Report of the Literature Review Committee

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

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Deterioration of Fatty Materials

During production, processing, storage, and culinary treatment, food fats may deteriorate through autoxidation, polymerization, lipolysis, pyrolysis, and uptake of foreign odors and flavors. In addition to the obvious organoleptic degrading, there is great concern about how some of these changes affect the nutritive value of the fat and the vitamins in the diet, also whether the fat becomes deleterious or toxic. These aspects all enter into the study of deterioration of fats. For a brief review of such read the publication of Brown (*Nutrition Revs. 17*, 321).

Recent works from many laboratories have described the deleterious effects of oxidized and/or polymerized fats. A polymer fraction from thermally oxidized (48 hrs. at 200°C.) corn oil, when fed at 30% of the diet to rats, was fatal to all the animals within seven days although feeding fresh corn oil with this fraction considerably counteracted the effect (Perkins and Kummerow, *J. Nutrition, 68*, 101; *J. Am. Oil Chemists' Soc. 36*, 371). In similar work where lard and cottonseed oil were oxidized 210 hrs. at 100°C. the polymer fraction from the oil was more deleterious than that from lard (Kaunitz *et al., J. Am. Oil Chemists' Soc., 36*, 611). The criteria in this work included growth, water intake, organ weights, total liver lipids, and serum and liver cholesterol. When heated 100 min. at 610°F. in vacuum, soybean oil, which polymerizes, became deleterious to rats at a 15% dietary level whereas, under the same conditions, cottonseed oil or lard were much milder (Alfin-Slater *et al., ibid.*, 638). Decrease in growth and interference with reproductive performance in the female rats were the criteria. In a comparison of peanut, cottonseed, and corn oils, oxidized with air 24 hrs. at 200°C., at a 15% dietary level with rats, the animals on the corn oil did not grow as well as the others, and they lost considerable hair (Dangouman *et al., Rev. franc. corps gras 5*, 613). Pure methyl oleate oxidized to the hydroperoxide was rapidly fatal to mice at as low as a 1.5% level in the diet (Khan, *Pakistan J. Biol. Sci., 1*, 119). Autopsies of the mice revealed enlarged hearts and fatty infiltrations. Studies in Japan showed details of the development of like severe toxicities from fish oils when autoxidized and/or heated (*Matsuo, Seikagaku, 29*, 769, 773, 807, 812, 816, 885; *Eiyô to Shokuryô 10*, 255; Kaneda *et al., ibid., 7, 188*).

Mortality and severity of hydropericardium resulting from feeding autoxidized fats to chickens may be somewhat relieved by the inclusion of Santaquin (a commercial antioxidant) or α-tocopherol in the diet (Machlin *et al., Poultry Sci., 38*, 579; *J. Nutrition, 67*, 333). Presence of antioxidants in those dietary fats most susceptible to autoxidation helped to sustain reproductive capabilities in female rats (Draper *et al., Brit. J. Nutrition, 12*, 89) and protected rats from liver damage (Valberg *et al., Can. J. Biochem. and Physiol., 37*, 493).

An hydrogenated cottonseed oil from a commercial deep-fry fryer under actual production conditions for as long as 24 days showed no deleterious effects on rats even when fed as high as a 20% level of the diet (Keane *et al., J. Nutrition, 68*, 57).

TESTS FOR DETERIORATION AND ANTIOXIDANTS. The iodometric method for the determination of active oxygen in fats was studied in several laboratories. Improvements recommended by Heaton and Uri (*J. Sci. Food and Agr., 9*, 781) were related to de-aeration, which would reduce errors caused by induced oxidation, and to the development of a micro-spectrophotometric method for samples of very low peroxide concentrations. For very rancid corn germ oil, del Pazo and Alemany (*Galenica Acta, 11*, No. 3, 7) recommended using a 2:1 mixture of acetic acid and chloroform as the solvent in the method; for lard samples Drozdov and Materanskaya (*Khim. Nauka i Prom., 4*, 133) preferred the same solvent, also the addition of some mineral acid.

Various determinations of carbonyl substances served to evaluate spoilage changes in fats. Berry and McKerrigan (*J. Sci. Food Agr., 9*, 693) favored an empirical method for the determination of the volatile carbonyl compounds in the form of a "carbonyl index" because it was more closely related to oxidative rancidity than either peroxide values or the total carbonyl values. The Lea method of this type was considered unsuitable for application to autoxidizing milk fat because the carbonyls developing in this fat differ from those of other common fats (Tamsma and Powell, *J. Agr. Food Chem., 7*, 643). Lower aldehydes present in rancid oils yield a polarographic wave with the half-wave potential of −1.9 volts. which has been used in studying the rancidity of fish oils (Kikuchi and Okada, *Nippon Suisangaku, 23*, 41, 177, 181). In an application of the determination of rancidity through the reaction of carbonyls with diphenylcarbazide, color scales were developed to rate the intensity of rancidity in butter and lard (Sedlacek and Rybin, *Fette, Seifen, und Anstrichmittel, 61*, 134). Work on the thiobarbituric acid value method involved development of a simple spectrophotometric method (Schmidt, *ibid.*, 127, 881), improvement with use of isoamyl acetate and ethanol as solvent (Dzikowski, *Roczniki Panstwowego Zakladu Hig. 9*, 461), and its application to the evaluation of rancidity

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in soup preparations (Sedlacek, *Z. Lebensm.-Untersuch. und -Forsch., 109,* 480), fishery products (Ryan and Stansby, *Com. Fisheries Rev., 21,* No. 1, 21), lard and walnut kernels (Sedlacek, Nahrung, *2,* 655). Wode (*TVF, 29,* 141) considered the aldehyde value determination most suitable for evaluating deodorized fats because it gave a good indication of the taste-retaining ability.

The lowering of the aniline point of lard obtained by the presence of free fatty acids, ketones, peroxides, and epoxides has been plotted as a basis for using the aniline point for evaluating the deterioration of lards (Wurziger and Lindemann, *Fette, Seifen, und Anstrichmittel 61,* 257).

In two comprehensive surveys on the analytical detection of antioxidants the reviewers have also listed the countries that approve each (Seher, *Fette, Seifen, und Anstrichmittel, 60,* 1144; Solomon, *Rev. franc. corps gras, 6,* 627). Seher (*Fette, Seifen, und Anstrichmittel, 61,* 345) developed a chromatographic method for scanning fats for antioxidants and tabulated color-development characteristics of 24 antioxidants as basic information for the method. A scheme developed for the determination of ascorbic acid, gallates, butylated hydroxyanisole, and butylated hydroxy toluene was based on segregation by selective solvents and by adsorption on alumina, and measurement by ultraviolet spectrophotometry (Wolff, *Rev. franc. corps gras., 6,* 630). The four American food-approved antioxidants have been completely separated by unidimensional chromatography (Dehority, *J. Chromatography, 2,* 384). A collaborative study of the methods of Anglin *et al.* for the quantitative determination of the four commonly used antioxidants found it unsuitable for official adoption (Conroy *et al., J. Assoc. Offic. Agr. Chemists, 42,* 358). A rapid, direct spectrophotometric determination of butylated hydroxy anisole, in lard, in the absence of other antioxidant, was devised (Hansen *et al., J. Am. Oil Chemists' Soc., 36,* 193). Reaction at 50–60° in the presence of a small amount of alkali accelerated the indophenol reaction method for the determination of butylated hydroxytoluene (Wurziger and Chandra, *Die Fleischwirtschaft, 11,* 926). Rapid spot tests have been designed to screen imported lards for those antioxidants which are forbidden in Germany (Roos, *ibid.,* 667). Complex formation with mercuric acetate served in a semimicro method for the determination of propyl gallate in lards (Sedlacek, *Z. Lebensm.-Untersuch, und -Forsch,* 111, 108).

Gordon and Machlin (*Poultry Sci., 38,* 1463) evaluated the efficiency of antioxidants biologically based on Vitamin A protection. The samples with low levels of Vitamin A were administered to vitamin A-depleted chicks. The criteria were growth and survival.

DETERIORATION MECHANISM. The recent reviews in the field treated the following: the subject in general (Russell, *J. Chem. Ed., 30,* 111), the mechanism of peroxide formation, decomposition, rates, and analysis (Paquot, *Actions chim. et biol. radiations No. 4,* 3), and the effect of light radiation on autoxidation of fats (Kaufmann and Vogelmann, *Fette, Seifen, und Anstrichmittel, 61,* 206; *Farbenchemiker,* 61, 6).

Patterns formed by the analytic characteristics of autoxidizing fats were developed and interpreted for practical and fundamental aspects. Curves of the iodine and peroxide value of ghee, lard, refined and crude peanut oils, and sesame oil were interpreted from the standpoint of rates of autoxidation, induction period, character at rancidity, stability, etc. (Kartha, *J. Sci. Res., India, 17B,* 237). In similar work peroxide value, carbonyl content, and epihydric aldehyde content were plotted to show the relative rates of spoilage of tallow and lard (Purenas *et al., Kauno Polotech Inst. Darbai, 9,* 19). Rates of peroxide development and decomposition at various temperatures were determined for saury, sardine, whale, linseed, sesame, and camella oils in a comparison of the stability of saury oil with other comparable oils (Watanabe and Toyama, *Mem. Fac. Eng. Nagoya Univ. 10,* 95). Peroxide development curves obtained from unsaturated fatty acids exhibited a straight-line relationship between the logarithms of the time of maximum peroxide content and storage temperature, also between the logarithm of time of maximum rate of peroxide formation and temperature of storage (Pietrzyk, Roczniki, *Tech. i Chem. Zywnosci, 3,* 77). In similar work on oleic acid at 20–60°C. the temperature at which the tests were run caused considerable difference in the character of the curves (Drozdov and Materanska, *Nauk Doklady Vysshei Shkoly. Khim. i Khim. Tekh. 1958,* No. 3, 536).

More intensive work of the same type pertained to the reaction occurring in the process and the products formed. Analysis of the products developed during oxidation of methyl oleate showed that a monohydroperoxide formed initially and that some polymerization took place on autoxidation at 60–100°C. (Khan, *Pakistan J. Sci. Res., 10,* No. 4, 149). The same progress of reaction was demonstrated with methyl linoleate in another investigation in which rate constants were also determined and the hydroperoxide was identified as the *cis,trans*-form (Kern and Dulog, *Mikromol. Chem., 29,* 199). In confirmation of the latter it was shown that there were two positional isomers (9- and 13-) for this *cis-trans* product, indicating two possibilities of attack by oxygen on the two 3-C segments in the 5-C system of methyl linoleate containing mobile π electrons (Khan, *Can. J. Chem., 37,* 1029). The oxidation mechanism of the oxidation of petroselenates was found to be fundamentally the same as for the oleates (Gold and Skellon, *J. Applied Chem., 9,* 389).

The carbonyls which form during autoxidation were analyzed for composition. The steam-volatile types found in autoxidized fats by Gaddis *et al.* (*Food Res., 24,* 283) included saturated aldehydes, methyl ketones, 2-en-1-als, and 2,4-dien-1-als. Generally, saturated carbonyls increased in unheated fats, and unsaturated carbonyls increased when the fat was heated. A thorough analysis of the steam-volatile monocarbonyls in rancid freezer-stored pork fat showed the presence of C_1, C_2, C_3, C_6, and C_9 *n*-alkanals; three unknown carbonyls; C_7 C_8, C_{10}, and C_{11} alk-2-enals; and C_7, C_9, C_{10}, and C_{11} alk-2-,4-dienals (Gaddis and Ellis, *ibid.,* 392). Here hexanal and deca-2,4-dienal were the dominant compounds present. The same compounds plus 2-octenal were the principal carbonyl compounds formed during the oxidation of ammonium linolcate (Badings, *J. Am. Oil Chemists' Soc., 36,* 648). It was believed that the oxidation taste reversal in soybean oil arises from 2,4-heptadienal and/or 2,4-octadienal (von Pezold, *Fette, Seifen, und Antsrichmittel, 61,* 1018). The methyl ketones with odd numbers of carbon atoms in the *n*-alkyl group from C_3 through C_{15} were identified in the steam volatiles of butterfat and were discussed in terms of origin of tastes and flavors (Patton and Tharp, *J. Dairy Sci., 42,* 49). In commercial evaporated milk the casein and lactose may also serve as precursors of some of these carbonyl compounds (Dutra *et al., Food Res., 24,* 688). Many obvious volatile carbonyl compounds were also isolated from fish and fish oils (Onoe, *Nippon Kagaku Zaashi 79,* 801; Toyama *et al., Mem. Fac. Eng. Nagya Univ. 9,* 125; Mangan, *Com. Fisheries Rev. 21,* No. 7, 21). One group of these investigators (Toyama *et al.*) did not think that the characteristic odor of fish oil could be attributed to these compounds. The unsaponifiables from rancid butcher's fat contained components with two conjugated double bonds which were believed to have arisen from α- and β-carbonyl compounds formed as a result of the oxidation of unsaturated fatty acids (Mirna, *Fette, Seifen, und Anstrichmittel, 61,* 1163).

STABILITY OF FATTY MATERIALS. The free fatty acid formation (lipolysis) in the course of storage at −10 and 10°C. of five species of commercially frozen fish has been determined as fundamental data on the stability of the fish during storage (Dyer *et al., Fisheries Res. Board Can. Progr. Repts. Atlantic Coast Stas., No. 71,* 17). In similar work on cod stored in crushed ice, hydrolysis of fat, phospholipids, and cholesterol esters were determined over an eight-week period (Lovern *et al., J. Sci. Food Agr., 10,* 327).

The amount of hydrolytic decomposition during the vacuum deodorization of fats at 180° appeared to be a function of the free fatty acid content, and it also increased in direct proportion to the absolute pressure (Sarkadi, *J. Am. Oil Chemists' Soc., 36, 143*).

Five drums of solvent-extracted, refined-in-miscella, and three drums of screw-pressed cottonseed oils were held 28 months under different types of storage and examined subjectively and objectively (Baumann, *ibid.,* 28). There was no relationship between graders' judgments of the oils for "off flavor and odor" and the tests for peroxide value and AOM stability.

Uncured cooked meat, which contains ferric denatured globin hemochromogen, showed high thiobarbituric acid values shortly after cooking whereas no such increase was apparent in cured meat in which the pigment was present as pink ferrous nitric oxide hemochromogen (Younathan and Watts, *Food Res., 24,* 728). Fresh blood catalyzed the oxidation of unsaturated fats greatest at pH 7.0; with boiled blood there was no such optimum pH, and the catalytic activity was decreased to two-thirds (Shimizu and Fukuhara, *Nippon Suisangaku Kaishi, 24,* 760). In a study of the kinetics of the oxidation of unsaturated fatty acids catalyzed by hematin compounds the rate of oxidation increased with increased hemoglobin concentration to a maximum rate at 7.5×10^{-5} moles (Amier and Tappel, *J. Am. Oil Chemists' Soc., 36,* 8). In this work the rate constants for reactions were determined for several hematin compounds. The products developed in this reaction in the absence of oxygen were principally oxirane, hydroxyl, and carbonyl compounds;

in addition, cleavage of the carbon chain, loss of conjugated double bonds, and polymerization also took place (*ibid.*, 12).

The autoxidation reactions of unsaturated fatty acids in the presence of chlorophyll was explained on the basis of an oxidizing chlorophyll-oxygen complex forming at an α-methyl group adjacent to the double bond under light sensitization (Khan, *Pakistan J. Sci. Ind. Res. 1,* 258). In an aqueous carotene-sodium linoleate system a minimum of carotene destruction occurred at the critical micelle concentration of sodium linoleate; below this concentration the oxidation of carotene did not appear to be coupled to that of linoleate; and above the critical micelle concentration the rate of oxidation of both carotene and linoleate was increased by raising the level of either (Lohmar and Tookey, *Arch. Biochem. and Biophys., 84,* 297). In a study of the stability of carotene in some oils, either crude or refined soybean oils were better solvents than rapeseed or peanut oils (Maciag, *Acta, Polon. Pharm., 15,* 447). Fox and Michelsen (*J. Nutr., 67,* 123) have recommended that the ferric phosphate, copper sulfate, and manganese sulfate ingredients of dietary salts of synthetic experimental rations be replaced by the citrates of the metals to reduce autoxidation. In certain emulsions, autoxidation was dependent on heavy metals in the oil phase and was inhibited by complexing the metal into the water phase with alanine (Loncin *et al., Fette, Seifen, und Anstrichmittel, 61, 1055*). The storage life of dry milk was enhanced three to four times in this way. The unsaturated fatty acids in their urea adducts were quite stable; this stability was reduced at lower pressures and disappeared at 80°C. (Makita, *Rev. Phys. Chem. Japan, 28,* 31).

ANTIOXIDANTS. Several studies were on natural antioxidants. The phospholipids extracted from sunflower and soybean oils stabilized raw-pressed vegetable oils more efficiently than expeller-produced oils, possibly because cold-pressed oils were phospholipid deficient (Rzhekhin and Preobrazhenskaya, *Masloboino Zhirovaya Prom., 25,* No. 7, 20). Among the natural antioxidants of butter, cephalin was best when tested individually, but in combination with tocopherol the efficiency was considerably increased (Haab, *Milchwissenschaft, 14,* 16). Among the seven tocopherols the δ-, γ-, and η-methylated forms were most efficient for protecting carotene in mineral oil solution; the δ-, γ-, η-, and β-forms were best for lard and methyl linoleate; and the δ-form most efficient for lard esters exposed to light (Lea and Word, *J. Sci. Food Agr., 10,* 537). In hydrogenated soybean oil the presence of residual hydrogenated catalyst markedly increased the oxidation of oil and the destruction of tocopherol (Evans *et al., J. Am. Oil Chemists' Soc., 36,* 73). This effect was eliminated by the addition of a small amount of citric acid. In a study of tocopherol oxidation in soybean oil it was observed that partial removal of the tocopherols improved the stability of the oil and that in soybean oils freed of natural antioxidant none of the food antioxidants were effective stabilizers (Frankel *et al., Fette, Seifen, und Anstrichmittel, 61,* 1036). Small amounts of the antioxidant, α-tocopherol, were equally effective in lard in either one complete dose or in successive portions during oxidation; however with large amounts a single complete dose caused inversion of activity whereas this large amount in successive portions was more effective than a single optimal dose (Heiman and von Pezold, *Z. Lebensm.-Untersuch. und. -Forsch., 111,* 1). The butterfat produced from the milk of cattle on tocopherol-poor diets was considerably improved by supplementing the cows' rations with wheat germ; but Vitamin E preparation supplements were ineffective. (Sukhnova, *Trudy Vsesoyuz. Sel'skokhoz. Inst. Zaochn. Obrazovaniya, 1957,* No. 1, 206). A greater antioxidant effect from the unsaponifiable fraction of rapeseed oil than that of sunflower oil was attributed to greater tocopherol content (Täufel *et al., Nahrung, 2,* 853). The juices of onions and garlic, particularly from the skins, exhibited antioxidant and copper-chelating properties (Lewis and Watts, *Food Res., 23,* 274). These were attributed to the flavonoid compounds present. Among flavonoid compounds tested for inhibition of the copper-catalyzed oxidation of ascorbic acid through chelation, quercitin was effective and rutin poor; catechol and dehydroquercetin formed no chelates (Heimann and Heinrich, *Fette, Seifen, und Anstrichmittel, 61,* 1024).

Some materials were tested for antioxidant activity in various substances. Chlorpromazine in small concentrations inhibited formation of lipid peroxides in liver and brain homogenates (Bernheim, *Proc. Exptl. Biol. Med., 102,* 660). Phosphates, green-tea extracts, trihydroxybutyrophenone, and tomato soup in combination with ascorbic acid were effective antioxidants for irradiated cooked pork (Tarladgis, *Food Tech., 13,* 635). Among distilled fractions of smoke components the most antioxygenic portion distilled at 119–126°C. and was composed of methyl esters of pyrogallol homologs (Kurko, *Meat Ind. U.S.S.R., 30,* No. 3, 19). An antioxidant combination of ascorbic acid and yellow flavones was very effective for stabilizing butter and margarine (Akkerman, *Izvest. Vysshikh Ucheb. Zavedenii Pishchevaya Tekh., 1959,* No. 2, 51). A combination of tannin, ascorbic acid, and Vitamin E was recommended for the stabilization of sunflower oil under conditions of prolonged heating (Fam-Yung and Varnas, *Trudy Odessk. Tekhnol. Pishchevoi i Kholodil. Prom., 9,* No. 1, 43). Dipping fresh fish in 1% butylated hydroxy anisole emulsion improved their storability (*Liljemark et al., Fette, Seifen, und Anstrichmittel 61,* 465). The stability of cooked oysters was considerably improved by cooking in a 0.1% ascorbic acid solution (Schwartz and Watts, *Com. Fisheries Rev. 21,* No. 3, 1). In other communications on the stabilization of fats, conventional antioxidants were evaluated for use in sunflower and cottonseed oils (Lebedeva, *Masloboino-Zhirovaya Prom. 25,* No. 7, 24), animal fats, dried milk, and baked goods Tollenaar, *Tluszcze i Srodki Pioarce 2,* 273), lard (Knorre *et al., Zhur. Priklad. Khim., 32,* 1359), margarine (Saito *et al., Eiyô to Shokuryô, 7,* 128), and butter (Kotova, *Izvest. Vysshikh Ucheb. Zavedenii, Pishchevaya Tekh., 1958,* No. 5, 53), *Zalashko, Molochnaya Prom., 20,* No. 2, 25). Reviews, lectures, and general discussion on antioxidants contained information on their uses in foods in general (Dugan, *Am. Perfumer Aromat., 73,* No. 2, 47; Lea, *J. Sci. Food Agr., 9,* 622; Stuckey, *Am. Perfumer Aromat., 73,* No. 2, 35), in emulsions (Thorvik, *Medd. Norsk Farm. Selskap, 20,* 147), in pharmacy (Soos, "*Osterr. Apoth. Ztg. 10,* 547; Dal Brollo *et al., Farmaco, Pavia, Ed. prat. 13,* 615; Jaminet, *ibid., 14,* 73; Marcuse, *Deut, Apoth. Ztg., 98,* 962) and in the manufacture of cosmetics (Peereboom, *Am. Perfumer Aromat., 73,* No. 2, 27; Williams, *ibid.,* 39; Privett and Lundberg, *ibid.,* 44).

Patents have been issued for the following antioxidants: a powdered mixture of butylated hydroxytoluene and anhydrous sodium lauryl alcohol sulfate (for fish) (Tsuboi and Tsuboi, *Japan, 2832,* '59), 2,2'-dihydroxy-3,5,3', 5'-tetramethyldiphenyl methane (De Benedictis, *Ger. 942,584, C123c*), an extract from yeast (Forbes *et al., Brit. 803,898*), acyclic amines (Hampson and Freeman, *Brit. 807,326*), mixtures of four conventional antioxidants (for feeds) (de Brochard *et al., Fr. 1,110,038*), and the condensate of 4-methalyloxyphenol and hydroquinone (Gleim, *U. S. 2,870,021*). Metal surfaces of containers were coated with inorganic phosphates to minimize oxidation of fat drippings (Holman, *U. S. 2,871,130*). The use of antioxidants to stabilize buffing compounds was patented (Gibson and Bogdanoff, *U. S. 2,897,075*). Reversion of hardened soybean oil was inhibited by treatment with a mild oxidizing agent in the water wash after the refining before hydrogenation (Sims *et al., U. S. 2,872,465*).

A comprehensive review of the biochemical and pharmacological aspects of antioxidants was compiled by Ward (*Chemistry and Industry, 1959,* 498). The toxicity of 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinolene was comprehensively determined on laboratory animals and cattle as basic information for use in alfalfa feed as an antioxidant to preserve Vitamin A (Wilson *et al., J. Agr. Food Chem., 7,* 203, 206). After feeding the antioxidant, α-phenylisobutyric acid, labelled with C^{14}, to rats, 78–88 and 2,3–5.6% of the activity appeared in the urine and feces, respectively (Bernhard and Thommen, *Helv. chim. Acta, 41,* 536).

EFFECT OF IONIZING RAYS. Ionizing irradiation of fats and oils caused bleaching, viscosity increase, and changes in the configuration of unsaturated groups present in the chemical structure (Koenecke, *Offic. Digest Fed. and Varnish Production Clubs, 31,* 1395; Clubb and Wills, *Biochem. J., 71,* 16P; Lück and Kühn, *Z. Lebensm.-Untersuch. und -Forsch, 110,* 430; Sedlacek, *Nehrung 2,* 547; Tarusov, *Proc. All-Union Conf. Radiation Chemistry 1st, Moscow 1957,* Pt. 4, 185). Irradiation of lard was detrimental to its digestion by the dog (Schreiber and Nasset, *J. Applied Physiol., 14,* 639; *Fed. Proc. 18,* 139).

LIPOXIDASE OXIDATION OF FATS. A stepwise oxidation of cottonseed oil with navy bean lipoxidase showed a straight-line relation for increases of peroxide value, carbonyl value, and diene conjugation (Koch *et al., J. Am. Oil Chemists' Soc., 36, 205*). This oxidized oil had a better caloric efficiency than cottonseed oil air-oxidized to the same peroxide value. Oxidation of linoleate by the lipoxidase of fish tissue was inhibited in decreasing order by hydroquinone, Vitamin C, sodium cyanide, copper sulfate, and mercuric chloride but not by sodium fluoride (Nagayama and Toyoki, *Nippon Suisangaku Kaishi, 24,* 853). The hydrolysis of soybean and corn oils with the lipase of the castor oil plant stimulated the rate of oxidation by lipoxidase (Kretovich *et al., Izvest. Vysshikh Ucheb. Zavedenii Pishchevaya Tekl. 1958,* No. 5, 23). Only a slight stimulation of the oxidation takes place with oat oil.

Nutrition and Biochemistry

FAT NUTRITION. Reviews and lectures concerning lipids in nutrition have been presented on the following topics: essential fatty acids, diet and atherosclerosis, the fat-soluble vitamins (Olson, *Am. Rev. Biochem., 28,* 467) parakeratosis and essential fatty acids (*Nutri. Revs., 17,* 43), essential fatty acid deficiency in infants (*ibid., 136*), dietary effects on human milk fatty acids (*ibid.,* 203), high-fat diets and blood changes (*ibid., 218*), nutrition and lipogenesis (*ibid., 316*), dietary fat and energy and protein metabolism in man (*ibid., 332*).

Fat nutrition in various age groups has been studied. There was no apparent relationship between diastolic blood pressures and fat contents of the diets of older women; data were reported on blood pressures and body weights (Burrill *et al., J. Am. Diet. Assoc., 35, 935*). Studies of the essential fatty acids in infant nutrition indicate that in young infants optimum caloric efficiency is obtained when linoleic acid comprises about 4% of the caloric intake (Adam *et al., J. Nutr., 66,* 555), the addition of saturated fatty acids to the diets of infants with clinical symptoms of essential fatty acid deficiency did not alleviate the condition whereas the addition of linoleic acid as trilinolein to constitute 2% of the daily caloric intake restored the skin to a normal texture within one to two weeks (Hansen *et al., ibid., 66,* 565). Fat in human milk was more completely utilized by premature infants than fat in cow's milk. Enrichment of human milk did not increase fat utilization (Ocklitz *et al., Z, Kinderheilk, 82,* 321). Studies on fat and growth during childhood (Corn *et al., ibid.,* 129, 1615) have been reported.

The identification of dietary protein and energy as affected by fat and carbohydrate was studied. Two diets furnishing equal amounts of protein and energy but differing widely in fat content were fed to male subjects. The differences in total daily energy expenditures and nitrogen balance between the two diets were insignificant (Swift *et al., J. Nutr., 68,* 281). Increases in dietary fat of lactating women were found to be accompanied by increases in milk fat as well as in lipase, esterase, and alkaline phosphatase contents of milk (KarmaMear *et al., J. Nutr., 69,* 274).

A study of the influence of dietary fat on the incorporation of fatty acids into body and egg fat of the hen suggested that in cases of dietary insufficiencies of polyenoic acids the hen selectively draws upon its body depot fat for the production of egg fat. Under normal conditions it appeared that the polyunsaturated acids not synthesized *de novo* were transferred directly from the diet (Feigenbaum *et al., Arch. Biochem. Biophys., 79,* 302). Diets containing various levels of safflower, linseed, cottonseed, corn, or soybean oils were fed to hens. The results of total fatty acid determinations on the yolk fat suggested that there was an antagonistic or inhibitory effect of linolenic acid toward incorporation of linoleic acid into yolk fat (Wheeler *et al., J. Nutr., 69,* 253). The effects of feeding malvolic and sterculic acids to laying hens on the production of pink whites in eggs was reported (Shenstone *et al., Poultry Sci., 38,* 1055). Isolation and characteristics were described for a lipophosphoprotein which apparently transports most of the plasma lipid and almost half of the plasma phosphoprotein included in egg yolks (McIndoe, *Biochem. J., 72,* 153).

The rate of growth of poultry was improved by the addition of 0.5–2.0% of sodium and potassium soaps of C_{12} to C_{22} fatty acids to the feed (*U. S. 2,899,308*). The effect of dietary fat, caloric intake, and protein level on caged layers was studied from the point of view of rate of egg production, atherogenesis, and serum cholesterol levels. Mono-beta-aminopropionitrite-fumarate (BAPN) was fed to chicks on low- and high-fat diets, with and without cholesterol supplements. The investigators concluded that the high levels of dietary fat increased the rate of internal hemorrhage due to BAPN in some way other than by increasing serum cholesterol (Barnett *et al., Poultry Sci., 38,* 589). Experiments were carried out to determine the value of tallow and the effect of protein level in all mash broiler diets based on wheat meal. Added fat did not affect growth rate but improved feed conversion. Stabilization of the fat had no effect on body weight or feed efficiency (Beilharz *et al., ibid., 38,* 519). In experiments evaluated with chicks, highly significant growth increases were obtained by increasing the vegetable oil content of purified diets, or soybean oil in the diet gave better growth rates than did hydrogenated vegetable oil or lard. A fraction of corn oil composed chiefly of glycerides was found to promote growth to approximately the same extent as the intact oil (Dam *et al., J. Nutr., 68,* 615). It was suggested by one group of investigators that soybean oil contains an organic growth-promoting and antipotic factor[s]. (Kratzer *et al., Poultry Sci., 38,* 1094). The edible tissues of chickens treated with stilbestrol were found to contain measurable amounts of estrogenic activity because of the stilbestrol treatment four to five weeks after the treatment (Umberger *et al., ibid., 38,* 118).

Lack of dietary fat in the feed of guinea pigs caused retarded growth, dermatitis, skin ulcers, loss of fur, and some mortality (Reid *et al., J. Nutr., 67,* 611). The addition of 10% of rapeseed oil meal to the diet of swine and of rats depressed the growth rate and feed efficiency in both species. (Hussar *et al., Can. J. Animal Sci., 39,* 84). Rapeseed oil meal in the diet of swine and rats significantly depressed apparent digestibility of dry matter and nitrogen in rats only. Female rats were more susceptible to rapeseed oil meal toxicity than were male rats (Hussar *et al., ibid. 39,* 96). The back fat of castrated male pigs was lower in water but higher in fat than that of non-castrated females, and the type of feed had little effect on such differences (Schon *et al. Fleischwrotschoft, 11,* 467).

Increased knowledge of the role of the essential fatty acids has been obtained from nutritional studies with rats. Young male rats received a diet enriched with sunflower oil. An increase of up to 40% in the linoleic acid content of organs and fat depots was observed. When these animals were placed on a fat-free diet, a gradual decrease in linoleic acid occurred while there was an increase in the arachidonic acid content (Nagner, *Z. Physiol. Chem., 212,* 235). Linoleyl alcohol relieved the symptoms of essential fatty acid deficiency when fed to rats. Octadecatriene and octadecadiene acted as severe skin irritants, induced fatty livers, and also caused changes in the polyunsaturated fatty acid pattern of the heart lipids in rats. Changes in the polyunsaturated fatty acid content of the heart lipids were also induced by linoleyl and linolenyl alcohols. Both these compounds stimulated growth, but the linolenyl alcohol had no effect on the deficiency symptoms (Aaes-Jørgensen *et al., J. Nutr., 67,* 413). Concentrates of polyunsaturated fatty acids from tuna oil were fractionated according to chain length and were fed to essential fatty acid-deficient rats. All the fractions except the C_{16} fraction showed marked stimulation of growth, but none relieved the symptoms of essential fatty acid deficiency (Privett *et al., ibid., 67,* 423). The fecal unsaturated fatty acids appear to be in part of an essential nature since the prevention of coprophagy in the rat hastened the production of essential fatty acid-deficiency symptoms (Barnes *et al., ibid., 68,* 121). An interrelation between the cholesterol palmitic acid to unsaturated fatty acid content of diets was established in the growing mouse and rat (Bosshardt *et al., ibid., 69,* 185). The absorption of corn and rapeseed oil fed to rats *ad libitum* was 95 and 92%, respectively (Beare *et al., Can. J. Biochem. and Physiol., 37,* 613). It was suggested that as a response to the stimulus of caloric deficiency in the tissues of rats a nonglycogenolytic, ketogenic, fat-mobilizing hormone[s] is produced (Moyes, *Biochem. J., 71,* 459). The pattern of depletion and repletion of polyunsaturated fatty acids in the serum and tissues of Cebus monkeys, which were changed from diets rich in linoleic acid to fat-free diets was reported (Portman *et al., J. Nutr., 69,* 245). The addition of lard to the diet of rats gave some protection against selenium poisoning (Romanowski *et al., Proc. S. Dak. Acad. Sci., 37,* 76).

The feeding of restricted roughage with high levels of protein concentrates produced significant declines in milk fat in lactating cows and goats. Associated with the decline in milk fat were a significant increase in the rumen propionic acid and a decrease in blood ketone bodies. The feeding of sodium acetate increased the low milk fat while sodium propionate tended to lower it further (Soest *et al., J. Dairy Sci., 42,* 1977). As the preparation of concentrate in the ration of ruminants was increased, the relative proportion of acetic acid decreased whereas that of propionic and butyric acids increased (Elliott et al., ibid., 42, 843). Compared to a ration of alfalfa, hay, and corn, a ration of ground corn or ground corn and minerals resulted in a marked decrease in rumen acetate and an increase in rumen propionate in Holstein heifers. Dissimilation of C^{14}-labelled glucose by incubated rumen contents demonstrated that rations which induced a marked change in molar proportions of rumen volatile fatty acids also induced a marked change in rumen microbial metabolism (Eusebio *et al., ibid., 42,* 692). Animal and vegetable fats prevented acute legume bloat in cattle, apparently by clearing the cardia of foam (Colvin *et al., ibid., 42,* 333). The addition of 10% brown grease, 10% hydrogenated cottonseed oil, or 5% of each to calf starters reduced feed consumption and weight gain. The major part of the reduction in growth was due to the reduction in starter intake (Miller *et al., ibid., 42,* 153).

