2% in [18], and no more than 1% in the remainder.

Other Acids. At least nine oils are unsuited for analysis by the isomerization method. Four [29,31, 34,35] contain enough preformed conjugation to cast doubt on the measurement of conjugation after isomerization. Three [8,17,18] contain constituents that absorb ultraviolet light and prevent measurement of preformed conjugation. These constituents were lost during the high-temperature isomerization and may have been essential oils. One [15] gave a negative value for saturated acids. The final oil in this group is from the *Ipomoea* species [20] already mentioned.

Other listed oils not specifically discussed contain varying proportions, within the usual ranges, of the common fatty acids.

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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

The majority of papers published on this subject dealt with the theoretical and practical aspects of autoxidation and its prevention. A significant step was made in the elucidation of many of the products of autoxidation of various lipids subjected to different reaction conditions. Increased effort in the identification of reaction products, and in the development of the required isolation and analytical procedures, should result in a substantial clarification of the mechanisms and kinetics of autoxidation within the next few years.

Papers on the nutritional aspects of oxidized fats are not included, as this literature is adequately covered in the "Nutrition, Physiology and Biochemistry" section. This section is further restricted to edible materials except for those cases where the investigations and findings on similar materials were considered significant.

REVIEWS

General reviews appeared on the development and inhibition of oxidative rancidity in foods (Dugan, *Food Tech. 15*, 10), autoxidation and analysis of oxidized fats (Debrus, *Riv. ital. sostanze grasse 38*, 229), and recent problems in rancidity and oxidation of fats and oils (Shimamura, *Yukagaku 10*, 129). The following reviews were presented at a symposium on flavor chemistry (*Proceedings Flavor Chemistry Symposium - 1961.* Campbell Soup Company, Camden, New Jersey): Kummerow, "Introductory remarks - fats and oils"; Evans, "Chemical changes accompanying flavor deterioration of vegetable oils"; Privett, "Some observations on the course and mechanism of autoxidation and autoxidant action"; Chang, "Isolation and characterization of reversion flavor of soybean oil"; and Jacobson, "Some aspects of chemical assessment of fat and oil flavors." Another symposium devoted exclusively to the oxidative deterioration of food lipids was held at Oregon State University. Extensive consideration was given to the mechanisms and products of lipid oxidation, factors affecting lipid oxidation, autoxidation in foods, and the biological significance of autoxidized lipids. These proceedings will be published by the AVI Publishing Co., Inc., Westport, Conn.

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OXIDATIVE STABILITY TESTS

The selection of a panel for the organoleptic testing of rancidity in fats was discussed (Gutierrez, *Grasas y Aceites 11,* 3). In a study on the relation of sensory to chemical methods for measuring the oxidized flavor of milk fats, the thiobarbituric acid number, peroxide number, total saturated and unsaturated carbonyls, volatile saturated and unsaturated carbonyls, individual volatile mono-carbonyls, and the absolute flavor threshold (FTV) of samples oxidized to different degrees were determined (Lillard and Day, *J. Dairy Sci. 44,* 623). Correlation coefficients between all the chemical tests for oxidized flavor intensity and 1/FTV value were significant at the 1% level; the volatile unsaturated carbonyls giving the highest at 0.996. Of the individual carbonyls, the correlation coefficient between 2-nonenal and 1/FTV was the highest at 0.942. The thiobarbiturac acid reaction was also employed in determinations of taste and odor threshold values of carbonyl compounds in aqueous solution as a measure of the degree of rancidity of food-fat products (Täufel and Zimmermann, *Nahrung 4,* 1010). Sensitivity of the reaction paralleled organoleptic threshold values, which were found to be about 0.005, 0.010, and 0.025 ppm for heptanal, 2-heptanal, and Me-heptyl ketone, respectively.

Edible olive oil was aged in an oven at 70C, in a Swift stability apparatus, and at room temp in the presence of light. The process of oxidation was followed by means of organoleptic examination, peroxide number, Kreis test, Watts and Major aldehyde test, thiobarbituric acid test, and diacetone test. All showed an initial induction period during which little change occurred, followed by rapid development of oxidation products (Gutiérrez and Vargas, *Grasas y Aceites 11,* 67). The Kreis reaction and peroxide no. were determined on samples of seed and olive oils after 20, 35, 60 and 100 days of storage in direct sunlight, diffused light, and darkness. (Valentinis and Romani, *Boll. lab. chim. provinciali* [Bologna] *11,* 351 and 358). It was confirmed that, in the absence of air, direct sunlight causes a decrease in the two values.

The Schaal oven test was found fairly reliable as an accelerated method for determining storage life of sunflower oil (Kozin and Ermakova, *Masloboĭno-Zhirovaya Prom. 27,* 20). One year of storage life was roughly equivalent to 11–12 hr in the Schaal oven test.

The chemistry of the color reaction between 2-thiobarbituric acid (TBA) and carbonyl compounds was investigated (Täufel and Zimmermann, *Fette, Seifen, Anstrichmittel 63,* 226). TBA reacts with aldehydes and is thus not specific as a test for autoxidation of fats. The reaction gives a colorless compound which is analogous to an aldol, with the activated H atom migrating from the TBA molecule to the CO group of the aldehyde. The compound is oxidized by oxygen, ferric ion, and light acting as accelerators to the yellow TBA dye. The formation of malonic dialdehyde during oxidation of fats was not confined to the non-conjugated unsaturated fatty acids but also occurs with saturated and conjugated acids. The Kreis test for rancidity was modified by substituting a 5% alcohol solution of resorcinol for phloroglucinol and comparing test solutions against a set of color standards prepared by mixing an alkaline, bromophenol blue solution in alcohol with an aqueous solution of Bordeaux R. and 50% alcohol at different volume ratios (Romani and Valentinis, *Boll. lab. chim. provinciali* [Bologna] *10,* 355).

Pieces of filter paper impregnated with a solution of metallic salt followed by soaking in a fatty oil and storage in the dark was utilized as a technique for studying the influence of metallic salts on the development of rancidity in fats (Vasquez, et al., *Grasas y Aceites 11,* 26). Odor, peroxide number and iodimetric titration followed the development of rancidity. A unique apparatus consisting of a series of electrically driven rotating glass cylinders was used for the study of the autoxidation of drying oils. (Kaufmann and Büscher, *Fette, Seifen, Anstrichmittel 62,* 944). Each cylinder dips into the sample to pick up a thin film which is removed by adjustable blades for analysis. The operation of the apparatus was illustrated by the study of different ferric ion complexes for catalyzing the drying of linseed oil.

In following the oxidation of fats, changes in physical properties were also utilized. The progress of accelerated oxidation was followed by plotting n_D^{25} *vs.* time (Popov and St. Ivanov, *Compt. rend, acad. bulgare sci. 11,* 275), and the end of the induction period is indicated by the break of the plot. The results agreed closely with the peroxide number method when an immersion type refractometer accurate to 0.00001 was used.

The dielectric behavior of oxidized linseed oil at a frequency of 1000 kc/sec was found useful for measuring the oxidation of the oil (de Marchi Gherini, *Bol. dept. quim. escota politec.* [Univ. Sao Paulo] *13,* 49). The oxidized oil showed anomalous dispersion phenomena between 207.5 and 5000.0 kc/sec. The significance of polarographic changes during the autoxidation of rapeseed oil was reviewed (Niewiadomski, et al., *Oléagineux* 16, 175).

Oxidative Stability of Fats and Oils

A study of the influence of the method of rendering and storage on the stability of lard related differences in stability to differences in the degree of contamination with iron and copper from the processing equipment (Romero, et al., *Grasas y Aceites 11,* 243). Lard and beef fats of higher stability were obtained on rendering in vacuo rather than in contact with air (Emanuel, et al., *Trudy Vsesoyuz. Nauch.-Issledovatel Inst. Myasoĭ. Prom. 1958,* No. 8, 195). Trienoic absorption at 268 mμ decreased in beef tallow but increased in lard upon oxidation. Bleaching-earth treatment did not affect the extinction coeff. at 268 mμ (Mirna, *Fette, Seifen, Anstrichmittel 62,* 577).

Organoleptic and chemical changes of goose fat during storage under various conditions were investigated. In comparison with changes in subcutaneous fats, the internal fats underwent more intensive chemical and organoleptic changes at 100C (Afanas' eva *Sbornik Nauch. Robot, Leningrad. Inst. Sovet Torgovli im. F. Engel'sa 1959,* No. 15, 100). Stabilities of the dienoic acids isolated from hydrogenated whale and fish oils were between those of linoleate and oleate (Watanabe and Toyama, *Nagoya Sangyô Kagukû Kenkyûjo Kenkyu Hôkoku 12,* 39).

The influence of processing on the AOM stability of olive oil was studied. The addition of fatty acids, lye refining and bleaching in air decreased stability. Vacuum bleaching had no effect on the stability of refined oils but lowered the stability of crude oilve oil. Deodorization always increased stability. Except for the very poor quality oils, processed olive oil always had less oxidative stability than the crude oil from which it came (Nosti and de la Borbolla, *Grasas y Aceites, 11,* 139). Light frequencies between 320 and 720 mμ were very detrimental for crude oilve oils, whereas refined and bleached olive oils were sensitive only to radiation between 320 and 450 mμ. (Vazquez and de la Borbolla y Alcalá, *Grasas y Aceites, 11,* 163).

Interest continued in chemical changes in animal and vegetable fats during heat treatment. Nonurea adduct-forming monomers and dimers were formed when cottonseed oil was heated at 225C in the presence of air for long periods of time (Firestone, et al., *JAOCS, 38,* 253). This fraction was toxic to rats and contained moderate amounts of carbonyl and hydroxyl and unsaturation difficult to remove by hydrogenation. Cyclic structures were present in the dimer fraction. Changes in the acid number, peroxide value, carbonyl value and iodine value were measured for soybean oil, lard, hydrogenated soybean oil and hydrogenated lard after heating for 12 hr at 180C in the presence and absence of air (Wurziger and Ostertag, *Fette, Seifen, Anstrichmittel 62,* 895). Changes in the characteristics of soybean oil during frying indicated slow oxidation and polymerization, with foaming becoming vigorous after an induction period. Alkali refining, bleaching, deodorization and molecular distillation had little effect on the heat stability of the soybean oil (Toi and Oue, *Yukagaku 6,* 87). Repetitious frying of fish paste in soybean oil resulted in increased viscosity and molecular weight and decreased iodine number (Kaneda and Tanaka, *Tokaiku Suisan Kenkyû Hokoku, No. 24,* 47). Grapeseed oil resisted deterioration at least 2 to 3 hr during cooking at 180 to 200C. Pressure refined grapeseed oil seemed to have better cooking stability (Silvestre, *Trav. soc. pharm. Montpellier 20,* 182). The effects of the heat treatment of frying fats were reviewed (Custot, *Acta Chim. Acad. Sci. Hung. 23,* 201).

The air oxidation of solutions of corn oil sterols and cholesterol in films of either corn oil or corn oil fatty acids were followed during storage up to 3 months at room temp and two weeks at 60C in the dark. The stability of these sterols was dependent upon the stability of the lipid film, with more than 90% conversion of cholesterol to oxidation products occurring in some systems (Norcia, *JAOCS 38,* 238). After aeration for 24 hr the peroxide value of sweet almond oil containing polyoxyethylene and sunflower oil remained about the same. The peroxide value of slightly deodorized cod liver oil was much greater than the deodorized oil after this period (Doee, *Cosmetologie 3,* 14). The treatment of an edible vegetable oil dissolved in a nonpolar solvent with activated aluminum was reported to improve keeping quality of the oil (Roylance, et al., *U. S. 2,976,156*). After removal of the solvent the oil was steam deodorized and an antioxidant added.

Oxidative Stability of Fats in Complex Systems

Oxidation rates of dispersed lipids in model dehydrated systems were influenced by concentration of the components, type of dispersing medium and position of the lipid film with respect to the dispersing medium. Generally, proteins decreased, and polymeric carbohydrates accelerated oxidation rates. Phospholipids had a stabilizing influence when dispersed with the fat and the dispersing medium prior to freeze-drying or when applied as a film between the dry medium and the fat film. When the positions of the fat and the phospholipid films were reversed, the protective action of the phospholipids decreased markedly (Bishov, et al., *J. Food Sci. 26,* 198).

The literature on the autoxidation of emulsified oils was reviewed (Jauslin and Leupin, *Pharm. Acta Helv. 35,* 148). In this article, oxidation absorption rates of emulsions of sodium linoleate, oleate, and stearate were determined. Results indicated that not only the oil phase but the emulsifier influences stability. Experiments with Tweens 20, 60, 80, and 85 showed similar results to a lesser degree.

Vitamin A contents and the peroxide numbers were determined monthly on 15 commercial household margarines kept at room temp for 12 months. Lauric acid had a good effect and liquid fatty acids a bad effect on vitamin A retention. There was no correlation between the loss of vitamin A and the increase in peroxide number (Nakazawa, et al., *Yukagaku 10,* 179). Exposure to air in the presence of irradiated methyl linoleate quickly destroyed vitamin A acetate (Hilda, *Vitamin 16,* 149).

Unsalted butter made from sweet cream and buffered to pH 6.6 showed lower keeping quality, owing to proteolytic changes. The addition of β-carotene, tocopherol acetate or diacetyl did not affect the rate of fat oxidation (Pijanowski, et al., *Przemyst Spozywcz 14,* 393). The rate of surface oxidation of the fat in sweet-cream butter was the same as the rest of the butter and was not affected by moderate drying or water uptake (Pont and Rogers, *J. Dairy Research 28,* 151). With severe surface drying, fat oxidation was inhibited while water uptake of the more heavily salted butters promoted surface oxidation.

Direct contact of fat with atmospheric oxygen was essential for oxidation to occur in cheddar cheese. Bleaching and the development of tallowy flavor was localized on the areas surrounding slits in the cheese (Riddet, et al., *J. Dairy Research 28,* 139).

The problems of oxidation in milk fat were reviewed (Thome, *Nord. Jordbrugsforskn. 42,* 40). The effects of direct stream heating and vacuum treatments on the chemical composition of milk with respect to oxidized flavor development was extensively studied (Kleyn, *Univ. Microfilms, L. C. Card* No. Mic 60-2343). On the basis of flavor score, TBA test, and induction period, milk from oat hay was much more resistant to oxidation than that from alfalfa hay (Dunkley, et al., *J. Dairy Sci. 43,* 1766). The susceptibility of milk towards the development of oxidized flavors was greatest when the xanthine-oxidase activity was high (Nilsson, *Kgl. Lantbruks-Hogskolans. Ann. 26,* 339). The stability of milk fat was related to the total amount of conjugated acid and the amount of diene and triene acids present (Zalashko, *Izvest. Timiryazev. Sel'skokhoz. Akad. 1960,* 209). Summer butter had the best keeping qualities and spring, the poorest.

Adequate deaeration markedly lessened flavor deterioration in concentrated sweetened cream during storage for six months at 40 and 70F (Anderson, et al., *J. Dairy Sci. 44,* 10). The effectiveness of deaeration increased as the temp at which it was initiated increased from 135 to 170F and as the period of deaeration was lengthened from 0 to 60 min.

The influence of several surface active agents on the activity of milk lipase was studied (Packard, *Univ. Microfilms, L. C. Card No. Mic. 60-5622*). The addition of an anionic reagent, sodium heptadecyl sulfate, produced a slight stimulation of lipolysis in normal milk incubated at 37 and 5C. A cationic reagent, alkyl tolyl methyl trimethyl ammonium chloride, stimulated both non-activated and, to a greater extent, induced lipolysis. Several surface active agents, both anionic and cationic, were observed to markedly stimulate lipolysis in uncooled "spontaneous" milk.

During the autoxidation of potato granules, the oxidative degradation of linoleic and linolenic acids was fairly closely correlated with the actual volume of oxygen absorbed and with the degree of off-flavor of the reconstituted products (Buttery, et al., *J. Agr. Food Chem. 9,* 245). Some of the volatile compounds present in the autoxidized dehydrated potato were identified.

Among various untreated English walnuts, those having a moisture content of 3.1% exhibited the greatest stability against rancidity development. Maximum stability was exhibited by kernels containing 3.3% and treated with antioxidant (Rockland, et al., *Food Tech. 15,* 112).

The oxidation of protein-bound phospholipids contribute to rancidity in cooked pork. The TBA test performed directly on meat tissue correlated with rancidity (Younathan and Watts, *Food Research 25,* 538). Ham smoked above 110C had a higher color, less pronounced aroma and more undesirable green spots and deteriorated areas (Kemp, et al., *Food Technol. 15,* 267). Oxidation of lipids in the lean tissue of roast beef slices preserved by refrigeration, freezing, or irradiation was followed by the TBA test and organoleptic evaluations. Oxidized products accumulated very rapidly during storage in the refrigerator. Lipid oxidation was not an important factor in irradiated beef stored at room temp. Antioxidant combinations of ascorbate and polyphosphate, used either as dips or as cover solutions, eliminate lipid oxidation and greatly improve the odor of refrigerated and frozen beef but do not benefit irradiated beef (Chang, et al., *Ibid.,* 168). Lipid oxidation in heat sterilized beef decreased as the internal temp of the ground beef round increased (Zipser and Watts, *Ibid.,* 445). The production of an antioxidant-active substance was reputed to be responsible for the increase in oxidative stability.

No significant differences were found between the spoilage rates of Pacific rockfish species *Sebastodes alutus, S. melanops* and *S. pinniger* during ice storage. The patterns of spoilage as indicated by the relationship between organoleptic scores and chemical indexes was similar to that of gadoid fish (Liston, et al., *Ibid.,* 19). The muscle lipids of mullet began to oxidize very rapidly after cooking as shown by increases in TBA number and rancid odors (Zipser and Watts, *Food Tech. 14,* 318). Oxidation was retarded by limited oxygen supply and by freezer temp. Inhibition was more complete with the use of sodium tripolyphosphate and sodium ascorbate, either alone or in combination with curing salts. Storage of herring meal at −20C promoted a more rapid decrease in the ether extractables and in the iodine number of the ether extract than did storage at 25C. Antioxidant treatment prevented decreases in both (March, et al., *JAOCS 38,* 80). Binding of the lipid in a complex from which it was extractable by acetone, only after HCl treatment, occurred early in the storage period. After six weeks, the amount of lipid in this fraction decreased, presumably as a result of further oxidation and polymerization into more refractory compounds.

White flour treated with 20 times the present usage rate of chlorine dioxide suffered loss of essential fatty acids (E.F.A.) on storage for 12 days in air. When this flour was stored under nitrogen the loss was considerably reduced, suggesting that heavy treatment destroyed the protective tocopherols, thereby exposing the E.F.A. to atmospheric oxidation. At the lower level of treatment the presence of chlorine in the chlorine dioxide gas did not have any significant effect on the E.F.A. (Daniels, et al., *J. Sci. Food Agr. 11,* 658).

The results of a study on the yellowing of oil films indicated that yellowing was a side reaction unrelated to the drying process, whereby colorless precursors of the yellow compounds are formed as a result of oxidation (Privett, et al., *JAOCS 38,* 22). Peroxide values as a measure of the autoxidation of anhydrous lanolin are useful only if values of surface and lower layers are compared (Clark and Kitchen, *J. Pharm. and Pharmacol. 13,* 121). The P.V. increases greatly if anhydrous lanolin refined from the grease is bleached by oxidation.

The oil of feed meal undergoes hydrolysis during grinding. Storing the feed meal at room temp for five months showed no further hydrolysis, but oil oxidation began which affected the meal flavor (Petrovskii, et al., *Myasnaya Ind. S.S.S.R. 1959,* 10). It was recommended that the meal be stored at 18C for no longer than 1½ months.

ANTIOXIDANTS

General

A series of review articles on pro- and antioxidants in fats was presented by Kaufmann et al. on natural and synthetic compounds affecting the oxidation of unsaturated fats and oils (Kaufmann, *Fette, Seifen, Anstrichmittel 63,* 331); the chemistry of antioxidants found in animal fats (Kaufmann, *Ibid.,* 334); the systems of unsaturated fatty acids—sulfhydryl compounds, unsaturated acids—ascorbic acid, vitamin A, and carotenoids, unsaturated acids—vitamin B and iron porphyrin compounds (Kaufmann and Garloff, *Ibid.,* 509); and the antioxidant effects of solutions of cyclic ureides on buffered linolenate (Kaufmann and Garloff, *Ibid.,* 697). In the latter two articles research was reported dealing with the antioxidant effects of the following materials on potassium linolenate in a phosphate buffer: adrenalin and noradrenalin bitartrates, 3-hydroxy-tyramine hydrochloride, serotonin cre-

atininsulfate, dihydroxyphenylalanine, cytochrome C, uric acid, xanthine, hypoxanthine, trimethylxanthine, guanine, adenine, uracil, thymine, cytosine, methyl cytosine, and thiobarbituric acid. Barbituric acid showed a strong prooxidant effect. A general discussion of the inhibitors of oxidation in the food industry was presented (Gerchuk, *Zhur Vsesoyuz Khim. Obshchestva im. D. I. Mendeleeva 5,* 395).

The Food and Drug Administration published regulations permitting the use of butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT) in potato flakes, alone or in combination with each other at a maximum level of 20 ppm (Anonymous, *Fed. Reg. 26,* 8283); a maximum level of 0.1% BHA in active dry yeast as an antioxidant (Anonymous, *Ibid.,* 3247); and BHA and BHT in dry breakfast cereals, alone or in combination at a maximum level of 50 ppm (Anonymous, *Ibid.,* 7127).

A number of new antioxidants were investigated for use in edible fats. The juice and juice sediment of *Emblica officinalis* fruit (Amla) has antioxidant properties, 0.25–2.75% retarding rancidity in edible fats to a greater degree than equivalent amounts of synthetic antioxidants (Ahmad, et al., *Pakistan J. Sci. Research 12,* 71). An antioxidant was prepared by dehydration and precipitation of the mucilage-producing portion of okra pods in iso-propyl alcohol (Hervey, *U. S. 2,950,975*). One percent of the dried okra product gave leaf lard a stability equivalent to about 15 hr by the active oxygen method as compared with 3 hr for lard without antioxidant. Methanol extracts of certain grain seeds or parts of seeds which were allowed to sprout and malt showed antioxidant activity in corn oil when added at a 5% level (Baker and Dockstader, *U. S. 2,975,066*). The natural antioxidants (polyphenols) in olives and olive oils were partly characterized (Cantarelli, *Riv. ital sostanze grasse 38,* 69).

Derivatives of acylaminothymol and of benzoylthymol were tested for antioxidant activity. Galloylaminothymol was most active among the acylaminothymols, being as effective as butylated hydroxyanisole and nordihydroguaiaretic acid (NDGA) in preventing oxidation of safflower oil and vitamin A (Fukuds, et al., *Yakugaku Kenkyu 32,* 335). Low toxicity was observed against mice. The antioxidant activity of 2-methyl-3,4,4',5'-tetrahydroxy-5-isopropylbenzophenone was excellent, but it colored the oil reddish yellow. In a similar study, ten derivatives of thymol were prepared and their antioxidant capacity determined (Naito and Urano, *Kyoto Yakka Daigaku Gahuko 8,* 38). Benzamidothymol showed good antioxidant activity in linolenic acid and was nontoxic, tasteless and odorless.

Patents were granted for an antioxidant composition made up in a corn oil base in which citric acid had first been dissolved, and 10–33% of butylated hydroxytoluene and combinations of other antioxidants added (Hall, *U. S. 2,981,628*); N-Hydroxyphenylureas as antioxidants for edible fats and oils (Zienty, *U. S. 2,967,775*); and the addition of from 0.2–2% butylhydroquinone as a stabilizer for high molecular weight fatty acid esters of vitamin A (Stieg and Nielson, *Ger. 1,058,211*).

Propylgallate was more efficient than octyl or dodecylgallate, or BHA and BHT, for inhibiting the oxidation of fats (Lyaskovskaya and Piul'skaya, *Trudy Vsesoyuz. Nauch.-Issledovatel. Inst. Myasnoĭ Prom. 1959,* 80). The antioxidant action and interactions of tocopherol, cetyl and octyl gallate and ascorbyl palmitate in the inhibition of oxidation of sunflower oil, peanut oil, and butter fat were discussed (Somogyi and Kundig, *Intern. Z. Vitaminforsch 31,* 221). Ascorbyl palmitate showed marked antioxidative action on sunflower seed and peanut oils, but had no action upon lard when subjected to prolonged storage at room temp. The maintenance of food quality with ethylenediaminetetraacetic acid (EDTA) and its di- or tetrasodium salts was reviewed (Aamoth and Butt, *Ann. N.Y. Acad. Sci. 88,* 526).

Methionine, alanine, proline, valine, glycine, aspartic acid, serine, phenylalanine, arginine, lysine, threonine, tryptophan, and histidine inhibited the rate of oxygen uptake of herring oil emulsions in decreasing order. Their inhibiting effect was strongly enhanced in the presence of phosphate (Marcuse, *Fette, Seifen, Anstrichmittel 63,* 547). Cysteine acted as a prooxidant in the same system. An extension of this study to the action of the same amino acids on the autoxidation of linoleate was made with similar findings (Marcuse, *Ibid.,* 940).

Evaluation and Analysis

The following methods were proposed for the evaluation of the protective action of antioxidants on fats and oils. Oils containing antioxidants were heated on a water bath with a current of oxygen sparging through at a definite velocity. Conjugated diene values were determined spectrophotometrically on aliquots of the oil every hr. Results agreed approximately with those obtained by peroxide value and viscosity measurements (Tsukamoto, *Pharm. Acta Helv. 35,* 431). The Swift stability test was modified so that the color produced by cresol red indicator served as the end point rather than using a peroxide value. This test was applied to the evaluation of antioxidants in tallow and to their detection in edible fats (Loury, *Rev. francs. corps gras 7,* 662). Peroxide number increase on standing at room temp, the active oxygen method, the lipoxidase method, and the thiobarbituric acid method, were evaluated as methods for studying the antioxidant activity of 8 commonly used antioxidants (Fukuba, et al., *Eiyô to Shokuryô 8,* 266). The active oxygen method was the most suitable.

Reverse-phase partition chromatography on Silastic 181 was used to separate BHA and gallate esters from fat (Berger, et al., *Analyst 85,* 341). The gallate esters were determined colorimetrically at 532 mμ after reaction with ferrous tartarate, and butylated hydroxyanisole at 610 mμ after reaction with 2,6-dichloroquinone chlorimide. Mixtures of ethyl propyl, nonyl, dodecyl and hexadecyl gallates, gallic acid, and nordihydroguaiaretic acid were separated by ascending paper chromatography with a mixture of ligroine, benzene and acetic acid (2:2:1). The compounds were detected by spraying with a 1% silver nitrate solution in ethanol and quantitatively analyzed comparing with a series of standards and equating the areas of the various spots (Salazar, *Galencia Acta* (Madrid) *13,* 333.) The comparison-chromatogram technique was also used with thin layer chromatography for the analysis of 3-BHA, 2-BHA, 4-hydroxyanisole, and hydroquinone (Seher, *Nahrung 4,* 466). Extraction with 95% methanol followed by the addition of acetone and ferrous ammonium sulfate to an aliquot of the dried, filtered extract and determination of the optical density at 580 mμ was used for the quantitative determination of total gallates in fats (Cassidy and Fisher, *Analyst 85,* 295).

A method for the analysis of antioxidants in polyethylene materials was based on dissolving the ether extract of the powdered material in a mixture of cyclohexane and ethanol (4:3) and determining its spectra between 220 and 350 mμ, (Cieleszky and Nagy, *Z. Lebensm.-Untersuch u. -Forsch. 114,* 13). A simple rapid modification of the method of Anglin (CA51, 4591h) was presented wherein an alkaline scrubber was used to remove volatile acidic interferences during the steam distillation of potato flake samples for the determination of BHT in the presence of BHA (Filipic and Ogg, *J. Assoc. Offic. Agr. Chemists 43,* 795). A quantitative test for the determination of BHA in fats was based on measuring the color (535 mμ) developed from the reaction of the extracted BHA with diazotized sulfanilic acid in alkaline solution (Laszlo and Dugan, *JAOCS 38,* 178). This method provides an accurate specific determination of BHA in fats even when other antioxidants are present.

A new semimicro method for the complexometric determination of nordihydroguaiaretic acid in lard was described (Sedlacek, *Fette, Seifen, Anstrichmittel 62,* 669). The use of 4,7-diphenyl-1,10-phenanthroline and the addition of phosphoric acid to prevent photochemical reduction resulted in an improved accuracy in comparison with the Emmerie-Engel method for the spectrophotometric determination of tocopherols (Tsen, *Anal. Chem. 33,* 849). Polarographic method was described for the determination of tocopherols in fats (Koltunova, *Trudy Vsesoyuz. Nauch.-Issledovatel. Vitamin. Inst. 6,* 260). The tocopherols were first separated as nonspanonifiable fraction, the sterols percipitated with digitonin, and the filtrate then oxidized with 0.1 N alcoholic $Ce(NO_3)_4$ solution.

Effect on Stability

Tocopherol was effective in increasing the storage stability of goose fat (Afanas' eva., *Sbornik Nauch. Rabot, Leningrad. Inst. Sovet Torgovli im. F. Engel'sa 1959,* No. 15, 100). The addition of 1% corn oil containing 0.002% α-tocopherol to lard had a maximum stabilizing effect while simultaneous addition of ascorbic and citiric acids with corn oil was without effect (Blattna and Manoŭsková, *Prumsyl portravin 12,* 150). The pyrocatechol derivatives 1,4-bis(3,4-dihydroxybenzyl),- 1,3-bis(3,4-dihydroxylbenzyl),- and 1,4-bis(2,5-dihydroxybenzyl) benzene were reported to have excellent antioxidative activity in lard and vitamin A (Yamada and Matsuda, *Kogyo Kaguku Zasshi 61,* 1257).

The antioxidant action of quercetin in lard seems to be a combination of reaction with free radicals and metal ion complexing, with the former being more important (Crawford, et al., *J. Food Sci. 26,* 139). Selective methylation of the hydroxyl groups of the quercetin molecule generally decreases the antioxidant activity. A study of antioxidant protection against oxidation of ox-fat by measurement of the hydroperoxide index at different time intervals showed that a combination of decyl gallate and BHT was the most effective (Clotet,

Anales bromatol. 12, 341). Ascorbic acid, sodium D-isoascorbate, and L-ascorbyl palmitate inhibited fat autoxidation in butter. However, darkening of the butter was caused by a reaction between decomposition products of ascorbic acid and proteins (Budslawski, *Zesyty Nauk. Wyzszej Szkoly Rolniczej, Olsztyn No. 60,* 243). Long-term storage and accelerated active oxygen tests showed that autoxidative degradation of anhydrous lanolin was inhibited for at least 32 months by 100 ppm of BHT or BHA (Clark and Kitchen, *J. Pharm. and Pharmacol. 13,* 172).

A number of 3-(alkylthio) propionic acids, their oxidation products, mercaptals, thiol derivatives of $PrCO_2H$, and ω-alkoxy derivatives of lower fatty acids were evaluated for their antioxidant action on vitamin A in cod liver oil. Only the 3-thiol derivatives of $EtCO_2H$ and the mercaptal derivatives were effective (Akagi and Aoki, *Yakugaku Zasshi 81,* 492). The addition of BHT to dried fish meal before grinding and storage resulted in light colored, odor free oil upon extraction (Fiskeridirektoratets Kjemisk-Tekniske Forskingsinstitutt, *Norw. 96,* 116).

The stability of rapeseed oil does not improve by the addition of ethyl or propyl gallates when the oil is stored with exclusion of air, light, and high temp. Only limited protective value was observed in improper storage conditions (Stawowczyk, *Acta Polon. Pharm. 17,* 229). An investigation of the protective action of various antioxidants on cocoa butter showed that a combination of BHA and BHT was most effective. BHA alone was considerably less active and citric acid alone was inactive (Verdauger, *Galencia Acta* [Madrid] *12,* 165). Treating soybean oil with a 0.01–0.5% solution of $K_2Cr_2O_7$, $KMnO_4$, or HNO_3 was reputed to retard the flavor reversion in soybean oil (Sims, et al., *U. S. 2,872,465*). The oxidizing agent may be incorporated in the water wash during the degumming operation. The preferred temp is 120–160F.

