic acids (Cason and Miller, *J. Biol. Chem. 238*, 853)*. Several branched chain fatty acids were isolated from *Bacillus subtilis* (Kaneda, *Ibid.*, 1222). They were 12-methyltetradecanoic, 14-methylhexadecanoic, isopentadecanoic, isopalmitic, isoheptadecanoic and isomyristic. *Azobobacter agilis* was found to contain 3-hydroxydecanoic, 3-hydroxydodecanoic and 2-hydroxydodecanoic acids (Kaneshiro and Marr, *Biochim. Biophys. Acta 70*, 271)*.

The fatty acid composition of various cooking oils and fats was determined by gas chromatography (Fleischman et al., *J. Am. Dietet. Assoc. 42*, 394)*. The linoleic, linolenic and arachidonic acid content of a number of vegetable oils and animal fats was determined (Blattna and Manouskova, *Nahrung 962*, 332)*. A detailed analysis of the fatty acids present in commercially rendered lard was given. Evidence was obtained for 29 fatty acids (Herb et al., *JAOCS 40*, 83)*. A quantitative comparison of commercially rendered, laboratory rendered, and solvent extracted lard from the same batch of pig tissue indicated that the minor components of lard were not artifacts (Magidman et al., *Ibid.*, 86)*. The composition of lard from different locations in pigs and the composition of male human depot fat on different rations was tabulated (Heyes, *Chem. Ind. (London) 1963*, 660)*. The composition and glyceride structure of lard was studied (Arnold & Milloy, *JAOCS 40*, 298)*. The fatty acid composition of bovine semen was reported (Dietz et al., *J. Dairy Sci., 46*, 468)*. The fatty acid composition of different parts of ox eye was given (Bartley et al., *Biochem. J. 85*, 332)*. Five species of ciliated protozoa *(Tetrahymenidae)* were analyzed and four were found rich in

γ-linolenic acid (Erwin and Bloch, *J. Biol. Chem. 238*, 1618)*. The 2-hydroxy fatty acids of rat spleen, kidney, lung, sciatic nerve and skin, and in bovine plasma were determined. None was found in liver and epididymal fat. The 2-hydroxy acids of brain were amide bound but were not in the gangliosides or sphingomyelin (Kishimoto and Radin, *J. Lipid Res., 4*, 139)*. The lipid of rat epidermis was analyzed at various stages of the hair growth cycle (Carruthers and Heining, *Proc. Soc. Exptl. Biol. Med., 112*, 278)*. The light and dark meat of bluefin tuna *(Thunnus thynnus)* was analyzed and a number of isomeric monoenoates were identified (Roubal, JAOCS 40, 213)*. Commercial canning operations caused no change in the fatty acid composition (*Ibid.* 215)*. The position of the double bonds of the fatty acids fractionated from skipper oil by gas chromatography were determined by ozonolysis and found to be similar to those of other fish oils (Ito and Fukuzumi, *Yukagaku 12*, 272)*. Gas chromatography and oxidative ozonolysis were used to examine the fatty acid composition of four species of fish from the La Plata River (Brenner et al., *Rev. Arg. Grassas Aceites 3*, 65)*. The species examined were *Pimelodus maculatus, Prochilodus lineatus, Salminus maxillosus*, and *Pseudoagenius brivifiliswere*. The composition of sardine, cuttlefish, oil-flat fish (*Cynopsetta dubia*), goby, tuna, sword-fish, cod-liver and shark-liver oils were reported (Ito and Fukuzumi, *Yukagaku 12*, 278)*. Proximate analyses were given for *Harengula Zunasi, Saurida argeyrophane* and half-beak oils (Ueno and Hamada, *Ibid., 11*, 526)*, and for the oil obtanied on broiling *Pagrosumus major* and drying *Engraulis japonica* (*Ibid.*, 630). The glyceride structure of *Mystus stenghala* visceral fat was investigated (Pathak and Reddy, *J. Sci. Food Agr. 14*, 395)*.

Heat treated butter was shown to contain the odd chain methyl ketones from 3–15 carbons while unheated butter contained only acetone (Taufel et al., *Fette Seifen Anstrichmittel 64*, 957)*. Butter fat was shown to contain six even carbon β-ketoacids believed to be precursors of the methyl ketones (Van der Ven et al., *J. Lipid Res. 4*, 91)*. Milk fat from the eland antelope (*Taurotragus Oryx*) was analyzed (Cmelik, *J. Sci. Food Agr. 13*, 662)*. Analyses were given for fourteen samples of hydrogenated rape seed oil suitable for margarine stock (Jakubowski et al., *Rev. Franc. Corps Gras 9*, 678)*; Russian margarine (Khomutov and Zolutareva, *Maslob.-Zhir. Prom. 12*, 15); cooking fats and oils (Fleishman et al., *J. Am. Dietet. Assoc. 42*, 394)*; and mayonnaise (Eastwood et al., *Ibid.*, 518)*. The composition of heat bodied linseed oil was examined by gas chromatography (Gast et al., *JAOCS 40*, 287)*.

FRACTIONATED LIPIDS. The sterol compositions of hop seed (*Humulus lupulus L.*) (Roberts, *Chem. Ind. (London) 1963*, 609)*, and rice bran oil (Takeshita and Watanabe, *Yukagaku 11*, 459) were examined. Squalene was isolated from olive leaves (Vazquez and Janer, *Grasas Aceites (Seville, Spain) 13*, 242), and the squalene content of 268 different olive oils was measured (Gracian et al., *Ibid. 14*, 101). Homo-olestranol (Martel and Gracian, *Ibid. 13*, 212) oleanic acid, and a triterpenoic acid similar to crategolic acid (Vioque and Maza, *Ibid. 14*, 9) were identified in olive oil. The D-glucosides of stigmasterol, sitosterol, and campestrol were isolated from tobacco and tobacco smoke (Kallianos et al., *Biochem. J. 87*, 596). Cholesterol was detected in the plants *Solanum tuberosum* and *Dioscorea spiculiflora* (Johnson et al., *Science 140*, 198). A ketone with properties similar to but not identical with calciferol was isolated from rice bran oil (Takeshita et al., *Yukagaku 11*, 269). Myristicin was isolated from parsley seed oil (*Petroselinum sativum*), and coriander seed (*Coriandrum sativum*) was found to be rich in petroselinic acid (Privett et al., *JAOCS 40*, 28). A detailed analysis was given of the hydrocarbons and unsaponifiables of *Neurospora crassa* (Davies et al., *Biochem. J. 87*, 326). The sterol precursors of corn seedlings and tare (*Vicia sativa*) were analyzed (Mercer et al., *Ibid.*, 317). Dried bran oil was analyzed and shown to contain oryzanol (Tanaka and Tsuchiya, *Yukagaku 12*, 26). Cytochrome and coenzyme Q were demonstrated in *Thiobacillus thiooxidans* and *Thiobacillus thioparus* (Cook and Umbreit, *Biochemistry 2*, 194).

The glyceride and phosphatide composition of barley, oats, and rye were reported (Showler, *J. Sci. Food Agr. 13*, 494)*. The phosphatides of sugar cane juice were examined (Friloux and Cashen, *J. Agr. Food Chem. 10*, 509). The composition of the glycerides and phosphatides of baobab seed (*Adansonia digitata*) was investigated (Cmelik, *J. Sci. Food Agr. 14*, 287)*. The unsaponifiable fraction, especially the glyceryl ethers, of cod liver oil was examined (Emmerie and Engel, *Fette Seifen Anstrichmittel 64*, 813). Ten volatile fatty acids were identified in apple wine (Sugisawa et al., *J. Food Sci. 27*, 435).

Analyses were reported on the following lipids: human adrenal gland (C. Riley, *Biochem. J. 87*, 500)*; pig epiphyseal plates (Bolognani, *Experientia 18*, 318)*; canine adrenal gland (Chang and Sweeley, *Biochemistry 2*, 592)*; and embryonic chick adipose tissue (Feldman et al., *Poultry Science 41*, 1851)*. An inverse relation was reported between the cholesterol content of egg yolk and yolk size (Nichols et al., *Proc. Soc. Exptl. Biol. Med. 112*, 378). A cholesterol-like material was found to be a major component of the lipid of *Trichomonas foetus* (Halevy, *Ibid. 113*, 47). Analyses on the complex lipids of the following materials were reported: ascites-carcinoma cells (Gray, *Biochem. J. 86*, 350)*; the whole tissue and cytoplasmic fractions of immature rat liver, kidney, uterus, and ovaries (Biezenski et al., *Biochem. Biophys. Acta 70*, 75); human blood thromboplastin (Bentley, *Proc. Soc. Exptl. Biol. Med. 111*, 757); human adult and infant erythrocytes and erythrocyte stroma (*Ibid.*, 591); human cerebrospinal fluid (Hack and Helmy, *Ibid.*, 421); rat brain (Long et al., *Biochem. J. 85*, 251); and hog carcass (Kuchmak and Dugan, *JAOCS 40*, 734). Egg lecithin was shown to be mainly α-saturated β-unsaturated (Privett et al., *J. Food Sci. 27*, 463)*. α-Glyceryl ether phospholipids were shown to be a constituent of terrestrial slug (*Arion ater*) (Thompson and Hanahan, *J. Biol. Chem. 238*, 2628). Glyceryl alkenyl ether diester and monoester have been demonstrated in the neutral lipid of starfish diverticulum (*Asterias forbesi*) (Bilbertson and Karnovsky, *Ibid.*, 893). The fluorescent lipid from human cardiac age pigment was analyzed (Hendley et al., *J. Gerontol. 18*, 144; 250). The distribution of fatty acids in milk glycerylphosphoryl choline and ethanolamine was determined (Hawke, *J. Lipid Res. 4*, 255)*.

The carotenoid content of sorghum was reduced 50% by weathering (Blessin et al., *Cereal Chem. 39*, 389). The ubiquinone, α- and γ-tocopherol, vitamin A, cholestra-3,5-dien-7-one and ergosterol contents of egg yolk during different stages of embryonic development were reported (Rennock et al., *Biochem. J. 85*, 530). The α- and γ-tocopherol content of filberts, walnuts, brazil nuts, pecans, almonds, peanuts, coconuts and chestnuts was measured (Lambertsen et al., *J. Sci. Food Agr. 13*, 617). The cell membrane of *Escherichia coli* was found to contain vitamin K and ubiquinone while that of *Micrococcus lysodeikticus* contained only vitamin K (Bishop and Kind, *Biochem. J. 85*, 550).

PHYSICAL PROPERTIES. Solubilities of trilaurin, trimyristin, tripalmitin, tristearin, and triolein in 90, 95.4, 98, and 100% ethanol were reported (Arnold et al., *JAOCS 40*, 33). Intercrystal forces were shown to be responsible for the consistency of plastic fats (Haighton, *Fette Seifen Anstrichmittel 65*, 479). The phase behavior of six binary mixtures of palmito-oleo triglycerides was studied as a means of predicting the physical properties of fat blends (Moran, *J. Appl. Chem. (London) 13*, 91). Mixtures of 2-oleo-palmitin and 1,3-di-oleopalmitin, particularly, showed evidence of steric factors favoring association. This may account for graininness and enhanced crystallization in certain palm oil-lard blends. The effect of hydrogenation on certain physical properties of peanut oils was discussed (Pokorny, *Papers Inst. Chem. Tech. Prague 3*, 347). X-ray diffraction studies on kettle wax show that it is not a simple phase but a mixture of curd, neat and lye (Vincent and Skoulios, *JAOCS 40*, 20). X-ray diffraction data was reported on single crystals of urea and thiourea adducts of various fatty acids, and this method was advocated for determining the structure of the fatty acid (Nicolaides and Laves, *Ibid.*, 400). Melting points were reported for alkyl and sterol esters of ferulic acid (Tamura et al., *Yukagaku 11*, 454). Electron diffraction studies on fatty acid monolayers were reported (Banerjee et al., *Nature 193*, 873). Surface pressure and surface potential vs. area measurements for monolayers of the myristyl and cetyl esters of oxalic, malonic, succinic, glutaric, adipic and pimelic were reported (Shereshefsky et al., *J. Phys. Chem. 66*, 1846). The properties of monolayers of fatty alcohols was studied and the existence of alcohol hydrates was demonstrated (Brooks and Alexander, *Ibid.*, 1851). The solubility behavior of monoglycerides in dilute, micellar bile-salt solutions was determined (Hofman, *Biochim. Biophys. Acta 70*, 306). The uptake by human α-lipoprotein of various lipids dispersed on Celite was examined, and the competition of various types of lipids and dyes for the protein binding sites was studied (Ashworth and Green, *Ibid.*, 68). The conductance, surface tension and solubilizing ability of 2-ethyl-, 2-butyl-, 2-isobutyl, 2-tertiary butyl- and 2-phenylmyristic acid was measured (Haque and Saha, *J. Indian Chem. Soc. 39*, 485). The IR spectrum of the hydroxyl group of several lipid compounds was shown to be related to the intramolecular environment (Eddy et al., *JAOCS 40*, 92). The effect of refining on the

visable spectra of oils was examined (Giordano and Pennati, *Olearia 5–6,* 168). The spectra of an oxidation product of γ-tocopherol believed to have been produced by reaction with autoxidized fats was reported (Shone, *Chem. Ind. (London) 1963,* 335). Raman spectra were given for a number of essential oils (Mohan, *Indian Oil Soap J. 28,* 171). A theory of monolayer rheology was described with particular reference to the problem of wave damping by surface active substances (Goodrich, *J. Phys. Chem. 66,* 1858). The rheology of olive paste and the effect of surfactants on it was studied (Brabender et al., *Grasas Aceites (Seville, Spain) 13,* 197).

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 composition and various proximate analyses of sunflower oils cultivated in different regions of Bulgaria were reported (Rankov et al., *Izv. Inst. Obsta. Neorg. Khim. Org. Khim., BAB 8,* 193). The differences were not great enough to influence technological properties. A study of the seed fats of *Acacia arabica, Citrullus vulgaris, var. fistulosus, Sida cordifolia, Sesamum undicum,* and *Ricnius communis* which were collected in the arid zone of India showed that the fatty acid composition reflects the genetic character of the plant and that environmental effects are quite limited (Gupta and Kapur, *Indian Oil Soap J. 28* (4), 83). Medium chain length hydroxy fatty acids which are components of the lipid of *Serratia marcescens* grown at 30C were greatly decreased in cells grown at 37C. There were also changes in a number of unidentified fatty acids with the temp of incubation (Bishop and Still, *J. Lipid Res. 4,* 87). The variation in the cholesterol content of White Leghorn hen eggs during the interval from August to February was determined. There were significant differences in the yolk cholesterol of the hens in the experiment and a significant change with season. The cholesterol content seemed to increase with the seasonal increase in yolk size, but for each sampling period there was a strong negative correlation between yolk size and cholesterol concentration (Harris and Wilcox, *Poultry Sci. 42,* 182). The I.V. of cod liver oil varied over a range of 20–30 units during an annual cycle (DeWitt, *J. Sci. Food Agr. 14,* 92). Max values were in winter with an abrupt decrease in March when the fish spawn. There was a slow increase during the summer. The change is a reflection of different levels of monoenoates and polyenoates in the oil. The saturated acid remains constant (DeWitt, *J. Sci. Food Agr. 14,* 92). The unsaturated fatty acids of milk fat were determined during an annual cycle on samples representing different regions of Canada (Riel, *J. Dairy Sci. 46,* 102).