In an attempt to determine some of the factors responsible for the poor condition and deaths of calves receiving rations containing a high content of unsaturated acids, the results of feeding corn oil-filled milk to dairy calves were studied. The animals were found to be in very poor physical condition, symptoms of anorexia and muscular involvement were observed, poor

feed utilization and unsatisfactory weight gains were observed (Adams *et al., ibid., 42,* 1552). The above studies were extended to determine the fecal characteristics and digestibility in calves receiving various filled milks. Abnormally large amounts of feces were voided by animals fed the corn oil-filled milk when the diet was prepared weekly, but the diarrhea was reduced when the diet was prepared daily or when hydrogenated codn oil was fed. Undoubtedly the toxicity symptoms were due largely to effects of the deterioration of the unsaturated fatty acids (Adams *et al., ibid., 42,* 1562). Further studies by the same group on the blood plasma tocopherol and Vitamin A levels were carried out. Low blood plasma tocopherol levels were found in calves fed corn oil-filled milk despite a relatively high tocopherol intake. Low levels of plasma tocopherol were also observed in calves receiving lard-filled milk and oxidized butter-filled milk. Vitamin A levels in plasma were also reduced during the experimental period. Oral supplementation of tocopherol increased plasma tocopherol levels (Adams *et al., ibid., 42,* 1569). The utilization of fat in milk replacers by the dairy calf has been studied. When a milk replacer composed of dried skim milk was supplemented with tallow, coconut fat grease, or butter, in the unhomogenized state the fat was poorly digested. The inclusion of crude soybean lecithin in the milk replacer improved the utilization of tallow coconut fat and grease (Hopkins *et al., ibid., 42,* 1815).

A low-fat milk was produced when cows received rations made up of cooked high-starch feeds while only a slight decrease in milk fat was produced when the more commonly fed concentrates with even lower levels of roughage were fed (Shaw *et al., J. Nutr., 69,* 235). Estrogenic activity in cow's milk and in the bile of calves was found after feeding of low levels of diethylstilbestrol (Herrick *et al., J. Dairy Sci., 42,* 1966). It was found that the tissues of steers, hogs, lambs, and poultry contained dieldrin when this substance was fed in the diet. Laying hens stored the greatest quantity, but their eggs contained very little dieldrin (Gannon *et al., J. Agr. and Food Chem., 7,* 826).

A number of reports were presented in the area of heated and toxic fats. The results of one study indicated that thermally oxidized butterfat or the thermally oxidized acetone-insoluble protein of butter did not exhibit any differences in the growth of rats when compared with those fed fresh fats. When rats were fed either corn or hydrogenated soybean oil thermally oxidized at 200°C. for 26 hrs., they gained significantly less weight than those fed the fresh fats. When these oils were mixed with 30% of the acetone-insoluble butterfat and thermally oxidized at 200°C. for 24 hrs., the growth-depressing effects on rats was not observed (Bhalerao *et al., J. Dairy Sci., 42,* 1057). The nutritional effect of polymers isolated from thermally oxidized corn oil was studied. The nondistillable residue from the nonurea-adduct-forming fatty acids of corn oil which had been heated at 200° for 48 hrs. was found to be the most toxic to rats (Perkins *et al., J. Nutr., 68,* 101). Hydrogenated cottonseed oil heated in a commercial fryer for 24 days had no deleterious effects of rats when fed at levels as high as 20% of the diet. The results indicated that during heating unsaturation occurred in the fat (Keane *et al., ibid., 68,* 57).

The fresh ethyl esters of purified, highly unsaturated fatty acids from sardine oil were not toxic to rats, but they became very toxic after auto-oxidation. The nonurea-adduct-forming fraction appeared to contain the most toxic compounds. The peroxides of the auto-oxidized fatty acids were found in livers and muscles of rats (Kaneda *et al., Eiyô to Shokuryô, 7,* 188). The toxicity of certain fats in chicken feeds received considerable attention. Mortality and other signs of toxicity, mainly hydropericardium, were produced in chickens in as little as six to seven days after the addition of certain toxic fats to a purified diet (Moehlin *et al., Poultry Sci., 38,* 573). Sixty-seven per cent of birds receiving a toxic fat for the full growing period died during the growing and laying period. Autopsy revealed hydropericardium, hydroperitoneum, and pale swollen kidneys. Egg production ceased in 55 days, and hatchability was greatly decreased (Dunahoo *et al., ibid, 38,* 663).

In more recent studies it has been found that the toxic principle in certain poultry feeds is concentrated in a particular portion of the nonsaponifiable fraction of the fat (Potter *et al., J. Am. Oil Chemists' Soc., 36,* 214). Immature mice fed a highly purified, low-fiber diet containing 7.5% of polyoxyethylene (20) and sorbital monostearate (Tween 60) exhibited retardation of growth, diarrhea, and decreased survival. These effects were largely overcome by the concurrent administration of alfalfa meal or dehydrated rye grass, orchard grass, wheat grass, or fescue grass at 10% level in the diet or carrageenin, sodium alginate, or agar at a 5% level (Ershoff *et al., J. Nutr., 69,* 172). The toxic factor in trichloroethylene-extracted soybean oil meal may be an S-dichlorovinyl derivative of cysteine or reduced glutathione McKinney *et al., J. Am. Chem. Soc., 81,* 909).

Intestinal Absorption and Intermediary Metabolism of Glycerides and Their Component Fatty Acids. Reviews on lectures on these subjects include the transport of fatty acids (Fredicken *et al., Physiol. Revs., 38,* 585); absorption studies with iodine 131-labelled fat and fatty acid (*Nutrition Revs., 17,* 211); the thoracic duct in lipid absorption (*ibid.,* 345); adipose tissue and fat oxidation (*ibid.,* 280); metabolism of unesterified fatty acids (*ibid.,* 241); nutrition and lipogenesis (*ibid.,* 316); and lipemia clearing after vegetable and animal fats (*ibid,* 102).

The gastro-intestinal digestion of fats in dogs fed triglycerides, partial glycerides, and free fatty acids was studied. The composition of lipid recovered from the various fats of the small intestine of dogs fed test meals of four lipid mixtures, the composition of which represented stages in the *in vitro* hydrolysis of triolein, closely resembled that obtained during *in vitro* hydrolysis. It was concluded that triglycerides may be hydrolyses in much the same manner *in vivo* as *in vitro* (Knoebel, *J. Nutr., 68,* 393). Studies on the glyceride-glycerol precursors in the intestinal mucosa indicated that the intestinal mucosa does not contain glycerol kinase, or at least it was not demonstrable under the conditions of the experiment. It appeared that L glycerophosphate was the immediate precursor of glyceride-glycerol (Buell *et al., J. Biol. Chem., 234,* 217). The synthesis of glycogen and glyceride-glycerol was studied by the use of rat adipose tissue *in vitro.* Insulin stimulated glycogen synthesis approximately 10–15-fold whereas glycerol synthesis was stimulated only 2–3-fold. Recovery of specifically labelled glycose carbons revealed that glycerol originates in part from metabolites derived through the phosphogluconate oxidative pathway (Cahill *et al., ibid., 234,* 2540). Gastric secretion in the intact rat is inhibited by fat in the small intestine. Under these same conditions, addition of bile salts to administered fat results in the same degree of inhibition as in the intact animal (Menguy, *Proc. Soc. Exptl. Biol. Med., 102,* 274). The incorporation of carboxyl-labelled C^{14} sodium acetate into total fat, neutral fat, and phosphatides was studied, using a soluble enzyme system from livers of normally fed and starving pigeons. The results indicated that there is a preferential formation of phosphatides in starvation in agreement with previous *in vitro* studies (von Brand, *Biochem. Z., 331,* 162). Replacing fats in the normal diet with a triglyceride of capric, caprylic, and lauric acids resulted in increased excretion of acetone and β-hydroxybutyric acid in the blood (Schön, *Gastroenterologia, 91,* 199). Analysis of thoracic duct lymph lipids indicated that in man one-fifth to one-half have been completely hydrolyzed before dietary glycerides reach the thoracic duct (Blomstrand *et al., Proc. Soc. Exptl. Biol. Med., 102,* 204).

Experiments were carried out to study the resorption of various edible fats (Haubold, *Fette, Seifen, und Anstrichmittel, 61,* 669). With the use of a preparation *in vitro,* labelled fatty acids continuously absorbed from the mucosal solution and transferred the serosal compartment over a 2-hr. period. The activity of the serosal solution was found predominantly in the triglycerides with small amounts in the diglyceride and free fatty acids. The distribution of glyceride activity in the intestinal wall was similar to that in the serosal solution (Johnston, *J. Biol. Chem., 234,* 1065). A study was made on the comparative effects of dietary fatty acids and triglycerides on lymph lipids in the rat. Butyric and lauric acids given with bile salts and cholesterol either as the free acid or the triglyceride had little effect on total lymph lipids or on absorption of exogenous cholesterol. Palmitic acid gave a marked increase in lymph lipids due entirely to a rise in neutral fat, cholesterol was simultaneously depressed. Stearic, oleic, and linoleic acids produced large increases in lymph levels of neutral fat and phospholipid. This increase was less marked when tristearin or triolein were fed but was comparable to the rise given by the free acid when trilinolein was fed. Trilinolein caused significant elevation of total lymph cholesterol (Vahouny *et al., Am. J. Physiol., 196,* 881). As a result of experiments on the absorbability of stearic acid when fed as a simple or mixed triglyceride, it was concluded that the coefficient of absorbability of a fat is inversely proportional to its content of simple triglycerides made up of saturated fatty acids with a chain length of 18 carbons or greater and is influenced by the level of such saturated fatty acids only insofar as they are present as saturated triglycerides (Mattson, *J. Nutr., 69,* 338). The presence of unsaturated fatty acids in the diet of rats caused a marked increase in lipid-soluble calcium and phosphorus in the feces. A structure for a calcium phosphate fatty acid complex in the feces was confirmed (Richards *et al., Can. J. Biochem. and Physiol., 37,* 725).

Cellular components of human blood were shown to be a convenient system for studies of fatty acids and lipid synthesis and exchange. After incubation of whole blood with C^{14} methyl-labelled acetate all the common saturated and unsaturated fatty acids including arachidonic, linoleic, and linolenic were found to be labelled. The long-chain fatty acids were incorporated into triglycerides, some neutral lipids of undefined origin, and phospholipids but not into cholesterol esters. The neutral lipids were rapidly secreted into plasma where they were incorporated into the plasma α-lipoproteins but not into the β-lipoproteins. The phospholipids were readily incorporated into the plasma β-lipoproteins (James *et al., Biochem. J., 73,* 106). Studies on intact rats with carboxyl-C^{14} labelled acetate indicated that the phospholipids and triglycerides were synthesized independently of each other (Handwerek *et al., Helv. Chim. Acta, 42,* 496).

A number of studies on fatty acid synthesis and factors affecting their synthesis have been reported. It was demonstrated that the fat body of the moth *Prodenia eridania* contains a system which incorporates acetate into long-chain fatty acids. The system required the presence of malonate ATP, coenzyme A, and glutathione or cysteine. The fatty acids formed by the system proved to be predominantly palmitic acid with small quantities of stearic, oleic, myristic, and lauric acid (Zebe *et al., Biochim. Biophys. Acta, 31,* 513. The role of TPNH in fatty acid synthesis from acetate by normal and diabetic rat liver homogenate fractions was reported (Abraham *et al., ibid., 36,* 556). It was shown that the majority of butyryl units of butyryl CoA are incorporated intact into palmitic acid by the pigeon liver fatty acid synthesizing system. The results were suggestive, but not conclusive, that butyryl CoA is a true intermediate in the biosynthesis of fatty acids (Long *et al., J. Biol. Chem., 234,* 1406). The operation of the various glycolytic schemes in the lactating rat mammary gland was considered, and it was concluded that at this time only the hexose-monophosphate oxidative pathway and the Embden-Meyerhoff pathway need be considered. The rate of conversion of glucose to CO_2 and nonvolatile fatty acids was found to be much higher in the mammary glands of lactating rats than in those of non-lactating rats (Abraham *et al., ibid.,* 2246).

General observations on the biochemistry of long-chain fatty acids were reported. Over a period of 4 to 18 hrs. after feeding a single dose of corn or olive oil to adult rats, the fatty acid distribution between various individual neutral and phospholipid components of rat liver was found to be reasonably constant (Dittmer *et al., ibid.,* 1976). After oral feeding of palmitic acid-1-C^{14}, oleic acid-1-C^{14}, linoleic acid-1-C^{14}, and stearic acid-1-C^{14} in corn oil to adult rats, a study was made of the incorporation and distribution of these individual fatty acids into major lipid components of the liver. The conversion of these fatty acids into other fatty acids was observed and related to the general metabolic pathways of the ingested fatty acids. The pathway of the individual fatty acids was found to be complex and, although similar, not necessarily the same (Dittmer *et al., ibid.,* 1983). After fractionation of rat liver cell particles the existence of a dehydrogenase in the supernatant which converts C^{14} labelled stearic acid into oleic acid was proven. The mitochondrial fraction showed no activity (Bernhard *et al., Helv. Chim. Acta, 42,* 152). Although leucine was rapidly incorporated as such into milk proteins, it acted neither as a precursor of fatty acids nor as an energy-yielding substrate. Isovaleric acid however was rapidly metabolized and acted as a precursor of fatty acids and of certain nonessential amino acids (Verbeke *et al., Biochem. J., 73,* 24). Studies of the assimilation and dissimilation of fatty acids by the rat liver were reported (Stein *et al., Am. J. Physiol., 196,* 1238).

Further information has been reported regarding the synthesis and interconversion of the essential and other polyunsaturated fatty acids. It was shown that in the fat-deficient rat oleic acid is the precursor of 5,8,11-eicosatrienoic acid (Fulco *et al., J. Biol. Chem., 234,* 1411). It was demonstrated that essential fatty acids are required for the proper utilization of fat calories and furthermore that high ratios of saturated fat to EFA promote the onset of EFA-deficiency symptoms in the rat (Peifer *et al., J. Nutr., 68,* 155). The oxidation of linoleic and linolenic acids by the normal and diabetic rat was studied (Lossow *et al., Proc. Soc. Exptl. Biol. Med., 101,* 857). It was found that the dienoic acid level of egg yolk triglycerides and phospholipids approaches an upper limit of 30% by the inclusion of not more than 7.5% of linoleic acid in the diet. The trienoic acid level of phospholipids increased from a basal value of 1.0% toward a maximum of 4% on a dietary level of 7.5% linoleic acid but did not maintain the increase even at the 15% level in the diet (Choudhury *et al., J. Nutr., 68,* 457).

A number of studies have appeared on the fatty acid oxidizing systems. Carp liver mitochondria were shown to contain an enzyme system that will oxidize fatty acids in the presence of ATP cytochrome C and Mg^{++}. Butyric, octanoic, palmitic, oleic, linoleic, linolenic, archidonic, eicosapentaenoic, and docosahexaenoic acids were readily oxidized (Brown *et al., Arch. Biochem. Biophys., 85,* 149). In the livers of female rats injected with large doses of DL-ethionine, the increases in neutral fat were not accompanied by a decrease in the levels of choline-containing or total phospholipids. Likewise, in such animals, the rates of synthesis of liver phospholipids were not significantly different from the rates measured in rats not receiving ethionine or receiving both ethionine and methionine. Besides the fat infiltration, homogenates of the livers of ethionine-treated female rats exhibited very considerable decreases in the ability to oxidize stearate-1-C^{14} added *in vitro*. Similar results were obtained for the production of isotopic carbon dioxide and acetoacetate from shorter-chain fatty acids, from acetates, and from pyruvate. The ethionine effect was completely prevented by administration of methionine along with ethionine; injections of choline chloride were ineffective. Addition *in vitro* of adenosine triphosphate, with or without diphosphopyridine nucleotide or coenzyme A, did not restore the ability of the tissue to oxidize the added fatty acid at a high rate. No impairment of fatty acid oxidation was apparent when ethionine was added *in vitro* to liver homogenates of untreated female rats. Neither the inhibition of fatty acid oxidation nor the fat infiltration of the liver was observed in the livers of male mature rats receiving ethionine. Under the conditions of these experiments, identical effects were observed when, instead of DL-ethionine, L-ethionine was injected in female mature rats (Artom *et al., J. Bio. Chem., 234,* 2259). B-Methylenecyclopropyl—aminopropionic acid (hypoglycine A) caused an increase in oxidation of uniformly labelled C^{14}-glucose to $C^{14}O_2$ in normal and alloxan diabetic rats to about twice the values observed in untreated animals. At the same time the oxidation of 1-C^{14}-capronate remained unchanged (Holt *et al., Biochem. Z., 331,* 430).

The effects of carnitine on fatty acid oxidation by muscle were studied. It appears possible that carnitine in muscle in some manner serves the function of facilitating fatty acid transfer to the fatty acid oxidase sites (Fritz *et al., Science, 129,* 334). The propionyl coenzyme A (CoA) carboxylation system was purified about 500-fold from pig heart extracts with no resolution into separate enzymes for CO_2 activation and propionyl-CoA carboxylation. The reaction is probably catalyzed by a single enzyme which is referred to as propionyl carboxylase. The purified enzyme contained no biotin, and no dissociable cofactors appeared to be involved in the reaction (Tietz *et al., J. Biol. Chem., 234,* 1394). Twenty-four normal and 16 obese mice were injected with radio-active propionate, and the tag was followed into CO_2, nonsaponifiable lipids, fatty acids, glycogen, urine, and feces. Propionate is a precursor for body fat synthesis in the whole animal. Conversion of propionate to lipids was greater in obese than in lean mice (Feller, *Proc. Soc. Exptl. Biol. Med., 102,* 605). Data were reported which were consistent with the substrate for the support of resting respiration of rat diaphragm *in vitro*. However these findings did not exclude some active role for carbohydrate metabolism in this tissue (Neptune *et al., J. Biol. Chem., 234,* 1659). Concentration of unesterified fatty acid (UFA) in blood plasma of fetal sheep and newborn man was consistently much lower than in that obtained simultaneously from their mothers. The levels were comparable to those found in man after administration of glucose or insulin. In both species the plasma level of UFA rose rapidly within 2 hrs. of birth with an associated hypoglycemia. These findings support previous studies which suggested the occurrence of a rapid shift from carbohydrate to fat catabolism in the neonatal period (Van Duyne *et al., Proc. Soc. Exptl. Biol. Med., 102,* 599). Repeated doses of ergotamine and hexamethonium had no effect on increase in plasma nonesterified fatty acid (NEFA) concentration which occurs during fasting. Dibenzyline was similarly without effect on this response. These findings suggested that the stimulus to increased NEFA mobilization during fasting may not be mediated by the adrenergic nervous system (Goodman *et al., ibid.,* 493). Fatty acid compositions of human milk from 11 mothers on diets taken *ad lib.* were determined by gas-liquid chromatography and by ultraviolet spectrophotometry after isomerization of polyenoic acids with alkali. A major proportion (88.4%, calc. as methyl esters) was made up of eight components: oleic 29, palmitic 21, myristic 9, linoleic 7, stearic 7, lauric 7, iso-oleic 7, and palmitoleic 2. At least 30 other acids were found in minor amounts, including odd-numbered and branched-chain acids as well as a wide variety of C_{19}–C_{20} unsaturated acids. The fatty acid composition of milks of mothers in the early and late postpartum periods were very similar although the fat concentration of the samples differed greatly (Insull *et al., Biochem. J., 72,* 27).

A modified ferric chloride-thiobarbituric acid reaction is described for the estimation of fatty acid peroxide contents of blood and tissues. Fatty acid peroxides were found in normal human blood and rat tissues (Kibrick *et al., Proc. Soc. Exptl. Biol. Med., 101,* 137). The lipid content of a human being was found to decrease with age. It was not apparently a function of body size or nutritional state (Briscoe *et al., ibid., 102,* 71). The effects of storage of serum at various temperatures on nonesterified fatty acid (NEFA) concentrations were studied. Appreciable increases in NEFA occurred within 24 hrs., even in frozen sera. Refrigerated heptane extracts of sera for NEFA analysis are stable for at least several days. Sera for NEFA analyses should be extracted promptly after collection (Forbes *et al., ibid., 102,* 709). From studies on oxidation and activation of unsaturated fatty acids it was shown that: a) small amounts of unsaturated fatty acids are oxidized to CO_2, both *in vivo* and *in vitro*, at a greater rate than the corresponding saturated fatty acids; b) fat-feeding to rats stimulates the rate of oxidation of unsaturated fatty acids; c) cholesterol-feeding to rabbits, but not to rats, stimulates the oxidation of all fatty acid by mitochondria, especially unsaturated fatty acids; d) long-chain unsaturated fatty acids are more readily activated by mitochondrial enzymes than most saturated acids (Lynn *et al., Arch. Biochem. Biophys., 81,* 353).

The *in vivo* incorporation of C^{14} from uniformly labelled glucose into glycogen, fatty acids, and respiratory CO_2 was studied with groups of pigeons that had received a fat-free diet, with and without thiamine, for 10 days. These three metabolic pathways were all found to be partially blocked in the thiamine-deficient animals. The decreased incorporation did not appear to be caused by a slower absorption of the glucose from the digestive tract. These observations were attributed to a diminished activity of pyruvic dehydrogenase and possible α-ketoglutaric dehydrogenase *in vivo* (Wiener *et al., Biochim. Biophys. Acta, 35,* 473). Brain is the only tissue which appears to contain large amounts of aminobutyric acid. Pyridoxine is involved in forming aminobutyric acid from glutamate. This acid is an important source of oxidative energy for the brain (*Nutr. Revs., 17,* 278). Gamma-aminobutyric acid-4-C^{14} was prepared and administered to male rats. The labelling patterns observed in isolated tissue glutamate, aspartate, alanine, and glycogen indicated that the succinate pathway (1, 2) can account for all the catabolism of α-aminobutyric acid *in vivo* (Wilson *et al., J. Biol. Chem., 234,* 347). By using the methods of paper chromatography and high-potential paper electrophoresis and by comparing the pattern of prepared extracts with that of synthesized β-hydroxy-γ-aminobutyric acid, it was found that free β-hydroxy-γ-aminobutyric exists in the brains of mice, rabbits, cattle, and human beings (Ohara *et al., Science, 129,* 1125). Gamma-guanidinobutyric acid has also been isolated from calves' brain in pure form by a series of fractionations on ion exchange resins. Its identity was established by chromatographic behavior, melting point, degradation, and enzymatic reaction, elemental analyses of the free acid as well as the flavianate, and by infrared absorption spectra (Irreverre *et al., J. Biol. Chem., 234,* 1438). Studies have been made on the biosynthesis of glycolipids and other brain lipids. C^{14}-labelled glucose or galactose was administered intraperitoneally to Swiss Albino mice ranging in age from 3 days to 6 months. After 1 hr. the animals were killed by decapitation, and the brain lipids were isolated. The activity present in cerebroside galactose exhibited the greatest variations with age. Maximal synthesis of this component occurred in animals 22 days old. When the administered glucose was labelled in carbon-6, almost all of the activity of the cerebroside galactose was found in that carbon. The patterns of labelling of other sugar components of brain lipids were determined and compared with those of liver glycogen and blood glucose. Glucose carbon was incorporated into all brain lipid components more readily than was galactose carbon except in the case of the gangliosides (Moser *et al., J. Biol. Chem, 234,* 1990).

It is known that lipid metabolism comes under hormonal control, and a number of reports have appeared regarding the effects of various hormones on fat metabolism. Purified corticotropin and growth hormone both stimulate lipid mobilization from the epididymal fat depots of fasting mice. Hydrocortisone failed to induce lipid loss in intact mice and inhibited fat mobilization in adrenalectomized mice, indicating that the lipid-mobilizing effect of corticotropin is not mediated by adrenal stimulation. Furthermore inhibition of fat loss by hydrocortisone in adrenalectomized mice could be overcome by simultaneous administration of corticotropin. The results demonstrated an extra-adrenal action of corticotropin on fat mobilization in the intact animal similar to that induced by growth hormone (White *et al., Proc. Soc. Exptl. Biol. Med., 102,* 272). Unsaturated vegetable fats promoted growth of thyrotoxic rats, an effect which appeared to be directly related to the linoleic acid supplied by the fats. Hydrogenated coconut oil was ineffective, and butterfat was marginal. The fat fraction of liver residue was however more active than other fats supplying more linoleic acid. The defatted fraction of liver residue promoted growth over and above all levels of fats. Growth stimulation by defatted liver residue and linoleic acid containing fats, or liver fat, appeared to be additive (Overby *et al., J. Nutr., 68,* 251). The release of nonesterified fatty acids (NEFA) from epididymal fat bodies removed from normal, hypophysectomy inhibits and growth-hormone administration stimulates NEFA release from adipose tissue *in vitro*. These findings are taken as direct evidence for the concept that growth-hormone treatment causes NEFA mobilization from fat stores (Knobil, *Proc. Soc. Exptl. Biol. Med., 101,* 288).

Concentrations of nonesterified fatty acids and of glucose in plasma were determined before and after infusion of epinephrine and norepinephrine into dogs. At the same concentration and rate of infusion, norepinephrine produced a greater increase in nonesterified fatty acids (Schotz *et al., ibid.,* 624). Male albino rats were kept on a fat-free diet for 23 weeks. During this time progressive reproductive system changes were observed by serial autopsies at 9, 12, 15, 20, and 23 weeks. The results showed that testicular weight was maintained in spite of decelerated body weight gain until the 10th week on the diet, after which progressive degeneration occurred, resulting in failure of spermatogenesis (Panos *et al., J. Nutr., 68,* 509). Two pairs of rumen-fistulated identical twin cows were used to determine some of the effects of low-level feeding of diethylstilbestrol (DES) on the rumen microflora. One twin of each pair was fed 10 mg. of DES per 1,000 lb. of body weight daily. Rumen-ingesta samples were collected, and determinations were made on pH, surface tension, total free volatile fatty acid concentration, percentage acetic, propionic, and butyric acids, *in vitro* gas production, and *in vitro* cellulose digestion as indirect indications of rumen microorganism activity. DES did not cause a marked change in any of these measurements. It was concluded that the oral administration of DES by the addition of "Stilbosol" to the feed in amounts to furnish 10 mg. per 1,000 kg. of body weight daily did not have a marked effect of the microbial activity in the rumen of the cows studied (Browning *et al., J. Dairy Sci., 42,* 1857).

Sterol Metabolism. Reports cited in this section are concerned with the absorption and intermediary metabolism of cholesterol and related sterols. Many of the studies on cholesterol metabolism are considered under the section on lipids in diseased states since the experimentation in these reports was primarily directed toward the metabolism and role of cholesterol in atherosclerosis. Studies on the mechanism of cholesterol absorption indicated that the free cholesterol pool of mucosa in rats expands with large amounts of fed cholesterol. It is approximately 5 to 6 g. and turns over once in 24 hrs. in the fasting animal. Examination of the data indicated that a complex of cholesterol and bile salts is formed in the lumen and enters the intestinal wall. A tentative scheme for the mechanism of cholesterol absorption was presented (Swell *et al., Am. N. Y. Acad. Sci., 72,* 813). Effects of various bile acids on intestinal absorption of dietary cholesterol were investigated in lymph-fistula rats. Cholanic, lithocholic, and deoxycholic acids, with none, one, and two hydroxy radicals, respectively, produced no increase in lymph cholesterol over the control group. Conjugated bile salts, glycocholate, and taurocholate gave comparable significant elevations in total lymph cholesterol/24 hrs. Cholic acid with three free hydroxyl groups and an unconjugated carboxyl radical was the most effective bile acid in promoting cholesterol absorption from the intestine (Vahouny *et al., Proc. Soc. Exptl. Biol. Med., 101,* 538). In studies with lymph-fistula rats, small doses of fed cholesterol-4-C^{14} led to labelling of cholesterol fractions of mucosa and lymph without an increase in level or turnover rate of the free cholesterol pool of the mucosa or in the amount of cholesterol in lymph. Simultaneous feeding of sodium taurocholate and oleic acid increased the amount of both labelled and unlabelled cholesterol in lymph (Swell *et al., ibid., 101,* 519).

β-Sitosterol reduces the absorption of cholesterol *via* the thoracic lymph. The percentage of β-sitosterol absorbed was about one-tenth that for cholesterol. β-sitosterol delayed the absorption of that cholesterol which was absorbed. Under the conditions of these experiments the total amount of β-sitosterol, rather than the ratio of the 2 sterols administered, determined the effectiveness of the β-sitosterol. In four groups of animals given different amounts of β-sitosterol and cholesterol, lymphatic cholesterol was about 70% esterified whereas lymphatic β-sitosterol was only about 25% esterified (Dunhan *et al., Arch.*

Biochem. Biophys., 82, 50). Oral administration of the methyl ether of cholesterol-4-C^{14} to lymph-duct-cannulated rats resulted in approximately 20% recovery of C^{14} activity in a 24-hr. lymph collection as compared to 35% recovery in the case of free cholesterol-4-C^{14}. Simultaneous administration of cholesterol methyl ether with cholesterol-4-C^{14} had no effect on cholesterol-4-C^{14} absorption in the cannulated rat. In the rabbit, dietary cholesterol methyl ether had a negligible effect on sterol levels when given alone or in conjunction with cholesterol (Gordon *et al., Proc. Soc. Exptl. Biol. Med., 102,* 679). The distribution of total sterols, 7-dehydrocholesterol, and lathosterol between the various cell fractions of intestinal mucosa of fasting rats and animals dosed with 7-dehydrocholesterol was determined. In both groups of animals about 50% of the total sterols in the tissue was associated with microsomes and mitochondria, 30% with the supernatant, and 10% with "cell debris and nuclei" fraction. Since the various sterols were uniformly distributed, dietary sterol apparently exchanges or mixes freely with endogenous sterols of the cell components. A large part of the 7-dehydrocholesterol absorbed from the intestine was reduced within the mucosa to lathosterol (Glover *et al., Biochem. J., 72,* 79).

Studies on the absorption of cholesterol, 7-dehydrocholesterol, and Vitamin A by guinea pig and rat intestinal mucosa indicated that esterification of single doses of cholesterol occurs mainly in the connective tissue before entry into lymph vessels. The action of esterase, although not obligatory for absorption, greatly accelerated the process (Glover *et al., ibid., 72,* 82). The influence of a purified diet containing 20% fat as hydrogenated cottonseed oil (Crisco, iodine no. 73), corn oil (Mazola, iodine no. 123), or coconut oil (iodine no. 10) on fecal excretion and tissue deposition of C^{14} was studied in rats injected with cholesterol-4-C^{14}. There was no significant difference between the three groups in amount of C^{14} excreted in feces or deposited in liver and in pooled internal organs (Anderson *et al., Proc. Soc. Exptl. Biol. Med., 102,* 155). In three patients with complete obstruction of biliary and pancreatic ducts cholesterol "secretion" by intestinal mucosa was 250–400 mg./day (Cheng *et al., ibid., 101,* 223). The absorption of cholesterol-4-C^{14} and 4-cholestenone-4-C^{14} was studied in rats provided with thoracic-duct cannulas. When the labelled internally cholestenal was administered, about 35% of the C^{14} was recovered in lymph in 24 hrs. The absorbed C^{14} was completely accounted for by free and esterified cholestanol. Less than 10% of the C^{14} of fed cholestenone-4-C^{14} was recovered in lymph in 24 hrs. The C^{14} was completely accounted for by cholestenone, free and esterified cholestanol, and cholestenone. About one-third of the cholestenone was unchanged in its passage from the intestinal lumen to the lymph (Chapman *et al., J. Biol. Chem., 234,* 273). The digitonides of various cholesterol esters were shown to have different specific activities 30 min. after injection of sodium acetate-1-C^{14}. In order of decreasing activity were esters of oleic, linoleic, saturated, and arachidonic acids. After 4 hrs. all esters had approximately the same activity, indicating that comparable portions of each of the esters had been replaced. It was concluded that the turnover rates of cholesterol esters in liver are heterogeneous (Klein *et al., ibid, 234,* 1684).

