Report of the Literature Review Committee

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

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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 hydroxyl value, soap in oil, nonvolatiles (solids), consistency penetration method, analysis of sodium alkyl benzene sulfonate, and neutral oil in alkyl benzene sulfonate. Minor revisions of several existing methods were also described (Hopper *et al., J. Am. Oil Chemists' Soc., 37,* 327). A review of the 1958 and 1959 literature on composition and characteristics appeared as part of the "Annual Review of the Literature on Fats, Oils, and Detergents," compiled by the American Oil Chemists' Society Literature Review Committee (Rusoff *et al., J. Am. Oil Chemists' Soc., 37,* 351). The 1958 literature on the chemistry, composition, characteristics, and analysis of oils and fats was reviewed by Rao (*Literature Review on Oils and Fats, 1958,* Central Food Technol. Res. Inst., Mysore, India, 1960). An excellent and comprehensive book on analytical methods for fats and oils appeared during the year. The standardized and nonstandardized methods and analytical information useful to the fat and oil analyst concerned with problems of production, control, and trading specifications were presented (Mehlenbacher, *Analysis of Fats and Oils,* Garrard Press, Champaign, Ill., 1960). The same author has discussed newer analytical methods pertinent to the fats and oils industry (Mehlenbacher, *J. Am. Oil Chemists' Soc., 37,* 613). A program based on statistical analysis was described for periodically checking the accuracy of analytical techniques within and between control laboratories(Waters *et al., ibid., 34,* 527).

Variety, maturity, environment, and feeding effects on the composition of fat sources were subjects of numerous papers. The iodine value of butterfat was found to vary with the breed of cow; the Parthenay breed was lower or at most equal to that of the Normandy breed, and both of these were lower than that from the Friesian breed (Kuzdzal-Savore, *Intern. Dairy Congr., Proc. 15th Congr., London, 2,* 1094). Five Russian breeds showed a range in milk fat content of 3.4 to 3.8% and a range of 34.8 to 39.2 in the iodine number of the butterfat (Davidov. *Vestnik Sel'skokhoz. Nauki, Vsesoyuz. Akad. Sel'skokhoz. Nauk im. V. I. Lenina 1959,* 81). Queensland butterfats from 12 centers varied in iodine value from 31.7 to 42.5 and softening point from 31.0 to 36.4° over a two-year period. Maximum values in total vitamin A content were found to take place between mid-winter and early spring and minimum values between mid-summer and early autumn (Mitchell, *Queensland J. Agr. Sci., 16,* 31; Gartner, *ibid.,* 1). Selection and seed breeding of sunflower resulted in varieties with oil contents up to 49% (Zhdanov, *Vsesoyuz. Nauch.-Issledovatel. Inst. Maslich. i Efiromaslich. Kul'tur, Vsesoyuz. Akad. Sel'skokhoz. Nauk, Kratkii Otchet 1956,* 21). Sunflower families and plants were isolated with the oil content of seeds of 15 to 56% (Zhdanov, *Agrobiologiya 1955,* 59). A significant variation in the linolenic acid content was found in linseed oil from different plants within a given variety grown in the same location. In this study 1,175 samples of flaxseed from Idaho, California, South Dakota, Missouri, Saskatchewan and other ports of Canada ranged in oil content from 32.4 to 45.8%, in iodine number from 166 to 198, in saponification number from 182 to 192, in linolenic acid content from 45 to 65%, in linoleic acid 7 to 19%, and in oleic acid from 10 to 28% (Zimmerman and Klosterman, *Proc. N. Dakota Acad. Sci., 13,* 71).

The effect of seasonal variation on the component fatty acids of yellowish viscera oil was described (Yukagaku, *8,* 31). A comparison of summer and winter milk fat showed that the entire C-18 fraction increased in the summer sample, mainly at the expense of palmitate (Patton *et al., J. Dairy Sci., 43,* 1187). Studies on the fat content of milk from 57 dairy centers in the north of Poland showed the lowest fat percentages in April and the highest in November (Budslawski *et al., Intern. Dairy Congr., Proc. 15th Congr., Landon, 1,* 232). A high degree of seasonal variation in the fat content of milk, with a peak in early winter, was also found in a semi-arid area in South Africa (Bakalor and Labuschagne, *ibid.,* 225).

The effects of maturity on the composition and characteristics of the lipids of fat sources were studied. The iodine number of olive oil increased during ripening (Uzzan *et al., Oléagineux, 11,* 705), and the unsaponifiable matter decreased from 4.5 to 1.0% (Cururachi, *Olii minerali, grassi e saponi, colori e vernici, 37,* 45). Although the fat content of rapeseed increased with ripening, the fat composition did not vary significantly (Steinbach and Franzke, *Nahrung, 3,* 95). The unsaponifiable content of sunflower seed oil decreased with maturity for two varieties; the relative content of sterols in the oil remained practically constant during this period. Changes in the composition of the oil were also discussed (Dublyanskaya, *Vsesoyuz. Nauch.-Issledovatel. Inst. Maslich. i Efiromaslich. Kul'tur, Vsesoyuz. Akad. Sel'skokhoz. Nauk, Kratkii Otchet 1956,* 129). The lecithin level in the yolk of normal

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TABLE OF CONTENTS

fertilized eggs was relatively constant (7–9%) over 16 days of incubation, after which it fell to 3 to 4% (Meduski and Orlowska, *Bull. acad. polon. sci., Ser. sci. biol., 6,* 455).

The influence of fats and carbohydrates in feeds on the triglyceride composition of milk was determined. The quantity of fat in the feed influenced the yield and composition of milk fat. Feed fat content equivalent to 40% of the milk fat caused a 28% decrease in the unsaturated fatty acid content. An intake of 65% of fat, based on the fat in the milk, increased fat content 0.15% and caused an increase in the unsaturated level to 30.5%. Rations with a fat content of 80 to 100% of the fat in the milk raised the unsaturated fatty acid content still higher (Kniga, *Intern. Dairy Congr., Proc., 15th Congr., London, 1,* 218). Another study showed that the iodine number was inversely proportional to milk fat production of the cows and to the fat content of the milk (Hietaranta and Holopainen, *Finnish J. Dairy Sci., 20,* 37). A relationship was found between the iodine addition product (I.P. = I. no. × % fat in feed/10) of the feed and the consistency of the back-fat of the pig. A firm back-fat was generally found if the I.P. was less than 27.5 (Kroeske and Hart, *Landbouwk, Tijdschr., 70,* 423).