PROOXIDANTS

The kinetics of the initial phase of the metal-catalyzed aerobic oxidation of linoleic acid were examined in detail, and a number of mechanisms postulated (Heaton and Uri, *J. Lipid Research, 3,* 152). The evidence supported the hypothesis that trace-metal catalysis and the initiation of autoxidation are intimately connected. A method of studying the influence of metallic salts on the development of rancidity in fats entailed treating the filter paper with a salt of the metal followed by impregnating the paper with a fat with or without an antioxidant. Rancidity development was detected by odor since the metals may interfere with peroxide determinations. Good agreements were obtained with triplicates (Ladrón, et al., *Grasas y Aceites 11,* 26).

Residues of oxidized oil, present as contamination in the manufacture and storage of vegetable oils, catalyze the deterioration of fresh oils (Kozin and Ermakova, *Masloboino-Zhirovaya Prom. 27,* No. 2, 12). Determination of the peroxide and acid numbers of sunflower seed oils containing added phosphatides showed that significant damage to the oil was caused by temp fluctuation in the iron storage containers, which resulted in condensation of moisture and increased water content of the oil. Phosphatides in the oil absorbed moisture from the air and precipitated along with other mucilaginous substances; this also increased microorganism activity (Kozin and Sitnikova, *Izvest. Vysshikh Ucheb. Zavedenii, Pishchevaya Tekhnol. 1960,* 24).

Autoxidation rates of methyl linoleate emulsions in aqueous phosphate buffer solutions were found to increase with increasing concentrations of glucose, fructose and sucrose. It was postulated that the effect of the sugar lies in an activation of the decomposition of linoleate hydroperoxide and on the acceleration of the autocatalysis. Additions of NDGA, propylgallate and hydroquinone were ineffective in stopping oxidation when they were added after oxidation commenced; however, they effectively reduced the rate of oxidation when added at zero time (Mabrouk, et al., *J. Am. Chemists' Soc. 38,* 692). In contrast to other amino acids, cysteine acted as a prooxidant in the oxidation of herring oil emulsions (Marcuse, *Fette, Seifen, Anstrichmittel 63,* 547). Both sodium chloride and curing salt accelerated oxidative deterioration and inhibited hydrolytic deterioration in the fatty tissues of back fat from bacon pigs when stored under various conditions (Dahl and Persson, *Acta Agr. Scand. 10,* 289).

Iron in olive oil was determined by combustion with $Mg(NO_3)_2$ and polarography. The prooxidative properties of iron in olive oil were discussed (Foresti, *Riv. ital sostanze grasse 38,* 121). A similar combustion technique with $Mg(NO_3)_2$ followed by solution in HCl, extraction with organic reagents, concentration, and burning the extracts to obtain emission spectra was used for the trace analysis of Fe, Cu, Mn, Zn, Al, Ni, and Sn in edible fats (Vioque and del Pilar, *Grasas y Aceites 11,* 71). Various olive oils were examined by this method and found to contain traces of Fe, Cu, Mn, Zn, and Al. An analysis of 107 samples of ice cream and mellorine showed that the chocolate-flavored samples usually contained more copper and iron than the vanilla or strawberry samples. However, no relation could be established between the oxidative flavor defects and the copper or iron content of the samples (Yanderzant and Miah, *Food Tech. 15,* 515).

A general review on the effects of irradiation of foods was presented (Mendes, *Rev. cienc. vet. 54,* 109). It was concluded that the important effect of ionizing radiations on the lipids in foods was to hasten oxidation and rancidity. *Gamma*-irradiation of solutions of DL-α-tocopherol caused extensive destruction of this vitamin and gave rise to products similar to those obtained by autoxidation. Irradiation in mineral oil or methyl myristate destroyed more tocopherol than did irradiation in unsaturated solvents; methyl oleate, methyl linoleate. (Rose, et al., *J. Food Sci. 26,* 153). When α-tocopherol was irradiated in iso octane, the main product appeared to be a 5-exo-methylene tocopher-6-one derived by the abstraction of two hydrogen atoms from tocopherol. When tocopherol was irradiated in tributyrin, transesterification was found to be a major reaction. In peroxidizing linoleic acid, α-tocopherol was oxidized to α-tocopherol quinone, but no radical-tocopherol addition products were detected (Knapp and Tappel, *JAOCS 38, 151*). Experiments on the storage of irradiated beef and beef fatty tissue at chilling temp showed that microbial spoilage was considerably retarded. However, yellow carotenoid pigments bleached noticeably and peroxide accumulated more rapidly in the irradiated than in the control fat, with changes most marked near the surface exposed to air (Lea, et al., *J. Sci. Food Agr. 11,* 690). Irradiating salmon meat by *gamma*-rays with a weaker source for longer periods or from a greater distance caused more extensive deterioration in the natural oils (Kaneda, et al., *Nippon Suisangaku Kaishi 26,* 823). Considerable inhibition was obtained by vacuum packaging, treatment with CO_2 or soaking in L-ascorbic acid solution prior to irradiation.

PRODUCTS OF AUTOXIDATION

Increased attention was given to the quantitative and qualitative identification of the primary and secondary products of the autoxidation of lipids. Liquid-partition chromatographic methods were developed to determine dimers (Frankel, et al., *JAOCS 38,* 130) and hydroperoxides (Frankel, et al., *Ibid.,* 134) in autoxidized fats, fatty acids and their metal esters. By the use of benzene containing 2–4% methanol as the mobile solvent, the hydroperoxides were separated from unoxidized fatty acids or methyl esters and from secondary and polymeric decomposition products. Saponification of autoxidized fatty esters destroyed the peroxides as determined iodimetrically, but the resulting acids contained a fraction which was eluted in the same position as the hydroperoxide acids. Chromatographic separation of free fatty acids from oxidized deodorized oils gave three well isolated fractions composed of unoxidized acids, dimeric or polymeric fatty acids, and a polar fraction (ethyl ether eluate). A linear relationship was found between the dimer content of deodorized soybean oil and the peroxide value of the oil before deodorization. The method was proposed as a means of estimating the degree of oxidation an oil received before deodorization and of following various phases of fat oxidation, polymerization, and processing.

During the decomposition of methyl linoleate hydroperoxide at 4C in oxygen, dimers of varying polarities, scission acids, and isolated trans bonds were formed. One mole of oxygen absorbed by the hydroperoxide was accompanied by the destruction of 1 mole of cis, trans diene conjugation, ½ mole peroxide group, and 1 mole linoleate hydroperoxide. Destruction of diene conjugation was one-fourth as rapid in a nitrogen atmosphere as in oxygen. Once methyl linoleate hydroperoxide is formed, regardless of storage atmosphere, dimerization and attendant destruction of double bonds and peroxides proceed (Johnston, et al., *Ibid.,* 367).

A method for determining total oxidized fatty acids in oils and fats was based on partition chromatography with inversed phases. High mol wt polyethylene was used as a substrate with mixtures of water and acetone as the mobile phases and hydrocarbons as an immobile phase (Naudet, et al., *Peintures, pigments, vernis 36,* 638). Detailed descriptions were given for the preparation of chromatography columns employing a similar polyethylene carrier for the analysis of autoxidized fatty acids (Perrot and Desnuelle, *Rev. franc. corps gras 7,* 429).

Further attention was given to polarography as a technique for the identification of peroxides and allied materials in autoxidized fats. It was found that oleate and linoleate hydroperoxide can not be well distinguished, because their half wave

potentials are too close. Methyl elaidate hydroperoxide, cis-, and trans,trans-linoleate conjugated hydroperoxides and methyl linoleate 11-hydroperoxide also behaved the same way (Khan *Pakistan J. Sci. Ind. Research 2*, 287). The analytical procedures and the limits of detection for the analysis of various organic peroxides with KI, $Ti_2(SO_4)_3$, and 4,4'-tetramethyldiaminodiphenylmethane reagents were reported (Foxley, *Analyst 86*, 348).

Adsorption chromatography on thin layers of silicic acid were shown to be a powerful tool in the studies of epoxy acids and hydroxy acids. Gas-liquid chromatography of epoxy esters, using both polar and nonpolar columns, was also used in the detection and analysis of these compounds in mixtures (Morris, et al., *J. Lipid Research 2*, 68). A new method for the detection and examination of long-chain epoxy acids in seed oils was based on increased absorption at 2.795 μ in the near infrared spectrum caused by chlorohydrins produced from epoxides by treatment with anhydrous ethereal hydrogen chloride (Morris, et al., *Ibid.*, 77). The method is sensitive to approximately 0.2% of epoxy acid in an oil and is specific for epoxides.

A simple method for the determination of the carbonyl value of thermally oxidized fats, using hydroxylamine hydrochloride, was based on titrating the liberated HCl with 0.5 normal sodium hydroxide solution. The sensitivity of the method is only about 5 millimoles per kilogram of fat (Bhalerao, et al., *JAOCS 38*, 689). However, more sensitive procedures for carbonyl are also listed. Allowances must be made for the decomposition of hydroperoxides under the acid conditions maintained in the 2,4-dinitrophenylhydrazine method of estimating carbonyl compounds in fats (Bishop, et al., *Food Technology in Australia 13*, 654). Lillard and Day (*J. Dairy Sci. 44*, 623) also recognized that the reaction conditions for the carbonyl value test as well as the TBA test are conducive to hydroperoxide decomposition and each is largely measuring products of this reaction.

New techniques were described for the isolation of flavor components from fats and oils. An all-glass, high-vacuum (10^{-5} to 10^{-6} mm) laboratory apparatus was used for the quantitative recovery of volatile constituents from fats and oils at moderately elevated temp (de Bruyn and Schogt, *JAOCS 38*, 40). Another isolation process involving countercurrent contact of the oil with steam in a 30 plate Oldershaw column, condensation of the steam carrying the flavor compounds in a trap cooled with liquid nitrogen, continuous liquid-liquid extraction of the condensate with a small amount of ethyl ether, and removal of the solvent with a 6 plate Oldershaw column, results in very little decomposition during isolation (Chang, *Ibid.*, 669).

A method for estimating the nonurea adduct-forming acids (urea filtrate) content of fats and fatty acids was described (Firestone, et al., *J. Assoc. Offic. Agr. Chemists 44*, 465). Analyses of the nonurea-adduct monomers that were isolated from a fatty acid by-product distillate by this technique indicated the presence of cyclic structures (Firestone, et al., *JAOCS 38*, 418).

The paper chromatographic analyses of the autoxidation products of saturated fatty acids, oxidized without catalysts at 150C, showed that a continuous homologous series of aldehydes and aliphatic mono- and dicarboxylic acids were formed. This supports the theory that oxygen attack takes place simultaneously on all the CH_2-groups of the fatty acid chain (Thaler and Saumweber, *Fette, Seifen, Anstrichmittel 63*, 1045). The formation of methyl azelaldehydate in the methyl esters prepared from oxidized soybean and linseed oils, or linoleic, linolenic, and oleic acids was observed. It was suggested that the semialdehyde arises from decomposition of the hydroperoxide methyl ester with the hydroperoxy groups in the C-9 position by cleavage of the C-9–C-10 bond. Unpublished evidence that hydrocarbons and alcohols may be constituents of the volatile fraction of reverted soybean oil was also obtained (Frankel, et al., *JAOCS 38*, 161). Upon heating trilinolein at 210, 240, 270 and 300C, isolated trans-type and conjugated trans-trans type acids appeared in amounts increasing with increased temp and duration of heating (Kurita, *Yukagaku 7*, 186).

The volatile monocarbonyls produced by mildly oxidized esters of oleate, linoleate, linolenate acids and fats were characterized by paper chromotography of their 2,4-dinitrophenylhydrazones (Gaddis, et al., *JAOCS 38*, 371, and Ellis, et al., *J. Food Sci. 26*, 131). The major aldehydes in oleate and linoleate were those that might be expected from the scission of reported monomeric hydroperoxide isomers. The predominance of 2,4-heptadienal and propanal in linolenate suggested that the major monomeric hydroperoxides were 12- and 16-hydroperoxy conjugated dienoic isomers. Heating at 165C increased the proportions of the most unsaturated major aldehydes in all samples.

The neutral volatile products obtained by stripping oxidized soybean oil were fractionated by gas-liquid chromatography. Identification of the "green bean" odor fraction indicated that a 3-cis-hexenal was responsible for the "green" reversion flavor of soybean oil (Hoffmann, *JAOCS 38*, 1). n-Hexanal and 3-trans-hexenal, also found in this fraction, were not important to the reversion flavor. Two isomeric 2,4-hepta- and 2,4-decadienals were isolated from volatile decomposition products of soybean oil (Hoffman, *Ibid.*, 30). The stereoconfiguration of the isomers appeared to be 2-trans,4-cis, and 2-trans,4-trans. Another study on the characterization of compounds isolated from reverted (but not rancid) soybean oil showed the presence of ethyl formate, ethyl acetate, ethyl alcohol, n-butyraldehyde, 2-heptanone, and 2-heptenal. The presence of alcohol, ester, and possibly dimethyl amino compounds in the fractions with higher chromatographic retention times were indicated (Chang, *Ibid.*, 671).

Badly deteriorated dried milk can be distinguished by the large number and concentrations of aldehydes present (Parks and Patton, *J. Dairy Sci. 44*, 1) and (Parks, *Univ. Microfilms, L.C. Card* No. Mic. 60–5452). Lipid oxidation as well as Maillard-type browning occurred in instant nonfat dry milk stored at room temp for one year. Compounds identified were H_2CO, AcH, Me_2CO, butanone, methylpropanal, 3-methylbutanol, furfural, diacetyl, hexanal, and nonanal (Basette and Keeney, *J. Dairy Sci. 43*, 1744). The flavor constituents of butter oil oxidized at 40C were isolated by molecular distillation, extraction of the trapped contents with petroleum ether, and fractionation by gas-phase chromatography. The carbonyl compounds produced in relatively large amounts made only a limited contribution to the oxidized flavor; the main contributor appeared to be a minor constituent eluting in the vicinity of octanal and heptanal (El-Negoumy, et al., *J. Dairy Sci. 44*, 1047). This same flavor component was found in flavor concentrates from linseed, safflower, and herring oils.

A distinct codliver oil flavor appeared after storage of commercial butter fat samples containing NDGA and citric acid dissolved in propylene glycol. The condition could be reproduced by the combined effect of NDGA with either citric or lactic acids. The acids alone in butterfat gave oily and less clearly defined fishy flavors after storage (Pont, et al., *J. Dairy Research 27*, 205). Two fractions with distinct fishy flavor were obtained from the gas chromatographic separation of the flavor concentrate from fishy butter fat. One had an oily flavor and contained hexanal, heptanal, 2-hexenal, and 2-heptanone; the other of metallic flavor contained a single carbonyl compound present in a relatively small amount (Forss, et al., *Ibid.*, 211). Other compounds isolated included propanal, pentanal, octanal, nonanal, decanal, acetone, 2-pentanone, 2-nonanone, 2-undecanone, acraldehyde, 2-pentenal, 2-heptenal, 2-octenal, 2-nonenal, 2,4-heptadienal, a compound resembling 2,4-octadienal, a compound with a mushroom flavor and a compound that formed the 2,4-dinitrophenylosazone of methylglyoxal.

The stale-flavor components developing in dry whole milk were concentrated by a carbon tetrachloride or carbon disulfide extraction of the steam distillate. Infrared spectra of the extracts indicated the presence of CH_3, CH_2, ester or lactone carbonyl, aldehyde or ketone carbonyl, aliphatic ether, unsaturated ether, cis-CH:CH-, or :C:CH_2 groups (Whiteny and Tracy, *U. S. Dept. Com., Office Tech. Serv. PB Rept.* 143 776, 35 pp).

In irradiated beef, pork, and chicken, isooctane-sol. carbonyl compds. came from the lipid, and the H_2O-sol. carbonyl compds. from the protein portion of the meat. Many long-chain aldehydes and ketones, which appeared to be derived from plasmalogens and other lipids, resulted from irradiation (Monty, et al., *J. Agr. Food Chem. 9*, 55).

At least 28 carbonyls were found in the volatile portion of rancid salmon oil. Positive identification was made on C_2, C_3, C_5, C_6, C_7, C_8, and C_9 alkanals; the C_5, C_4, C_5, C_6, C_8, C_9, and C_{10} 2-enals; and hept-2,4-dienal (Yu, et al., *J. Food Sci. 26*, 192). Epoxy-β-cyclocitral was considered as the most probable oxidation product resulting from the exposure of vitamin A acetate to irradiated methyl linoleate in the presene of air (Hida, *Vitamin 16*, 149).

AUTOXIDATION: MECHANISM AND THEORY

In a study of the autoxidation of lard samples with different iodine numbers exposed to air at 20C in diffused sunlight the rates of oxygen absorption were directly proportional to the ratio of the average molecular weight of the triglycerides to the number of double bonds. Contrary to earlier concepts, the molar oxygen absorption depended not on the iodine number, but on the intramolecular distribution of double bonds in the triglycerides of the fat (Drozdov and Materanskaya, *Doklady Akad. Nauk S.S.S.R. 137*, 603). The

theories of the autoxidation of unsaturated fats and the mechanism of the inhibition and retardation of autoxidation by benzoquinone and hydroquinone were reviewed (Taufel, et al., *Fette, Seifen, Anstrichmittel 62,* 1061).

Peroxide formation in the autoxidation of pure lauric and stearic acids as well as their esters, carried out without catalysts at 120 to 150C, followed practically the same course as in the case of unsaturated fatty acids (Thaler and Saumweber, *Fette, Seifen, Anstrichmittel 63,* 945). Unexpectedly, total cis to trans isomerization did not take place during a high level of autoxidation of oleic acid with a current of air at 100C (Loury, *Comp. rend. 251,* 1643). Cis-trans conjugated linoleate was not found in any of the samples of cottonseed oil, methyl oleate, or methyl linoleate aerated at 0–180C for 24 hours. The total trans isomer content of the lipids did not increase much further on heating up to 180C from the critical temp which was 90C for cottonseed oil, 120C for methyl oleate, and 60C for methyl linoleate (Fukuzumi, *Yakagaku 10,* 143).

The kinetics of the initial phase of the metal-catalyzed aerobic oxidation of linoleic acid were examined in detail, and a number of mechanisms postulated. Experimental data was in fair agreement with theoretical considerations and supported the hypothesis that trace-metal catalysis and the initiation of autoxidation are intimately connected (Heaton and Uri, *J. Lipid Research 2,* 152). The catalytic activity of heme compounds on lipid oxidation in muscle tissues was reviewed (Tarladgis, *JAOCS 38,* 479). Existing data indicates that the ferric heme compounds initiate lipid oxidation, while the ferrous heme compounds are inactive in the absence of preformed peroxides. The latter are decomposed by both types of heme compounds, but by a different mechanism. Evidence favoring hematin catalysis over autoxidation as the dominant mechanism of lipid peroxidation in animal tissues was presented (Tappel, et al., *Ibid.,* 5). It was postulated that a tocopherol-ascorbate-glutathione-triphosphopyridine nucleotide couple could act synergistically to inhibit lipid peroxidations in animal tissues.

Neutral methemoglobin and acid hematin possessed high catalytic activity towards vitamin A and fat oxidation *in vitro* and *in vivo* (Dmitrovskiĭ, *Biokhimiya 26,* 225). Hemoglobin and hemin were found to catalyze the simultaneous oxidation of linoleic acid and various hydrocarbons of naphthalan crude oil and the decomposition of their peroxides (Babaev, *Sbronik Trudov Azerbaĭdzhan. Med. Inst. 1958,* 290). The breakdown of unsaturated fatty acid peroxides into inactive products was catalyzed by cytochrome c which was denatured during the process. The denatured cytochrome c catalyzed olefinic oxidation (Banks, et al., *Nature 190,* 908).

Rate of oxygen uptake increased with increasing concentrations of glucose, fructose, and sucrose in methyl linoleate emulsions in aqueous phosphate buffer solutions. The sugars seemed to be effective only in combination with the resulting methyl linoleate hydroperoxide. The data suggested concurrent oxidation of methyl linoleate and sugars; the sugars activating the decomposition of linoleate hydroperoxide and accelerating the autocatalysis (Mabrouk, et al., *JAOCS 38,* 692).

Many articles appeared dealing with kinetics studies of the autoxidation of non-fat materials. Attention is directed to a few selected papers because of the similarity of mechanisms involved. Photochemical reactions in solution were shown to be of two distinct types, the reversible photoreductions-photooxidations, where the system is at equilibrium in the dark, and light disturbs this equilibrium while darkness restores it, and the irreversible photoreductions-photooxidations where the system is not at equilibrium in the dark and light acts only as a catalyst by accelerating the reaction in the dark. A mechanism was presented for reactions where the photochemically active light is absorbed by an oxidized reaction component (Havemann, *Z. wiss. Phot. 54,* 220). Disproportionation and dimerization of tert-butyl and tert-amyl hydroperoxides and their corresponding radicals were increased in the presence of Mn, Fe, Co, or Ni. It was theorized that these reactions proceeded through a metal ion-free radical complex of relatively longer life than a simple alkyl radical (Kochi and Rust, *J. Am. Chem. Soc. 83,* 2017). Considerable dehydrogenation was observed in the autoxidation of dipentene at 80.5C with $Co(OAc)_2$ as a catalyst. Total oxygen absorption was independent of Co concentrations up to very high levels (29.2 mmoles/mole), where oxygen uptake was sharply decreased (Mitskevich and Sheerbak, *Sbornik Nauch. Rabot, Akad. Nauk. Belorus, S.S.R., Inst. Fiz.-Org. Khim. 1960,* 205). The kinetics of the autoxidation of α-fenchene and bornene were studied at 60–159C. The hydroperoxides formed as intermediates were mostly decomposed during oxidation. The primary oxidation products were ketonic compounds (Saito and Fushizaki, *Bull. Univ. Osaka Prefecture,* Ser. A 8, 67). The liquid phase autoxidation of 5-methylnonane at 90C was investigated to determine the effect of a methyl group on the position of radical attack during autoxidation. The susceptibility of various carbon positions to attack was in the expected order (tertiary > secondary > primary, Arndt, et al., *J. Chem. Soc. 1959,* 3258). In the radiochemical oxidation of liquid hydrocarbons by molecular oxygen, the rate was constant at 45 and 63C and the oxygen absorbed was all converted into peroxides (ROOR prime), thus ruling out initiation by decomposition of hydroperoxides, which was the mechanism of thermal and photochemical oxidation (Montarnal and Brun, *Large Radiation Sources in Ind., Proc. Conf., Warsaw, 1959* 2, 29). The efficiency for inhibiting the autoxidation of a white mineral oil was with 2,6-di-tert-butyl-4-substituted phenols was studied. Electron-releasing 4-substituents increased the efficiency of the inhibitors, provided the substituents were small. For bulky alkyl substituents in the 4-position, the relative inhibiting efficiencies were related to the Taft steric substituent constants (Ingold, *J. Phys. Chem. 64,* 1636).

The mechanism of the liquid-phase oxidation of cyclohexanol at 110 to 130C was investigated by the method of inhibition of free radicals with 1-naphthol in the course of the reaction. The rate of the inhibitor consumption was proportional to the rate of the chain initiation (Denisov and Kharitonov, *Zhur. Fiz. Khim. 35,* 444). In a study of the effects of antioxidants on the kinetics of the oxidation of a kerosene fraction (synthane), it was found that the inhibiting action was determined not only by the structure of the inhibitor, but, in accordance with the chain-position theory, by the type of the hydrocarbon undergoing oxidation (Rozhkov and Kornilova, *Khim. i Tekhnol. Topliv i Masel 6,* No. 5, 54).

The decomposition and secondary oxidation of hydroperoxides was the subject of many investigations. The addition of α-naphthol during oxidation of n-decane at 130C resulted in an immediate lowering of the hydroperoxide concentration. This was followed by continuous decrease in hydroperoxide concentration, indicating a nonchain decomposition mechanism. When the reaction was inhibited by phenol, the hydroperoxide was quantitatively decomposed to the corresponding alcohols, indicating that the major radical involved is RO. The number of links in the chain reaction was established as < 20 and is dependent upon hydroperoxide concentration (Maĭzus, et al., *Doklady Akad. Nauk S.S.S.R. 131,* 351). A detailed kinetic study was made of cumene hydroperoxide decomposition (to phenol and acetone) as catalyzed by acid ion exchange resin (Marberry, *Univ. Microfilms, L.C. Card* No. Mic 60-6903). The rates of iodine value decrease in comparison to the rates of peroxide decomposition during various stages of the autoxidation of fats by aeration at 99C showed that the decrease in iodine value is not caused by the interaction of fat hydroperoxides with double bonds (Kartha, *J. Sci. & Ind. Research* [India] *19B,* 199). The concentration of primary alcohols resulting from the thermal decomposition of hydroperoxides formed by the oxidation of n-undecane decreased with increased reaction temperature (Tsyskovskii and Shchleglova, *Khim. Prom. 1961,* 325).

An enzyme solution extracted from defatted soybean meal and added to linoleic acid emulsions caused the destruction of hydroperoxides formed during oxidation. The hydroperoxide-destroying factor was more active than hemoglobin, catalase, peroxidase, or cytochrome c (Blain and Barr, *Nature 190,* 538).

In a study of secondary deterioration in partially oxidized lipids, it was established that the free amino groups of glycine, phenylalanine and the ε-amino group of lysine were sites of reaction in the insulin-autoxidizing methyl linoleate interaction. Polarographic analysis indicated that at least 4 compounds are involved in the reaction with protein amino groups (Quartermaster Food & Container Inst. for the Armed Forces, *L-400-Rept.* Nos. 5 and 6). In the final stage of the autoxidation of methyl esters of linseed oil fatty acids, the secondary oxidation products as well as the remaining hydroperoxides decompose and form, in part, polymers. A free radical mechanism for chain formation was theorized and C-C as well as C-O bonds were indicated (Helme, et al., *Peintures, pigments, vernis 36,* 574). The absorption rate of oxygen was measured during the oxidation of hexadecane at 140C and the change in the concentration of hydroperoxides determined iodometrically. At first, the absorption of oxygen was autocatalytic; however, above 25–30% oxidation, the absorption rate decreased rapidly. The formation of oxidation products which could break the reaction chain was indicated (Yur'ev, et al., *Doklady Akad. Nauk. S.S.S.R. 125,* 1301).

Linoleic acid was oxidized at 37C on filter paper containing various amounts of lipoxidase, stearic acid, or light. The principal primary compounds formed were n-hexanal, 2-octenal, and 2,4-decadienal, in almost the same proportion under all condi-

tions (vander Poel, *Neth. Milk Dairy J. 15,* 98). A model system of thin films of lipids supported on dry gelatin plates was used for studying rates of lipoxidation. Variations in the nature of the gelatins used showed significant differences in protective action against lipid oxidation. Gamma-tocopherol and phospholipid in large concentration were effective inhibitors (Togashi, et al., *J. Food Sci. 26,* 186). The effects of surface-active agents and antioxidants on legume lipoxidase activity were studied. Observations supported the existence of at least two lipoxidases in legumes, one specific for linoleic acid or other fatty acids with metylene-separated dienoic systems, and the other for glycerol esters of these fatty acids (Dillard, et al., *J. Biol. Chem. 236,* 37).

COMPOSITION AND CHARACTERISTICS

OFFICIAL METHODS AND REVIEWS

The Uniform Methods Committee of the American Oil Chemists' Society reported minor changes in the methods for seed and meal analysis and the determination of Lovibond color (Henery, et al., *JAOCS, 38,* 224). The results of a joint study of the determination of crude fiber by the American Oil Chemists' Society and the American Official Agricultural Chemists were reported (Holt, *Ibid,* 151). The Uniform Methods Committee of the American Oil Chemists' Society adopted changes in the methods for determining crude fiber and oxirane content (Sallee, et al., *Ibid,* 404). The American Official Agricultural Chemists also modified their method for determining crude fiber (Philbeck, *J. Assoc. Offic. Agr. Chem., 43,* 536). The Spectroscopy Committee of the American Oil Chemists' Society reported the status of the determination of *trans*-isomer by infrared absorption (O'Connor, et al., *JAOCS, 38,* 180). A study was made of the American Oil Chemists' Society's tentative method for determining *trans*-isomer using three different instruments (Firestone and Villadelmar, *J. Assoc. Offic. Agr. Chemists, 44,* 459). It was found that triglycerides interfered with the determination but that a correction was possible. A collaborative study of the determination of fat content of expanded dog food revealed that there was a significant drop in the fat extractable by the AOAC method 22.033 with ethyl ether after expansion but none in the 22.035 method. It was requested that method 22.035 be amended to cover expanded dog food (Hoffman, *Ibid,* 556).

A review of the 1959-60 literature of composition and characteristics was compiled by the American Oil Chemists' Society Literature Review Committee (Rusoff, et al., *JAOCS, 38,* 423). The American Oil Chemists' Society Short Course for 1960 was devoted to "Newer Lipid Analyses." For details regarding topics covered and for methods of interest to the lipid biochemist, the reader is referred to the "Analytical and Methodology" part of the section on "Nutrition, Physiology, and Biochemistry."

ANALYSIS OF FAT SOURCES

A rapid method was reported for the determination of lipids in brewing adjunct cereals based on blending the sample with a solvent in a Servall Omnimixer for 2.5 min (Haas and Fleischman, *Cereal Chem., 38,* 198). The extract was filtered and the solvent removed at 103–5C for 75 min. The results were not significantly different from two other methods. The Gerber, DPS, TeSa, Schain, Babcock, and Mojonnier procedures were compared for the determination of the butterfat content of raw milk (O'Dell, *J. Dairy Sci., 44,* 47). The Babcock, Gerber, and DPS averaged higher than the Mojonnier and the TeSa and Schain tests were lower. All the differences were significant except between the DPS and Mojonnier. The TeSa and Schain tests were significantly lower than the Babcock method. The Gerber, DPS, TeSa, and Schain test were more closely correlated with the Babcock test than with the Mojonnier. A method for estimating the total body fat of animals by measuring the postassium-40 content in a whole-body scintillation counter was devised. It is assumed that the potassium-40 content of the lean body mass is const. The method correlated well with the estimation of fatness by skin-fold thickness and wt/ht ratios for children and young adults (Forbes, et al., *Science, 133,* 101 and 1917).

A simple graphic method was presented to determine which of two or three refining-cup results is the settlement cup according to the National Cottonseed Products Association Rule No. 201 (Sikes, *JAOCS, 38,* 266). Laboratory test on United States soybean oils showed no relationship between crude free fatty acid and refining loss. The use of excess lye tended to increase refining losses without improving refined oil color (Castro and Nosti, *Grasas y Aceites [Seville, Spain], 11,* 213). In an investigation of oven methods for determining the moisture content of shelled peanuts, the results obtained with ground, sliced, and whole nuts were compared (Hart, *JAOCS, 38,* 190). Whole nuts were found to give the most accurate results. A sample heated in a forced draft oven at 130C for 3 hr gave values which agreed with the Karl Fischer method.

Changes in the official methods of determining crude fiber and fat content will be found under the Official Methods and Reviews part of this section.