The enzyme system from rat liver, consisting of a soluble-protein fraction and microsomes, which synthesizes cholesterol from mevalonic acid, was investigated for stability of the microsomal enzymes (Gosselin *et al., Biochem. J., 71,* 660). The formation of two phosphorylated derivatives of mevalonic acid by liver enzymes in the presence of adenosine triphosphate and Mg^{2+} ions was described. The two substances were purified by various chromatographic procedures. One of the compounds, MV_1, was thought to be 5-phosphomevalonate. The second, MV_2, contains two atoms of phosphorus per molecule of mevalonate. Both of these substances were converted into cholesterol more efficiently than is DL-mevalonate, but adenosine triphosphate was still needed in this conversion (de Waard *et al., ibid., 73,* 410). An ammonium sulfate precipitated fraction of the water-soluble, supernatant enzyme system from rat liver was found to form at least nine intermediates between mevalonic acid and squalene. Three of these were identified as 5-phosphomevalonic acid, 5-pyrophosphomevalonic acid, and isopentenyl pyrophosphate (pyrophospho-3-methyl-but-3-ene-1-ol). The other six were water-soluble, acid-labile derivatives of higher terpenoid compounds. Farnesol was identified as a component of these compounds (Witting *et al., J. Biol. Chem., 234,* 2841). The influence of ATP on biosynthesis of cholesterol and precursors from mevalonic acid was studied with relatively crude (200 xg supernatant) and more refined (9000 xg supernatant) fractions of rat liver homogenate under aerobic and anaerobic conditions, following preincubation with or without added ribonuclease. Mevalonic acid incorporation occurred under conditions favorable for oxidative phosphorylation or under conditions where the ATPase of tissue fragments had been removed by centrifugation (9000 xg). Ribonuclease inhibition of mevalonic acid utilization occurred only under oxygen and in the presence of tissue fragments relatively rich in ATPase (Wright *et al., Proc. Soc. Exptl. Biol. Med., 102,* 540). Ribonuclease-treated homogenates of liver that do not incorporate mevalonic acid into non-saponifiable material or cholesterol were devoid of ATP. ATP was present, and mevalonic acid incorporation occurred when ribonuclease pretreatment was carried out in the presence of an autoclaved extract of liver. The results were interpreted as indicating existence of a polynucleotide factor essential for maintenance of a positive balance with respect to ATP in liver homogenates (Wright *et al., ibid., 101,* 866). The decarboxylation of mevalonic acid and conversion to squalene and enzyme fractionation and coenzymes for this system were described (Markley *et al., Biochim. Biophys. Acta, 31,* 287). Enzymes catalyzing the formation of mevalonic acid 5-pyrophosphate and of isopentenylpyrophosphate were purified by fractionation of yeast autoysates. Phosphomevalonic kinase catalyzed the phosphorylation of phosphomevalonic acid to mevalonic acid 5-pyrophosphate by adenosine triphosphate in the presence of a divalent metal ion. Stoichiometric amounts of adenosine diphosphate were formed in the reaction (Bloch *et al., J. Biol. Chem., 234,* 2595). 2-C^{14}-5-T-DL-Mevalonic acid and 2-C^{14}-5-D_2-DL-mevalonic acid were synthesized and converted to squalene in yeast extracts. The squalene formed from the deuterium-labelled mevalonic acid was chemically degraded, and the ratios of D to C^{14} in the various products were determined. It was concluded that the head-to-tail condensations of isoprenoid units take place without loss of hydrogen bound to C-5 of mevalonic acid but that two hydrogens were removed from C-5 positions when the central carbon-carbon bond of squalene was formed (Rilling *et al., ibid., 234,* 1424). C^{14}-Ergosterol was biosynthesized from 1-C^{14}-acetate, and the distribution of the labelled atoms was studied. By conversion of ergosterol to progesterone it was shown that the distribution of label between the side-chain and the nucleus was that predicted on the basis of the squalene hypothesis. The specific carbons, C-3, C-4, C-11, and C-12 were obtained by degradation of appropriate precursors, and it was found that C-4, C-11, and C-12 were derived from the carboxyl of acetate, again as predicted by the squalene hypothesis. These results strongly supported the concept of the utilization of the intact acyclic triterpene, squalene, in the biosynthesis of all steroids (Dauben *et al., J. Am. Chem. Soc., 81,* 403). The effects of Δ1-testololactone, Δ4-androstene 17 α-ol-3-one-17 β-oic acid, and fluoromevalonic acid upon *in vitro* conversion of labelled acetate and labelled mevalonate into cholesterol were studied. All of these compounds inhibited conversion of acetate while only the latter two inhibited conversion of mevalonate. Fluoromevalonic acid was the most potent inhibitor studied. Fluoroacetate, fluoride ions, and zinc ions had no effect on the mevalonate system. Of these substances only zinc ions inhibited conversion of acetate to cholesterol (Singer *et al., Proc. Soc. Exptl. Biol. Med., 102,* 370). The incorporation of 2-C^{14} mevalonic acid into cholesterol and fatty acids of brain and liver *in vitro* was reported (Garattini *et al., Arch. Biochem. Biophys., 80,* 210). The biosynthesis of cholesterol in isolated rat liver cells was studied (Albers, *Univ. Microfilms* [Ann Arbor, Mich.], L. C. Card No. *Mic 58*-7960, 156 pp.; *Dissertation Abstr. 19,* 1909). The feeble stimulation of 11-deoxycorticosterone 11 B-hydroxylation in mitochondria from ox-adrenal cortex by citrate was not due to failure of this substance to enter the mitochondria or to be oxidized *via iso*-citrate but may be related to the fact that *iso*-citrate oxidation, which appears to be predominantly diphospropyridine nucleotide-linked in these mitochondria, failed to produce adequate amounts of reduced triphosphopyridine nucleotide. The reduction of diphosphopyridine nucleotide by the mitochondrial enzymes in the presence of *iso*-citrate, under the condtions described, could not be attributed to the activity of a pyridine nucleotide transhydrogenase. The failure to oxidize reduced triphosphopyridine nucleotide by flavoprotein-cytochrome systems in the mitochondria and the possible significance of the transhydrogenase reaction in relation to biosynthetic reactions were discussed (Grant *et al., Biochem. J., 71,* 34). Rats showed an increase in plasma cholesterol as the linoleic acid content of the diet was increased. The liver cholesterol esters were increased in fat deficiency, low in the normal ranges of dietary fat, and increased again at high levels of linoleic acid. The second increase was specifically related to the polyunsaturated fatty acid content, not to the fat level. The polyunsaturated acid content of liver cholesterol esters bore a simple relation to the dietary polyunsaturated fatty acids; the level of ester found in the liver was not related to the content of polyunsaturated fatty acid in the ester. Plasma cholesterol esters differed markedly in composition from liver esters and did not follow the same

relation to the dietary fat as did the latter. Possibilities for the derivation of plasma cholesterol esters from liver cholesterol were discussed (Klein, *Arch. Biochem. Biophys., 76,* 56).

In rats, addition of 0.5% cholesterol to the diets containing various levels of linoleic acid resulted in the accumulation of liver cholesterol ester; the magnitude of the accumulation was not related in an apparent manner to the amount of linoleic acid. There was little or no increase in plasma cholesterol except in animals on a fat-free diet. The multi-unsaturated fatty acid content of the plasma esters decreased markedly, and cholesterol arachidonate largely disappeared. The influx of cholesterol in the liver was accompanied by increases in the amounts of dienoic and trienoic ester, but tetraenoic and pentaenoic esters did not change. The fact that feeding a diet containing 2% cholesterol for 28 days decreased incorporation of acetate into the octabromide fraction of liver lipids suggested that the formation of arachidonic acid from linoleic acid was impaired (Klein, *ibid., 81,* 382).

A study of sequence of events during initiation and regression of inhibition of cholesterol synthesis by dietary cholic acid was made in rats. In the initiation study, increases of liver cholesterol and serum bile acid levels paralleled decreases of liver cholesterol-4-C^{14} activity. There was a decrease in liver phospholipid during the same time interval. In the regression study, serum bile acid and liver cholesterol returned to control levels more rapidly than the rate of liver cholesterol synthesis. The results suggested that dietary cholic acid initially elevates liver cholesterol, which in turn leads to the inhibition of acetate-1-C^{14} incorporation into liver cholesterol (Beher *et al., Proc. Soc. Exptl. Biol. Med., 101,* 214). Young male rates were fed synthetic diets containing either no fat or various individual fatty acids for 3–4 weeks. They were then killed, and the incorporation of acetate-1-C^{14} into cholesterol and fatty acids was measured in liver slices and in scrapings of intestinal mucosa. Acetate incorporation into cholesterol by liver slices was much greater in animals fed erucic acid than in those fed no fat, palmitic, stearic, oleic, or linoleic acid. A marked differential was not observed in fatty acid incorporation, but values tended to be higher on the fat-free and erucic acid diet. Erucic acid did not stimulate acetate incorporation into cholesterol by mucosa, and, in general, mucosa seemed to be less sensitive to changes in diet (Carrol, *Can. J. Biochem. and Physiol., 37,* 803). When linoleic acid was added to the diet of the rat, there was an average increase in incorporation of radiocarbon into liver cholesterol of 259%. By contrast, the addition of coconut oil to the diet did not increase the incorporation of radiocarbon into liver cholesterol.

The addition of linoleic acid to a stock diet was associated with an increased fecal excretion of Liberman-Burchard chromogens, 3-β-hydroxy sterols, and bile acids (Merrill, *Circulation Res., 7,* 709). Yellow mice fed *ad libitum* exhibited a greater rate of lipogenesis and cholesterogenesis than their controls. Females showed fatty acid content in the liver and in extrahepatic tissues which was twice as high as the males. Unlike the males, they were hypercholesterolemic and also showed an elevated rate of lipogenesis after an 18-hr. fast (Zomzely *et al., Am. J. Physiol., 196,* 611). Feeding various bile acids to mice for three weeks had the following effects. a) Cholic acid increased hepatic and intestinal cholesterol levels but had no effect on kidney cholesterol. It decreased hepatic cholesterol synthesis rates but had no effect on intestinal synthesis rates. b) Hyodeoxycholic and lithocholic acids decreased liver cholesterol levels. Hyodeoxycholic acid had no effect on intestine and kidney cholesterol or on intestinal cholesterol-4-C^{14}. Both acids effected large increases in hepatic cholesterol synthesis. (c) Deoxycholic acid significantly decreased liver, small intestine, and kidney cholesterol levels. It also decreased hepatic cholesterol synthesis. Metabolism in tissues other than liver is independent of hepatic control. d) Results of this study suggested homeostatic control of cholesterol (Beher *et al., Proc. Soc. Exptl. Biol. Med., 102,* 317).

The mechanism by which ingestion of large amounts of nicotinic acid lowers serum cholesterol in rabbits was investigated. The rate at which cholesterol is synthesized from acetate-1-C^{14} by liver slices from animals on control or cholesterol-supplemented diets, with or without nicotinic acid, was measured. There was marked inhibition of the rate of cholesterol synthesis by liver slices from animals fed nicotinic acid on both control and supplemented diets. Since the principal detoxication product of large doses of nicotinic acid is nicotinuric acid, it is possible that this inhibition of cholesterol synthesis occurs as a direct result of competition of lipid synthesizing and detoxication systems for a limiting amount of CoA in the liver cell (Schade *et al., ibid., 102,* 265). For periods up to six weeks rats were fed diets containing either 0.05 or 2.0% of cholesterol labelled with cholesterol-4-C^{14}. In these animals the relative contribution of dietary and endogenous cholesterol to the composition of the cholesterol in liver, mucosa, and serosa of the small intestine, adrenal gland, and testis was determined (Morris *et al., J. Biol. Chem., 234,* 1095).

The capacity to synthesize cholesterol from C^{14}-labelled acetate, mevalonic acid, and squalene was studied in cell-free preparations of liver from rats subjected to x-rays, Triton WR 1339, food deprivation, administration of a cholesterol-rich diet, and Δ^7-cholesten-3-one. In each experimental group large deviations from the normal level were found in all segments of the reaction sequence, but the extreme changes appearing before the point of entry of mevalonic acid far outweighed those occurring at later stages. Tissue fractionation studies showed that the activity of the system was determined principally by the microsomes. The soluble components were also affected, but to a lesser extent (Bucher *et al., ibid., 234,* 262). It was shown that a 1:1 cholesterol:sitosterol mixed crystal is formed not only in aqueous *in vitro* systems but also *in vivo* (Hudson *et al., Proc. Soc. Exptl. Biol. Med., 102,* 461). Sixteen samples of serum from 11 subjects were studied for the capacity to esterify naturally occurring free cholesterol alone and in the presence of dimyristoyl, distearoyl, or dioleyl lecithin of synthetic origin. All sera showed "normal" esterifying capacity when incubated for three days. This effect was regularly and significantly enhanced by the prior addition of dimyristoyl lecithin but not by the addition of either of the other two lecithins. The quantity of lecithin present seemed to bear some relation to the degree of esterification (Wagner, *Circulation Res., 7,* 818).

By *in vitro* studies with liver tissue, cholic acid conjugates were found to depress oxidation of cholesterol but not of sodium pyruvate or 3α, 7α, 12α-trihydrocoprostane. Preparations of liver mitochondria from livers of rats which had been deprived of bile salts by interruption of enterohepatic circulation oxidized cholesterol to a greater extent than controls. The mechanism was discussed briefly (Whitehouse *et al., Proc. Soc. Exptl. Biol. Med., 101,* 439). Rats were maintained on normal diets or on diets containing 20% corn oil or on commercial shortening for 40 days. The liver mitochondria of the rats fed saturated fat oxidized cholesterol-26-C^{14} to $C^{14}O_2$ to a much greater extent than did liver mitochondria from rats fed unsaturated fat. In general, liver mitochondria from control rats also oxidized more cholesterol-26-C^{14} than did mitochondria from rats fed unsaturated fat, but this difference was not as consistent. The results were the same with rats of either sex. Oxidation of sodium pyruvate-2-C^{14} did not vary with diet. The results could not be attributed to differences in liver lipid or cholesterol content. It was shown that the homologous combination of liver mitochondria and boiled supernatant from the livers of the unsaturated fat-fed rats greatly impairs cholesterol oxidation. Addition of boiled supernate from the livers of the other dietary groups to liver mitochondria from the rats fed unsaturated fat restored the cholesterol oxidation to normal levels as did substitution of a 10% sucrose solution for the boiled supernate. Addition of the boiled liver supernate from the animals fed unsaturated fat to liver mitochondria from the normal or saturated fat-fed rats had no appreciable inhibitory effect (Kritchevsky *et al., J. Lipid Res., 1,* 83). In the absence of mitochondria there was no oxidation of cholesterol-26-C^{14}. Mitochondrial oxidation of the terminal carbon atoms of cholesterol was much greater in rats fed large amounts of saturated fats than in rats fed equivalent amounts of unsaturated fats (Kritchevsky *et al., Arch. Biochem. Biophys., 80,* 221). Rat liver mitochondria were able to oxidize the terminal methyl groups of the cholesterol side-chain to carbon dioxide in the presence of a soluble cofactor prepared from either liver tissue or heart muscle. Glutathione, adenosine 5-monophosphate, adenosisne 5'-triphosphate, diphosphopyridine nucleotide, magnesium ions, and sodium had to be added for optimal activity. Evidence was presented that this oxidation required the functioning of the tricarboxylic acid cycle (Whitehouse *et al., J. Biol. Chem., 234,* 276).

The preparation of cholic acid-7-B-H^3, 24-C^{14} was described. During the conversion of this acid to deoxycholic acid in the rabbit intestine the tritium remained in the molecule. Evidence was presented that the tritium is located in the 7-position in the deoxycholic acid which was formed. 7-Ketodeoxycholic acid could be transformed into deoxycholic in the rabbit intestine but remained unchanged after one passage through the liver (Bergström *et al., ibid., 234,* 2022). The tritium label in cholic acid 7-B-H^3, 24-C^{14} was almost completely retained in the molecule during the conversion to deoxycholic acid by the microorganisms in the large intestine. The tritium label in the isolated deoxycholic acid was lost in the 7a-hydroxylation to cholic acid in the liver; this suggests a shift of the H^3-label from the 7B- to the 7a-position (Lindstedt *et al., ibid., 234,* 2026). The

synthesis of cholesterol-6-H^3-4-C^{14} was described. Cholesterol-6-H^3 together with cholesterol-4-C^{14} was administered to bile fistula rats, and chenodeoxycholic acid was isolated with the H^3:C^{14} ratio unchanged. With the aid of enzymatic 6a- and 6B-hydroxylations it was found that the 6-H^3- label was in the 6α-position, *i.e.*, the reduction of the cholesterol double bond takes place by a stereo-specific reaction in which the new hydrogen atoms at C-5 and C-6 are brought in B-position (Samuelsson, *ibid.*, *234*, 2852).

Radio-active cortisol was recovered from the urine of guinea pigs that had been fed, for five days, cholesterol labelled with tritium in both the tetracyclic nucleus (61%) and in the side chain. The cortisol was identified by its chromatographic behavior, by its ultraviolet absorption and blue tetrazolium reaction, and by characterization of its derivatives. Radiochemical purity of the urinary cortisol was established by the attainment of constant specific activities of this compound as the acetate derivative after repeated chromatographic separations. Evidence was cited in support of the view that the H^3-cortisol isolated from the urine was derived from conversion of the intact rings of the fed tritiated cholesterol (Werbin *et al.*, *ibid.*, *234*, 282). Lymph fistula rats were given a test meal containing 48 mg. of C^{14}-phytosterol. There was considerable uptake of C^{14}-phytosterol by the intestinal wall with virtually all of it present as free sterol. Most of the total C^{14} activity was present in the proximal portion of the intestine. The lymph, at 24 hrs., contained only 2% of the fed C^{14}-phytosterol, which produced an increased fecal excretion of cholesterol and related sterols and a lower lymph cholesterol level than were found in a control group. This study provided evidence that the principal block in phytosterol absorption occurs within the mucosa at a step in the transfer mechanism from mucosa to the lymph; this could be esterification or chylomicron formation (Swell *et al.*, *ibid.*, *234*, 2286).

A study was made of the distribution of radio-activity in the tissues and excreta of a bile-fistulated steer after a single small oral dose of tritium-labelled stilbesterol, and of an intact steer after stilbesterol administration for 11 days. Approximately 20% of the administered radio-activity was excreted in the urine, and 30% was recovered from the feces. Only traces of activity were found in the bile of the fistulated animal. Separation of the radio-activity into free phenolic and conjugated fractions revealed that stilbesterol was present in urine and bile primarily in conjugated form. The radio-active phenolic material was identified as stilbesterol, and its concentration in parts per billion was 0.30 for lean meat, 0.35 for fat, 9.12 for liver, and 4.15 for kidney (Mitchell *et al.*, *Agr. and Food Chem.*, *7*, 509). It appears that obese animals retain proportionally more steroid hormones than nonobese animals. The retention of these hormones does not appear to be a function of the nature of the obesity syndrome but simply a function of the increased fat content (Zomzely *et al.*, *Science*, *129*, 1546). The sterol and wax alcohol fractions of the unsaponifiable matter of human forearm sebum (skin surface fat) were studied. The alcohols formed a homologous series (C_8 to C_{20}) similar to that of the fatty acids of sebum. There were fewer unsaturated and highly branched alcohols in comparison with the acids, and the average chain length of the major alcohols appeared to be longer than that of the major acids. In addition to cholesterol, the sterol fraction contained traces of 7-dehydrocholesterol, isocholesterol, and unidentified keto steroids (Boughton, *Biochem. J.*, *73*, 144).

Glycerophosphatides, Sphingolipids, and Other Complex Lipids

In this section studies pertaining to structure, isolation intestinal absorption, and intermediary metabolism of glycerophosphatides, sphingolipids, and such other complex lipids as inositol lipids, plasmologens, etc., are considered. Phospholipids from human red blood cells were chromatographed on silicic acid and found to consist chiefly of ethanolamine- and serine-containing phospholipids, lecithin, sphingomyelin, and lysolecithin (Phillips *et al.*, *Proc. Soc. Exptl. Biol. Med.*, *100*, 489). Body weights and blood lipid values were determined periodically in male and female miniature swine fed high-tallow diets supplemented with soybean phosphatides. Weight changes were not related to sex or variations in diet. The phosphatides appeared to counteract the hypercholesterolemic effect of the tallow diet. The alcohol-insoluble phosphatides (inositol phosphatides) may have been most active in this respect. The tetraenoic acid concentration of plasma tended to be higher in females than in males. There was no apparent correlation between changes in plasma polyunsaturated acids and changes in other plasma lipids (Hill *et al.*, *ibid.*, *99*, 586).

Chromatography on silicic acid was employed for the separation of phospholipid components of human serum, using diethyl ether and chloroform-methanol solutions as eluting agents. The components identified by paper chromatography were cephalins, lecithin, sphingomyelin, and lysolecithin. Chemical analyses and infrared spectra were employed to confirm the identification of the compounds isolated. The quantities of the phospholipid component were also determined (Gjone *et al.*, *J. Lipid Research*, *1*, 66). Rabbits fed cholesterol for one to five months showed gradually increasing amounts of aortic phospholipid, preceded by increases in plasma phospholipid concentration. Cholesterol feeding appeared to increase the turnover of plasma as well as aortic phospholipid. Curtailment of plasma phospholipid synthesis to one-tenth of normal by evisceration did not diminish the incorporation of P^{32} into aortic lipids. In these animals the aortic phospholipid specific activity was 15 to 90 times as great as that of plasma so that practically all of the labelled aortic phospholipid must have been synthesized *in situ*. Lowering the plasma lipid levels by removal of cholesterol from the diet did not diminish the cholesterol content of the aortic lesion or alter its phospholipogenesis (Zilversmit *et al.*, *ibid*, *1*, 118). Hack's (1953) rapid method of disk chromatography was used for orientation analysis of lipids of the sebum obtained from the skin of the forehead of healthy white adults. The detection of individual components was extended by the use of additional reactions. This method detected, besides other previously demonstrated substances, the presence of phosphatides in the human sebum (Horacek *et al.*, *Biochem. J.*, *71*, 417). Sheep brain lipids were treated with 1-fluoro-2,4-dinitrobenzene and then with diazomethane. Products were fractionated by countercurrent distribution between aqueous ethanol and petroleum ether. A derivative of a complex phospholipid was obtained in a yield of 28% of the total phospholipids. It had a molecular weight of 2030 and contained 2 moles of glycerol 1-phosphate, 1 mole each of dinitrophenylethanolamine, choline, a sugar, and a fatty alcohol, 2 moles of fatty acids, and two unidentified nitrogen-containing materials (Collins, *ibid.*, *72*, 532). In the growing brown adipose tissue of young rats it was found that there is a completely paralleled relationship between the phosphatide content and the amount of lipid-free dry substance. At birth almost the same relative proportions were already present as in the mature animal whereas the storage of depot fats ocurred later. In starvation the total lipid content of the tissue was reduced to about a tenth of normal; the phosphatide content stayed almost unchanged. This confirmed the assumption that phosphatides in brown adipose tissue are present in the cytoplasm and its preformed constituents and not in the vacuoles as storage material (Langer *et al.*, *Z. für Physiol. Chem.*, *315*, 80).

Several methods were compared for recovery and purification of mixtures of phospholipids from dried buttermilk. The amounts of unsaturated fatty acids were estimated from spectrophotometric data and iodine values. A representative sample of milk phospholipids (3.72% phosphorus, 2.23% nitrogen, iodine value 53.8) contained 26.3% monoene, 1.5% conjugated diene, and 5.8, 2.4, 1.3, and 1.5% non-conjugated diene, triene, tetraene, and pentaene acids, respectively. Percentages of each of these acids in the total fatty acids (with corresponding percentages for milk fat from the same source given in parentheses) were 39.8(32.1), 2.3(1.4), 8.8(1.6), 3.6(0.9), 2.0 (0.3), and 2.2(0.2), respectively. The phospholipids contained 41.3% saturated fatty acids and the milk fat 63.5% (Smith *et al.*, *J. Dairy Sci.*, *42*, 767). Acetone precipitation of an etheral solution of commercial rapeseed lecithin yielded a crude phospholipid which contained 2.1% of a sterol glycoside. A similar substance isolated from linseed phospholipid contained 2.9% of a sterol glycoside. It is probable that in each case β-sitosterol was the major sterol component, but minor proportions of other phytosterols may also be present (Aylward *et al.*, *Nature*, *184*, 1319). The mixed calcium-magnesium salt of phosphatidyl inositol was obtained, by a relatively simple solvent-fractionation procedure, from lyophilized frozen peas which were stored at −17.8° for 1 month before lyophilization.

Methods for preparing the soluble sodium and potassium salts are reported. Elementary analyses and data on the hydrolysis products show that the parent compound is a monophosphoinositide which probably possesses a phosphatidyl inositol structure. The fatty acids were shown to possess C_{16} and C_{18} chain lengths (Wagenknecht *et al.*, *J. Biol. Chem.*, *234*, 2265). The total phosphatides and the lecithin and phosphatidylethanolamine fractions of beef heart were isolated and reduced with hydrogen. These lipids were degraded to give a 68-72% yield of long-chain α-glycerol ethers. Hence the major part of the plasmalogens of beef heart have the aldehyde attached to the α-position and the fatty acid attached to the β-position of glycerol. The

unreduced beef heart lecithin fraction was hydrolyzed by snake venom lecithinase A to yield a mixture of the typical monoester type of lysolecithin and the monoether type of lysoplasmalogen. These lyso-phosphatides were reduced with hydrogen and hydrolyzed with acid. A 68% yield of long-chain α-glycerol ether was obtained from the lysoplasmalogen. These data demonstrated that snake venom lecithinase A can hydrolyze the β-linked fatty acid on the plasmalogen. Data on the reactivity of the plasmalogens and lysoplasmalogens toward the Schiff reagent and methanolic iodine were given (Marinetti *et al., J. Am. Chem. Soc., 81,* 861). Plasmalogen content in brains of newborn rats was about 2 μmoles/g. of wet tissue and represented about 10% of total phospholipid. During growth, and after major increase in brainweight, phospholipid composition shifted to one containing about 20 to 25% plasmalogen (12 μmoles/g.). Maximal rate of accumulation of plasmalogen in brain occurred in 20 to 24-day-old rats (Erickson *et al., Proc. Soc. Exptl. Biol. Med., 102,* 512). Crude sphingomyelin was purified by treatment with dilute alkali, column chromatography on alumina, and several recrystallizations from ethyl acetate. After refluxing with sulfuric acid in methanol, three fractions of methyl esters were isolated. The fatty acid of fraction 1 (0.5 g.) was identified as nervonic, fraction 2 (0.7 g.) as a mixture of stearic and palmitic acids, and fraction 3 (1.4 g.) as behenic acid (Fujino *et al., Nature, 184,* 817).

The brains of from 11 to 30 rats of various age groups (23 to 418 days old) were pooled and analyzed for total lipids, total cerebrosides, and the individual cerebroside acids. Cerebroside deposition was evident over the range of ages studied, and its contribution to the total deposition became increasingly important with increasing age. Cerebronic acid was by far the major cerebroside acid, but appreciable amounts of the α-hydroxy C_{23} and C_{22} acids are also present. The unsaturated acids constituted a minor element, and the contribution of the hydroxy unsaturated acids was least. The odd-numbered acids showed the greatest increases with age, compared to the other acids, while the α-hydroxy unsaturated acids showed little accumulation except during the earliest period studied. Degradation experiments with the saturated hydroxy acids showed that the hydroxyl groups are in the *alpha* position (Kishimoto *et al., J. Lipid Research, 1,* 79).

Specific radio-activity of Dawson's phospholipids in rat liver was determined 30 min. after the intraperitoneal injection of P^{32}-phosphate: diphosphoinositide >> phosphatidylethanolamine >> phosphatidylserine >> phosphatidylcholine. At 12 hrs. after the P^{32} injection those four fractions showed approximately equal specific activities. Similar results were also demonstrated by mild alkaline hydrolysis, paper chromatography, and autoradiography successively (Sakagawa *et al., J. Biochem. (Tokyo), 46,* 51). P^{32}-Lecithin of high specific activity was prepared biosynthetically with baker's yeast. Activities of *Penicillium notatum* phospholipase were estimated from the rate of transfer of counts from a surface film of this lecithin to a supporting aqueous phase. The enzyme had no effect on a film of the pure lecithin but did hydrolyze films containing dicetylphosphoric acid. It was concluded that a net surplus of negative groups on a lecithin surface is prerequisite for lecithinase attack (Dawson *et al., Biochem. J., 72,* 493). Electrophoretic measurements of aqueous emulsions of various lecithin preparations indicated that pure lecithin particles have a net positive charge at the optimum pH for lecithinase action. Activating lipids (monophosphoinositide, cardiolipin) and anionic amphipathic molecules which activate the enzyme system were found to confer a net negative surface to the enzyme. Cationic materials caused complete inhibition of the enzyme when their concentration was sufficient to reduce the mobility of the emulsion particles below the critical activation value. Electrophoresis of *Penicillium notatum* phospholipase β indicated that lysolecithinase and lecithinase activity resided in the same protein band and that these enzymes are identical (Bangham *et al., ibid., 72,* 486).

Studies of the hydrolysis of lecithin by phospholipase β preparations from *P. notatum* showed that the enzyme requires certain activating lipids in the system. Most effective lipids were cardiolipin, liver polyglycerolphospholipid, and monophosphoinositide. Tripalmitin and tristearin were less effective. A variety of other lipids were tested. The lecithinase was inhibited by bivalent metal ions and by fluoride. It was suggested that cardiolipin and monophosphoinositide are held at the surface of the lecithin particles and thereby introduce certain polar groups which are necessary for the enzymatic attack (Dawson, *ibid., 70,* 559). Red cells of the camel were like those of true ruminants in being resistant to lysis by cobra venom but dissimilar in containing lecithin. Lipids in these cells were more difficult to extract with ether-ethanol mixtures than were the phosphatides in red cells from man and ox (Turner *et al., Proc. Soc. Exptl. Biol. Med., 99,* 547).

Phosphatidic acid was synthesized from diglyceride and adenosine triphosphate-P^{32} in soluble extracts of deoxycholate-treated brain microsomes. The most effective substrate was diglyceride from cabbage phosphatidic acid; diglyceride from brain lecithin, and 1-palmityl, 2-oleyl diglyceride were much less effective. Glycerophosphate was not an intermediate in this reaction. Diglyceride kinase activity was present in the intact microsomes, but added substrate was not utilized unless surface-active agents such as deoxycholate were added (Hokin *et al., J. Biol. Chem., 234,* 1381). C^{14}-labelled glucose or galactose was administered intraperitoneally to Swiss Albino mice ranging in age from 3 days to 6 months. After 1 hr. the animals were killed by decapitation, and the brain lipids isolated. The activity present in cerebroside galactose exhibited the greatest variations with age. Maximal synthesis of this component occurred in animals 22 days old. When the administered glucose was labelled in carbon-6, almost all of the activity of the cerebroside was found in that carbon. The simultaneous incorporation *in vitro* of P^{32}-orthophosphate and Me-C^{14} acetate into the phospholipids of human blood cells was measured. The fatty acids of the fractionated phospholipids were analyzed by vapor-phase chromatography, and different distributions of fatty acids for each fraction were observed. Two ethanolamine-containing phospholipid fractions were isolated. These differed in the rates of phosphate and acetate incorporation and in fatty acid composition (Rowe, *Biochem. J., 73,* 438). A method was described for the determination of the radio-activity of C^{14}-phospholipids, using a liquid scintillation counter. The incorporation of L-C^{14} serine into mitochondrial phospholipids was studied. CMP, ATP, CoASH, glutathione, glycerophosphate, and magnesium ions were required for optimal incorporation. CTP was less effective than CMP in stimulating the incorporation. Phosphoryl serine did not produce an isotope dilution effect and does not seem to be an intermediate biosynthesis of phosphatidyl serine.

The effects of pH, magnesium, fluoride, and pyrophosphate ions on this system were studied. High concentrations of calcium ions inhibited the incorporation of L-serine whereas low concentrations brought about a marked stimulation in the rate of incorporation. The radio-active phospholipid was identified as phosphatidyl serine by means of mild alkaline hydrolysis and ion-exchange chromatography of the resulting diesters (Hübscher *et al., Biochimica et Biophysica Acta, 36,* 518). The preparation and purification of phospholipase A from aged ox pancreas was described. No phospholipase A activity could be demonstrated in fresh ox pancreas (Rimon *et al., Biochem. J., 71,* 620). A particular enzyme system which decomposes lecithin and lysolecithin into glycerylphosphoryl-choline and fatty acids was isolated from the mucosa of rat intestines. The properties of the enzyme processes were discussed (Epstein *et al., ibid., 71,* 615; Moser *et al., ibid., 234,* 1990). The enzymatic synthesis of sphingosine seems to occur by a reaction involving the addition of palmitic aldehyde to the activated methylene carbon atom 2 of serine in the presence of pyridoxal phosphate and manganese ions. Ethanolamine is not a precursor of sphingosine and does not readily form a Schiff base-metal complex with pyridoxal and nickel ions. The enzyme preparations used in these studies catalyzed the oxidation of palmitic aldehyde in the presence of diphosphopyridine nucleotide. The conversion of dihydrosphingosine to sphingosine has been observed in an enzyme system obtained from rat brain tissue (Brady, *ibid., 233,* 1072).

The enzymatic synthesis of sphingomyelin has been found to occur by the transfer of the phosphorylcholine moiety of cytidine diphosphate choline to the free primary hydroxyl group of a ceramide. The enzyme (PC-ceramide transferase) catalyzing this reaction is highly specific both for cytidine diphosphate choline and ceramide. The sphingosine of active ceramides must have the *trans* configuration of the double bond, and the hydroxyl group on carbon 3 must have the threo relationship to the amino group on carbon 2. Ceramides of dihydrosphingosine are inactive, but derivatives of sphingosine containing a triple bond rather than a double bond at carbon 4 are active if the hydroxyl group on carbon 3 is threo. The enzyme is widely distributed in various animal tissues and is particularly active in chicken liver (Scribney *et al., ibid., 233,* 1315). Structural studies were described on phytoglycolipid, a complex phytosphingosine-containing glycolipid obtained from plant seeds. Alkaline degradation of corn or soybean phytoglycolipid gave, among other products, cerebronylphytosphingosine phosphate and an oligosaccharide which contained inositol, glucosamine, hexuronic acid, galactose, arabinose, and mannose. Acid hydrolysis of the oligosaccharide gave a high yield of a trisaccharide which contained hexuronic acid, glucosamine, and inositol. On the basis of these data a tentative structure for phytoglycolipid was proposed (Carter, *et al., ibid., 233,* 1309).

Lipoproteins. In this section reports concerning any aspect of lipoprotein chemistry and lipids in cellular and subcellular particles are considered.

A study was made of the interaction of human low-density lipoproteins with long-chain fatty acid unions. Five fatty acids were compared both in terms of the absolute values of the association constants and in terms of the relative tightness with which they bind to lipoprotein compared to their binding to albumin. Two lipoprotein fractions were shown to be qualitatively similar in their interaction with the different fatty acids; there was a slight quantitative difference however in that the low-density lipoprotein bound all five fatty acids somewhat more tightly. Increasing the ionic strength was shown to alter the distribution in such a way that relatively more fatty acid became associated with lipoprotein. In contrast, altering the pH within the range to 6.8 to 7.7 had very little effect on the distribution (Goodman *et al., J. Am. Chem. Soc., 81,* 364). It was shown that cholesterol dispersed on Celite dissolved in the presence of whole human or rat serum and of isolated serum lipoproteins. Most of the cholesterol dissolved by serum was associated with lipoprotein fractions. Like cholesterol, some other lipids could be solubilized in serum or serum lipoproteins. Cholesterol incorporated into serum lipoproteins by the described method behaved, when administered intravenously, more like cholesterol incorporated biosynthetically than did labelled cholesterol in the form of a suspension (Avigan, *J. Biol. Chem., 234,* 787). By sedimentation and viscosity measurements it was shown that not more than one albumin molecule interacts with one molecule of Δ^{4}/3-ketosteroid (progesterone). The results of a comparison of the influence on protein interaction of α and β substituents in the steroid molecule were interpreted by assuming an attachment of the protein to the rear side of the steroid ring system (Westphal *et al., ibid., 234,* 2847).