ANALYSIS OF FAT SOURCES. Modern methods of analysis of oils, neutralization foots and gums by simple and low-cost methods were discussed (Estellés, *Inform. quím. anal. [Madrid], 4,* 101). An apparatus was described for the rapid extraction and analysis of fatty materials, based on the density of the solvent–fat solution as a function of fat content (Hahn and Ruyssen, *Agron. trop. [Nogent sur Marne], 14,* 721). The calculation was made by reference to standard curves prepared from the various oil-bearing materials. Good results were obtained with peanuts, palm nuts, and karite seeds. Copra gave varying results. A rapid method for determining fat in foods and industrial products consisted of extracting 5 g. with 30 cc. of benzene, centrifuging, removing the solvent from 15 cc. of the benzene solution, and weighing. Results on bouillon cubes, cocoa, and chocolate products, also on confectionery and soap were comparable with those obtained by Soxhlet extraction (Invernizzi and Sampietro, *Boll. lab. chim. provinciali [Bologna], 10,* 27). A revision of the A.O.A.C. method for crude fat in meat and meat products provided for weighing and drying the sample before extraction (Philbeck, *J. Assoc. Offic. Agr. Chemists, 43,* 536). Moisture and fat determinations in various meat products were made in 3 hrs. (as compared to 24 by the air-oven method) by refluxing the sample with an azeotrope-forming fat solvent in a specially designed compartmented flask (Wistreich *et al., Anal. Chem., 32,* 1054). Kamala seed kernels were satisfactorily extracted with petroleum ether and water as co-solvents by violent stirring for 4 hrs. at 400 r.p.m. Presoaking the meal cut down the time to 1.5 hrs. The optimum weight ratios of meal: petroleum ether: water is 3:3:2.5, respectively (San Gupta and Varma, *J. Proc. Oil Technologists' Assoc. India, Kanpur, 14,* 8). Methods for estimating fats in cocoa products were discussed (Loft, *Mfg. Confectioner, 40,* 51). The best method was a 30-hr. ethyl ether Soxhlet extraction of a finely-ground material. A method was developed for estimating oil content of a single cottonseed from the area of the oil spot on filter paper after it was pressed in a 10-ton hydraulic press (Ahmad and Qurashi, *Pakistan J. Sci. Ind. Research, 2,* 13). A rapid method for the neutral oil content of crude vegetable oils consisted of shaking 2 to 3 g. of crude oil sample, 25 g. of silicic acid, and 50 ml. of chloroform for 10 min., filtering it through a sintered glass funnel *in vacuo,* washing it with five 50-ml. lots of chloroform, and evaporating the combined filtrates to a constant weight. The results check well with the conventional silicic acid column chromatographic method (Roy and Arnold, *J. Am. Oil Chemists' Soc., 37,* 87).

Experiments with cottonseed oil and soybean oil showed that results obtained by the Wesson method and the cup test for refining loss could be satisfactorily correlated only when the oils had the same phosphatide content(Pardun and Werber, *Fette, Seifen, und Anstrichmittel, 61,* 1010). Tests have shown that the National Cottonseed Processors Association's refining tests for hydraulic oils, when used on current Expeller oils, resulted in a substantial under-refining of the oils as per the requirements of commercial practice. Adequate refining in the test is met by the use of 16° and 20° Bé. NaOH (Henry, *J. Am. Oil Chemists' Soc., 37,* 182). An apparatus is described for the identification and determination of small amounts of fat by application of the material to the surface of a liquid with high surface-tension (Röth, *Ger. 1,027,429,* April 3, 1958). A rapid detergent method was detailed for the detection of fat in milk, cream, ice cream, frozen desserts, and cheese. The method avoids the use of sulfuric acid or other corrosive chemicals to liberate the fat. Results by this method were in excellent agreement with those of the Roese-Gottlieb and Majonnier methods. The method was adopted by the A.O.A.C. for the detection of fat in raw milk only (Anderson *et al., J. Assoc. Offic. Agr. Chemists, 43,* 399; Washburn, *ibid.,* 746). This method was originally reported by Macdonald (Macdonald, *Analyst, 84,* 747). A study of the fat content of 413 samples of milk, covering a period of one year, by both the Gerber butyrometer method and the Roese-Gottlieb gravimetric method showed that a pipette delivering 10.74 ml. of water at 20°C. should be used in the Gerber test if the two methods are to agree. The influence of density and solids-not-fat content of samples was statistically significant, though remaining within the tolerances permitted (Labuschagne and Vogt, *S. African J. Agr. Sci., 3,* 83). Results using the Gerber method varied with the total quantity of milk from which the sample was taken, with the height from which the sample was drawn in the weighing container, and with the rate at which milk was drawn from the weighing vessel (Kirchhübel and Meyer, *Deut. Milch. Wirtsch., 6,* 163). The use of sulfuric acid (d. 1.7744) gave satisfactory values for the butterfat content of sweetened condensed milk by the standard Gerber procedure (Gerber, *Ind. Lechera, 41,* 252). A comparison was made with the Babcock test, and the Gerber test was recommended (Levowitz, *J. Milk and Food Technol., 23,* 69).