GRADING AND VITAMIN TESTS

A chromatographic method for separating vitamins A and D and cholesterol which uses a polytetrafluoroethylene column was reported (Areus and Dunckley, *J. Chromatography, 5,* 272). A procedure was described for the separation of the carotenoids of yellow corn into seven fractions by chromatography on magnesia (Quackenbush, et al., *J. Agr. Food Chem, 9,* 132). Subsequent spectral examination gave values for three provitamin A's and the eight predominant biologically inactive carotenoids. In addition to the *cis*-isomers of the major polyenes, a number of minor components were recognized. The carotenoids and chlorophylls of marine algae were separated by a two-dimensional paper chromatography with 1-propanol-petroleum ether and chloroform-petroleum ether (Jeffrey, *Biochem. J., 80,* 336). The pigments separated included chlorophylls a, b, and c; their pheophytins; the carotenes; the xanthophylls lutein, violaxanthin, neoxanthin, fucoxanthin, and isomers, peridinin, esterified astaxanthin, and a number of minor xanthophyll components.

An improved hydrochloric acid color test was reported for carotenoid, 5,6- and 5,8-epoxides (Curl and Bailey, *J. Agr. and Food Chem., 9,* 403). The reaction was carried out in ether-methanol and followed spectrophotometrically. Diepoxides give greenish blue; monoepoxides give yellowish green. Two epoxides of unknown structure, persicaxanthin and sinensiaxanthin, gave red violet colors. Citric acid may be used instead of hydrochloric to detect 5,6-epoxides. Contrary to earlier reports, neither β-apo-2-carotenal nor capsorubin gave a blue color in the test. Vitamin A aldehyde may be determined with thiobarbituric acid (Futterman and Saslaw, *J. Biol. Chem., 236,* 1653). The assay does not distinguish isomers of Vitamin A aldehyde.

A procedure for the determination of α-tocopherol was reported which depends on chromatography on a paraffin coated kieselguhr column developed with aqueous ethanol (Csallany and Draper, *Arch. Biochem. Biophys., 92,* 462). An improvement for the Emmerie and Engle method was proposed (Tsen, *Anal. Chem., 33,* 849). Substances were found in fat solvents which reacted with ferric chloride-bipyridyl to give false tests for tocopherol (Booth, *Ibid.,* 1224). These materials made recovery experiments look better than they really were. The substances were found in the solvents even after they had been percolated through various adsorbents. The substances were separated from tocopherol by paper chromatography but not always by column chromatography.

FUNCTIONAL GROUP ANALYSIS. The reader is referred to the Official Methods and Reviews part for several reviews pertinent to this subject. A rapid colorimetric method was developed for determining the fat acidity in grain (Baker, *Cereal Chem., 38,* 7). The method depends on the reaction of a benzene solution of the fatty acids with aqueous cupric acetate. The copper soaps were soluble in the benzene and gave a blue color. The long chain free fatty acids of blood plasma and tissues were isolated for microdeterminations by a single extraction with heptane-isopropyl alcohol (Dole and Meinertz, *J. Biol. Chem. 235,* 2595). The long chain fatty acids were found in the heptane phase. If large amounts of lactic, acetic, acetoacetic or β-hydroxybutyric acids were present, a second extraction eliminated their interference.

The iodine value of fatty acids and oils was determined with ozone (Maggiolo and Tumolo, *JAOCS, 38,* 279). A stream of ozonized air containing a known amount of ozone was passed through the sample until the ozone was no longer taken up. The iodine value of fats was determined radiometrically with iodine-131 (Jaky and Kaffka, *Fette, Seifen, Anstrichmittel, 62,* 682). This method may be used on paper chromatograms. The Wijs method was adapted to high molecular weight fatty nitrogen derivatives (Milun, *Anal. Chem., 33,* 123). The procedure entails acetylation of primary and secondary amines, use of acetic acid as a solvent for tertiary amines and addition of sodium lauryl sulfate before titration for quaternary ammonium compounds. The determination of the quantity of fatty acids with isolated double bonds was accomplished with a single spectrogram in the Schumann ultraviolet region (Schauenstein and Benedikt, *Fette, Seifen, Anstrichmittel, 62,* 687).

Fats and synthetic triglycerides with only saturated fatty acids present were found to absorb at 10.3 μ and interfere with the determination of *trans*-unsaturation (Kaufmann, et al., *Ibid.*, *63*, 8). Absorption in this region was also found when fatty esters of polyols and fatty esters of varying chain lengths were examined. This report was confirmed and a correction procedure was reported (Firestone and Villadelmar, *J. Assoc. Offic. Agr. Chemists*, *44*, 459). The infrared spectra of 35 different fatty materials (including triglycerides, both saturated and unsaturated, normal and hydroxylated, free fatty acids and methyl esters) were presented to aid in the determination of *trans*-unsaturation (Jart, *Oleagineux*, *16*, 101). The reader is also referred to the Official Methods and Reviews part for information about the official method for determination of *trans*-unsaturation.

A new method for the detection and estimation of long-chain epoxy acids was described based on the absorption at 2.795 μ of the chlorohydrins produced from epoxides by anhydrous hydrogen chloride (Morris and Holman, *J. Lipid Research*, *2*, 77). The method is sensitive to 0.2% epoxy acid. Hydroxy acids unless they are vicinally unsaturated and cyclopropenoid fatty acids do not interfere. The adoption of a number of separation procedures for the determination of the epoxy components in seed oils was made (Morris, et al., *Ibid.*, *2*, 68; *JAOCS*, *38*, 316). These included distillation, urea, countercurrent distribution and a variety of partition and adsorption chromatography techniques. Some of the methods in combination provide a total analysis of the epoxy acids.

The halphen test for the cyclopropene configuration present in sterculic and malvalic acids was studied and conditions were found which gave a reproducible color which may be used as a spectrophotometric method (Deutschman and Klaus, *Anal. Chem.*, *33*, 1809).

A new modification of the hydroxamic color test for ester groups was reported (Antonis, *J. Lipid Research*, *1*, 485). The reaction and color development were carried out under essentially nonaqueous conditions to give a higher color yield and more stable color. The average error of the method was 1.7%.

A spectrophotometric method for the determination of fatty acid amides in lipids was devised (Haskins, *Anal. Chem.*, *33*, 1445). With the method used, fatty acid esters and amides both gave the same absorbance per equivalent of acid. The fatty acid ester was determined by the method of Tauber, and the amide was determined by difference.

The reaction between ozone and oleic and erucic acids was studied under a variety of different solvent and cleavage conditions (Pasero and Naudet, *Fette, Seifen, Anstrichmittel*, *62*, 190). A new method for detecting double bonds was suggested which is less likely to give false positive tests (Sharefkin and Shwerz, *Anal. Chem.*, *33*, 635). The olefin is reacted with peracetic acid to form the glycol monoacetate which can then be detected as an ester with the hydroxamate-iron test. The epxodides formed may also be rearranged with boron trifluoride to carbonyl compounds which may be characterized as 2,4-dinitrophenylhydrazones. Acetylenes do not interfere. Acetylenes may be detected and differentiated from olefins by hydrating with a mixture of boron trifluoride, mercuric oxide, and trichloroacetic acid (Sharefkin and Boghosian, *Ibid.*, 640). The carbonyl compounds that are formed may be detected as 2,4-dinitrophenylhydrazones.

Nuclear spin resonance was applied to the analysis of the isomers obtained by hydrogenation of linolenic acid (Willard and Martinez, *JAOCS*, *38*, 282). Mass spectrometry was explored as a method for locating double bonds in monoenoates and for identifying the volatiles from reverted soybean oil (Selke, et al., *Ibid.*, 614).

Small amounts of iron and copper in vegetable oils were determined by ashing the oil at 800C in the presence of acid and determining the iron and copper colorimetrically with o-phenanthrolin and diethyldithiocarbamate, respectively (Takeuchi and Tanaka, *Kôgyô Kagaku Zasshi*, *64*, 305). The amount of iron, copper, manganese, zinc, aluminum, nickel and tin in fat was determined by ashing the fat in the presence of magensium nitrate, dissolving the residue in hydrochloric acid, extracting the solution with various organic reagents, and burning the extracts to obtain emission spectra (Vioque and del Pilar, *Grasas y Aceites* [*Seville, Spain*], *11*, 71). The method was quantitative for these metals.

ANALYSIS OF LIPID CLASSES

The reader is referred to the Official Methods and Reviews part for several reviews pertinent to this area.

The lipids of wheat flour were fractionated into sterol esters, triglycerides, phosphatidylethanolamine, lecithin, lysolecithin, and an ethanolamine—containing lipid (not phosphatidylethanolamine) by chromatography on silicic acid using a concave gradient elution system of methanol in chloroform (Wren and Elliston, *Chem. & Ind.* [*London*], *1961*, 80). Chromatography on Florisil (magnesium silicate) was used to separate a model mixture of hydrocarbon, cholesterol ester, triglyceride, free sterol, diglyceride, monoglyceride, and free fatty acid (Carroll, *J. Lipid Research*, *2*, 135). The order of elution was the same as with silicic acid except free acids were eluted after monoglyceride. Phosphatides were not eluted under the conditions used, but Florisil offered several advantages in the fractionation of the above classes. A method for separating the major lipid classes on silicic acid, while avoiding the use of low boiling solvents, was worked out (Horning, et al., *Ibid.*, *1*, 482). Mixtures of benzene in hexane were used effectively in separating cholesterol ester and triglyceride from the other lipid classes. Alumina was investigated as an adsorbant for the separation of polar and nonpolar lipids (Sims and Mes, *JAOCS*, *38*, 229). None of the alumina systems tried gave satisfactory separation when the lipid phosphorous load approached 0.4 mg./g. of alumina, although silicic acid columns resolved such mixtures completely. Gamma alumina had greater resolving power than alpha alumina monohydrate, and Brockmann Grade I alumina with a "strong" solvent system gave better separation than less activated aluminas with "weaker" solvents. The elution of lipid compounds from silicic acid columns was monitored by the absorption of the eluate at 1745 cm^{-1} (Wren and Lenthen, *J. Chromatography*, *5*, 370).

It was found that the separation of lipoproteins by ultracentrifugation at a density of 1.063 was hindered by failure of the high density fraction to accumulate in the bottom of the centrifuge tube (Sakagami and Silversmit, *J. Lipid Research*, *2*, 271). This interfered with the recovery of the low density lipoproteins. Dextran sulfate precipitation was suitable for the quantitative separation of β-lipoprotein. The β-lipoprotein prepared in this way could be shown to be free of α-lipoprotein by electrophoresis.

Molecular distillation was used to determine the proportions of mono-, di-, and triglyceride in a mixture (Privett, et al., *JAOCS*, *38*, 312). The mono- and diglycerides undergo considerable acyl migration during this treatment, but the method was useful if there was nothing present which catalyzed disproportionation. The same authors reported that thin-layer chromatography could be used to determine α- and β-monoglycerides and 1,2- and 1,3-diglycerides on a microscale. The relation between the amount of hydrolysis of a fat, the free fatty acid, saponification value, neutralization number, hydroxyl number and content of mono-, di-, and triglycerides was analyzed mathematically (Ceballos, *Grasas y Aceites* [*Seville, Spain*], *11*, 79). The 1- and 2-monoglycerides may be differentiated and mixtures may be analyzed by investigation of the overtones and stretching vibration of the hydroxyl group near 1.4 μ (Susi, et al., *JAOCS*, *38*, 199). A new method for the microdetection of diglycerides on paper chromatograms was discovered (Clark, *J. Chromatography*, *5*, 368). A rapid method for the direct determination of the glycerides in human blood was devised (Blankenhorn, et al., *J. Lipid Research*, *2*, 281). A Florisil column was used to remove phosphatides from the lipid extract and the glycerides in the eluate were saponified and analyzed for glycerol by periodate oxidation. A similar method was devised for the determination of liver triglycerides (Maling, et al., *Ibid.*, 95). In this method the phospholipids were removed with a zeolite column. A fluorimetric method for the determination of glycerol has been devised which was useful in such triglyceride determinations (Mendelsohn and Antonis, *Ibid.*, 45). The glycerol was reacted with o-aminophenol in the presence of concentrated sulfuric acid and an oxidizing agent to form 8-hydroxyquinoline. The latter compound fluoresced on chelation with a divalent cation in alkaline solution. The method was also useful in determining phosphatide glycerol.

A method for the complete separation of phosphatidyl ethanolamine and phosphatidyl serine by chromatography on silicic acid-silicate-water columns with chloroform and methanol as solvents was described (Rouser, et al., *JAOCS*, *38*, 14). The same authors reported a rapid paper chromatographic method for the separation of phosphatides using paper impregnated with disodium hydrogen phosphate or silicic acid and developed with mixtures of chloroform and methanol. A procedure for the fractionation of butter phosphatides on silicic acid, using gradient elution with methanolchloroform was used in the study of the effect of phosphatides on blood coagulation (Billimoria, et al., *Biochem. J.*, *78*, 185). A method combining silicic acid chromatography and differential hydrolysis was used to determine the fatty acid composition of several phosphatide fractions of blood (Bottcher and van Gent, *J. Atheroscler. Res.*, *1*, 36). The combined use of thin-layer chromatography, paper chromatography, countercurrent

distribution, and infrared spectroscopy enabled the identification of various phosphatides with certainty (Wagner, *Fette, Seifen, Anstrichmittel, 62,* 1115). Phosphatidylserine, mono- and diphosphoinositides, lecithin, cephalin, cerebroside, sphingomyelin, and cardiolipin were studied. An improved method for the separation of phosphatides on silicic acid impregnated paper which avoided loss of lecithin at the point of origin was described (Zieve, et al., *Proc. Soc. Exptl. Biol. Med., 105,* 508). Methods for the saponification of lecithin and hydrogenation of the resulting fatty acids that may be carried out on paper chromatograms were described (Kaufmann and Wessels, *Fette, Seifen, Anstrichmittel, 62,* 1020). The methods were useful in studying the fatty acid composition of lecithin by paper chromatography. A method for the hydrolysis of phosphatides, into water soluble phosphates, which could be separated and estimated by ion exchange chromatography, was reported (Hubscher, et al., *J. Lipid Research, 1,* 423). The effect on the hydrolysis reaction of alkali concentration, organic solvents, and time was studied. Lecithin, phosphatidyl ethanolamine, phosphatidyl serine, and phosphatidyl inositol in liver were determined by this method. The sphingolipids of brain were fractionated on silicic acid columns after a preliminary mild alkaline treatment (Schwarz, *Ibid., 2,* 208). Cerebrosides containing hydroxy fatty acids were eluted after cerebrosides without hydroxy fatty acids. Pure sphingomyelin was isolated and the recovery of hexose and alkali stable phosphorus was high.

An improved procedure for converting cerebroside to ceramide and sphingosine was devised (Carter, et al., *Ibid.,* 228). The glycosidic ring was oxidized with periodate followed by reduction with sodium borohydride and mild acid hydrolysis to ceramide. Alkaline hydrolysis then gave erythrosphingosine.

The separation of mixtures of steroids containing 19, 21, and 27 carbon atoms was investigated by gas phase chromatography using various stationary phases (Lipsky and Landowne, *Anal. Chem., 33,* 818). Relations were discovered between the order of elution on the various stationary phases and the chemical structure of the steroid. Silicone rubber gums and grease were used at low concentrations and 240–400C for the separations of sterols, tocopherols, fatty acid methyl esters and waxes (Nicolaides, *J. Chromatography, 4,* 496). Five polyesters were used as stationary phases in the separation of steroids by gas chromatography (Haahti, et al., *J. Org. Chem., 26,* 626). Steroids containing a polar group had increased retention times on these phases compared to the SE-30 phase originally used. The fluorinated silicone polymer QF-1, was recommended as a thermostable polar liquid phase for the gas chromatography of steroids and other natural products (Vanden Heuvel, et al., *J. Am. Chem. Soc., 83,* 1513). A gas chromatographic method for the determination of cholesterol and squalene in sebum was published (O'Neill and Gershbein, *Anal. Chem., 33,* 182). The cholesterol determination can be carried out on the unsaponifiable fraction from the sebum or on a fraction enriched in sterol by liquid column chromatography. The squalene was analyzed directly from the sebum or from the unsaponifiable fraction. The method was also used on various other biological mixtures. The acetic acid esters of estrone, estradiol, and estriol have been quantitatively analyzed by gas chromatography (Wotiz and Martin, *J. Biol. Chem., 236,* 1312). Evidence for quantitative acetylation and thermal stability of the hormones was presented. Gas-liquid chromatography was used to analyze bile acids (Blomstrand, *Proc. Soc. Exptl. Biol. Med., 107,* 126).

A paper chromatographic method for the separation and detection of sterols on paper chromatograms was developed and used for distinguishing between animal and vegetable fats (Harzopoulos, *Rev. franc. corps gras., 7,* 575). The paper was impregnated with 2% paraffin oil—10% butanol—88% ethanol and dried for 15 min at 120C. The sterols were separated as their respective acetates using methanol or ethanol as a developing solvent. The spots were detected with silicotungstic acid. Over 30 color tests suitable for detecting sterols on paper chromatograms were studied (Katz, *Arch. Biochem. Biophys., 91,* 54). The Carr-Price reagent and a system of p-anisaldehyde, sulfuric acid, antimony trichloride, and chloroform were the most promising reagents with respect to sensitivity and range. Various structural groups and isomers could be distinguished on the basis of their reactions with several reagents, and a scheme for the tentative identification of steroids was developed from this. Thin-layer chromatography was used to fractionate the cholesterol esters of 15 saturated and unsaturated fatty acids (Kaufmann, et al., *Fette, Seifen, Anstrichmittel, 63,* 235). Both plates impregnated with paraffin and unimpregnated plates were used. Tetralin-hexane and methyl ethyl ketone-acetonitrile were used as developing solvents and the spots were detected with phosphomolybdic acid.

A reagent consisting of 4.5–22.5% sulfuric acid, 1% acetic acid, and at least 40% acetic anhydride for estimation of cholesterol in serum was patented (Hopper, *U. S. 3,001,950*). A rapid method for the extraction and spectrophotometric determination of cholesterol in small samples of blood and cerebrospinal fluid was described (Shin and Lee, *Anal. Chem., 33,* 1220). The sample was extracted with chloroform-methanol and the color was developed by heating with acetic acid and ferric chloride. A spectrophotometric method for the determination of cholesterol and coprosterol in feces was reported (Gerson, *Biochem. J., 77,* 446). The method involved the precipitation of the sterols with digitonin and the formation of color with ferric chloride-acetic acid-sulfuric acid. A modified Liebermann-Burchard reagent for the determination of cholesterol was reported (Huang, et al., *Anal Chem., 33,* 1405). It is stable for 2 weeks at room temp and more than 4 weeks under refrigeration. A rapid one step procedure with the reagent was described. The cholesterol content of blood serum could be determined directly by the turbidity produced when sodium alcoholate was added to the serum (Kingsley and Robnett, *Ibid.,* 561). The results obtained by photometric measurement of the turbidity agreed well with established methods.

The pigments of cottonseed oil were fractionated by means of molecular sieves and countercurrent distribution (Verberg, et al., *JAOCS, 38,* 33). Gossypol and pigments which have an absorption maxima below 375 mμ are trapped by 13X molecular sieves and appear in the hydrophilic phase when cottonseed oil is distributed between 95% ethanol and isooctane. These pigments are found in the liquid fraction when cottonseed oil is crystallized from acetone at —63C. Pigments not admitted to the 13X sieve were found in the hydrophobic phase on countercurrent distribution and in the solid fraction from low temp crystallization.

The reader is referred to the Grading and Vitamin Tests' part for additional methods for the analysis of the unsaponifiable fraction of lipids.

FATTY ACID ANALYSIS. The reader is referred to the Official Methods and Reviews' part for several reviews pertinent to this section. A rapid and simple procedure for the conversion of fatty acids to their methyl esters for gas chromatography, was devised which consisted of boiling the fatty acids in boron trifluoride-methanol for two min (Metcalfe and Schmitz, *Anal. Chem., 33,* 363). A quantitative comparison of four methods for the preparation of methyl esters for gas chromatography was reported (Vorbeck, et al., *Ibid., 33,* 1512). The reagents were: diazomethane, methanol-hydrochloric acid (with sublimation), methanol-hydrochloric acid on ion exchange resin, and methanol-boron trifluoride. The best method depends upon the nature of the sample. The use of thin-layer chromatography as a complement to gas-liquid chromatography was advocated (Mangold and Kammereck, *Chem. & Ind. [London], 1961,* 1032). Hydroxy fatty acid methyl esters may be removed from the gas chromatography sample by this technique. The sample can also be separated into saturated and unsaturated esters by thin-layer chromatography of the mercuric acetate addition products either before or after separation by gas-liquid chromatography.

The relative response of a thermal conductivity cell for fatty acid methyl esters of C_2 to C_{22} saturated fatty acids and oleate, linoleate, linolenate, and elaidate was measured (Horrocks, et al., *J. Lipid Research, 2,* 92). The data was used to determine correction factors that could be applied to the usual peak area measurements. It was possible, by using gas chromatography, to partially separate the positional isomers of the monoene, diene, and triene methyl esters obtained from the hydrogenation of linoleic acid with hydrazine (Scholfield et al., *JAOCS, 38,* 208). A preliminary separation by countercurrent distribution was necessary. Free fatty acids from C_6 to C_{20}, both saturated and unsaturated, were separated by gas-liquid chromatography on Reoplex 400 at 180C. Under the conditions used the separation was as good as with methyl esters (Stuve, *Fette, Seifen, Anstrichmittel, 63,* 325). Cyclopropenoid fatty acids were analyzed by gas chromatography after hydrogenation (Wilson, et al., *JAOCS, 38,* 616). A quantitative method for determining the steam volatile fatty acids in biological materials was reported (Gherke and Lamkin, *J. Agr. Food Chem., 9,* 85). The technique included vacuum concentration of the sample and removal of residual water on the column. A quantitative procedure for the analysis of milk fat by gas chromatography was reported (Smith, *J. Dairy Sci., 44,* 607). The milk fat was converted to methyl esters and extracted from the reaction mixture with ethyl chloride. Known mixtures were analyzed under carefully controlled conditions to supply correction factors for the evaporation loss of short chain fatty esters and variations in the thermal conductivity cell response. The limitations of gas-liquid chromatography with some naturally occurring fatty acids were investigated (Morris, et al., *J. Lipid Research, 1,* 412). Con-

jugated trienoates were found to undergo *cis-trans* isomerization. The esters of vicinally unsaturated hydroxy acids were dehydrated and acetylation of the hydroxy group provided no protection. Unsaturated hydroperoxides were converted to more highly unsaturated derivatives. Conjugated dienoates and hydroxy esters which were not vicinally unsaturated were stable. The changes, believed to occur in the flash heater, were promoted by the metal surfaces present.

A reproducible numerical constant for characterizing the migration of mono- and dicarboxylic methyl esters on gas-liquid chromatography was suggested (Miwa, et al., *Anal. Chem., 33,* 1739). The constant was called the "equivalent chain length" and depended on the nature of the stationary phase. Constants obtained on both polar and non-polar stationary phases were sufficient to characterize most fatty acids. A punched-card method for analysis of gas chromatography data was suggested which eliminates nearly all manual arithmetic and has advantages of ease and data storage (Tandy, et al., *Ibid.,* 665). A method of expressing the separation of components by gas-liquid chromatography as a numerical separation function was described (Giddings, *Ibid., 32,* 1707). The effect of various operating variables on this function was treated theoretically.

When capillary columns are used in gas chromatography, a stream splitter must be employed to divide the sample into two parts. It is important for the splitter not to discriminate against any sample component. A splitter was described and its linearity of discrimination demonstrated over a wide range (Ettre and Averill, *Ibid., 33,* 680).

Ortho-phthlate-ethylene glycol polyester was suggested as a new stationary phase for gas-liquid chromatography (Craig, *Chem. & Ind.* [*London*] *1960,* 1442). The separation factor for oleic/stearic was 1.11. The "polarity" could be varied by the mesh size of the support and the ratio of stationary phase to support. It had three to six times the capacity of aliphatic polyesters making it useful for preparative work. Silicone polymers were suggested as high temp-stable stationary phases for the separation of methyl esters and other lipids (Nicolaides, *J. Chromatography, 4,* 496, Vanden Heuvel et al., *J. Am. Chem. Soc., 38,* 1513). Adsorption on the gas-liquid interface was reported to be an important factor affecting the retention times of substances undergoing separation by gas chromatography (Martin, *Anal. Chem., 33,* 347) The influence of the type of column support on the separation factors, retention volumes, and theoretical plates for methyl esters during chromatography was reported (Hornstein and Crowe, *Ibid.,* 310).

The analysis of several natural fats and oils by gas-liquid chromatography and alkali isomerization were compared (Kaufmann, et al., *JAOCS, 38,* 495). The agreement for linoleic was good for unhydrogenated samples, but the alkali isomerization method gave low results for linolenic acid. With hydrogenated samples the results for both linoleic and linolenic acids were low by alkali isomerization. Most of the difference could be accounted for by the difference in rate of isomerization of the polyenoate isomers. The determination of linoleic and linolenic acids by alkali isomerization under both air and nitrogen was compared with gas-liqiud chromatography (Wolff, *Rev. franc. corps gras., 8,* 68). The results by gas chromatography were reproducible if carried out by the same operator, the chromatogram had symmetrical peaks, and it was not necessary to change sensitivity during the analysis. An internal standard enhanced the accuracy. It was concluded that alkali isomerization was more precise for oils containing only linoleic acid or having only minor amounts of linolenic acid compared to linoleic. In linseed oil, chromatography gave less accurate values for linolenic but more accurate values for linoleic than did alkali isomerization.

Circular paper chromatography was used for the separation of fatty acids and glycerides with petroleum hydrocarbon, dodecylbenzene, and tetralin as stationary phases and aqueous acetic acid and acetic acid-methanol as mobile phases (Noda and Hirayama, *Yukagaku, 10,* 24). The fatty acids were detected by successive treatment with lead acetate and ammonium sulfide solutions. Unsaturated fatty acids were detected with iodine vapors. Low-temp development was used effectively. The unsaturated glycerides were chromatographed as mercuric acetate addition compounds and detected by spraying with a solution of diphenylcarbazone in ethanol or by soaking in Sudan black. The 8,12-linoleic acid formed during the hydrogenation of linoleic acid was separated from the 9,12-isomer by paper chromatography of the rhodan derivatives (Moller and Gabrielsson, *Fette, Seifen, Anstrichmittel, 62,* 936). A paper chromatographic procedure for the separation of even numbered wax acids up to C_{36} was devised (Kaufmann and Das, *Ibid., 63,* 614). The paper was impregnated with a 10% solution in petroleum ether of a petroleum fraction boiling at 230C. The mobile phase was isopropanol-acetic acid-ethanol (96%)— water (8:2.5:4:1.25) saturated with the stationary phase. The column partition method of Zbinovsky was adapted to paper chromatography for the separation of C_6-C_{12} dibasic acids in the presence of monobasic acids (Occolomitz, *J. Chromatography, 5,* 373).

Thin-layer chromatography (chromatostrip) was used to separate the methyl esters of various fatty acids (Applewhite, et al., *JAOCS, 38,* 609). Starch was used to bond the silicic acid to the glass plates. Conjugated unsaturates were detected with fluorescent minerals, unsaturates with fluorescein-bromine, and 2',7'-dichlorofluorescein served to detect a variety of compounds. Thin-layer chromatography was used in conjunction with gas chromatography to fractionate methyl esters (Mangold and Kammereck, *Chem. & Ind.* [*London*], *1961,* 1032). Thin-layer chromatography could be used to remove esters of hydroxylated fatty acids which might interfere with gas chromatography. Mixtures of saturated and unsaturated fatty esters which were not resolved by gas chromatography could be separated by thin-layer chromatography as mercuric acetate addition products. The separation of hydroxy-, epoxy-, and episulfido-fatty acids as well as other lipids by thin-layer chromatography was reported (Kaufmann and Makus, *Fette, Seifen, Anstrichmittel, 62,* 1014). Different solvent systems and different methods of detecting the spots were evaluated.

A liquid partition column was used for separating the short chain fatty acids from C_1 to C_{10} (Gordillo and Montes, *Revista Argentina de Grasas y Aceites, 3,* 31). Silicic acid was used as a support with propylene glycol as a stationary phase, and n-butanol-petroleum ether was the mobile phase. An apparatus for the separation and purification of fatty acids on a scale of 10 g or more by reverse phase partition chromatography was invented and applied to the separation of long chain fluoro-fatty acids (Hall, *J. Chromatography, 5,* 93). The stationary phase was liquid paraffin on a cellulose powder support. A reversed phase liquid partition column was used to separate mixtures of saturated, unsaturated, and oxygenated fatty acids (Gunstone and Sykes, *J. Sci. Food Agr., 12,* 115). A simple automatic valve for const volume liquid column chromatography, in which the volume collected was easily varied, was invented (Nelson, *Anal. Chem., 32,* 1724).

A solvent system of petroleum ether for an upper phase and dimethylsulfoxide-1-octanol (9:1) as a lower phase was used to separate fatty acids from C_{10} to C_{18} by countercurrent distribution (Will, *Ibid., 33,* 647). A system of heptaneisopropanol-water was suggested as a solvent for countercurrent distribution of plasma and tissue extracts (Dole and Meinertz, *J. Biol. Chem., 235,* 2595). This system separated the extract into long chain fatty acids, cephalins and polar organic acids in eight transfers. Countercurrent distribution was used to fractionate radioactively labeled soybean fatty acid esters (Dutton, et al., *J. Lipid Research, 2,* 63). The process yielded pure linoleate, 97% pure linolenate, a mixture of palmitate and oleate, 83% pure stearate and concentrates of C_{20} and higher esters. Countercurrent distribution was used to separate the methyl esters obtained in the hydrazine reduction of linolenate (Scholfield, et al., *JAOCS, 38,* 208). Countercurrent distribution was also used to fractionate the methyl esters obtained by the hydrazine reduction of linoleate as the mercuric acetate addition products (Schilling, *Fette, Seifen, Anstrichmittel, 63,* 421). Esters of specific configuration could be prepared since the configuration and position of the double bonds remained unchanged.

GLYCERIDE STRUCTURE ANALYSIS. The specificity of pancreatic lipase for the alpha positions of glycerides was exploited in several studies of the glyceride structure of fats. A series of animal and vegetable fats were hydrolyzed with pancreatic lipase and the glyceride composition of the original fat was calculated by the method of Coleman and Fulton (Coleman, *JAOCS, 38,* 685). In the case of shea, illipe, and cocoa butters, the beta position was occupied by an unsaturated fatty acid. In lard palmitic acid occupied the beta position. From a study of the glyceride structure of a series of lard samples, it was concluded that the glyceride structure of a sample of lard could be predicted from its fatty acid composition alone. The glyceride structure of milk fat was examined with pancreatic lipase (Ast and Vander Wal, *Ibid.,* 67). It was found that although the individual acyl groups were not distributed at random on the alpha and beta positions, when the saturated and unsaturated fatty acids were considered as groups and not as individuals, they appear to be distributed randomly or nearly so. The fatty acid distribution of 18 vegetable oils was investigated with pancreatic lipase and certain fatty acids were found to exhibit specific distributions (Mattson and Volpenhein, *J. Biol. Chem., 236,* 1891). Fatty acids with a chain length of more than 18 carbon, regardless of whether they were saturated or unsaturated,

were esterified almost exclusively at the alpha positions. Palmitic and stearic acids were preferentially esterified at the alpha positions. As a result of the specific distribution of these acids, the 2-position contained a high proportion of oleic, linoleic, and linolenic acids. Improvements in the digestion of glycerides with pancreatic lipase were suggested which increase the accuracy and simplicity of the method (Mattson and Volpenhein, *J. Lipid Research, 2,* 58). By acylation of partial glycerides with a "marker" fatty acid, the method could be used for determining the structure of mono- and diglycerides. They presented evidence that partial glycerides could be acylated with fatty acid chlorides without causing rearrangement. The fatty acids released from several fats with pancreatic lipase and lipoprotein lipase were compared with the fatty acid composition of the original fat (Korn, *J. Biol. Chem., 236,* 1638). Unlike pancreatic lipase, lipoprotein lipase seemed to hydrolyze the ester groups of the three positions of glycerol at the same rate. Both enzymes seemed to hydrolyze all fatty acids at a given position at the same rate. The glycerides 2-oleo-dipalmitin and 2-palmito-diolein were hydrolyzed with milk lipase (Gander and Jensen, *J. Dairy Sci., 43,* 1762). The milk lipase preferentially hydrolyzed the alpha positions.