Changes in the lipid classes of Raja flax and Indian safflower oil were followed during maturation of the plants (McKillican and Sims, *JAOCS 40,* 108). Phosphatides showed the greatest amt of change with maturity. An almond variety, *Prunus amygalus S* showed a diminishing iodine value during ripening (Galoppini and Lotti, *Olearia 5–6,* 164). This was caused by a decrease in linoleic acid. *Citrus sinensis* (orange) oil samples analyzed at different stages of maturity had a similar fatty acid composition (Hendrickson and Kesterson, *JAOCS 40,* 746). The oil content of pineapple orange seed was correlated with the moisture content. Free (+) threo-12,13-dihydroxy-*cis*-9-octadecenoic acid was the major component of *Vernonia anthelmintica (L) willd.* seed at intermediate stages of maturity, and is believed to be converted to an epoxy form before incorporation into glycerides (Miwa et al., *Ibid.,* 225). The normal course of fat accretion in male rats from birth to 3 years was followed (Zucker and Zucker, *J. Nutr. 80,* 6). The relative amt of fat and size of fat pads increased steadily throughout life. Similar studies on the phosphatides and cholesterol of rats showed that total cholesterol and phosphatides maintained a constant ratio for the first year and then the ratio increased (*Ibid.,* 20). The free cholesterol decreased steadily throughout life. These changes were influenced very little by the diets.

Yolk cholesterol but not serum cholesterol of White Leghorn hens was increased significantly by inclusion of cholesterol in the diets (Harris and Wilcox, *Poultry Sci. 42,* 186). The effect of the inclusion of cottonseed oil, acidulated cottonseed soap stocks and cottonseed still bottoms in the diets of hens on the quality of the eggs was tested. None of these materials was recommended for inclusion in hen's diets (Pepper et al., *Poultry Sci. 41,* 1943). The essential and saturated fatty acid content of eggs from hens fed either peanut oil or tallow was followed during the laying cycle of the hens. The amt of essential fatty acids declined for six months, rose slightly and then fell again. There was a difference of 2% in the essential fatty acid content of the eggs produced on the two diets. There was little change in the saturated fatty acid during the laying cycle or as a result of the two diets, but there was a difference of ca. 7% in the saturated fatty acid content of the body fat on the two diets (Coppock and Daniels, *J. Sci. Food Agr. 13,* 459). Hen's diets containing 0.2% or more cottonseed oil and 3 mg/day or more of gossypol resulted in eggs which discolored on storage, but diets with 0.1% cottonseed oil and 3 mg/day of gossypol did not (Kemmerer et al., *Poultry Sci. 42,* 893). A xanthophyll-rich material from the Aztec marigold, *Tagetes eracta,* gave good pigmentation of poultry skin and egg yolks when it was the only source of pigment in the diet. At 0.25% of the diet it supplied 13.6 mg% of xanthophyll/lb feed (Pino and Mendoze, *Ibid.,* 294). The fatty acid compositions of different pieces of chicken on diets containing corn oil, beef tallow and menhedan oil were compared. Appreciable quantities of long chain polyenoates were found in breast and thigh tissue but not in skin or adipose tissue. Each tissue tended toward a fatty acid composition reflecting the fat in the diet (Marion and Woodroof, *Ibid.,* 1202). The eggs and tissue lipids of hens on a low fat diet were compared with those supplemented with corn oil (Marion and Edwards, *Ibid. 41,* 1785). Corn oil increased egg wt, lowered serum triglyceride, lowered liver cholesterol, changed the ratio of free to esterified cholesterol, and increased the hatchability and progeny growth of fertile eggs. The inclusion of fat in hen's diets was found to have no effect on body wt, mortality, egg production, albumin quality or shell thickness. Egg size was reduced when 10% fat was added to the ration without increasing the energy level. This was attributed to decreased food intake on the high fat diet (March and Biely, *Ibid. 42,* 20). High level intakes of protein and corn oil by growing female chickens increased body wt and decreased the size and lipid content of the livers. The changes in liver lipid were largely in the glyceride and cholesterol ester fraction. Dietary protein level did not affect the composition of sexually mature birds. Corn oil diets increased the linoleic acid content of eggs, plasma, heart, liver and depot fat of mature birds at the expense of oleic and palmitoleic acids. Egg wt and production was not influenced by the dietary protein level but was increased by the added corn oil (Marion and Edwards, *J. Nutr. 79,* 53). A synergistic effect was found between selenium and tocopheryl acetate, selenium and DPPD, and selenium and cytine in the prevention of muscular dystrophy in chicks. These effects were markedly reduced by the presence of unsaturated fat in the rations (Hutchesen et al., *Ibid.,* 846). A study was made to determine if there was a relation between the deposition of vitamin A and copper in swine liver, and whether this would be effected by whole body gamma irradiation (Shirley et al., *J. Nutr. 78,* 454). Vitamin A supplementation decreased the deposition of copper in liver and copper supplementation increased the level of liver vitamin A. Gamma irradiation increased deposition of liver copper.

Nutritional factors affecting milk fat production were reviewed (Van Soest, *J. Dairy Sci. 46,* 204). Ingestion of soybean oil by cattle incraesed the stearic acid content of both milk fat and adipose tissue and the oleic acid of milk fat. Linoleic acid increased only slightly. Intravenous infusion of cottonseed oil caused dramatic increases in milk fat linoleate indicating that dietary unsaturated fatty acids are hydrogenated by rumen microflora (Tove and Mochrie, *Ibid.,* 686). Experiments reporting that either urea or linseed meal supplments in cattle rations caused a lowered milk fat production when compared with soybean meal supplements could not be confirmed (Loosli et al., *Ibid.,* 825).

Yolk cholesterol was found to vary among individual White Leghorn hens (Harris and Wilcox, *Poultry Sci. 42,* 178). NMR spectroscopy was used to evaluate the oil content of individual corn kernels in order to select kernels for breeding high oil content (Bauman et al., *Science 139,* 498). The oil content of the individual kernels was significantly correlated with that of their progeny indicating that this was a heritable trait.

The fat, protein, and solids-not-fat content of milk taken in the morning was lower than that of evening milk. Samples taken at intervals during a milking process showed that the fat percentage was lower in the first milk drawn. The interval between milkings had a highly significant effect on the percentage of fat (Gilmore and Gaunt, *J. Dairy Sci. 46,* 680). The milk, milk fat, and total solids production of cows milked at different intervals was followed throughout

the lactation period. No significant differences were found for milk fat and total solids yield. (Schmidt and Trimberger, *Ibid.*, 19). Castration caused a slight increase in the I.V. of cattle fat (Dahl, *J. Sci. Food Agr. 13*, 520). The increase was caused by a somewhat higher content of oleic acid and a lower content of stearic acid. Steers deposit a slightly yellower external fat than bulls and contain 16% fat as compared to 10% for bulls. External fat was more unsaturated than internal fat and this was caused by a higher conen of oleic and palmitoleic acid and a lower content of stearic acid in external fat. The effect of hair growth on the epidermal fat of plucked mice was examined (Carruthers and Heining, *Proc. Soc. Exptl. Biol. Med. 112*, 278). At the time of most rapid cell division in the epidermis there appears to be no significant change in triglyceride fatty acid composition.

DETECTION OF ADULTERATION

A reverse phase chromatographic procedure was developed which separated cholesterol acetate from phytosterol acetate. It could be used for the detection of mixtures of animal and vegetable fats (Peereboom and Beekes, *J. Chromatog. 9*, 316). A method was reported for detecting castor oil in vegetable oil. One ml filtered oil is dissolved in 10 ml petroleum ether containing 2% by volume of conen HCl. To this solution add one drop of a reagent composed of 1.25 g ammonium molybdate in 100 ml H_2SO_4. Castor oil is indicated by immediate turbidity while unadulterated oil remains clear (Kumar, *Oils, Oilseeds J. (Bombay) 16*, 6 and *JAOCS 40*, 80). The presence of 5% or more of animal fat in vegetable oils was detected by the presence of arachidonic acid (Gallardo and Sameh, *Rev. Arg. Grasas Aceites 3*, 105). The fatty acids to be tested are purified by formation of urea adducts. The arachidonic acid is eventually determined by alkali isomerization. The theory and use of the Boemer value was discussed (Meskens and Vanderkelen, *Lab. Tech. 8*, 103). Bellier indexes for 13 fats were determined (Arniges, *Lipidos 22*, 36). Different values were obtained when acetic acid was substituted for HCl in the procedure.

Gas chromatography of the fatty acids was used to determine the presence of more than 10% cottonseed oil in olive oil (Synodinois et al., *Rev. Franc. Corps Gras 10*, 285). The *trans* acid content of olive oil was determined by gas chromatography of the brominated methyl esters, but this method was not able to distinguish esterified oil from solvent extracted (Bigoni, *Riv. Ital. Sostanze Grasse 9*, 428). Yellow-colored virgin olive oil may be distinguished from deep green varieties by spectrophotometry (Giancaspro and Florio, *Ibid. 40*, 121). The absorption constant at 268 mμ after correction for background is never higher than 0.01 in virgin oil. The adulteration of virgin olive oil with re-esterified oil could be detected by pancreatic lipase hydrolysis of the glycerides (Mazuelos, *Grasas Aceites (Seville, Spain) 13*, 239). The re-esterified oil contained fewer saturated fatty acids on the 1 and 3 positions. Vizern's test detected as little as 5% sunflower seed, grape seed, or soybean oil and 10% cottonseed, corn or rapeseed oil in olive oil (Macchi, *Rev. Arg. Grasas Aceites 3*, 60).

The addition of 5% or more of hydrogenated fats to cocoa butter could be detected by the presence of *trans* fatty acids (Pokorny et al., *Papers Inst. Chem. Tech. Prague 4-II*, 313). Pressed cocoa butter can be distinguished from solvent extracted cocoa butter by their differential thermal cooling curves. The method requires only 0.3 g of sample and will detect the presence of 12% or more of extracted cocoa butter in pressed cocoa butter (Mathieu et al., *Rev. Franc. Corps Gras 10*, 123). UV spectrophotometry was used to differentiate lard and refined pork fat. The absorbancy of lard at 268 mμ was lower than that of refined fat, and the ratio of absorbancies at 232/268 mμ was lower for lard. As little as 15–20% adulteration of lard with refined white grease could be detected (Paolini, *Riv. Ital. Sostanze Grasse 6*, 294). The measurement of minor fatty acid constituents in lard by gas chromatography with a flame ionization detector was used to detect the adulteration of lard with tallow (Wolff, *Rev. Franc. Corps Gras 10* (4), 187). The method depends on the relative amt of C_{14}, C_{15}, C_{16}, C_{14} unsaturated and C_{15} branched fatty acids in the two fats. It is applicable for mixtures containing 5–50% tallow and has a precision of ca. 10%.

A number of paper dealt with the detection of pesticide residues in fats, especially milk fat. A summary of prseent methods was given. Separation of the pesticides from the lipid material is often the hardest step (Cook, *JAOCS 40*, 313). The limitations of current techniques were discussed and comparative analyses were performed (Henderson, *J. Assoc. Offic. Agr. Chemists 46*, 209). Microcoulometry tended to give higher results than Mills' paper chromatography technique, and electron capture gas chromatography gave lower results than either of the former tests. A procedure was given for the detection of DDT in fats and oils based on electron capture gas chromatography. As little as 0.1 ppm could be detected in a 1-g sample. A variable amt of an artifact having the same retention time as DDT limits the sensitivity of the method (Klein et al., *Ibid.*, 165). A simple method for separating the insecticide from fat was reported which depends on chromatographic separation on Florisil columns. Methylene chloride-petroleum ether mixtures were used to develop the column and give better separation than previous methods. The pesticides in the eluate was detected by paper chromatography (Moats, *Ibid.*, 172). A method for total chlorinated hydrocarbons was reported which depends on the reduction of organic chloride in the sample with sodium in liquid ammonia (Krzeminski and Landmann, *J. Agr. Food Chem. 11*, 81). The chloride ion was determined potentiometrically. A preliminary removal of inorganic chloride ion was required. A method for the detection of Kelthane was reported which depends on the alkaline decomposition of Kelthane to chloroform which can be determined colorimetrically (Gunter et al., *Ibid. 11*, 72). A comparison was made of two colorimetric methods for the determination of DDT in dairy products (Blinn and Gunther, *J. Assoc. Offic. Agr. Chemists 46*, 191). The method using an oxidative cleanup step was favored. A rapid method for determination of total organic chlorine was reported which depends on the neutron activation of organic chloride in a nuclear reactor and measuring the induced Cl^{38} which is formed (Schmitt and Zweig, *J. Agr. Food Chem. 10*, 481). Kelthane fed to dairy cattle at 1 ppm for 39 days gave no detectable residue in milk or body fats (Zweig et al., *Ibid.*, 72). When fed at 2 ppm for 71 days it gave 0.23–0.40 ppm Kelthane in the milk, and there was 1.07–2.70 ppm in the tissue fat at the end of the experiment. Small amt of heptachlor epoxide residue were found in the fatty tissues of cattle grazing on pastures treated with 0.25 lb heptachlor/acre. Gradually decreasing amt of the residue were found in biopsy samples of omental fat taken at intervals after the beginning of grazing. Residues were also found in cows allowed to graze at the time of aerial application of heptachlor (Rusoff et al., *Ibid. 11*, 289). The incorporation of the toxic factor in chick diets caused growth depression and accumulation of fluid in the pericardial sac, abdominal and subcutaneous regions (Flick et al., *Poultry Sci. 42*, 855). There was an inverse relation between hematocrit and level of toxic factor fed and a direct relation between hydropericardial fluid and toxic factor fed.

NUTRITION, PHYSIOLOGY, BIOCHEMISTRY

NUTRITION

MAN. Clinically healthy subjects with elevated serum cholesterol were placed on a diet high in linoleic acid. A decrease in serum cholesterol occurred (from 293–230 mg %). The diet produced an increase in percentage of linoleic acid in the serum cholesterol esters and triglycerides, with concomitant drops in percentages of saturated and oleic acids (Swell et al., *Proc. Soc. Exp. Biol. Med. 111*, 48). Substitution of a palatable mixed diet containing 45% of the calories as fat, 50% of this derived from PUFA, for an average American diet, produced a significant decrease in the level of plasma cholesterol and phospholipids in normal young women, as tested by values less than the critical level of 15%. No significant change occurred in the plasma glycerides. The increase in cholesterol linoleate which occurred in all subjects can be used as an index of short term diet adherence (Gunning et al., *J. Nutri. 79*, 85). Serum cholesterol, serum total and polyunsaturated fatty acids and diet of three populations were compared. Of two groups of African Negroes examined, one took 6.8 and the other 37.8% of its calories as lipids of vegetable origin. The third group (Negroes from Nashville) consumed 48.3% of its dietary calories as animal fats. The Nashville group had the highest serum cholesterol level, but there was little difference in the serum cholesterol of the other two groups (Roels et al., *Ibid. 79*, 211). Cholesterol fed to Guatemalan Indian children did not increase their low serum lipid and cholesterol levels (Mendez et al., *Ibid. 79*, 200). The effect of the level of dietary protein with and without added cholesterol on plasma cholesterol levels in man was investigated. Only the diets low in protein (5% of calories) caused a significant change in plasma cholesterol and this was in the form of an increase (Beveridge et al., *Ibid. 79*, 289). Marine trainees on 16 hr rigorous daily activity were fed a high calorie, high saturated-fat diet.

511). The total phospholipids from both the entire erythrocyte and the erythrocyte stroma of the newborn infant have been shown to be identical to the adult (Bentley, Jr., *Proc. Soc. Exp. Biol. Med. 111,* 591). The reasons for the lower morbidity of atherosclerosis of the aorta among the indigenous population of the Fergana Valley, Uzbek SSR were attributed to the predominance of carbohydrates and vegetable oils in the diet and in the climatic conditions peculiar to the region (Laufer, *Federation Proc. 22,* T59; *Arkhiv Pathologii 24* (4), 38).