The finding that intestinal mucosal cells incorporated amino acids into proteins with the same electrophoretic mobility as the chylomicron A and B proteins indicated that the intestine was a possible source of the proteins of chylomicrons as well as the protein in the high density lipoprotein fraction. During the disappearance from the plasma of chylomicrons containing labelled proteins, there was an immediate appearance of radioactivity in the high density lipoproteins, suggesting a rapid equilibration of the α-protein with this fraction. The behavior of the labelled β-protein suggested that it disappeared with the chylomicrons and reappeared in the plasma in a small pool of soluble lipoproteins (Rodbell *et al., ibid., 234,* 567). The amount of glycoprotein, expressed in terms of hexosamine concentration, was determined in lipoprotein fractions of 4 human sera, separated in the ultracentrifuge at various densities. On an average, no more than approximately 5% of total serum hexosamine was detected in lipoprotein fractions (Epstein *et al., Proc. Soc. Exptl. Biol. Med., 101,* 740). Different fractions of serum lipoproteins and glycoproteins were determined by paper electrophoresis in aminopterin-treated, folic acid-deficient, and paired fed normal rats. Total lipid, free cholesterol, ester cholesterol, and neutral fat were also estimated in the serum of these animals. In aminopterin-treated animals there was a significant increase in β-lipoprotein and O-fraction, no change in the α-lipoprotein, and increase in total lipid, neutral fat, and free and ester cholesterol. There was no change in the glycoprotein fractions of serum. It appeared that lipid metabolism is much more disturbed than carbohydrate metabolism in folic acid deficiency (Rohatgi *et al., ibid., 102,* 761). It was observed that human low-density S_f 3-9 lipoproteins can be frozen and thawed, stored at —20° C., and heated to 56° C. for 45 min. without altering their immunological characteristics in precipitin ring and agar diffusion tests.

Lipoprotein fractions stored at 4° C. were decomposed by bacterial action. Aqueous merthiolate added to final dilution of 1:10.000 prevents bacterial contamination in lipoprotein fractions stored at 4° C. without altering immunological characteristics of the lipoproteins. Freezing and thawing increased turbidity of lipoprotein solutions but did not alter lipid and protein patterns obtained with paper electrophoresis (Briner *et al., ibid., 101,* 784). The S_f20-400 and the high density lipoproteins of human sera were degraded by partially extracting their lipid content with ethyl ether. The resultant degradation products were analyzed both chemically and ultracentrifugally. In the case of the S_f 20-400 class lipoproteins, the ether extracted more than 60% of the total lipids. The composition of the extracted lipids (mostly glycerides) was approximately constant for each of four successive extractions. On the other hand, the main lipid constituent of the lipoprotein fragments was phospholipid. In the case of the high-density lipoproteins, the ether extracted only a very small amount of lipids. In spite of this resistance to ether degradation an essentially lipid-free protein fragment was produced, the molecular weight of which was calculated to be approximately 40,000 (Hayashi *et al., J. Am. Chem. Soc., 81,* 3793). An ultracentrifugal method described provides a means for determining all major classes of lipoproteins in serum. The serum is added to a solution of sodium bromide and centrifuged at 40,000 r.p.m. for 24 hrs. at 18° to 20° C. The procedure is rapid and inexpensive and requires only 2 milliliters of serum (Del Gatto *et al., Anal. Chem., 31,* 1397).

The average lipid composition of the three broad lipoprotein classes in the serum of nine fasting adults was determined. Great variations were found in glyceride, free cholesterol, and cholesteryl esters because of differences in lipoprotein distribution. In the S_f 20-400 band the dominant lipid was glyceride. Analysis of narrow band S_f 6-8 lipoprotein fractions showed greater uniformity of composition although considerable variability remained. There were lipoproteins of high S_f values by glyceride hydrolysis and fatty acid removal. A low-density lipoprotein model with a simple glyceride core containing small amounts of cholesterol, cholesteryl ester, and phospholipids was proposed. With an electron microscope, studies indicated that these lipoproteins (S_f 6-8) are composed of two or three asymmetrical submits approximately 350° A. in length with molecular weights from 2.8 to 3.1 x 10^6. Calculations from dissymmetry data yielded prolate ellipsoids approximately 160 x 360 $\Delta^{\prime}$A. rather than the spherical shape given for chemically isolated lipoprotein (S_f 2-10) (Lindgren *et al., Ann. N.Y. Acad. Sci., 72,* 826). It was shown that by electrophoresis on paper of human serum in barbital buffer of pH 8.6, the α and β-lipoproteins are reduced to only one line (stained by Sudan Black B) when 0.2% (a nonionic detergent) is added to the buffer solution. This decrease of the α-lipoprotein and the disappearance of the α-globulin, as previously shown by Downs *et al., (C.A., 52,* 647*d),* seem to be related. Sodium oleate in place of Sterox did not produce the effect (Cardoso *et al., Rev. brasil. biol., 19,* 43). The polyunsaturated acid contents of the total fatty acids of particulate enzyme preparations were determined by alkaline isomerization. The polyunsaturated fatty acids varied from one-third to two-thirds of the total fatty acids. In one case the polyunsaturated acids were found to be 34.9% by weight. Fractionation of the mitochondria into separate submitochondrial particulate fractions possessing different enzyme activities did not fractionate the polyenoic acids. Analyses for the fatty acids from cytochrome oxidase and interchain lipoprotein were given (Holman *et al., J. Biol. Chem., 234,* 2269).

Further studies have been reported in the role of lipids, lipoproteins, and coenzyme Q in the electron transport system. Digestion of the electron transport particle with phospholipase A resulted in the loss of its oxidative capacity. Evidence was presented to indicate that this is primarily due to the cleavage of the phospholipid-cytochrome *c* complex within the mitochondria (Ambe *et al., Science, 129,* 98). A number of surface-active agents were tested in combination with sodium deoxycholate for the purification of cytochrome oxidase. From among these agents, only sodium cholate improved the spectrum. The resulting preparation had however a markedly reduced cytochrome oxidase activity. Reactivation was accomplished by adding either a 2% deoxycholate extract of heart muscle particles or any one of a number of crude and partially purified phospholipids. The most active preparation was a phosphatidyserine fraction from brain (Greenless *et al., J. Biol. Chem., 234,* 658). The isolation of lipoflavoprotein from beef heart muscle mitochondria was described. Lipid accounted for 85 to 88% of the dry weight of the enzyme. The flavin (flavin adenine dinocleotide) content of the flavoprotein was 13.6 mimoles per mg. of protein which corresponds to a minimal molecular weight of 74,000 in terms of protein and 474,000 in terms of both protein and lipid. Both the Straub diaphorase and Mahler's cytochrome *c* reductase appeared to be derived from this lipoflavoprotein. A method of fractionating lipoproteins with organic solvents was described (Zeigler *et al., ibid., 234,* 1916). Many species of animals, plants, and microorganisms have been examined for the presence of coenzyme Q and related lipids. It was found that coenzyme Q is widespread in nature but not ubiquitous. As a rule, in highly aerobic tissues, there occur large amounts of coenzyme Q. It was suggested that, in those tissues which are apparent exceptions to this rule, a different electron transport mechanism, possibly utilizing other quinones such as vitamin K, is operative (Lester *et al., ibid.,* 234, 2169).

Other studies describing the chemical and physical properties of the coenzyme Q family of compounds were reported (Lester *et al., Biochim. Biophys. Acta, 33,* 169). A further study gave the properties of a soluble lipoprotein dissociated from the succinic dehydrogenase complex (Basford *et al., ibid., 185).* The lipid composition of coenzyme Q lipoprotein was reported

(Basford, *ibid., 195*). Procedures have been described for the isolation of three crystalline compounds of the coenzyme Q family. One of these compounds was isolated from the non-saponifiable fraction of cells of *Azotobacter vinelandii*. Two distinct compounds were isolated from *Torula utilis* by direct solvent-extraction with or without saponification. Chromatographic procedures, followed by crystallization techniques, are used for the final purification of these three compounds (Lester *et al., Biochim. Biophys. Acta, 32,* 492). Coenzyme Q (Q 275) has been isolated from beef heart and beef heart mitochondria (Crane *et al., Biochim., ibid., Biophys., 32,* 73). Coenzyme Q can be extracted from the electron transport particle and other particles with *iso*-octane. Such extracted particles lose the capacity to oxidize succinate, and this capacity can be restored by the addition of coenzyme Q and other lipid supplements. The coenzyme role of Q275 is highly specific. A large number of quinones including vitamin K_1 and tocopherol quinone were unable to replace coenzyme Q. No evidence could be found that coenzyme Q is a component of the DNPH chain. On the other hand, cytochrome *c* is also released in an insoluble form ETP by *iso*-octane, and the addition of cytochrome *c* fully restores succinoxidase activity of the *iso*-octane-extracted particle. The requirement for both cytochrome *c* and coenzyme Q in oxidation of succinate can be demonstrated by treating ETP with deoxycholate and *iso*-octane (Crane *et al., ibid., 31,* 476). The sequence of components in the succinic chain of the Mitochondrial electron transport system has been described (Green *et al., Arch. Biochem. Biophys., 85,* 280). The effects of adenosine diphosphate and inorganic phosphate on the steady-state oxido-reduction level of coenzyme Q has been reported (Hatefi, *Biochem. Biophys. Acta., 31,* 502). The isolation of a soluble form of cytochrome c_1 has been described. Cytochrome c_1 can be extracted in reasonable yields only from particles which are free of cytochrome *a*. Such particles when exposed to butanol in the presence of deoxycholate and ammonium sulfate liberate cytochrome c_1 in either of two soluble forms and in yields of as high as 75%. Cytochrome c_1 can also be isolated in the form of a soluble lipoprotein complex. This complex contains about 50% by weight of lipid. Duponol efficiency separates the hemoprotein from the lipoprotein with which it is complexed (Green *et al., ibid., 31,* 34). The net synthesis of cytochrome *c* in calf-heart mitochondria was reported (Bates *et al., ibid., 32,* 597).

Decreased lipoprotein lipase response in obstructive jaundice in man was confirmed by complete ligation of the common bile duct in dogs. Subsequent increased lipoprotein lipase response in these dogs, after chronic complete cholestasis of 4 to 10 weeks, was attributed to the development of biliary cirrhosis. While lipoprotein lipase response was generally inversely proportional to total serum lipids, cholesterol, and lipid phosphorus levels, this relationship was by no means absolute. Possible pathogenetic mechanisms which decreased lipoprotein lipase response in obstructive jaundice were discussed (Baker *et al., Proc. Soc. Exptl. Biol. Med., 101,* 464). Many studies indicate that unsaturated fats may lower serum cholesterol whereas saturated fats of animal origin usually increase it. The mechanism of these differing actions is unexplained however. It has also been demonstrated that alimentary lipemia is cleared largely by an enzymatic lipolytic mechanism in which heparin is involved (heparin lipoprotein lipase). In one paper differences in hydrolysis of saturated and unsaturated fats by the heparin lipolytic factor were reported (Engelberg, *Circ., 19,* 884). Plasma samples obtained from rats which had been injected with dextran sulphate were found to contain active lipase. Inhibition of clearing activity by the high-molecular-weight dextran sulphates was thought to be caused by their interaction with the chyle (Robinson *et al., Biochem. J., 71,* 286). Isotonic sucrose solutions of carbon tetrachloride and other fat solvents produce an extensive transformation in adenosine-triphosphatase properties of rat liver mitochondria *in vitro*. The effectiveness of the fat solvents appears to be related inversely to their water solubilities. Magnesium-activated adenosinetriphosphatase activity was elicited by carbon tetrachloride only in the mitochondrial fraction and was associated with insoluble mitochondrial material. The effects *in vitro* on adenosinetriphosphatase properties produced by carbon tetrachloride were accompanied by loss of pyridine nucleotide-dependent oxidative function. Carbon tetrachloride treatment *in vitro* had no effect on three microsomal enzymes. The same signs of mitochondrial degeneration produced by carbon tetrachloride *in vitro* were observed *in vivo* after carbon tetrachloride administration to rats (Rechnagel *et al., J. Biol. Chem., 234,* 1052). The conversion of Δ^7-cholesterol-H^3 to cholesterol by cell-free homogenates of rat liver was demonstrated. Up to 15% of the added substrate was converted (60% if it is assumed that three-fourths of the label is lost). The cellular factors necessary for the conversion were found to reside in the 105,000 x *g* supernatant and in the microsomes. Of the latter, the sediment collected at 30,000 x *g* after removal of the mitochondria at 9000 x *g* proved most active. The conversion was not inhibited by cholesterol feeding (Frantz *et al., ibid., 234,* 2290.

Acetate-$2C^{14}$ was administered to normal and partially hepatectomized rats, and to rats bearing a transplanted adenocarcinoma; and the incorporation of radio-activity into the liver and tumor lipids was measured. The data suggested an altered phospholipid metabolism in the tumors over that seen in mitotic phase liver regeneration and indicated that the relative availability of fatty acids and glycerol for phospholipid metabolism as compared to glyceride metabolism is dependent upon the physiological state of the tissue under consideration (Johnson *et al., ibid., 234,* 22). The oxidation of albumin-bound palmitate-1-C^{14} by rat liver and adipose tissue has been demonstrated *in vitro*. Normal adipose tissues are about one-eighth as active as equal wet weights of liver. Prolonged fasting and alloxan diabetes depleted the fat depots and markedly elevated their capacities for fatty acid oxidation. Livers from such animals show normal rates of oxidation. Insulin administration depressed oxidation of the fat complex in the liver, but it had no significant effect on its catabolism in adipose tissue (Milstein *et al., ibid., 234,* 19). The removal of palmitate-1-C^{14} and cholesterol-4-C^{14} by the isolated perfused rat liver was studied. From 34 to 43% of the C^{14} in the incoming perfusate was removed in a single passage through the liver. The loss of C^{14} from the perfusate was similar regardless of whether the liver-donor rats had fasted or had been fed. The addition of large amounts of glucose to the perfusate did not affect the C^{14} disappearance. Of the C^{14} that disappeared from the perfusate, 58% was recovered as phospholipids and triglycerides in the liver (Hillyard *et al., ibid., 234,* 2240). The latent phase of necrotic liver degeneration in the rat, produced by dietary deficiency of vitamin E and Factor 3, is characterized by respiratory decline, *i.e.*, a failure of respiration of liver slices in the Warburg. It has been shown that tocopherol readily reverses this defect when injected intravenously but not when added to the Warburg medium. The *in vitro* effect of various tocopherol derivatives on respiratory decline was investigated (Simon, *Biochem. Biophys. Acta, 32,* 484). Vitamin K substances and FMN appear to catalyse separate pathways of cyclic photophosphorylation. The FMN pathway shows a dependence on added TPN and greater sensitivity to inhibition by dinitrophenol and *o*-phenanthroline than the vitamin K pathway. Both pathways are inhibited by *p*-chloromecuribenzoate, gramicidin, and methylene blue but not by arsenite or antimycin A. The role of vitamin K in phosphorylations by plant and animal tissues is reviewed. A possible physiological role for cyclic photophorylation in photosynthesis of green plants has been suggested (Whatley *et al., ibid., 32,* 32).

The Vitamins. Reports cited in this section include those on nutritional and metabolic aspects of the fat-soluble vitamins and also those on the effects of water-soluble vitamins on lipid metabolism. The addition of unsaturated fatty acids, even in small amounts, to diets low in vitamin E, selenium, and the sulfur-containing amino acids greatly accelerated the development of acute liver necrosis in rats (Valberg *et al., Can. J. Biochem. and Physiol., 37,* 493). Codliver oil showed 10 mg. of DL-α-tocopherol per 100 g. by chemical tests. Other forms of tocopherol were not present. Even the inclusion of 10% codliver oil in the diet of rats failed to prevent the abnormalities caused by avitaminosis E. These were readily prevented by a small weekly dose of DL-α-tocopherol acetate. The failure of whole codliver oil to act as a good source of vitamin E in accordance with the results of chemical tests is explained by the antagonistic action of its other components, particularly its highly unsaturated fatty acids (Moore *et al., Brit., J. Nutr., 13,* 100). Chops from swine fed either animal or plant protein, and 0, 6, or 12 g. daily of a Vitamin E feed supplement for a period of 2, 4, or 6 weeks, were analyzed fresh and after 3, 6, 9, and 12 months of frozen storage. Feeding 12 g. of supplement was more effective than 6 g., and a 6-week period was more effective than either a 4- or a 2-week period in retarding peroxide development (Zachringer *et al., Food Techn., 13,* 313). Addition of glucose-6-phosphate or glucose-1-phosphate did not prevent the depletion of glycogen supplies in skeletal muscle treated with deoxycorticosterone. These enzymes concerned with the oxidation of glucose-6-phosphate to lactic acid seemed to remain unimpaired in the presence of deoxycorticosterone. Similar finds were observed for both Vitamin E-supplemented and Vitamin E-deprived rabbits (Rosenkrantz, *J. Biol. Chem., 234,* 35).

Two groups (males and females) of weanling rats were given a Vitamin E-free basal diet for 127 days with a to-

copherol supplement. Two similar groups were given the unsupplemented diet and, when killed, were Vitamin E-deficient. The unsaponifiable fractions from liver, heart, and kidney were chromatographed on alumina. Ubiquinone contents of approximately 120, 110, 230, and 25 μg./g. were found for liver, kidney, heart, and testes. The Vitamin E status had no striking influence on the amounts of hydrocarbon, sterol, or ubiquinone obtained from the different tissues (Morton *et al., Biochem. J., 73,* 427). Chicks were fed two different muscular dystrophy-producing diets, with and without Vitamin E, and determinations of glycogen, phosphorylase, dry matter, ash, sodium, potassium, and creatine were made on the breast (white) and leg (red) muscles after 4 to 5 weeks of feeding. Gross lesions in the dystrophic muscle were more apparent in the white than in the red muscle although microscopic detection of lesions in the latter was possible. The changes in creatine and potassium content of dystrophic muscles were variable, and any decrease in these constituents in the dystrophic muscles could be explained mainly on the basis of increased water content. The sodium content of dystrophic muscles was increased when expressed on either a wet or dry weight basis (Nesheim *et al., J. Nutr., 68,* 359).

Large supplements of *alpha*-tocopherol failed to prevent the muscular dystrophy of aging rats. Other chronic diseases, longevity, and growth were also unaffected (Berg, *J. Gerontology, 14,* 174). The gastrocnemius muscles of 30-day-old guinea pigs subjected to a Vitamin E-deficient diet for 21 or 30 days underwent a statistically protein nitrogen. Masseter muscles were more resistant to the effects of Vitamin E-lack on the protein components. Sarcoplasmic nitrogen was not altered by 21- or 30-day dietary deficiency regimens. (Bender *et al., Proc. Soc. Exptl. Biol. Med., 102,* 362). A marked decline in myoglobin concentration was demonstrated in gastrocnemius and masseter muscles of guinea pigs maintained longer than 15 days on a Vitamin-E-deficient diet. Supplementation with Vitamin E after 14 days on a deficient diet reduced the magnitude of concentration change and indeed stabilized pigment concentration at control levels. No significant difference between control and experimental animals in regard to water content, muscles weight, or body weight was evident from 21- and 30-day dietary regimen studies. Noncollagen was significantly depleted only in the gastrocnemii of the 21-day deficient animals (Schottelius *et al., ibid., 102,* 581). Administration of α-lipoic acid to guinea pigs on a scorbutogenic diet appeared to afford about the same protection as a suboptimal amount of ascorbic acid. When a small amount of ascorbic acid and of α-lipoic acid were given, the combination protected the guinea pigs better than either compound alone. Male and female rats reared since weaning on a diet low in Vitamin E did not reproduce. Administration of α-lipoic acid brought about reproduction although not to the same extent as that attained by an optimal amount of α-tocopherol. The efficacy of α-lipoic acid in alleviating symptoms of ascorbic acid and of tocopherol deficiencies can best be explained on the basis of a protective action exerted by α-lipoic acid or its dihydro-derivative on the two vitamins (Rosenberg *et al., Arch. Biochem. Biophys., 80,* 86). Tocopherol was shown to activate the *in vitro* synthesis of ascorbic acid by liver extracts of tocopherol-deficient rats (McCay *et al., Arch. Biochem. Biophys., 82,* 472).

Liver preparations from Vitamin E-deficient rats and rabbits were shown to have an inhibited rate of ascorbic acid synthesis and an increased lipid peroxide-forming activity. The agents which activated ascorbic acid synthesis inhibited the formation of peroxides. The addition of synthetic organic peroxides inhibited the synthesis of ascorbic acid. Succinic and tertbutyl peroxides greatly enhanced the destruction of ascorbic acid. Peroxides produced by irradiating cod liver oil and linoleic acid did not show a clear inhibition of ascorbic acid synthesis (Carpenter *et al., J. Biol. Chem., 234,* 2814). Added tocopherol was effective both in inhibiting lipid peroxidation and stabilizing DPNG-cytochrome *c* reductase in isolated mitochondria. Vitamin E-deficient rabbits had livers showing *in vivo* lipid peroxidation and increased liability of their isolated mitochondria. It was suggested that this liver damage may be a primary cause of other Vitamin E-deficiency syndromes (Tappel *et al., Arch. Biochem. Biophys., 80,* 333). The DPNH-cytochrome *c* reductase activity was determined in heart-muscle preparations from normal and Vitamin E-deficient chicks. Although no α-tocopherol was demonstrable in the deficient preparations, the enzyme activity was not significantly different from the control preparations which contained α-tocopherol. Substances which react with $FeCl_3$-bipyridyl reagent, other than α-tocopherol, were found in Florex-treated extracts of unsaponifiable matter from tissues. No unequivocal evidence was obtained for the presence of tocopherylquinone in tissues (Pollard *et al., Biochem. Biophys. Acta, 35,* 420). The decline in succinate oxidation in the presence of DPN was observed to be greater in liver mitochondria from rats deficient in Vitamin E than it was in mitochondria from Vitamin E-supplemented animals. The decline in the deficient mitochondria was reversed in 30 to 40 min. after α-tocopherol was given by intraportal injection but not by addition *in vitro* (Corwin *et al., J. Biol. Chem., 234,* 191).

Through gas-liquid chromatography it was shown that increasing the ingestion of linoleic acid-containing fats increased the depostion of linoleic acid in erythrocytes and in brain tissue. Such changes were probably casually related to the tocopherol requirement, the incidence of chick encephalomalacia, and the peroxide hemolysis test. Whether similar mechanisms are involved in the ability of unsaturated oils to lower serum cholesterol levels was not determined (Horwitt *et al., Science, 130,* 917). Corn oil or lard, from which tocopherol had been removed, promoted chick encephalomalacia whereas coconut oil, butter, linseed oil, and cod liver oil did not produce symptoms, and olive oil had a questionable effect. Dietary combinations of 2% corn oil with either 8% coconut oil, lauric acid, myristic acid, or a mixture of saturated fatty acids like coconut oil significantly increased the incidence of encephalomalacia over 2% corn oil alone while 6% linseed oil, cod liver oil, or oleic acid inhibited the effect of 4% corn oil. Olive oil, butter, fatty acids like butter, palmitic acid, and stearic acid had no net effects upon the incidence of encephalomalacia induced by corn oil. The intake of linoleic acid appears to be a primary factor in the etiology of encephalomalacia, but some of the other fatty acids may secondarily increase or decrease this effect (Century *et al., Proc. Soc. Exptl. Biol. Med., 102,* 375). A casein cerelose purified diet low in Vitamin E, containing 3% unsaturated fish oil, failed to induce Vitamin E-deficiency symptoms in either depleted or nondepleted chicks. When the cerelose was replaced by degerminated white corn meal, both depleted and nondepleted chicks developed encephalomalacia. Water washing of the white corn meal was without effect, but solvent extraction of degerminated white corn meal decreased the incidence of encephalomalacia to a low level in some unknown manner. When corn starch served as the carbohydrate source in this diet, symptoms of Vitamin E deficiency were not observed (Amato *et al., Poultry Sci., 38,* 176). Concomitant with the onset of exudates in chicks fed a diet deficient in Vitamin E and selenium, changes occurred in the electrophoretic patterns of the serum proteins. The decrease in albumin: globulin ratio did not appear to be of sufficient magnitude to account for the edema. Total serum protein declined only slightly, if at all. The most marked alterations in protein patterns developed after the chicks had spontaneously recovered, when increases in α_{2}-, α_{3}-, β- and Y- globulins occurred (Bieri *et al., J. Nutr., 69,* 301).

Curative tests of Vitamin E activity of ubiquinone, its hydroquinone and acid reduction product in relation to muscular dystrophy in the rabbit were negative. Similarily solanachromene exhibited no biological activity with respect to dystrophy in rabbits or sterility in rats. Vitamin E-deficient dystrophic rabbits underwent a remission of gross symptoms within approximately 5 days following oral treatment with N, N′-diphenyl-p-phenylenediamine. The elevated plasma concentration of unesterified cholestehol declined to near-normal levels within three weeks while skeletal muscle concentration responded more slowly. The results confirmed previous findings, indicating that the Vitamin E requirement of rabbits and rats represents a need for suitable antioxygenic compounds (Draper, *Proc. Soc. Exptl. Biol. Med., 102,* 737).

The effects of furazolidone on turkey reproduction when fed continuously at levels of 0.011% and 0.022% from 10 weeks of age were studied. Two levels of Vitamin E (a basal with approximately 6 I.U./lb. and basal supplemented with 34 I.U./lb.) were also included in one trial to study any possible relationship between the vitamin and furazolidone in respect to fertility, hatchability, and egg production (Smyth *et al., Poultry Sci., 38,* 288). Thirty-six Holstein male calves, 64 days of age and partially depleted of their tocopherol and Vitamin A stores, were fed, in addition to a basal ration low in tocopherol and Vitamin A, one of the three levels of tocopherol acetate, equivalent to 1, 5, or 25 mg. of free tocopherol per pound of live weight per day and one of three levels of Vitamin A palmitate, equivalent to 10, 100, or 1,000 Y of Vitamin A alcohol for a four-wk. period. Upon completion of a 28-day tocopherol-Vitamin A supplementation period, one-half of the calves were slaughtered and the other 18 calves were maintained on only the basal ration until plasma Vitamin A decreased to 4.0 Y/100 ml. Based on plasma and liver Vitamin A concentration at the termination of supplementation and on Vitamin A at the highest intake of Vitamin A, decreases at the lowest intake of Vitamin A, and inappreciable change at the middle intake. Across all levels of tocopherol intake

the tocopherol concentration of the tissues decreased with the increasing intake of Vitamin A. This decrease was greater between the 10 and 100 Y intake of Vitamin A than between the 100 and 1,000 Y intake. These results indicated a need to establish levels or ratios of intake of Vitamins A and E (Dicks *et al., J. Dairy Sci., 42,* 501). Low blood plasma tocopherol levels were found in calves fed corn oil-filled milk despite a relatively high tocopherol intake. Both plasma tocopherol and Vitamin A values declined progressively during the experimental period in the corn oil-filled milk group. Low blood plasma tocopherol levels also occurred in animals fed lard-filled milk and a ration containing butter oil, which was prepared from oxidized butter. Oral supplementation of the corn oil and lard-filled milk diets with 500 mg. of DL-*alpha* tocopherol or *alpha* tocopherol acetate daily resulted in high plasma tocopherol values (Adams *et al., ibid., 42,* 1569).

In a study on the metabolic pathway of carbon-14-labelled α-tocopherol, a firmer basis for the use of accurate dosages of *alpha*-tocopherol was established (Sternberg *et al., Can. Med. Assoc. J., 80,* 266).

Studies were reported on the transport of carotenoids, Vitamin A, and cholesterol across the intestines of rats and chickens (Genguly *et al., Biochem. J., 71,* 756). An important factor in the differential utilization of carotene in oils is Vitamin E. Another factor is hydrogenation. In most Indian diets the edible oils are used for frying at a high temperature for the preparation of vegetable diets. It was found that frying markedly reduced the utilization of carotene subsequently added to the fried oils. Frying evidently caused the development of compounds which interfered with the proper utilization of Vitamin A. Coconut oil, which is devoid of Vitamin E, gave the best response (Rangnekar, *Sci. and Culture (Calcutta), 24,* 330). *Beta*-Carotene, given orally to chicks as an aqueous suspension containing Tween 60, appeared in the intestinal tissue as Vitamin A within ½ hr., following administration of the dose, and in the liver in about 1 hr. The peak level occurred in the intestinal tissue of approximately 4 hrs., in the liver at approximately 8 hrs. A very small but definite deposition of carotene occurred in the liver, but this disappeared within 24 hrs. *Beta*-Carotene administered by cardiac injection in the form of a suspension in chick plasma was apparently not converted to Vitamin A by the chick. About 1/3 of the injected carotene was found in various organs of birds killed during the first 2 hrs. after injection, and this amount decreased to about 3% after 4 days. No carotene was found in the circulating plasma 1 hr. after injection or at any subsequent sampling to 21 days (Olsen *et al., Poultry Sci., 38,* 688).

The conversion of β-carotene to Vitamin A in a ligatured duodenal loop of a living chick has been found to take place in the duodenal wall. Some factor(s) present in a duodenal loop of a living bird but absent in an excised loop appear to be essential to the absorption of β-carotene by duodenal tissue. It has been shown that the continuity of the alimentary canal is not essential to the successful operation of the carotene-converting mechanism. When the blood supply to a duodenal loop is ligated, the formation of Vitamin A from β-carotene is prevented (Sibbald *et al., ibid., 38,* 701). It would appear that the crop does not possess the ability to convert β-carotene to Vitamin A within a 4-hr. period even though carotene may be absorbed by the crop wall in the presence of bile (Sibbald *et al., ibid., 38,* 698). Four experiments were conducted to compare the availability to chicks, and stability during storage in mixed feeds, of several commercial dry Vitamin A products, cod liver oil, and dehydrated cereal grass, using liver storage of Vitamin A, growth, and mortality as criteria. The results indicated that, in short term experiments, growth may not be a reliable criterion for evaluation of Vitamin A availability. Judging by liver storage of Vitamin A, gelatin-coated preparations were superior to preparations in which the vitamin was coated with wax or fat or was absorbed in vegetable protein. Feeding oils and dehydrated cereal grass were the poorest sources. The data from stability tests indicated some deterioration of the vitamin in all preparations, and, although definite conclusions were difficult to draw, the superiority of gelatin preparations was indicated (Olsen *et al., ibid., 38,* 929).

A diet deficient in Vitamin A and carotene was fed to 10 White Leghorn cockerels at 10 mos. of age; five others were used as controls. The adverse changes are not irreversible, and the administration of 30,000 I.U. of Vitamin A per kgm. of food resulted in complete recovery in two weeks. It was shown that in cockerels alterations in the semen are first signs of Vitamin A-deficiency (Paredes *et al., ibid., 38,* 3). Intermittent feeding of Vitamin A, as compared with feeding the nutrient continuously, resulted in no significant difference in chick growth. Growth was significantly better in lots receiving chemical Vitamin A material than in those lots receiving dehydrated alfalfa meal. Feed conversion, in general, was better with continuous Vitamin A feeding. More Vitamin A-containing feed was consumed than the basal ration even though each was offered for the same length of time (Hastings *et al., ibid., 38,* 385). Oral administration of 100,000 I.U. of Vitamin A acetate for 4 to 6 months significantly reduced the elevated serum cholesterol levels in atherosclerotic patients but had no effect on individuals with normal cholesterol levels (Kinley *et al., Proc. Soc. Exptl. Biol. Med., 102,* 353). The influence of the protein and energy level of the diet on the utilization of Vitamin A and carotene, as judged by liver storage, was studied in two experiments. The findings showed an inverse relationship between protein level and vitamin storage, indicating a higher Vitamin A requirement at a higher level of protein. Increasing the energy level resulted in better growth and a greater storage of the vitamin in the liver. However this increased storage may not be due to the higher energy level as such, but to the higher fat content of the diets. The diet variations had little effect on the composition of breast muscle except that the fat content of the muscle of birds on low-energy diets was the lowest of the various groups. Judging from growth data, the Vitamin A requirement of the birds in these experiments was met by a level of 600 I.U. per pound of diet (Olsen *et al., Poultry Sci., 38,* 942). When the intake of Vitamin A was controlled, rats were able to store an ample amount of Vitamin A in the liver despite amino-acid imbalance or in complete absence of dietary protein. If the vitamin intake was not controlled however, the lack of or imbalance in the dietary protein seemed to result in a lower storage of Vitamin A because under these conditions the animals usually lose appetite, a trend which will result in lowered consumption of Vitamin A (Recheigl Jr. *et al., Nature, 184,* 1404).

Four experiments involving 226 pigs carried out over a period of two years were described. These pigs were from sows reduced considerably in their Vitamin A reserves by previously feeding a ration low in Vitamin A. They were weaned at seven days of age, and the requirement for Vitamin A was estimated in the first eight weeks of life. Several criteria of adequacy were investigated and their sensitivity, precision, and validity discussed. These criteria were weight gain, feed efficiency, blood plasma and liver Vitamin A, and cerebrospinal fluid pressure. The minimum requirement of the young pig for a stabilized source of Vitamin A palmitate on a dry carrier was judged to be 800 I.U./lb. of feed under the conditions which existed. Normality in weight gain occurred at as low as 100 I.U./lb. of feed. Acute paralysis of the hindquarters was the most striking deficiency symptom (Frape *et al., J. Nutr., 68,* 173). Thyroid function was investigated in the young pig. It was shown that dietary Vitamin A within the range tested has considerable influence upon the rate of thyroxine secretion. Insufficient and excessive intakes of Vitamin A lowered the rate of secretion. It was shown later that the relationship between this secretion rate and growth rate in the pig is rather small so that a more direct effect of Vitamin A upon thyroid function is postulated. When thiouracil was used, the rate of thyroxine secretion was shown to increase with time (Frape *et al., ibid., 68,* 333).

The occurrence of *iso-a* and *iso-b*, the 6-*cis*, and the 2, 6-di-*cis* isomers of Vitamin A in a number of fish liver oils was investigated by means of reaction with the retinal protein, opsin. These isomers were found to constitute about 20% of the Vitamin A in the cod, shark, and mixed fish liver oils examined. As measured by rat bioassay, the isomers had only about one-fourth the growth-promoting activity of all-*trans* Vitamin A. The possibility of the isomers originating from *in vivo* isomerization of all-*trans* or neovitamin A to form an equilibrium mixture was discussed (Brown *et al., Nature, 184,* 1377). It was observed that carrot extracts can form significant amounts of carotene from glucose as well as from acetate although the acetate was shown to be a somewhat superior substrate. Yeast extract stimulated synthesis in both media, possibly because of the presence of coenzyme A and adenosine triphosphate in the yeast (Modi *et al., ibid., 184,* 983). It has been shown that rhodopsin is synthesized from 11-*cis* retinene (Vitamin A aldehyde) but releases all-*trans* retinene when bleached by light. In the frog both isomers of Vitamin A are stored in the eye. Total ocular Vitamin A, including that bound as retinene in rhodopsin, remains constant during light and dark adaptation. Stores of 11-*cis* Vitamin A however diminish in the light and are replenished in darkness (Hubbard *et al., Science, 130,* 977). The biological activity of 11-*cis*-Vitamin A in the rat has been shown to be caused by its conversion predominantly into the all-*trans* form in the small intestine and at other sites (Plack, *Brit. J. Nutr., 13,* 111).