Countercurrent distribution was employed to fractionate the glycerides of cocoa butter (Dutton, et al., *JAOCS, 38,* 96). Tripalmitin and tristearin labeled with C^{14} were distributed along with the cocoa butter to help elucidate the incomplete separation achieved. These results were compared with the distribution of cocoa butter randomized with sodium methoxide and a synthetic "cocoa butter". The results indicated that cocoa butter contained oleic acid on the beta position and stearic and palmitic acids randomly arranged on the alpha positions. Corn oil was also partially fractionated by countercurrent distribution (Scholfield, et al., *Ibid.,* 175). The fatty acid composition of the fractions and the amounts of the more unsaturated triglycerides that were found agree with a random distribution pattern. The apparent disagreement between these results and those obtained with pancreatic lipase was discussed.

A method for quantitative determination of the six glyceride types: SSS, SSU, SUS, USU, UUS, and UUU was described (Youngs, *Ibid.,* 62). The unsaturated acyl groups were oxidized to the corresponding dicarboxylic acids with permanganate-periodate. The oxidized fat was then separated on a liquid partition column into a fraction containing zero or one free acid group and a fraction containing more than one free acid group. The free carboxylic acid groups were converted to the methyl esters and the glycerides analyzed by pancreatic lipase. Fatty acid analysis of the various fractions by gas phase chromatography allowed calculation of the six glyceride types. The results were reproducible and gave the proper values with synthetic glycerides. The results for five natural fats agreed with the distribution pattern proposed by Vander Wal. A micromethod for determination of the composition of mono-, di-, and triglycerides was reported (Privett and Blank, *J. Lipid Research, 2,* 37). The double bonds in the glycerides were oxidized by ozone and then reduced to the corresponding aldehydes with hydrogen. The aldehydo-glycerides were then fractionated by thin-layer chromatography and determined quantitatively by charring with sulfuric acid. The method resolved four of the possible monoglyceride types, six of the seven possible diglyceride types, and four of the six possible triglyceride types. A number of samples of milk fat were analyzed for trisaturated triglyceride by the mercaptoacetic acid method (Boatman, et al., *J. Dairy Sci., 44,* 544). The amount of trisaturated glyceride was found to agree well with the amount calculated by random distribution. The fatty acid composition of the trisaturated glyceride was compared with that of the original fat and no preferential inclusion or exclusion of the saturated fatty acids from the trisaturated glyceride could be detected. The results were explained in terms of Vander Wal's distribution theory. The same authors show that the relation of the melting point of a fat, the melting point of the trisaturated glyceride and the amount of trisaturated glyceride, which has been found to hold for most fats, does not hold for milk fat.

The glyceride structure of cocoa butter was studied by fractionating it in a thermal gradient column (Jones and Hammond, *JAOCS, 38,* 69). From a fatty acid analysis of the partially resolved fractions, no difference was found in the ratio of palmitic to stearic acid in the monosaturated and disaturated glycerides. Ideal solution theory predicts that the components of the disaturated glyceride will form ternary and binary eutectic mixtures. When this was taken into account a calculation of the individual glycerides present agreed with that predicted by restricted random distribution. It was also shown that when triolein was mixed with cocoa butter and separated by this method, that the separation of the triolein from the disaturated glyceride was incomplete. A differential cooling curve technique which measured the difference in cooling rates of crystallizing and noncrystallizing fat under specific conditions gave a rapid means of determining the trisaturated glyceride content of modified lard and related fats (Jacobsen, et al., *Ibid.,* 399).

Simple triglycerides were separated by gas phase chromatography using a short column with "SE-30" as a stationary phase (Pelick, et al., *Ibid.,* 506). Quantitative results were not claimed. The separation of triglycerides by gas phase chromatography was reviewed and the separation of synthetic and natural mixtures was reported (Huebner, *Ibid.,* 628). A programmed temp apparatus was used and quantitative results accurate enough for many purposes were reported.

Paper chromatography was used to separate triglyceride mixtures using undecane as a stationary phase and acetic acid or acetone-acetonitrile as a mobile phase (Kaufmann and Makus, *Fette, Seifen, Anstrichmittel, 63,* 125). The spots were detected with Sudan black or iodine vapor, and the composition of the individual spots was determined by hydrolysis of the spot on the paper and further chromatography of the fatty acids in a direction perpendicular to the first development. Results with synthetic mixtures, linseed, corn, cottonseed, and soybean oils are given. The separation of the glycerides of cocoa butter and Borneo tallow by paper chromatography using liquid paraffin and methanol-acetone as stationary and mobile phases, respectively, was reported (Steiner and Bonar, *J. Sci. Food Agr., 12,* 247). The spots were detected by iodine vapor and a starch spray. The mixed glycerides of acetic, butyric, stearic, palmitic, and oleic acids were resolved by chromatography on glass fiber paper by development with ether-isooctane (Ory, *J. Chromatography, 5,* 153). Circular paper chromatography with petroleum hydrocarbons, dodecylbenzene, and tetralin as stationary phases and aqueous acetic acid and methanol-acetic acid as mobile phases, was used to separate the mercuric acetate addition compounds of unsaturated glycerides (Noda and Hirayama, *Yukagaku, 10,* 24). The spots were detected with Sudan black or diphenylcarbazone.

Column chromatography on silicic acid was used to fractionate the glycerides of several natural fats (Sahasrabudhe and Chapman, *JAOCS, 38,* 88). Gradient elution with increasing concentrations of ether in hexane was used. The elution of the glycerides depended both on their chain length and degree of unsaturation.

ANALYSIS OF PHYSICAL PROPERTIES. A modified dilatometric method for determining the ratio of solid/liquid fat was proposed (Loncin, *Rev. franc. corps gras., 7,* 569). The specific density of the fat was determined at various temperatures and compared with standard samples. The method was said to have greater speed and require less sample handling. A differential cooling-curve technique which measured the differece in cooling rate of crystallizing and non-crystallizing fat was applied to several glyceride mixtures (Jacobsen, et al., *JAOCS, 38,* 399). The significance of the data was discussed and a method for determining trisaturated glyceride in lard was developed. The relation between the melting point of a fat and the melting point and amount of trisaturated glyceride which holds for many fats did not hold for milk fat (Boatman, et al., *J. Dairy Sci., 44,* 544). This indicated that the melting point of the last glyceride to melt changed from sample to sample.

The thermal conductivity of several types of food and fat was measured between 5 and −25C (Lentz, Food Technol., *15,* 243). The conductivity of the fat varied only slightly with temp. An apparatus was devised for measuring the vapor-liquid equilibrium of fatty acid mixtures (Lengyel, *Fette, Seifen, Anstrichmittel, 62,* 913). This was applied to oleic-ricinoleic, palmitic-oleic and palmitic-stearic mixtures and the results were found to deviate from the Raoult-Dalton law.

COMPOSITION AND CHARACTERISTICS

Many reports on the composition and physical properties of fats and lipids were published in 1961. 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. The list has been subdivided into sections. The first includes analyses on fats, oils, and unfractionated lipid mixtures. An asterisk appearing after the reference indicates that the fatty acid composition is given. The second section of the list includes reports in which there was at least partial fractionation into lipid groups. An asterisk after the reference indicates that the fatty acid composition of the lipid groups is given. The sections on waxes, vitamins and physical properties include reports that deal with these categories.

In addition, many of the references in the previous sections

on methodology contain information on the composition and characteristics of certain materials. An attempt has been made to include the most important of these in this section. All reports on the glyceride structure of fats have been included in the section on glyceride structure analysis.

All reports that deal primarily with the effect of environmental, dietary, and genetic factors on composition and characteristics will be found in the section so named.

FATS, OILS, AND UNFRACTIONATED LIPID MIXTURES. In a study of seed oil from 37 species of the family Cruciferae, the oil, protein, and fatty acid composition of the following species were examined: *Alyssum saxatile, Arabis alpina, A. virginica, Brassica campestris, B. carinata, B. juncea, B. napus, B. nigra, B. oleracea* var. *capitala, Cakile edentula, Camelina sativa, Capsella bursa-pastoris, Cheiranthus cheiri, Crambe abyssinica, Descurainia sophia, Eruca sativa, Erysimum perofskianum, Herperis matronalis, Iberis amara, I. umbellata, Isatis tinctoria, Lepidium lasiocarpum, L. montanum* var. *angustifolium, L. sativum, L. virginicum, Lobularia maritima, Lunaria annua, Matthiola bicornis, Nasturtium officinale, Nerisyrenia camporum, Raphanus sativus, Selenia grandis, Sisymbrium irio, Sophia ochroleuca, Stanleyella texana, Thlaspi arvense,* and *Tropaeolum majus* (Mikolajczak, et al., *JAOCS, 38,* 678)*. Several tropical seed fats were examined: *Pinang mabuk, Monodora myristica, Xylopia aethiopica, Anogeissus schimperi, Mangifera indica,* and *Carapa procera* (Mackie and Mieras, *J. Sci. Food Agr., 12,* 202)*. The protein and lipid of five plants native to Pakistan were examined: gram, mung, mash, masur, and lobia (Baker, et al., *J. Sci. Food Agr., 12,* 205)*. Other vegetable sources examined were: *Ankistrodesmus braunii* (Williams and McMillan, *Science, 133,* 459)*, *Bulimus sriatulus japonicus* (Mitsuhashi, *Nippon Kagaku Zasshi, 82,* 465)*,*Dichapetalum toxicarium* (Peters, et al., *Biochem. J., 77,* 17)*, *Hibiscus mutabilis* (Kato, *Yukagaku, 10,* 174)*, *Limnanthes douglasii* (Smith, et al., J. Org. Chem. *25,* 1770)*, *Matthiola incana* R. Br. (Rahman and Khan, *JAOCS, 38,* 281)*, orujo oil (sulfur olive oil) (Vioque et al., *Ibid.,* 489)*, rice bran oil (Mehta and Meshramkar, *Indian Oil and Soap J., 26,* 18)*, spinach (Mattick and Lee, *J. Food Sci., 26,* 356)*, *Trichosanthes cucumeroides* (Kato, *Yukagaku, 10,* 174)*, and wheat gluten (Moruzzi, et al., *Arch Biochem. Biophys., 91,* 328)*.

Animal fats analyzed were: human depot fat (Kingsbury, et al., *Biochem. J., 78,* 541)*, kiwi depot fat (*Apteryx australis mantelli*) (Shortland and Gass, *J. Sci. Food Agr., 12,* 174)*, male and female rat tissues (Kirschman and Coriglio, *Arch. Biochem. Biophys., 93,* 297)*, elastin (Loomeijer, *J. Atheroscler. Res., 1,* 62)*, mitochondria from chicken liver, beef heart, rat liver, and several fish species (Richardson et al., *Arch. Biochem Biophys., 94,* 1)*, subcellular fractions from rat liver (Getz and Bartley, *Biochem. J., 78,* 307)*, fecal lipids (James, et al., *Biochem. J., 78,* 333)*, feces and fecaliths (Williams, et al., *Proc. Soc. Exptl. Biol. Med., 105,* 192)*, and *trans*-isomer in pork, horse, beef, and butter by infrared (Firestone and Villandelmar, *J. Assoc. Offic., Agr. Chemists, 44,* 459)*.

Marine fats analyzed were: sperm blubber oil (Tateishi, et al., *Kôgyô Kagaky Zasshi, 64,* 1028)*, herring oil (Klenk and Bricker-Voight, *Z. physiol. Chem., Hoppe-Seyler's 324,* 1)*, and mitochondria from catfish liver, carp liver, salmon liver, and salmon heart (Richardson, et al., *Arch. Biochem. Biophys., 94.* 1)*.

Analyses were reported on milk fat: (Hansen, et al., *Biochem. J., 77,* 64)*, (Kaufmann, et al., *Fette, Seifen, Anstrichmittel, 63,* 261)*, and (Patton, et al., *J. Dairy Sci., 43,* 1107)*.

Analyses reported on processed fats were: eleven commercial margarines (Ostwald, *J. Am. Dietet. Assoc., 39,* 313)*, twenty commercial ice cream emulsifiers (Jensen, et al., *J. Dairy Sci., 44,* 1057)*, flour treated with chlorine dioxide, (Daniels, et al., *J. Sci. Food Agr., 11,* 658 and 664)*, and chages in soybean during the production of tempeh (Wagenknecht, et al., J. Food Sci., *26,* 373)*.

LIPID GROUPS AND FRACTIONS. The phosphatides of cabbage leaf were separated by silicic acid chromatography into phosphatidyl glycerol and an unknown glycero-phosphatide (Wheeldon, *J. Lipid Research, 1,* 439)*. The acetone-soluble lipids of red clover were found to consist largely of the galactolipids, galactosyl-l-glycerol- and digalactosyl-glycerol linolenate (Weenink, *J. Sci. Food Agr., 12,* 34)*. No evidence for the presence of triglycerides was obtained. The acetone soluble lipids from rye-grass, cocksfoot, white clover, and rape were 70.5–76.5% fatty acids. (Shorland, *J. Sci. Food Agr., 12,* 39)*. Dialysis of the rye-grass acetone soluble lipid through rubber membranes indicated that none of the fatty acids were dialyzable and were therefore not in triglycerides. The triterpenoid acids isolated from sulfur olive oil were shown to be oleanolic, 3-β-hydroxyolean-12-en-28-oic and an unknown dihydroxy compound (Vioque and Morris, *JAOCS, 38,* 485). Changes taking place in the fatty acid composition of the phosphatides and neutral lipids of raw and enzyme-inactivated peas during storage were followed (Lee and Mattick, *J. Food Sci., 26,* 273)*. The sulfolipid found in the photosynthetic tissues of plants was isolated after deacylation to sulfodeoxyhexopyranosyl glycerol (Lepage, et al., *JAOCS, 83,* 157). The phospholipids of *Pseudomonas aeruginosa* were fractionated by paper chromatography, hydrolyzed, and rechromatographed to reveal 13 ninhydrin-positive compounds (Silberman and Gaby, *J. Lipid Research, 2,* 172). The fatty acid composition of soy lecithin was examined by paper chromatography methods involving hydrogenation and saponification of the lecithin on the paper (Kaufmann and Wessels, *Fette, Seifen, Anstrichmittel, 62,* 1020)*. The monogalactosyl- and diagalactosylglycerol lipids and cerebrosides of wheat flour were isolated by solvent extraction and silicic acid chromatography (Carter, et al., *J. Lipid Research, 2,* 215). The galactosylglycerol lipids of wheat flour were found to be 2,3-diglycerides (probably in the D-configuration) with a carbohydrate moiety attached to the l-position (Carter, et al., *Ibid.,* 223). The monogalactosyl glycerol yielded 2,3,4,6-tetra-O-methyl-D-galactose after methylation and hydrolysis and the digalactosyl glycerol gave 2,3,4,-tri-O-methyl-D-galactose. A cerebroside fraction from wheat flour was found to contain dihydrosphingosine, phytosphingosine, dehydrophotosphingosine, and a new long chain base (Carter, et al., *J. Biol. Chem., 236,* 1912)*. Glucose and α-hydroxystearic acid were also present. The cerebrosides found in Gaucher's disease were shown to contain normal erythrosphingosine (Carter, et al., *J. Lipid Research, 2,* 228).

The fatty acid composition of the adrenal cholesterol ester fraction was determined in rats, dogs, men, geese, chickens and rabbits (Dailey, et al., *Proc. Soc. Exptl. Biol. Med., 105,* 4)*. The phospholipid composition of erythrocyte ghosts and plasma from men, pigs, horses, cows, sheep, and goats was determined (Dawson, et al., *Biochem. J. 77,* 226). The nature of the neutral lipids and phosphatides of bovine and human erythrocytes and plasma was reported (Hanahan, et al., *J. Lipid Research, 1,* 421)*. The nonphosphatide aldehydrogenic lipids in milkfat, beef tallow, and ox heart were found to have the aldehye bound at the alpha position as an enol ether (Schogt, et al., *Ibid.,* 446). Branched chain aldehydes were released from both the phosphorus-free portion and phosphatides of milk fat and ox heart lipid (Schogt, et al., *Ibid., 2,* 142)*. A complex phospholipid containing ethanolamine, serine, and choline was found in rats, egg yolk, allantoic membrane, influenza virus, human plasma, cabbage leaf, human brain, yeast, and a fresh-water crustacean (Collins and Shotlander, *Biochem. J., 79,* 316). Analyses of cytolipin H were reported (Rapport, et al., *J. Lipid Research, 2,* 148).

Beef brain phosphoinositide was subjected to structural analysis and it was concluded that it is a triphosphoinositide with the phosphates on the 1, 4, 5 (6) positions on L-myoinositol (Grado and Ballou, *J. Biol. Chem., 236,* 54). Further work on methodology indicated that the phosphates of inositol released from phosphoinositide by alkaline hydrolysis were the 1,4,5-, 2,4,5-, 4,5-, and 1,4-phosphates (Tomlinson and Ballou, *Ibid.,* 1902). D-2,3-Diglyceride was obtained by the enzymatic cleavage of beef liver phosphoinositide thus establishing that the original phosphatide had the L-configuration (Brockerhoff, *Arch. Biochem. Biophys.. 93,* 641)*. The lipid composition of bovine serum lipoprotein fractions was determined (Evans, et al., *J. Dairy Sci., 44,* 475)*. The fatty acid composition of the phosphatides of bovine adrenals was investigated and several polyunsaturated fatty acids shown to be present (Klenk and Eberhagen, *Z. physiol. Chem., Hoppe-Seyler's, 322,* 258)*. Cardiolipin and phosphatidylinositol were identified in ox liver (Macfarlane, *Biochem. J., 78,* 44)*. The phosphatides of ox spleen were fractionated by silicic acid chromatography and the fatty acid and fatty aldehyde composition of the various fractions were obtained (Gray, *Ibid., 77,* 82)*.

The phospholipids of three developmental stages of the blowfly, *Phormia regina,* were fractionated by silicic acid chromatography (Bieber et al., *J. Biol. Chem., 236,* 2590). The fatty acid and glycerol ether composition of the alkoxydiglycerides of dogfish liver oil were reported (Malins, *Chem. and Ind. [London], 1960,* 1359)*. The lipid components of the lipoproteins of egg yolk were characterized (Evans and Bandemer, *Poultry Sci., 40,* 597).

The fatty acid composition of the lipid fractions of human blood was determined in health and during certain diseases (Bohle and Biegler, *Fette, Seifen, Anstrichmittel, 62,* 673)*. The fatty acids bound to human serum albumin were determined (Saifer and Goldman, *J. Lipid Research, 2,* 268)*. The plasmalogen content of human blood platelets was assayed (Zilversmit, et al., *J. Biol. Chem., 236,* 47). Fatty acids of human platelet phosphatides have been studied by silicic

acid and gas-liquid chromatography (Marcus, et al., *Proc. Soc., Exptl. Biol. Med. 107*, 483). The lipoprotein content of human pathological serous fluids was reported (Kellogg, *Proc. Soc. Exptl. Biol. Med., 107*, 102). The fatty acid composition of the cholesterol ester, triglyceride, and phosphatide fractions from aortic media, thickened intima and plaque material was determined in six human subjects (Swell, et al., *Ibid., 105*, 662)*. The lipids of the intima and media of aortas in progressive stages of atherosclerosis were analyzed (Mead and Gouze, *Ibid., 106*, 4). The lipid and fatty acid composition of human aorta were reported (Bottcher and van Gent, *J. Atheroscler, Research, 1*, 36)*. The bile acid composition of human bile was determined by gas chromatography (Blomstrand, *Proc. Soc. Exptl. Biol. Med., 107*, 126).

The lipid composition of the fat globule membrane of milk was determined by chromatography on silicic acid (Thompson, et al., *J. Dairy Sci., 44*, 1589). The lipoproteins isolated from milk fat globule membrane protein were fractionated and their lipid and phospholipid content reported (Alexander and Lusena, *Ibid.*, 1414). The fatty acid composition of the monoglycerides isolated from lipolyzed milk was reported (Jensen and Gander, *Ibid., 43*, 1758).

The phosphatides of pig liver were determined by deacylation of the phosphatides and separation by ion exchange chromatography (Hubscher, et al., *J. Lipid Research, 1*, 433).

The fatty acid composition of mouse plasma lipid and lipoprotein was reported (Rehnborg, et al., *Proc. Soc. Exptl. Biol. Med., 106*, 547)*. The lipids of rat brain, brain mitochondria, and brain microsomes were fractionated into lipid classes and the fatty acid composition of each class was reported (Biran and Bartley, *Biochem. J., 79*, 159)*. The phosphatides of rat liver mitochondria and microsomes were examined by countercurrent distribution after dinitrophenylation and methylation (Collins and Shotlander, *Ibid.*, 321). The lipid composition of rat liver mitochondria and microsomes was compared in another publication (Macfarlane, et al., *Ibid., 77*, 626). The fatty acid composition of the principal lipid components of rat liver was determined under various dietary conditions (Okey, et al., *J. Nutrition, 75*, 51)*.

WAXES. The fatty acid composition of carnauba, montan, and beeswax was determined by paper chromatography (Kaufmann and Das, *Fette, Seifen, Anstrichmittel, 63*, 614)*. The paraffin and primary alcohol fractions of a number of plant waxes were examined and in some cases even numbered paraffin compounds were found (Waldron, et al., *Biochem. J., 78*, 453). Peat wax was examined and evidence was found for the presence of aliphatic monohydroxy fatty acid with a chain length of 20 and 21 carbons (Rauhala, *JAOCS, 38*, 233)*. Cuban Palm cuticle wax was investigated (*Copernicia hospita*) (Kitzke and Wilder, *Ibid.*, 699).

VITAMINS. Analyses on vitamins included: the vitamin A content of 15 commercial margarines during storage (Nakazawa, et al., *Yukagaku, 10*, 179), the content of various types of carotenoids in various races of corn (Quackenbush, et al., *J. Agr. Food Chem., 9*, 132), carotenoid and other pigments in marine algae (Jeffrey, *Biochem. J., 80*, 336), lycopene (shown to be the pink pigment in the Sarah variety) in Shamouti (Jaffa) orange (Monselise and Halevy, *Science 133*, 1478), and the blood tocopherol of 197 factory workers in Rochester, New York (Harris, et al., *Proc. Soc. Exptl. Biol. Med., 107*, 381).

PHYSICAL PROPERTIES

Infrared data was given for the following: Methyl punicate, alpha-eleostearate and beta-eleostearate during isomerization with iodine and light (Tolberg, et al., *JAOCS, 38*, 102); *trans*-10-hydroxy-2-decenoic acid (Koga, et al., *Nippon Kagaku Zasshi, 81*, 1782); 10-hydroxystearic acid and its methyl ester (Nakajima and Toyama, *Ibid.*, 1472); D-12-hydroxystearic acid, *Ibid.*, 1474); stearic, palmitic, myristic, and lauric acids (Kawano, *Ibid., 82*, 161 and 427); methyl stearate and elaidate, *Ibid.*, 432). The Schumann ultraviolet spectrum of unsaturated fatty acids was given (Schauenstein and Benedikt, *Fette, Seifen, Anstrichmittel, 62*, 687). See also the section on the determination of *trans*-isomer.

X-ray diffraction data was presented on sodium soaps of C_8-C_{18} fatty acids (Ogino, *Kogyo Kagaku Zasshi, 64*, 1021). The emulsion system of oilve oil in water was studied with light microscopy, electron microscopy, X-ray diffraction, and electron diffraction (Marquez, *Grasas y Aceites* [*Seville, Spain*], *11*, 83). The solubility of linoleic acid in aqueous solutions and its reaction with water were investigated (Mabrouk and Dugan, *JAOCS, 38*, 9). The following physical property data was recorded: freezing point, X-ray and infrared data for the system acetamine-palmitic acid-stearic acid (Mod, et al., *J. Phys. Chem., 11*, 1613), thermal conductivity of various kinds of fat (Lentz, *Food Tech., 15*, 243), melting point, infrared, and other data on 2,3-dibutyro, 2,3- isobutyro, and 2,3-di-isovalero-1-olein (Taufel, et al., *Fette, Seifen, Anstrichmittel, 62*, 926), melting point data on fatty acid esters of diglycerol (Kaufmann and Forster, *Ibid., 62*, 796), vapor-liquid equilibrium data for oleic-ricinoleic, palmitic-oleic, and palmitic-stearic acids (Hollo and Lengyel, *Ibid.*, 913), the crystalline organization of cocoa butter in various stages of autoxidation (Sterling, *Food Research, 25*, 770), dilatometric data on 1-oleodistearin, 2-palmito-oleostearin and mixtures of 2-oleopalmitostearin with 2-palmito-oleostearin, 1-oleodistearin, and 2-oleodistearin (Landmann, et al., *JAOCS, 38*, 681), X-ray diffraction, cooling curve, dilatometric, photomicrographic data on sodium methoxide treated lard (Wiedermann, et al., *Ibid.*, 389), X-ray diffraction, cooling curve, aniline point and melting point data on sodium methoxide treated lards (Weiss, et al., *Ibid.*, 396), and data showing a relation between the *trans*-isomer content of a hydrogenated oil, the congeal point, Wiley melting point, and solid index (Stingley and Wrobel, *Ibid.*, 201).

THE EFFECT OF DIETARY, ENVIRONMENTAL AND GENETIC FACTORS ON COMPOSITION AND CHARACTERISTICS. Many experiments in this area will be found in the section on Nutrition. The present section is restricted to reports which are primarily of technological interest. The fatty acid composition, iodine value and tocopherol content of 39 cottonseed oils grown in various parts of Argentina were correlated with area, climate, and variety (Karman, et al., *Revista Argentina de Grasas y Aceites, 3*, 7). Cotton varieties with no gossypol pigment glands or few glands have been developed and the oil from these was compared with that from normal cottonseed (Thaung, et al., *JAOCS, 38*, 220). Iodine values, unsaponifiables, cloud-point, pour-point, Halphen test, fatty acid composition, ultraviolet and infrared spectra were compared. The nutritive value for rats of the meal from such cottonseed was also investigated (Smith, et al., *J. Agr. Food Chem., 9*, 82). The linoleic and linolenic acid content of various varieties of soybean oil was measured (White, et al., *JAOCS, 38*, 113). Crosses and introductions, "high" and "low" in linolenic acid, indicated that inheritance of both acids was quantitative rather than qualitative. The lowest value for linolenic observed was 3.35%. Environmental effects markedly influenced the quantity of both acids. The effect of variety and maturity in flax and safflower on the fatty acid content and a number of other analytical values were studied (Sims, et al., *Ibid.*, 273 and 276). Fat content and unsaturation increased with maturity. Changes in the ratios of the various fatty acids during ripening were noticed. The changes in the composition of peanuts during storage under different moisture conditions were the subject of one study (Davis, *Ibid.*, 516). A high moisture content increased the tendency to hydrolysis of the oil and oxidative rancidity. The moisture, fat, and ash content and fatty acid composition of peanuts undergoing germination were studied (Rabari, et al., *Ibid.*, 4). The fatty acids seemed to be converted to sugars and starch. The saturated fatty acids were converted faster than the unsaturated and the rate of conversion of the saturated acids decreased with chain length. The α-tocopherol content of the green leaves of a wide variety of plants growing under a variety of conditions was determined (Booth and Hobson-Frohock, *J. Sci. Food Agr., 12*, 251). They concluded that the tocopherol content was inversely related to the growth rate.

A comparison of the lipid composition of rat epidermis and dermis, during the active and quiescent stages of the hair growth cycle, showed no differences (Carruthers, et al., *Proc. Soc. Exptl. Biol. Med., 105*, 259). The composition of the neutral fat of rats fed long- and short-chain triglycerides, in conjunction with various levels of linoleic acid, indicated that linoleic acid regulated the type of fat deposited (Kaunitz, et al., *J. Nutrition, 73*, 386). Feeding linoleic acid led to a decrease in neutral fat in relation to body weight and facilitated the laying down of a depot fat more representative of that in the diet.

The effect of season on the fatty acid composition of milk fat was investigated by gas chromatography of the methyl esters (Patton, et al., *J. Dairy Sci., 43*, 1187). The effect of lactation number, calendar year, stage of lactation, and month of year on the ratio of fat to solids-not-fat in milk was determined with 243 Holstein and 276 Jersey cows (Johnson, et al., *Ibid., 44*, 658). The ratio decreased as lactation number increased, the ratio reached a low level 60–90 days after parturition and increased thereafter until the end of lactation. The vitamin A, carotenoid, iodine value, thiocyanogen value, and refractive index of milk fat were recorded for different breeds of cows on different kinds of feed (Krukovsky, *J. Agr. Food Chem., 9*, 326). The results indicated an inverse relation between the iodine value and carotenoid content. The conversion of carotene to vitamin A may vary

with the degree of unsaturation of the fat and may be regulated by the same process. The ability of Holstein calves to absorb and store α- and γ-tocopherol from their diets was investigated (Chatterton, et al., *J. Dairy Sci. 44,* 1061). The total tocopherol and the ratio of α to other forms of tocopherol was measured in plasma, liver and heart. The calves receiving γ-tocopherol had a considerably higher proportion of other forms of tocopherol in their tissues, indicating a possible dietary interaction between the different forms.

Contrary to previous studies, it was found that hens cannot synthesize linoleic acid *de novo* (Murty, et al., *J. Nutrition, 72,* 451). Feeding either *Sterculia foetida* seeds or crude cottonseed oil to laying hens caused them to lay eggs with an increased proportion of saturated fatty acids and a decreased proportion of monounsaturated acids (Evans, et al., *Ibid., 73,* 282). The fatty acid composition of the phosphatides was fairly constant. The storage of eggs from hens fed 20% cottonseed oil meal, under air, oxygen, carbon dioxide, or nitrogen failed to change the incidence of pink whites (Deutschman, et al., *Poultry Sci., 40,* 1305). Treatment of the cottonseed meal with sulfur dioxide destroyed the capacity of cottonseed meal to discolor eggs.

DETECTION OF ADULTERATION

A paper chromatographic method to distinguish animal from vegetable fats was reported which depends upon the separation and detection of the types of sterols present in the fats (Hatzopoulos, *Rev. franc. corps gras., 7,* 575). The paper, impregnated with paraffin oil, was developed with ethanol or methanol, and the spots were detected by spraying the paper with a solution of silicotungstic acid and heating to 115C. The spots were identified by comparison with standard phyto- and zoosterols. Adulteration at the 5% level could be detected. The adulteration of butter with hydrogenated fats could be detected by infrared spectrophotometry (Bartlet and Chapman, *J. Agr. Food Chem., 9,* 50). Butter was found to contain a constant ratio of isolated *trans-* to conjugated *cis-trans*-unsaturation. The addition of hydrogenated fats increased the amount of isolated *trans*-unsaturation. Adulteration at the 7% level could be detected. A method for measuring the amount of stearine in cottonseed oil was reported (Macchi and Crespo, *Revista Argentina de Grasas y Aceites, 3,* 3). The oil was mixed with hexane in a ratio of 1:2 by volume and cooled to 0C for 20 hrs. The formation of a precipitate indicated that the oil contained more than 5% stearine. Pure, table grade olive oil could be differentiated from solvent extracted or adulterated oil by determination of the hydroxyl number of the unsaponifiable fraction (Gracian and Martel, *Grasas y Aceites [Seville, Spain], 11,* 59). Pressed oil had a hydroxy number between 30 and 50 while extracted olive, almond, peanut, soy, and cottonseed oils gave values above 65. Bellier's test for the amount of alcohol insoluble fatty acids in olive oil showed wide variations from year to year (Gracian and Arevalo, *Ibid., 11,* 261). Such data must be carefully interpreted when used to test olive oil adulteration.