ANIMALS. Citrus pectin with a methoxyl content of 10.7% when fed at a 5% level in the diet, largely counteracted the increment in plasma and liver cholesterol and liver total lipids induced by cholesterol feeding in the rat but was without significant effect in the cholesterol-fed rabbit, guinea pig and hamster (Wells and Ershoff, *Proc. Soc. Exp. Biol. Med. 111,* 147). Suggestive evidence that cholesterol feeding may increase the number of thymidine-H^3 labaled cells in the heart tissues of rabbits was presented (Spraragen et al., *Circulation Res. 11,* 982). Addition of 9% corn oil to the diet of rats receiving suboptimal protein and corn starch as a source of carbohydrate decreased food consumption and depressed growth. These effects were not observed if glucose replaced starch in the diet. Replacement of corn oil by other fats caused growth depression of rats receiving a starch diet. Linseed oil and cod liver oil also caused a growth depression in glucose low-protein diets (Howe and Gilfillan, *J. Nutri. 79,* 395). Lipid antioxidant activity of lipid-free tissue fractions, from control and selenium-fed chickens, rats and sheep was studied (Hamilton and Tappel, *Ibid. 79,* 493). Fat-fed rats exhibit increased ketone bodies in plasma, liver, kidney and heart muscle and this increase was greater in saturated fat-fed animals than those fed unsaturated fat. The citric acid level also was elevated in the saturated fat-fed animals (Brahmankar and Nath, *Proc. Soc. Exp. Biol. Med. 112,* 670). The concn of serum cholesterol in rats fed a diet containing cholesterol, cholic acid and hydrogenated cocoanut oil was reduced when ethionine was included in the diet. The addition of equal amt of thionine and methionine to this diet further reduced the cholesterol levels (Seidel and Harper, *J. Lipid Res. 4,* 75). The effects of feeding soybean oil oxidized at 180C for ten and twenty hr upon the body fat of the rat was investigated, with and without vitamin E supplementation. The body fats contained oxidation products of unsaturated fatty acids. The diene, triene and total polyenoic acid content of the body fat are discussed (Degkovitz and Long, *Fette Seifen Anstrichmittel 64,* 893). Cholesterol concn in serum, liver and carcass, as well as acetate-1-C^{14} incorporation rates, were investigated in a comparative study on mice, rats, hamsters and guinea pigs, using a single standard basal diet supplemented in various ways with cholesterol and bile acids (Beher et al., *J. Nutri. 79,* 523). Data are presented on the content of phosphatides, free and total cholesterol, and neutral fat in 152 male rats from birth to three years of age (Zucker and Zucker, *Ibid. 80,* 20). The normal course of fat accretion in male rats from birth to three years is described. The relative amt of fat and relative size of fat pads increased steadily throughout life in a manner closely related to body wt and independence of age (Zucker and Zucker, *Ibid. 80,* 6). Suckling rats show a level of serum cholesterol about twice as high as the level present in adult rats. No changes were observed in liver cholesterol (Bizzi et al., *J. Atheroscler. Res. 3,* 121). The effects of palmitic, oleic and linoleic acids on hepatic and vascular lipid deposits of rats are described (Morin et al., *J. Lipid Res. 4,* 351). Pure triglyceride from fresh menhaden oil was equivalent to lard in supporting growth of rats. Experimentally oxidized and bleached-oxidized oils caused steatitis, enlarged livers, anorexia and even death (Rasheed et al., *J. Nutri. 79,* 323). 22,25-Diazacholestanol caused a lowering of serum cholesterol and an accumulation of desmosterol in the serum liver and adrenals of rats (Dvornik and Kraml., *Proc. Soc. Exp. Biol. Med. 112,* 1012). Diets supplemented with shrimp caused hypercholesterolemia and aortic atherosclerosis in rabbits. Aortic cholesterol was as much as 60 times greater than in control rabbits (Conner et al., *J. Nutri. 79,* 443). A notch has been seen in lipid phosphorus, total cholesterol, lactic dehydrogenase or malic dehydrogenase, but showed increases in triglycerides and isocitric dehydrogenase (Calvy et al., *J. Am. Med. Assoc. 183,* 1). A study of total serum cholesterol, and its relation to certain factors, particularly age, wt and somato type was conducted on 476 healthy men between the ages of 17 and 44 years. There appeared to be a general increase in cholesterol level with aging (Fischer, *J. Am. Dietet. Assoc. 12,* They did not show significant changes in serum uric acid, the electrocardiograms of rats who were fed diets deficient in essential fatty acids. It can be prevented by the addition of small amt of linoleate, linolenate or arachidonate to the diet (Caster and Ahn, *Science 139,* 1213). Dietary linoleate fed in excess of 1% of calories maintained good growth and cured fat deficiency. Dietary arachidonate was three times more effective than linoleate. Dietary linolenate did not support wt gain or cure fat deficiency symptoms as much as linoleate or arachidonate (Mohrhauer and Holman, *J. Lipid Res. 4,* 151). Di-α-tocopherone, a metabolite of α-tocopherol, was shown to have only 5% of the activity of α-tocopherol (Lee et al., *Proc. Soc. Exp. Biol. Med. 113,* 242). Sesame oil (I.V. 110), mustard oil (I.V. 104) and cocoanut oil (I.V. 9) were fed to Rhesus monkeys at a 20% level in the diet for eight months. Some of the monkeys were also fed cholesterol along with the oils for eight months. Plasma cholesterol of monkeys fed different oils did not change considerably during the experiment; plasma cholesterol of monkeys fed cholesterol along with the oils increased considerably but the increase was highest when sesame oil was fed (Banerjee and Bandyopadhyay, *Ibid. 113,* 541). Intravenous administration of heterologous DNA to rats did not cause any change in serum cholesterol; nor did it alter the increase in cholesterol in rats receiving a hypercholesterolemic diet (Lamonthezie et al., *Ibid. 113,* 27). The influence of dietary linoleate, arachidonate and linolenate on erythrocytes and depot fats of rat was investigated. Linoleate and linolenate are incorporated into depot fat. All three dietary fats caused a decrease in the 20:3 to 20:4 ratio. The composition of the unsaturated fatty acids of erythrocyte lipids is readily altered. The concn of 20:3 is lowered by all three (Mohrhauer and Holman, *J. Lipid Res. 4,* 346). It was found that the polyunsaturated fatty acids of cuttlefish oil could not substitute for linoleic acid for its role in growth and cholesterol metabolism (Kaneda and Alfin-Slater, *JAOCS 40,* 336). Fish were readily depleted of tissue polyunsaturated acids. Linoleic and linolenic acid were converted to more highly unsaturated acids when fed at 1% level in the diet, but only to a slight degree when fed at 5% level (Reiser et al., *Ibid. 40,* 507). Amylase stearate, amylase palmitate, amylase oleate, distearin adipate and glycerol adipate were found to be low in digestibility and caloric availability. Diolein fumarate was completely digested but poorly utilized (Booth and Gros, *Ibid. 40,* 551). Acetostearin fed rats showed retarded growth and poor survival (Coleman et al., *Ibid. 40,* 737). Growth, nitrogen balance, carcass analysis and liver fat studies were made on rats fed low protein, low fat diets with sucrose or dextrin as the dietary carbohydrate (Wiener et al., *J. Nutri. 80,* 279). Two experiments are reported comparing linseed meal with soybean meal, combinations of linseed meal and urea or a combination of several other protein-rich feeds (Loosli et al., *J. Dairy Sci. 46,* 285). The ingestion of soybean oil by cows resulted in a distinct increase in stearic acid of both milk fat and adipose tissue, but oleic acid increased only in the milk fat. The results show that dietary unsaturated fatty acids are hydrogenated by rumen microflora, then deposited by the animal (Tove and Mochrie, *Ibid. 46,* 686). Literature on certain environmental and physiological factors known to affect the fat content of milk are reviewed and these are compared in light of their apparent effects on the over-all efficiency and performance of cattle (Van Soest, *Ibid. 46,* 204). Variations in percentage of protein, milk fat and solids-not-fat between milkings and the milking process in three breeds of cows were studied (Gilmore and Gaunt, *Ibid. 43,* 680). The effect of a fat-free diet and of different dietary fatty acids (palmitate, oleate and linoleate) on the fatty acid composition of fresh-water fish lipids is described (Brenner et al., *J. Lipid Res. 4,* 341). A marked depigmentation was observed in the skin of chinook salmon-fry fed a fat-free diet since hatching. A similar effect was observed when triolein or linolenic acid was included in the diet but was prevented by trilinolein (Nicolaides and Woodall, *J. Nutri. 78,* 431). Wt gains and vitamin A storage studies in rats fed β-carotene, treated with N_2O_4, indicate that the material was neither toxic nor had any vitamin A activity (Emerick and Lievan, *Ibid. 79,* 168). A study was made to determine 1) whether dietary vitamin A had an effect on the level of copper deposition in the liver; 2) whether dietary copper had had an effect on the deposition of vitamin A in the liver; and 3) whole body γ irradiation had an effect on the deposition of vitamin A and copper in the liver of swine (Shirley et al., *Ibid. 78,* 454). In vitamin E deficient but not in starved rabbits, a significant increase in inulin and sucrose space of skeletal muscle was found (Diehl and Birset, *Proc. Soc. Exp. Biol. Med. 112,* 173). The influence of a fat-deficient diet and *N,N'*-diphenyl-*P*-phenylenediamine (DPPD) on vitamin E deficiency was studied in the rhesus monkey. Fat deficiency did not prevent the monkeys from developing the full syndrome of vitamin E deficiency. DPPD had a beneficial effect on the vitamin E-deficient monkeys (Fitch and Dinning, *J. Nutri. 79,* 69).

BIRDS. Genetic parameters in respect to serum cholesterol level were estimated on random bred White Leghorns (Wilcox et al., *Poultry Sci. 42,* 37). The effects of adding fat, with and without a concomitant increase in energy level, to the ration fed to 11-months old laying birds are reported (March and Biely, *Ibid. 42,* 20). Genetic variation of egg cholesterol and the relationship between yolk cholesterol and a number of physiological traits have been studied in a random-bred strain of White Leghorns (Harris and Wilcox, *Ibid. 42,* 178). The influence of season on egg yolk cholesterol was determined throughout the laying year at bimonthly intervals. The conen of yolk cholesterol increased from August to February after which it decreased in April and June (Harris and Wilcox, *Ibid. 42,* 182). Egg yolk and serum cholesterol levels were determined before and after 11 days of feeding crystalline cholesterol to White Leghorns. The addition of 1, 2 and 4% dietary cholesterol resulted in increases of 49, 42 and 50% yolk cholesterol. Serum cholesterol was increased 20, 38 and 35%, respectively (Harris and Wilcox, *Ibid. 42,* 186). Serum cholesterol was significantly higher in chicks fed vitamin B_6-deficient diets than in those receiving diets with adequate vitamin B_6. Aortas of chicks fed vitamin B_6-deficient diets weighed more than those from chicks fed adequate vitamin B_6 (Daghir and Ballorin, *Ibid. 41,* 1868). The capacity of sterols of different structural types to spare the normal dietary cholesterol requirement of *Dermestes vulpinus* had been studied (Clayton and Block, *J. Biol. Chem. 238,* 586). A high level of quaternary ammonium anion exchange resin (cholesteryamine) when fed to hens decreased plasma cholesterol and egg yolk pigmentation but did not significantly affect egg yolk cholesterol levels (McGinnis and Ringer, *Poultry Sci. 42,* 394). It has been shown that fatty acid esters can be added to chicken diets satisfactorily (Dangouman and Debruyne, *Rev. Franc. Corps. Gras. 10*(5), 259). Results of studies on the nutritive value of raw passion fruit *(passiflora edulis sims.)* seed oil and its blend with peanut oil (50/50) have been presented and discussed. Feeding the raw oil or blended oil at the 10% level in a 15% casein diet resulted in a better retention of calcium and phosphorus than in the fat-free diet. No difference was noticed in nitrogen assimilation, blood analysis, liver wt, liver lipids, ash content, etc. (Pruthi, *Indian Oileeds J. 7*(1), 60). 5% Corn oil sterols in the diet of cockerels retarded the atherogenesis that was accentuated by the addition of egg oil or egg powder in the diet. It was suggested that the beneficial effects were not entirely due to interference with cholesterol (Fisher et al., *Proc. Soc. Exp. Biol. Med. 113,* 415). Synergistic relationships exist between selenium and tocopherol acetate, selenium and DPPD, selenium and L-cysteine in the prevention of muscular dystrophy in chicks. An inhibitory effect of the presence of linoleic acid in the diet was shown (Hutcheson et al., *Poultry Sci.42,* 846). The basal diet of hens was supplemented by 4% arachis oil or 4% tallow. The arachis ration supplied 2.52% EFA and the tallow ration, 1.45%. The influence of this diet and husbandry on the nutritional value of the egg is described (Coppock and Daniels, *J. Sci. Food Agr. 13,* 459). White Leghorn pullets were fed practical diets containing different cereal grains with and without corn oil. During the first 6–8 weeks of production, corn oil addition improved average egg wt but did not stimulate egg wt significantly when compared to an isocaloric diet containing tallow (Shutze, *Poultry Sci. 41,* 1846). The effects of the dietary inclusion of cottonseed oil, acidulated cottonseed soapstocks and cottonseed still bottoms on the interior quality of eggs stored at either 30 or 60F. On the basis of the data collected, it would seem unwise to incorporate any of the three products into diets for laying hens (Pepper et al., *Ibid. 41,* 1943). Two trials, each of 70 days duration, have been conducted with fat deficient laying hens to determine the influence of isocalorically adding corn oil to a low fat diet on various measures of lipid metabolism (Marion and Edwards Jr., *Ibid. 41,* 1785). A high level of protein and the presence of corn oil in the diet of growing female chickens each increased body wt, decreased the size and lipid content of the liver and decreased the incorporation of sodium acetate-1-C^{14} into liver lipids. Egg wt and egg production were not influenced by dietary protein level but higher values for these were noted when corn oil was added to the diet (Marion and Edwards, *J. Nutri. 79,* 53). Using a technique for separate urine and feces collection, it has been demonstrated that urinary lipid excretion was not increased by feeding a ration containing 10% rape seed oil to growing chickens. Apparent digestibility and utilization coefficients for total lipids in the rapeseed oil ration were 84 ± 1.3 and 83 ± 1.3%, respectively (Sell and McKirdy, *Poultry Sci. 42,* 380). A detailed description of a biological assay which has proved to be satisfactory for measuring the metabolizable energy content of poultry feed ingredients is given. The results show that available metabolizable energy values for feed fats are subject to a number of errors and are therefore of doubtful practical value (Sibbel and Slinger, *Ibid. 42,* 313). Two-tenths per cent or more cottonseed oil in the diet and 3 mg/day or more of gossypol resulted in discoloration of stored eggs but 0.1% cottonseed oil and same amount of gossypol did not (Kemmerer et al., *Ibid. 42,* 893). The effect of age of chicks on their sensitivity to raw and under-processed soybean oil meals was studied (Bornstein and Lipstein, *Ibid. 42,* 61). The amt of vitamin A acid required to prevent vitamin A deficiency symptoms in chicks depended upon the time dosing began relative to the onset of symptoms. Experiments with C^{14} vitamin A acid confirmed the rapid metabolism or destruction which begins in the digestive tract (Krishnamurthy et al., *J. Nutri. 79,* 503). A comparison was made of utilization by chicks of α-carotene and vitamin A with and without added furazolidone and ethoxyquin (Parrish et al., *Ibid. 79,* 9). *Coccidiosis* did not appear to increase the chicks' requirement for vitamin K (Harms et al., *Poultry Sci. 41,* 1836). The nutritional requirement of a *Lactobacillus plantarum* variant found in the intestinal flow of chicks for unsaturated acids in the presence of biotin is specific. The significance of this in chick nutrition is discussed (Zygmunt et al., *Ibid. 42,* 194).