The rises in ubiquinone and substance concentration SC which occur in the livers of Vitamin A-deficient rats may be

hastened if at an early stage in the deficiency the rats are bilaterally adrenalectomized. If however the deficiency syndrome is already far advanced, adrenalectomy does not noticeably influence the concentrations of minor constituents of liver unsaponifiable matter. In rats on a stock diet adrenalectomy brought about no striking changes in liver Vitamin A (range 137-216 i.u./g. of liver) ubiquinone content (range 110-133 μg./g.) or sterol content (range 1.80-2.23 mg./g.). Substance SC was not present in more than trace amounts (Phillips *et al., Biochem. J., 73,* 430). Weanling rats placed on a Vitamin A-deficient diet reached a weight plateau after a variable time. Groups of deprived animals were killed at 5, 11, 20, 28, and 39 days while still growing; other groups were killed at the weight plateau and others at a time when they were losing weight. Chromatographic examination of liver unsaponifiable matter showed a rise in sterol content only at or beyond the weight plateau; a rise in substance SC which became marked when growth had slackened or ceased; and a progressive rise in ubiquinone (Morton *et al., ibid., 73,* 416). The preparation of homogenates of tomatoes which can synthesize radio-active lycopene from 2-C^{14}-mevalonic acid was described. For optimal incorporation of tracer, ATP, pyridine nucleotides, glutathione, manganese ion, and incubation in air are necessary (Schneour *et al., J. Biol. Chem., 234,* 770).

Weanling rats kept for 29 or 36 days on a diet containing 1% of sulphadiazine showed, as compared with control rats, small livers, kidneys, intestines, and very much smaller spleens. Weaning rats kept for 26 days on a diet containing dicoumarol (30 mg./kg.) differed from control rats slightly but significantly in body weight. No other signs of Vitamin K deficiency were seen. Rats which had been on a Vitamin A-deficient diet for 45 days after weaning had exhausted their liver reserves of Vitamin A, and the liver concentration of sterol, ubiquinone, and substance SC had all risen (Morton *et al., Biochem. J., 73,* 421). Consumption of diets containing *gamma* ray-irradiated (2.79 or 5.58 x 10^6 rad) beef resulted in internal hemorrhages and prolonged prothrombin times in growing male rats. Generally the female rat did not show this syndrome. The lesion was induced by freshly irradiated beef as well as irradiated beef which has been stored for more than six months at room temperature. Supplementation with Vitamin K prevented the hemorrhagic diathesis in rats consuming irradiated beef (Metta *et al., J. Nutr., 69,* 18). Experiments were conducted on factors affecting the Vitamin K requirement of chicks 30 to 34 days of age. The onset of prolonged blood-clotting time was advanced and the time required for clotting increased by supplying excessive quantities of sulfaquinoxaline (0.1% to 0.125%) as a stress factor. The number of hemorrhages was greater, the mortality was increased, and the weight gains were reduced in the presence of the drug (Nelson *et al., Poultry Sci., 38,* 1094).

A diet containing 65% sucrose with added cholesterol resulted in hypercholesteremia and increased tissue cholesterol deposition in rats. Ten per cent corn oil with added cholesterol in Purina laboratory chow had no apparent effect on the serum total cholesterol but increased cholesterol deposition in tissues. Ovariectomy was without significant effect. Thyroidectomy or hypophysectomy aggravated the hypercholesteremia and the increased tissue cholesterol deposition induced by the sucrose diet. The effect of hypophysectomy was not due to the loss of thyrotrophic hormone alone. Addition of Vitamin D aggravated hypercholesteremia and increased liver cholesterol deposition in normal, thyroidectomized, or hypophysectomized rats fed the sucrose diet. In ovariectomized rats the tissue cholesterol deposition was also augmented. When corn oil diet was fed, the supplementary Vitamin D increased the serum total cholesterol level of the thyroidectomized rat (Lee *et al., Circulation Research, 7,* 354). The response of total liver lipids, phospholipid, plasmalogens, and cholesterol to thiamine deficiency was studied in the rat. Neutral lipid, except for cholesterol, fell rapidly to well below the normal control levels. Phospholipids were mainly unaffected during the deficiency, and plasmalogens showed a tendency to be maintained at a high level regardless of the deficiency. Cholesterol was higher in thiamine-deficient rat liver than in the normal controls. Sudden reintroduction of thiamine by injection caused total lipids to rebound to a high normal level and cholesterol, when expressed in terms of body weight, to reach a level almost twice that of normal. Phospholipids and plasmalogens followed patterns after thiamine injection that can be explained in terms of the maintenance of important cellular components that resist dietary changes (Williams Jr. *et al., J. Nutr., 69,* 229). Riboflavin deficiency was produced in cats fed *iso*-nitrogenous purified diets varying in carbohydrate and fat content and containing different quantities of riboflavin. In a number of instances cataracts developed in cats receiving amounts of riboflavin slightly below their minimal requirement. Fatty livers and testicular hypoplasia were present as well as skin changes which were less dramatic than in other species. Anemia and central nervous system changes were not observed. High-carbohydrate, low-fat diets appeared to exert a sparing influence on the cats' riboflavin requirement (Gershoff *et al., ibid., 68,* 75).

Rats fed a ration containing 20% of fat and varying levels of riboflavin had a higher concentration of cholesterol in liver than did corresponding animals fed a ration containing 5% of fat. An increase of supplementary riboflavin from 10 to 30 μg or 30 to 100 μg per day decreased the deposition of cholesterol in liver for rats on 20% of fat. The level of dietary fat had no effect on serum cholesterol, which increased with increased riboflavin intake at both fat levels (Harrill *et al., ibid., 69,* 356). Liver CoA concentration in the rat was decreased 50% by Vitamin B_6 deficiency and 66% by pantothenic acid deficiency. The decrease caused by Vitamin B_6 deficiency was independent of food intake and was not remedied by cystine supplementation of a 20% casein diet (Williams *et al., Arch. Biochem. Biophys., 80,* 367). The effect of the level of dietary fat on the development of Vitamin B_6 deficiency and on the body composition of Vitamin B_6-deficient and pair-fed control rats was studied with diets in which the ratio of protein/food energy remained constant as the level of fat increased. In both deficient and control animals, the percentage of body fat increased as the percentage of dietary fat increased. Whether Vitamin B_6 deficiency reduced body fat depended upon the level of dietary fat; there was no significant difference between the deficient and control groups at 5 or 40% of cottonseed oil, but a significant decrease occurred in the deficient animals fed a 10 or 20% of cottonseed oil diet. Vitamin B_6 deficiency also decreased the storage of liver cholesterol in cholesterol-fed rats (Williams *et al., J. Nutr., 68,* 25). Fourteen patients with congestive heart failure and 14 control patients without heart failure were studied with the tryptophan load test. Urinary xanthurenic acid (XA) excretion following tryptophan load was significantly greater in the cardiac group than in controls. Mean XA excretion of the group with congestive failure was 35.6 mg/24 hrs. with standard deviation of 22.8. Mean excretion of the control group was 12.9 mg/24 hrs. with standard deviation of 5.2 mg/24 hrs. The heart-failure group, which attained a higher level of XA excretion than the control group in the post-tryptophan period, showed a greater decline in XA excretion after pyridoxine than the controls. Since pyridoxine is required for orderly catabolism of tryptophan, and pyridoxine corrects this metabolic aberration of tryptophan, it is not unreasonable to state that in congestive heart failure availability of Vitamin B_6 is limited (Levy *et al., Proc. Soc. Exptl. Biol. Med., 101,* 617).

Total phospholipid and the three fractions, lecithin, cephalin, and sphingomyelin, were decreased in the Vitamin B_{12}-deficient chick embryo. A decrease in the synthesis of sphingosine was noted in the B_{12}-deficient embryo. However the decreased synthesis was not a limiting factor for sphingomyelin formation (Yesair *et al., Arch. Biochem. Biophys., 79,* 168). A purified diet containing isolated soybean protein was developed for the study of Vitamin B_{12} in the nondepleted chick. The Vitamin B_{12}-sparing effect of methionine in day-old New Hampshire chicks receiving 0, 4, and 24% fat in the diet was determined with the new diet. In confirmation of earlier studies with a crude corn-soybean meal diet, increasing the fat content of the diet increased the severity of the Vitamin B_{12} deficiency that was obtained during 4-week experimental periods. This effect of high dietary fat was lost in the presence of supplemental methionine; however both Vitamin B_{12} and methionine were necessary for maximum growth (Fox *et al., J. Nutr., 68,* 371).

Lipids in Micro-organisms, Insects, and Higher Plants. Cells of *Lactobacillus arabinosus* and *Lactobacillus casei* failed to produce measurable amounts of *cis*-vaccenic acid when lactobacillic acid replaced biotin in their culture medium. Attempts to demonstrate the presence of "fatty acid dehydrogenase" activity in three lactic acid organisms were unsuccessful (Hofmann *et al., J. Biol. Chem., 234,* 1672). Twenty-five pure cultures of lipolytic micro-organisms sometimes found in market milk were inoculated into samples of high-quality pasteurized whole milk subsequently held at 4 ± 1° C. for a period of 12 days. At four-day intervals during the hold-period the samples were examined for microbial content, free-fat acidity, and developed flavors. Eighteen cultures grew reasonably well and brought about increases in free-fat acidity. Seventeen of these produced a rancid flavor sometimes accompanied and/or followed by bitterness whereas one caused the milk to be criticized as sour and unclean (Overcast *et al., J. Dairy Sci., 42,* 1479). The possible involvement of methionine in bacterial fatty acid

synthesis was investigated. *Lactobacillus arabinosus* and a methionine-stimulated mutant of *Escherichia coli* were shown to incorporate appreciable amounts of methyl carbon from exogenous methionine-methyl-C^{14}. In each species 20 to 25% of the total radio-activity in the cells was contained in the mixed cellular fatty acids. Chromatographic analysis showed that there was a selective accumulation of methionine methyl carbon in the C^{18-19} fraction of *L. arabinosus* and in the chromatographic fraction of *E. coli* acids that is usually found to contain palmitic acid. The possible involvement of one-carbon transfers in the bacterial synthesis of certain unusual fatty acids is discussed (O'Leary, *J. Bacteriol., 78,* 709).

Oxidation of paraffins by a gram-negative coccus resulted in the accumulation of relatively high molecular weight esters (waxes). Octadecane yielded a 1:1 mixture of octadecyl stearate and octadecyl palmitate. Tetradecane yielded tetradecyl (myristyl) palmitate. The ester produced from the dodecane was not completely identified but was shown to possess a palmityl moiety. The waxes produced always had an alcohol with the same carbon skeleton as the paraffin from which it was derived while the acid moiety was palmitic except in the case of octadecane, where half of the total ester was made up of octadecyl stearate (Stewart *et al., ibid., 78,* 726). The occurrence of tocopherols in the lipid fraction of a range of micro-organisms was investigated. Of some 11 organisms studied, α-tocopherol was detected in *Chlorobium thiosulphatophilum, Ochromonas malhamensis,* and *Euglena gracilis.* When grown in the daylight, *Ochromonas* contained about six times as much tocopherol as did the same organism grown in the dark. These results together with the observation that tocopherol occurred only in organisms containing chlorophyll support the suggestion that there is a close relationship between tocopherol and chlorophyll synthesis and that tocopherol might be formed from the same phytol precursors as chlorophyll (Green *et al., Nature, 184,* 1339).

The degradation of fatty acids of medium molecular weights to the methyl ketones under the influence of enzymes as *Phycomycetes* and *Ascomycetes* was established. The presence of fatty acid dehydrase in the extracts of mold fungi was proved with the help of the Thunberg method. It was indicated that the degradation of enzymes takes place over coenzyme A derivatives. The addition of DPN is considered to be necessary. A highly stable *beta*-ketocarboxylase was detected in the extracts of methyl ketone components (Franke *et al., Fette, Seifen und Anstrichmittel, 61,* 264). Measurement of the action spectrum of fluorescent of *Rhodopseudomonas spheroids* and *Rhodospirillum rubrum* showed no difference between intact bacteria and chromatophores prepared therefrom in energy transfer from carotenoids to bacteriochlorophyll. The efficiency of light absorbed by carotenoids in *R. spheroides* was found to be 90% and in *R. rubrum* 30%. Extraction of a large fraction of the carotenoids from chromatophores does not result in an increase in efficiency of the remaining fraction. It seemed unlikely that the low value of energy transfer in *R. rubrum* could be explained by the assumption of two different carotenoid systems (Goedheer, *Biochem., Biophys. Acta, 35,* 1).

The ability of protozoa from cow rumen to hydrogenate various dietary unsaturated fats was studied. Incubation with a suspension of rumen protozoa caused hydrogenation of both linoleic acid and linseed oil. A suspension of chloroplasts prepared by grinding freshly picked red clover leaves was also incubated. There was considerable conversion of the C_{18} triene acid to diene and the monoene to stearic acid; only slight conversion of diene to monoene appeared to have occurred. The nonsaponifiable material exhibited a spectrum typical of a carotene-xanthophyll mixture. Little hydrogenation of the highly-unsaturated pigments had occurred however (Wright, *Nature, 184,* 875). Component fatty acids of fat produced by *P. spinulosum* grown in aerated deep culture were found to be (% by wt. of total acid): palmitic 18.0, stearic 11.9, arachidic 1.4, hexadecenoic 3.8, oleic 43.4, linoleic 21.1, linolenic 0.3, eicosenoic 0.2 (Shiml *et al., Biochem, J., 72,* 184).

Among lipids obtained from *Drosophila* were hydrocarbon, wax ester, cholesterol ester, triglyceride, cholesterol, glycerophosphatidic acid, phosphatidylglycerol, inositol phospholipids, phosphatidylethanolamine, ethanolamine plasmalogen, phosphatidylserine, cerebroside, lysophosphatidylethanolamine, lysophosphatidylserine, phosphatidylcholine, choline plasmalogen, and lipids containing bound amino acids. Twelve different bound amino acids and perhaps peptides were found in lipid fractions, but no fraction contained more than 0.1 mole of amino acid per kg. of lipid (Wren *et al., J. Biol. Chem., 234,* 2823). The utilization of dietary ergosterol by nymphs of the German cockroach *Blatella germanica* was investigated. When these insects received uniformly labelled ergosterol, the formation of a new radio-active sterol was observed. This conversion product could not be demonstrated with ergosterol labelled at C-28 only, which showed that it no longer contains the C-28 methyl substituent of ergosterol. By isotopic techniques the demethylation product of ergosterol was shown to be 22-dehydrocholesterol. A small amount of crystalline sterol was isolated from a large number of ergosterol-fed nymphs and shown to be identical with authentic 22-dehydrocholesterol (Clark *et al., ibid., 234,* 2589). Larvae of the beetle *Dermestes vulpinus* reared on diets containing 1-C^{14}-acetate or randomly labelled C^{14}- fructose failed to form radio-active squalene or sterols. The nonsaponifiable matter isolated from these insects contained two radio-active fractions. One of them was shown to be saturated aliphatic hydrocarbon, or mixture of hydrocarbons, with an average molecular weight of 346 and an unbranched carbon chain. The second fraction was characterized as a primary aliphatic alcohol with an average molecular weight of 395 ± 17. The cholesterol necessary for the growth of *Dermestes* larvae cannot be replaced or spared by mevalonic acid, squalene, lanosterol, or Δ^8-4,4-dimethylcholestenol (Clark *et al., ibid., 234,* 2578). Larvae of the hide beetle *Dermestes vulpinus* were reared on synthetic diets, and the quantities of cholesterol necessary for supporting growth of these organisms were determined. Diets containing subminimal quantities of cholesterol were found to support larval growth if supplemented by β-sitosterol or by various other sterols which by themselves cannot meet the sterol requirement of *Dermestes.* This ability to spare cholesterol was shown by cholestanol, Δ^7-cholestenol, 22-dehydrocholesterol, Δ^7-ergostenol, and to a somewhat lesser extent by stigmasterol, 22-dihydroergosterol, and $\Delta^{7,22}$-ergostadienol. Ergosterol was inactive as a sparing agent. Attempts to satisfy part of the cholesterol requirements of *Dermestes* by supplementing the diets with bile acids, Δ^5-pregnenolone, and vitamin D_3, gave negative results; a combination of the same steroids with β-sitosterol also failed to support growth (Clark *et al., ibid., 234,* 2583).

Carbon-14 from both acetate-1- and -2-C^{14} was rapidly incorporated into free sugars and intermediates of the tricarboxylic acid cycle by excised cotyledons from etiolated peanut and sunflower seedlings. The observed distributions of the carbon-14 within malic acid and the glucose moiety of sucrose were consistent with the operation of the glyoxylate cycle in the conversion of fat into carbohydrate in these tissues (Bardbeer *et al., J. Biol. Chem., 234,* 498). An α-oxidation pathway in higher plants was postulated to explain long-chain acids (from lauric to stearic acids) undergoing a succession of carbon-1 cleavages in contrast to the carbon-2 cleavage of the conventional β-oxidation pathway. The isolated reactions shown were a peroxidative decarboxylation of the fatty acid to yield CO_2 and an aldehyde with 1 less carbon atom. This reaction is catalyzed by an enzyme which has been named the long-chain fatty acid peroxidase and a DPN-linked oxidation of the long-chain aldehyde to the corresponding acid, catalyzed by an aldehyde dehydrogenase (Martin *et al., ibid., 234,* 2548). The plant growth-regulating activity of a tobacco isolate as well as that of 60 long-chain fatty alcohols and related compounds were measured. The tobacco isolate and C_{18} to C_{22} alcohols and their acidic esters exhibited significant activity (Vlitos *et al., Nature, 184,* 462).

Lipids in Diseased States. The greatest interest in lipids in the diseased state in 1959 involved the role of linoleic acid in the regulation of serum cholesterol levels. Recent findings of clinical research have amply demonstrated a close relation between level of cholesterolemia and development of atherosclerotic disease on the one hand, and between the dietary pattern and the level of cholesterolemia on the other (Stamler, *J. Am. Dietetic Assoc., 34,* 701). The overwhelming success achieved in producing atherosclerosis in every important laboratory species (a success achieved primarily by dietary means involving the addition of fat and cholesterol to the diet) is a major piece of evidence which cannot be ignored. From this experience in rabbit, chick, rat, dog, monkey, etc., it would seem valid to conclude that man is in all likelihood not decisively exceptional, *i.e.*, that diet, dietary fats, and cholesterol play an important role in the pathogenesis and etiology of atherosclerosis in man as well (Stamler, *Food Tech., 13,* 50). In rats an increase was noted in plasma cholesterol as the linoleic acid content of the diet was increased. The liver cholesterol esters were also increased at high levels of linoleic acid. The second increase was specifically related to the polyunsaturated fatty acid content, not fat level. The polyunsaturated acid content of liver cholesterol esters was believed to bear a simple relation to the dietary polyunsaturated fatty acids; the level of ester found in the liver was not related to the content of polyunsaturated fatty acid in the ester. Plasma cholesterol esters differed markedly in composition from liver esters and did not follow the same relation to the dietary fat as do the latter (Klein, *Arch.*

Biochem. Biophys., 76, 56). Rats which had been maintained on various low-fat diets were injected with acetate 1-C^{14}, and the rate of incorporation of C^{14} into cholesterol was determined. Methyl linoleate or sunflower seed oil (10% of total calories of a standard low-fat diet) resulted in a decrease in the half-life of the serum cholesterol to 60% of the control and an increase in the rate of synthesis to 160% of the control. On feeding methyl stearate (10% of calories), the "half-life" increased and the rate of synthesis decreased. Serum cholesterol levels were also lower for the unsaturated acid-fed animals. In all cases the rats grew normally and showed no signs of essential fatty acid deficiency (Boyd *et al., Biochem. J., 73,* 9).

Weanling rats were fed for seven weeks at a concentration of 10% in an adequate synthetic diet: menhaden, safflower, corn, soy, straight and hardened cottonseed, peanut, olive, lard, coconut, and butter oils, butterfat, and shortening. Each fat was given to two groups of 10 males and 10 females; in addition, one group also received 1% cholesterol in the diet. No significant effects on growth were noted among these fats. In general, lower liver cholesterol contents were found in rats fed fats of low iodine number and *vice versa.* Females fed cholesterol tended to have higher serum cholesterol and lower liver cholesterol concentrations than did males. Very high serum cholesterols were seen only in females fed safflower, cottonseed, olive, and coconut oils. The fatty acid moiety of the liver cholesterol esters reflected the composition of the dietary fat though the percentage of cholesterol linoleate was always lower than that of linoleate in the diet; oleic acid was always an important constituent of the ester (Okey, *Metabolism, Clin. and Exptl., 8,* 241). The prolonged feeding of a diet containing 20% of highly saturated fat led to experimental atherosclerosis and shortened the coagulation factors (bleeding time, coagulation time, and prothrombin time) as well as increased the liver fat and number of blood platelets and the ester cholesterol in blood and liver and heart tissues (Saikia, *J. Nutr., 69,* 403). The feeding of a diet rich in unsaturated fats to rabbits produced no significant alteration of the serum cholesterol level while the feeding of a diet rich in saturated fats produced an elevation of the serum cholesterol without development of experimental atherosclerosis. The addition of unsaturated fats to a diet rich in cholesterol failed to prevent hypercholesterolemia and experimental atherosclerosis in rabbits (Steiner, *Circulation Res., 7,* 448). Rabbits, treated with cholesterol dissolved in the unsaturated fatty acid, revealed a significantly higher hypercholesterolemia and more marked atherosclerosis than the rabbits that were treated with cholesterol dissolved in the same amount of olive oil (Rona *et al., Can. J. Biochem. and Physiol., 37,* 479). Linoleic acid appeared to be the responsible constituent of those vegetable fats which, when taken in the diet, lower plasma levels of cholesterol and other lipids.

Marked improvement had been noted in certain diabetic and nondiabetic patients with vascular disease when administered large amounts of linoleic acid in their blood. The fatty acid composition of the diet specifically influenced the composition of the plasma cholesterol esters. High linoleate intake resulted in markedly increased plasma cholesterol linoleate, and high oleate intake resulted in markedly increased plasma cholesterol oleate (Kinsell *et al., Diabetes, 8,* 179). Short- and long-term studies were carried out on 15 atherosclerotic, hypercholesterolemic patients aged 50–70 years, who ingested 1.5 ml. of oil before each principal meal. After 6–11 days mean total plasma cholesterol had fallen 16%, esterified plasma cholesterol had fallen 18%, and free plasma cholesterol had risen 15%. After 24–164 days results were more variable; although total plasma cholesterol levels had diminished in most cases, they had risen in several above control levels (Wolff *et al., Presse méd., 66,* 1706). Nine patients in a mental hospital with control plasma cholesterol values between 247 and 331 mg./100 ml. were given during successive periods a low-fat diet (57 ± 10 g. daily), the low-fat diet plus 90 ml. of corn oil daily, the routine hospital diet plus 20 ml. of corn oil, and the routine hospital diet without corn oil. In all subjects, plasma cholesterol values decreased with the low-fat diet, decreased further with the low-fat diet plus corn oil, increased but not to control levels with the routine diet plus corn oil, and returned to pretreatment levels when the routine diet without corn oil was resumed. The subjects lost weight on the low-fat diet but regained this weight when the corn oil was added (Rhoads *et al., Proc. Staff Meetings Mayo Clinic, 34,* 225). A diet modified by substituting a margarine containing 64.2% of nonhydrogenated corn oil for the ordinary solid fats over a period of nine months reduced blood cholesterol levels in 301 institutionalized patients (Boyer *et al., J. Am. Med Assoc., 170,* 257). A significant lowering of the serum cholesterol level was believed to be brought about only when safflower and corn oil were used as replacements for and not as supplements to saturated fats of the diet (Perkins *et al., J. Am. Med. Assoc., 169,* 1731). On the other hand, six middle-aged men fed capsules containing a concentrate of arachidonic acid during experimental periods or oleic acid during control periods did not show a change in the concentration of total cholesterol during the first few days. It tended to rise thereafter however and remained elevated after the withdrawal of the arachidonic acid (Keys *et al., Am. J. Clin. Nutr., 7,* 444). In cockerels fed diets containing 8.2% of vegetable fat and 2% of cholesterol there was a close connection between plasma lipid pattern and the composition of the fat fed. Plasma cholesterol concentrations were inversely proportional to the percentage of polyenoic acids in the fat (or directly proportional to saturated plus monoenoic). Plasma lipid phosphorus concentrations were inversely proportional to the total amount of unsaturation. Aortic lesion scores were not closely related to the composition of the fat fed. It appears that a given plasma cholesterol concentration in birds fed some fats may not be so injurious as the same concentration would be in birds fed other fats (Tennent *et al., J. Nutr., 69,* 283). Fasted chicks on fat-free diet without cholesterol showed significantly higher plasma cholesterol than nonfasted chicks on the same diet. There was no difference in cholesterol content of tissues between fasted and nonfasted chicks. Without dietary cholesterol there was no difference in tissue cholesterol values between cod liver oil- and linseed oil-fed chicks; with 1% dietary cholesterol, linseed oil caused greater cholesterol values for plasma and tissues than did cod liver oil. Fat-free diets caused a higher polyenoic fatty acids content in liver of fasted chicks than of nonfasted chicks; with all other diets there was no significant difference in polyenoic fatty acids values between fasted and nonfasted groups (Dam *et al., Acta Physiol. Scand., 45,* 31). When dietary cholate was added with cholesterol to natural and hydrogenated cottonseed oil, an increase in serum cholesterol and phospholipid was noted. Dietary lipid saturation or cholate alone had no effect on serum cholesterol. The serum cholesterol/phospholipid ratio was elevated only when both dietary cholate and cholesterol were added to hydrogenated fat. In the rat, highly saturated fat (Iodine No. 43) fed with cholesterol reversed the *alpha* to *beta* lipoprotein ratio and *beta* became dominant. Combination of dietary cholate with cholesterol permitted reversal of *alpha* to *beta* lipoprotein with moderate (Iodine No. 78) or no hydrogenation (Iodine No. 107) of the dietary fat. The serum cholesterol ester fatty acid composition changed with greater saturation of dietary fat and added cholate and cholesterol. High oleic acid levels were noted in serum cholesterol ester fraction of rats fed saturated fat (Iodine No. 78) with cholate and cholesterol. The authors conclude that not only serum cholesterol level but also qualitative and quantitative composition of cholesterol esters, as influenced by dietary factors, is likely to be significant in understanding elevation of serum lipids and deposition of lipids in arteries (Seskind *et al., Proc. Soc. Exptl. Biol. Med., 102,* 90). In swine, the degree of incorporation of injected 1-C^{14}-acetate into tissue cholesterol on cholesterol-free diets was much higher in the liver and plasma of swine ingesting saturated than unsaturated fat. Levels of cholesterol in all tissues increased with the presence of cholesterol; cholesterol levels were highest in the presence of unsaturated fat. The combination of unsaturated fat with cholesterol in the diet produced the greatest degree of typical atheromatosis and the highest levels of cholesterol in the tissues while saturated fat with cholesterol produced diffuse atypical lesions (Reiser *et al., Circulation Res., 7,* 833). The effects of supplementary oils, saturated and unsaturated, were examined in groups of cholesterol-fed cockerels. Unsaturated oils failed to suppress hypercholesterolemia and atherogenesis. When various oils and fats high in oleic acid were added to the mash, slight lowering of serum cholesterol occurred. Oleic acid *per se* given to a group was accompanied by a diminution in atherosclerosis (Stamler *et al., ibid.,* 398). There was an increase in serum cholesterol levels with age in man, to about age 50 in males and 60 in females and a decrease thereafter to values close to those of young individuals. Cholesterol:fatty acid ratios appear to be more dependent on absolute lipid values than on age (Ackermann *et al., Clin. Chem., 5,* 100).

A study of serum cholesterol levels in relation to diet and some physical and biochemical variables in the armed forces of the Philippines on 403 persons picked at random, 19–58 years of age, average 30, showed serum cholesterol to vary from 73–353 mg./100 ml., mean 171 ± 35. Dietary fat intake of the subjects was low compared to the U. S. so a correlation was not possible. Serum cholesterol increased 0.812 mg. % per year of age and was significant at the 1% level (Camara-Besa, *J. Philippine Med. Assoc., 35,* 137). Very little information is available

about the incidence of coronary heart disease in India or of factors considered responsible for it. The incidence of atherosclerosis as judged by electrocardiogram, the fat intake, and serum cholesterol levels in two low-income groups (industrial and rural) in Delhi were investigated. The data in high-income groups in Delhi have now been studied and the results compared. There were considerable differences in the findings in the two groups. The higher income groups had the higher rate of atherosclerosis (Padmavati *et al., Circulation, 19,* 849). The natural history of aortic atherosclerosis in the Bantu, of Durban, South Africa, age 1 to 40 years, was compared to that of the New Orleans Negro and white man in the same age group. Fatty streaks are present universally in all three groups to some extent after the first decade. The average surface involvement with fatty streaks was not appreciably different in the three groups. The prevalence and quantitative extent of fibrous plaques were much less in the Bantu than in the New Orleans Negro and New Orleans white man. This difference does not appear to be due to sampling bias by selection of autopsies. The degree of aortic fatty streaking in early life was not correlated with reported group differences in dietary fat intake, serum cholesterol levels, and the incidence of clinical manifestations of atherosclerosis in later life. The degree of aortic fibrous plaques in the fourth decade does parallel the reported racial and geographic differences in these factors (Strong *et al., ibid., 20,* 1118).

The mean serum cholesterol level of members of Australian aborigines was significantly lower than the mean level for controls of European descent. This difference was related to differences in dietary fat intake. Serum cholesterol levels were not elevated in a series of atherosclerotic patients in this study (Schwartz *et al., Med. J. Australia, 2,* 84). The serum cholesterol level of five tribes of American Indians who were examined during the Indian Health Survey was significantly lower than that of the Cleveland Clinic group, whose mean level is similar to that found in other surveys of American non-Indian populations. The findings of a higher serum cholesterol level among one subgroup of the Navajos and a significantly lower mean level among other subgroups suggests important environmental factors at work among this ethnic group (Abraham *et al., Public Health Repts. (U. S.), 74,* 392). Total cholesterol, α- and β-cholesterol, and other lipid fractions were estimated by paper electrophoresis in healthy young adult Yemenithis and Ashkenazi Jews. Contrary to the view that cholesterol levels rise with age from the early twenties to middle age, no such trend was found in Yemenite Jews. Young Ashkenazi Jews from the upper middle social classes had higher total cholesterol levels than the Yemenites, but these were lower than those of the middle-aged Ashkenazi group of mixed social classes. With regard to low average values of β-cholesterol and the high percentage of α-cholesterol, the young Ashkenazis, unlike the middle-aged Ashkenazis, were identical with Yemenites of all ages (Brunner *et al., Lancet,* 1071, 1959). There were no significant differences between two tribes of American Indians in total serum fatty acids despite their vastly different dietary fat intake. The differences between the sums of the polyunsaturated fatty acids for both groups were however highly significant when this sum was expressed either in milligrams per 100 ml. of serum or as a percentage of the total serum fatty acids. The significantly higher level of polyethenoid fatty acids in the serum of the group with the greater fat intake was due to their considerably higher serum levels of dienoic and tetraenoic fatty acids although their levels of trienoic, pentaenoic, and hexaenoic serum fatty acids were significantly lower than those of the group with the low fat diet (Roels *et al., J. Nutr., 69,* 195). Total, free, and ester cholesterol, lipid phosphorus, and total cholesterol to lipid phosphorus ratio were determined in 40 Indian subjects with coronary disease and in 100 normal Indian subjects. In normal subjects lipid phosphorus rose by 0.022 mg.% for every unit increase of total cholesterol, and by 0.017 mg. % in the coronary- disease patients. In normal subjects total cholesterol concentrations increased significantly as body weight was significantly greater in those subjects whose weights were over 2.40 lb./in. (Gupta, *Metabolism. Clin. and Exptl., 7,* 349).

The mean serum-cholesterol levels of 842 Eskimo men did not appear to be unusual, but there were significant differences in men from different localities in Alaska. Measurements of blood pressure showed much less variation (Scott *et al., Lancet,* 778, 1958). There was a significant increase in the serum triglycerides in patients with coronary arterial disease. An error in the metabolism of triglycerides may be the lipid abnormality operative in this disease (Albrink *et al., A. M. A. Arch. Internal Med., 103,* 4). From a review of the literature the following normal values for the concentration of various lipids in human plasma in mg.% were derived: total fatty acids 190–450; neutral fatty acids 0–200; neutral fat 0–200; total cholesterol 150–260; free cholesterol 35–90; lipid phosphorus 6–10; total phospholipids 150–250; saponifiable phospholipids 120–230; lecithins 120–230; cephalins 0–20; sphingomelins (non-saponifiable phospholipids) 10–30; plasmalogen 2.2–3.0; carotenoids 0.025–0.250; total lipids 400–700; and free fatty acids in milliequivalent per liter of 0.31–1.21 (Zollner, *Deut. Med. Wochschr., 84,* 386).

Other dietary components than unsaturated fatty acids served to influence serum cholesterol values. Two separate series of rabbits of the New Zealand White strain were fed a 0.35% cholesterol-containing diet with either 29% sucrose or lactose as the carbohydrate for periods of 7 or 8 weeks. At all time-intervals studied, the serum cholesterol levels of the lactose-fed groups were consistently higher than the corresponding values of the sucrose-fed controls. The total liver cholesterol concentration of the lactose-fed groups was more than double that of the sucrose-fed animals. The atherosclerosis score for lactose-fed rabbits was 40.6% and 56.5% involvement in contrast to 11.8% and 11.5% for the sucrose-fed controls (Wells *et al., J. of Nutr., 68,* 541). When rats were fed a diet containing 25% of hydrogenated coconut oil, 1% of cholesterol, and 0.5% cholic acid, substitution of wheat flour for sucrose caused a reduction in serum cholesterol concentration. Diets containing sucrose, dextrin, and anhydrous glucose produced the same degree of hypercholesterolemia. When sucrose was replaced in part with lactose or sorbitol, hypercholesterolemia was enhanced. Nicotinic acid at high levels and inositol-hexanicotinate at 0.57% level in the diet failed to lower serum cholesterol concentration. High doses of nicotinic acid had no effect on pyridine nucleotide content of the liver but raised the oxidized pyridine nucleotide content of blood significantly (Nath *et al., Proc. Soc. Exptl. Biol., Med., 102,* 571).