The chick edema-producing factor in certain commercial fats was the subject of some investigation. An assay procedure based upon the measurement of the pericardial fluid volume was described (Ott, et al., *Poultry Sci., 40,* 1016). A level of 7 ppb of pure edema factor in the diet was detectable after 20 days feeding. A number of distillates and residues from the production of commercial fatty acids were assayed for edema factor using rats as a test animal (Firestone, et al., *JAOCS, 38,* 418). The toxic material was found in the non-urea-adduct-forming portion. The presence of cyclic structures was indicated. A crystalline material containing halogen was isolated which produced symptoms of chick edema at a level of 0.1 ppm. (Yartzoff, et al., *Ibid.,* 60).

NUTRITION, PHYSIOLOGY, BIOCHEMISTRY

NUTRITION

MAN. A vegetable oil food pattern as served from the research kitchen that effectively reduced serum cholesterol levels in both normal and hypercholesterolemic individuals was described. The diet provided 12–13% calories as protein and 30–40% as fat. The total fat calories consisted of 3–6% animal fat, 20–30% as vegetable oil (cottonseed), and 2–6% basic fat (fat present in low-fat foods) (Brown, *J. Am. Dietet. Assoc. 38,* 536). Use of the vegetable oil food pattern is possible in the home with some modifications in standard recipes to provide palatable and acceptable diets to all members of the family. The author cites a need for more commercial products available through regular markets which would meet the requirements of a vegetable oil diet (Meredith, *J. Am. Dietet. Assoc. 38,* 543). A method of modifying "exchange" values for calculating the nutrients supplied by diets rich in an unsaturated fat (safflower oil) and a saturated fat (coconut oil) was described and used in a study in which safflower oil and coconut oil respectively were substituted for 80% of the fat in a prison diet (Hampton and Lee, *J. Am. Dietet. Assoc. 37,* 562, 566). Feeding experiments with animals and man have demonstrated two special conditions under which milk fat rates higher nutritionally than coconut oil—during the growth period and when the diet is otherwise marginal (Rice, *J. Agr.* and *Food Chem. 8,* 488).

It is reported that fat may contribute to the aroma of mutton and lamb broths (Hofstrand and Jacobson, *Food Research 25,* 706). Fats should make a positive flavor contribution to foods by blending flavors. It is suggested that animal and vegetable fats might be given unusual flavors by altering the molecular compositions of the fat (Angeline and Sjostrom, *Perfumery and Essent. Oil Record 12,* 681).

Wheat gluten was found to contain 5.3 to 5.6% total lipid. GLC analysis of fatty acid composition showed 60.1% linoleic and 19.8% palmitic acids. It is suggested that the cholesterol lowering effect of diets having wheat gluten as the protein source may be related to the composition of the fatty acids of the wheat gluten (Moruzzi, et al., *Arch. Biochem. Biophys. 91,* 328).

In a fat balance and metabolism study in healthy infants it was found that from a chemical and metabolic point of view, a diet containing ½ and ⅔ cows' milk with addition of 2% cottonseed oil surpassed the method of feeding with ½ and ⅔ milk (Droese and Stolley, *Fette, Seifen, Anstrichmittel 63,* 264). Investigation of the blood lipids of infants fed with (a) cows' milk substitute based on corn oil, (b) normal cows' milk and (c) human milk showed no changes in the total serum lipid content during the different feeding periods. Serum cholesterol levels in the case of feeding human and substitute milk are significantly increased. The highest cholesterol, lipid phosphorus, and iodine values occurred with the use of human milk (Löhr and Wolf, *Fette, Seifen, Anstrichmittel 63,* 269). The effect of animal protein and vegetable protein diets, (50 gm), having the same fat content of vegetable origin, (95 gm), on the serum lipid levels was studied in 12 young women. At the end of two weeks and five weeks on the dietary regimen serum cholesterol levels were significantly lower in subjects taking the vegetable protein diet than in those taking the animal protein diet (Walker, et al., *J. Nutrition 72,* 317). Serum cholesterol levels were studied in healthy university students (four men and four women) maintained 15 days on diets which contained seven different fats: butter, margarine, cottonseed oil, hydrogenated cottonseed oil, lard, hydrogenated lard, and corn oil. The fat provided 35% of total calories. Significant decreases in cholesterol levels occurred on the diets containing margarine, cottonseed oil, hydrogenated cottonseed oil, and corn oil. The decrease was 22% on the corn oil diet and 12% for the other fats. Cholesterol levels changed only slightly on the butter and two lard diets. The change in serum cholesterol depended on the initial serum cholesterol value and the cholesterol content in the test diet (Wilcox and Galloway, *J. Am. Dietet. Assoc. 38,* 227 (1961). In a study with six young men, the linoleic acid content was raised from 10 to 20 and 30% of the dietary fat in a mixed diet in which fat provided 40% of the calories. No significant effects of linoleic acid level were observed on the retention of calcium, magnesium, and phosphorus. Magnesium retention showed a quadratic trend as the proportion of linoleic acid in the dietary fat increased. The effect on nitrogen metabolism and fecal fat excretion was not clear cut. There was a downward but not significant trend in serum cholesterol and total fatty acids levels with increasing amounts of dietary linoleic acid. Increasing dietary linoleic acid resulted in greater proportions of dienoic acid in fatty acids of serum lipid fractions (Irwin and Wiese, *J. Nutrition 74,* 217). Rigidly controlled experiments on middle-aged men subsisting on diets of natural foods with and without supplements of 15 gm daily of either cellulose (fiber) or pectin failed to show any significant effect on serum cholesterol concentration from the cellulose but they did consistently show an effect from the pectin. The pectin effect was apparent in three weeks and amounted to an average fall of about 5% below the level on the same diet without pectin supplement (Keys, et al., *Proc. Soc. Exptl. Biol. Med. 106,* 555). Three ounces each of corn oil and hydrogenated coconut oil were added daily to the diets of two groups of six men for a period of a month. Serum cholesterol concentration fell an average of 9% in the men fed corn oil but did not change significantly in the others. Liver cholesterol concentration, as measured by liver biopsy, fell an average of 25% in the men fed corn oil. No consistent effect was observed in the other group. The fall in serum cholesterol produced by corn

oil feeding in man is probably not due to a shift of cholesterol from the blood to the liver (Frantz and Carey, *Proc. Soc. Exptl. Biol. Med. 106,* 800). In a seven-year study in man tocopherol requirements have been found to vary from a minimum of 5 mg per day for a diet high in animal tissue components to 20 mg α-tocopherol for diets providing 60 gm of stripped corn oil per day. The time of erythrocyte survival was shortened in man when a diet with a relatively low tocopherol to linoleic acid ratio was fed for prolonged periods (Horwitt, et al., *J. Am. Dietet. Assoc. 38,* 231). The mean tocopherol concentration in 197 factory workers in Rochester, N.Y., was 1.05 ± 0.32 mg/100 ml plasma. About 7% of the subjects had less than 0.50 mg tocopherol/100 ml, the level below which erythrocyte hemolysis tests become positive, indicating vitamin E deficiency (Harris, et al., *Proc. Soc. Exptl. Biol. Med. 107,* 381).

Diet and heart disease—facts and unanswered questions are discussed by Van Itallie and Hashim (*J. Am. Dietet. Assoc. 38,* 531). Effect of diet on serum lipids, especially manipulation of the fatty acid pattern of the diet and its effect in lowering serum cholesterol, is studied and discussed. In view of the fact that partial information now available is affecting and will continue to affect American dietary patterns and on the food industry, the authors stress the need for filling in quickly the major gaps in our knowledge. Nutritional problems of lipids are reviewed by Yasuda (*Yukagaku 10,* 338) and nutritionally essential fatty acids are reviewed by Tomono (*Ibid. 10,* 344).

OTHER MAMMALS. A number of studies dealt with the essential fatty acids and the fat deficiency syndrome. See preceding subsection for a review. Hill, et al. (*J. Nutrition 74,* 335), in studies in 66 swine fed purified diets varying in linoleate content from zero to 12.9% of calories, observed characteristic high levels of tissue trienoic acids and low levels of tetraenoic acids in unsupplemented swine. This relationship was rapidly reversed as dietary linoleate was increased. From a plot of tissue triene/tetraene ratio versus dietary linoleate and from the wt gains, the dietary linoleate requirement was deduced to be near 2% of calories. Guinea pigs were fed plant oils containing diets supplemented with either oleic or elaidic acid. The oleic acid-fed group showed signs of essential fatty acid deficiency after four weeks and severe signs after eight weeks. The elaidic acid-fed group did not show these symptoms. It is proposed that oleic acid competes with linoleic acid as a substrate for the enzymes involved in linoleate transformations when only a very limited supply of dietary linoleic acid is available to the animals and when oleic acid is made available in relatively large amounts (Dhopeswarkar and Mead, *JAOCS 38,* 297). A number of statistical techniques were applied to the study of tissue analysis, dermal score, and weight gain data obtained from rats maintained with controlled intakes of linoleic acid and total fat. The correlation between each of these variables and dietary linoleate as well as all other variables was presented. The correlation matrix thus generated was used for multiple regression and factor analysis studies (Caster and Holman, *J. Nutrition 73,* 337). Fat deficient rats had higher liver-lipid concentrations owing to increases in cholesterol esters and triglycerides. Both effects were more marked in males than in females. The total wt of dienoic and tetraenoic fatty acids fell and the wt of trienoic acid rose, in livers of fat deficient rats. These effects were due mostly to phospholipid fatty acids. Sex differences were observed for the dienoic and tetraenoic acids changes in different classes of lipids (Morton and Horner, *Biochem. J. 79,* 631). Fatty acid composition of lipids extracted from rats fed milk fat, corn oil, and lard was determined. Effect of dietary fat on body fat was shown by deposition in body fat of branched chain acids from milk fat. The major component fatty acids in milk fat, corn oil, and lard were selectively deposited in the carcass and liver tissue. The most drastic selectivity involved linoleic acid, which varied from 2% in those fed milk fat to 39% in those fed corn oil while tissue arachidonic acid content for these groups showed a small difference (Bhalerao, et al., *J. Dairy Sci. 44,* 1283). In monkeys with single and combined deficiencies of essential fatty acids and vitamin B_6, fatty acid patterns of plasma and erythrocytes were studied with GLC. EFA deficiency alone or combined with B_6 deficiency led to marked increases in palmitoleic and oleic acids in both plasma and erythrocytes while stearate, linoleate, and arachidonate were decreased. A peak, identified as eicosatrienoate, was observed for erythrocytes after four months EFA deprivation. The fatty acid patterns of vitamin B_6 deficient monkeys were the same as the controls (Greenberg and Moon, *Arch. Biochem. Biophys. 94,* 405). The fatty acid composition of cholesterol esters of livers of male and female rats was determined under various dietary conditions (Okey, et al., *J. Nutrition 73,* 117 and *Ibid. 75,* 51). The composition of the neutral fat of rats fed long- and short-chain acid triglycerides in conjunction with various levels of linoleic acid was studied. Linoleate regulated the type of fat deposited; it leads to a decrease in neutral fat in relation to body wt and facilitates the laying down of a depot fat more representative of that in the diet (Kaunitz, et al., *J. Nutrition 73,* 386).

The nutritive values of several different fats were studied. A number of partially hydrogenated rapeseed oils fed with purified basal diet to rats produced variable wt gain responses compared with those in rats fed the unhydrogenated rapeseed oil. The alterations in the C_{18} fatty acids resulting from hydrogenation of rapeseed oil appeared to be responsible for differing responses in wt gain (Beare, et al., *JAOCS, 38,* 310). In three growth experiments in rats, the Brazil nut oil had a nutritive value comparable to that of butterfat, olive oil, cottonseed oil, and corn oil. The digestibility of the Brazil nut oil averaged 98% and was not affected by heat treatment up to 120 min at 140C. The heat treatment did not reduce its nutritive value (Elias and Bressani, *JAOCS, 38,* 450).

Decreased survival times were shown by total body X-irradiated, male mice fed high fat diets, i.e., 20 to 30% of cottonseed oil or margarine fat in a purified ration (Ershoff, *Proc. Soc. Exptl. Biol. Med. 106,* 306).

A number of researches entered the common meeting ground of dietary lipid, plasma and tissue cholesterol, and experimental atherosclerosis. In rabbits fed commercial diet with added fat containing varying percentages of saturated acids (as glycerides) there were no significant differences in serum cholesterol, but the concentration of liver cholesterol varied inversely with the saturation of the fat. Addition of 0.5% of cholesterol to the diets gave the expected high levels of serum cholesterol and formation of aortic plaques, but the degree of saturation of the fat did not alter the magnitude of these effects significantly (Beare, et al., *J. Nutrition 73,* 17). Endogenous hypercholesterolemia was produced in adult, male rabbits fed a purified, fat-free and cholesterol-free diet *ad libitum* for 12 weeks (Diller, et al., *J. Nutrition 73,* 14). With rabbits fed cholesterol supplemented chow basal diet, alcohol-insoluble phosphatides supplement caused less hypercholesterolemia and less hyperphospholipemia than alcohol-soluble phosphatides or hydrogenated vegetable oil supplements (Van Handel, *J. Nutrition 73,* 259). Addition of different fish oils to basal carbohydrate or fat diets induced hypercholesterolemia and increased liver cholesterol in the rats. Reversal of these effects was observed upon addition of antioxidants to the diets (Nimni, et al., *J. Nutrition 73,* 243). The method for the study of cholesterol metabolism in hypercholesterolemic rats was used by which previous authors had found that cod liver oil had a distinctly stronger cholesterol-depressive effect than linoleic acid. A comparison was made between various marine oils, vegetable oils, egg yolk fat, whole egg yolk, and synthetic arachidonic acid (Nicolaysen and Ragard, *J. Nutrition 73,* 299 (1961). Studies on interrelationship effects of dietary protein, sulfur containing amino acids, and choline on the serum cholesterol concentration of the rat suggest that the serum cholesterol lowering effect of protein supplements is due largely to the sulfur containing amino acids they contain. Hypercholesterolemic effects were produced with choline supplements. Methionine appeared to have two opposing effects on serum cholesterol concentration in rats rendered hypercholesterolemic with a diet lacking choline: a) a cholesterol elevating effect attributable to its ability to spane choline by providing a supply of preformed methyl groups; and b) a cholesterol lowering effect common to sulfur containing amino acids and not dependent upon the lowering of methyl groups.

The vitamin K dietary requirement of normal adult mink was established as being less than 13 mg of menadione sodium bisulfite (U.S.P.) per ton, or 6.5 μg, per pound of feed (Travis, et al., *J. Nutrition 74,* 181). The antisterility activity of *alpha-toco*-hydroquinone in vitamin E-deficient female rats has been re-examined. An *alpha*-tocopherol-free preparation of *alpha*-tocohydroquinone injected at a level of 5 mg daily exhibited antisterility activity (Mackenzie and Mackenzie, *J. Nutrition 72,* 322).

In the area of vitamin and other deficiencies, studies on tocopherol and pyridoxine predominated in number. Survival of Cr^{51} tagged erythrocytes, autologously transfused, is severely shortened in monkeys maintained on vitamin E-deficient diets. Maximal survival times of 35, 45, and 49 days were obtained after monkeys had been on the diet for 349 or more days, as compared with 100 days for replete controls. The shortened survival time, associated with anemia, could be reversed upon addition of vitamin E. The mechanism is discussed (Marvin, et al., *Proc. Soc. Exptl. Biol. Med. 105,* 461). The tocopherol content of maternal and fetal tissues as related to vitamin E intake during gestation was studied in rats. The material serum tocopherol level was lowest in the vitamin E-sufficient group, highest in the vitamin E-deficient group, and intermediate in the mar-

ginally deficient group (groups with abnormal young). Values for liver had the same trend as serum but values for skeletal muscle were opposite for E-deficient and E-sufficient groups (Cheng, et al., *J. Nutrition 74,* 111). The distribution of vitamin E in the rat and the effect of *alpha*-tocopherol and dietary selenium on ubiquinone and ubichromenol in tissues was studied. The distribution of vitamin E, vitamin A, ubiquinone, and ubichromenol has been studied in 14 tissues of the rat. High concentrations of vitamin E and ubiquinone were found in adrenal gland, heart, uterus, and nerve. Tissues lost their vitamin E at different rates during depletion. Female rats contained higher amounts of the 4 substances than male rats of the same age. Vitamin E deficient rats had lower concentrations of ubiquinone in their tissues than rats receiving dietary vitamin E, and oral administration of vitamin E increased the ubiquinone concentration in tissues. Dietary selenium increased the concentration of ubiquinone in the same way as did *alpha*-tocopherol (Green, et al., *Biochem. J. 79,* 91). In similar studies with rabbits, the uterus in the rabbit is little affected by tocopherol. Vitamin E-deficient rabbits have lower concentrations of ubiquinone in heart, liver, and skeletal muscle than animals on the same diet supplemented with *alpha*-tocopherol acetate. In most tissues except nerve and brain, ubichromenol was also lower. Administration of single doses of vitamin E to deficient animals increased ubiquinone in all tissues except fat and also decreased ubichromenol. After 15 weeks on the vitamin E-deficient diet skeletal muscle contained little tocopherol and became depleted to low levels; however, even after 15 weeks the rabbits still contained large reserves of vitamin E in their adipose tissue (Green, et al., *Ibid. 79,* 108). The occurrence of vitamin E in the liver of dystrophic and antioxidant-fed rabbits was studied by other workers. No *alpha*-tocopherol was detected in the liver tissues of the deficient animals or of those cured of dystrophic symptoms with the synthetic antioxidant, N,N′-diphenyl-p-phenylenediamine. The authors feel that these results provide evidence for the nonspecificity of the vitamin E requirement of animals and relegate against a possible role for tocopherols as cofactors in enzymic reactions (Csallany and Draper, *Arch. Biochem. Biophys. 92,* 462). With respect to pyridoxine deficiency the emphasis was on fatty acid composition. In rats under *ad libitum* feeding conditions the percentage of stearic acid was higher and of linoleic acid lower in carcass fats of the deficient animals compared with fats of the supplemental controls. When the feed consumption of both groups was the same, carcass fat of the deficient animals contained a higher percentage of stearic acid and a lower percentage of palmitoleic acid than carcass fat of the controls, but the degree of unsaturation of the fat from rats in both groups was the same (Johnston, et al., *J. Nutrition 74,* 96). Vitamin B_6-deficient young male rats, in which the liver arachidonate had been depleted by feeding a fat-free diet, were fed daily for six days a supplement of 40 mg of methyl arachidonate and 360 mg of cottonseed oil, with and without the addition of 3 μg of pyridoxine per day. The lipid supplement increased the percentage of liver arachidonate equally in the vitamin B_6-deficient and the vitamin B_6-supplemented groups although the net gain in liver arachidonate by the former group was less because of smaller size (Williams and Scheier, *J. Nutrition 74,* 9). A study of the influence of pyridoxine deficiency on the fatty acid composition (GLC) of cholesterol ester, triglyceride, and phospholipid fractions of serum, liver, and adrenal tissue lipids showed that only the triglyceride fraction of the serum and liver and the phospholipid fraction of liver showed declines in the level of arachidonic acid and increases in linoleic acid. Pyridoxine deficiency had no effect on serum lipid levels, but there was a significant decline in the liver free cholesterol and a large decline in the liver phospholipid fraction (50%). Adrenal corticoid hormone synthesis was not impaired in the pyridoxine-deficient animal, nor was there any change in the level of the adrenal polyunsaturated fatty acids (Swell, et al., *J. Nutrition 74,* 148). Others observed in pyridoxine deficient rats a significant degree of hypercholesterolemia and a deposition of cholesterol in the aortae (thoracic and abdominal). The tetraenoic fatty acid level of serum was lowered in pyridoxine-deficient hypercholesterolemia, but the unsaturated fatty acid spectrum of aortae was not significantly altered. The authors conclude that the hypercholesterolemia was probably due to increased mobilization of cholesterol from the liver (Goswami and Sadhu, *Biochem. J. 78,* 732). The effect of a single dose of choline on total lipid phosphorus, lecithin P, and cephalin P of mitochondria, nuclei, and homogenates of the liver was studied in choline-deficient rats. A statistically significant increase in total lipid P and lecithin P of liver mitochondria and homogenates occurred in 3, 6, 10, and 18 hours following administration of a single dose of choline. Diets low in protein reduce the level of total lipid P, lecithin P, and cephalin P in liver mitochondria (Cornatzer and Gallo, *Proc. Soc. Exptl. Biol. Med. 107,* 383). The effect on lipid phosphorylation of a single dose of choline was compared in rats maintained on low protein-high fat diet or similar diet supplemented with methyl acceptor, guanidoacetic acid. Administration of a single dose of choline stimulated lipid phosphorylation in the kidney, heart, and aorta (Cornatzer, et al., *Proc. Soc. Exptl. Biol. Med. 107,* 463). The greater susceptibility of the male rat than the female rat to vitamin K deficiency was attributed by the authors to a true sex difference under hormonal control (Metta and Johnson, *J. Nutrition 72,* 455). Plasma free fatty acids were studied in fasting vitamin C-deprived guinea pigs. Such variables as length of the fast and administration of vitamin C before the fast influenced changes of FFA concentrations (Mueller and Cardon, *J. Lipid Research 2,* 83).

Other nutritional studies involved feeding of oxidized fats, minor components of lipids, or other unusual lipid substances. The influence of lauroyl and myristoyl peroxides and oxidized cottonseed oil on depot fat and liver lipid composition were studied on rats. Observations were made on fatty acid composition and cholesterol levels (Kaunitz, et al., *JAOCS 38,* 301). Chemical changes in animal and vegetable fats during heat treatment were determined. Rats were fed the various oxidized fats at the 10–20% level. The carbonyl value of the rat body fat was 0.04–0.13. The authors have shown that carbonyl compounds from highly oxidized fats are deposited in rat carcass fat (Wurziger and Ostertag, *Fette, Seifen, Anstrichmittel 62,* 895). The effects of ingestion of thermally oxidized corn oil on fat composition in the rat were studied. The results indicated that hydroxy acids, originating from oxidized fats, are deposited and influence the character of the normal mixed fatty acid compositions of the carcass fat (Perkins, et al., *Proc. Soc. Exptl. Biol. Med. 106,* 370) The above findings were reinforced by an investigation on the influence of ingested ricinoleic acid, 12-hydroxystearic acid and their corresponding triglycerides on rat growth and on carcass fat composition when compared with the effects of a commercial hydrogenated shortening and corn oil (Perkins, et al., *J. Nutrition 73,* 291). Butylated hydroxy anisole was fed at various levels to groups of weanling Cocker Spaniel pups. General health, weight gains, hemoglobin and blood cell counts, urine studies, and microscopic study of tissues at autopsy yielded results which indicate that dogs can ingest BHA for a long period without harm, at a level at least 220 times the maximum allowable level for the antioxidant in lard (Wilder, et al., *J. Agr. Food Chem. 8,* 504). A dietary evaluation of cottonseed protein of varying gossypol content was made. The excellent growth when meals were prepared from certain genetic lines of cotton having relatively high levels of gossypol suggests that other components may have been improved through breeding (Smith, et al., *J. Agr. Food Chem. 9,* 82). Subacute toxicity was observed in rats fed the non-adducting (with urea) fatty acids obtained from a "tarry" feed by-product of the manufacture of oleic and stearic acids (Munn, et al., *J. Assoc. Offic. Agr. Chemists 44,* 615). Further studies of this type of toxicity in weanling rats were also reported (Firestone, et al., *JAOCS 38,* 418). Rats fed a purified diet supplemented with 1% of orotic acid developed an extensive accumulation of triglycerides in the liver, an effect nullified by dietary adenine and diminished if the animals were receiving dog chow instead of purified diet. Incorporation of radioactivity derived from acetate-2-C^{14} into the liver triglycerides *in vivo* was increased 15-fold in animals ingesting orotic acid with a purified diet (Creasey, et al., *J. Biol. Chem. 236,* 2064).

Fat utilization in the fluoride-fed rat has been studied. Fluoride ingestion had no effect on the level of metabolic fat. Although all fractions were elevated in fluorotic rats, the neutral portion of the fecal lipid was raised to a greater extent than free or soap-bound fatty acids. Dietary free fatty acids were efficiently utilized by fluoride-fed rats. When the fluoride intake was equalized, more fat was observed in the feces of animals receiving fluoride by stomach intubation than by intraperitoneal injection. The high level of fecal lipid in fluorotic animals can be explained in part on the basis of a partial inhibition of lipase activity in the intestine (Suttie and Phillips, *J. Nutrition 72,* 429).

BIRDS. The largest group of studies in these species dealt with diets and dietary lipids for food, energy, and performance. A dietary fat requirement was shown in young chicks by growth responses when fed "fat free" (0.05% fat) basal diets supplemented with various fats and oils or with methyl linoleate (Ross and Adamson, *J. Nutrition 74,* 328). Three experiments have been conducted to measure the influence of calcium levels, antibiotics, pantothenic acid, phosphorus levels, and phosphorus sources on the metabolizable energy content of diets fed to chicks or poults. An increase in dietary

metabolizable energy associated with a decrease in calcium when diets contained Aureomycin or Penicillin was observed (Sibbald, et al., *Poultry Sci. 40,* 945). Studies on energy utilization were conducted with two-to-four-week-old chicks fed *ad libitum* amounts of four rations containing widely varying levels of glucose (Cerelose), corn oil, and soybean protein plus amino acids. Chicks which received a low-fat diet (2% corn oil), showed slightly lower gains in weight, higher gross energy content per gram of carcass gain, greater retention of metabolizable energy intake than chicks fed a ration containing 20% corn oil. No measurable differences in heat increment or carcass composition were attributed specifically to the carbohydrate or protein level of the rations (Bossard and Combs, *Poultry Sci. 40,* 930). To obtain maximum efficiency in the use of unextracted soybean products in chick rations, some such means as flaking must first be employed to increase the availability of the oil (Carew, et al., *JAOCS 38,* 249). A comparison of two antioxidants and two sources of xanthophyll in a pigmentation study with broilers was made. The addition of 0.0125% ethoxyquin to broiler rations significantly enhanced pigmentation in three out of four instances. BHT, at the same dietary level, significantly improved pigmentation in one instance but significantly depressed pigmentation in another instance (Ratliff, et al., *Poultry Sci. 40,* 716). A 32-week experiment concerning the effect of free fatty acid content in fats upon the performance of laying hens showed that there was no difference in the performance of laying hens receiving a diet supplemented with 2.5 or 5% animal fat or a mixture of hydrolyzed animal and vegetable fats ranging from 14.2 to 90.60% free fatty acids (Treat, et al., *Poultry Sci. 39,* 1550). Variations in dietary protein, energy, and cholesterol failed to influence serum cholesterol levels in growing chicks when fed a diet very low in fat. Increases of serum cholesterol were obtained by the addition of corn oil to a diet containing cholesterol. An inverse relationship between dietary protein and serum cholesterol was noted only when corn oil was present in the diet. Greater growth response to the addition of corn oil occurred with diets low in protein which suggests that the protein level of the diet may greatly influence fatty acid deficiency (Marion, et al., *J. Nutrition 74,* 171). Egg yolk cholesterol level is not influenced by feeding the laying hen choline either separately or with different fats. Serum cholesterol levels of the groups receiving no added fat showed a tendency toward an increase as the choline level of the diet was increased (Daghir, et al., *Poultry Sci. 39,* 1459). The effects in cockerels of dietary fats and cholesterol on serum and liver cholesterol concentrations, liver lipid concentration, the degree of saturation of tissue fats, and endogenous cholesterol synthesis were measured after 10-, 16-, and 22-week feeding periods. Serum cholesterol concentrations were increased only when both fat and cholesterol were fed. Increased liver cholesterol and total lipid concentrations were also observed under specified conditions (Adamson, et al., *J. Nutrition 73,* 247).

Requirements for vitamins A and K were studied in chickens and turkeys. About 1,200 USP units of stabilized vitamin A per pound of turkey breeder diet were sufficient for optimum egg production, hatchability of fertile eggs, and maintenance of the breeding hens; however, a vitamin A level of approximately 2,400 USP units per pound of starter diet were needed in order to produce satisfactory liver storage of vitamin A and blood uric acid levels in the progeny (Stoewsand and Scott, *Poultry Sci. 40,* 1255). The minimum requirements of laying hens for maximum egg production, maintenance of body weight and health, and minimum incidence of the blood spotting defect was 1,200–1,600 USP units of vitamin A per pound of diet (Hill, et al., *Poultry Sci. 40,* 1245). Data are presented on the vitamin A reserves of newly hatched chicks. The rate of mortality of chicks fed a deficient ration indicated considerable variation in vitamin A reserves among individuals hatched from eggs obtained from the same flock. This same high variability was apparent when the studies were extended to individual observations on chicks from different hens and among chicks of the same hen (Squibb, *Poultry Sci. 40,* 1197). The effect of sulfaquinoxaline on the quantitative requirements of the chick for vitamin K_1, Menadione, and Menadione sodium bisulfite were studied. The effect of sulfaquinoxaline on the vitamin K requirement of the chick varied with age. Vitamin K_1 was more effective than either Menadione or Menadione sodium bisulfite in overcoming the toxic effects of sulfaquinoxaline (Nelson and Norris, *J. Nutrition 73,* 135). The vitamin K content of six foods which had been frozen, canned and irradiated was obtained using the chick assay procedure (Richardson, et al., *J. Nutrition 73,* 369).

The level of dietary fat as well as the type of fat have a definite effect on calcium utilization by the chick as measured by growth rate and percentage of ash in the tibiae (Dunahoo, et al., *Poultry Sci. 39,* 1389). Shell quality, indicated by specific gravity of chicken eggs, was not affected by the addition of tallow to the diet at levels up to 12%. Shell quality was depressed by low dietary calcium, but tallow did not augment this effect (Hunt, et al., *Poultry Sci. 40,* 1193).

A crystalline halogen containing material producing chick edema symptoms at 0.1 part per million in the diet has been isolated from a sample of triolein which was toxic to monkeys. The material was similar to that reported by Harman, et al., but differed somewhat in ultraviolet spectral properties (Yartzoff, et al., *JAOCS 38,* 60). The chick edema disease factor was found to be present in a number of distillates and residues that were obtained during the production of commercial fatty acids. The raw materials from which the toxic samples were produced included inedible animal tallows, acidulated vegetable oil foots, and oils recovered from tin plate manufacture. The chick edema factor was found to be present in several oleic acids and in a triolein. Twenty stearic acid samples which were examined were non-toxic. A chick bioassay procedure for the factor is described (Firestone, et al., JAOCS *38,* 418). A chick assay procedure based upon measurement of pericardial fluid volume was described for the determination of the edema-producing factor in toxic fat (Ott, et al., *Poultry Sci. 40,* 1016).

The chromogen that appears in yolks of eggs produced by hens fed rations containing cottonseed meal and is responsible for the brown coloration that develops in such eggs (stored under refrigeration in the shell) is a pH indicator. Correlations between intensity of coloration in the yolks from cottonseed meal-fed hens and total gossypol, "free" gossypol, chemically uncombined gossypol, and gossypol-like pigments were all poor. Evidence indicates that the chromogen in cottonseed meals responsible for the brown coloration in stored shell eggs is heat-stable (Frampton, et al., *J. Agr. Food Chem. 9,* 59). The correlation between the available gossypol unit (AGU) values of cottonseed meals and coloration in yolks of stored shell eggs produced by the meals is virtually zero. The AGU method may not be relied upon for grading cottonseed meals for laying rations (Frampton and Piccolo, *J. Agr. Food Chem. 9,* 129). The fatty acid and lipid distribution in egg yolks from hens fed cottonseed oil or *Sterculia foetida* seeds is described. Feeding either *S. foetida* seeds or crude cottonseed oil to laying hens caused them to lay eggs with an increased proportion of saturated fatty acids and a decreased proportion of monenoic acids. A substance giving the Halphen reaction was concentrated in the triglyceride fraction of egg yolk lipids from hens fed *S. foetida* seeds. Fatty acid composition of phospholipides and triglycerides and changes therein is described (Evans, et al., *J. Nutrition 73,* 282). A process is described for elimination of pink white discoloration in stored eggs from hens fed cottonseed meal or sterculic acid (Deutschman, et al., *Poultry Sci. 40,* 1305).