Patented preparations of interest to the nutritionist were: A lecithinated, spray dried, free flowing powder containing 30–60% lecithin, 10% edible shortening oil and 30–60% of a coating material (Obenauf and Taber, *U.S. 3,060,030*). Radioactive iodinated (I^{131}) fatty material admixed with wax-like material in capsule (Numerof and Knoll, *U.S. 3,061,510*). A dicalcium-phosphate medicant tablet containing a fatty acid monoglyceride (Goldman, *U.S. 3,054,723*). Stable fat-soluble vitamin compositions (Hochberg and Ely, *U.S. 3,067,104*). Stable and biologically available and effective vitamin-containing compositions (Ralish and Hochberg, *U.S. 3,067,105*). An excipient of dermatological use comprising a mixture of hydrogenated tallow (15–40%) and perhydrosqualene (85–60%) (Monot, *U.S. 3,069,324*). An aqueous dispersible lecithin composition (Cogswell, *U.S. 3,069,361*). Parenteral aqueous solutions of fat-soluble vitamins (Mullins and Macek, *U.S. 3,070,499*). A stable alcoholic emulsion of grape sugar (Buer, *U.S. 3,070,500*). Sterile aqueous solutions of vitamin D and calcium salts and method for making the same (Schenk, *U.S. 3,089,822*). A stable aqueous vitamin A oil emulsion (Czarnecki, *U.S. 3,089,823*). Stabilization of carotenoid material (Reiners and Morgan, *U.S. 3,081,171*). Method for preparing a superior phosphatide emulsifying agent suited for use in parenteral preparations (Elenbagen, *U.S. 3,081,320*). Oil-in-water emulsion for oral administration and process for preparation (Wruhle, *U.S. 3,085,939*). Method of increasing blood clotting time with a liver lipid (Dailey and Colescott, *U.S. 3,089,820*).

PHYSIOLOGY

Digestion, Intestinal Absorption, and Excretion

The absorption of oils thermally oxidized at 180C, protein efficiency and liver function were determined. Other physiological effects were measured and discussed in detail (Kieckebusch et al., *Fette Seifen Anstrichmittel 64,* 1154). A measure of the lymph fatty acids in rats indicate that thermal oxidation decreases the absorption of unsaturated fats (Bhalerao et al., *J. Dairy Sci. 46,* 176). Experiments were conducted to study *in vivo* the over-all fatty acid specificity of the mechanisms involved in chylomicron cholesterol ester and triglyceride formation during fat absorption in the rat. The process of fatty acid absorption and chylomicron triglyceride fromation displaced no specificity for one fatty acid relative to another. In contrast, chylomicron cholesterol ester formation showed marked specificity for oleic acid (Karmen et al., *J. Lipid Res. 4,* 312). Chylomicron lecithin formation showed a marked specificity for stearic acid and a lesser specificity for linoleic acid. Oleic acid was incorporated least of all into lecithin (Whyte et al., *Ibid. 4,* 322). In contrast to earlier results no impairment of fat absorption was found in rats with subcutaneous growth of Walker Carcinoma 256, as measured by the amt of I^{131}-labeled triolein in thoracic duct lymph (Baker, *Cancer Res. 23,* 928). Guinea pigs given more than 5 ml 50% glycerol solution in saline daily died with acute toxic symptoms. Rabbits tolerated at least 10 ml daily. No change in plasma or cell cholesterol levels was observed in either species of animals. Probably, intake of glycerol is accompanied by anemia in the guinea pig (Ostwald, *Proc. Soc. Exp. Biol. Med. 111,* 632). Rats were maintained on a purified diet containing 20% fat as hydrogenated cottonseed oil, corn oil or cocoanut oil and then injected intravenously with a tracer dose of cholesterol-4-C^{14}. In the six days following injection excretion of C^{14} was observed in animals maintained on hydrogenated cottonseed oil. Cocoanut oil-fed rats excreted smaller amt of cholesterol (Coniglio et al., *Ibid. 112,* 140). Effects of adding animal tallow, minerals or

both to a basal non-fat milk solids diet for calves were determined. Added minerals increased incidence of diarrhea in calves whereas animal tallow decreased its incidence. Added fat delayed the rate of abomasal evacuation (Bush et al., *J. Dairy Sci. 46,* 703). α-Tocopherol-C^{14} was administered orally to rats and chicks, and its distribution in the body and rate of excretion were determined at short intervals up to 24 hr and at longer intervals to 21 days (Krishnamurty and Bieri, *J. Lipid Res. 4,* 330). The absorption of dietary vitamin A and cholesterol by 6- to 7-week old chickens was studied under various conditions. It was found that there is mutual interference between vitamin A and cholesterol during the course of absorption across the intestinal wall (March and Biety, *J. Nutri. 79,* 474). The direct effect of several blood cholesterol-lowering agents on intestinal absorption of cholesterol-4-C^{14} was studied in the lymph-fistula rats. MK-135 and pectin caused reductions in lymph total cholesterol and absorption of cholesterol-4-C^{14}. Cholesterol trimethyl acetate, nicotinuric acid and pyridine-3-acetic acid had no effect on cholesterol absorption or lpmph cholesterol levels. The percentage esterification of absorbed cholesterol was constant, irrespective of the extent of absorption (Hyun et al., *Proc. Soc. Exp. Biol. Med. 112,* 496). Coprostanol-C^{14}, biosynthetically prepared, was administered orally to two patients and cholestanol-4-C^{14} was administered to one of them 10 months later. The dynamic behavior of absorbed radioactive cholestanol was identical with that of orally ingested cholesterol-4-C^{14}, including the esterification process. After five days, over 50% of both compounds had been excreted in the feces (Rosenfeld et al., *J. Lipid Res. 4,* 337). A method for studying the turnover of deoxy cholic acid in the rabbit is described. The mean values for half-life, pool size and daily production of deoxy cholic acid were 6.8 days, 700 mg and 73.4 mg, respectively. Of the bile acid pool, 97–98% was present in liver, gall bladder and G.I. tract. A large amt (10%) was present in the stomach. Fecal excretion was the main excretory pathway for bile acids (Hellström and Sjövall, *Ibid. 3,* 397). The half-life, pool size and daily synthesis of deoxy cholic acid in ten rabbits on the control diet and on a semisynthetic diet containing hydrogenated cocoanut oil have been determined. Fecal excretion of bile acids was reduced in rabbits fed the semisynthetic diets, whereas the urinary excretion was the same as on the control diet. The dietary effects on bile acid metabolism are discussed (Hellström et al., *Ibid.* 412). A palatable, stable, fluid oil-in-water emulsion for oral administration comprising a protective colloid, an edible unsaturated oil and sitosterol was described (Wruble et al., *U.S. 3,085,939*). An antithromboplastic liver lipid which is nontoxic on oral and intravenous administration, prolongs *in vitro* whole blood clotting time, and inhibits thromboplastic generation (Dailey and Colescott, *U.S. 3,089,820*).

Lipid Transport and Body Fat

Triglyceride increase in the liver after ethionine administration is shown to depend on both blocking of the release from, and fat mobilization into the liver from the periphery (Campagnari et al., *Proc. Soc. Exp. Biol. Med. 111,* 479). Unsaturated fatty acids in the diet exert a profound influence on the level of alkaline phosphatase in the plasma of rats with biliary obstruction but not in dogs (Butenandt, *Ibid. 111,* 409). Oil emulsions containing a fluorescent dye were given intravenously to rats. Rate of removal from the blood steram was typical for reticuloendothelial clearance of a colloid. The emulsion accumulated in the liver but appeared in the bile duct and duodenum within minutes after injection. One half of the dye was found in the intestinal contents (Wilkins, *Ibid. 112,* 953). The hypothesis of an impaired transport of neutral fat from the liver of fasting choline deficient animals is sustained by the isotopic data showing a persistently high activity in the liver neutral fat and low plasma triglyceride activity (Arvidson and Borgström, *Ibid. 112,* 676). It is suggested that the adenine analogue, 4-aminopyrazalopyrimidine causes fatty livers in mice by inhibiting the secretion of triglycerides from the liver (Henderson, *J. Lipid Res. 4,* 68). Chylomicrons labeled with C^{14}-glycerol-H^3-palmitic acid obtained from the cannulated thoracic duct of a rat given C^{14}-glycerol triolein and H^3-palmitic acid, were injected intravenously into male rats. The major part of the chylomicron glyceride leaves the circulating blood without hydrolysis. The liver triglycerides showed a C^{14}/H^3-ratio close to 1.0 during the first ten min and the ratio declined rapidly. The conclusion is that chylomicron glyceride is taken up intact by the liver, but is rapidly metabolized with re-esterification of the fatty acids to unlabeled glycerol (Olivecrona, *Ibid. 3,* 439). When fat-body tissue from locusts was incubated with palmitate-1-C^{14} in phosphate-saline the acid was readily taken up by the tissue: 80–90% of that taken up was esterified and recovered in the glyceride fraction. When the prelabeled tissue was incubated in hemolymph, glycerides were released from the tissue into the medium (Tietz, *Ibid. 3,* 421). Palmitic acid-1-C^{14} was given intravenously to volunteer subjects before, and toward the end of 35-45 min exercise. The results indicate that exercise accelerates the afflux of plasma free fatty acids and then, secondarily, increases immobilization of free fatty acid from depots (Friedberg et al., *Ibid., 4,* 34). A major metabolic defect induced by CCl_4 administration to the rat appears to be inhibition of the outward transport of hepatic triglycerides. This inhibition of outward triglyceride transport may be the etiology of the fatty liver associated with CCl_4 poisoning (Heimberg et al., *J. Biol. Chem. 237,* 3623). A chylomicron-containing, low-density lipoprotein fraction of thoracic duct chyle obtained from rats fed either cholesterol-4-C^{14} or palmitic acid-1-C^{14} was injected intravenously into rats. The disappearance curves for the labeled cholesterol differed strikingly from those for the labeled triglyceride. The latter first declined rapidly and then more slowly. The former consisted of three phases: 1) rapid decline, 2) rising and 3) leveling off (Naidoo et al., *J. Lipid Res. 3,* 309). Lipemia clearance was observed in dogs of four age groups after a meal containing 1 g lard, in a meat patty/pound body wt (Sobel and Thomas, *Proc. Soc. Exp. Biol. Med. 112,* 206). The accumulation of triglycerides in liver of choline deficient rats is not due to an impairment of the movement of triglyceride from the liver to the plasma, unlike that which follows administration of hepatotoxic agents (Lombardi and Schotz, *Ibid. 112,* 400). The effects of insulin and epinephrine on free fatty acid and glycerol production by rat epididymal tissue in the presence and absence of glucose were studied (Tingas and Ball, *Biochemistry 2,* 283). The influence of hair growth cycle on the triglyceride fatty acid composition of mouse epidermis was studied. At the time of most rapid cell division in the epidermis (two-four days post-plucking) there appears to be no significant change in triglyceride fatty acid composition (Carruthers and Heining, *Proc. Soc. Exp. Biol. Med. 112,* 278). A stable heat sterilizable aqueous solution of a vitamin D compound and a non-toxic calcium salt suitable for parenteral administration is described (Schlenk, *U.S. 3,822*). Arteriovenous measurements across the mammary glands of lactating goats showed that most of the fatty acids of the milk which comes from the blood lipids are derived from the triglycerides of the chylomicron and low-density lipoproteins (Robinson et al., *Biochem. J. 87,* 238). Uptake and release of plasma free fatty acids by the tissues of the rabbit were investigated by measuring the arteriovenous difference in plasma FFA concn across the perirenal fat depot intestines and mesentery, leg, kidney and liver (Hirsch et al., *J. Lipid Res. 4,* 289). The influence of *D*- and *L*-triiodothyronine and propyl thiouracil (PTU) on turnover rate and pool size of bile acids in rats on a normal diet has been investigated. No significant difference was observed between half-lives of bile acids in normal thyroid hormone-treated or PTU-treated rats (Strand, *Ibid. 4,* 305). Liver slices from rats which had been made deficient in vitamin E were unable to reaccumulate potassium removed by leaching in the cold (McLean, *Biochem. J. 87,* 164). The contents of 2-hydroxy fatty acids in rat spleen, kidney, lung, seratic nerve and skin and in bovine plasma were determined. Two groups of hydroxy acids, short chain (C_{16}) and long chain (C_{24}) were present. The presence or absence of these in other tissues is discussed (Kishimoto and Radin, *J. Lipid Res. 4,* 139). The effect of castration on composition of the depot fats of monozygous twin cattle is described. Castration is followed by a slight increase of the I.V. of perinephric, mesenteric and subcutaneous fats. Differences between fatty acid composition of external and internal fats are given (Dahl, *J. Sci. Food Agr. 13,* 520). In rats during pregnancy plasma vitamin A decreased and liver vitamin A increased but both returned to prepregnant values by the second postpartum day. It was suggested that changes in plasma vitamin A levels were related to transport and secondarily to the hyperlipidemia of pregnancy (Dannenburg et al., *Proc. Soc. Exp. Biol. Med. 113,* 605). The fatty acid composition of embryonic adipose tissue resembles that of egg fat and not of the adult chicken. Triglyceride fatty acids do not reflect the changes associated with embryonic development. The phospholipid fatty acids fluctuate widely and appear to be related to the onset of triglyceride synthesis and piping of the shell (Feldman et al., *Poultry Sci. 41,* 1851). Under aerobic conditions rat epididymal adipose tissue from fasted refed rats produced glycerol at a rate ten times greater than tissue from normally fed animals but had a lower tissue FFA level. Anaerobic incubation reduced the formation of FFA and glycerol but increased lactate production in both types of tissue (Ball and Jungas, *Biochemistry 2,* 586).

Lipid Metabolism In The Intact Animal

Pigs irradiated with 350 γ bilaterally at the rate of 11 γ/min nine ft from source survived an average of 254 hr. A moderate vitamin A deficiency had no effect on irradiation damage; in

fact, the vitamin A-low pigs lived an average of two days longer (Meacham et al., *Proc. Soc. Exp. Biol. Med. 111*, 30). Adult male Swiss mice sustained a profound fat loss during growth of Krebs-2 tumor. Fat loss occurred in three stages (Costa and Holland, *Cancer Res. 22*, 1081). Western Wethers with ligated reticulo-omascular orifices were used to conduct ruminal fat studies using linoleic-1-C^{14} acid. Tagged compounds appeared in the jugular blood about four hr after dosing and decreased rapidly between the eighth and twelfth hr. Most of the activity appeared in the non-steam distillable fraction, indicating the compound absorbed from the rumen contained 10 or more carbons (Wood et al., *J. Nutri. 79*, 62). Studies were performed concerning the relative distribution of fatty acids in the blood serum of adult beagle dogs, German shorthair and mongrel puppies that received diets deficient in linoleic acid or vitamin B_6 or both. The presence or absence of linoleic acid caused marked changes in the fatty acid spectrum in the serum whereas the presence or absence of pyridoxine in the diet did not influence the relative content of arachidonic acid in the blood serum. Apparently the conversion of linoleic to arachidonic acid is possible without the presence of vitamin B_6 in the diet (Söderhjelm, *Ibid. 78*, 438). The conen of linoleic acid in milk lipids was increased by infusing two cows with a cottonseed oil emulsion. Infusing 150 g cottonseed oil tripled the linoleic acid content of the milk fat in the next milking. Fatty acid distribution and oxidative stability of milk are discussed (Smith et al., *J. Dairy Sci. 46*, 7). *N*-methyl derivatives of ethanolamine were markedly effective in preventing the elevation of liver fat due to 1 mg 2-amino-2 methyl propanol (Longmore et al., *J. Nutri. 78*, 295). Castrate female beagles were found to have a greater maximum percentage of administered triolein I^{131} in circulation than intact females. Curves representing blood radioactivity plotted against time were similar after castration to those of some conditions in the human in which deranged lipid metabolism is implicated (Dost and Dickson, *Proc. Soc. Exp. Biol. Med. 111*, 674). The total quantities of fatty acids released during a four-hr incubation period of epididymal adipose tissue from various groups of rats fed crude and purified diets containing different fats were not significantly different. However, adipose tissue fatty acids were released in proportions different from those initially present in the parent tissue (Di Giorgio et al., *J. Nutri. 78*, 384). Dexamethasone at very low conen increased the release of fatty acid by incubated parametrial and mesenteric adipose tissue. The effects of several glucocorticoids on metabolism of adipose tissue are described (Fain et al., *J. Biol. Chem. 238*, 54). A simplified technique for measuring plasma cholesterol-C^{14} in dogs after intravenous injection, using liquid scintillation counting of an alcohol-acetone extract of plasma, is described. Excretory rate constants of 1.5 and 1.6%/day of the retained dose were derived. The size of the "exchangeable cholesterol pool" increased as serum cholesterol rose, either as the result of a high fat or of thyroidectomy (Casdorph et al., *Proc. Soc. Exp. Biol. Med. 112*, 191). Plasma FFA conen under the influence of a sympathetic blocking agent, guanethidine, and the effect of this blocking agent on the rise in FFA evoked by administration of epinephrine and norepinephrine in rabbits were studied. The rise of plasma FFA evoked by administered epinephrine was somewhat counteracted by the blocking agent (Konttinen and Rajasalmi, *Ibid. 112*, 723). Studies on the thermogenic role of brown fat in rats exposed to cold suggested that arousal of the animal by cold is induced by sympathetically activated thermogensis in areas of brown fat so located, relative to the vasculature, that heat is transferred to the thoracic structures and cervical spinal regions (Smith and Hock, *Science 140*, 199). Turnover rate of free fatty acid was studied during rest and exercise in normals and diabetics using Palmitate-1-C^{14}. During rest the turnover rate of free fatty acid did not differ in normals and diabetics (Ford et al., *Proc. Soc. Exp. Biol. Med. 113*, 177).