Chicks were fed herring oil, corn oil, Crisco, lard, butter, and chicken fat in diets containing 20 and 26% of protein. The chicks fed the higher protein level showed lower serum cholesterol levels than those fed the lower protein level whether or not the diet was supplemented with fat and regardless of the type of fat added to the diet. After the experiment the weights of the thyroid glands of chicks fed the various diets were determined. The data suggest that the effect of the diet upon serum cholesterol level may be mediated to some extent through the thyroid gland (March *et al., J. Nutr., 69,* 105). High-protein intake suppresses hypercholesterolemia and atherogenesis in young cockerels on high-cholesterol, high-fat diets. This happens with supplementation by any nutritionally good protein and is manifest irrespective of source of cholesterol and type of fat. In mature roosters or hens, as in young growing cockerels, low protein intake intensifies and adequate protein intake suppresses hypercholesterolemia and atherosclerosis occurring with high-cholesterol, high-fat diets. However inhibition of coronary atherogenesis induced in hens by endogenous estrogen secretion continues to supervene despite low-protein intake. Oviduct ligation prevented intensification of hypercholesterolemia and atherosclerosis with low-protein intake (Pick *et al., Circulation Res., 7,* 866).

Adult swine were fed either a human type of diet of mixed foods or a purified diet with changes in the amount and type of fat or the amount of protein. In both types of diet, fat caused the serum cholesterol to rise with the greatest increase resulting from the most saturated fat. The human type of diet consistently gave higher serum cholesterol values, but the response to different fats paralleled the results obtained with the purified diet (Barnes *et al., J. Nutr., 69,* 261). Four groups of weanling pigs were fed either high- or low-protein and either high- or low-fat diets for 36 weeks. Evidence of protein malnutrition was most marked in the low-protein, high-fat group. These animals exhibited signs that have been interpreted as resembling the human infant disease, kwashiorkor. Fat in the form of beef tallow in the diet caused a rapid rise in serum cholesterol. Low-protein intakes also resulted in an increase in serum cholesterol (*ibid,* 269). Newly weaned male and female rats were fed a hypercholesterolemic diet with supplements of different protein concentrates or amino acids. Lowest levels of serum cholesterol were obtained with dried whole egg, wheat gluten, fish meal, and meat meal. Casein, gelatin, and soybean protein were less active. Low-cholesterol levels were also obtained when a mixture of amino acids based on the composition of wheat gluten was substituted for the intact protein. The addition of different combinations of three amino acids (at concentrations of 0.2, 0.4, and 0.6%, respectively) lowered the cholesterol level if methionine was one of the acids (de Groot, *Nature, 184,* 903).

Rats and rabbits were fed a hypercholesteremic diet until elevated serum cholesterol levels were observed in all animals.

The animals were then divided into groups, one group remaining on the original diet and the others receiving the same hypercholesteremic diet fortified with taurine, glycine, or *beta*-sitosterols. In the rat 4% taurine in the diet significantly decreased the serum, liver, and aorta cholesterol concentrations, but glycine was without significant effect. In the rabbit 2% β-sitosterol in the diet significantly reduced the serum, liver, and aorta cholesterol while taurine or glycine produced no significant effect. Taurine, glycine, or β-sitosterols did not produce any signs of toxicity (Herrmann, *Circulation Res., 7,* 224). Deficiencies in the amino acids, arginine, lysine, methionine, and tryptophan, were studied at different levels of protein intake in relationship to growth rate and plasma cholesterol of the young chick. It was shown that, at suboptimal protein intakes, a hypercholesteremia resulted which could be modified by supplementing the deficient protein with amino acids such that more protein would become available to the bird. At optimal or supernormal protein intakes arginine, when added to a casein diet deficient in this amino acid, continued to exert a cholesterol-lowering effect which could be explained on the basis of greater protein availability; lysine and tryptophan, when added to proteins deficient in these amino acids, exerted no effect on the plasma cholesterol level, and methionine produced a cholesterol-lowering effect which was not related to any improvement in protein quality or growth of rats (Johnson *et al., J. Nutr., 66,* 367). Milk contained 12 mg./liter of cholesterol, the lowest of all foodstuffs of animal origin. The body synthesized 10–20 times this amount of cholesterol on a normal milk diet. The lecithin content of milk is 20 times that of cholesterol. Milk fat in a balanced diet leads to a significant decrease of cholesterol in the blood of humans (Holden, *Intern. Dairy Congr., Proc. 15th Congr., London, 1,* 27). Inositol, vitamin B_{12}, and glucose cycloacetoacetate (GCA), both hydrolyzed and nonhydrolyzed, have been found to check the rise of total cholesterol, ester cholesterol, and cholesterol to lipid phosphorus (C/P) ratio in the serum as well as in the liver and kidney of cholesterol-fed animals. The effect is the most pronounced in the case of the GCA-injected group (Nath *et al., Arch. Biochem. Biophys., 79,* 216). Serum, bile, and liver cholesterol levels of nine species of laboratory animals and 4 species of amphibians were reported. Dogs, monkeys, hamsters, and mice have relatively higher serum cholesterol concentration than cats, rats, rabbits, and guinea pigs. Total cholesterol concentration in liver varies only slightly among these species. Gall-bladder bile of monkeys contained large amounts of cholesterol. Guinea pigs excreted very small amounts of cholesterol in bile. Serum and liver cholesterol concentrations of the pigeon were very high. Total cholesterol concentration in liver varied slightly among the four species of amphibians used (Lee *et al., Proc. Soc. Exptl. Biol. Med., 102,* 542). The addition of cholesterol to a stock diet increased the concentration of cholesterol in the serum by one-third and in the liver 2–5 times. Addition of peanut oil to the stock diet greatly increased serum and liver cholesterol. In a high-fat diet containing cholesterol, cholic acid, choline chloride, and peanut oil, cholic acid and choline chloride were both necessary to produce maximum concentrations of cholesterol in the serum and liver. Hydrogenated peanut oil was more effective that the raw oil in producing hypercholesterolemia; peanut oil was likewise more effective than corn oil (Cuthbertson *et al., Brit. J. Nutr., 13,* 227).

The cholesterol content of the blood plasma of 194 laying chickens was determined. One hundred forty-four of the chickens were kept in cages and fed six different isonitrogenous, isocaloric diets that contained, respectively, 3.64, 5.81, 7.98, 10.16, 12.33, and 14.50% of tallow. Fifty of the chicks were kept on good grass range and fed a diet that contained no added fat of any kind. No statistically significant difference in average cholesterol content of the blood plasma was found among the six lots of birds kept in cages and fed the different fat-containing diets, among the five lots of birds kept on range and fed the same diet, or between the birds kept in cages and those kept on range (Johnson *et al., Poultry Sci., 38,* 1109). Ordinary and unsaturated hen's eggs were fed to adult Bantus. The unsaturated eggs had been laid by hens fed a mixture containing 43% sunflower seed. The iodine number of their yolk lipids was 100 in comparison with 73 in control eggs. Serum cholesterol levels of subjects fed 10 boiled unsaturated egg yolks daily rose from 188 mg./100 ml. on a basal diet very low in fat to 218 mg. Next the basal diet plus 3 g. of cholesterol (*i.e.*, the amount in 10 egg yolks) was fed, and serum cholesterol returned to basal level. In another experiment a minor effect was produced by feeding unsaturated eggs. The effect of dietary fats on serum cholesterol is not therefore simply a function of their degree of saturation (Gordon *et al., Lancet, 255,* 1958).

In the majority of rhesus monkeys fed a high-fat diet (by mixing the various lipids with ground monkey food) the serum cholesterol values reached 300–600 mg. % within three months (Cox, *et al., Arch. Pathol., 66,* 32).

C^{14}-Ergosterol was biosynthesized from 1-C^{14}-acetate, and the distribution of the labeled atoms was studied. By conversion of ergosterol to progesterone it was shown that the distribution of label between the side chain and nucleus was that predicted on the basis of the squalene hypothesis. The specific carbons, C-3, C-4, C-11, and C-12 were obtained by degradation of appropriate precursors, and it was found that C-4, C-11, and C-12 were derived from the carboxyl of acetate, again as predicted by the squalene hypothesis. These results strongly support the concept of the utilization of the intact acyclic triterpene, squalene, in the biosynthesis of all steroids (Dauben *et al., J. Am. Chem. Soc., 81,* 403).

Hormone and drug therapy led to a variable response. Results of dose- and time-response studies in quantitation of atherogenic effect of pharmocologic amounts of estrogen on young cockerels have been used in formulating a method for the study of atherosclerosis. The method is based upon the response of immature birds to 2.5 mg. of estradiol cyclopentylpropionate dissolved in cottonseed oil (ECP) administered intramuscularly at the beginning of a seven-day experiment. Results suggest that a feminizing effect and some degree of atherosclerosis result from ECP dosage levels lower than those required to produce hypercholesterolemia and other blood serum changes associated with atherosclerosis. There are indications that atheroma may regress with time (Caldwell *et al., Proc. Soc. Exptl. Biol. Med., 101,* 299). Testosterone in large doses in intact cholesterol-fed cockerels partially inhibited hypercholesteremia, without affecting aorta or coronary atherosclerosis. Castration, with and without testosterone administration in young male and female chicks, was without influence on hypercholesteremia and atherogenesis (Pick *et al., Circulation Res., 7,* 202). Male mice of the C57B1 strain on the high-fat diet had a higher incidence of *arthrosis deformans* than those on the stock diet, but the castration had no significant effect on the results (Silberberg *et al., Schweiz. Z. allgem. Pathol. u. Bakteriol., 22,* 447). Addition of β-sitosterol to a diet containing corn oil enhanced the hypocholesteremic effect of corn oil. Calciferol and Q275 (coenzyme Q), when fed to rats, had no significant effect on serum cholesterol concentration, but calciferol caused an increase in the deposition of cholesterol in the liver (Nath *et al., Am. J. Physiol., 197,* 102). Rats and rabbits were fed a hypercholesteremic diet until elevated serum cholesterol levels were observed in all animals. The animals were then divided into groups, one group remaining on the original diet and the others receiving the same hypercholesteremic diet fortified with taurine, glycine, or β-sitosterols. In the rat 4% taurine in the diet significantly decreased the serum, liver, and aorta cholesterol concentration, but glycine was without significant effect. In the rabbit 2% β-sitosterol in the diet significantly reduced the serum, liver, and aorta cholesterol while taurine or glycine produced no significant effect. Taurine, glycine, or β-sitosterols did not produce any signs of toxicity (Herrmann, *Circ. Res., 7,* 224).

Neomycin was given orally to 18 patients at daily doses of 1.5 to 2 g. for 4 to 20 weeks. Mean serum-cholesterol levels were decreased significantly in each patient by 17 to 29% and were maintained low for the duration of administration of neomycin. Intramuscular administration to five patients for three weeks failed to alter serum-cholesterol levels. Oral administration of 12 g. of *para*-amino-salicylic acid to two patients lowered serum cholesterol concentrations significantly while doses of 6 g. had no effect. No appreciable changes in serum cholesterol resulted when phthalysulfathiazole, isoniazid, dihydrostreptomycin, oxytetracycline, polymyxin B sulfate, bacitracin, and novobiocin were given orally (Samuel, *Proc. Soc. Exptl. Biol. Med., 102,* 194). Benzmalacene (N-[1-methyl-2,3-di-*p*-chloro-phenylpropyl-maleamic acid]) was claimed to lower blood cholesterol levels in most hypertensive patients but with some weight loss. Cholesterol was lowered in some hypercholesteremic patients but not in all, and this occurred without weight loss. In two hypercholesteremic patients a sharp rise in triglycerides occurred while the cholesterol:phospholipid ratio fell and the free:total cholesterol ratio rose. Liver function as measured by bromsulfalein after four months' treatment had deteriorated in eight of the 12 patients studied. Nausea, epigastric discomfort, and diarrhea were on occasion sufficiently discomforting to require discontinuing the drug. Drugs that interfere with cholesterol synthesis should be studied with great care for long periods before their widespread use in an attempt to prevent atherosclerosis (Page *et al., Circulation, 20,* 1075). MER-29 is 1-[(4-dimethylaminoethoxy) phenyl]-1-*p*-

tolyl)-2-(*p*-chlorophenyl) ethanol. In preliminary tests this compound was found to produce a profound lowering of blood cholesterol in rats. Further studies indicated that it inhibited the biosynthesis of cholesterol in liver and intestine of the intact rat. This inhibition is specific for cholesterol and occurs at a late stage in the synthetic pathway since incorporation of acetate-1-C^{14} into total digitonin-precipitable matter of rat liver is not affected, but incorporation into cholesterol purified *via* the dibromide is reduced (Blohm *et al., Arch. Biochem. Biophys., 85,* 245).

The effects of MER-29 on the blood and tissue lipids of the rat and monkey were also studied. Chronic administration significantly reduced the cholesterol levels of the following tissues in the rat: plasma, erythrocytes, liver, skeletal musele, lung, adrenal, and aorta. The cholesterol of brain and adipose tissue was not reduced. Liver total lipid was unchanged, and liver and plasma unsaponifiable matter were reduced. Plasma phospholipid was also reduced but less than cholesterol. A linear relationship exists between the logarithm of the dose and reduction of plasma cholesterol and phospholipid in the rat. In monkeys oral administration of MER-29 over a six-month period reduced plasma total and α- and β-lipoprotein cholesterol, erythrocyte cholesterol, and plasma unsaponifiable matter. It had no effect on plasma phospholipid or α- and β-lipoproteins stainable with Oil Red O (Blohm *et al., ibid., 85,* 250). Results of administering a resin with bile acid-sequestering properties are reported. The preparation was administered to 26 patients for periods of two to 34 weeks. Serum total cholesterol levels were lowered by more than 10% in 23 of 26 patients. Average decrease in serum total cholesterol for all subjects was 20% ($p<0.001$). No systematic side-effects were observed. Six subjects treated for six weeks or longer maintained lowered values of serum total cholesterol (Bergen *et al., Proc. Soc. Exptl. Biol. Med., 102,* 676). Atherosclerosis was induced in chickens by adding cholesterol to their food. They developed liver hypertrophy with high cholesterol and lipid contents. Another set of chickens was fed cholesterol-enriched food and simultaneously injected with glutathione. Both the cholesterol and lipid content were considerably lower (Fridlyand Byull, *Eksptl. Biol. i Med., 46,* 55).

Familial hyperlipemia is an inherited disease associated with the early onset of coronary atherosclerosis. In a survey of a student population in Sweden an estimated case incidence of 2 to 3% was discovered. This study also demonstrates that there is probably a heterogeneity of causes for the primary elevation of blood triglycerides in man (Hirschhorn *et al., Science, 129,* 716). Supervised exercise showed no effect in three months on subjects with normal initial serum levels. Three high-level subjects showed a decrease following exercise. Change in total serum cholesterol generally accompanied a change in body weight regardless of exercise status. Free serum cholesterol showed the same trends as total cholesterol, but it was so closely related to weight changes (Montoye *et al., Am. J. Clin. Nutr., 7,* 139). Plasma concentration of unesterified fatty acids in the arterial blood and in the femoral veins was determined at rest and during exercise in six healthy human subjects. During exercise the arterial unesterified fatty acids concentration decreased. The exercising leg extracted unesterified fatty acids from plasma (Carlson *et al., J. Lab. Clin. Med., 53,* 833). Patients with abnormal lipid metabolism were compared with normal controls both before and after a fatty meal to determine whether the coaguability and fibrinolytic activity of the blood was affected by hyperlipemia (*Nutrition Revs., 17,* 263). A significant increase in the *in vivo* coagulability of blood after a fat meal has not been demonstrated with certainty, nor has the presence of an increased amount of lipid in an available form that can promote clotting been demonstrated after fat feeding. It must be borne in mind that the methods of study are crude and that no reliable technique for the evaluation of the *in vivo* status of the coagulability of the blood is available (Rouser, *Am. J. Clin. Nutrition, 6,* 681). The reported inhibition of fibrinolytic activity of blood plasma after ingestion of fat has been confirmed. It is shown that the chylomicrons present in such plasma are responsible for this phenomenon (Merigan *et al., Circulation Res., 7,* 205.

Pretreatment of fatty acid with human plasma, plasma albumin or globulin, unesterified cholesterol, or cyanide retards or prevents hemolytic activity. Once lytic quantities of fatty acid are bound by human erythrocytes, the above substances reverse their protective activity and accelerate hemolysis. An hypothesis is suggested for linking increases in mammalian plasma unesterified fatty acid with *in vivo* hemolysis (Greisman, *Exptl. Biol. Med., 101,* 725). Following intake of 85 g. of butter by healthy adult male humans, mean clotting time of the blood was significantly lower than the fasting values; prothrombin time, circulation time, and serum-cholesterol concentration were not affected (Ramanathan and Gopalan, *Ind. J. Med. Res., 46,* 466). Intravenous administration of a fat emulsion has been found to accelerate substantially the formation of a fibrin clot in isolated venous segments of anesthetized dogs. This finding is interpreted as implying that induced lipemia may contribute to, or result in, an *in vivo* hypercoagulable state (Roth *et al., Proc. Soc. Exptl. Biol. Med., 101,* 516). Pure egg phosphatidylethanolamines show strong platelet-like activity in blood coagulation systems consisting of purified protein components The clot-promoting activity of egg phosphatidylethanolamines is a property of particles in colloidal suspension. The colloidal behavior of these particles determines their clot-promoting activity and depends upon a) the degree of unsaturation of the fatty acids in the phospholipid and b) the temperature, pH, and ionic composition of the suspending medium. The behavior of phosphatidylethanolamines in the system studied here suggests that the active micelles are bimolecular leaflets of limited size, thickness, and surface configuration (Wallach *et al., J. Biol. Chem., 234,* 2829). Bishydroxycoumarin-induced hypoprothrombinemia may result in a slight delay of fibrin clot in isolated venous segments of anesthetized dogs. The hemorrhagic state produced by the drug fails to prevent acceleration of intravascular clotting caused by intravenous infusion of fat emulsion. This clot acceleration therefore may not operate through the mechanism of prothrombin conversion (Fahimi *et al., Proc. Soc. Exptl. Biol. Med., 102,* 398).

Fat tolerance tests with radio-active triolein were performed on a group of patients with coronary atherosclerosis or hypercholesterolemia, and significant differences from normal subjects were observed (Likoff *et al., Circulation, 18,* 1118). ϵ-Aminocaproic acid competitively inhibited the activation of human or bovine plasminogen by streptokinase, urokinase, and probably finrinokinase but inhibited plasminogen activation by trypsin noncompetitively. ϵ-Aminocaproic acid in concentrations exceeding 0.06 M was a noncompetitive inhibitor of the proteolytic activities shown by plasmin or trypsin. In lower concentrations it enhanced the proteolytic action of plasmin. The results support the view that plasminogen activation may occur by two mechanisms yielding plasmins with similar biochemical activities but of different molecular size (Alkjaersig *et al., J. Biol. Chem., 234,* 832). Supernatant from MK cell cultures activated plasminogen and was also proteolytic in the absence of plasminogen. These cell cultures had a third property, inhibition of proteolysis. The activator described here resembles "tissue activator" in that it is not proteolytic in the absence of serum and can be dissolved by KSCN. It also resembles activator from exercised rat lung in that it is the size of microsomes. The interpretation that release of activator from cell culture is a metabolic process and not from cell breakdown seems warranted since only a small fraction of the activity found in the supernatant can be extracted from the cells (Barnett and Baron, *Proc. Soc. Exptl. Med., 102,* 308). Yellow mice fed *ad libitum* exhibit a greater rate of lipogenesis and cholesterogenesis than their controls. Females show fatty acid content in the liver and in extrahepatic tissues which is twice as high as the males. Unlike the males, they are hypercholesterolemic and also show an elevated rate of lipogenesis after an 18-hr. fast (Zomzely and Mayer, *Am. J. Physiol., 196,* 611). No significant correlation was found between the serum cholesterol level and weight, weight corrected for frame size, or thickness of the fat shadow in medical students (mean age, 22 years) (Thomas and Carn, *Science, 131,* 42).

The role of lipoprotein in atherosclerosis is receiving increasing attention. It has been found that three proteins are associated with both dog and human chylomicrons. In both species, one of these proteins has been shown to have the same N-terminal amino acid, paper electrophoretic mobility, and "fingerprint" pattern as does the major protein found in the density 1.063 to 1.21 plasma lipoproteins. Another protein associated with the chylomicrons has been described, which is probably identical with a protein found throughout the density spectrum of human plasma lipoproteins. It is suggested from the results that these proteins may play a fundamental role in the transport and metabolism of exogenous triglycerides (Rodbell and Frederickson, *J. Biol. Chem., 234,* 562). The influence of fat emulsion infusions on the serum lipoprotein spectrum of atherosclerotic rabbits was investigated. After administration of the lipid infusion a fluctuation of the cholesterol and the lipids in the *alpha-* and *beta*-globulins was noted (Felt and Frafnetter, *Experientia, 15,* 113). Administration of a diet rich in animal fat to 10 normal subjects produced significant increases in serum cholesterol and α-lipoproteins and a decrease in serum albumin (Copinschi, *Comp. rend. soc. biol., 162,* 1212). The normal variability of lipo-

protein and cholesterol levels in 107 normal men who developed myocardial infarction were studied for 7 years. Among these 11 developed infarction and six angina pectoris. Little change in lipid levels was associated with these events. Electrophoretic patterns early after infarction showed certain relatively characteristic changes, especially increase in α-2 and β globulins and fibrinogen. Low-density lipoprotein also increased. While α-2 globulin was usually greatest in patients with the most extensive myocardial damage, there was little over-all correlation between lipoprotein pattern and severity of infarction. The "coronary profile" can be more sharply delineated by repeated lipid measurements because as a group those with atherosclerosis and infarction exhibit slightly elevated values (Page and Lewis, *Circulation, 20,* 1011).

An abnormal type of cholesterol lipoprotein curve is associated with a history of recovery from a previous myocardial infarction (Toro *et al., Proc. Soc. Exptl. Biol. Med., 101,* 34). In rats, cholesterol feeding apparently decreased the proportion of triglycerides in lymph and increased those of sterol ester and phospholipid containing oleic-1-C^{14}. Although glyceride fatty acid distribution was similar to that of the fed acids, both sterol ester and phospholipid fatty acids were diluted with endogenous oleic, linoleic, and stearic acids (Clement and Mead, *Proc. Soc. Exptl. Biol. Med., 100,* 285). Parenteral administration of DL-α-tocopherol acetate during the development of experimental atherosclerosis in rabbits produced progressive hypercholesteremia and β-lipoproteinemia with a marked rise of the total cholesterol lipide P ratio and cholesterol from the β-zone of an electrophoretogram. In the untreated animals fed with cholesterol in olive oil, the peak of hypercholesterolemia was noted at six weeks, which was maintained up to 12 weeks. Feeding of olive oil alone did not give rise to an increase of serum cholesterol level but produced relative increase of serum β-lipoproteins (Krishna *et al., J. Sci. Ind. Res. (India), 18C,* 57). For a group of women aged 30–97 yr., fat calories represented 35 to 41% of total calories and were supplied chiefly by butter, dairy products, meats and gravies. There was no apparent relationship between diastolic pressures and fat contents of the diets (Burrill *et al., J. Am. Dietet. Assoc., 35,* 935).

The most important factor in atherosclerosis involves the aorta itself. Studies of cholesterol metabolism in the walls of arteries of normal intact cockerels indicate that plasma cholesterol is the major precursor of the cholesterol in the artery wall, but that local synthesis may contribute a significant fraction. The fractional turnover of cholesterol in the wall of the abdominal aorta is faster than in either the thoracic aorta or the brachiocephalic arteries. However, considered in relation to endothelial surface area, the rate of cholesterol transfer from plasma is smallest in the abdominal aorta. The abdominal aorta also has a lower cholesterol concentration in this species than do the other large arteries (Dayton, *Circulation, 7,* 468).

Two breeds of pigeons (White Carneau and Silver King) have been found to have a a high incidence of spontaneous atheromatous lesions in the aorta while two breeds of homing pigeons (Racing Homers and Show Racers) seemed to be resistant to atherosclerosis. The weight of the aorta, and its cholesterol content, paralleled the severity of the disease. The levels of serum cholesterol and phospholipids and the cholesterol:phospholipid ratio however appear to be unrelated to the incidence and severity of atherosclerosis in pigeons. The differences observed among the four breeds of birds showed little correlation with age, sex, diet, or physical activity (Lofland and Clarkson, *Circulation Res. 7,* 234). The development of spontaneous aortic arteriosclerosis was studied in four generations of standard-fed, uniformly treated, genetically similar group of 114 White Leghorns ranging up to 5 years of age. Progressively more severe arteriosclerosis, as measured by score, cholesterol concentration, and aortic weight increment, developed in both males and females although the diet can be considered nonatherogenic, *i.e.*, contained approximately 0.025% cholesterol and 4–5% fat (Weiss, *J. Gerontology, 14,* 19). Segments of both normal and atherosclerotic rabbit aortas were placed in the anterior chamber of host rabbits according to the Higginbotham technique. Normal aorta segments placed in the eyes of cholesterol-fed rabbits quickly became infiltrated with fat and cholesterol. The intensity of this infiltration exceeded that observed in the host's own aorta. Atherosclerotic aorta segments placed in the eyes of normal rabbits quickly lost both lipid and cholesterol (Friedman and Byers, *Circ. Res., 7,* 179). In the normal dog the rate of movement of cholesterol from the serum into the inner layer of the aortic wall is greatest at the proximal end of the aorta and decreases progressively along the length of the aorta until in the terminal aorta the rate is only about one-sixth that in the proximal aorta. A similar gradient of rates was previously demonstrated for albumin, which enters the aortic wall about three times as fast as cholesterol. The similarity of the gradients and the relative magnitude of the rates for cholesterol and albumin support the concept that cholesterol is carried into the aortic wall of the normal dog by the passage of the lipoproteins of which it is a part (Duncan *et al., ibid.,* 765).

The feeding of highly purified cholesterol to rabbits for 12 weeks resulted in high serum cholesterol and atherosclerotic lesions which appeared more severe than those obtained with commercial cholesterol. Impurities isolated from mother liquors after treatment of commercial cholesterol with anhydrous oxalic acid in organic solvents did not produce atherosclerotic lesions but, when given together with cholesterol, caused less extensive lesions than those obtained with pure or commercial cholesterol (Schwenk *et al., Proc. Soc. Exptl. Biol. Med., 102,* 42). Feeding of highly purified cholesterol to rabbits for 12 weeks resulted in high serum cholesterol and atherosclerotic lesions, which appear more severe than those obtained with commercial cholesterol. The latter contained about 2% impurities isolated from mother liquors after treatment of commercial cholesterol with anhydrous oxalic acid in organic solvents. Such impurities did not produce atherosclerotic lesions when fed to rabbits but, when given together with cholesterol, caused less extensive lesions than those obtained with pure or commercial cholesterol (*ibid.,* 42).

The dietary production of experimental arterial thrombosis, with resultant myocardial and renal infarction, has been accomplished in six separate experiments. Of the many dietary constituents omitted, none were found to be absolutely essential to the development of infarcts, but omission of any of the important ingredients (propylthiouracil, sodium cholate, cholesterol, and fat) lowered the incidence of infarcts. The areas of infarction in these rats are grossly visible, well circumscribed, almost always single, and often associated with thrombi in the supplying artery. These infarcts are to be distinguished from "metabolic" areas of necrosis or "infarctoid" lesions obtained in other experimental models of myocardial disease (Thomas *et al., J. Nutrition, 69,* 325). Levels of total cholesterol in plasma of rats fed several variations of a basal infarct-producing diet containing 40% butter, butter-oil, or lard were higher than in rats of other groups fed vegetable fat (corn oil or Crisco). Levels of rats in groups offered a diet not containing exogenous cholesterol were far lower than those of the control group. A significantly high level was observed when the amount of salt mix in the diet was increased three-fold to 12%. In a group of 30 rats fed the basal diet, cholesterol in plasma of those that developed infarcts rose neither more rapidly nor to significantly higher levels than did cholesterol in plasma of rats in the same group that did not develop infarcts within the experimental period of four months (Suzuki, *Proc. Soc. Exptl. Biol. Med., 102,* 544). Lipid plaques in the corneas of patients with lipid keratopathy, in corneas of hypercholesteremic rabbit are described. An interesting and thought-provoking analogy is drawn between the plaques that can be seen and followed in the eye during life with the more occult plaques of blood vessels in atheromatosis (Cogan *et al., Circ., 18,* 519). A substance which seems to be in the nature of a mucopolysaccharide protein complex has been obtained from the aorta of cattle by a technique described as "enzymatic dissection." This material seems to have an amino acid composition resembling that of elastin and therefore may constitute a pool for substances required for the replacement of destroyed or degenerated elastic fiber. It further has the property of inhibiting elastolysis by elastase, and this seems to be accomplished by a union with substrate rather than with the enzyme. Certain observations suggest a physical coating of the elastic fiber and the exclusion of the enzyme from access to susceptible chemical bonds. This mucopolysaccharide exhibits an anticoagulant ability similar to heparin although considerably less potent than the latter on a mg.-for-mg. basis (Yu and Blumenthal, *J. Gerontology, 13,* 366).

Two metachromatically staining fractions of acid mucopolysaccharides separated through paper electrophoresis from material isolated from 30 samples of human aortic tissues were subjected to chemical analyses. Determinations were made of the total hexosamine, galactosamine, glucosamine, uronic acid (colorimetric), and sulfate content of the fractions. The faster-moving fraction, which possessed a mobility similar to that of chondroitin sulfate, showed the following mean values: total hexosamine, 24%; uronic acid, 27%; and sulfate, 22%. All the amino sugar of this fraction was found to be galactosamine. The average composition of the slower-moving fraction was: total hexosamine, 22%; uronic acid, 28%; and sulfate, 16%. Galactosamine constituted 56% and glucosamine 44% of the hexosamine of this fraction. No significant variation with age

in the composition of the two fractions was observed (Kirk *et al., ibid.*, 362). The metabolism of the acid sulfomucopolysaccharides in human aortas was studied *in vitro* by means of S^{35} labeled inorganic sulfate. Sixteen samples were incubated with the radio-sulfate, the mucopolysaccharides were isolated by chemical methods, and the uptake of S^{35} in the isolated material was determined. A decreasing uptake with increasing age and arteriosclerosis was observed (Dyrbye, *J. Gerontology, 14,* 32). During an eight-week period cockerels were fed diets enriched with cholesterol (2%) and cottonseed oil (5%). Parallel to increased levels of serum and liver lipids and in cholesterol/phospholipid ratios, there was a significant increase in the S^{35} uptake by sulphated mucopolysaccharides in aorta but not in bones (Kowalewski, *Proc. Soc. Exptl. Biol. Med., 101,* 536).

Lipids have also been implicated in other diseases. Serum glycoproteins, mucoproteins, phospholipid, total lipid, total cholesterol, and lipoproteins were determined in patients suffering from cholera and in normal subjects. In cholera serum glycoproteins, mucoproteins, phospholipids, total lipids, and β-lipoproteins were significantly increased, and β-lipoproteins and total cholesterol were diminished (Banerjee *et al., ibid.*, 340). The isolation and chemical properties of lipopolysaccharide phosphoric acid ester endotoxin from *N. Gonorrhoeae* have been described. The high toxicity and other properties of the preparation indicate that indotoxic activity resides in the lipopolysaccharide phosphoric acid ester molecule (Tauber *et al., J. Biol. Chem., 234,* 1391). Five experiments were conducted to study the effects of inhibiting growth of an implanted fibrosarcoma in randomly bred and inbred Irish gray rat by a) adding essential fats to otherwise ineffective diets and by b) giving intraperitoneal injections of guinea pig serum. Statistical analysis revealed that cottonseed oil in the diet, without guinea pig serum injections, had significant effects in a) reducing the number of tumor "takes," b) slowing tumor growth, or c) bringing about regressions unless the tumor was too rapidly growing to allow sufficient time for the production of possible immunologic, inhibitory agents. Considering all experiments together, the results were significant at the 1% level. Guinea pig serum injections greatly enhanced the effect of fat in the diet, statistically significant at less than 0.01 or less than 0.001 level, except in experiments in which only one series of injections was given. Fat in the diet increased the activity of guinea pig serum injections significantly. The final conclusion must be reached that essential fatty acids are necessary for the fullest resistance of rats to this tumor (*Jameson et al., J. Nutr., 67,* 469).

Esters of acids in the C_{14} to C_{18} range, from five lots of tubercle bacillus, have been analyzed by use of gas chromatography, and certain fractions have been separated and examined. Three lots of virulent strains of the organism and two lots of an avirulent strain were represented. The pattern of acids in the several lots of lipids was found to be quite similar qualitatively but not quantitatively. There were present each normal fatty acid from C_{14} to C_{26}, also a branched isomer at each molecular weight above C_{21}; therefore a total of 11 acids was detected in this narrow molecular-weight range. A similar pattern probably continues for the much smaller amount of acids in the C_{20} and C_{21} range (Agre *et al., J. Biol. Chem., 234,* 2555). The fraction of esters previously termed the C_{18}–C_{19} fraction, from four lots of tubercle bacillus, has been examined by use of gas phase chromatography. In each, similar components were found although in varying ratios. In addition to palmitic, stearic, and tuberculostearic acids, there were also present smaller amounts of three other acids whose esters had retention times between palmitic and stearic esters. These components are probably a branched C_{17} acid, the normal C_{17} acid, and a branched C_{18} acid. Ozonolysis of the C_{18} fraction showed that the unsaturated C_{18} acid is oleic acid in each instance, not vaccenic acid as found in other microorganisms (Cason *et al., ibid.*, 1401)

Composition and Characteristics

COMPREHENSIVE AND GENERAL INFORMATION. The Uniform Methods Committee of the American Oil Chemists' Society reported the adoption of new tentative methods for congeal point, viscosity of transparent liquids by bubble-time method, moisture in alkylbenzene sulfonates by Karl Fisher and distillation methods, and the analysis of fatty alkyl sulfates. In addition, minor changes recommended for a number of existing methods were described (Andrews *et al., J. Am. Oil Chemists' Soc., 36,* 114, 352). The British Standards Institute (*British Standard* 684, 1958) listed a number of physical and chemical methods for the analysis of fats and oils. The 1956 and 1957 literature on the chemistry, composition, characteristics, and analysis of oils and fats was reviewed (Rao, *Lit. Rev. Oils and Fats,* Council of Scientific and Industrial Research, New Delhi, India, 1958, 1959).