A butyrometric acid procedure was reviewed, and suitable calculations for detecting the fat content in milk were presented (van Houten, *Ind. Lechera, 41,* 480; Gerber, *ibid.,* 482). A routine method applicable to all types of dried milk was described by use of the van Gulick butyrometer. The difference between duplicates was less than 0.3% fat (Vester, *Neth. Milk Dairy J., 14,* 44). A butyrometric fat determination in cream employed two types of butyrometers. The required solution temperature was obtained by mixing concentrated sulfuric acid with the combined cream, water, and amyl alcohol. Both the solution temperature and holding time were important variables (Roeder, *Milchwissenschaft, 14,* 194). The detection of fat in milk by the Roese-Gottlieb extraction of the ammoniated sample is more rapidly done in the Mojonnier apparatus than by the standard Weibull-Stoldt procedure (Mrozek, *Deut. Molkerei Ztg., 80,* 895). Certain free fatty acids evaporating during drying caused an error of 0.4 to 1.7% in the determination of dry matter in blue cheese. The error in the calculation of fat in dry matter was of the same magnitude, and consequently high values were obtained. Methods for diminishing this error were discussed (Sjöström and Willart, *Intern. Dairy Congr., Proc., 15th Congr., London, 3,* 1474). A portable apparatus was described for the determination of acidity and fat in milk (Yavel'berg, *U.S.S.R. 120,679,* June 19, 1959).

GRADING AND VITAMIN TEST. The theory of color and the factors utilized in color measurement on fats and oils were reviewed (Naudet, *Rev. fermentations et inds. aliment., 14,* 154). A simple photocolorimeter with four interferential filters at 624, 552, 496, and 444 mμ was used in place of a spectrophotometer for characterizing the color of oils. Factors were developed that led to results similar to those obtained by spectrophotometry (Sambuc and Naudet, *Rev. Franc. Corps Gras, 7,* 21). A "color index" for cottonseed oil, determined by the area under the absorption curve in the region of 400 to 500 mμ, was preferred over the A.O.C.S. photometric method as a more accurate measure of the relative chromagen concentration (Pons *et al., J. Am. Oil Chemists' Soc., 37,* 671). With this method the panel score for cottonseed oil color intensity agreed better with the "color index" than with the photometric color. A uniform color-scale and a colorimeter for grading olive oils were described (Cruz *et al., Anales Real Soc. Espan. Fis. y. Quím., 52A,* 173). The color reversion of refined and deodorized soybean oil was discussed in a series of articles (Harada *et al., Nippon Nôgei-kagaku Kaishi, 34,* 545, 551, 558). Color changes were followed with the Lovibond colorimeter.

The important analytical details in the vitamin A assay were studied by the Vitamin Assay Commission of the Food Division of the International Union of Pure and Applied Chemistry (Brunius, *J. Assoc. Offic. Agr. Chemists, 42,* 657). The interlaboratory error for a duplicate assay was 6 to 8% for oils with unsaponifiable matter with an absorption maximum lying within the region 325 to 327 mμ and at ratio $E_{300}:E_{325}$ not exceeding 0.3. The corresponding error was 10 to 14% for oils not meeting these criteria and requiring chromatographic purification. Isopropyl alcohol was recommended as the most suitable solvent and *beta*-carotene as the best standard for the spectrophotometric method of vitamin A analysis (Castrelos, *Rev. Farm* [Buenos Aires], *101,* 15). A new spectrophotometric method for the determination of vitamin A was described. The unsaponifiables were extracted with benzene, an aliquot of the extract was diluted with chloroform, acetic anhydride was

added to a portion of the chloroform solution, and a phosphotungstic acid reagent was added to develop a blue color, which was read after 1 min. at 620 mμ (Jakovljevic, *Pharm. Weekblad, 95,* 549). Review articles appeared on the determination of vitamin A in margarine (Naito, *Bitamin, 13,* 371), and paper partition chromatography methods for the microdetermination of vitamin A and carotene (Suzuki, *ibid.,* 212).

In the colorimetric determination of tocopherols after separation by paper chromatography, traces of water in the paper gave high and variable blank values (Hobson-Frohock, *Analyst, 84,* 567). Evidence was presented in favor of ε-tocopherol as 2,5,8-trimethyl-2-(4,8,12-trimethyl trideca-3,7,11-trienyl)-chroman-6-ol (Green, *Chem. and Ind. 1960,* 73).

ANALYSIS OF CHEMICAL PROPERTIES. A new potentiometer was described for the titration of free fatty acids in dark-colored media and emulsions or in nonaqueous solutions (Hadicke, *Fette, Seifen, und Anstrichmittel, 62,* 584). The outline area method of paper chromatography was modified for the detection of fatty acids. Oils and acids separately had smooth index-constitution curves; for mixtures the curves were inflected (Poxon, *J. Oil & Colour Chemists' Assoc., 43,* 245). A gas chromatographic method for the analysis of the amount and type of free fatty acids in fat was developed. It consisted of the addition of n-heptadecanoic acid as an internal standard to a petroleum ether solution of the fat and fatty acid mixture, stirring this mixture with strong anion base exchange resin, filtering, washing with petroleum ether to remove fat, directly converting the adsorbed fatty acids to methyl esters by stirring 20 min. with anhydrous methanol-HCl, extracting the methyl esters with petroleum ether, concentrating, and analyzing an aliquot by gas-liquid chromatography on a column of Chromosorb coated with polyvinyl acetate. Results were based on the percentage of recovery of added internal standard (Hornstein *et al., Anal. Chem., 32,* 540). Gas chromatography was also used for the analysis of the lower volatile fatty acids in foods. Isolation methods for the volatile fatty acids in foods, milk, butter, and cheese were included (Diemair and Schams, *Z. Lebensm.-Utersuch. u-Forsch., 112,* 457). Other methods reported for the determination of volatile fatty acids in dairy products were a circular paper chromatography method for milk (Hadland and Johnsen, *Intern. Dairy Congr., Proc., 15th Congr., London 1959, 3,* 1539) and a combination of column and paper chromatography methods for the fatty acids formed on the aging of cheese (Palo, *Chem. zvesti, 13,* 602). The acid number of crude vegetable oils cannot be set according to the general consumption of alkali upon neutralization when phospholipids were present. The true acid number of crude vegetable oils that did not contain gossypol was calculated according to the formula: $AV_1 = AV - 0.33\ P$ where AV is the apparent acid value and P is the % of phosphatides present (Rzhekhin and Semenov, *Trudy Vsesoyuz. Nauch.-Issledovatel. Inst. Zhirov, 1958,* 59).