BIOCHEMISTRY

Analytical and Methodology

Cholesterol was identified in *Solanum tuberosum* and *Dioscorea spiculiflora* plants (Johnson et al., *Science 140*, 198). Circulating thyroid hormones were shown to occur predominantly bound to proteins of density higher than 1.23 g/ml (Toro-Goyco and Cancio, *Ibid. 139*, 761). Distillation of body water and extraction of fat are performed in one operation based on the lower specific gravity and higher boiling point of toluene as compared with water (Wolff and Bakay, *Proc. Soc. Exp. Biol. Med. 112*, 524). Non-polar lipid classes are separated by column chromatography using factice, a hydrophobic polymer as stationary phase and aqueous acetone as the mobile phase (Hirsch, *J. Lipid Res., 4*, 1). Improvements are described for the isolation of phosphatidylethanolamine from hen's egg and the preparation of racemic dioleoyl-phosphatidyl-ethanolamine. Both preparations were accelerators in the Hicks-Pitney test and moderately active in the thromboplastin generation test (Turner et al., *Ibid. 4*, 52). Milk lipase was purified 250-fold. Some physical constants are given (Chanadan and Shahani, *J. Dairy Sci. 46*, 275). Deacylation of phospholipids with lithium hydroxide in chloroform methanol (2:8) will cause the hydrolysis of not more than 0.1% phosphate ester bond (Brockerhoff, *J. Lipid Res. 4*, 96). Studies on the stereochemistry of phytosphingosine established the structure as D-ribo-1, 3, 4-trihydroxy-2-amino-octadecane and dehydrophytosphingosine as D-ribo-1, 3, 4-trihydroxy-2-amino-8-*trans*-octadecene (Carter and Hendrickson, *Biochemistry 2*, 389). A method for the determination of vitamin A levels using trifluoroacetic acid is described. A typical Carr-Price color without the turbidity and film forming properties of $SbCl_3$ is formed (Neeld and Pearson, *J. Nutri. 79*, 454). A method for the isolation of 2-hydroxy fatty acids is described. The acids are precipitated as the copper chelate. The copper can then be determined by a colorimertic determination of copper or the acids can be liberated and determined by gas chromatography of their methyl ester acetates (Kishimoto and Radin, *J. Lipid Res. 4*, 130). A rapid and sensitive method for the analysis of plasmalogen in the presence of free aldehydes is proposed (Warner and Lands, *Ibid. 4*, 216). A method is presented for the quantitative gravimetric isolation of cholesterol and cholestanol as the digitonides and for the quantitative recovery of these sterols (Sperry, *Ibid. 4*, 221). The correlation of GLC behavior and structure of 90 steroids was made (Brooks and Hanaineh, *Biochem. J. 87*, 151). Liver slices from vitamin E deficient rats were unable to reaccumulate potassium removed by leaving in the cold, and also showed a falling off of oxygen uptake. These effects were not reversed by feeding selenium but were reversed by feeding α-tocopherol or by the addition of antioxidants to the incubation solution (McLean, *Ibid. 87*, 164). All cis-docosa-, 4,7,10,13,16,19-hexaenoic acid has been isolated from cattle retina (Hands and Bartley, *Ibid. 87*, 263). Adrenal lipids of the dog were found to be approx 90% neutral lipids. The cholesterol esters were mainly unsaturated containing high proportions of 8,11,14-eicosatrienoic, arachidonic acid and adrenic acid (7,10,13,16-docosatetraenoic acid). Arachidonic and adrenic acids were also found in all fractions of the phosphatides (Chang and Sweeley, *Biochemistry 2*, 592). A method was given for gas chromatographic analysis of BHA and BHT (Anderson and Nelson, *Food Technol. 17*, 95).

Approx half of the fatty acids of *azotobacter agilis* are hydroxy fatty acids. These were identified as 3-hydroxydecanoic and 2- and 3-hydroxydodecanoic acids (Kaneshira and Marr, *Biochim. Biophys. Acta 70*, 271). An apparatus and mathematical treatment of linear and non-linear gradient elution and its application to the column chromatography of phosphatides is described (Walloch and Nordby, *Ibid. 70*, 188). A simplified procedure for the isolation of docosahexaenoic acid was reported (Stout, *JAOCS 40*, 40). The quantitative analysis of short chain fatty acids by gas chromatography was described (Craig et al., *Ibid. 40*, 63). The ozonalysis of small samples of lipids and their thin-layer and gas chromatography and application to structural analysis of triglycerides and lecithins were reported (Privett and Blank, *Ibid. 40*, 70). Fatty acids of lard were studied by silicic acid and gas chromatography (Herb et al., *Ibid. 40*, 83, 86). Changes in lipid classes were studied in maturing Flax and Safflower oils (McKillican and Sims, *Ibid. 40*, 108). The use of NMR for the determination of the structure of the fatty acids of fish oils was described (Hashimoto et al., *Ibid. 40*, 124).

TLC of bromo- and hydroxystearic acids was presented (Sgoutas and Kummerow, *Ibid. 40*, 138). A significant advance in chromatography was the quantitative separations of higher fatty acid methyl esters by adsorption chromatography on silica impregnated with silver nitrate (DeVires, *Ibid. 40*, 184). The preparation of highly purified fatty acids by column partition and silver nitrate-silica partition was reported (Privett and Nickell, *Ibid. 40*, 189). TLC methods for the separation of brain phosphatides was reported (Horracks, *Ibid. 40*, 237).

A symposium on methodology of fats and oils included the following: The chemical and biological assay of essential fatty acids (Williams and Reiser, *Ibid. 40*, 237), the determination of glyceride structure (VanderWal, *Ibid. 40*, 242), the determination of polymers in fats and oils (Firestone, *Ibid. 40*, 247), the analysis of *cis-trans* fatty acid isomers using GLC (Litchfield et al., *Ibid. 40*, 302). Identification of peaks in GLC chromatography (Miwa, *Ibid. 40*, 309) and assay of insecticides and herbicides in fats and oils (Cook, *Ibid. 40*, 313). A symposium on *in vivo* antioxidants and polyunsaturated acid metabolism included the following: the application of GLC to the determination of vitamin E and K (Nair and

Turner, *Ibid. 40,* 353); effects of *in vivo* antioxidants in feeding menhaden oil to swine (Oldfield et al., *Ibid. 40,* 357); dietary antioxidants in young swine (Hill, *Ibid. 40,* 360); metabolic effects of selenium as related to vitamin E (Bieri and Andrews, *Ibid. 40,* 365), and the biological consequences of feeding polyunsaturated fatty acids to antioxidant-deficient animals (Machlin, *Ibid. 40,* 368). Near IR spectroscopic differentiation of 1, 2- and 1, 3-diglycerides was presented (Susi et al., *Ibid. 40,* 329). A symposium on special methods in lipid chemistry included the following: Fractionation of Lipids by Countercurrent distribution (Therriault, *Ibid. 40,* 395); The use of X-ray diffraction studies of urea and thiourea adducts (Nicolaides and Laves, *Ibid. 40,* 400); Acid-Treated Florisil as an adsorbent for column chromatography (Carroll, *Ibid. 40,* 413); Special methods of purifying fatty acids (Pelick et al., *Ibid. 40,* 419); Lipid composition of beef brain, beef liver and the sea anemone (Rouser et al., *Ibid. 40,* 425) and the lipids of the myelin sheath of nerve (Vandenheuvel, *Ibid. 40,* 455).

Butterfat triglycerides were determined by direct gas chromatographic analysis (Kuksis et al., *Ibid. 40,* 530). For the syntheses of mono-, di- or triglycerides acyl migration was minimized by detritylation in petroleum ether (Jackson and Lundberg, *Ibid. 40,* 502). Diethyl ether Soxhlet extraction of liver was shown to be inadequate for extraction of lipids for gas chromatography (Sheppard, *Ibid. 40,* 545).

Using gas chromatography it was found that the equilibrium composition of *cis* and *trans* isomers obtained by isomerizing oleic, linoleic and linolenic acids with selenium or nitrous acid was 75–80% *trans* double bonds (Litchfield et al., *Ibid. 40,* 553). Structural correlation of unsaturated fatty acids with gas chromatographic retention times was made (Ackman, *Ibid. 40,* 558). Triglyceride mixtures were separated by TLC on silica impregnated with silver nitrate (Barrett et al., *Ibid. 40,* 580).

The separation of glycerides was accomplished by liquid-liquid column partition chromatography (Black and Hammond, *Ibid. 40,* 575). The relationship between optical activity and structure of triglycerides was discussed. (Litchfield and Reiser, *Ibid. 40,* 751).

Lipid Biosynthesis and Biooxidation

Tetrolyl-CoA and propiolyl-CoA have been shown to be strong non-competitive inhibitors of fatty acid synthesis. Palmityl CoA and free CoA have also been shown to inhibit fatty acid synthesis and to block the condensation of acetyl-CoA with malonyl CoA and the reduction of Crotonyl CoA to butyrate (Robinson, et al., *J. Lipid Res. 4,* 144). A study of the cerebroside fatty acids in weanling rats indicated that the cerebroside fatty acids are made by a chain lengthening process from one or more shorter acids. The C_{23} acid is made by two enzyme systems, one being a 1-carbon degradation of the C_{24} acid. The 18:0 of cerebrosides and 16:0 of total brain were apparently made directly from acetate while the 18:0 of whole brain resembled the cerebroside longer acids (Hajra and Radin, *Ibid. 4,* 270). Intraperitoneal injection of methyl linolenate-1-C^{14} into help bass, *Paralablax clathratus,* indicated that linolenic acid was converted to eicosapentaenoic acid and the latter converted to docosahexaenoic acid (Kayama, et al., *JAOCS 40,* 499). The rapid incorporation of citrate into fatty acids has been demonstrated with high-speed supernatant preparations of lactating mammary gland. Malonate is incorporated into fatty acids slower than citrate or acetate. The rate-limiting step is the activation of malonate to malonyl CoA (Spencer and Lowenstein, *J. Biol. Chem. 237,* 3640).

The effect of phenethylbiguanide on triglyceride synthesis has been studied *in vitro.* In intact tissue, PEBG interferes with the conversion of diglyceride to triglyceride. In homogenates, it increases the incorporation of labeled fatty acids into triglyceride, thus differing from insulin in its effect on triglyceride synthesis (Long et al., *Proc. Soc. Exptl. Biol. Med. 111,* 775). Incorporation of acetate-1-C^{14} into lipids of vacuolated rat liver cells occurs at a normal rate (Gaje and Bernelli-Tazzera, *Experientia 18,* 122).

The action of hypoglycemic compounds on the biosynthesis of cholesterol has been investigated *in vitro* with both acetate-1-C^{14} and mevalonate-2-C^{14}. Their incorporation into cholesterol is inhibited by all compounds tested (McDonald and Dalidowicz, *Biochemistry 1,* 1187). The biosynthesis of squalene has been demonstrated in peas *(Pisum sativum),* a flowering plant, for the first time (Capstack, et al., *Ibid. 1,* 1178). Mevalonic acid-2-C^{14} was injected into the yolk of incubating fertile hen's eggs on the sixth day of incubation. A high level of labeled squalene was found in the yolk sac non-saponifiable fraction (Camerino and Wright, *J. Lipid Res. 3,* 416). Brain slices were shown to incorporate potassium mevalonate to a greater extent than mevalonolactone into unsaponifiable and digitonin-precipitable material. Similar data were observed in brain homogenates. In liver, this effect was not observed (Fumagalli, et al., *Arch. Biochem. Biophys. 99,* 529).

Rat liver microsomes catalyze the transfer of acids from their acyl coenzyme A derivatives to the β-hydroxyl group of α′-acylglycerophosphoryl choline to form lecithin. The unsaturated acyl CoA ester react more rapidly than the saturated derivatives (Lands and Merkl, *J. Biol. Chem. 238,* 898). Stereospecifically labeled radioactive diacylglycerophosphoryl ethanolamine may be synthesized from either α′- or β-acylglycerophosphoryl ethanolamine by use of a rat liver microsomal preparation. Linoleate was a better substrate than stearate in esterifying α′-acylglycerophosphoryl ethanolamine, whereas the reverse was true with the β-acyl-precursor (Merkl and Lands, *Ibid. 238,* 905). Approx 50 and 25% of the isotope in sphingosine after administration of DL-serine-3-C^{14} was on carbon atoms 1 and 2, respectively; when tritium oxide was the source of label, the activity in sphingosine, sphingolipid fatty acids and cholesterol was greatest during the period of myelination and diminished soon afterward (Weiss, *Ibid. 238,* 1953). In rats, (C^{14}-glucose and (C_2)-C^{14} pyruvate give rise to labeled inositol, both free and lipid bound, in brain, liver and kidney, but no incorporation was obtained with (1-C^{14}) acetate or (6-C^{14}) glucuronate (Hauser, *Biochem. Biophys. Acta 70,* 278). A simple method for the preparation of DL-palmityl-, DL-octanoyl-, and DL-butyryl carnitine is reported. On incubation with mitochondria from tissues of the rat and catalytic amt of succinate, the acyl group of one of the optical isomers (L isomer) undergoes complete oxidation to CO_2 and H_2O (Bremer, *J. Biol. Chem. 237,* 3628). The oxidation of cholesterol-26-C^{14}, sodium propionate-1, -2 or -3-C^{14} and sodium octanoate-1-C^{14} by liver mitochondrial preparations from intact and gonadectomized rats of both sexes, and from intact and gonadectomized rats of both sexes treated with androgens and estrogens, has been studied. Androgen treatment of female rats slightly depressed cholesterol oxidation, but ovariectomy had no effect. Cholesterol oxidation by preparations of normal male or female rat liver mitochondria was inhibited by sex hormones added *in vitro* (Kritchevsky et al., *J. Lipid Res. 4,* 188).