The effects of variety, maturity, and environment on the fat content and composition of various fat sources were studied. Composition and characteristics of tropical animal and vegetable fats as influenced by species, climate, nutrition, or fertilization, etc., were reviewed (Zaheer and Achaya, *Nahrung, 2,* 710). The seed oils of *Brassica campestris,* flax, *Carthamus tinctorius,* sunflower, sesame, and soybean at three stages during a month's growth showed an increase in oil content, decrease in free acid, and increase in I. V. (Vidal, *Rev. Fac. Agron., La Plata, 32,* 159). Fertilizer treatment was found to have no effect on oil content and iodine values of linseed oil (Damaty and Kobbia, *Ann. Agr. Sci., 1,* 77). Oil accumulations in the Baladi and Hindi varieties of flax seeds were slow and similar (about 0.5% daily) during the first three weeks after flowering. Maximum amounts of 37.72 and 43.72% were reached after 48 and 51 days, respectively. The iodine value increased during the entire period but at a lower rate toward the end. The greatest rate of increase occurred after the oil content was the maximum value, suggesting unsaturated acid formation in the late period (Hashad *et al., Ann. Agr. Sci., 1,* 37). On the other hand, Karatha (*J. Sci. Ind. Research 17C,* 104) isolated the neutral oils in linseed at four germination stages corresponding to 15, 32, 67, and 73% of the mature oil content and found the respective iodine numbers to be 180, 179, 179, and 173. The poppy seed oil isolated at three germination stages corresponding to 62, 72, and 85% oil had iodine numbers of 62, 72, and 85, respectively. This showed the absence of selective mobilization of the more unsaturated acids in the germinating seeds. The oil content during the pod-filling stage of soybeans was found to increase with temperature (Howell and Cartter, *Agron. J., 50,* 664). The oil formation, relation of carbohydrates to oil formation, and the effect of storage of soybeans has also been investigated (Tremazi, *Texas J. Sci., 11,* 17). The effect of different localities on the fatty acid composition and characteristics of sunflower oil was studied (Rankov and Popov, *Bulgar. Akad., Nauk. Izvest. Khim. Inst., 5,* 203). Similar studies were made on the effect of climate and ecological zone on 22 strains of corn grown in Buenos Aires (Burquete, *Rev. Fac Cienc. Quim. Univ. Nacl. La Plata, 29,* 61). Those samples grown at the highest (coldest) latitude had higher contents of linoleic and saturated acids and lower contents of oleic acids.

The factors affecting the iodine number of fat from lamb were found to be local temperature of the tissue, rate of fat deposition, and level of fatness (Calow, *J. Agr. Sci., 51,* 361). The fat of winter herring varies considerably and diminishes during the catching season; the average figures decline from 12.5 to 9.6% (Flood, *Fiskeridirektorat. Skrifter, Ser. Teknol. Undersk., 3* [5], 1). The *n*, I.V., free fatty acid, saponification number, nonsaponifiable matter, and Gardner color number of 126 menhaden and 14 herring body oils and 12 tuna and 12 salmon by-product oils collected during each of two consecutive seasons were reported (Simmons, *Com. Fisheries Rev., 20, No. 11a,* 15). The changes in the phospholipids, sterols, and fatty acids of cod during storage in ice were studied (Lovern *et al., J. Sci. Food Agr., 10,* 327).

The data on milk and butterfat are extensive. The effect of the stage of lactation and the lipid nature of the food on the composition and characteristics of milk fat were studied (Bienfait, *Ann. Méd. Vet. 101,* 45, 102). The fatty acid content of cow butter without feed and water supplements in cool summer weather and those with these supplements during hot summer weather were compared (Haab, *Schweiz Milchztg., 84,* 454). The influence of fat and carbohydrates of feeds on the quantity and composition of milk fat was studied by Kniga (*Intern. Dairy Cong., Proc., 15th Congr., 1,* 218), Georgea (*Doklady Vsesoyus. Konf. po Moloch. Delu, 1958,* 162), and Witt (*Z. Teierenahr, 10,* 375). Others have investigated the effect of seasonal variations on these and also physical properties (Nagae, *Kenkyusho Hokoku,* 47, 22; Purenas and Griniene, *Kauno Politech. Inst. Darbai,* 7, 33). The latter also includes the effects of lactation and the animal's age. Seasonal variations in the solid fat content of butterfat indicated large differences in the content of higher melting glycerides (DeMann and Wood, *J. Dairy Research, 26,* 17). The influence of various stages of lactation on the fat content of milk of Iskar and Zebu cows was also reported (Ikonomov, *Sbornik Nauch. Trudone Vet. Inst. Minist. Zenedel, 6,* 481), (Labouche and Peytavin, *Rev. Elev., 10,* 373).

Significant seasonal variation in the Reichert-Meissl value and Kirschner values were found for ghee prepared from cow's milk in different parts of India (Kehar, *Studies of Fats, Oils, and Vanaspatis, 1956,* 5). Values for a sample of buffalo milk

are also given. The fat contents of milk in the north of Poland (Budlawski *et al., Intern. Dairy Congr., Proc. 15th Congr., London, 1,* 232), in the Netherlands (Schiere, *Offic. Orgaan, Koninkl. Ned. Znivelbond, 50,* 601), and in Manitoba (Reinart and Nesbitt, *Proc. Intern. Dairy Congr., 14th, Rome, 1,* 911) are presented.

The Vitamin A and carotene content of these samples from eight breeds of cows were found to be the same under identical feeding conditions (Reinart and Nesbitt, *ibid.,* 13). However there were significant seasonal and breed variations in the Vitamin A and carotene content of milk from Manitoba cows (Reinart and Nesbitt, *ibid.,* 934). The variation between breeds was influenced by diet. Other studies on the effects of feeds on the Vitamin A and carotene content of milk fat discussed (Ezdakova, *Byall Nauch. Tekh. Inform., 1,* 48), (Thompson, *Intern. Dairy Congr., Proc. 15th Congr., London, 1,* 247). In a similar study the seasonal variation of these materials was attributed to changes in cow fodder (Purenos and Griniene, *Kauno Politech. Inst. Darbai, 9,* 25).

ANALYSIS OF FAT SOURCES. A rapid method for the determination of free fatty acids and neutral fats in seeds and various foods was described (Akiya, *Shokuryo Kenkyujo Kenkyu Hokoku, No. 9,* 3). Perchlorethylene, CCl_4 and 1,1,1-trichloroethane were compared to Et_2O for the determination of crude fat in feeds and meat products. The advantages of chlorinated solvents are that they extract more completely, are nonflammable, and chemically stable, do not require drying of the samples, are not limited by particle size, and are cheap (Sherman, *J. Assoc. Offic. Agr. Chemists, 42,* 646). A rapid method for the determination of fat in meat products involves homogenizing the sample with gypsum and trichlorethylene, filtering, and determining the specific gravity on the cooled sample (Gipr *et al., Myarnaye Ind. S. S. R., 29, No. 6,* 53). A similar method for oil in seed cake, grist, and husks was presented (Grynbergowa, *Tluszcze i Srodki Piorace, 2,* 240).

Modifications of the Gerber test for butterfat and improvements in the use of detergents of neutral or alkali reagents were critically reviewed (Bramer and MacWalter, *J. Soc. Dairy Technol., 12,* 105). Fat values for 40 samples of lactose were highest by the Deniges method, lowest by the Roese-Gottlieb method, and intermediate by a modified Gerber method. Only the Gerber method yielded the exact percentage of fat from a synthetic preparation (Marini *et al., Ind. Lechera, 41,* 16, 30). A method using a Kohler volumetric butyrometer was described for cream fat, which gives results that compare closely with those obtained by the longer gravimetric method with the Roeder butyrometer (Zühlsdorf, *Deut. Milch. Wirtschaft, 1959,* 63). Emulsified fat in milk and dairy products can be demulsified and collected in a measurable layer with the aid of a nonionic surface-active agent, polyoxyethylene sorbital monolaurate (Tween), and an anionic surface-active agent, dioctyl Na phosphate (Tergitol). The method must be standardized for each type of product tested by comparison with an absolute method, such as the Roese-Gottlieb method (Schain, *U. S. 2,863,734*). A semi-micro Roese Gottlieb procedure was used for the fat content in milk, and the accuracy of the results was assessed from recoveries of fat. The influence of varying the quantities of ammonia and alcohol was studied. The Roese-Gottlieb and Alla-Laval methods were compared, and the difference between results was shown (Stigen *et al., Proc. Intern. Dairy Congr. 14th, Rome, 1956,* 3, 580). A gravimetric method for the fat content of casein included a pretreatment with alcoholic ammonia, followed by precipitation with alcoholic HCl and the addition of alcohol in order to obtain quantitative extraction (Peter, *ibid.,* 374). Data were given, showing agreement between results by the photometric test and by the Majonnier test of fat for ice-cream mix (Pettinatti and Haugaard, *Ice Cream Trade J., 55,* 16). An improvement in the accuracy of the Van Gulik rapid method for fat in cheese was achieved by the use of a butyrometer of new design graduated from 5% to 30% in 0.5% scale divisions (Ludwig and Funke, *Deut. Milch. Wirtschaft 1959,* 62). A comparison made among the Babcock, Gerber, and Mojonnier methods for testing fat in fresh raw milk and preserved milk indicated that fat percentages obtained by the Gerber test were significantly higher than the Babcock and Mojonnier methods. The difference between the Babcock and Mojonnier methods were not significant (Lewallen, *Milk Products J., 49,* 14, 28). The methods used in New York State for determining the accuracy of volumetric glassware used in determinations of fat in milk were described (Robertson *et al., J. Milk and Food Technol., 21,* 309). A modification of Gentilini's method for butterfat used on 20 samples of milk averaged 0.14% higher than the Gerber method (Iannucci, *Acta Med. Vet., 1,* 263).

A rapid, modified Babcock method (details given) was adopted for fat in canned fish (Risley, *J. Assoc. Offic. Agr. Chemists, 42,* 261). Fat in fish meal was determined by refractometry of a single extract with 1-chloronaphthalene. The results agree well with fat determination by the solvent-extraction method (Treiber, *Fette, Seifen, und Anstrichmittel,* 60, 488).

In determining the fat in soybean, sunflower, flax seeds, and oil cakes with various solvents, the use of diethyl ether gave results which were 0.19–0.86% higher than with petroleum ether (Rzhekhin and Pogonkina, *Trudy Vsesoyuz. Nauch. Issledovatel. Inst. Zhirov, 1954,* 73). On applying the method of van de Kamer to the determination of fat in wet liver tissue, a better recovery was obtained if 34 cc. 10% HCl were added to 50 cc. saponified material for its hydrolysis (Matsushita, *Naika Hokun 3,* 516). Swahn's triolein test was examined and found unsuitable for the accurate determination of the lipid content of serum (Woller, *Klin Wochschr., 36,* 563).

A method was described for determining the oil content of cereal adjuncts. The procedure is based on a column extraction with petroleum ether (West and Lautenbach, *Proc. Am. Soc. Brewing Chemists, 1957,* 56). Fat in rice meal was determined by extraction with CCl_4 in a Soxhlet apparatus (Luykx, *Intern. Tijdschr. Brouw. en Mout., 17,* 222).

Extracted plant balances show 0.44–0.72% more oil than analytical results on the original soybeans. This is attributed to decomposition of lipoproteins and sugar lipids which release phospholipids into oil and solvent during plant processing but not by analytical Soxhlet extraction in the laboratory (*Semenov, Trudy Vsesoyuz. Nauch-Issledovatel. Inst. Zhirov, 1954, No. 15,* 94). A rapid estimation of the oil content of sunflower seeds consists of placing a given number of seeds between layers of filter paper, pressing in a laboratory hydraulic press, and measuring the weight of oil absorbed by the filter paper. The oil values were approximately 73% of those obtained by the standard Soxhlet procedure (Sherfelt and Putt, *Can. J. Plant Sci., 38,* 419).

GRADING AND VITAMIN TESTS. A comparison was made of the specificity of methods for determination of Vitamin E by Furter and Meyer (I), Emmerie and Engel (II), Nair and Magas (III), and Stiller (IV). According to the decreasing specificity, the methods were arranged in the following order, I> III> IV> II. The sensitivity of these methods decreased in the following order, III> II> IV> I (Vendt and Drokova, *Vitaminy, Akad Nauk Ukr. S. S. R. Inst. Biokhim, 3,* 75). A method for determination of Vitamin E in blood serum was described (Epel'baum and Lushchevskaya, *ibid.,* 95).

A collaborative study of "Woelm" Al_2O_3 resulted in its acceptance as an optional absorbent in the official method for Vitamin A in mixed feeds (Parrish, *J. Assoc. Offic. Agr. Chemists, 42,* 520). Other literature which deals with vitamin testing can be found in the Analysis of Composition section below.

A colorimeter with two filters, having transmittance between 400 and 700 mμ, was used to measure the color of olive oil. The two-filter system characterized the oils as well as the complete absorption spectrum determined with a spectrophotometer. Other color standard methods were discussed (Brobolla y Alcalá *et al., Grasas y Aceites, 9,* 302). A F-6 Moskip photocolorimeter was recommended for determining the color of cottonseed oil. Comparative data and means of calculating adustment for obtaining comparable results are given for the method, the Lovibond-Wesson Tintometer, and the Russian V.N.I.I.S.H. colorimeter (Khalimova and Markman, *Izvest. Akad. Nauk Uzbek, S. S. R., Ser. Khim Nauk., 1957, No. 2,* 77). The ultraviolet spectra of the nonsaponifiable fractions of lard and tallow of satisfactory quality were found to be essentially identical while those of spoiled, deodorized, and bleached lard showed maxima at 230 and 270 mμ. This indicates that spoilage brings about structural changes in the unsaponifiable fraction (Grau and Mirna, *Fette, Seifen, und Anstrichmittel, 60,* 553). The procedure of Kaufman *et al.,* for ultraviolet spectrophotometric analysis of lard treated with bleaching earth was studied in detail. Suggestions were made for improving sensitivity (Grau and Mirna, *Fleischwirtschaft, 11,* 23). The influence of various bleaching earths and temperature on the spectrophotometric technique for grading color in tallow and lard were reported (Trizis and Uzzan, *Rev. Franc. Corps Gras, 5,* 499). Analytical procedures for determining glyceride losses in alkali refining were discussed (Danowski, *Tluszcze i Srodki Piorace, 2,* 285).

ANALYSIS OF CHEMICAL PROPERTIES. Schowell *(J. Am. Oil Chemists' Soc., 36,* 363) proposed that the iodine number, saponification number, acid number, hydroxyl number, acetyl number, peroxide number, carbonyl oxygen, and oxirane oxygen be reported in basic units, moles and grams. Equations for obtaining and calculating these specific numbers are presented. Molchanov (*Trudy Krasnodor. Inst. Pishchevol Prom., 1955,* 109) presented formulas for the relation of the neutralization

number of fatty acids with molecular and weight parts and also with theoretical saponificated numbers of mixtures of mono-, di-, and triglycerides.

The apparatus and method for determining the acid number of vegetable oils by potentiometric titration were described (Akimov, *Izvest. Vysshikh Ucheb. Zavedenii, Pishchevaya Tekhnol., 1958*, 162). This method was recommended for dark-colored oils.

The halogenated fatty acids formed in the iodine number determination on oleic, linoleic, and ricinolcic acids were identitfied (Awe and Grote, *Fette, Seifen, und Anstrichmittel, 61*, 1). The effect of certain variables on the determination of iodine values of waxes was studied (Waller and Fussenegger, *Trans. Kentucky Acad. Sci., 19*, 6). The quantitative aspects of the iodine number by the addition of Hg (II) acetate were also investigated (Awe and Grote, *Fette, Seifen, und Anstrichmittel, 59*, 733). A method for determining the iodine value of fats in aqueous medium without emulsifiers was described (Suprum, *Aptechnoe Delo, 7*, 58). This analysis was also extended to oil in emulsions (Gengrinovich, *Med. Prom. S.S.S.R., 12*, 38).

A simplified method for the acetyl value was based on the detection of excess Ac_2O as AcOH after acetylation (Hirayama and Inomata, *Nippon Daigaku Yakuyaku Kenkyu Hokoku 2*, 29). Kartha (*J. Sci. and Ind. Research, 18B*, 217) developed an acetyl value method for deeply colored fats by extracting the hydrolyzed acetic acid before titration with alkali. Increasing temperature of acetylation from 105 to 130–150°C. was found to shorten the time of the hydroxyl number determination from 60 to 10 min. without affecting the accuracy (Vasilescu, *Fette, Seifen, und Anstrichmittel, 60*, 541). A new method of determining hydroxyl numbers by near-infrared absorption analysis in the region from 2.0 to 3.2 μ was described (Hilton, *Anal. Chem., 31*, 1610). A savings of 2.4 hrs. per duplicate determination was claimed, and an average difference of less than 1.0% relative was found.

The saponification method was improved by the use of ion exchange to obtain the free acids which were titrated potentiometrically (Swann, *Anal. Chem., 30*, 1830). A micro-method for the saponification number was described (Lee, *J. Assoc. Off. Agr. Chemists, 41*, 899). The saponification velocity of Chrysalis oil was investigated and found to be influenced by the solvent for KOH, decreasing with the carbon number of the alcohol (Katsuya, *Kagoshima Daigakar Nogakubu Gakiyutsu Hokoku, No. 7, 1958*, 156).

ANALYSIS OF PHYSICAL PROPERTIES. The physical chemical methods of analysis of fatty materials and their derivatives was reviewed by Wolff (*Inds. Aliment et Agr., 75*, 639). Woerfel and Bates discussed the use of dilatometry and rheology of fats in the formulation and testing of shortenings (Food Tech., *12*, 674). A symposium on the rheology of fats was presented in the Journal of Japan Oil Chemists' Society (Kuwata, *Yukagaku, 7*, 455).

The measurement of hardness of fats received considerable attention. A formula for the yield value was developed for cone penetrometer measurements and found to apply for cones with angles 15–90° over a wide hardness range (Haighton, *J. Am. Oil Chemists' Soc., 36*, 345). A modified Brinell test for hardness was developed and applied to various fats (Feuge and Guice, *J. Am. Oil Chemists' Soc., 36*, 531). Hardness was found to increase as the components of a fat were converted to higher melting polymorphs and to decrease as the crystal size increased. The extent of hardness increase of butter during storage was shown to be directly dependent on the initial hardness (DeMan and Wood, *J. Dairy Sci., 42*, 56). Other factors important to the hardness of butter were also discussed.

A simplified method for the determination of the softening point of butterfat utilized the temperature at which a standard ball-bearing has fallen half-way through the fat (Dixon, *Australian J. Dairy Technol., 14*, 22). A study was made of the effect of various methods of pretreatment on the melting point of cocoa butter (Reith, *Rev. Intern. Chocolat., 13*, 466). The four temperatures at which an oil becomes cloudy, immobile, fluid, and clear were used to identify various oils (Martinenghi, *Olearia, 12*, 97).

Dilatometric curves for hydrogenated whale and vegetable oils, milk fat, coconut oil, beef tallow, and milk margarine were obtained and their application to technical control discussed (Grauerman *et al., Masloboĭno-Zhirovaya Prom., 24*, 10). The use of low-resolution nuclear magnetic resonance was found to provide a new method for determining the liquid-solid content of fats (Chapman *et al., Nature, 183*, 44). Advantages are that fats can be examined without altering crystal structure and results are independent of the composition of the fat and of the polymorphic form of the solids. The influence of the temperature treatment and season on the dilatometric behavior of butterfat was investigated (DeMan and Wood, *J. Dairy Research, 26*, 17). The relationship between the solid fat content of chocolate coating and its viscosity was established (Duck, *Mfg. Confectioner, 38*, 9). This was used to measure the solid fat in the coating during tempering.

The solubilities of several fatty acids and their methyl esters in SO_2 have been determined as a function of temperature (Schlenk and Ener, *J. Am. Oil Chemists' Soc., 36*, 145).

The effect of the polymorphic history on the refractive index was felt to invalidate the use of 40° measurements corrected to 25° for cocoa butter (Sachsse and Sachsse, *Fette, Seifen, und Anstrichmittel, 59*, 1063). The application of refractometry in studying changes during the hardening of lard, cottonseed, and palm oil and its relationship to the character of butter and margarine was discussed (Kaufmann and Theieme, *ibid.*, 832). Melting-point curves and x-ray examination of mixtures of fatty acids were determined and used to show molecular aggregations between the fatty acids, phosphatides, and cholesterol (Dervichian, *Olii Minerali, Grassi e Saponi, Colori e Vernici, 35*, 229). The molecular interaction in mixed monolayers of fatty acids were studied (Durham, *J. Applied Chem., 8*, 724). These intermolecular forces were related to the excess thermodynamic properties of the system (Goodrich, *Proc. Intern. Congr. Surface Activity, 2nd, London, 1957, 1*, 85). The structure of fatty acid monolayers was studied by film balance and electron microscope techniques and a mechanism for collapse postulated (Ries and Kimball, *ibid.*, 75).

Newer instrumentation techniques for studying fats include the use of the dielectric and refractodensimetric behavior to determine the state of oxidation (Ludde, *Fette, Seifen, und Anstrichmittel, 61*, 1157) and the application of nuclear magnetic resonance spectra to structural studies of fatty acids, their esters and glycerides (Hopkins and Bernstein, *Can. J. Phys., 37*, 775).

COMPOSITION ANALYSIS. The literature concerned with analytical methods for determining the composition of fats are divided below into three main categories: fatty acid composition, glyceride composition, and lipid composition. General review articles on the subject were the application of spectrophotometry in the study of vegetable oils (Minutilli, *Rass, Chim. 10, No. 3*, 2), the application of infrared spectroscopy to fats (Kaufmann *et al., Fette, Seifen, und Anstrichmittel, 61*, 547), the identification of fatty acids by functional derivatives (Lefort, *Oleagineux, 12*, 685), the separation of fatty acids (Holman, *Experientia, 14*, 121), and the determination of fatty acid composition by gas chromatography and ultraviolet spectrophotometric methods (Malin, *Soap, Perfumery, and Cosmetics, 32*, 597).

Fatty Acid Composition. A few articles dealt specifically with the determination of the volatile fatty acids present in fats. A gas-liquid chromatographic method for volatile fatty acids in milk was described (Hankinson *et al., J. Dairy Sci., 41*, 1502). Data were presented to show the behavior on steam distillation of a mixture of normal saturated fatty acids from C_4 to C_{10} (Weenink, *New Zealand J. Sci., 1*, 18). The volatilities of the C_4–C_{10} acids were considerably reduced by the presence of higher M. W. acids. A study was made of the fatty acid composition of the Reichert-Meissl and Polenske fractions of butterfat and its admixtures with other fats (Sengupta, *Univ. Microfilms, L. C. Card No.* Mic *59–138*). A method for the determination of volatile fatty acids in bovine blood by isotope dilution was described (Holter *et al., J. Dairy Sci., 42*, 358).

A rapid enzymatic method was developed for determining polyunsaturated fatty acids by using lipoxidase. The enzyme produces the conjugated diene hydroperoxide which is measured at 234 mμ (MacGee, *Anal. Chem., 31*, 298). In the spectrophotometric determination of polyunsaturated fatty acids in butter, higher absorption values of butter triglycerides, using 21% KOH rather than 7–10% KOH, were found (Mattsson and Swartling, *Milk Dairy Research Rept. 55*, 8). Increasing the concentration of the KOH used for isomerization was found to make the micro-alkaline isomerization technique for unsaturated fatty acids more sensitive (Michaels, *Am. J. Clin. Nutrition, 6*, 593). For accurate results by alkali-isomerization it was found necessary to establish special constants of the various polyunsaturated fatty acids by alkali isomerizing under the conditions that will be used in analysis measurement of ultraviolet absorption at various wavelengths, then establish constants which may be used in calculating the original composition of alkali isomerized mixtures of polyunsaturated fatty acids (Lundberg, *ibid.*, 592). The effects of isomerizing methyl linolenate in an alkaline glycol solution for 7 hrs. at 200°C. were studied (Scholfield and Cowan, *J. Am. Oil Chemists' Soc., 36*, 631). Extensive polymerization was found; the characteristics of other fractions were discussed.

Detailed methods were given, and comparisons made between micro- and macro-methods of determining fatty acids in plasma and lipid components of tissues by ultraviolet spectroscopy (Reimenschneider *et al.*, *Am. J. Clin Nutrition, 6,* 587) and (Leupold and Eberhagen, *Klin. Wochschr., 36,* 484). The detection and measurement of *cis*-unsaturation in fatty acids by near-infrared spectroscopy (at 2.14, 2.15 μ) was the subject of two articles (Holman *et al., Arch. Biochem. Biophys., 80,* 72) and (Fenton and Crisler, *J. Am. Oil Chemists' Soc., 36,* 620). Methods for the determination of *trans*-unsaturation by spectrophotometric measurements in the infrared were also discussed (Callen and Pace, *Anal. Chem., 30,* 2066; Miller, *ibid.,* 1884). Polyene fatty acids were separated from saturated and weakly unsaturated fatty acids in blood serum by urea fractionation (Leupold and Eberhagen, *Fette, Seifen, und Anstrichmittel, 60,* 809). The influence of varying amounts of urea and solvent (EtOH) on the fractionation of cottonseed-oil fatty acids was studied (Kats and Uarkman, *Doklady Akad. Nauk. Uzbek. S.S.R., 1957, No. 4,* 45). The efficiency of urea fractionation can be improved by use of an homogeneous system or lowering the interaction by esterification of the fatty acids (Sakurai, *Kogyo Kagaku Zusshi, 60,* 506). The effects of urea concentration, crystallization time and temperature on the fractionation of a fatty acids mixture and yields of fractions from cottonseed soapstock were discussed (Markman and Kats, *Masloboino-Zhirovaya Prom. 24,* 12). A potentiometric method was proposed for determination of high M.W. fatty acids by titrating with alcohol KOH, followed by 0.10 N. Ag NO_3 (Sass, *Fette, Seifen, und Anstrichmittel, 61,* 93). Linoleic and linolenic acids were determined by means of the different solubilities of their Br addition products (Franzke and Ittrich, *Fette, Seifen, und Anstrichmittel, 59,* 740). In the determination of isoleic acids in hydrogenated fats the Pb salts method gave results that were 1–9% lower than those of the spectral method (Pokorný and Kakác, *Prumysl Potravin, 10,* 19). The detection of arachic acid in edible oil by means of partition chromatography on filter paper was described (Briski and Brodarec, *Kem. i ind., 7,* 1). This and other high-molecular weight acids can be spotted on the dried chromatograms in the form of their green-colored Cu salts. The relative evaporation, equilibration curves, number of trays, and reflux ratio for the separation of fatty acids by fractional distillation were determined (Perédi, *Elelmezesi Ipar, 11,* 189).

A simplified Ramsey-Patterson partition chromatographic method was described for use on short chain fatty acids (Kemp and Hetrick, *J. Dairy Sci., 41,* 1494). Resin column chromatography was employed for the separative determination of fatty acids (Seki, *J. Biochem.* (Tokyo), *45,* 855). Normal C_{13}, C_{15}, C_{17}, and C_{19} carboxylic acids in menhaden oil were determined by using paper chromatography in an acetic acid/peracetic acid system with silicone-treated paper (Schlenk, *Experientia, 15,* 387). Several reversed-phase paper chromatographic systems for separation of fatty acids were described, and the relationship between structure and R_f was determined for more than 40 pure fatty acids (Ballance and Crombie, Biochem. J., *69,* 632). Combination of two temperatures in 2-dimensional ascending paper chromatography impregnated with paraffin and paraffin oil was used to separate fatty acids containing more than 24 C atoms (Fiker and Hájek, *Chem. Listy, 52,* 549). The method and results of separation of higher fatty acids by paper chromatography by using continuous change of mobile phase was also described (Palo *et al., Chem. Zvesti, 12,* 525). A combination of paper-chromatographic and polarographic methods for the separation and determination of fatty acids was described (Kaufmann and Deshpande, *Fette, Seifen, und Anstrichmittel, 60,* 537). Paper chromatography of the thiocyanogen derivatives of fatty acids was recommended because their R_f values were greater than the free acids or their bromo-derivatives (Kaufmann and Arens, *ibid.,* 803) Fatty acids separated by paper chromatography were separately identified as Cu and Hg soaps and as Hg additional compounds of the unsaturated acids by determining the areas colored by HgS and Cu $(OAc)_2$, respectively (Kaufman and Schnurbusch, *ibid.,* 1046). The detection of fatty acids on paper chromatograms by means of ninhydrin was also described (Burness and King, *Biochem. J., 68,* 32). In the analysis of fatty acids in natural fats by paper chromatography it was found best to use the method of 2,4-dinitrophenylhydrazide formation and reversed-phase chromatography of hydroxamic acids for higher saturated fatty acids and the ordinary hydroxamic acid method for volatile fatty acids (Noda *et al., Nippon Nogei-Kagaku Kaishi, 30,* 106). Inversed radial paper chromatography was used to determine the fatty acids of lard, hydrogenated lard, palm oil, coconut oil, and hydrogenated coconut oil (Sulser, *Mitt. Gebiete Lebensm. und Hyg., 49,* 264). Polyunsaturated fatty acids were determined quantitatively by photometric evaluation of the paper chromatograms on which the fatty acids have been analyzed (Miyakawa, *Fette, Seifen, und Anstrichmittel, 61,* 850). The possible sources of error in this method were discussed, and methods were suggested to avoid them (Seher, *ibid.,* 55). The paper chromatographic separation of linoleic, oleic, stearic, and palmitic acids was investigated with four types of paper, 13 solvent combinations, and two developing reagents (Mirzakarimov and Yakubov, *Doklady Akad Nauk Uzbek. S.S.R., 1958, No. 3,* 29). For their separation and identification the mixed fatty acids of dehydrated castor oil were acetylated, reacted with maleic anhydride, and subsequently hydrogenated and subjected to paper chromatographic analysis (Chowdhury, *Fette, Seifen, und Anstrichmittel, 61,* 924). A preliminary fractionation of fatty acids by molecular distillation was found to aid subsequent polarographic paper chromatographic analysis (Niewiandomski *et al., ibid.,* 897). Ozonolysis and chromatographic procedures were examined for determining the position of double bonds in olefinic acids (Benton *et al., J. Am. Oil Chemists' Soc., 36,* 457). Ozonolysis was found to be seriously limited because of the secondary reactions undergone by the ozonide.

The interest in gas chromatography for separating fatty acid mixtures continued. Two bibliographies on the subject were compiled for those articles appearing before 1958 (Zahn and Langer, *Bureau of Mines Circ. 7856,* reprinted by Fisher Scientific Company, 1959), and during 1958 (Fisher Scientific Company, *The Laboratory, 27,* 5). A separate bibliography on the gas chromatography of fatty acids was also published *(ibid.).* An inexpensive and simple apparatus which permits automatic analysis of γ-quantities of long-chain fatty acids was described (James, *Am. J. Clin Nutrition, 6,* 595). Columns prepared with diethylene glycol succinate and adipate were placed in series with separate detectors. The succinate column separated stearic and oleic acid esters: the adipate separated linoleic and arachidic acid esters (Craig and Murty, *Can. J. Chem., 36,* 1297). The even-numbered free fatty acids C_{12} to C_{22} and their methyl esters were determined in menhaden oil and in Ximenia caffra oil (Beerthuis *et al., Ann. N. Y. Acad. Sci., 72,* 616). It was shown that methyl esters of highly unsaturated long-chain fatty acids are not significantly altered in chemical structure during gas liquid chromatography with the stationary phase Apeizon M at 197° (Stoffel *et al., Proc. Soc. Exptl. Biol. Med., 99,* 238). By collecting fractions from the gas chromatography columns and identifying their components by mass spectrometry, complex mixtures of fatty acids present in butter and margarine were analyzed (Hallgren *et al., Acta Chem. Scand., 12,* 1351). The lower fatty acids (C_1–C_5) were separated on diactyl sebacate containing 15% sebacic acid (Raupp, *Angew. Chem., 71,* 284). Fatty acids from eight to 24 carbons in length were separated quantitatively (Jart, *Fette, Seifen, und Anstrichmittel, 61,* 541). The fatty acid composition of a number of vegetable oils and of two synthetic mixtures of methyl esters were compared by gas-liquid chromatography and by standard methods (Craig and Murty, *J. Am. Oil Chemists' Soc., 36,* 549). Standard methods gave lower values for linolenic acid than did G.L.P.C. A technique was devised for the quantitative formation of methyl esters of long-chain fatty acids (Stoeffel *et al., Anal. Chem., 31,* 307). The esters were separated in pure form by sublimation. Alteration of the chemical composition of the stationary liquid as a method of facilitating the analysis of higher fatty acid esters was studied (Lipsky *et al., Biochem. et Biophys. Acta, 31,* 336). A method for the determination of column efficiency in gas liquid chromatography (Johnson, *Anal. Chem., 31,* 357) and a calculation for locating the carrier gas front of a gas-liquid chromatogram (Peterson and Hirsch, *J. Lipid Research, 1,* 132) were presented.