A comparison of the Hübel method and the Kaufmann method for the iodine value of unsaturated fats found that the Kaufmann method gave more reproducible results and required much shorter time for completion of the reaction. For fats with high iodine values the reaction could be speeded up by immersion of the flask in a water bath at 40 to 50°C. for 30 min. (Shteĭndel, *Biul. Inst. Roslin Leczniczych, 2,* 160). The Kaufmann-Budwig method for the radioactive iodometric determination of the iodine number was investigated with respect to its reproducibility and limits of error. Sources of error were radioactive decay, the uneven geometry of the grease spots on the paper, and oscillation of the voltage. The total of these errors could be kept under 1% (Jáky and Kaffka, *Élelmezési Ipar, 13,* 333; Jáky and Kaffka, *Fette, Seifen, und Anstrichmittel, 62,* 682). A spectrophotometric method for the microdetermination of the iodine value employs a reagent containing 2% iodine in glacial acetic acid and 2% mercuric acetate in glacial acetic acid 1:1 (vol.-vol.) and further dilution so that the amount of iodine in 0.1 ml. of the solution is 2–2.5 times the amount of iodine to be consumed. The addition to the sample and to a blank is made under nitrogen and in the dark. After 0.05 molar KI in 75% MeOH is added to both samples and blank, the absorbancies are measured at 357–8 mμ, and the iodine number is calculated from the difference. Values in good agreement with the Wijs method were obtained (Smits, *Rec. trav. chim., 78,* 713). A direct volumetric determination of the iodine number employed a 0.15*N* bromo-acetic acid solution and a Pt double electrode with a transistor indicator. Results were consistent but lower than other methods (Napoli, *Boll, lab. chim, provinciali* [Bologna], *10,* 346). A commercial bleaching solution, Clorox, was used as a reagent in the determination of unsaturation of oils (Choudhury, *J. Am. Oil Chemists' Soc., 37,* 198). The use of N-bromosuccinimide in the iodine number determination resulted in an accuracy of ±0.8% (Jovtscheff, *Nahrung, 3,* 153). The fatty acids of olive oil and their methyl esters reacted with 0.2 *N* solutions of iodine in anhydrous MeOH to add I and MeO radicals to the double bonds. The acids were not esterified in anhydrous MeOH. If the MeOH contained 5% water, the acids were esterified and the esters not only added MeO and I to the double bond but also had an H of the —C replaced by an MeO group (Tyutyunnikov and Novitskaya, *Ukrain. Khim. Zhur., 26,* 218). A comparative study of the infrared spectra of milk fats of different mammals showed that fats with a high I number were characterized by a doublet at 9 μ. The extinction at 3.33 μ was proportional to the I number (Lück and Kühn, *Milchwissenschaft, 14,* 339). A continuous absorption of I by hydrogenated castor oils over a 12-hr. period resulted from unknown causes (Droste, *Rev. Franc. Corps Gras, 7,* 396). The Winkler method was modified to include destruction of the peroxide groups in oxidized fat by a cold reduction with $SnCl_2$ (Galanos and Voudouris, *Chim. Chronika* [Athens, Greece], *24,* 165). A linear relationship was found between the iodine number and the refractive index of 253 samples of milk fat (Hietaranta, *Finnish J. Dairy Sci., 20,* 3), and of 46 samples of olive oils with slightly differing physical and chemical properties (Frenguelli, *Olearia, 11,* 59). The causes of disagreement in saponification values and iodine values of wool grease were studied (Miyakawa and Omori, *Abura Kagaku, 3,* 195).

Saponificaion values varied with the method of determination. Refluxing of the glycerides with 3*N* ethanolic KOH or with 0.5 *N* KOH in higher-boiling alcohol gave higher values. The fatty acids recovered after refluxing had a lower iodine number and an increased percentage of conjugated diene. Such alteration of the acids does not take place if the determination is carried out with 0.5*N* ethanolic KOH (Paquot, *Olearia, 11,* 5). A semi-micro determination of the saponification number employed a potentiometer for titration of the excess base. The saponification number of fat samples as small as 0.4 g. were determined by this technique (Gutierrez and Martin, *Grasas y Aceites* [Seville, Spain], *10,* 12).

A semi-micro determination of unsaponifiable matter and total fatty acids involved recovery of total fatty acids, including unsaponifiables, removal of the fatty acids with an anionic exchange resin, and determination of unsaponifiable matter by weight. Since the ion exchange method was a direct determination of the nonionic components, the results were claimed to be more accurate than with the macro-extraction method. The method showed a standard deviation of 0.06 for the unsaponifiable matter and 0.21 for the total fatty acid content (Benedict, *J. Am. Oil Chemists' Soc., 37,* 415). Khan compared distillation and chromatographic separations of the unsaponifiable matter of oils and concluded that solvent extraction was the best available method. This paper also described the isolation of unsaponifiables in sufficient quantities for testing (Kahn, *Oleagineux, 15,* 85). The application of fluorescence indicators in the electro-optical titration for acid and saponification values of dark-colored waxes was described (Hessler and Marsen, *Fette, Seifen, und Anstrichmittel, 62,* 579).

The interference of certain unsaturated hydroxy acids in the Durbetaki method of epoxide determination was demonstrated (Morris *et al., J. Am. Oil Chemists' Soc., 37,* 323). The concentrations of these acids were determined concurrently with those of epoxy components by measurement of the near-infrared spectra of samples before and after treatment with anhydrous ethereal HCl. A method was also reported for differentiating between epoxy acids and those without epoxy groups which react similarly in the determination of oxirane O in seed oil according to Durbetaki (Smith *et al., ibid.,* 320).