A *Lactobacillus plantarum* variant, isolated from chicks, requires unsaturated fatty acids in the presence of biotin for growth (Zygmunt, et al., *Poultry Sci. 42,* 194). Single doses of dinitrophenol, oligomycin, arsenate and bilirubin inhibit the synthesis of phospholipids in the liver of rats (Youngs and Cornatzer, *Proc. Soc. Exp. Biol. Med. 112,* 308). Polymorphonuclear leukocytes and monocytes from guinea pig exudate show an uptake of oxygen and palmitate when palmitate is added to the medium. The uptake of oxygen and palmitate was inhibited by sodium fluoride and iodoacetic acid, but unaffected by cyanide and dinitrophenol (Evans and Mueller, *J. Lipid Res. 4,* 39). A partially purified preparation from rat brain reduces acetoacetyl CoA, D(−)-β-hydroxybutyryl CoA and crotonyl CoA to butyrate and on the addition of malonyl CoA, palmitate is formed. Degradation studies indicate the four carbon units are found in carbon atoms 13–16 of the palmitic acid molecule (Robinson et al., *J. Biol. Chem. 238,* 528). Palmitic acid was incorporated into liver phosphatides, with the exception of phosphatidylserine. A pronounced decrease in palmitate incorporation into phosphatidylethanolamine occurred in fatty liver after cerium administration (Glenn et al., *Ibid. 238,* 1249). Soluble bacterial synthetase systems *Clostridium kluyveri* and *Escherichia coli* catalyze the synthesis of long chain fatty acids from malonyl CoA, acetyl CoA, and TPNH. Over-all syntheses is dependent on the presence of the enzymes of the condensation reaction (Goldman et al., *Ibid. 238,* 1255). Lipid antioxidant activity of lipid-free tissue fractions was increased by the addition of dietary selenium to chickens, rats and sheep (Hamilton and Tappel, *J. Nutri. 79,* 493). Extracts of either *Serratia marcescens* or *Clostridium butyricum* form of either *Serratia marcescens* or *clostridium butyricum* form labeled cyclopropane fatty acids when they are incubated with S-adenosylmethionine labeled in the methyl group. Methionine, formate, formaldehyde and serine are not effective precursors (Zalkin et al., *J. Biol. Chem. 238,* 1242). Essential fatty acid deficiency in rats results in an increased oxidation of practically all of the citric cycle intermediates, pyruvate and caprylate by liver homogenates (Smith and De Luca, *J. Nutri. 79,* 416). Using intramammary infusion it was demonstrated that cholesterol esters, glycerides and phospholipids are all synthesized from a common pool in the gland. A remarkably active role is indicated for cholesterol esters (Patton and McCarthy, *J. Dairy Sci. 46,* 396). Fatty acids were synthesized in a medium enriched with tritiated water. The major portion of radioactivity was found in the odd carbons of palmitic and stearic acids. This result suggests an exchange reaction occurs between reduced triphosphopyridine nucleotide and water (Foster and Bloom, *J. Biol. Chem. 238,* 888). A phospholipid

fraction was isolated from heart muscle which activates a partially purified preparation of cytochrome C oxidase. The active fraction is composed primarily of lysophosphatides (Cohen and Wainio, *Ibid. 238,* 879). The liver of the fat-fed rat has a diminished capacity to incorporate acetate carbon into fatty acids. The depressed conversion was observable within two hr after the enteral administration of 2 ml corn oil. After four hr, the depression was pronounced (Bortz, *Ibid. 238,* 1266). The rates of lipolysis and of fatty acid esterification were estimated by measurement of net changes in glycerol fatty acids in epididymal fat pads of rats. Epinephrine, glucagon, adrenocorticotropic hormone and thyroid stimulating hormone stimulate both lipolyses and fatty acid esterification. Growth hormone and norepinephrine increased the rate of lipolysis (Vaughan and Steinberg, *J. Lipid Res. 4,* 193). Androgen treatment of female rats slightly depresses cholesterol oxidation but ovariectomy had no effect. Cholesterol oxidation by preparations of normal male or female rat liver mitochondria was inhibited by sex hormones added *in vitro* (Kritchevsky et al., *Ibid. 4,* 188). Various derivatives of hemoglobin and myoglobin were found to be active catalysts for unsaturated lipid oxidation. There were induction periods with some of the ferrous forms which were found to be in the ferric state as soon as oxidation started (Brown et al., *Arch. Biochem. Biophys. 101,* 14). Phytoene and not eycopensene is the first C_{40} compound formed in cartenoid biosynthesis. Lycopersene could not be found in maize seedlings (Mercer et al., *Biochem. J. 87,* 317) or in *Neurospora crassa* (Davies et al., *Ibid. 87,* 326). Cartilaginous rudiments of chick embryo grown in the presence of 3.3 μg vitamin A/ml release an acid protease into the culture medium. Vitamin A also released a protease from a particulate preparation obtained from bovine and chick cartilage. Vitamin A when added in excess of normal requirements causes a change in the permeability or stability of intracellular particles (Fell and Dingle, *Biochem. J. 87,* 403). Four of five species of the ciliated protozoa (family *Tetrahymenidae*) contain 30% or more of γ-linoleic acid. Evidence is presented for the metabolic pathway stearate → oleate → linoleate → γ-linolenate. Fatty acid biosynthetic reactions of both plant and animal tissues are pointed out (Erwin and Bloch, *J. Biol. Chem. 238,* 1618). A pure homogenous milk lipase was found to have the following characteristics: pH optimum of 9.0–9.2, opt temp of 37, unstable at temp of 20,30,37 and 45, the enzyme hydrolyzes both milk fat and tributyrin but not ethyl acetate or ethyl decanoate. Simultaneous loss of milk fat and tributyrin activities indicate that both substrates may be catalyzed by a single enzyme (Chandan and Shahani, *J. Dairy Sci. 46,* 503). It is suggested that catalytic stimulation by carnitine of long-chain fatty acid oxidation is mediated via acylcarnitine, with subsequent transfer of the acyl group to CoA at the site of the fatty oxidase system (Fritz and Yue, *J. Lipid Res. 4,* 279). Studies of the incorporation of (C^{14}) palmitate in subcellular particles of normal and regenerating rat liver suggest a multiplicity of pathways of incorporation of fatty acid into glycerolipids and changes in metabolism with altered tissue physiology (Johnson and Kerur, *Biochem. Biophys. Acta. 70,* 152).

Propionate labeled with C^{14} in each of its carbons was incubated with slices prepared from lactating rat mammary glands. In the absence of added glucose, carbon 2 of propionate was more rapidly incorporated into fatty acids than either carbons 1 or 3. The addition of glucose to the medium increased fatty acid synthesis from all three carbon atoms. Carbons 2 and 3 were incorporated at identical rates and exceeded that from carbon 1 of propionate. The results suggest that there are three pathways of conversion of propionate to fatty acids, but direct condensation with malonyl CoA is by far the predominant pathway (Cady et al., *Biochim. Biophys. Acta 70,* 118). ω-oxidation of fatty acids has been shown with cell-free systems of guinea-pig liver. Hydroxy-fatty acid was found to be the intermediate compound and the hydroxylating reaction occurs in the microsomes (Wakabayashi and Shimazona, *Ibid. 70,* 132). Acetate incorporation into fatty acids of lettuce chloroplasts depends on non-cyclic photophosphorylation which results in the formation of TPNH, ATP and O_2 (Stumpf et al., *Ibid. 70,* 260). Incorporation of tritium from position 4 of the pyridine ring of TPNH and DPNH into fatty acids was shown. Tritium from TPNH was preferentially incorporated (Matthes et al., *Ibid. 70,* 242). The incorporation of α-glycero (P^{32}) phosphate into mitochondrial phospholipids was greater in ATP induced contraction of swollen mitochondria than during swelling. The chief labelled phospholipid was tentatively identified as phosphatidic acid. It is suggested that incorporation of α-GP into phospholipids and contraction are related (Wojtczak et al., *Ibid. 70,* 306). In a study of the biosynthesis of fatty acids of leaf lipids two separate pools of long chain fatty acids are suggested. In one pool, myristic acid is converted to palmitic and stearic acids which are then esterified to give galactolipid and phospholipid. In the second pool myristic acid is converted to palmitic, stearic and oleic acid. Only the latter is freely available for esterification to combined lipids or for conversion to linoleic and linolenic acids (James, *Ibid. 70,* 9). Monoglyceride transacylase occurs in rat intestinal mucosa in a soluble and bound form. A partially purified enzyme of mitochondria was studied. Palmitic acid, ATP and CoA can be replaced by palmityl CoA. The reaction product is diglyceride (Clark and Hubscher, *Ibid. 70,* 43). Phosphorylcholine-glyceride transferase and diglyceride acyl transferase are similar in distribution to glucose 6-phosphatase and are thus microsomal enzymes. The highest specific activity of phosphatidic acid phosphatase was found in an intermediate fraction suggesting that it might be a lysosomal enzyme. Phosphorylcholine-cytidyl transferase is found both in microsomes and soluble supernatant (Wilgram and Kennedy, *J. Biol. Chem. 238,* 2615). Stearolic acid-1-C^{14} (9-octadecynoic acid) was incorporated into lipids of anaerobically grown yeast. In the phosphatidylcholine fraction stearolate was found exclusively at the β position. When yeast was grown anaerobically on stearolic-1-C^{14} and subsequently aerated, a significant shift of the acetylene acid from phospholipids to neutral lipids occurred (Myer and Bloch, *Ibid. 238,* 2654).

GENERAL LIPID BIOCHEMISTRY. The principal phospholipids from lyophilized fresh sugar cane juice were isolated by solvent fractionation and silicic acid column chromatography. Phosphatidyl ethanolamine and lecithin were identified (Friloux and Cashen, *J. Agr. Food Chem. 10,* 509). Lecithin containing a radioactive acid located specifically in either the β- or α'-position was synthesized enzymatically. Incubating these labeled lecithins with *Crotalus adamanteus, Crotalus atrox* and *Bothrops atrox* venoms demonstrated the selective β-esterase activity of the venoms (Robertson and Lands, *Biochemistry 1,* 804). Rat-brain lipid was chromatographed in an alumina column using $CHCl_3$–CH_3OH–H_2O. The neutral lipids, together with choline-galactose-ethanolamine-, and inositol containing lipids, passed through the column. Phosphatidyl serine was then eluted with an alkaline solvent system (Long et al., *Biochem. J. 85,* 251). Incubation of human serum or plasma at 38C for four hr results in conversion of 10% of the lecithin to lysolecithin. Serum from patients with acute pancreatitis and postheparin plasma was no different than normal serum or plasma in this respect (Vogel and Zieve, *Proc. Soc. Exptl. Biol. Med. 111,* 538). The glycerides and phosphatides in barley, oats and rye, as well as their fatty acid composition, have been partially identified (Aylward and Schowler, *J. Sci. Food Agr. 13,* 494). Electrophoretic mobilities and thromboplastic activities of emulsions of mixtures of phospholipids derived from beef brain and egg yolk were determined (Papahadjopoulos et al., *Proc. Soc. Explt. Biol. Med. 111,* 412). Human cerebrospinal fluid had a high content of phosphatidyl ethanolamine and ethanolamine plasmalogen as compared with blood plasma. Other phospholipids and neutral lipids were also detected (Haek and Helmy, *Ibid. 111,* 421). The phospholipids from *Mycobacterium tuberculosis* (Var. bovis, Strain BGG) have been deacylated, and the water-soluble material has been separated to yield four products containing myo-inositol: glycerol myoinositol phosphate, glycerol myoinositol phosphate mannoside, glycerol myoinositol phosphate dimannoside and glycerol myoinositol phosphate pentamannoside (Ballou et al., *J. Biol. Chem. 238,* 69). A series of lysophosphatides was prepared from the plasmalogens phosphatidal choline and phosphatidal ethanolamine and their lipolytic activity compared with that of α-palmitoyl lysolecithin (Gottfried and Rapport, *J. Lipid Res. 4,* 57). Improvements are described in the preparation of phosphatidyl ethanolamine from hen's egg and in the preparation of intermediates used in the total synthesis of racemic dioleoyl-phosphatidyl ethanolamine. Both preparations have been found to be potent accelerators in the Hicks-Pitney test and moderately active in the thromboplastin generation test (Turner et al., *Ibid. 4,* 52). Lipids from blood thromboplastin were identified by column and paper chromatography as inositol phosphatide, lecithin, sphingomyelin, phosphatidyl serine, phosphatidyl ethanolamine and cholesterol (Bentley, Jr., *Proc. Soc. Exptl. Biol. Med 111,* 757). Liver and kidney mitochondria catalyze a rapid incorporation of radioactive phosphate into their lipid fraction. An unidentified mitochondrial lipid is labeled more rapidly than is phosphatidic acid (Garbus et al., *J. Biol. Chem. 238,* 59).

A method is described for determination of lipid α-glyceryl ethers in animal tissues. They are a minor component of all of the tissues except bone marrow (Wakagawa and McKibbin, *Proc. Soc. Exptl. Biol. Med. 111,* 634). The presence of glycerol alkenyl ether diesters in the neutral lipid fraction of the lipids from the diverticulum of the starfish has been established. A number of mammalian tissue lipid extracts have been shown to contain glycerol ethers of both alkyl and alkenyl types (Gilbertson and Karnovsky, *J. Biol. Chem. 238,* 893). The assign-

ment of *cis* configuration to the double bond in the α,β-unsaturated ether linkage was made as a result of IR data (Norton et al., *J. Lipid Res. 3,* 456). α-Glyceryl ether phospholipids have been found to account for more than 25 male % of the phospholipids of two species of terrestrial slugs. The major glyceryl ether is chimyl alcohol (Thompson and Hanahan, *Ibid. 238,* 2628). A new naturally occurring member of the coenzyme Q group has been isolated and crystallized from cells of *Gibberella fujchenroi.* This new compound is designated coenzyme Q_{10}(H-0) (Gale et al., *Biochemistry 2,* 196). A newly characterized naphthoquinone has been isolated from *Mycobacterium pheli.* This compound is designated vitamin Kg (H) (Gale et al., *Ibid. 2,* 200). The existence of a cylochrome system and coenzyme Qs has been demonstrated in *Thiobacillus thiooxidaus* and *Thiobacillus thioparus* (Coole and Umbreit, *Ibid. 2,* 194).

Epididymal adipose tissue was incubated with glycine-1-C^{14}, glycine-2-C^{14}, glyoxylate-1-C^{14} and CO_2. Glycine-1-C^{14} was converted to labeled fatty acids to a greater extent than glycine-2-C^{14}. The path of conversion of glycine to fatty acids is discussed (Fetter and Feist, *Proc. Soc. Exptl. Biol. Med. 111,* 18). The metabolism of palmitate by polymorphonuclear leukocytes (PMN) from guinea pig exudate in the presence of physiological conen of human serum albumin has been studied (Evans and Mueller, *J. Lipid Res. 4,* 39). C^{14}-β-Carotene is converted into retinol ester by the isolated perfused rat liver (Zachman and Olson, *J. Biol. Chem. 238,* 541). Macroscopically visible lipid plaques were produced on the surface of human blood agar plates and on 10% plasma agar plates when they were inoculated centrally with certain strains of coagulase-positive *S. aureus* or exposed to active sterile agar segments. The lipid plaques consist of octadecenoic acid, both free and in triglycerides (Weld et al., *Proc. Soc. Exptl. Biol. Med. 112,* 448). Pancreatic lipase hydrolysis of cow milk fat showed that the majority of the fatty acids were uniformly distributed within the glycerides, except for C_4 and C_6, which are predominantly in the external positions and C_{16}, which tends to conc in the two position (Jack, et al., *J. Dairy Sci. 46,* 284). Seasonal and regional variations in ethenoid acids of Canadian milk fat have been established. Ca. 30% of the fatty acids in milk fat were unsaturated; 12% of these were polyunsaturated acids, with one-third being of the conjugated type (Riel, *Ibid. 46,* 102). Marked activity in carbon atoms 3 and 4 of glucose from glycogen was observed when D-glyceraldehyde-3-C^{14} was a substrate. This indicates that an initial step in D-glyceraldehyde metabolism is reduction to glycerol (Landan and Merlevede, *J. Biol. Chem. 238,* 861). The rates of lipolysis and of fatty acid esterification were estimated in rat epididymal fat pads under various conditions *in vitro* (Vaughan and Steinberg, *J. Lipid Res. 4,* 193). The α- and γ-tocopherol contents of different nuts have been determined spectrophotometrically (Lambertsen et al., *J. Sci. Food Agr. 13,* 617).