Glyceride Composition. A review of the progress in the synthesis of glycerides of fatty acids from 1940 to 1958 included methods of glyceride synthesis, characteristics of the products, and techniques used in the estimation and definition of glycerides (Hartman, *Chem. Revs., 58,* 845). A theory was presented for the formation of fats which gives the amounts of the various glyceride types equivalent to a random or modified, restricted-random distribution and at the same time gives predominance of specific positional isomers (Young, *J. Am. Oil Chemists' Soc., 36,* 664). Cocoa butter was fractionated by countercurrent distribution between pentane-hexane and 2-furaldehyde-nitroethane solvent phases with 1100 transfer-stage equipment and was found to follow neither a "random" nor an "even" pattern of glyceride structure. (Scholfield and Dutton, *ibid.,* 325). The apparatus of Baker and Williams for crystallization in a thermal gradient was tested for separation of various binary mixtures of tripalmitin, trilaurin, 1-oleo-2,3-dipalmitin, and trimyristrin. The apparatus was able to separate model mixtures efficiently (Magnusson and Hammond, *ibid.,* 339). To study the nature of the fatty acids in the 2-position of natural triglycerides, oils and fats were partially

hydrolyzed with pancreatic lipase, 1-monoglycerides were destroyed with periodic acid, and the 2-monoglycerides were recovered chromatographically (Savary and Desnuelle, *Biochem. et Biophys. Acta, 31,* 26). In beef tallow, sunflower oil, and cacao butter the saturated acids preferentially occupy the external positions (Savary *et al., Rev. Franc. Corps Gras, 5,* 493). The position of fatty acids in the glycerides appears to be dependent on the chain length of the saturated acids as well as the degree of unsaturation of the unsaturated acids (Desnuelle and Savary, *Fette, Seifen, und Anstrichmittel, 61,* 871). The manner in which the location of fatty acid in the glyceride molecules affects the biological and physical properties of the glycerides was discussed. In a study of the glyceride structure of swine depot fat the data obtained by hydrolysis with pancreatic lipase indicated that unsaturated acids are predominately in the 1- and 3-positions and the saturated in the 2-position (Reiser and Reddy, *J. Am. Oil Chemists' Soc., 36,* 97). It was postulated that the structure of endogenous animal fat is a result of the specificity of the acylating enzyme and of the nature and relative proportion of the available fatty acids. The paper chromatographic separation of synthetic and natural triglyceride mixtures was studied (Kaufmann and Makus, *Fette, Seifen, und Anstrichmittel, 61,* 631). The authors separated various mixtures consisting of 12 synthetic and 17 natural fats. A method was described for determining the position of fatty acids on lecithin with the use of pancreatic lipase (Tattrie, *J. of Lipid Research, 1,* 60).

The analysis of mono- and diglycerides by gas chromatography was investigated (Huebner, *J. Am. Oil Chemists' Soc., 36,* 262). Using the acetylated mixture for separation, a straight-line relationship between the log of the retention time and molecular weight was found for each class of compounds. Preliminary studies could not differentiate between mono-olein and monostearin. Paper chromatography by using 80% aq. Me_2CO as solvent from a 1% solution in C_6H_6 and identifying the spots by means of Rhodamine B permitted separation of mono-, di-, and triglycerides (Jaky, *Fette, Seifen, und Anstrichmittel, 61,* 6). A comparative determination of α-monoglyceride and glycerol and calculation of errors in the methods by Pohle and Mehlenbacher and Kruty gave absolute errors of ±0.9 and ±0.3%, respectively (Becker and Krull, *Fette, Seifen, und Anstrichmittel, 60,* 449). The authors claimed that the determination of β-glycerides based on isomerization by means of perchloric acid is not applicable to commercial sample of monoglycerides. The method of Kruty for determining monoglycerides + glycerol, and glycerol alone in fats was found more accurate and convenient for monoglycerides in baking fats (Croes, *Conserva, 6,* 164).

Lipid Composition. Many of the composition methods dealt with the qualitative and quantitative detection of the various lipid classes. The structure of fatty acids, the incorporation of fatty acids into lipids, the synthesis of glycerol-based lipids, and interrelations of classes of lipids were discussed (Lovern, *J. Sci. Food Agr., 9,* 773). The microdetermination of the three major lipid classes (cholesterol, triglycerides, and phospholipids) was reviewed (Van Handel, *J. Am. Oil Chemists' Soc., 36,* 294). A method and apparatus were described for the separation of complex lipid mixtures into chemical classes by elution from a single column of silicic acid (Hirsch and Ahrens, *J. Biol. Chem., 233,* 311). A similar method utilized a mixture of equal parts of silicic acid and celite (Michaels, *Am. J. Clin. Nutrition, 6,* 604). Hack's (*C.A., 47,* 12486) rapid method of disk chromatography was used for orientation analysis of lipids (Horacek and Cernikova, *Biochem. J., 71,* 417). The detection of individual components was extended by the use of additional reactions. Total blood serum lipids were fractionated into two groups, phospholipids in one and a mixture of glycerides, steroids, and cholesterol in the other (Cormier and Jouan, *Bull. Soc. Chim. Biol., 40,* 171). Immobile neutral fat, free chylomicron lipid, and lipoproteins in fractions corresponding to albumin, α_1-, α_2-, β-, and γ-globulins were found to be separable by two-dimensional electrochromatographic methods (Keler-Bacoka *et al., Clin. Chim Acta, 3,* 335). A cytochemical method for the demonstration of masked lipids *in situ* consisted of a specific coloration of lipids after suitable fixation, coupled with an incipient separation of the protein from the lipid in the complexes by means of reagents which mildly attack the proteins and not the lipid (Serra, *Science, 128,* 28). Column chromatography of a number of plant waxes by means of Al_2O_3 led to the isolation of fractions containing paraffins, esters, alcohols, or acids identifiable by means of x-ray analysis (Wiedenhof, *J. Am. Oil Chemists' Soc., 36,* 297). To facilitate detection, fatty acids were esterified with radioactive diazomethane, and lipids containing amino or hydroxy groups acetylated with radio-active acetylation mixtures before chromatographic separation (Mangold, *Fette, Seifen, und Anstrichmittel, 61,* 877). The lipid and protein-staining properties of Sudan II, III, IV, and Sudan Black B were investigated (Kutt *et al., Stain Technol., 34,* 197, 203). The commercial materials all showed some protein-staining properties.

Many papers dealt with the analysis of phospholipids. Egg lecithins were practionated as their Hg $(OAc)_2$ addition compounds by reversed-phase paper chromatography (Inoue and Node, *Arch. Biochem. Biophys., 76,* 271). The distribution of fatty acids in egg lecithins was discussed on the basis of these results.

A rapid chromatographic procedure for the separation of sphingomyelin, phosphatidyl choline, ethanolamine, phosphatidyl serine, phosphatidyl inositol, and free fatty acids on glass paper coated with sodium silicate was described (Muldrey *et al., J. Lipid Research, 1,* 48). Crude phosphatides were separated by using a specially prepared formaldehyde paper and a butanol-glacial acetic acid-water mixture as solvent (Hornkammer *et al., Biochm. Z., 331,* 155). Malachite green was found to be a new specific reagent for detection of lysophosphatides. Lecithin and cephalins in soybean phospholipids were isolated at purities of 95% and 84%, respectively, on a column of cellulose powder (De Zotti, *Olii Minerali, Grassi e Saponi, Colori e Vernici, 36,* 114). Chromatography on silicic acid was employed for the separation of phospholipid components of normal serum (Gjone *et al., J. of Lipid Research, 1,* 66). Milk phospholipids were fractionated into the major phospholipid classes by successive elution from a silicic acid column with $CHCl_3$, acetone, MeOH (20% and 40%) in $CHCl_3$, and MeOH and analyzed by infrared spectrophotometry as well as by chemical methods (Smith and Freeman, *J. Dairy Sci., 42,* 1450). Lipids from rat liver and sheep brain were dinitrophenylated and methylated before fractionation by countercurrent distribution (Collins, *Biochem. J., 72,* 281). A procedure for the alkaline hydrolysis of phosphatides and fractionation of the hydrolyzates on ion exchange columns was described (Hawthorene and Hübscher, *ibid., 71,* 195). In another study the hydrolysates of sphingolipids were oxidized by sodium metaperiodate and the fatty aldehyde reaction products isolated and analyzed by gas-liquid partition chromatography (Sweeley and Moscatelli, *J. of Lipid Research, 1,* 40). Florisil columns were used to separate the cerebroside hydroxy and normal acids from fresh brain. The esters within each class were determined by gas chromatography (Kishimoto and Radin, *ibid.,* 72). Thaler's method for the determination of phosphorus content of lipids was improved. The sensitivity of the new method makes it possible to determine one microgram of phosphorus in one gram of fat (Korpaczy, *Fette, Seifen, und Anstrichmittel, 61,* 748). A perchloric acid digestion procedure for digesting lipids for the determination of phosphorus was also described (Smith *et al., J. Dairy Sci., 42,* 552).

A rapid, accurate pptn. test for β-lipoproteins, using buffered K-agar was described (Boyle and Moore, *J. Lab. Clin. Med., 53,* 272). The flotation principle was utilized to develop a more convenient ultracentrifugal method for the separation of serum lipoproteins (Del Gatto *et al., U. S. At. Energy Comm.,* UCRL 8476). An electrophoretic method for the determination of lipoproteins was based upon direct staining of the electrophoretogram with oil-soluble dyes. Sudan Black was found to be superior as it clearly shows each protein fraction designated as α-, β-, γ-lipoproteins (Vysotskaya and Livshits, *Lab. Delo, 1959,* No. 3, 31).

A method was described for the determination of total fatty acids of serum by microtitration. The method was designed to permit also the determination of cholesterol, lipid phosphorus, and triglycerides by difference (Albrink, *J. of Lipid Research, 1,* 53). Micro amounts of higher unesterified fatty acids (C_{12}–C_{18}) in blood were determined by reaction of the extracted acids with rosaniline and analyzing at 520 mμ (Mendelsohn, *S. African J. Med. Sci., 23,* 75). A method was presented for the extraction of free fatty acids from lipid material, which eliminates the troublesome emulsions formed during the extraction of soap solutions with ether (Mattick and Lee, *Food Research, 24,* 451). Fatty acids were readily isolated from extracts containing dissolved proteins by placing the hydrolyzed mixture on an Amberlite IRA 400 column, washing with distilled water and eluting with MeOH (Nowotny, *Naturwissenschaften, 43,* 519).

Lipoproteins were separated by paper electrophoresis, identified by reference to marker strips stained with Sudan Black B, eluted with 2:1 $CHCl_3$:MeOH and their cholesterol estimated by means of the $FeCl_3$ reaction (Crawford, *Clin. Chim. Acta., 4,* 494). A comparative study of the ferric chloride method with others for the determination of total cholesterol and cholesterol esters was made (Chiamori and Henry, *Am. J. Clin. Pathol., 31,* 305). Cholesterol esters reacted in the Riffart-Keller reaction (*C.A., 28,* 6857) with greater speed and higher

intensity than the corresponding equivalent amount of cholesterol Brieskorn and Herrig, *Z. Lebensm.-Untersch, und Forsch., 110,* 15). The esters should first be saponified in order to reduce the error. Three methods, the Schoenheimer-Sperry, the Grigaut, and the Delsal for the estitmation of serum chlosterol were evaluated (Bargeton *et al., Rev. Franc. Études Clin. et Biol., 4,* 326). The Schoenheimer-Sperry method (*C.A., 28,* 6752) showed the smallest standard error in replicate analyses.

Gossypol in cottonseed oil was separated by paper chromatography, and the spots were developed by spraying with a 2% solution of stannic chloride and heating at 105–10°C. for 15 min. (Rakhmanov and Yakubov, *Doklady Akad. Nauk. Uzbek. S.S.R., 1957* [9], 51). Gossypol, not luminescent in itself, quenches the luminescence of $CHCl_3$ and Me_2CO. The degree of quenching increased with concentration (Vilkova and Markman, *Zhur Priklad. Khim., 31,* 1548). This was used as a basis for the quantitive determination of gossypol in cottonseed oil. A new method of determination of gossypol in oils consisted of extracting from oil by aqueous KOH and determine gravimetrically by pptn. or volumetrically with Fehling's solution (Markman and Zalesov., *Uzbek. Khim. Zhur., Akad., Nauk Uzbek, S.S.R., 1958,* No. 6, 91). A simple method of extracting gossypol was based on the extraction of an ethereal extract of cottonseed with an equeous solution of borax, removal of gossypol in the form of a soluble borate complex, and regeneration by treatment with acid (Chander and Seshadri, *J. Sci. Ind. Research, 17B,* 279).

A method of determining α-tocopherol and self-nitrating tocopherols from the same extract was developed (Devyatnin and Solunina, *Med. Prom. S.S.S.R., 13,* No. 2, 38). An over-all procedure for the determination of tocopherols in oils, foods, and feeds was discussed in detail (Anon., *Analyst, 84,* 356). A chromatographic method to remove interfering peroxides was used prior to determining tocopherol in more highly oxidized fats as well as in Me esters of fatty acids which are distillable under conditions of heating (Frankel *et al., J. Am. Oil Chemists' Soc., 35,* 600).

Methods for the paper chromatographic separation of sterols from vegetable and animal fats were compared. Spraying of chromatograms with phosphomolybdic acid to develop spots gave better results than an antimony trichloride spray. Stigmasterol can be detected in mixtures with other sterols by chromatographing bromides of sterol acetates (Riemersma and Stoutjesdijk, *Mitt. Gebiete Lebensm. und Hkg., 49,* 115). The infrared determination of hydroxyl equivalent in steroids was discussed (Kabasakalian *et al., Anal. Chem.,* 31, 375). Using the Pavolini-Isidoro reaction (*C.A., 47,* 2515) a more rapid analytical method for sesamin was described (Chindemi, *Boll. Lab. Chim. Provinciali, 9,* 297).

COMPOSITION AND CHARACTERISTICS. The investigation of the chemical and physical properties and the fatty acid and lipid composition of a wide variety of fats and oils was the subject of numerous communications. The data are so extensive as to prohibit the detailed listing of specific properties. Consequently only a general description of the types investigated and the corresponding references are presented below. An asterisk appearing after the reference indicates that the fatty acid composition of the particular fat or fats is presented. A double asterisk indicates that the glyceride composition is also given.

A few articles dealt with a wide variety of oils. An extensive characterization of selected oils from 24 plant families was made in a search for new industrial oils (Earle *et al., J. Am. Oil Chemists' Soc., 36,* 304).* Oil yield, acid number, saponification number, iodine number, unsaponification content, and the fatty acid composition of each is given. A similar study of other vegetable oils and fats was published in a series of articles in the J. Chem. Soc. Japan (Kashimoto, *Nippon Kagaku Zassi, 78,* 123; *ibid., 79,* 403, 873).* The seed oils from 15 species were studied for appearance at room temperature, d^{20}, n^{20}, acid number, saponification number, iodine number, and percentage of unsaponifiables (Koyama *et al., Mem Fac. Eng., Nagoya Univ., 10,* 88). The *trans*-olefin content of various natural fats and their changes by U.V. irradiation were investigated)Kuhn and Luck, *Z. Lebensm. Untersuch. und Forsch., 109,* 306). The fatty acid composition of food fats was the subject of three tabulations (Coons, *J. Am. Dietetic Assoc., 34,* 242),* (Harding and Crooks *ibid.,* 1005), and (Mattil *et al., J. Am. Oil Chemists' Soc., 36,* 181).* The composition and characteristics of cramb oil, perilla oil, German sesame oil, safflower oil, purging oil, and fats suitable for cultivation in Poland were reported (Grynberg *et al., Fette, Seifen, und Anstrichmittel, 61,* 908).*

Individual animal fats investigated for fatty acid composition and the common physical and chemical properties were: Indian bear fat (Pathok *et al., J. Chem. Soc., 1959,* 1645),** camel fat (Siddiqui and Bari, *Scientist, Pakistan, 3,* 67), pork liver lipids (Privett *et al., J. Am. Oil Chemists' Soc., 36,* 443),* Hungarian lard (Szeredy, *Elelmezesi Ipar, 10,* 253),** various portions of sow body fat (Dagawa, *Kenritu Noka Daigaku, Gakyzyutu Hokoku, 7,* 98), and milk phosphatides (Kaufmann and Mohr, *Fette, Seifen, und Anstrichmittel, 61,* 285).* The polyunsaturated acid content of butterfat, lard, goat tallow, cow tallow, and the dermal seborrheic secretion of man was determined (Kajanne, *Suomen Kernistilehti, 31B,* 213).*

A comparison was made of the plasma cholesterol fatty acids in human subjects as determined by alkaline isomerization and by gas chromatography (Michaels *et al., Ann. N. Y. Acad. Sci., 72,* 633).* The volatile and unsaturated fatty acids of wool fat were determined (Janecke and Senft, *Deut. Apoth.-Ztg., 97,* 820),* and the variations in composition were reviewed (Nitschke, *Faserforsch. und Texliltech., 10,* 380).* The fatty acids of blood was the subject of another review (Garton, *J. Sci. Food Agr., 10,* 12).* The fatty acids of Tubercle Bacillus were studied (Agre and Cason, *J. Biol. Chem., 234,* 2555).* The isooleic acids in beef liver and goat milk fats were identified (Schenolikar and Subbaram, *J. Sci. Ind. Res., 17C,* 208).

Specific fatty acids in butterfat received extensive study. These were unsaturated fatty acids (Scott *et al., J. Agr. and Food Chem., 7,* 125), monoethenoic fatty acids (Backderf, *Arch. Biochem. Biophys., 76,* 15), 14-methyl pentadecanoic acid (Hansen *et al., J. Dairy Research, 26,* 190). The fatty acid composition of butter phospholipids was also reported (Deutsch, *Milk Dairy Research, Rept. No. 54,* 9)* and (Smith and Jack, *J. Dairy Sci., 42,* 767).*

The polyunsaturated acids of herring oil were studied (Klenk and Steinbach, *Z. Physiol. Chem., 316,* 31),* as were those of some marine plankton (Kelley *et al., J. Am. Oil Chemists' Soc., 36,* 105).* The fatty oil of a snail (Tanaka and Tomama, *Mem. Fac. Eng., Nagoya Undiv., 10,* 84)* and a northern elephant seal oil (Tsuguki, *Sci. Repts. Whales Research Inst., 13,* 323)* were analyzed for their fatty acid compositions.

The individual vegetable oils which were characterized were olive-kernel oils (Francois and Heinrich, *Compt. Rend. 247,* 222), β-rectified olive oils (Sissi, *Olii Minerali, Grassi e Saponi, Colori e Vernici, 368,* 214), several U.S. varieties of soybeans (Collins and Sedgwick, *J. Am. Oil Chemists' Soc., 36,* 641),* oils of five varieties of sunflower plants cultivated in Latvian S.S.R. Putnina, *Trudy Inst. Eksptl. Med., Akad. Nauk Latv. S.S.R., 14,* 149), Hyderabad and Bombay varieties of cottonseed oil (Harwalkar *et al., The Oil and Oilseeds J., 11,* 11),* cottonseed oil from Azerbaijanian seeds type 01298 and Central Asian seeds (Artamonov and Mamedow, *Maslogoĭno-Zhirovaya Prom., 25,* 8),* rapeseed oil in Okayama (Shinozaki and Ohara, *Okayama Daigaku Nogakubu Gakujutsu Hôkoku, 11,* 15), Brazilian peanut oils (Cattaneo *et al., Anales Assoc. Quim. Arg., 46,* 179),* kenaf seed oil (Hopkins and Chisholm, *J. Am. Oil Chemists' Soc., 36,* 95; Ramos, *Grasas y Aceites, 9,* 176),* carrot seed oil (Prakash *et al., J. Proc. Oil Technologists Assoc. India, Kanpur, 13,* 42),* Zelkova-seed oil (Hopkins and Chisholm, *J. Am. Oil Chemists' Soc., 36,* 210),* oil of walnut (Aĭzenberg, *Trudy Kishinev. Sel'skokhoz. Inst., 11,* 63),* oil of sesame, a review (Varna, *Oleagineux, 13,* 793), Perilla oil (Tischer, *Fette, Seifen, und Anstrichmittel, 59,* 313),* earth almond (*Chuta*) oil (Franzke, *ibid.,* 328), oils of the fruits of caraway and anise (Zaraĭskaya and Broisyuk, *Sbornik,* 1956, 185),* Bulgarian grape oil (Gerasimov *et al., Khim. i Ind., 29,* 7),* Indian lantanas seed oil (Nigam and Kaul, *J. Sci. and Ind. Research, 17B,* 472),* seed oil of *Eruca Sativa* and *Lepidium Sativum* (Popov and Mazhdrakov, *Compt. Rend Acad. Bulgare Sci., 11,* 279),* seed oil of some species of *Malva* (Shelud'ko and Goikhman, *Nekotorye Voprosy Farmatsii, Sbornik Nauch. Trudov. Vyssh. Farm. Ucheb Zavedenĭĭ Ukr. S.S.R., 1956,* 190), oil of *Xanthium strumarium, Xanthium spinosum,* and *Xanthium italicum* (Popov and St. Ivanov, *Bulgar. Akad Nauk., Izvest, Khim. Inst., 5,* 377),* oil from seeds of *Citrullus fistulosus* (Agarwal *et al., Indian Soap J., 24,* 257),* *Momordica dioica* and *Trichosanthes cucumerina* (Chakrabarty *et al., Naturwissenchaften, 43,* 523),* *Argemone mexicana seed* (Chiniwala and Desai, *J. Indian Chem. Soc., Ind. and News Ed., 20,* 70), seed oil of *Cardiospermum halicacabum* (Chisholm and Hopkins, *Can. J. Chem., 36,* 1537),* pulp and seed oil of *Pithecolobium dulce* (Gamo and Cruz, *Philippine J. Sci., 86,* 131), seed fat of *Caesalpinia digyna* (Gupta *et al., J. Indian Chem. Soc., Ind.* and *News Ed., 20,* 112),* seeds of *Sapium indicum* (Khan and Ahmad, *Pakistan J. Biol. Agr. Sci., 1,* 59), *Myristica beddomei* seed fat (Kartha and Narayanaan, *J. Sci. Ind. Research, 17B,* 283),** seed oils of *Podocarpus nagi* and nine other Japanese plants (Koyama and Toyama, *Mem. Fac. Eng., Nagoya Univ., 9,* 140; Koyama and Toyama, *Nippon Kagaku Zasshi, 78,* 1223), fruit of *Rhus trichocarpa* (Takai

and Toyama, *Nagoya Sangyo Kagaku Kenkyujo Kenkyu Hokoku, 10,* 62),* fatty oil of *Incillaria confusa* (Tanaka and Toyama, *Nippon Kagaku Zassi, 78,* 1101), seed oil of *Cassia tora* (Tiwari and Gupta, *J. Proc. Oil Technologists' Assoc. India, Kanpur, 10,* 111),* seed oil of *Clitoria ternates* (*ibid., 13,* 9), seed oil of *Bryonia laciniosa* (Tiwari and Gupta, *Indian J. Appl. Chem., 21,* 31), oil of *Euonymus verrucosa* (Simonova, *Zhur. Priklad. Khim., 32,* 1637),* seed oil of *Chrysanthemum coronarium* (Smith *et al., Chemistry and Industry, 1959,* 259),* *Tragopogon porrifolius* seed oil (Chisholm and Hopkins, *ibid.,* 1154),* seed fat of the *Moringaceae* (Patel *et al., Indian J. Appl. Chem., 21,* 85),* Himalayan pine (*Pinus griffithi*) seed oil (Prakash *et al., J. Proc. Oil Technologists Assoc. India, Kanpur, 13,* 47),* fatty oil of the fruit of *Pimpinella anti-setum* (Borisyuk and Makarova, *Nekotorye Voprosy Farmatsii, Sbornik Nauch. Trudov Vyssh. Farm. Ucheb. Zavedenii Ukr. S.S.R., 1956,* 179),* seed oil from *Oenothers lamarckiara* (Mazhdrakov and Popov, *Bulgar. Akad. Nauk., Izvest. Khim. Inst., 5,* 2091),* cocoa beans from Yap Island and Papua (Spoon, *Zucker- und Süsswarenwirtsch, 12,* 64), isooleic acid in cacao butter (Pelz, *Deut. Lebensm-Rundschas, 54,* 228), and seed oil of *Sapium indicum* (Khan and Ahmad, *Pakistan J. Biol. Agr. Sci., 1,* 59).

Saturation solubilities of mowraah, safflower, peanut, and cottonseed oils in aqueous EtOH solution at 7 temperatures between 40 and 100°C. were tabulated (Kaparthi and Chari, *J. Am. Oil Chemists' Soc., 36,* 77). The variation in viscosity on mixtures of oil of *Moringa concanensis* with various solvents was reported (Patel *et al., Indian J. Appl. Chem., 21,* 87).

The nontriglyceride composition of a large variety of fats and oils was investigated. These papers are organized with respect to the type of fat studied. The general articles include a review on the sphingolipides (Fujino, *Kagaku no Ryoiki, 11,* 214, 297), the distribution of tocopherols during the life cycle of various plants (Green, *J. Sci. Food Agr., 9,* 801), and the existence of bound Vitamin E in vegetable oils and concentrates (Savinov *et al., Sbornik, 1958,* 85). A comparative study of the squalene content in Pataua and olive oils were made (Padua, *Olearia, 11,* 262). An average of 56.4 mg. of squalene per 100 g. of oil were found in the crude Pataua oil, 49.5 in the refined, and 220.3 in the olive oil. The minor lipid constituents of Italian olive oil were investigated (Vitagliano and Turri, *Olearia, 12,* 145). Seventeen amino acids, liberated on acid hydrolysis of the lipids isolated from three Australian flours, were identified (Bottomley *et al., Chem. and Ind., 1958,* 1476). The lipids of corn pollen were found to contain large amounts (48%) of unsaponifiable materials (Barr *et al., J. Am. Oil Chemists' Soc., 36,* 33). The fatty acid composition of this fraction was reported. The composition of the unsaponifiable fraction in wool wax was also studied (Horn, *J. Sci. Food Agr., 9,* 632).

The fatty oils of the following aquatic invertebrates were studied with particular reference to their sterol components: *Cynthia roretzi* and *Pinna pectinati japonica* (Takagi *et al., Nippon Kagaku Zassi, 78,* 88), one species of *Ophiuroidea,* three species of *Asteroidea,* and three species of *Loricata* (Takagi and Toyama, *ibid.,* 93), *Tonna luterostoma* (*ibid.,* 366), and *Brachidontes senhousia* (*ibid.,* 1236). In a similar study the fatty oil of *Tonna luterostoma* was investigated for its sterols and other unsaponifiable constituents (Tanaka and Toyama, *Mem Fac. Eng., Nagoya Univ., 9,* 116, and *10,* 77). The composition of the nonsaponifiable fraction of menhaden oil was also studied (Mosher *et al., Comm. Fisheries Rev., 20, 11a,* 1). Sperm whale head oil was segregated into wax and glyceride by elution chromatography and molecular distillation (Sakurai and Fujiwara, *Mem. Inst. Sci. and Ind. Research, Osaka Univ., 15,* 229; Sakurai and Tozaki, *ibid., 15,* 235). The composition of fatty alcohols in *Beradius bairdii* blubber oil (whale oil) was reported (Saika *et al., Bull. Japan Soc. Sci. Fisheries, 24,* 578). The phospholipids of the sea anemone, Anthopleura elegantissima, were isolated and analyzed (Bergmann and Landowne, *J. Organic Chem., 23,* 1241). The difference in distribution of oil and Vitamin A in the skin, flesh, and liver of different species of edible fishes of Karach waters was discussed (Khan and Haq, *Pakistan Sci. Ind. Research 1,* 309).

The Vitamin A and carotene content of butter (Khotsko, *Doklady Vessoyuz. Konf. po Moloch. Delu, 1958,* 296) and of buffalo milk (Vermesanu *et al., Analele Inst. Cercet. Zootch. Bucarest, 15,* 263) were studied with respect to seasonal effects. Phosphatides isolated from butter serum had the ratio lecithin: cephalin: sphingomyelin 30: 45: 25 (Koops, *Neth. Milk Dairy J., 12,* 226). The electrophoretic and enzymic properties of membrane lipoprotein and its relation to other lipoproteins in cream were studied (Sasaki and Koyama, *Intern. Dairy Congr., Proc. 15th Congr., London, 1,* 308).

Beta-sitosterol was identified as a component of an oil obtained from *Cassia absus* (Johnson, *J. Org. Chem., 23,* 1814). It was concluded that the C-(24) ethyl group of stigmasterol is of the *alpha* configuration when it is expressed in Plattner's convention (Tsuda *et al., Chem. and Ind., 1959,* 1411).

DETECTION OF ADULTERATION. The infrared absorption spectra between 6 and 15 μ of 15 vegetable oils and derivatives are discussed in regard to interpretations of structure and adulteration (Favier, *Compt. Rend., 247,* 295). A method for the direct identification of sesame oil by the Villavecchia-Fabris reaction in samples of edible oils and fats colored with fat-soluble azodyes was developed (Polzella, *Boll. Lab. Chim. Provinciali, 9,* 162). As little as 5% coconut oil in other fats and oils can be detected by the "fruity" odor resulting from shaking with an equal quantity of alcoholic KOH (Gash, *J. Am. Oil Chemists' Soc., 36,* 400). By examining the absorption at 280 mμ marine oils can be detected in mixtures of at least 10% with olive, linseed, etc. (D'Arrigo, *Olii Minerali, Grassi e Saponi, Colori e Vernici, 35,* 111). Peanut oil can be detected in butter within $\pm$2.5% and cottonseed oil in lard within $\pm$2.67% by means of the critical mixing temperature (Fisher and Kartnig, *Fette, Seifen, und Anstrichmittel, 60,* 904). A method based on the lauric acid content was proposed for the detection of palm kernel oil in cacao butter (Wachs and Petscha, *Z. Ledensm. Untersuch. und-Forsch., 108,* 244). Five different methods for the analytical detection of adulterant fats in cacao butter were described (Purr, *Fette, Seifen, und Anstrichmittel, 61,* 675).

Another method for the determination of foreign fats in cacao butter was based on the estimation of nonhydrogenated fatty acids obtained on low-temperature crystallization (Purr, *ibid.,* 119).

Most of the adulteration tests which appeared in the literature were concerned with olive oil. A method for detection of adulteration with olive oil based upon tests for oleuropein was proposed. (Blasco and Pizzorno, *Anales Direc. Nacl. Quim., 10,* 13; Schmidt-Hebbel, *Colegio Farm., 16,* 2). Olive oils showing an urea-fractionated fatty acid molecular weight over 280 may be considered as adulterated with an oil containing erucic acid glycerides. (Bhalerao and Mahon, *J. Assoc. Off. Agr. Chemists, 41,* 745). The spectrofluorimetric curves of olive oil were stated to be distinctive enough to identify easily the type of oil and any possible adulteration of virgin oil with as low as 5% refined oil (De Francesco, *Olearia, 12,* 19). The ultraviolet spectrographic characteristics of Italian olive oils can be used to distinguish virgin oils and refined oils and to detect small percentages of refined oils in virgin oils. Adulteration of esterified oils with animal oleins can not be detected by this method (Mattei and Volpi, *Olearia, 13,* 55). Examples of the use of the iodine number of the unsaponifiable matter in the differentiation of olive oil from other oils were given (Frontero, *Olii Minerali, Grassi e Saponi, Colori e Vernici, 35,* 227). The infrared spectra between 2.80 and 3.3 μ and between 8.5 and 13 μ was used to distinguish the oils of peanut, sesame, sunflower, first pressing of olive oil and chemically refined olive oil, and refined solvent-extracted olive oil, and synthetic oil in olive oil (Bottini and Sapetti, *Ann Sper. Agrar., 12,* 1007). The ratio of extinction coefficients at 470 and 365 mμ was used to detect adulteration by refined olive or by animal and vegetable fats (Fabbietti, *Boll. Lab. Chim. Provinciali, 9,* 287). One study characterized the polyunsaturated acids in 31 virgin olive oils and discussed these with respect to identifying adulteration of virgin oils with refined oils (Albonico and Vitalgliano, *Olearia, 12,* 5). The adulteration of the olive oil can be established by comparing the number of triglyceride spots on the paper chromatograms (Kaufmann and Aparicio, *Fette, Seifen, und Anstrichmittel, 61,* 768). A more accurate technique was proposed for the Fitelson reaction (*C.A., 41,* 299) for the detection of tea oil in olive oil (Anselmi, *Olii Minerali, Grassi e Saponi Colori e Vernici, 36,* 210). A test for *lentiscus* oil was described where a red color is obtained with diazoic reagent if at least 0.5% is admixed with olive oil (Condorelli, *Boll. Inform. Ind. Olearia e Sapon., 4,* 49). The use of paper chromatography to detect the presence of linolenic acid was recommended for detecting grape oil in β-rectified olive oils (Petruccioli, *Olii Minerali, Grassi e Saponi, Colori e Vernici, 36,* 260). The higher amounts of tetradecenoic and hexadecenoic acids in animal fats formed the basis of one test for detecting animal fats in olive oil (Bigoni, *ibid.,* 1). The determination of forgeries of butter and of olive oil was made with the use of the extinction coefficients of the pure materials at various wavelengths (Strivek and Droppelman, *Anales Univ. Catalica Valparaiso,* No. 2, 129).

Animal fat was detected in ghee by the critical temperature of solution technique (Prakash *et al., J. Proc. Oil Technologists Assoc. India, Kanpur, 12,* 131). The Baudouin test was modified for the detection of adulteration of butter and ghee with vanaspati (Kapur *et al., J. Sci. and Ind. Research, 17B,* 471). The difficulty of determining whether ghee samples were adul-

terated with vanaspatis or lard was discussed (Kehar *et al., Studies of Fats, Oils, and Vanaspatis 1956,* 23–30). A comparison was made of several methods for detecting the addition of extraneous fats to butter (Vitagliano and D'Ambrosio, *Ann. Fac. Agrar. Univ. Napoli Portici, 22,* 35).

Methods for the detection of other extraneous materials included those for epoxides in oils and fats (Krull, *Farbenchemiker, 61,* 23) and surfactants derived from polyethylene glycol (Carbowax) added to edible fats (Anselmi *et al., Chem. e. Ind.* (Milan), *41,* 421). Methods also appeared for the detection of trace amounts of Cu, Fe, and Ni in fats by emission spectography (Taufel and Barthel, *Fette, Seifen, und Anstrichmittel, 60,* 534) and a micromethod for nickel in hydrogenated fats (Znamenskaya and Titova, *Trudy Krasnodar Inst. Pishchevoĭ Prom., 1955,* No. 11, 47).

Book Review

Several reviews have appeared during the past year. Klenk and Debuch (*Ann. Rev. Biochem., 28,* 39) prepared a review on the chemistry of lipids with emphasis on fatty acid analysis and synthesis, structure of unsaturated fatty acids, and chemistry of phospholipids.

A new volume (No. 5) of "Progress in Chemistry of Fats and Other Lipids" contains reviews on standard methods in the fat and oil industry, processes, soap manufacture, oilseed residues, composition properties and utilization of fish oils, drying oils, technology of soybeans, edible animal fats, and margarine manufacture. Each of these reviews is written by outstanding authorities in their fields.

The new books of interest to fat, oil, soap, and detergent chemists are given below.

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