The determination of total and *beta*-monoglycerides by isomerization with perchloric acid was slightly modified to improve reproducibility (Hartman, *J. Sci. Food Agric., 11,* 191). With this technique the content of *beta* monoglycerides in commercial monoglyceride preparations stored for 1½–5 years was in the range of 5–9% of the total monoglycerides. This is contrary to a recent report that *beta* monoglycerides are present in freshly prepared products only and disappear on prolonged storage (Hartman, *Fette, Seifen, und Anstrichmittel, 62,* 271). Gas-liquid partition chromatography was applied to the quantitative estimation of monoglycerides. The monoglyceride mixture was mesylated with mesyl chloride in the presence of pyridine, and the resulting dimesyl derivatives were converted to allyl esters of the constituent fatty acids by treatment with sodium iodide in anhydrous acetone at 100°C. The allyl esters were then analyzed quantitatively by gas-liquid partition chromatography at 240°C. on a column of Apiezon M-Celite. Both

alpha and *beta* monoglycerides were quantitatively converted to allyl esters by this procedure. *Beta* monoglycerides in a mixture of *alpha* and *beta* isomers may be determined separately after removal of the *alpha* isomers by oxidation with periodic acid. The analytical procedure was also applicable to monoglycerides in the presence of free fatty acids, diglycerides, and triglycerides (McInnes *et al., J. Am. Oil Chemists' Soc., 37,* 7). A general method for the paper chromatographic analysis of mono-, di-, and triglycerides and the mono- and diesters of ethylene glycol and polyethylene glycol was reported (Papariello, *ibid.,* 396).

ANALYSIS OF PHYSICAL PROPERTIES. Low-resolution nuclear magnetic resonance (NMR) was applied to the determination of the liquid/solid content of fats (Chapman, *J. Am. Oil Chemists' Soc., 37,* 243). A comparison of NMR and dilatometric results on margarine fat showed that dilatometry indicated higher solids between room temperature and 100% solids. This may result from the fact that NMR determination is of the polymorphic form. This method was also discussed in two review articles (Hopkins and Bernstein, *Can. J. Chem. 37,* 775; Chapman, *Chem. and Ind., 1960,* 707). A viscometric method was used to determine the quantity of solid fat in tempered chocolate (Duck, *Fette, Seifen, und Anstrichmittel, 62,* 705). The method is based on the direct relationship between the viscosity of the tempered chocolate and the quantity of solid fat present. The curves of the expansion *vs.* temperatures for several fats were recorded and illustrated, and the use of volumetric and gravimetric dilatometers considered (Casella, *Olii minerali, grassi e saponi, colori e vernici, 37,* 195). The melting and dilatometric behavior of 2-oleopalmitostearin and 2-oleodistearin were recorded. Mixtures of these glycerides behaved in some respects as single components (Landmann *et al., J. Am. Oil Chemists' Soc., 37,* 638). Dilatometric data were also presented for a sample of cocoa butter and sweet milk chocolate of the coating type. Specifications for the spreadability of butter were established in objective terms with the use of a modified Huebner-Thomsen apparatus (Riel, *J. Dairy Sci., 9,* 1224).

The melting point of fat determined by the "rising point" method depended considerably on the chilling time and temperature. A micro-dilatometric study of this transformation showed that melting-point values of high reproducibility can be obtained when the fat is thoroughly solidified and its crystal content "tempered" by heating at a comparatively high temperature prior to the actual determination (Soeltoft, *Chim. et Ind.* [Paris], *82,* 75). One melting-point method for fats consisted of forming small spheres by dripping molten fat in 65–70% ethyl alcohol and adding these to vegetable oil and heating. The temperature at which the solid fat sphere disappeared was recorded as the melting point (Vadachkoria, *Shornik Trudov Nauch.-Issledovatel. Iust. Zhivotnovodstva Gruzin., U.S.R.R. 2,* 278). An apparatus was described for the rapid melting-point determination of waxes by the cooling-curve method (Walker, *Tappi, 43,* 668). The method was found to be useful with a wider variety of waxes than the A.S.T.M. cooling-curve method. It had almost the same precision and required much less time.

The crystalline structure of fat globules in butter was studied by polarized light and x-ray diffraction. The symmetrical 4-part halo seen on the circumference of fat particles in milk and butter, when viewed in polarized light, could not be attributed to crystallinity. It was postulated that the differential light refraction observed was caused by a liquid fat fraction enveloping the solid milk fat globule. Photomicrographs under polarized light and x-ray diffraction spectra were presented (Knoop and Samhammer, *Deut. Molkerei Ztg., 80,* 1031). Hoerr discussed the general relationship of the physical behavior and performance of fats to their crystal structure and molecular configuration (Hoerr, *J. Am. Oil Chemists' Soc., 37,* 539).

A method was reported for determining the initial cloud, solidification, flow, and clear points, in that order, on a single sample while progressively lowering the temperature by 2°C. intervals. The critical temperature at which an oil stays clear indefinitely is between the initial cloud and the clear points. The method was confirmed with tests on 24 samples of refined and semi-refined oils, acid oils, and fatty acids (Martinenghi and Balestrini, *Olearia, 10,* 192). An improved "four-temperature test" gave reproducible pour- and cloud-point values. These were correlated with the chemical nature of the fatty acid and were used to characterize the source of the oil (Martinenghi and Balestrini, *Olearia, 11,* 126). A filterless photoelectric colorimeter was recommended for the determination of clouding in whale oil (Khalina, *Izvest. Tikhookean. Nauch.-Issledovatel. Inst. Rybnogo Khoz. i Okeanog., 44,* 265).

Experiments on the flow properties of waxes indicated that these could be used for characterization (Brotz, *Fette, Seifen, und Anstrichmittel, 62,* 31). The influence of KW-waxes on the solubility of wax mixtures was studied by determination of the solubility of different wax types and their mixtures at 25°C. Eutectic mixtures were postulated (Lindemann, *ibid.,* 924). Other communications on physical properties were about the permeability of some fat products to moisture by the permeability cup method (Landmann *et al., J. Am. Oil Chemists' Soc., 37,* 1), the heat in and volume of mixing of binary mixtures of rapeseed, soybean, camellia, and coconut (Kusano, *Yukagaku, 8,* 57), the ionization constant solubility product and solubility of lauric and myrstic acid (Nyren and Back, *Acta Chem. Scand., 12,* 1305), and the influence of the nature and position of side chains on the physical properties of alkanes and saturated fatty acids (Hager, *Fette, Seifen, und Anstrichmittel, 62,* 7).

COMPOSITION ANALYSIS. The literature dealing with methods of analysis for the constituents of lipids is divided into three categories: Fatty Acid Composition Analysis, Glyceride Composition Analysis, and Lipid Composition Analysis.