Steroids

The relative retention time of a steroid on gas chromatography may be expressed by the equation: $\text{Log } r = \Sigma \Delta R \text{ mg} + \log r_N$ where r_N is the relative retention time of the steroid nucleus and RMg is the change in Log r brought about by the introduction of the group (g) into the nucleus (*Chem. Ind.* (London) *1963,* 43). A TLC procedure for steroids is described using silica gel mixed with starch. The relative mobility of 55 steroids is given for four different solvent mixtures (Smith and Foell, *J. Chromatog. 9,* 339). A method of partition TLC using Celite No. 545 and Zafarom's solvent systems is described. The method has a development time of 3–7 min. The separation of steroids of widely differing polarities is satisfactory (Vaedtke and Gajewska, *Ibid. 9,* 345). Eight 3-β-sterols differing in unsaturation in ring B and in the side chain were separated by TLC. Differences in resolving power between polar and non polar systems are given (Bennett and Heftmann, *Ibid. 9,* 359). UV and non UV absorbing steroids were separated by TLC and determined quantitatively (Mathews et al., *Ibid 9,* 331). The separation of 19-nor-steroids by TLC on silica gel and subsequent identification of the individual compound is described. Chromatographic mobilities and colors developed are listed for 38 19-nor-steroids (Golab and Layne, *Ibid. 9,* 321). The separation of 18 corticosteroids and pregnane derivatives in submicrogram quantities by TLC is presented. Positional isomers, axial-equatorial hydroxyl epimers and A/B *cis-trans* isomers were successfully resolved (Bennett and Heftmann, *Ibid. 9,* 348). A method for the quantitative analysis of cholesterol and coprostanol in feces by gas chromatography is presented. The technique appears to be useful in measuring these substances in sterol balance studies (Rosenfeld et al., *Ibid. 7,* 293). The presence of cholesterol in *Solanum tuberosum* and *Dioscorea spiculiflora* plants was demonstrated (Johnson et al., *Science 140,* 198). Retention data, relative to cholestane, for GLC of 90 steroids of the androstane, pregnane, cholane and cholestane series have been recorded for two silicone stationary phases, SE-30 and QF-1, on Gas Chrome P at 200C. Data have been applied to the tentative identification of keto steroids derived from urine (Brooks et al., *Biochem. J. 87,* 151). The digitonin-anthrone indirect determination of cholesterol was revised to produce more reliable data and to provide separate final aliquots on all fractions that were suitable for C^{14} counting (Goodman et al., *Anal. Chem. 35,* 760). Procedures are described for the quantitative gravimetric isolation of cholesterol and cholestan-3β-ol as the digitonides and for the quantitative recovery of these sterols from their digitonides in the range of 0.5–4.0 mg (Sperry, *J. Lipid Res. 4,* 221).

The retention volumes of 12 sterol acetates relative to cholesteryl acetate were determined on four well-characterized silica gels of different body structure. The application of these values to the fractionation of model and biological mixtures of sterol acetates is illustrated (Klein and Szczpanik, *J. Lipid Res. 3,* 460). The synthesis of 3β,7α-dihydroxy, 3β,12α-dihydroxy and 3β,7α,12α-trihydroxy-5β-cholamic acid has been isolated from rabbit feces and found to be a metabolite of deoxylcholic acid (Danielsson et al., *J. Biol. Chem. 237,* 3657). A summary of the personal views on the nomenclature of steroidal sapogenins is presented. Errors have been found in the nomenclature rules of 1960 (Mueller and Pettit, *Experientia 18,* 404).

Solvent systems for the TLC of bile acids are described (Eneroth, *J. Lipid Res. 4,* 11). TLC of several sterols that are metabolic precursors of cholesterol is presented (Avigan et al., *Ibid. 4,* 100). The anaerobic reduction of lanosterol to dihydrolanosterol has been demonstrated with rat liver homogenates. The reduction required reduced triphosphopyridine nucleotide (Avigan et al., *J. Biol. Chem. 238,* 1283). The time course of distribution of radioactivity in rat liver non saponifiables showed activity in a C_{28} zone containing both saturated and unsaturated compounds. No significant radioactivity was found in $\Delta^{7,24}$-cholestadienol or in zymosterol (Goodman et al., *Ibid. 238,* 1287). There was a reduction in the incorporation of radioactivity from acetate into the Δ^5-stenol fraction and an increase in the incorporation into lanosterol and Δ^7-sterols in skin from triparanol-treated rats (Horlick and Avigan, *J. Lipid Res. 4,* 160). Evidence is presented for at least two effects of the drug triparanol in rats: 1) in causing the accumulation of the Δ^{24}-analogues of sterols and 2) marked alterations in the proportions of Δ^7- to Δ^8-isomers of these intermediates (Clayton et al., *Ibid. 4,* 166).

In a comparative study using mice, rats, hamsters and guinea pigs, diets supplemented with cholesterol caused a greater accumulation in hamsters and guinea pigs than rats and mice; in all species dietary cholesterol inhibited incorporation of acetate-1-C^{14} into liver cholesterol. Bile acids that are present in a given species inhibited incorporation of acetate-1-C^{14} into liver cholesterol whereas other bile acids showed a varying effect. Hyodeoxycholic limited the accumulation of liver and blood cholesterol of animals fed atherogenic diets (Beher et al., *J. Nutri. 79,* 523). An enzyme system has been found in rat liver which converts cholesterol into 3α, 7α, 12α-trihydroxycoprostane (Mendelsohn and Staple, *Biochemistry 2,* 577). Evidence for a sterol diol in human adrenal esters was found. When human adrenal glands are stimulated sterol esters are depleted and phospholipids increased. Free sterols and triglycerides are not appreciably changed. The fatty acids of the gland with and without stimulation is recorded (Riley, *Biochem. J. 87,* 500). In a study of the biosynthesis of skin sterols a highly labeled companion of lanosterol was observed which appeared to be a metabolic intermediate in the formation of skin sterols. Homogenates of rat liver converted the labeled metabolites into cholesterol (Gaylor, *J. Biol. Chem. 238,* 1643). The D-glucosides of stigmasterol, sitosterol and campesterol have been isolated from tobacco and cigarette smoke (Killianos et al., *Biochem. J. 87,* 596). No significant difference was found between half-lives of bile acids in normal, thyroid hormone-treated or propylthiouracil treated rats (Strand, *J. Lipid Res. 4,* 305).

Lipoproteins

Stereomodels were used to form hypotheses about the structure of lipoproteins (Vandenheuvel, *JAOCS 40,* 455). The interaction of low-density lipoproteins of human serum with hemin and the nature of the oxidative denaturation of low-density lipoproteins of serum catalyzed by hemin was studied by means of spectrophotometric and ultracentrifugal analyses and by manometric methods. The results indicated that a complex was formed *in vitro* between hemin and isolated serum low-density lipoproteins (Nishida and Kummerow, *J. Lipid Res. 3,* 448). Slices of adipose tissue from rabbits in different nutritional states were incubated under various conditions with plasma very low-density lipoproteins ($d > 1.006$)

in which triglyceride fatty acids had been biologically labelled with palmitate-1-C^{14}. Lipoprotein lipase activity, released into a heparin-containing medium, was assayed in the same tissues. The results show that the incorporation of TGFA into the slices is dependent on the nutritional state of the animal and is positively correlated with the lipoprotein lipase activity released from the tissue under the influence of heparin, which in turn probably correlates with the total lipoprotein lipase activity of the tissue (Bezman et al., *Ibid. 3,* 427). Results of clinical investigations on the influence of safflower and olive oil ingestion on a) the lipid composition of the major serum lipoprotein classes, b) the fatty acid composition of the lipoprotein lipids and c) the fatty acid composition of the ultracentrifugal protein residue fraction, are presented. Significant glyceride increases occurred in $S_f20–10^5$ and the high-density lipoproteins (HDL). Fatty acid composition changes occurred in the glyceride moieties of the $S_f20–10^5$, $S_f0–20$, and HDL fractions. Marked alterations in the composition of the fatty acids associated with the ultracentrifugal protein residue fraction occurred following oil ingestion. The origin of the HDL lipid following oil ingestion is discussed in relation to the metabolism of the $S_f20–10^5$ lipoproteins (Nichols et al., *J. Lipid Res. 3,* 320). A chylomicron-containing, low-density lipoprotein fraction of thoracic duct chyle (S_f classes 20 and higher) obtained from rats fed either cholesterol-4-C^{14} or palmitic acid-1-C^{14} was injected intravenously into rats. The disappearance curves for the labeled cholesterol differed strikingly from those for the labeled triglyceride. The latter first declined rapidly and then more slowly. The former consisted of three phases: 1) rapid decline, 2) rising and 3) leveling off (Naidoo et al., *Ibid 3,* 309). A comparison of cholesterol levels of α- and β-lipoprotein of human serum obtained by dextran sulfate from two different sources gave identical results. The presence of oxalate may interfere with the analysis of β-lipoprotein cholesterol, but this can be avoided by using analytical methods which entail digitonide precipitation (Kritchevsky et al., *Proc. Soc. Exp. Biol. Med. 112,* 259). In patients with atherosclerosis, mainly of the coronary arteries, the initial β-lipoprotein level was high. A connection between the development of the atherosclerotic process and the presence in the blood of high β-lipoprotein levels was noted and confirms the diagnostic importance of these fractions in clinical examination of the patient. Under treatment with large doses of iodine, decrease in the β-lipoprotein and β-globulin levels was observed in more than one-third of the patients, attended by a rise of the α-lipoprotein and alubmin levels (Pitel, *Federation Proc. 22,* T 135; *Terapevticheskii Arkhiv 34,* 47, 53). Lipoproteins from patients receiving therapeutic doses of iodine-131 were isolated by density-gradient techniques. The binding of circulating thyroid hormones by β-lipoproteins (those of low density) was negligible. Alpha-lipoproteins (high density) bound appreciable amt. The bulk was bound by proteins of density higher than 1–23 g/ml (Toro-Groyes and M. Cancio, *Science 139,* 761). The uptake of celite-dispersed lipids by human α-lipoprotein can bind many lipids but shows a considerable degree of discrimination amongst sterol and vitamin A derivatives. It binds a number of anionic dyes. β-Lipoproteins ($S_f2–10$) are severely damaged by incubation with lipid-coated celite (Ashworth and Green, *Biochem. Biophys. Acta 70,* 68). Oxidized lipids containing *trans*-isomers were found in lipids on the lipid-protein complexes from atherosclerotic aorta (Fukuzumi and Iwata, *Yukagaku 12,* 92). Regardless of the character of the lesion, the protein-lipid metabolism is grossly disturbed in hypertensive patients in the sclerotic stage and in patients with generalized and cerebral atherosclerosis. This is manifested by an increase in the β-lipoprotein fraction of the serum and its cholesterol concentration (Mittelshtedt et al., *Federation Proc. 22,* T 240; *Zhurnal Nevropathologii i Psikhiatri imeni S. S. Korsokova 62*(1), 59, (1962). Growing chicks were fed either a low fat diet or this diet supplemented with cocoanut, olive or corn oil, with and without added cholesterol. The percentage of plasma cholesterol and lipid phosphorus bound to the plasma α lipoproteins decreased with increasing plasma cholesterol levels. The absolute amt of cholesterol bound to the α-lipoproteins remained essentially unchanged, demonstrating that the increase in cholesterol occurs exclusively in the β-lipoprotein fraction (Leveille and Sanberlich, *Proc. Soc. Exp. Biol. Med. 112,* 300). Prolonged feeding of ethionine to rats receiving a diet containing cholesterol, cholic acid and hydrogenated cocoanut oil leads to ca. a 10-fold rise in serum triglyceride conen which is primarily confined to triglycerides bound to low density lipoproteins (Seidel and Harper, *Ibid. 111,* 579). Low density lipoproteins ($d < 1.019$) of blood plasma were shown to provide the fatty acids of milk fat (Robinson et al., *Biochem. J. 87,* 23P). Peanut lipoprotein has been shown to be suitable as a substitute for or as an additive for lipoproteins present in processed foods. It appears to have similar functions and play a corresponding role in various food formulations (Waldt et al., *Food Technol. 17,* 927). A method is presented for the analysis of peanut lipoprotein in comminuted meats based on differences in the conen of behenic acid as analyzed by GLC (Waldt et al., *Ibid.* 657). The conen of lipoprotein lipase of guinea pig mammary gland increases markedly just prior to parturition, reaching a max level within 2-hr post-partum. This level is maintained until the cessation of suckling, when the enzyme becomes undetectable within 18 hr. Most of the lipoprotein lipase of the mammary gland would appear to be accounted for by that in the retained milk (McBride and Korn, *J. Lipid Res. 4,* 17). Lipoprotein lipase (LPL) activity was demonstrated in canine pancreatic juice and pancreas. The possible relationship of the LPL activity and its inhibition (s) to the lipemia accompanying acute pancreatitis is discussed (Kessler et al., *Proc. Soc. Exp. Biol. Med. 113,* 127). The effect of more than 25 different sulfated polysaccharides and of a few other polyanions on the activity *in vitro* of mouse heart LPL was measured. All polysaccharide sulfates which contained no sulfoamino groups and which had at least 0.6 sulfate ester group per repeating unit proved to be potent inhibitors of the enzyme (Bernfeld and Kelly, *J. Biol. Chem. 238,* 2136).

LIPIDS IN DISEASED STATES

MAN. The significance of serum triglycerides and its importance in evaluating the impact of modern diet on vascular disease were outlined (Albrink, *J. Am. Dietet. Assoc. 42,* 29). Observations on 107 patients with atherosclerosis, mainly of the coronary arteries, showed that in half the subjects atherosclerosis was accompanied by hypertensive disease. After treatment for 20–30 days with iodine, increase of phospholipids and of the phospholipid-cholesterol ratio was observed, while blood cholesterol tended to fade. A connection between the development of the atherosclerotic process and the presence in the blood of high β-lipoprotein levels was noted. Under treatment with large doses of iodine, decrease in the β-lipoprotein and β-globulin levels was observed in more than one-third of the patients, attended by a rise of the α-lipoprotein and albumin levels (Pitel, *Federation Proc. 22,* T135). The effects of prolonged intake of sunflower oil (as the sole dietary fat) on experimental conditioned-reflex adrenalin hypertension did not show a consistently beneficial influence on higher nervous activity (Braksh et al., *Ibid. 22,* T132). A daily ingestion of sunflower oil (100 and 50 g/day) in addition to a diet containing up to 40 g animal fat leads to a rapid drop of the blood cholesterol level. The blood cholesterol level began to rise rapidly once the ingestion of sunflower oil was stopped. A weaker effect was noted with cottonseed oil (Pleshkov, *Ibid. 22,* T138). Lipids of atherosclerotic abdominal aorta were dialyzed to separate the residue (phospholipid conc) and the dialysate. The UV and IR spectra compositions and properties of fatty acids were examined.