PATENTED PREPARATIONS. The preparation of a number of vitamin products that may be of interest to nutritionists were reported. New stabilized vitamin A compositions in finely divided, rough shaped, non-spherical form consist of a source of vitamin A, a waxy solid, a vegetable flour, and a polyethylene gelling resin having a molecular weight in the range of 2,000–24,000 (Winsten, *U. S. 2,982,691*). A dry, free-flowing, mineral-stable vitamin preparation consists of vitamin A and/or D, a jellifiable, film-forming colloid, a carboyhydrate, and a stabilizer (Ten Ham and Kring [North American Philips Co., Inc.], *U. S. 2,980,587*). A stable solution of vitamin A-ascorbic acid complex in alcohol and the process of preparing said solutions is described (Pancrazio and Vitali [Orma Institute Terapeutico Romano I.T.R. S.r.l.] *U. S. 2,995,495*). Described is a process for making dry, discrete beads containing stabilized fat-soluble vitamin in a fatty core which is encased in an irreversibly heat-denatured, water-resistant, proteinaceous, digestible shell (Rosenberg, *U. S. 2,973,266*). A process is described for the production of a concentrated, stable, readily water-dispersible multivitamin preparation containing fat-soluble and water-soluble vitamins (Larde [Les Laboratories Francais de Chimothérapie, Paris] *U. S. 2,980,588*).

PHYSIOLOGY

Digestion, Intestinal Absorption and Excretion

Data on the digestibility, absorption, and *in vivo* oxidation in rats of two types of adipic acid esters of glycerides, a diglyceride adipate and a polymer of fatty acids, adipic acid, and glycerol have been presented. These compounds have high digestibility coefficients and the stearic acid moiety is well absorbed. The rate of oxidation of the stearic acid is greater when fed as the diglyceride adipate than as the poyester (Shull, et al., *JAOCS 38,* 84). The *in vivo* and *in vitro* incorporation of C^{14} labeled bicarbonate and acetate-2-C^{14} into

the ruminal volatile fatty acids of sheep receiving purified diets was compared (Van Campen and Matrone, *J. Nutrition 72,* 277). The influence of various feeds on *in vivo* and *in vitro* production of volatile fatty acids was studied. Rumen fluid was collected from two rumen fistulated animals on six different rations and was used to prepare washed cell suspensions of bacteria. Each suspension was used to ferment five of seven different substrates which were prepared from the same feeds as those fed the donor animals (Hinders and Ward, *J. Dairy Sci. 44,* 1129). The effect of environmental and other factors such as lactation number, calendar year, stage of lactation, and month of year on milk fat and solids-not-fat were studied. Age, stage of lactation and month of year appeared to be major sources of variation (Johnson, et al., *J. Dairy Sci. 44,* 658). Tissue storage and apparent absorption of *alpha-* and *gamma*-tocopherols by Holstein calves fed milk replacer was studied. The calves receiving *gamma*-tocopherol had a considerably higher proportion of other forms of tocopherol (particularly *alpha*) in their tissues than calves receiving *alpha*-tocopherol, thereby indicating a possible dietary interaction between the two forms of tocopherol (Chatterton, et al., *J. Dairy Sci. 44,* 1061).

Unusual fatty acids occurring in fecal lipids from humans with normal and abnormal fat absorption were studied. A variety of isomeric octadecenoic acids were present with varying double bond positions and about half of these with the *trans*-configuration. A new major component was found to be 10-hydroxystearic acid, together with lesser amounts of other isomeric hydroxystearic acids (James, et al., *Biochem. J. 78,* 333). The amount of fat in the diets and feces was determined for 35 girls, 7 to 9 years of age. In these studies, the fecal fat of preadolescent girls increased 0.05 gm as the fat intake increased 1 gm (Stier et al., *J. Nutrition 73,* 347). Feeding of bile acid-binding polymeric organic bases lowered plasma cholesterol concentrations in normocholesterolemic cockerels and dogs, and increased fecal bile acid and sterol output in the dog. The substances are thought to act by binding the bile acids in the intestinal tract (Tennent, et al., *J. Lipid Research 1,* 469). Fat excretion was studied in dogs lacking both bile and pancreatic juice. Apparently, in the dog, mechanisms other than the actions of bile and pancreatic juice can account for the assimilation of approximately 40% of the dietary fat (Cohen, *Proc. Soc. Exptl. Biol. Med. 107,* 40). Rats fed diets containing incompletely digested carboyhydrates, lactose, cellobiose, and raw potato starch, excreted larger amounts of organic acids and had a lower content of body fat than rats fed glucose, sucrose, or galactose. Organic acid urinary excretion could be increased by adding $CaCO_3$ but not $CaCl_2$ to the diet. This latter finding, in conjunction with the results of urinary analysis of ionic constituents, indicated that the increased urinary excretion of organic acid is not a consequence of an altered metabolism that decreases body fat deposition but is a renal acid-base mechanism for neutralization of increased urinary calcium, an increase resulting from the enhancement of gastrointestinal absorption of calcium by incompletely digested carbohydrates (Tomarelli and Bernhart, *Proc. Soc. Exptl. Biol. Med. 106,* 588).

The intestinal absorbability by chicks of palmitic and stearic acids present in mixtures of unsaturated fatty acids increased as the level of unsaturated fatty acids in the mixture increased and when fed in the form of mixed triglycerides present in intact fats (Renner and Hill, *J. Nutrition 74,* 254). Absorbability of single fatty acids fed at a level of 20% in a semipurified diet was studied. In the chick, utilization of the saturated fatty acids from C_{12} to C_{18} decreased as chain length increased. Palmitic and stearic acids were essentially unutilized. Oleic acid was found to be approximately 88% utilized by the chick. In the hen, utilization of the saturated fatty acids also decreased with increase in chain length. Absorbability of myristic, palmitic, and stearic acids, however, was significantly greater for the hen than for the chick (Renner and Hill, *J. Nutrition 74,* 259). The utilization by chicks of palmitic and stearic acids when fed in mixtures of free fatty acids was significantly improved as compared to the zero adsorbability of these fatty acids when fed singly. The degree of utilization of these saturated fatty acids was greater in mixtures containing higher amounts of the unsaturated fatty acids (Young, *Poultry Sci. 40,* 1225).

Lipid Transports and Body Fats

The turnover of fatty acids released by lipolysis in the body was estimated by measuring the lipolytic activity of plasma and the rise in fatty acid concentration after an injection of heparin. The mixture of fatty acids liberated endogenously appeared to be cleared at the same rate as has been previously reported for albumin-bound, C^{14}-labeled fatty acids (Dole and Rizack, *J. Lipid Research 2,* 90). The amount and timing of glucose administered to fasting post-partum women receiving intravenous insulin determines the amount and direction of change in plasma free fatty acids (FFA). The regulation of plasma FFA is a complex function that may involve fatty acid synthesis, utilization, and recycling as well as controlled release from fat depots (Burt, et al., *Proc. Soc. Exptl. Biol. Med. 106,* 330). Human serum FFA separated by column chromatography were analyzed for composition by gas chromatography. Data from analyses of blood serum FFA and other serum lipid fractions are given in detail for a large number of normal and abnormal patients and possible inter-relationships between disease and FFA composition discussed (Schrade, et al., *Deutsche Med. Wochenschrift 86,* 781). The effect of intra-arterial insulin in alloxan-diabetic dogs was to increase the artery tissue cholesterol and total fatty acids as well as muscle total fatty acids in the insulin injected hind limb compared with the contralateral saline injected hind limb (Cruz, et al., *Circulation Research 9,* 39). Fat transport in serum protein fractions in postprandial hyperlipemia was studied using starch electrophoresis. Normally a small content of low density lipoproteins is present in the *alpha*-2 fraction. Transport of chylomicrons was observed in the *alpha*-2 fraction. It was suggested that a high content of chylomicrons in the *alpha*-2 fraction of serum gives a possible indication of a disturbance in fat transport (Berg, *Fette, Seifen, Anstrichmittel 63,* 329). The regulation by heparin of the lipemia clearing reaction in biological processes, especially transport of blood fats to tissue fats, was investigated. The effects of heparin injections on the content of fat, phospholipid, and cholesterol was investigated as a function of time. The results indicated that the presence of a lipoprotein lipase could be responsible for hyperlipemia (Eggstein, *Fette, Seifen, Anstrichmittel 63,* 603). It was shown that the pituitary and adrenal glands play an important part in the response to the lipid mobilizing action of epinephrine in the rat, both in terms of FFA and lipoprotein responses (Shafrir, et al., *J. Lipid Research 1,* 459).

A lipid mobilizing composition has been described. It is prepared from a dialyzate of the blood plasma of nonspecifically stressed mammals. The lipid-mobilizer is a white, crystalline, solid polypeptide; soluble in water and dilute ethanol, insoluble in nonpolar organic solvents, and yields on hydrolysis alanine, glycine, leucine, glutamic acid and lysine (Seifter and Baeder [American Home Products Corp.] *U. S. 3,002,888*). A stable, sterile, nonpyrogenic, nontoxic fat emulsion for intravenous injection consists of: 10 to 35% w/v of a low melting, nontoxic, vegetable fat and a soybean phosphatide fraction in an amount of 6.7 to 9.3% by weight of the fat (Hainsworth *et al.,* [Don Baxter, Inc.] *U. S. 3,004,892*). A therapeutic fat product which is especially suitable for intravenous use in human beings is also described (Meyer, et al., [Upjohn Co.] *U. S. 2,977,283*). A concentrate composition suitable for admixture with physiologically acceptable aqueous media for intravenous administration consists of one part by weight of safflower oil, about 0.8 parts of glycerine, and about 1/120 as much lecithin w/v as oil (Zilversmit [Univ. of Tennessee Res. Corp.] *U. S. 2,972,565*). A phosphatide containing naturally associated neutral fat in an amount of 0.5 to 5% by weight of the phosphatide is stirred into an aqueous solution having a temperature of 70–80 C and containing 5–25% ethyl alcohol and a carbohydrate. The carbohydrate is present at a concentration of 6–35% by weight and the phosphatide at 5–15% by weight of the emulsion (Buer, *U. S. 3,004,922*).

Lipid transport was studied in the laying hen and in the incubating egg. Tracer doses of tripalmitin, C^{14}-labeled in both the glycerol and fatty acid moieties, were fed to laying hens over a 3-week period. Triglyceride transport through the intestinal mucosa and ovary was accompanied by replacement of an appreciable fraction of its glycerol. Phospholipids passed from ovary to eggs without change. The relatively high isotope concentration in the glycerol of carcass glycerides indicated that considerable accumulation of dietary glycerol occurred in adipose tissue. During incubation, triglycerides were transported from yolk to embryo without change in activity, while phospholipids underwent hydrolysis with loss of glycerol activity (Budowski, et al., *Arch. Biochem. Biophys. 93,* 483).

The following composition for lowering blood serum cholesterol was described: (parts by wt): vitamin B_{12}, 1; dessicated liver, 87; *dl*-methionine, 110; inositol, 40; choline bitartrate, 233; safflower oil, 415; *dl*-d-tocopherol acetate, 4; pyridoxine HCl, 2.1; excipients and fillers (Freedman and Shapiro, *U. S. 2,978,381*).

Palmitic-1-C^{14} and linoleic-1-C^{14} acids were infused into fasted dogs, and fractionation of serum lipids carried out at time intervals up to 72 hr. Although many similarities

were found in the metabolism of these two fatty acids, it was observed that linoleic-1-C^{14} appeared to (1) persist longer in the plasma as the FFA and (2) to be incorporated preferentially into cholesterol esters and phospholipides (Orth, et al., *Proc. Soc. Exptl. Biol. Med. 106*, 339). Normal carbohydrate fed rats and functionally hepatectomized rats were injected with C^{14}-labeled palmitic acid bound to albumin and the distribution of activity studied. The concentration of free fatty acid (FFA) in plasma rises rapidly after hepatectomy. In the normal rat, a considerable fraction of the injected FFA is recirculated in the blood in glycerides and to a lesser extent in phospholipids. This recirculation is almost completely abolished after hepatectomy, indicating that the liver is the chief organ for plasma lipoprotein synthesis. The peripheral tissues are capable of esterifying the FFA but do not release any esterified fatty acids into plasma, with the possible exception of adipose tissue (Borgström and Olivecrona, *J. Lipid Research 2*, 263).

On the assumption that the potassium content of the lean body mass is constant, it should be possible to estimate fat content in living man from a measurement of potassium-40 activity in the whole body scintillation counter. A series of such measurements on children and young adults shows good correlations with skin-fold thickness and wt/ht ratio as indices of fatness (Forbes, et al., *Science 133*, 101). A brief review is given of the development of methods for the determination of the total body potassium by whole-body scintillation counting and of studies to establish the utility of body potassium as a measure of gross body composition including fat (Angerson, et al., *Science 133*, 1917).

Lipid Metabolism in the Intact Animal

The serum cholesterol ester fatty acid (CEFA) and cholesterol level of children (6–10 years old) and older subjects (60–87 years old) of both negro and white races have been compared. Children of both races had significantly less oleic acid and significantly more linoleic acid in their serum CEFA fraction than older subjects. Arachidonic acid did not show significant changes with increasing age. Negro and white subjects of the same age group did not show significant differences in CEFA spectrum. Also children had a significantly lower total blood cholesterol level than older individuals (Swell, et al., *Proc. Soc. Exptl. Biol. Med. 105*, 129). The influence of age upon radioactive turnover and chromatographic separation of the individual phospholipides was studied in female mice. There was little change in percentage of total lipid phosphorus for the various phospholipid fractions with respect to age. However, the relative specific activities of each phospholipid fraction were greater in the younger mice (Cornatzer and Reiter, *Proc. Soc. Exptl. Biol. Med. 106*, 194). A new pharmaceutical product for reducing an excess of the lipids, especially cholesterol, in the blood, consists of a mixture containing at least 6% by weight of each of the following racemic amino acids: alanine, isoleucine, valine, serine, and glycine (Castaigne, *U. S. 2,965,542*).

Water and lipid composition of epidermis and dermis (including adipose and muscle layers) were determined during the growing and quiescent stages of the hair growth cycle in the mouse. Content of various lipids is not significantly different for the two stages of the cycle. However, independent of hair growth activity, epidermis has a lower water content and a much greater level of cholesterol, cholesterol esters, and phospholipid than dermis. The fast acting sterols of epidermis are limited largely to the esterified fraction (Carruthers, et al., *Proc. Soc. Exptl. Biol. Med. 105*, 259). Studies in humans showed that almost four times as much triglyceride occurs in a given volume of fasting blood serum as in the red cells. In humans four hr after fat meals and in dogs after being given intravenous fat emulsion, the amount of washed red blood cells glycerides and nonesterified fatty acids were not significantly different from those in the fasting state (Vacca, et al., *Proc. Soc. Exptl. Biol. Med. 105*, 100). The adrenal cholesterol ester fatty acid composition of seven different species were studied by gas-liquid chromatography. A Characteristic CEFA pattern for each species was apparent. The species were: man, rat, dog, goose, chicken, rabbit, and guinea pig (Dailey, et al., *Proc. Soc. Exptl. Biol. Med. 105*, 4).

Animals stressed by simple restraint for 30 min after injection of isotopic acetate exhibit patterns of cholesterol incorporation into liver and plasma esters that differ qualitatively and quantitatively from those of unstressed animals (Klein and Dahl, *J. Biol. Chem. 236*, 1658). Free and conjugated bile acids had qualitatively similar effects on mouse liver cholesterol levels and *in vivo* cholesterol-x-C^{14} synthesis rates. The effect of free bile acids on cholesterol metabolism are not due to exhaustion of amino acids used in formation of glycine and taurine conjugates, (Beher, et al., *Proc. Soc. Exptl. Biol. Med. 107*, 49). The effects of chain length upon the rates of metabolism of four C^{14}-carboxyl-labeled saturated fatty acids (butyric, caprylic, lauric, palmitic) were studied in the rat. The rates of metabolism of butyric and caprylic acids to respired $C^{14}O_2$ were highest and similar, that of lauric acid was intermediate, and that of palmitic acid was lowest (Kirschner and Harris, *J. Nutrition 73*, 397). Increasing levels of the diacetate of coumestrol, an estrogenic substance isolated from colver, were compared with estradiol benzoate, in the normal and castrate male rat, for their effect on food intake, growth, testicle and adrenal size, and liver and plasma lipid distribution. It appears, that although coumestrol has a definite estrogenic effect on the uterus of the young female rat, the compound is inactive and apparently nontoxic for the adult male animal (Lyman and Krueger, *J. Nutrition 73*, 391). Digitoxin was shown to increase the specific activity of heart ventricle phosphatides in rats receiving simultaneously radioactive orthophosphate and digitoxin as compared to control rats not receiving digitoxin. The increase in specific activity was demonstrated in both lecithin and phosphatidyl ethanolamine (Marinetti, et al., *J. Lipid Research 2*, 188). High fat feeding in the rat increased blood sugar, liver, fats, and tissue weights (liver and kidneys). It increases acetoacetate production by liver slices and ultilization by kidney slices, but this high rate of utilization is not maintained on prolonged feeding (19 weeks). High fat feeding augments hypothyroidism and suppresses hyperthyroidism to some extent. A beneficial effect of hydrolyzed glucose cycloacetate feeding with high fat diets to hyper- and hypothyroid rats is indicated (Khanade and Nath, *Proc. Soc. Exptl. Biol. Med. 105*, 566). The long-chain fluorofatty acid (fluoro-oleic) "ratsbane" is toxic to rabbits in a dose of 1 mg/kg. Death is delayed and usually occurs quite suddenly probably due to a heart attack. LD_{50} values are given for several species (Peters and Hall, *J. Sci. Food Agr. 11*, 608). It has been confirmed that weight maintenance of rats by means of caloric restriction is accompanied by a gradual decline in caloric requirements. This caloric-restriction-adaptation was retained even after a period of severe caloric restriction accompanied by weight loss. Body composition changes were slight or unaffected by caloric restriction (Lee and Lucia, *J. Nutrition 74*, 243).

Lipoprotein lipase was found in the post-heparin thoracic duct lymph of the seven dogs studied and in the post-heparin lymph aspirated from the chest in a patient with chylothorax. This enzyme reached a maximal concentration later and remained longer in the lymph than in the blood. Post heparin lymph also reflected the presence of heparin by greatly prolonged coagulation times. Evidence was given that lipoprotein lipase should be determined by measuring its lipolytic activity and not by "clearing" technics alone (Conner *Proc. Soc. Exptl. Biol. Med. 106*, 378).

Milk fat synthesis was studied in normal lactating dairy cows following intra-mammary infusion of C^{14}-labeled glycerol, glucose, acetate, propionate, or butyrate. Results indicate that glycerol is synthesized in the mammary gland from glucose but not from acetate, propionate, or butyrate. The newly formed glycerol is incorporated into milk fat. This strongly implies that one pathway of milk fat formation involves its *de novo* synthesis in the mammary gland from pools of free fatty acids and glycerol (Luick, *J. Dairy Sci. 44*, 652). Arithmetic and log functions of bovine and liver and plasma vitamin A and carotenoid were correlated in all possible combinations within each of four sampling periods. The correlation coefficients of the positive relationships that were found were so small that any known variable had little predictive value (Diven, et al., *J. Dairy Sci. 11*, 1632).

BIOCHEMISTRY

Analytical and Methodology

This section contains an annotated bibliography of methods of interest to the lipid biochemist and experimental biologist. The reader is also referred to the "composition and characteristics" section wherein analytical methods as applied to different lipid classes are dealt with.

"Optimum conditions for separation in gas chromatography" (Giddings, *Anal. Chem. 32*, 1707); "Ionization detectors for gas chromatography" (Stirling and Ho, *Ind. Eng. Chem. 52* [11], 61A); "Use of ortho-phthalic-ethylene glycol polyester in gas-liquid chromatographic analysis of fatty acid esters" (Craig, *Chem. and Ind. 1960*, 1442); "The use of silicone rubber gums or grease in low concentration as stationary phase for high temperature gas chromatographic separation of lipids" (Nicolaides, *J. Chromatography 4*, 496); "Alteration of some long chain esters during gas-

liquid chromatography'' (Morris, et al., *J. Lipid Research 1,* 412); ''Influence of column support on separation of fatty acid methyl esters by gas chromatography'' (Hornstein and Crowe, *Anal. Chem. 33,* 310); ''Quantitative determination of steam volatile fatty acids by gas-liquid chromatography'' (Gehrke and Lamkin, *J. Agr. Food Chem. 9,* 85); ''Absorption of the liquid phase in gas chromatography'' (Martin, *Anal. Chem. 33,* 347); ''Analysis of gas-liquid chromatograms by a punched card technique'' (Tandy, et al., *Anal. Chem. 33,* 665); ''Quantitative fatty acid analysis of milk fat by gas-liquid chromatography'' (Smith, *J. Dairy Sci. 44,* 607; ''Quantitative gas-liquid chromatography of fatty acid methyl esters with the thermal conductivity detector'' (Horrocks, et al., *J. Lipid Research 2,* 92); ''Gas chromatographic analysis of saturated and unsaturated fatty acids as the free acids from caproic up to arachidic acid (Stuve, *Fette, Seifen, Anstrichmittel 63,* 325); ''Preparation of methyl esters of fatty acids for gas-liquid chromatography'' (Vorbeck, et al., *Anal. Chem. 33,* 1512); ''A new liquid phase for gas chromatographic separations of steroids; Dow-Corning Corp. silicone polymer QF-1'' (Vanden Heuvel, et al., *J. Am. Chem. Soc. 83,* 1513); ''Determination of cholesterol and squalene by gas chromatography'' (O'Neill and Gershbein, *Anal. Chem. 33,* 182); ''Effects of varying the chemical composition of stationary phase on the separation of certain C_{19} and C_{27} steroids by gas chromatography'' (Lipsky and Landowne, *Anal. Chem. 33,* 818); ''Gas chromatographic analysis of estrogens'' (Wotiz and Martin, *J. Biol. Chem. 236,* 1312); ''Gas chromatography separations of steroids with polyester phases'' (Haahti, et al., *J. Org. Chem. 26,* 626); ''Gas-liquid separations of some simple triglycerides of long chain fatty acids'' (Pelick, et al., *JAOCS 38,* 506); ''Effects of relative concentrations on efficiency of separation of polar and nonpolar lipids by alumina column chromatography'' (Sims and Mes, *JAOCS 38,* 229); ''Separation of tissue cholesterol esters and triglycerides by silicic acid chromatography'' (Horning, et al., *J. Lipid Research 1,* 482); ''Application of reversed-phase chromatography to the analysis of seed oils'' (Gunstone and Syker, *J. Sci. Food Agr. 12,* 115); ''Separation of lipid classes by chromatography on florisil (Carroll, *J. Lipid Research 2,* 135); ''An apparatus for large scale preparative chromatography with especial application to the separation of a long chain fluoro-fatty acid'' (Hall, *J. Chromatography 5,* 93); ''Chromatography of some lipids on polytetrafluoroethylene'' (Areus and Dunckley, *J. Chromatography 5,* 272); ''Chromatography of lipids on silicic acid: infrared spectrophotometric elution curve'' (Wren and Lenthen, *J. Chromatography 5,* 370); ''Partition chromatography of short chain fatty acids'' (Gordillo and Montes, *Revista Argentina de Grasas y Aceites, 3,* 31); ''The separation of phosphatidyl ethanolamine and phosphatidyl serine by column chromatography'' (Rouser, et al., *JAOCS 38,* 13); ''Simple automatic valve for constant volume collection in column chromatography'' (Nelson, *Anal. Chem. 32,* 1724); ''The location and tentative identification of steroids on paper chromatograms by means of a system of color tests'' (Katz, *Arch. Biochem. Biophys. 91,* 54); ''An improvement in quantitative separation of phospholipides with silicic acid impregnated filter paper'' (Zieve, et al., *Proc. Soc. Exptl. Biol. Med. 105,* 508); ''The saponification and hydrogenation of lecithin on paper and the paper chromatographic analysis of its fatty acids'' (Kaufmann and Wessels, *Fette, Seifen, Anstrichmittel 62,* 1020); ''The separation of C_6–C_{12} dibasic acids in the presence of monobasic acids: a simple procedure by paper chromatography'' (Occolomitz, *J. Chromatography 5,* 373); ''The separation of glycerides of mixed fatty acid chain length by paper chromatography'' (Ory, *J. Chromatography 5,* 153); ''The paper chromatographic analysis of glycerides'' (Kaufmann and Makus, *Fette, Seifen, Anstrichmittel 63,* 125); ''Circular paper chromatography as a simple analytical method for fatty acids and glycerides'' (Noda and Hirayama, *Yukagaku 10,* 24); ''Qualitative and quantitative paper chromatographic analysis of wax acids'' (Kaufman and Das, *Fette, Seifen, Anstrichmittel 63,* 614); ''Separation of some glycerides of cocoa butter by paper chromatography'' (Steiner and Bonar, *J. Sci. Food Agr. 12,* 247); ''Thin layer chromatographic separation of cholesterol fatty acid esters'' (Kaufmann, et al., *Fette, Seifen, Anstrichmittel 63,* 235); ''Thin layer chromatography and its applications. A review'' (Vioque, *Grasas y Aceites 11,* 223); ''Separation, identification, and quantitative analysis of fatty acids by thin-layer chromatography and gas-liquid chromatography'' (Mangold and Kammereck, *Chem. and Ind.* (London) *1961,* 1032); ''Separation of lipids by thin layer chromatography'' (Kaufmann and Makus, *Fette, Seifen, Anstrichmittel 62,* 1014); ''Some new methods for separation and analysis of fatty acids and other lipides'' (Fontell, et al., *J. Lipid Research 1,* 391); ''A colorimetric method for determining fat acidity in grain'' (Baker, *Cereal Chem. 38,* 7); ''The ultraviolet absorption of fatty acids with isolated double bonds'' (Schauenstein, *Fette, Seifen, Anstrichmittel 62,* 687); ''The use of urea adducts to separate mixtures of fatty acids, esters, and waxes'' (Srivastava, *Indian Oil and Soap 3,* 51); ''Microdetermination of long-chain fatty acids in plasma and tissues'' (Dole and Meinertz, *J. Biol. Chem. 235,* 2595); ''Rhodanometric determination of the fatty acid composition of partially hydrogenated fats'' (Moller and Grabielsson, *Fette, Seifen, Anstrichmittel 62,* 936); ''Determination of trans fatty acids by infrared spectroscopy'' (Jart, *Oleagineaux 16,* 101); ''Various applications of ion exchange resins in the chemistry of fats and their derivatives'' (Ollero and Soto, *Grasas y Aceites 11,* 81); ''The application of ion exchange resins as catalysts in the chemistry of fats and related compounds'' (Ollero and Soto, *Grasas y Aceites 11,* 29); ''X-ray diffraction of binary systems of long-chain fatty acids'' (Saguchi and Asada, *Nippon Kagaku Zasshi 82,* 958); ''Dichroism of the infrared spectra of n-fatty acid crystals in the cesium bromide region'' (Kawano, *Ibid., 82,* 161); ''Infrared dichroism of n-fatty acids'' (*Ibid., 82,* 427); ''Infrared dichroism of methyl stearate and elaidic acid'' (*Ibid., 82,* 432); ''Synthesis of arachidonic acid'' (Rachlin, et al., *J. Org. Chem. 26,* 2688); ''Spectrophotometric determination of sterculic acid'' (Deutschman and Klaus, *Anal. Chem. 13,* 1809); ''Study of different methods for measuring linoleic and linolenic acid in oils'' (Wolff, *Rev. Franc. Corps Gras 8* [2], 68); ''A method for the determination of the water-insoluble combined lactic acid content of shortenings containing lactylated emulsifiers'' (Fett, *JAOCS 38,* 447); ''Isomerization of unsaturated fatty acids. V. Thermal change of pure methyl linoleate isomers'' (Nagano and Tanaka, *Yukagaku 10,* 146); ''Trans isomers in thermally oxidized lipids (Fukuzumi, *Ibid. 10,* 143); ''Isomerization of unsaturated fatty acids. IV. Effect of oxygen for thermal change of methyl linoleate'' (Nagano and Tanaka, *Ibid. 10,* 29); ''Determination of the glyceride structure of fats. Analyses are given for five natural fats'' (Youngs, *JAOCS 38,* 62); ''The structural components of milk triglycerides'' (Vander Wal, *JAOCS 38,* 67); ''Analysis of the glyceride structure of cocoa butter by thermal gradient crystallization'' (Jones and Hammond, *JAOCS 38,* 69); ''Glyceride structure of vegetable oils by countercurrent distribution. V. Comparison of natural, interesterified, and synthetic cocoa butter'' (Dutton, et al., *JAOCS 38,* 96); ''Glyceride structure of vegetable oils by countercurrent distribution. VI. Corn oil'' (Scholfield, et al., *JAOCS 38,* 175); ''The use of pancreatic lipase for determining the distribution of fatty acids in partial and complete glycerides'' (Mattson and Volpenheim, *J. Lipid Research 2,* 58); ''A new method for the analysis of component mono-, di-, and triglycerides (Privett and Blank, *J. Lipid Research 2,* 37); ''A fluorimetric micro glycerol method and its application to the determination of serum triglycerides'' (Mendelsohn, et al., *J. Lipid Research 2,* 45); ''A new method for the detection of diglyceride on a micro-scale'' (Clark, *J. Chromatography 5,* 368); ''Direct determination of liver triglycerides by an adaptation of the method of Van Handel and Zilversmit'' (Butler, et al., *J. Lipid Research 2,* 95); ''A method for the estimation of blood glycerides employing florisil'' (Blankenhorn, et al., *J. Lipid Research 2,* 281); ''The colorimetric determination of ester groups in lipid extracts'' (Antonis, *J. Lipid Research 1,* 485); ''Infrared spectroscopic investigation of fatty acid esters of polyalcohols'' (Kaufmann, et al., *Fette, Seifen, Anstrichmittel 63,* 8); ''Factors affecting extractability of cholesterol from lyophilized sera by cold chloroform'' (Forbes, et al., *Proc. Soc. Exptl. Biol. Med. 105,* 628); ''Turbidimetric determination of total serum cholesterol'' (Kingsley and Robnett, *Anal. Chem. 33,* 561); ''Spectrofluorometric determination of total bile acids in bile'' (Levin, et al., *Anal. Chem. 33,* 856); ''The determination of cholesterol and coprosterol in fecal lipides'' (Gerson, *Biochem. J. 77,* 446); ''Rapid spectrophotometric determination of total cholesterol in small amounts of blood and cerebrospinal fluid'' (Shin and Lee, *Anal. Chem. 33,* 1220); ''A stable reagent for the Liebermann-Burchard reaction application to rapid serum cholesterol determination'' (Huang, et al., *Anal. Chem. 33,* 1405); ''Reagent for determining the amount of cholesterol in serum and method of preparing same'' (Q. R. Hopper, *U. S. 3,-001,950*); ''Spurious recovery tests in tocopherol determinations'' (Booth, *Anal. Chem. 83,* 1224); ''An improved spectrophotometric method for the determination of tocopherols using 4,7-diphenyl-1,10-phenanthroline'' (Tsen, *Anal. Chem,* 33, 849); ''Determination of small amounts of iron and copper in vegetable oils'' (Takeuchi and Tanaka, *Kogyo Kagaku Zasshi 64,* 305); ''Mass spectrometry in lipide research'' (Ryhage and Stenhagen, *J. Lipid Research 1,* 361);

"Mass spectrometry and lipide research" (Selke, et al., *JAOCS 38,* 614); "Infrared spectroscopy of fatty substances. A review" (Barcelo, *Grasas y Aceites 11,* 34); "Infrared spectroscopy: application to fats" (Chouteau, *Rev. Franc. Corps Gras 8,* 268); "Chemistry of the color reaction of 2-thiobarbituric acid with carbonyl compounds" (Taufel and Zimmerman, *Fette, Seifen, Anstrichmittel 63,* 226); "The estimation of vitamin A aldehyde with thiobarbituric acid" (Futterman and Saslow, *J. Biol. Chem. 236,* 1653); "Diels-Alder reaction in the field of fats" (Kaufmann, et al., *Fette, Seifen, Anstrichmittel 63,* 633); "A new method of determining unsaturation values of fatty acids and oils by ozone" (Maggiolo and Tumolo, *JAOCS 38,* 279); "Measurement of lipoprotein lipase activity in post heparin plasma: description of technique" (Kern, et al., *J. Lipid Research 2,* 51); "Dehydration of fluid fatty mixtures, a process" (Greenfield, *U. S. 2,979,408*); "New results in the isolation and analysis of phosphatides and glycolipides" (Wagner, *Fette, Seifen, Anstrichmittel 62,* 1115); "The analysis of tissue phospholipides: hydrolysis procedure and results with pig liver" (Hubscher, et al., *J. Lipid Research 1,* 433); "Comparison of acid and nonacid volumetric methods for determining the percentage of butterfat in raw milk" (O'Dell, *J. Dairy Sci. 44,* 47); "Olive seed proteins. II Amino acids from acid hydrolysis" (Fernandez, *Grasas y Aceites 11,* 173); "New extraction methods of olive seed proteins" (*Ibid.,* 220). The following topics were covered in a short course entitled "Newer Lipid Analyses": Entenman, et al.: The preparation of tissue lipide extracts; Creech: Column chromatography; Creech: Isolations of neutral lipides; Rouser, et al.: Quantitative chromatographic fractionation; Schlenk and Gellerman: Column chromatography; Scholfield: Countercurrent distribution; Rouser, et al.: Paper chromatography of lipides; Hamilton and Muldrey: Glass paper chromatography; Horning, et al.: Separation of steroids; Huebner: Analysis of glycerides; Dutton: Monitoring eluates; Stein: Fatty acid structure; O'Connor: Near infrared spectroscopy; O'Connor: Infrared spectroscopy and lipides; Dutton: Mass spectroscopy and lipides; Hopkins: Nuclear magnetic resonance; Mangold: Thin-layer chromatography; Schlenk: Crystallization of fatty acids; (*JAOCS 38,* 534–588, 625–668, 708–736).