Fatty Acid Composition Analysis. New methods for the separation and analysis of fatty acids and other lipids were reviewed (Fontell *et al., J. Lipid Res., 1,* 391). A review of principles and authors' own unpublished work on applications of gas chromatography to fat research was presented (Craig, *Can. Food Inds., 31,* 41). Gas chromatographic separations of an artificial mixture of methyl esters and of 19 samples of butter and other fats were presented, and advantages of the method were discussed (Houghton and Lund, *Rev. Intern. Chocolat, 15,* 470). Reviews of the gas chromatographic determination of the composition of fatty acids and fatty alcohol mixtures (Kotel'nikov and Datskevich, *Masloboino-Zhirovayan Prom., 26,* 20), and of plasma fatty acids (Takahashi and Tanaka, *Saishin Igaku, 15,* 185) were presented. The carbon number was recommended as a parameter for the comparison of gas-liquid chromatography columns in the analysis of fatty acids (Woodford and van Gent, *J. Lipid Research, 1,* 188). Saturated straight-chain esters have integral carbon numbers whereas branched-chain and unsaturated esters have non-integral carbon numbers. The relative response of a thermal conductivity cell is terms of peak area per mole of compound was determined for the fatty methyl esters caproate through myristate. The values were found to be a linear function of molecular weight (Killheffer Jr. and Jungermann, *J. Am. Oil Chemists' Soc., 37,* 456). Retention volumes were tabulated for 36 methyl esters of saturated and unsaturated fatty acids as determined by gas chromatography of 200 and 220°C. on silicone grease, Apiezon M, and poly(diethylene glycol succinate). Included were esters of normal odd- and even-numbered saturated acids from C_5 to C_{26}, normal unsaturated acids from C_{18} to C_{22}, and iso- and (+)-anteiso acids from C_{13} to C_{18} (Hawke *et al., J. Chromatog., 2,* 547). An unique method for the gas chromatographic separation of fatty acid esters employed a stainless steel column 1,000 mm. long and 8 mm. in internal diameter, filled with silicone grease, on stainless steel Dixon rings, a catharometer, a dectector, a Dewar flask filled with a mixture of acetone and dry ice, a second column, catharometer, and detector. The two detectors were connected to a Wheatstone bridge and a 5-mv recorder. However the apparatus would not differentiate between saturated and unsaturated acids or between various unsaturated acids of the same chain-length (Jart, *Fette, Seifen, und Anstrichmittel, 61,* 541). Methyl esters of stearic, oleic, linoleic, and linolenic acids were quantitatively separated with an accuracy within 5% on a poly(vinyl acetate) column (Hornstein *et al., Nature, 184,* 1710). The number of double bonds and carbon atoms in an unknown fatty acid were determined by comparison of its relative retention volumes on a polar (polyethylene glycol adipate) and a nonpolar (Apiezon L) stationary phase in a gas-liquid chromatogram by use of a grid established with the pure acids. Retention volumes were tabulated for 40 saturated and unsaturated fatty acids (James, *J. Chromatog., 2,* 552). The amount of elaidate found by gas chromatography compared well with the *trans* acid found by infrared analysis. Good separation of Me oleate and elaidate was accomplished with a capillary column (Kauffmann and Lee, *J. Am. Oil Chemists' Soc., 37,* 385). The methyl esters of some naturally-occurring fatty acids or their auto-oxidation products were found to be altered during gas-liquid chromatography. Conjugated trienoates undergo *cis-trans* isomerization. The esters of vicinally unsaturated hydroxy derivatitves, with either ethylenic or acetylenic bonds, are dehydrated. Acetylation of the hydroxy group provides little or no protection against such changes. Unsaturated hydroperoxides are similarly altered to more

highly unsaturated derivatives. Conjugated dienoates and hydroxy esters that are not vicinally unsaturated are stable under the same conditions. These changes are probably caused in the flash heater owing to high temperature, with metal catalysts from components of the flash heater promoting dehydration and deacetylation (Morris *et al., J. Lipid Res., 1,* 412). A stationary phase of dioctyle sebacate, containing 15% sebacic acid, improved the symmetry of fatty acid peaks in gas chromatography. Retention volumes at 150°C. on Celite relative to pentane as 1 were: water 0.20, HCOOH 0.81, HOAc 1.30, EtCOOH 1.87, CH_2:CHCOOH 2.25, PrCOOH 3.27, iso-PrCOOH 2.65, MeCH:CHCOOH 5.16, BuCOOH 5.95 (Raupp, *Angew. Chem., 71,* 284). Fatty acids from human-and chicken-brain lipids were analyzed as their Me esters by gas chromatography on a 10-ft. diethylene glycol succinate polyester column. Twenty-five resolved peaks were obtained, representing normal fatty acids C-10 to C-24 and even-numbered *alpha*-hydroxy acids from C-18 to C-24 (Johnston and Kummerow, *Proc. Soc. Exptl. Biol. Med., 104,* 201). New methods for the preparation of methyl esters of fatty acids for gas chromatographic analysis included a small-scale methylation of fatty acids with diazomethane (Schlenk and Gellerman, *Anal. Chem., 32,* 1412), methylation of fatty acids with 2,2-dimethoxypropane in MeH and HCl (Radin *et al., J. Lipid Research, 1,* 250), and the direct conversion of lipid components to their fatty acid methyl esters with a large excess of sodium or potassium methoxide an absolute methanol (Luddy *et al., J. Am. Oil Chemists' Soc., 37,* 447). The last article also describes a silicic acid chromatographic adsorption column technique for the removal of methyl esters from unsaponifiables and free acids. Polyester stationary phases commonly used for the separation of fatty acid methyl esters were stabilized by the removal of polymerization catalysts and hydrogen ions from the polyesters by passing them in an ethanolic solution through Duolite A-4 ion exchange resin (Corse and Teranishi, *J. Lipid Res., 1,* 191).