Highly unsaturated acids are conen in the phospholipids. Consequently, they were also highly oxidized. *Trans-trans* conjugated diene hydroperoxide existed in the oxidized lipids. It was also shown that atherosclerotic aorta contained lipid-protein complexes. Oxidized lipids containing *trans*-isomers existed in the lipids of lipid-protein complexes (Fukuzumi and Iwata, *Yukagaku 12,* 93). Normal intima and different types of lesion were compared in the same aorta. In fatty streaks, the cholesterol ester increases while its linoleic content is lower than normal tissue and eicosatrienoic acid is higher than normal. The conen of lipid in large intimal plaques is much greater than in fatty streaks, but the cholesterol ester fatty acid pattern is closer to normal (Smith, *Biochem. Ibid. 88,* 49). Lipids were extracted from the tissue of bronchial carcinoma and from cancerous fluid and fatty acid methyl esters of the lipids were prepared. The fatty acid compositions were calculated from UV spectra. IR spectra indicated the presence to *trans-trans* or *cis-trans* conjugated diene to hydroperoxide in the methyl esters of fatty acids obtained from the lipids of all samples (Fukuzumi et al., *Yukagaku 12,* 165). Regardless of whether, at the moment of investigation, there was an acute disturbance of the cerebral circulation or not, and also regardless of the character of the lesion, the protein-lipid metabolism is grossly disturbed in hypertensive patients in the sclerotic stage and in patients with atherosclerosis. This is manifested by an increase in the total lipid conen, an increase in the β-lipoprotein fraction of the serum and an increase in the conen of the total cholesterol and the cholesterol in the β-lipoprotein fraction; the distribution of the phospholipids among the fractions also is abnormal. The increase in the fibrinogen conen in the patients' blood and the changes in heparin tolerance demonstrate that the clotting system of the blood is involved (Mittelshtedt et al., *Federation Proc. 22,* T240). Turnover rates of free fatty acids was studied during rest and exercise in eight normals and 28

diabetics using palmitate-1-C^{14}. During rest the turnover rate of free fatty acids does not differ in normals and diabetics. At higher and lower levels of blood glucose the turnover rate is increased (Ford et al., *Proc. Soc. Exp. Biol. Med. 113,* 177). Serum cholesterol and phospholipid determinations were made in four groups of subjects: men without evident disease; men suffering from chronic disorders, but without evidence of coronary artery or cerebrovascular disease; men who had recovered from a myocardial infarction; and men who had recovered from cerebral thrombosis. In the two atherosclerosis groups, a substantial and highly significant abnormality in the quantitative relationship between cholesterol and phospholipids was observed. This imbalance is thought to represent some important and consistent lipid abnormality characteristic of atherosclerosis (Moore et al., *Proc. Soc. Exp. Biol. Med. 113,* 350).

MAMMALS. A comparison of the specific activities of the individual phosphatides of Ehrlich ascites tumor-bearing mice, after injection of p^{32}, showed that they were increased above those of the normal livers. The specific activity of the total liver phosphatides tumor-bearing mice reached a maximum 8–10 hr after injection and then decreased. The specific activity of the total phospholipids of the ascites tumor cells rose more slowly and continued to rise for 72 hr (Lee et al., *Cancer Res. 22,* 1046). Total body composition of mice bearing Krebs-2 carcinoma transplanted subcutaneously was studied at several times during the course of tumor growth. Adult mice sustained a profound fat loss during growth of Krebs-2 tumor. Fat depletion occurred in three stages (Costa and Holland, *Cancer Res. 22,* 1081). A major metabolic defect induced by CCl_4 administration to the rat appears to be inhibition of the outward transport of hepatic triglycerides. This inhibition of outward triglyceride transport may be the etiology of the fatty liver associated with CCl_4 poisoning (Heimberg et al., *J. Biol. Chem. 237,* 3623). X-irradiation was shown to accelerate the formation of atherosclerotic lesions in the coronary and pulmonary arteries of rats fed on a supplemented high fat diet (Gold, *Proc. Soc. Exp. Biol. Med. 111,* 593). The mechanism by which 4-amino-pyrazolo-pyrimidine causes fatty livers in mice was investigated. Injection of 1 mg raised the total liver lipid 3- to 4-fold in 24 hr, which was almost neutral lipid. The plasma lipid concn was decreased by this treatment (Henderson, *J. Lipid Res. 4,* 68). Swiss-Webster mice were protected against lethal amt of exotoxins prepared from *staphylococcus, Cl. perfringens* and *Cl. tetani* when the toxins were mixed with oleic or linoleic unsaturated fatty acids prior to intravenous injection. Injection of a toxin and unsaturated fatty acid also gave protection. Unsaturated fatty acid also protected against *Crotalus terrificus* snake venom (Spink and Su, *Proc. Soc. Exp. Biol. Med. 112,* 463). The effects of choline deficiency in young rats fed diets containing various levels of beef fat with or without added cholesterol for seven or eight days were studied. Liver lipid levels always decreased and plasma lipid levels increased following choline supplementation. Injury to the kidney, coronary vessels, myocardium and aorta were produced by this short period of choline deprivation (Newherne and Salmon, *J. Nutri. 79,* 179). The *in vivo* incorporation of C^{14} palmitate into liver and plasma triglycerides of rats fed a choline deficient diet has been studied.

It was shown that choline-deficiency fatty liver has a pathogenetic mechanism different from that of fatty livers due to toxic agents (Lombardi and Schotz, *Proc. Soc. Exp. Biol. Med. 112,* 400). The different fractions of plasma lipids were estimated in normal, scorbutic, insulin treated scorbutic and ascorbic acid supplemented scorbutic guinea pigs (Banerjee and Bandyopadhyay, *Proc. Soc. Exp. Biol. Med. 112,* 372). EFA-deficient rats and those supplemented with linseed oil has 35% less fat in the intestine than the normal and starved rats. Compared with supplemented animals, the EFA-deficient rats contained 1% linoleic acid, 10% of arachidonic acid, 20% docosahexaenoic acid and 8% docosapentaenoic acid. Both EFA-deficient and supplemented rats had 50% more palmitoleic acid than rats on a normal diet of rat cubes (Ensen and Bartley, *Biochem. J. 85,* 607). Lipid was extracted from freeze-dried Landschutz acites-carcinoma cells and BP8/C3H ascites-sarcoma cells grown in mice and fractionated in silicic acid columns. The distribution of lipid phosphorus was not significantly different from that in the majority of normal tissues. GLC analysis showed a far less selective distribution of fatty acids in the phospholipids and glycerides than was usually found in normal tissues. A preponderance of C-18 saturated acid was found in most phospholipids. Small amt of two chromatographically-distinct carbohydrate-containing lipids were found in the phospholipid fractions from the tumor cells (Gray, *Biochem. J. 86,* 350). Rabbits were fed diets containing 1) 20% maize oil, 2) 24% butter, 3) 20% butter fat or 4) 1% maize oil and 42% wheat starch. No cholesterol was added to any diet. After nine months, the diets containing butter, butter fat or high wheat starch gave rise to hypercholesterolemia and atheromatous lesions of the aortas. The animals on the diet containing maize oil had a low plasma cholesterol and lesions were completely absent. Thus, in the rabbit, marked atheromatous lesions may arise on diets containing no saturated fat (Moore and Kon, *Chem. Ind.* (London) *165* (1963). Palmitic acid-1-C^{14} was rapidly incorporated into normal rat liver phosphatides, with the exception of phosphatidyl serine. A pronounced decrease in palmitate incorporation into phosphatidyl ethanolamine occurred in liver after cerium administration. This decrease occurred at a time when greater palmitate incorporation was observed into phosphatidyl inositol and cardiolipin (Glenn et al., *J. Biol. Chem. 238,* 1249). A diet supplemented with shrimp was fed to rabbits for 24 weeks. These animals developed a sustained hypercholesterolemia. At autopsy, all animals had evidence of aortic atherosclerosis. Aortic cholesterol in the shrimp-fed rabbits was as much as 60 times greater than that in control rabbits (Conner et al., *J. Nutri. 79,* 443). Young hamsters fed on a fat-free diet having an easily digestible sugar develop cholesterol gallstones. Adding small amt of fat to the diet prevents the gallstones but, especially in the presence of cholesterol, favors formation of amorphous pigmented gallstones which are more resistant to curative treatment. The more unsaturated fats are more effective in preventing formation of these amorphous gallstones (Dam, *Rev. Ital. Sostanze Grasse 10,* 510). In rats subjected to stress, an increase in the content of unesterified fatty acids in the blood serum is observed. A parallel increase in the lipolytic activity of the myocardium decreases, as also that of the aortic tissue. The depression of the lipolytic activity of the myocardium and aortic tissue under stress may be significant in the pathogenesis of the cardiovascular diseases that develop during this state (Leikes and Chou-Su, *Federation Proc. 22,* T244. *Part II Trans. Supp.*). The lipid composition of lipoid granules of Ehrlich ascites tumor cells was analyzed. Triglycerides formed the largest single pool. Oleic and linoleic acids formed the largest pools in the steroid ester fraction whereas palmitic, stearic, oleic and linoleic acids predominated in the triglyceride fraction (Dipaolo, *Proc. Soc. Exp. Biol. Med. 113,* 68). Edematous changes in the large artery are considered to be an important mechanism in atherogenesis and thrombogenesis. The administration of substances capable of inducing an atheromatous change experimentally, such as fats of animal origin, including cholesterol, was found to induce an edematous reaction; soybean oil or linoleic acid did not (Shimamoto, *J. Atheroscler. Res. 3,* 87). Hypercholesterolemia was induced in seven-month old rabbits by a semi-synthetic diet containing either hydrogenated cocoanut oil or trilaurin, but not cholesterol. The aorta and coronary arteries showed atheromatous changes resembling these in rabbits fed cholesterol. The morphological changes and their relation to human atherosclerosis are discussed (Stormby and Wigand, *J. Atheroscler. Res. 3,* 103). An attempt has been made to confirm the observations of Posner that subcutaneous growth of Walker Carcinoma 256 in rats results in defective fat absorption, as measured by the amt of I^{131}-labeled triolein in thoracid duct lymph. Posner's results could not be confirmed (Baker, *Cancer Res. 23,* 928). Fluorescence studies, both with the naked eye and with the microscope, are valuable methods for the diagnosis and investigation of atherosclerosis. In the parts of the aorta where atherosclerosis frequently occurs, even when no change is apparent to the naked eye, a bright luminescence can be seen in some cases. Fluorochroming with phosphine and promilin demonstrates deposits of lipids in recent unfixed film preparations of the intima (Anestradi, *Federation Proc. 22,* T463). Stress induced in rats by immobilization is accompanied by a reduction of the lipolytic activity of the aortic wall. Decreased lipolytic activity of the aortic wall is also observed in transient hypoxia and longstanding diabetes mellitus (Leites and Chzhou-Su, *Ibid. 22,* T466).

BIRDS. The effects of MER-29 were studied to determine whether this drug will lower the blood lipids as well as the severity of aortic and coronary atherosclerosis of 10-week old cockerels on a regimen of plain mash or on an atherogenic diet. After ten weeks of treatment, it was observed that the drug had no significant influence on blood cholesterol or phospholipid levels (Wong and Johnson, *Circulation Res. 11,* 843). Pigeons were maintained on cholesterol-free diets. When such diets were supplemented with 10% hydrogenated shortening, aortic atherosclerosis and the level of aorta cholesterol were less than in birds receiving no supplement or 10% Safflower oil (Lofland and Clarkson, *Proc. Soc. Exp. Biol. Med. 112,* 108). Dietary lithocholic acid alone, and in combination with cholic acid of cholesterol, has been shown to induce the ductular cell fraction in the livers of chickens. Dietary cholic acid, deoxycholic acid or cholesterol did not alter liver structure. Removal of lithocholic acid from the

diet resulted in regression of the reaction (Hunt et al., *Proc. Soc. Exp. Biol. Med. 113,* 139). Castrated adult pheasants were administered testosterone, protactin or corticosterone. They increased food intake 38%. Corticosterone reduced adrenal wt 21% and increased hepatic weight 100% and lipid content of the liver 168% (Nagra et al., *Poultry Sci. 42,* 770). The lipids from plasma, aortic intima-media and liver of control cockerels and of birds made hyperlipaemic either by the addition of 1% cholesterol plus 3% peanut oil to the diet or by subcutaneous implantation of stilbestrol tablets have been studied. The fatty acid compositions of cholesteryl esters, triglycerides and phospholipid fractions were determined by GLC. When the conen of any lipid fraction was considerably above the control value, the amt of oleic acid as percentage of the fatty acids of that fraction was increased; often the percentage was more than doubled relative to control values (Blomstrand and Christensen, *J. Atheroscler. Res. 3,* 142). In rooks, high serum lipid values were found, as well as macroscopically visible lipaemia without any symptoms of atherosclerosis. The hyperlipemia was accompanied by lipoproteins of high electrophoretic mobility. Reasons for not finding atherosclerosis in rooks, despite high plasma lipid levels, are discussed (Lelek et al., *Ibid. 3,* 137). The effects of hypercholesterolemia secondary to induced hyperthyroidism of hypercholesterolemia produced by cholesterol feeding on spontaneous atherosclerosis of the White Carnean pigeon was investigated. Feeding cholesterol to normal pigeons did not aggravate the degree of atherosclerosis unless plasma cholesterol levels greater than 1500 mg/100 ml were produced (Kottke et al., *J. Atheroscler. Res. 3,* 129). Leghorn cockerels were fed for 20 months on diets containing either no supplement, whole egg powder, egg oil or these diets in combination with corn sterols. The inclusion of corn oil sterol retarded the atherogenesis that was accentuated by the addition of egg or egg oil. Total fat deposition in the abdominal section of the aorta was reduced in the groups fed the sterols. Egg oil was similar in its atherogenicity to egg powder sterol excretion patterns suggesting that the beneficial effect of the corn sterol was not due to interference with cholesterol absorption alone (Fisher et al., *Proc. Soc. Exp. Biol. Med. 113,* 415). Two experiments were conducted to study the influence of dietary fat on the development of muscular dystrophy in the chick and to investigate whether synergistic relationships existed between selenium, DPPD, cystine and tocopheryl acetate in prevention of the disorder. Birds receiving a fat-free diet had severe breast muscle degeneration. The omission of 4% lard from the basal ration resulted in substantial protection against muscle degeneration by previously ineffective levels of selenium, DPPD and tocopheryl acetate. Replacement of 4% lard with equivalent amt of saturated or unsaturated fatty acids indicated that the inhibiting effect of lard on selenium activity was attributable to its content of linoleic acid (Hutcheson et al., *Poultry Sci. 42,* 846).

BOOK REVIEW

Several interesting reviews on various phases of the fats and oils field have appeared during the last year. Hanahan and Thompson, Jr. reviewed "Complex Lipids" (*Ann. Rev. Biochem. 32,* 215–240). The metabolism of lipids was reviewed by Mead (*Ibid. 32,* 241–268). A review on gas chromatography with special reference to advances in methodology and applications to problems in lipid biochemistry was prepared by Horning and Vanden Heuvel (*Ibid. 32,* 709–754). Mass spectrometry and its applications to lipid research was reviewed by Biemann (*Ibid. 32,* 755–765). A symposium on "Chemical Modification of Fats and Oils" was published (*JAOCS 40,* 237–255; 302–318). "In vivo Antioxidants and Polyunsaturated Acids Metabolism" was the subject of another symposium (*Ibid. 40,* 353–371). Another symposium dealt with "Special Methods in Lipid Chemistry" (*Ibid. 40,* 395–471). Lectures of the 1963 Short Course on "Advances in Soaps and Detergents" were published (*Ibid. 40,* 609–698). A review of the properties of organic solutions of heavy metal soaps appeared (*Pilpel, Chem. Revs. 63,* 221). In addition, several feature and review articles covering the sources and industrial applications of fats and oils appeared in the *JAOCS.*

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

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