Lipid Biosynthesis and Bio-oxidation

A review on the biosynthesis of lipids appeared (Fukuba, *Yukagaku 10,* 333) and another on the mechanism of fatty acid synthesis also appeared (Wakil, *J. Lipid Research 2,* 1). Studies were made on the mechanism of fatty acid synthesis with a highly purified enzyme system prepared from pigeon liver which converts malonyl and acetyl CoA to palmitate in the presence of TPNH (Bressler and Wakil, *J. Biol. Chem. 236,* 1643). The biosynthesis of palmitic acid was investigated with an enzyme preparation from rat liver which has been purified 580-fold from the supernatant solution obtained by high speed centrifugation of cell-free suspensions of liver tissue. The most highly purified fraction obtained exhibited marked malonyl coenzyme A decarboxylase activity which was noncompetitively inhibited by short chain acyl CoA derivative (Brady, et al., *J. Biol Chem. 235,* 3093). The biosynthesis of long chain fatty acids has been investigated with an enzyme preparation obtained from rat brain tissue (Brady, *Ibid.,* 3099). Enzymic synthesis and metabolism of malonyl coenzyme A and glutaryl coenzyme A were studied by enzymic coenzyme A transfer reactions in animal tissues and yeast (Menon and Stern, *J. Biol. Chem. 235,* 3393). It was shown that cell-free preparations of the fat-body of the migratory locust, *Locusta migratoria,* incorporated acetate into fatty acids in the presence of ATP, CoA, glutathione, Mg^{++}, TPN, malonate, *alpha*-ketoglutarate, and $KHCO_3$. Newly synthesized fatty acids were esterified by the system with glycerol as glycerides and phospholipides (Tietz, *J. Lipid Research 2,* 182).

The carboxyl carbon of propionate was found to be a poor precursor for the synthesis of fatty acids in the rat. The data indicate that propionate as a three-carbon unit is not incorporated into long-chain fatty acids by the intact rat to any appreciable extent. A small fraction of the propionate is converted to long-chain fatty acids in adipose tissue *in vitro* by a mechanism that involves the use of propionate as an intact three-carbon unit, but in any case, its significance in the intact animal would appear to be negligible (Masoro and Porter, *J. Lipid Research 2,* 177). The preparation of crystalline pig heart propionyl carboxylase is described. The enzyme is active, although to a lesser extent, with butyryl, acteyl, and crotonyl coenzyme A besides propionyl coenzyme A (Kaziro, et al., *J. Biol. Chem. 236,* 1917).

Nonsynthesis of linoleic acid *de novo* from acetate-1-C^{14} was shown in the laying hen (Murty, et al., *J. Nutrition 72,* 451). Orally administered *cis*-12-octadecenoic-1-C^{14} acid serves inefficiently, if at all, as a precursor of linoleic acid in the rat. Activity did appear in an isomeric octadecadienoic acid presumably derived by degradation of the fed acid. Evidence for the presence of two highly labeled octadecatrienoic acids was obtained (Fulco and Mead, *J. Biol. Chem. 235,* 3379). Analysis of fatty acids isolated from rats 4 hours after feeding the methyl ester of 8, 11, 14-eicosatrienoic-2, 3-C^{14} acid revealed that this substance is efficiently converted to arachidonic acid by dehydrogenation in the 5 position. This finding furnishes evidence for the validity of the last step in a proposed pathway of conversion of linoleate to arachidonate via *gamma*-linolenate and 8, 11, 14-eicosatrienoate (Howton and Mead, *Ibid.,* 3385). Mitochondrial *beta*-hydroxybutyric, isocitric, and malic dehydrogenase activities for essential fatty acid deficient rats were greater than those of control rats when the mitochondria were protected by 0.5 M sucrose (Hayashida and Portman, *Arch. Biochem. Biophys. 91,* 206). The biosynthesis of lignoceric, cerebronic, and nervonic acids has been studied in the rat using acetate C^{14}. Stepwise degradation of brain cerebroside C_{24} acids to determine the label distribution revealed that lignoceric acid is completely synthesized from acetate, with little dilution from acids of intermediate chain length during the chain elongation process. Cerebronic acid, in turn, is formed directly from lignoceric acid. Nervonic acid, on the other hand, is not derived from lignoceric acid by desaturation but rather appears to be formed by chain elongation of oleic acid (Fulco and Mead, *Ibid. 236,* 2416).

Requirements for the synthesis of neutral lipids by cell-free homogenates of rat epididymal adipose tissue are described (Steinberg, et al., *Ibid.,* 1631). A comparison of the utilization of labeled fatty acids and glycerol, both free and combined, in the formation of glycerides has been made employing an *in vitro* procedure with rat intestine. Small amounts of long-chain fatty acid glycerides were formed from glycerol-C^{14} or from monoacetin or monopalmitin, both labeled in the glycerol moiety, in increasing amounts in the order named. Evidence suggested that most of the fat is completely hydrolyzed prior to absorption (Tidwell and Johnston, *Arch. Biochem. Biophys. 93,* 546).

The effect *in vivo* and *in vitro* of the estrogens: estradiol, estrone, or estriol was to greatly increase lipide synthesis in rat uterus. Individual phospholipide fractions were increased in level *in vivo,* and surviving uterine segments of uterus showed increased incorporation of inorganic orthophosphate-P^{32} into the individual phospholipide fractions. Acetate-1-C^{14} incorporation into fatty acid, cholesterol, and nonsaponifiable fractions of surviving uterine segments was also stimulated by early estrogen administration (Aizawa and Mueller, *J. Biol. Chem. 236,* 381).

Biosynthesis of cholesterol and terpenes. A review on this subject appeared (Tamura, *Yukagaku, 10,* 361). In liver homogenates an isotope labeled 4-hydroxymethylene-*delta* 7-cholestene-3-one was converted to $C^{14}O_2$ and tritium-containing cholesterol. Under anaerobic conditions the principal product is *delta* 7-cholestenol rather than cholesterol (Pudles and Bloch, *J. Biol. Chem. 235,* 2417). Squalene biosynthesized from mevalonate-5-D_2 was examined for the disposition of deuterium atoms. It was found that the substance contained 11 atoms of deuterium instead of the theoretically possible number of 12. Labeling in the center of squalene was asymmetrical, $-CHD \cdot CH_2-$ (Popjak, et al., *Ibid., 236,* 1934). The incorporation of zymosterol-C^{14} and zymostenol-24,25H^3 into cholesterol by Bucher and Waring Blendor homogenates of rat liver has been demonstrated (Schroepfer, *Ibid., 236,* 1668). Preputial glands from rats, when incubated with C^{14} acetate showed a marked incorporation of C^{14} into squalene, a sterol precursor, and little into sterols (Patterson, *Proc. Soc. Exptl. Biol. Med. 105,* 461). It has been demonstrated that treatment of rats with MER-29 leads to an accumulation of desmosterol (24-dehydrocholesterol) in the serum and tissues to the extent of 27 to 79% of the total sterols present. The identity of desmosterol was proven by several independent methods (Avigan, et al., *J. Biol. Chem. 235,* 3123). The role of desmosterol as a precursor in the biosynthesis of cholesterol has been demonstrated in the rat and in man treated with the drug MER-29 (Steinberg and Avigan, *Ibid., 3127*). *beta*-Diethylaminoethyl diphenylpropylacetate hydrochloride (SKF 525-A) has been shown to inhibit the conversion, by rat liver homogenates, of mevalonate-2-C^{14} to cholesterol and other nonsaponifiable lipides. Evidence has been presented which shows that the drug interferes with the conversion of the C_5- alcohol pyrophosphates to nonsaponifiable lipides. (Holmes and Bentz, *Ibid., 235,* 3118) Three analogues of mevalonic acid have been found to inhibit cholesterol biosynthesis in liver homogenates in the following

order of efficacy: 3-methyl-3-hydroxypentanoic acid greater than, *delta* 2-3-methylpentenoic acid greater than, *delta* 3-3-methyl pentenoic acid (Weiss, et al., *J. Lipid Research 2,* 258). Nicontinic acid and related compounds (0.1 to 5 mg. per ml.) have no significant effect on conversion of mevalonic acid to nonsaponifiable material by rat liver homogenates. The incorporation of acetate into nonsaponifiable material (largely cholesterol) decreases with increasing amounts of nicotinic acid. The results suggest that the locus of the nicotinic acid effect may be somewhere between acetate and mevalonate (Gamble and Wright, *Proc. Soc. Exptl. Biol. Med. 107,* 160). Addition of oleyl alcohol to a pancreatic extract capable of cholesterol esterification resulted in esterification of the aliphatic alcohol and stimulation of cholesterol esterification. Correspondingly, the extent of the oleyl alcohol reaction was greater in the presence of cholesterol (O'Connell, *Proc. Soc. Exptl. Biol. Med. 106,* 848).

Lactating cows were injected intravenously with butyrate-1-C^{14}, and butyrate-3-C^{14}. At 3 and 10 hr after injection, milk was collected and used to prepare lactose and amino acids from casein. On a basis of rleative specific activities among these compounds, butyrate is a better precursor of glutamate and aspartate than it is for lactose, serine, or alanine. Results indicate that the cow metabolized butyrate in the classical fashion by *beta*-oxidation (Black, et al., *J. Biol. Chem. 236,* 2399). Washed, red blood cells from normal, adult rats were incubated *in vitro* in a modified Tyrode phosphate medium with palmitate-1-C^{14}, the oxidation of which was demonstrated and measured by following the incorporation of radioactivity in the respiratory CO_2. The effect of several variables on the rate of the palmitate to CO_2 was studied (Hrachovec, et al., *Proc. Soc. Exptl. Biol. Med. 107,* 205).

Oxidation of carbon atoms 25, 26, and 27 of the cholesterol side chain to carbon dioxide by rat liver mitochondria is demonstrated (Whitehouse, et al., *J. Biol. Chem. 236,* 68). Rat liver mitochondria readily oxidized 3 *alpha,* 7 *alpha,* 12 *alpha*-trihydroxycoprostane and, to a lesser degree, its 24-hydroxy and 24-keto derivatives. Coincubation of these compounds with cholesterol and with each other, indicate that 3 *alpha,* 7 *alpha,* 12 *alpha*-trihydroxycoprostane is the most nearly related to, if not actually on, the pathway of metabolism of cholesterol to form cholic acid (*Ibid.,* 73). Results demonstrate that side-chain cleavage and transformation of cholesterol to pregnenolone in bovine adrenal cortex occurs in the mitochondrial fraction and has an absolute requirement for TPNH (Halkerston, et al., *Ibid., 236,* 374). The oxidation of ergosterol-28-C^{14} and of ergosterol-U-C^{14} by mitochondrial preparations from rat or mouse livers has been investigated. Similarities between the oxidation of cholesterol and ergosterol by liver mitochondria *in vitro* suggest that enzyme systems involved are closely related to each other, if not identical (Kritchevsky, et al., *Proc. Soc. Exptl. Biol. Med. 106,* 704).

GENERAL LIPID BIOCHEMISTRY. The stability of orally administered stearic-9,10-H^3 appears comparable to that of stearic-1-C^{14} in its incorporation into lipide in rat liver and epididymal fat pad (Reid and Williams, *Proc. Soc. Exptl. Biol. Med., 105,* 151). The diffusion of monocarboxyllic acid organic anions into rat diphragm *in vitro* was investigated. Evidence obtained in kinetic studies indicates that these anions, in diffusing from the medium to cells, do not pass through the main bulk of the interstitial fluid of the tissue (Foulkes and Paine, *J. Biol. Chem. 236,* 1019).

In vitro studies on the influence of thyroid hormone on the epinephrine-induced release of free fatty acids (FFA) from rat epididymal tissue showed that the thyroid hormone is essential for the epinephrine-induced release of FFA from adipose tissue (Debons and Schwartz, *J. Lipid Research 2,* 86). Epididymal adipose tissues from epinephrine-treated rats release more fatty acids into the medium during *in vitro* incubation than do tissues from untreated rats. The effect of epinephrine was abolished when an adrenergic blocking agent was administered to the animals before epinephrine was injected. Experiments *in vitro* showed that increased release of free fatty acids from adipose tissue due to addition of ACTH, epinephrine, and norepinephrine was inhibited by the addition of an adrenergic blocking agent to the medium (Schotz and Page, *J. Lipid Research 1,* 466). The monoglycerides from eight samples of lipolyzed milk fat were converted to allyl esters and identified, by gas-liquid chromatography and the fatty acid composition of the monoglycerides was different from that reported in intact milk fat (Jensen and Gander, *J. Dairy Sci. 43,* 1758). Two symmetrical triglycerides, 2-oleyl dipalmitin and 2-palmitoyl diolein, were synthesized and then subjected to hydrolysis at pH 6.6 by milk lipase. The results indicate that milk lipases preferentially hydrolyze the primary hydroxyl esters of synthetic long-chain triglycerides (Gander and Jensen, *J. Dairy Sci. 43,* 1762). Chylomicrons derived from olive oil, corn oil, cream, and cocoa butter were incubated with lipoprotein lipase and pancreatic lipase. These, and other, data indicate that liproprotein lipase is similar to pancreatic lipase in having no specificity among glyceride bonds involving palmitic, stearic, oleic, and linoleic acids. Lipoprotein lipase, unlike pancreatic lipase, hydrolyzes all three ester bonds of a triglyceride molecule at the same rate, or at very similar rates (Korn, *J. Biol. Chem. 236,* 1638). A series of animal and vegetable fats has been subjected to hydrolysis with pancreatic lipase. From the results obtained, the triglyceride compositions of the original fats have been calculated by the method previously proposed by Coleman and Fulton (Coleman, *JAOCS 38,* 685). The lipolytic activity of aortic tissues of young and old rats and the influence of heparin *in vivo* was studied. Free fatty acid (FFA) production during incubation with an artificial triglyceride substrate was measured. A relationship was shown to exist between weight of aortic tissue and FFA production and the amount of FFA released with time of incubation of a constant amount of aortic tissue (Drury, *J. Gerontology 16,* 114). Human plasma, red blood cells, plasma protein fractions, and a hemoglobin preparation were found to inhibit lipolytic activity of a microbiological lipase preparation when an olive oil emulsion was used as a substrate (Phillips, *Proc. Soc. Exp. Biol. Med. 106,* 192). Clearing factor lipase activity was present in abundance in chicken adipose tissue extracts. No such activity was demonstrated in seven of nine samples of human adipose tissue removed at surgery. Slight lipolytic activity was found in the other two instances (Engelberg, *J. Lipid Research 2,* 169).

Polyunsaturated fatty acid concentrations of tissues were determined in male and female rats of weanling, 3 months, and 6 months of age. No significant differences were found in concentrations in corresponding tissues of the males and females. Concentrations did change with age (Kirschman and Coniglio, *Arch. Biochem. Biophys. 93,* 297). Polyunsaturated fatty acid concentrations have been determined and metabolism of linoleic acid-1-C^{14} has been studied in pyrodoxine deficient and in pair-fed, pyridoxine-supplemented rats. Fatty acid concentrations were only slightly different for pyridoxine supplemented rats compared with those rats not supplemented with pyridoxine (Kirschman and Coniglio, *J. Biol. Chem. 236,* 2200). The chemical synthesis of a metabolite of pyridoxamine, 2-methyl-3-hydroxypyridine-5-carboxylic acid, is described (Argoudelis and Kummerow, *J. Org. Chem. 26,* 3420).

The dietary effects of fats upon the fatty acid composition of liver and cerebellum mitochondrial lipids was studied. The incorporation of a high level of linoleic and arachidonic acids into the cerebellum precedes the incidence of encephalomalacia in vitamin E deficient chicks (Marco, et al., *Arch. Biochem. Biophys. 94,* 115). Analyses of milk fats collected at various seasons showed high summer levels of *trans*-octadecenoic acid, which progressively declined through fall and early winter. A comparison of summer and winter fat showed the entire C_{18} fraction to be increased, mainly at the expense of palmitate in the summer sample (Patton, et al., *J. Dairy Sci. 43,* 1187).

The synthesis of "chylomicron-like" bodies and maintenance of normal blood sugar levels by the isolated, perfused rat liver is described. When palmitic acid-1-C^{14}-labeled chylomicrons are injected into the perfusate, C^{14} appears in phospholipids and cholesterol esters of plasma and liver and in carbon dioxide (Kay and Entenman, *J. Biol. Chem. 236,* 1006). The interfacial tensions of control and lipolyzed milk fat-water systems were studied. It appeared that monoglycerides decreased interfacial tension to some extent (Duthie, et al., *J. Dairy Sci. 44,* 401).

The synthesis of C^{14}-lipides in rabbit atheromatus lesions has been studied. Rabbits were injected with sodium acetate-1-C^{14} to measure the incorporation of this label into the phospholipides and other lipides of plasma and intima (Newman, et al., *J. Biol. Chem. 236,* 1264).

Contrary to the original findings, it is established that cholesterol-C^{14} and cholesterol-H^3 are deposited in tissues of rabbits in exactly the same way. Deposition is different for different tissues and that of cholestanol is only a very small fraction of that for cholesterol (Schwenk, et al., *Proc. Soc. Exptl. Biol. Med. 107,* 83). Some of the factors influencing uptake of cholesterol by a strain of mammalian cells growing *in vitro* have been examined. When emulsions of free (nonprotein bound) cholesterol were added to the serum medium, relatively small increases in cholesterol content of the medium resulted in large increases in cholesterol content of the cells. It is concluded that the main factor controlling cellular cholesterol uptake may be the relationship between serum cholesterol and the binding power of the serum proteins,

rather than the cholesterol level itself (Bailey, *Proc. Soc. Exper. Biol. Med. 107,* 30). The cholesterol content of cultured cells grown on serum samples taken from normal and atherosclerotic humans and rabbits was determined. Cells grown on atherosclerotic sera had significantly higher cholesterol levels than those grown on normal sera (Bailey, et al., *Proc. Soc. Exptl. Biol. Med. 107,* 594).

Methemoglobin has been shown to react with deoxycholate to form an ionic type of complex involving the prosthetic group and detergent anion. The complex becomes incorporated into miscelles of deoxycholate. It has a characteristic absorption spectrum much like that of cyanmethemoglobin. It can be dissociated by dilution; the deoxycholate anion is readily displaced by cyanide (Turner, *J. Biol. Chem. 235,* 3426).

The effect of steroids on electron transport is reported. Further data are given on the effect of steroids on the DPNH-cytochrome *c* reductase reaction. More precise localization of the site of steroid inhibition has been made (Yielding, et al., *J. Biol. Chem. 235,* 3413). Evidence is presented for a stimulating action of steriods on the ATP-ase activity of lymphosarcoma and liver mitochondria. The data obtained establish that certain steroids should be added to thyroidal hormones as endogenously produced hormones which alter mitochondrial permeability and ATPase activity (Blecher and White, *Ibid. 235,* 3404).

The metabolic fate of a synthetic corticosteroid, triamcinolone-H^3, was studied in the dog after intravenous injection. Countercurrent distribution and paper chromatography revealed two major radioactive and blue tetrazolium-reducing components in the urine. The results of this and previous studies of triamcinolone are inconsistent with mechanisms of corticosteroid action which involve biochemical alterations of the steriod molecule (Florini, et al., *J. Biol. Chem. 236,* 1038).

The catalytic activity of the heme compounds on lipide oxidation in muscle tissues has been reviewed. A comprehensive mechanism of the different catalytic activities of the various heme compounds has been presented, based on both experimental evidence and theoretical considerations (Tarladgis, *JAOCS 38,* 479). Evidence favoring hematin catalysis over autoxidation as the dominant mechanism of lipide peroxidation in animal tissues is presented. A tocopherol-ascorbate-glutathione-triphosphopyridine nucleotide couple could act synergistically to inhibit lipide peroxidation in animal tissues (Tappel, et al., *JAOCS 38,* 5).

The effects of surface-active agents and antioxidants on legume lipoxidase activity have been studied in some detail. The observations further support the idea of the existence of at least two lipoxidases in legumes, one specific for linoleic acid or other fatty acids with methylene-separated dienoic systems, and the other for glycerol esters of these fatty acids (Dillard, et al., *J. Biol. Chem. 236,* 37). The effects of high concentrations of the lipoxidase extracted from defatted soy flour on a purified linoleic acid substrate was studied. Rapid hydroperoxide production was followed by equally rapid decomposition. The hydroperoxide breakdown factor can be eliminated by a high-temperature short-time treatment of soy extracts. KCN addition to reaction mixtures causes a partial inhibition of the activity. A possible optimum at pH 8–9 was indicated. Qualitative evidence for the presence of a lipohydroperoxidase was obtained with well-known reagents that produce a specific color reaction with peroxidase (Gini and Koch, *J. Food Sci. 26,* 359).

A review on naturally occurring antioxidants appeared (Kaufmann and Garloff, *Fette, Seifen, Anstrichmittel 63,* 509). Studies on the mechanism of vitamin E action involving selenite and tocopherol inhibition of lipid peroxidation in the chick are reported. *In vivo* and *in vitro* lipid peroxidation products in the tissues of the vitamin E deficient chick have been measured (Zalkin et al., *Arch. Biochem. Biophys. 91,* 117). The decline of *alpha*-ketoglutarate and succinate oxidation in vitamin E deficient rat liver homogenates can be completely prevented or limited to a low rate by dietary *alpha*-tocopherol and N,N′-diphenyl-*p*-phenylenediamine, or by supplementation *in vitro* of small quantities of *alpha*-tocopherol, menadione, N.N′-diphenyl-*p*-phenylenediamine, methylene blue, and the Simon tocopherol metabolite (Corwin and Schwarz, *J. Biol Chem. 235,* 3387). The mechanism of kidney transamidinase depression by vitamin E deficiency was investigated. The evidence obtained supports the hypothesis that the depression is secondary to excessive creatine excretion (Fitch et al., *Ibid., 236,* 490).

A review on some biochemical properties of vitamin A appeared (Harashima, *Yukagaku 10,* 333). The conversion of radioactive *beta*-carotene to vitamin A by the rat intestine *in vivo* has been studied (Olson, *J. Biol. Chem. 236,* 349). Ten to 20% of *beta*-carotene was destroyed by incubating C^{14}-labeled *beta*-carotene with various tissues from the rat. All tissues studied had the ability to transfer radioactivity from *beta*-carotene to sterols, saponifiable material and steam distillable compound or compounds (Krause, *Proc. Soc. Exptl. Biol. Med. 107,* 363). Vitamin A acid exhibited a curative action when administered to vitamin A deficient rats; it did not appear to exert this action through its prior conversion into vitamin A alcohol. The C_{25} homolog of vitamin A acid also exhibited a curative action, but in this case there had been a conversion into vitamin A (Redfearn, *Arch. Biochem. Biophys. 91,* 226). Vitamin A, carotenoid, iodine, thiocyanogen values, and refractive index of milk fat as influenced by feed and individual breed of milk cow has been studied. Data suggest that conversion of carotene to vitamin A in an animal body may vary with the degree of unsaturation of fat and is regulated by the same metabolic processes which control the degree of unsaturation of secreted fat (Krukovsky, *J. Agr. Food Chem 9,* 236). Free and ester-type vitamin A could be changed to kitol, photodimer, by light of longer waves than 300 millimicrons. Antioxidants could not prevent vitamin A dimerization by light. Retinene was also changed to the dimer by light (Kaneko, *Nippon Kagaku Zasshi 81,* 1876).

The reactivity of the methoxy groups of coenzyme Q_{10} has been utilized to prepare the diisoamoxy- and diisopropoxy-homologs of coenzyme Q_{10} (Shunk et al., *J. Am. Chem. Soc. 22,* 5914). A number of organs and tissues of 3 humans were examined for coenzyme Q content. The liver, heart, spleen, kidney, pancreas, and adrenals contained relatively high concentrations of CoQ_{10}; thyroid and brain contained quite low levels. The total body content appeared to be in the range of 0.5-1.5 gm. (Gale et al., *Arch. Biochem. Biophys. 93,* 211).

Phosphoglycerides, Phosphoinositides, Sphingolipids, and Other Complex Lipids

A review discusses recent work on the synthesis of lecithins and cephalins, structure of plasmalogens, inositol phosphatides, and the sphingolipids (Malkin, *Chem. and Ind.,* London, *1961,* 605). Plasmalogen in human blood platelets was measured by two independent, stoichiometric analytical methods. The plasmalogen content was found to be approximately 25 micromoles per gm. of dry wt. An indirect evaluation of the possible role of plasmalogen in blood coagulation was carried out. The results suggest that this group of phosphatides does not play an essential role in blood thromboplastin formation (Zilversmit et al., *J. Biol. Chem. 236,* 37). The addition of butter to plasma shortened the Stypven clotting time of human plasma by over 75%. Butter was more active than any other fat tested (margarine is the only other fat mentioned specifically). A preliminary separation of butter lipides indicated that the thromboplastic activity was confined to the phosphatides originally bound to the proteins of butter (Billimoria et al., *Biochem. J. 78,* 185).

Phospholipide hydrolysis in cod flesh stored at temperatures ranging from +20 to −29C appeared to be promoted entirely by tissue enzymes, with nonenzymic reactions and the bacteria present playing negligible roles. In all cases there seemed to be virtually simultaneous loss of both fatty acids from the phospholipide molecule (Olley and Lovern, *J. Sci. Food Agr. 11,* 644). Rat liver contains an enzyme that catalyzed the hydrolysis of the vinyl ether linkage of plasmalogen. The enzyme is located principally in the microsomal fraction. No additional cofactors are required for the reaction to proceed in dialyzed preparations (Warner and Lands, *J. Biol. Chem. 236,* 2404).

A synthetic route to "mixed acid" L-*alpha*-lecithins and D-*alpha*, *beta*-diglycerides is described. α-palmitoyl-β-oleoyl-L-*alpha*-lecithin was obtained in 12% over-all yield and α-oleoyl-β-stearoyl-L-*alpha*-lecithin was synthesized in 45% yield (Hanahan and Brockerhoff, *Arch. Biochem. Biophys. 91,* 326).

Beef brain phosphoinositide, isolated according to Folch, has been found to contain three phosphate groups per myo-inositol residue, and to yield on base hydrolysis mainly myo-inositol triphosphate. The total myo-inositol phosphate fraction contained two isomeric triphosphates, two diphosphates, and one monophosphate. All were optically active. The structures and configurational relationships of some of these materials were worked out in some detail (Grado and Ballou, *J. Biol. Chem. 236,* 54). Sphingolipides of human brain were prepared by solvent extraction of the tissues or from mixed brain extract subjected to mild alkaline hydrolysis, and separated on silicic acid columns. Pure galactocerebrosides were isolated and characterized. A ceramide was detected in the brain from a patient with multiple sclerosis and significant amounts of glucocerebrosides were found in the brain of an old patient. Pure sphingomyelins were isolated from brain tissue (Schwarz et al., *J. Lipid Research 2,* 208). An improved procedure for the conversion of cerebroside to ceramide and sphingosine has

been devised, consisting of periodate opening of the glycosidic ring, reduction with sodium borohydride, and milk acid hydrolysis to produce ceramide. Alkaline hydrolysis of ceramide gives *erythro*-sphingosine in good yield. Application of this procedure to Gaucher cerebrosides has shown it to contain "normal" *erythro*-sphingosine (Carter et al., *J. Lipid Research 2,* 228). Hydrophilic colloids have been prepared from brain cephalins. When a turbid water emulsion of inositol phosphatide fraction or phosphatidylserine from brain tissues is added to a solution of ethylenediaminetetracetate at pH10, the solution becomes transparent and remains so even after removal of the reagents added by dialysis. Such a phenomenon is not seen with phosphatidylethanolamine or with a brain lecithin fraction. Aqueous ammonia also has a clearing action at higher pH values, but the resulting clear solutions were far less stable (Kimura and Nagai, *Biochem. J. 77,* 1). Infrared spectra are reported for 4 forms of brain phosphatidylserine: the original preparation, metal-free mono- and di-sodium derivatives (Kimura and Nagai, *Ibid., 77,* 3).

The sulfolipids. A review discusses the chemistry, metabolism and the occurrence of sulfolipids (Goldberg, *J. Lipid Research 2,* 103). A review on the biochemistry of glycolipids appeared (Makita and Yamakawa, *Yukaguku 10,* 352).

Steroids

The conversion *in vitro* of cholesterol-4-C^{14} to estrone by human ovarian tissue has been described. Criteria for the identity of the radioactive metabolite and complete data on recovery and changes in specific activity have been provided (Ryan and Smith, *J. Biol. Chem. 236,* 2204). The conversion *in vitro* of acetate-1-C^{14} to neutral steroid intermediates is described and the relationship of these neutral steroid metabolites to the biosynthesis of estrogens was outlined (Ryan and Smith, *Ibid.,* 2207). In adult guinea pigs with biliary tract fistulae and stable enterohepatic bile acid pool, cholesterol-4-C^{14} was converted into 3 labeled bile acids. The conversions were correlated to the time, hours or days, of free flow and continuous enterohepatic cycling (Peric-Golia and Jones, *Proc. Soc. Exptl. Biol. Med. 106,* 177). The addition reaction of hypochlorous acid to epicholesterol derivatives has been studied. The reaction at the 3-*alpha* carbon belongs to the type of *anti*-Markownikoff addition (Mukawa, *Nippon Kagaku Zasshi 81,* 1348). Water-soluble vegetable oil sterol derivatives are described. The derivative is a water-soluble polyethylene glycol ester of a phytosterol acid ester of a dicarboxylic acid (Stern (Eastman Kodak Co.) *U. S. 3,004,043*). A process for purification of crude sterol mixtures containing not more than 20% nonsterol impurities is described (Cunningham et al., (Upjohn Co.) *U. S. 2,963,494*). A process for separation of substantially pure, crystalline phytosterol from unsaponifiables is described (Burns and McDaniel (Union Bag and Paper Corp.) *U. S. 2,957,891*).