The use of mass spectrometry in conjunction with gas chromatography for the analysis of fatty acids was described (Hallgren, *Acta Chem. Scand., 12,* 1351). By collection of fractions coming from the column and identification of their components by mass spectrometery, complex mixtures of fatty acids present in butter and margarine were analyzed. The position of the double bond in Me oleate was determined by comparison of the mass spectra of Me stearate and Me 9,10-dideuteriostearate, prepared by reduction of Me oleate, with H_2NNHD (Nguyen, *Acta Chem. Scand., 12,* 1350). The extension of this method to polyunsaturated methyl esters was indicated. High-resolution nuclear-spin resonance spectral analysis of the possible eight-component system, resulting from the hydrogenation of linolenic acid, was discussed, and the spectra of four fatty acid methyl esters were presented. It was theorized that the analysis would be possible if one of the constituents other than 9- or 12-oleic acid could be found by another method (Storey, *J. Am. Oil Chemists' Soc., 37,* 676.

The results of analysis of the component fatty acids of olive oil by gas chromatography agreed with the results on the same oil obtained by the A.O.C.S. spectrophotometric procedure, the traditional ester fractionation method, and from thiocyanogen values (Gracián *et al., Nature, 184,* 1941). Known mixtures of 9 fatty acid Me esters, and Me esters of olive, sesame, safflower oils, and lard were analyzed by conventional gas-liquid chromatography by using a thermoconductivity detector. Results with the known mixtures agreed with the composition and with analysis by the spectrophotometric method (Herb *et al., J. Am. Oil Chemists' Soc., 37,* 127). In a similar study of a synthetic mixture of methyl esters and of methyl esters of corn oil, sunflower seed oil, soybean oil, linseed oil, olive oil, and peanut oils, the A.O.C.S. spectrophotometric method gave lower values for linoleic acid and higher values for linolenic acid (Craig and Murty, *ibid., 34,* 549). Gas chromatographic analysis of higher fatty acids and their methyl esters was used to study the alkali isomerization of linoleic acid (Beerthius *et al., Ann. N. Y. Acad. Sci., 72,* 616).

The possibilities and limitations of ultraviolet spectrometry in fat research were reviewed (Schauenstein, *Nahrung, 3,* 1123). The ultraviolet spectra of mono- and polyunsaturated isolated fatty acids and their esters in Schumann-UV at 53,000 γ are discussed and compared with that of conjugated compounds (Schauenstein and Benedikt, *Fette, Seifen, und Anstrichmittel, 62,* 687). Isomerization with a 1.3 *N* KOH in absolute ethylene glycol quantitatively converted linoleic and linolenic acids to their conjugated isomers. Tertiary BuOH was found to be the best replacement for ethylene glycol when necessary (Franzke, *Nahrung, 3,* 238). A simplified technique was described for isomerizing fats with K *tert*-butoxide in *tert*-BuOH at 60°C. for 20 hrs. Analysis for linoleic and linolenic acids and five seed oils, isomerized by this "bottle method," agreed well with results obtained by the A.O.C.S. KOH-glycol method (White and Quackenbush, *J. Am. Oil Chemists' Soc., 36,* 653).

The application of infrared spectroscopy in fats analysis was thoroughly reviewed (Kaufmann *et al., Fette, Seifen, und Anstrichmittel, 61,* 547). The infrared spectra of several unsaturated fatty acids, their methyl esters, and barium salts were determined, and the *trans*-isomer content was calculated (*ibid.,* 463). An indication was found that the glyceride structure influenced results in a manner to give high values. Data and equations were presented for the simultaneous analysis of conjugated methyl *cis-trans-* and *trans-trans*-octadecadienoates by infrared spectrometry (Chipault and Hawkins, *J. Am. Oil Chemists' Soc., 36,* 535). Interference from —COOH band at 933 $cm.^{-1}$ was compensated for by the use of solvent solutions of stearic acid and methyl stearate, respectively, in a variable space cell in the reference beam (Cleverly, *Anal. Chem., 32,* 128). This paper also described the determination of nonconjugated *trans*-unsaturation in C-18 acids and esters from natural sources. The American Oil Chemists' Society Spectroscopy Committee reported collaborative analytical results for the determination of *trans*-isomers by infrared absorption spectroscopy on 11 samples (O'Connor *et al., J. Am Oil Chemists' Soc., 36,* 627). These results were discussed with regard to further work necessary to devise a satisfactory procedure.

Linoleic acid was determined in samples of butter, mutton tallow, and coconut oil by $KMnO_4$ oxidation, thiocyanogen number, and spectrophotometric methods. The values by the oxidation method were higher than those with the other methods, which are in fairly close agreement (El Said *et al., Egyptian Pharm. Bull., 40,* 221). A simple colorimetric method was reported for the detection of esters and glycerides of oleic, linoleic, and linolenic acids in fats. Characteristic absorption spectra were obtained when these materials were treated with ferric chloride or mercuric acetate in the presence of glacial HOAc and concentrated H_2SO_4 (Rhodes, *J. Appl. Chem.* [London], *10,* 122). Methods for the thiocyanometric determination of the fatty acid composition of partially-hydrogenated fats were detailed (Möller and Gabrielsson, *Fette, Seifen, und Anstrichmittel, 61,* 893).