Lipoproteins

A review on the quantitative aspects of lipids in plasma (Kasuga, *Yukagaku 10,* 347) and another on the chemical aspects of lipoprotein appeared (Hara, *Yukagaku 10,* 371).

Separation of dog serum lipoproteins by ultracentrifugation, dextran sulfate precipitation, and paper electrophoresis are described and compared (Sakagami and Zilversmit, *J. Lipid Research 2,* 271). The effect of molecular weights of colloidal dextran on human serum lipides was studied with respect to electrophoretic behavior of the lipoproteins on paper (Pucar and Keler-Bacoka, *Science 134,* 1369). The molecular complexes formed by the interaction of certain polysaccharides with plasma lipoproteins are discussed in great detail (Cornwell and Kruger, *J. Lipid Research 2,* 110). Lipoprotein prestaining and ultracentrifugal analysis in a density gradient are reported (Cornwell and Kruger, *Proc. Soc. Exptl. Biol. Med. 107,* 296). The effects of centrifugation at 20,000 × g for 2 hours on lipide distribution of various human sera have been studied (Forbes et al., *Proc. Soc. Exptl. Biol. Med. 107,* 224).

A rapid, simple method for preparing lipoprotein containing radioactive cholesterol is described. Cholesterol-4-C^{14} in a Tween 20 dispersion was found to exchange readily with cholesterol of the plasma lipoprotein of rats or humans. On the basis of electrophoretic, ultracentrifugal, and immunochemical characteristics it was concluded that it was a true isotope exchange and that the physical properties of the lipoproteins studied were not altered (Whereat and Staple, *Arch. Biochem. Biophys. 90,* 224). Recombination with lipids of the lipid-free protein from canine serum was reported. When I^{131}-labeled protein moiety was added to serum injected into dogs, the appearance of radioactivity in lipoprotein indicated a preferential interaction of the labeled protein with its own lipoprotein class. In the absence of serum the I^{131}-labeled protein moiety was mixed with isolated low density lipoproteins and chylomicrons and the products formed were studied. The radioactive products were injected into dogs and their disappearance from the circulation was compared with that of an artificial complex of I^{131} labeled lipoprotein and Lipomul, an artificial triglyceride emulsion similarly injected (Scanu and Page, *J. Lipid Research 2,* 161). The interaction of serum lipoproteins with the hydroperoxide of methyl linoleate was studied by means of ultracentrifugation and paper electrophoretic analysis. The results indicated that lipohydroperoxide seemed to have a marked effect on the stability of low density or beta lipoproteins. *In vivo* studies indicated that hydroperoxide inhibited intestinal absorption of lipide. Some lipohydroperoxide was absorbed from the intestinal tract, when diluted with methyl linoleate. The question of an exogenous source of lipohydroperoxide contributing to the *in vivo* denaturation or degradation of beta lipoproteins was posed (Nishida and Kummerow, *J. Lipid Research 1,* 450).

The beta/alpha lipoprotein ration and other serum lipid components have been studied in a population of full blooded Navajo Indians. The findings offer a basis for assessing the low degree of atherogenesis and are consistent with evidence for the low incidence of coronary heart disease in this ethnic group (Kositchek et al., *Circulation 23,* 219). Fatty acid composition of the lipid fractions from bovine serum lipoproteins was reported. A large concentration of linoleate in the total lipides and cholesteryl esters of the high-density lipoproteins was indicated. Low-density lipoproteins contained chiefly palmitate and stearate in all lipid fractions (Evans et al., *J. Dairy Sci. 44,* 475). Mouse plasma lipid and lipoprotein fatty acids were evaluated by gas-liquid chromatography (Rehnborg et al., *Proc. Soc. Exptl. Biol. Med. 106,* 547).

The membrane material obtained by freezing washed cream has been fractionated from suspensions in 2% sodium desoxycholate into five sedimentable fractions representing 75% of the total material and one soluble fraction (25%). Enzyme activities in and lipid composition of the various fractions is described (Alexander and Lusena, *J. Dairy Sci. 44,* 1414). Lipid components were extracted from fat-globule membranes prepared by two procedures. One method started with cold, pooled milk, whereas in the other procedure freshly drawn, uncooled milk served as the source. The lipids obtained were resolved by silicic acid chromatography. A number of different lipid substances were identified as components of the fat-globule membrane (Thompson et al., *J. Dairy Sci. 44,* 1589). The effect of stirring, pasteurization, homogenization, and condensing on the migration of the phosphatides from the fat globule membrane to the skim milk has been studied (Greenbank and Pallansch, *J. Dairy Sci. 44,* 1597).

The egg yolk lipoprotein complexes, lipovitellin and lipovitellenin have been studied. Amounts of lipid and protein material, lipid binding, and lipid composition were studied (Evans and Bandemer, *Poultry Sci. 40,* 597).

LIPIDS IN DISEASED STATES

The literature concerning the influence of food fats on heart disease is reviewed. The equilibrium between protein, fat, and carbohydrate metabolism and the intermediary metabolism of the sterols, especially cholesterol, is discussed. The authors discuss the requirements for and the effects of essential fatty acid deficiencies (Randouin and Brun, *Rev. Franc. Corps Gras 7,* 639). The incidence of deaths due to arteriosclerotic heart disease is being compared in men recovered from myocardial infarction and treated 75 days or more with either Premarin or no estrogen (control), allocation of treatments being randomized. Survival rate in those receiving Premarin is significantly higher than in controls (Marmorston et al., *Proc. Soc. Exptl. Biol. Med. 105,* 618). Serum cholesterol and relative body weight of coronary patients in different populations were studied. The serum total cholesterol concentration was significantly higher in each of the three groups of coronary patients than in their corresponding controls. Serum cholesterol values were correlated with *alpha-* and *beta*-lipoprotein values. The cholesterol data are compatible with the hypothesis that the populations have different frequency distributions of cholesterol values, the coronary patients in each population tending to be drawn from the upper end of the corresponding population distribution, with no general critical level distinguishing all coronary patients as a total group (Keys and Fidanza, *Circulation 22,* 1019). The disposal of intravenously administered fat in subjects with atherosclerosis and in normal controls was studied. Artificial oil emulsion (Lipomul I.V.) was given to five groups of subjects and disappearance of fat from their blood was observed by plasma optical density measurements. The control, untreated infarct and peripheral vas-

cular disease groups exhibited similar curves of optical density on time. Treated myocardial infarct groups had received heparin and phenoprocoumon (Mashford and Nestel, *Circulation Res. 9,* 7). The lipids extracted from the blood serum of 30 healthy male subjects of different ages, of 20 male atherosclerotic patients with hyperlipidemia and of 17 patients with idiopathic hyperlipidemia were separated into cholesterol esters, phospholipids, glycerides, and non-esterified fatty acids. The results of analyses indicated that in cases of hypercholesterolemia, hyperphospholipidemia, and hyperglyceridemia there appear in serum additional esters which contain more saturated and monounsaturated and fewer polyunsaturated fatty acids (Schrade et al., *J. Atheroscler. Res. 1,* 47). The quantitative complement fixation technique was used to study human low density lipoproteins *in vitro* and *in vivo*. The work done *in vivo* included 61 normal subjects as well as 24 patients with diabetes mellitus and 20 with atherosclerotic heart disease. Complement fixation curves of low density lipoproteins seem to change as the individual grows older, inhibition of fixation becoming less and less in antigen excess. Low density lipoproteins of diabetics show complement fixation curves similar to those of normal individuals in the same age bracket. The incidence of flat and irregular complement fixation curves is much higher in atherosclerotic patients than in normal individuals (Spitzer and Spitzer, *J. Gerontology 16,* 125). The oral administration of solid diiodotyrosine polypeptide (containing about 1 to 1.5 mg. of iodine) controls the cholesterol content of human blood by decreasing the concentration of *beta*-lipoprotein and increasing the *alpha/beta* lipoprotein ratio (Stambul, *U. S. 2,980,585*).

Phagocytosis of lipid-rich platelets by monocytes and the transformation of such cells into lipophages containing fat was observed in human thrombi. The lipophages are similar to lipophages in atherosclerotic plaques. This observation supports the idea that some atherosclerotic plaques are organized mural thrombi (Chandler and Hand, *Science 134,* 946). The role of the intimal mucoid substances in the pathogenesis of atherosclerosis has been studied. Complex formation *in vitro* between mucopolysaccharides (MPS) from atherosclerotic aortic intimas and plasma *beta*-lipoproteins and fibrinogen was shown. Some of these complexes were shown to be homogeneous to paper electrophoresis. It was concluded that the aortic deposition of plasma *beta*-lipoprotein and plasma fibrinogen may have a common cause: chemical and physicochemical changes in the MPS composition of the intimal ground substance which facilitate the formation of specific MPS-*beta*-lipoprotein and MPS-fibrinogen complexes (Gerö et al., *J. Atheroscler. Res. 1,* 67). Changes in the composition of phospholipids and of phospholipid fatty acids associated with atherosclerosis in the human aortic wall have been reported. The increasing percentage of sphingomyelins of human aorta (intima plus media) with increasing degree of atherosclerosis has been confirmed. The fatty acid compositions of seven phospholipid fractions were studied. It was shown that the compositions change in the direction of increasing saturation with increasing degree of athersclerosis, attributable mainly to a decline in the percentages of polyunsaturated acids, especially arachidonic acid (Böttcher and van Gent, *J. Atheroscler. Res. 1,* 36). Analysis of lipids of the intima and media of human aortas with progessive stages of atherosclerosis reveals an increase in sterol and sterol ester fractions and a decrease in phospholipids. It is proposed that these changes reveal a very low rate of lipid turnover in this tissue. The fatty acid composition of the cholesterol esters did not change with advancing atherosclerosis (Mead and Gouze, *Proc. Soc. Exptl. Biol. Med. 106,* 4). The cholesterol ester, triglyceride, and phospholipide fatty acid composition (GLC) of aortic media, thickened intima, and plaque material were determined in six human subjects. For comparison, the fatty acid composition of those fractions in serum was determined in six subjects (aged 55-70) with occlusive atherosclerosis in a good nutritional status. The triglyceride fraction of those tissues and serum were similar in their fatty acid composition. Some slight differences were noted in the fatty acid composition of the phospholipid fractions, most notably in the arachidonic and long chain fatty acids. The cholesterol ester fraction of the tissues studied showed the greatest differences in fatty acid composition. Both early and advanced plaques had significantly less linoleic and more oleic acid in that fraction than serum or media. Media and serum cholesterol esterified fatty acids were similar in their fatty acid composition. The significance of these findings in relation to atherogenesis is discussed (Swell et al., *Proc. Soc. Exptl. Biol. Med. 105,* 662). Deposition of C^{14}-labeled cholesterol in the human atheromatous aorta has been studied. Circulating (in the blood) cholesterol exchanges with intimal cholesterol only slowly and with the greatest difficulty with that in the atheromatous plaques. Exchange or depositions were greatest for the aortic arch, lesser for abdominal aorta. The media showed about the same rate of exchange of cholesterol through the length of the aorta. A disk technique suitable for various types of quantitative assays of intima, media, etc., of the aorta was described (Rabinowitz et al., *Proc. Soc. Exptl. Biol. Med. 105,* 241).

Human blood lipids were separated by dialysis and adsorption chromatography and the fatty acid composition (GLC) of all of the fractions determined. Characteristic changes in the ratio of essential to nonessential fatty acids in the case of infected (?) persons were observed (Schrade et al., *Fette, Seifen, Anstrichmittel 62,* 673). Lipids and proteins exist in peritoneal, joint, pleural, hydrocele, and pericardial effusions which have properties which are similar to the plasma lipoproteins. Classification of fluids according to traditional concepts of pressure and irritation reveals that irritative fluids tend to have higher concentrations of low density lipoproteins, total cholesterol, and globulin than do pressure fluids (Kellog and Mann, *Proc. Soc. Exptl. Biol. Med. 107,* 102). Serum lipids in a case of lipisarcoma have been studied. The authors have reported serum lipid values for one diseased and one normal patient, aged 45 and 24 years, respectively. The fact that the amount of phosphatides was very low, and that cholesterol-free unsaponifiable material was very high in lipisarcoma was discussed (Kaufmann and Garloff, *Fette, Seifen, Anstrichmittel 62,* 679).

Fasting plasma free fatty acid (FFA) concentrations were determined in 34 subjects with cirrhosis and 28 without cirrhosis. Mean fasting FFA concentrations were elevated in subjects with cirrhosis and this was most marked in those subjects with acute hepatic failure or hepatic coma. The increased FFA concentrations were not accompanied by a rise in blood ketone concentrations (Stormont et al., *Proc. Soc. Exptl. Biol. Med. 106,* 642). Cerium (2 mg/kg) causes an early decrease in serum glucose and a later significant increase in plasma FFA followed by fatty degeneration of the liver in rats. Conditions that prevent the FFA and fatty liver responses are (a) administration of the cerium as a bound complex or particle, and (b) use of hypophysectomized, diabetic, or male rats (Snyder and Stephens, *Proc. Soc. Exptl. Biol. Med. 106,* 202).

Gross steatorrhea has been induced experimentally in healthy human subjects by administration of a resin capable of sequestering bile acids in the intestinal lumen. This agent (MK-135) inhibited absorption of I^{131}-labeled triolein but not I^{131}-labeled oleic acid. In contrast to certain other forms of experimental malabsorption, MK-135-induced steatorrhea appears to be predictable and innocuous (Hashim et al., *Proc. Soc. Exptl. Biol. Med. 106,* 173).

In addition to the electronic factors, there is a steric factor responsible for the carcinogenicity of polynuclear aromatic hydrocarbons. A carcinogenic polynuclear aromatic hydrocarbon must bear steric resemblance to steroids. One possible implication to this requirement for carcinogenicity is that these hydrocarbons may act on the same sites as steroid hormones (Yang et al., *Science 134,* 386). The antigens of *Ricinus Communis* (castorbean) have been studied. It would appear that allergy to castor pomace may actually be sensitivity to any one or more of the antigenic components of the pomace including both pollen and female blossoms. The phenomenon of passive cutaneous anaphylaxis in guinea pigs was shown to be applicable in the laboratory determination of residual antigenicity in fractionated and chemically treated castor-seed proteins (Layton et al., *JAOCS 38,* 76, 597).

Three separate experiments in rabbits showed that cholesterol administration in the absence of added fat is more atherogenic than cholesterol fed together with corn oil (Kritchevsky et al., *JAOCS 38,* 74). Effects of an unsaturated and saturated lipid on experimental atherosclerosis was studied in rabbits. It was found that addition of the lipid to the diet increased absorption and retention of cholesterol and this intensified the cholesterol atherosis (Merrill, *Proc. Soc. Exptl. Biol. Med. 105,* 268). The effect of certain liquid organopolysiloxanes on cholesterol atherosclerosis of the rabbit has been studied. Varying effects on plasma, liver, and aortic cholesterol levels and on atheromatosis were produced by different test compounds (Gollan, *Proc. Soc. Exper. Biol. Med. 107,* 442).

The polyunsaturated fatty acid and cholesterol concentrations of plasma and aorta and their relationship to avian atherosclerosis has been studied (Feigenbaum et al., *JAOCS 38,* 93). The influence of dietary fatty acids and sterols on atherosclerosis in the avian abdominal aorta has been reported. In chickens, atherosclerotic severity tended to be greatest in the group given fatty acids and least on the sterol-containing diet (Fisher et al., *Proc. Soc. Exptl. Biol. Med. 106,* 61). Another study in cockerels showed that there is a relationship between serum free cholesterol not combined upon incubation of serum and development of atheroma in young cockerels

(Caldwell, *Proc. Soc. Exptl. Biol. Med. 106,* 893). Cockerels (cholesterol-fed) subjected to a conditioned emotional stress reaction compared with nonstressed controls gave no indication that the stress influenced weight gain, blood lipid level, or the development of atherosclerosis (Joyner et al., *Circulation Res. 9,* 69). The effects of androsterone and testosterone propionate on atheroma and plasma lipid shifts induced by cholesterol feeding in cockerels were compared. Both compounds produced significant inhibition of coronary arterial atheroma. Aortic atherogenesis was not affected. Effects on plasma lipids, testicular weight, and comb growth were described (Cook, *Proc. Soc. Exptl. Biol. Med. 105,* 586).

Rabbit antibodies were produced in response to cholesterol-induced chicken atheroma and chicken normal intima. Properties of these antisera were studied by complement fixation and described by isofixation curves (Bahler and Butler, *Proc. Soc. Exptl. Biol. Med. 106,* 383).

The effect of intravenous injection of oxidized methyl esters of unsaturated fatty acids on chick encephalomalacia has been reported. The data suggest that nutritional encephalomalacia may be initiated by accumulation of sufficient, though still immeasurable, levels of lipohydroperoxide *in vivo* (Nishida et al., *Proc. Soc. Exptl. Biol. Med. 105,* 308).

LIPIDS IN MICROORGANISMS, PLANTS, AND INSECTS

Ricinoleic and oleic acid derivatives were screened for their antimicrobial activity, under optimum growing-conditions, against several species of bacteria, yeasts, and molds. Several ricinoleic acid derivatives and petroselenic (iso-oleic) acid exhibited considerable activity; in fact, their activity against some micro-organisms was comparable to sorbic and 10-undecenoic acid, known antimocrobial agents, as indicated by this test (Novak et al., *JAOCS, 38,* 321). Two dimensional chromatograms of the aqueous acid hydrolyzate of phospholipides obtained from *Pseudomonas aeruginosa* resting cells indicated the presence of at least 13 ninhydrin positive compounds. There was a correlation between the uptake of DL-alanine-1-C^{14}, DL-leucine-1-C^{14}, and DL-phenylalanine-3-C^{14} by phospholipides of the resting cells and utilization of these amino acids by the cell as indicatd by manometric studies (Silberman and Gaby, *J. Lipid Research 2,* 172). Determination was made of the types and quantities of fatty acids released from lard, tallow, corn oil and coconut oil by lipases from psychrophilic strains of *Pseudomonas.* Much of the palmitic acid in lard was found to be esterified at the *beta* position (Alford et al., *J. Food Sci. 26,* 234).

Changes in the lipids of soybeans brought about by *Rhizopus oryzate* during the production of tempeh were studied. The mold possesses strong lipase activity and caused the hydrolysis of over one-third of the neutral fat of the soybean during three-day fermentation. The fatty acid composition of soybean tempeh was compared with that of cooked soybeans. Except for the depletion of some 40% of the linolenic acid in the later stages of the fermentation, there apparently was no preferential utilization of any fatty acid (Wagenknecht et al., *J. Food Sci. 26,* 373). *Torulopsis utilis,* a lipid-rich yeast known to contain large amounts of linoleate, has been found to form this acid efficiently from oleate. The paper also deals with the nature of the processes which introduce the second double bond of the diene system (Yuan and Bloch, *J. Biol. Chem. 236,* 1277).

The cellular lipids of *Ankistrodesmus braunii,* a green alga, which was grown to stationary phase on a chemically defined media, were analyzed and described (Williams and McMillan, *Science 133,* 459). Two-dimensional paper chromatography was used to separate the chloroplast pigments of marine algae belonging to several classes. A variety of chlorophylls, pheophytins, carotenes, xanthophylls, and other pigments were separated (Jeffrey, *Biochem. J. 80,* 336). The various carotenoid pigments of the flagellated alga, *Euglena gracilis* have been studied and measured (Krinsky and Goldsmith, *Arch. Biochem. Biophys. 91,* 271).

The rates of C^{14} incorporation into lipids and their components during steady-state photosynthesis in $C^{14}O_2$ by *Chlorella pyrenoidasa* reveal the metabolic importance of the galactolipids and phosphatidyl glycerol. The most rapidly labeled were galactosyl glycerol, digalactosyl glycerol, diglycerophosphate, sulfoglycosyl glycerol, and glycerophosphorylinositol. Labeling of the triglycerides proceeded more slowly than that of the surfactant lipids. The fatty acids of phosphatidylglycerol and the galactosyl diglycerides were more readily labeled than any other esterified fatty acids (Ferrarri and Benson, *Arch. Biochem. Biophys. 93,* 185)

Soybean plants were cultured (a) in presence of $C^{14}O_2$ and (b) with H^3OH in the nutrient medium. The oils were extracted from the seeds at various stages of maturation and preparations containing randomly C^{14}- and H^3-labeled fatty acid esters were obtained by transesterification. The labeled fatty acid ester preparations were fractionated in a 200-tube automatic countercurrent distribution apparatus. The products obtained included labeled linoleate, linolenate, 2:1 oleate-palmitate mixture, stearate, and concentrates of C_{20} and higher acid esters (Dutton et al., *J. Lipid Research 2,* 63). The behaviour during maturation of the seeds of Raja and Rocket flax and Indian safflower were compared and the points of difference and similarity were reported. Lipid synthesis was studied by incubating to 2-mm squares of tissue in water containing acetate-1-C^{14} (Sims et al., *JAOCS 38,* 273). Changes that occur in the fatty acid composition of the oil in flax and safflower seed during the seed-ripening period have been measured and described (Sims et al., *JAOCS 38,* 276). Evidence is presented that the cyclopropenoid acids, sterculic and malvalic acids, occur together in seed oils of *Sterculia foetida, Hibiscus syriacus,* and *Lavatera trimestris.* The authors discuss the biogenetic problem presented by the two acids occurring in the same oils together with their dihydro derivatives. Malvalic and sterculic acids differ in chain length by a single carbon atom. A scheme for the biosynthesis of cyclopropenoid acids is presented which involves formation of the cyclopropene ring by addition of a 1-carbon fragment (Wilson et al., *JAOCS 38,* 696). The presence of stigmasterol in tomato fruits was demonstrated. Labeled mevalonic acid was incorporated into this sterol, while sodium acetate was not. The identity of the isolated product was established by rigorous purification to constant specific activity, melting point determinations, and infrared spectrum (Bennett et al., *Science 134,* 671).

From a study of germinating peanut seeds it was concluded that saturated acids are metabolized at a greater rate than that of unsaturated acids during germination; some acids are not metabolized during the initial stages of germination; and fatty acids are converted into soluble and insoluble glucide during the course of germination (Rabari et al., *JAOCS 38,* 4). Changes in free fatty acid content of various tissues of wheat germinated for eight days were followed by a histochemical method using the base of acridine orange in xylol. The scutellum was indicated to be the main site of lipase activity. As the germination period increased, zones farther from the scutellum tissue gradually increased in fatty acids until after about eight days, measurable quantities of free fatty acids were present throughout the kernel (Pomeranz and Shellenberger, *Cereal Chem. 38,* 122).

The *alpha*-tocopherol content of leaves of a variety of different plants was measured. Variables such as amounts of tocopherol at the apex or base of leaves, amounts in leaves during spring and fall, dying and fallen leaves *vs.* green leaves, etc., were studied. The authors conclude that the *alpha*-tocopherol contents of leaves is inversely related to growth rate (Booth and Hobson-Frohok, *J. Sci. Food Agr. 12,* 251). Occurrence and inheritance of linolenic and linoleic acids in soybean seeds has been studied. The range of linolenic acid from 251 field-grown varieties and introductions of soybeans was 4.89 to 9.28% and that of linoleic, 35.8 to 53.4%. Crosses of varieties and introductions "high" and "low" in linolenic acid indicated that inheritance of both acids was quantitative rather than qualitative. Transgressive segregation, particularly to low values, was observed occasionally. However, 3.35% was the lowest linolenic acid value observed. Environmental effects markedly influenced the quantity of both acids (White et al., *JAOCS 38,* 113).

Changes taking place in the fatty acids of the lipids of edible peas on storage at −17.8C in the raw and in enzyme-inactivated condition were studied. All of the fatty acids in the phospholipide fraction showed large losses in the raw as contrasted with the enzyme-inactivated samples. The neutral fats in the raw sample showed lesser amounts of all the unsaturated fatty acids as contrasted with those extracted from enzyme-inactivated material (Lee and Mattick, *J. Food Sci. 26,* 273). The fatty acids in the extracted crude lipid of spinach were studied to determine changes during storage at −17.8C in the blanched and untreated condition. The total free fatty acids increased in the unblanched samples during storage. Palmitic acid concentration increased during storage, whereas that of the longer-chain fatty acid, particularly linolenic acid, decreased. A fatty acid containing 17 carbon atoms, n-heptadecanoic acid, was present in fair quantity (Mattick and Lee, *J. Food Sci. 26,* 356).

Isolation and characterization of the glycolipids, monogalactosyl- and digalactosylglycerol lipids and cerebrosides from wheat flour are reported. A comparative study was made of the composition of the lipid mixtures obtained from bleached and unbleached wheat flours (Carter et al., *J. Lipid Research 2,* 215, 223). The acetone-soluble lipids of red clover were found to consist largely of the galactolipides,

galactosyl-1-glycerol and digalactosyl-1-glycerol linolenate. The fatty acids from the galactolipids were shown to consist of 95.8% linolenic, 1.9% linoleic, and 2.3% palmitic acid (Weenink, *J. Sci. Food Agr. 12,* 34). Lipids of grasses and other plants soluble in acetone at 0° were studied by determining yield of fatty acids following saponification and by diffusion in light petroleum through a rubber membrane. Results of the studies showed that most of the fatty acids were combined in a nondialyzable form as galactolipids and that glycerides do not occur to any appreciable extent in leaves but are replaced by galactolipids. Fatty acid analysis showed that the dialyzable lipids contained palmitic, stearic, C_{20} and higher saturated acids, oleic, linoleic, and linolenic acids while the nondialyzable lipids contained mostly linolenic with small amounts of palmitic and linoleic acids (Shorland, *J. Sci. Food Agr. 12,* 39). Low temperature extraction of wheat flour lipids and gradient elution from silicic acid is described (Wren and Elliston, *Chem. and Ind. 1961,* 80).

The isolation and properties of sulfoglycosyl glycerol, a sulfolipid found in photosynthetic tissues, was reported on material isolated from deacylation products of alfalfa leaf lipides by anion exchange resin chromatography (Lepage et al., *J. Am. Chem. Soc. 83,* 157).

Grujo oil (sulfur olive oil) has been shown to contain several classes of oxygenated fatty acids amounting to more than 10% of the total oil. Epoxy acids amount to 3.6% of the fatty acids of this oil and monohydroxy acids constitute a further 6%. The major epoxy acid has been proven to be *trans*-9:10-epoxystearic acid (Vioque et al., *JAOCS 38,* 489). Two triterpenoid acids have been isolated from orujo oil (sulfur olive oil) and characterized in part (Vioque and Morris, *JAOCS 38,* 485). Fluoro-oleic acid from the seeds of *D. toxicarium* (Sierra Leone) has been isolated in pure form and its structure proved to be *omega*-fluoro-*cis*-9-octadecenoic acid. Another fluoro-fatty acid of much higher melting point was isolated in small amounts. Both acids were found to significantly inhibit citrate metabolism in guinea pig kidney particles (Peters et al., *Biochem. J. 77,* 17).

Effects of oleic and other fatty acids on the growth rate of an insect parasitoid, *Agria Affinis* (Fall.) (Diptera: Sarcophagidae), was reported. Oleic acid had the greatest effect. Linoleic, linolenic. palmitoleic, and arachidonic acid apparently are not required (House and Barlow, *J. Nutrition 72,* 409). The phospholipides of three developmental stages of the blowfly, *Phormia regina* (Meigen), have been examined by silicic acid chromatography. Ethanolamine phospholipides comprise 60% or more of the total phospholipide of each growth stage analyzed, and lipids of the lecithin type less than 25%. In this respect the blowfly differs markedly from vertebrates (Bieber et al., *J. Biol. Chem. 236,* 2590). The role of intestinal symbionts in the sterol metabolism of the German cockroach, *Blatella germanica* has been investigated. The principal sterol synthesized from acetate under nonaseptic conditions has been shown to be 22-dehydrocholesterol. Evidence is presented for the derivation of this material from ergosterol synthesized by the intestinal flora of the insect (Clayton, *J. Biol. Chem. 235,* 3421).

BOOK REVIEW

Several interesting reviews on various phases of the fats and oils field have appeared during the last year. Asselineau and Lederer reviewed "The Chemistry of Lipids", (*Ann. Rev. Biochem. 30,* 71-92). A symposium dealt with tall oil industry and tall oil products (*JAOCS, 38,* March, 31–37). Langdon and Phillips prepared a review on lipid metabolism (*Ann. Rev. Biochem. 30,* 189–212). A symposium on lipid metabolism held in Atlantic City was published (*Federation Proc., 20,* 921–955). A symposium held in England on the relative values of animal and vegetable fats in nutrition was also published (*Proc. Nutrition Soc., 20,* 138–173). Lectures given during a short course on newer methods of lipid analysis conducted by the American Oil Chemists' Society appeared (*JAOCS, 38,* 534–588, 625–668, 708–736).

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

"Castor Seed and Its Products." Indian Central Oilseeds Committee, Hyderabad (India), 1960.

"Soybean Blue Book." American Soybean Association, Hudson, Inc., 160 pp., 1961.

Rao, B. Y. "Literature Review on Oils and Fats 1958." Central Food Technological Research Institute, Mysore, *India. 96 pp., 1960.*

Devine, J. and P. N. Williams, Ed. "The Chemistry and Technology of Edible Oils and Fats." Pergamon Press, Inc., 154 pp., 1961.

Markley, K. S., Ed., "Fatty Acids, Their Chemistry, Properties, Production and Uses." Second completely revised and augmented edition. Interscience Publishers, Inc., Part 1, 714 pp., 1960, and Part 2, 715–1485 pp., 1961.

King, H. K., "The Chemistry of Lipids in Health and Disease." University of Liverpool Publication, C. C. Thomas. 120 pp., 1960.

Bloch, Konrad, Ed., "Lipid Metabolism." John Wiley and Sons, Inc., New York, 411 pp., 1960.

Mead, J. F. and D. R. Howton, "Radioisotope Studies of Fatty Acid Metabolism." Pergamon Press, 141 pp., 1960.

Heftmann, E., and E. Mosettig, "Biochemistry of Steroids." Reinhold Publishing Corporation, 231 pp., 1960.

Lundberg, W. O., Ed., "Autoxidation and Antioxidants." Vol. 1., Interscience Publishers, Inc., 464 pp., 1961.

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"Detergents and Emulsifiers." Fifth edition, John W. McCutcheon, Inc.

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Bishop, E., Ed., and Translator, "Technology of Nonmetallic Coatings" by A. Ya, Drinberg, E. S. Gurevich and A. V. Tikhomirov, Pergamon Press, 531 pp., 1960.

Well, F. V., "Soap, Perfumery and Cosmetics Year Book and Buyers' Guide." 18th edition, United Trade Press, Ltd., London, 327 pp., 1960.

Heftmann, E., Ed., "Chromatography." Reinhold Publishing Corporation, 753 pp., 1961.

Lederer, M., Ed., "Chromatographic Reviews." Vol. III, through 1960. D. Van Nostrand Co., 187 pp., 1961.

Scott, R. P., Ed., "Gas Chromatography—1960." Butterworth, Inc., 466 pp., 1960.

Noebels, H. J., Wa., R. F., and Brenner, N., Ed., "Gas Chromatography." Report of a symposium held under the auspices of the Analysis Instrumental Division of the Instrument Society of America. Academic Press, 463 pp., 1961.

Clark, George L., Ed., "The Encyclopedia of Spectroscopy." Reinhold Publishing Corporation, 787 pp., 1960.

"Infrared Spectroscopy; Its Use as an Analytical Tool in the Fields of Paints and Coatings." Chicago Society for Paint Technology, Chicago, 160 pp., 1961.