Paper chromatography analysis of mixtures of fatty acids and glycerides was reviewed, and experimental data were presented on the separation of mixtures containing odd- and even-numbered fatty acids from C-7 to C-28 in an undecane-acetic acid system (Kaufmann, *Fette, Seifen, und Anstrichmittel, 62,* 153). A thorough investigation of "critical pairs" of fatty acids was reported in the same paper. Quantitative determinations o polyunsaturated fatty acids was obtained by photometric evaluation of paper chromatograms that employed undecanactonitrile-glacial acetic acid solvent systems (Miyakawa, *ibid., 61,* 850). A similar procedure employed the extraction of chromatographic spots and titration of the extracts with 0.005 N NaOH, using bromphenol blue as the indicator. The paper chromatographic separation and analysis of monoglycerides from di- and triglycerides by use of Rodamine B as the color developer (violet-purple for monoglycerides) was also described (Jáky, *Élelmezési Ipar,* 11, 148). C_1 to C_4 acids were separated as their hydroxamic acid derivatives by paper chromatography (Obukhova, *Okislenie Uglevodorodov v Zhidoĭ Faze, Akad. Nauk U.S.S.R., Inst. Khim.-Fiz., Sbornik Stateĭ, 1959,* 249). A method was developed for quantitative paper chromatography of higher fatty acids in the form of the radioactive methyl esters. Autoradiography of the chromatograms and planimetric treatment of the photometric curves gave quantitative results (Smirnov *et al., Biokhimiya, 25,* 368). A 3-stage paper chromatography method for the separation and identification of mixtures of saturated, unsaturated, and 2-hydroxy fatty acids from C_{14} to C_{24} was described. Elevated temperatures during development of the chromatogram improved the separation and solubility (Skipski *et al., Arch. Biochem. and Biophys., 87,* 259). Fatty acid mixtures that contain myristic, palmitoleic, and linoleic acids in addition to other constituents were separated on paper by use of the system undecane-acetic acid-acetonitrile and were evaluated photometrically after the Cu soap spots were colored with dithiooxamide. Spots that contained more than one component were extracted and analyzed with a second chromatogram after either catalytic hydrogenation or by the so-called "hydrogenation difference method" (Kaufmann *et al., Fette, Seifen, und Anstrichmittel, 62,* 1). The photometric method of Seher for the quantitative evaluation of paper chromatograms of fatty acids was critically reviewed, and modifications were recommended (Seher, *ibid., 61,* 855). To identify the conjugated trienic acid of the seed oil of *T. anguina* the acid was isolated from the other fatty acids in the oil and oxidized with

permanganate; the two cleavage products that were obtained paper were chromatographed (Chowdhury, *Nahrung, 4,* 17). A modification of this method was described for the differentiation of components of fatty acids, including this conjugated trienic acid and eleostearic acid (*ibid.,* 230). Auto-oxidation and polymerization of parinaric acid during paper chromatography were overcome by use of an inert atmosphere of CO_2 (Kaufmann and Sud, *Fette, Seifen, und Anstrichmittel,* 62, 160). Circular paper chromatography was used to separate synthetic mixtures of pure oleic and linoleic acids. The spots were quantitatively determined with periodate-permanganate-benzidine development, having been photographed and reverse-developed to obtain a positive. The area and optical density were measured with a microphotometer (Sulser, *Mitt. Gebiete Lebensm. und Hyg., 50,* 275).

Simplified column chromatography methods were developed for the separation and analysis of C_4 through C_{12} straight-chain dicarboxylic acids. Good separation and quantitation were achieved on silicic acid columns, modified by the addition of either water or aqueous citrate buffer solutions (Smith, *Anal. Chem., 32,* 1301). A column of Mealorub rubber was used to separate the hydroxy acids in castor oil (Vezinet and Naudet, *Rev. Franc. Corps Gras, 7,* 85). A column packed with a 1:1 mixture of Dow Corning 200 silicone on Johns-Manville Celite and a 75% aq. CH_3CN mobile phase were used for the separation of odd-numbered fatty acids of menhaden oil (Gellerman and Schlenk, *Experientia, 15,* 387).

The fractionation of castor oil methyl esters by liquid-liquid extraction was described (Lakshmahan and Laddha, *J. Am. Oil Chemists' Soc., 37,* 466). Countercurrent fractionation of partially-hydrogenated fatty acids was improved by their transformation into mercury complexes. The detection of the fractions was accomplished by the dithizone method; the extinction was determined at 620 mμ, and the moles of fatty acids were calculated therefrom (Schilling, *Fette, Seifen, und Anstrichmittel, 61,* 765). The removal of unsaponifiable matter from fatty acids resulted in an improved purity of the fractions obtained by low-temperature crystallization from organic solvents (Grynberg *et al., Prace. Inst i Lab. Badawczych Przemyslu Sozywczego, 9, 111*). Methyl oleate and pure methyl linoleate from hazelnut oil and poppy seed oil, respectively, were obtained in excellent yields by purification on a 75% silica gel/25% Hyflo Super-Cel column eluted with petroleum ether. The naturally-occurring all-*cis* form of the acids was not affected by this isolation procedure (Franzke, *Fette, Seifen, und Anstrichmittel, 61,* 905).

The mode of distribution of the mixed solid and liquid fatty acids of peanut oil have been studied by the liquid-solid counter-current distribution with urea. A similar distribution study of the saturated acids obtained on bromination of the mixed fatty acids, followed by urea adduction, was also reported. The fatty acid composition could be calculated from these data (Mehta and Meshramkar, *Indian J. Appl. Chem., 21,* 211). A further description of the bromination method, followed by urea adduction, was given by the same authors (Mehta and Meshramkar, *ibid., 22,* 218). These methods were applied to the fractionation of chaulmoogra oil fatty acids and karanja oil fatty acids (Mehta and Dabhade, *Grasas y acentes* [Seville, Spain] *10,* 24; Mehta and Meshramkar, *Indian J. Appl. Chem., 23,* 23). The addition of biuret to urea was found to reduce the amount of adducts formed with fatty acids (Rigamonti and Riccio, *Fette, Seifen, und Anstrichmittel, 61,* 864). A combination of molecular distillation, followed by paper chromatography, was used to determine the fatty acid composition of Crambe abyssinica seed oil (Niewiadomski *et al.,* 897).

Glyceride Composition Analysis. Kartha described a method for calculating the configuration of mixed glycerides and natural fats, according to the rule of restricted random distribution, which states that the ratio between the amounts of symmetrical and unsymmetrical components in any glyceride type does not remain fixed but varies from 1:1 to 1:2 according to the component fatty acid composition (Kartha, *J. Sci. Ind. Research* [India], *18A,* 304). The progress of experimental studies on configuration was also reviewed in this article. Hammond and Jones disagreed with Kartha's calculation scheme and asserted that it is not correct to calculate from an equilibrium state to another by proportions. In Kartha's method the equations gave negative values for triunsaturated glycerides in cases where the saturated acids exceed 61.8% and the trisaturated glyceride is reduced to the smallest possible value (Hammond and Jones, *J. Am. Oil Chemists' Soc., 37,* 376). The authors proposed a "correct equation" for such conditions. Vander Wal reviewed the various theories of glyceride structure and their interrelationships (Vander Wal, 595). The same author used data that were obtained by the pancreatic lipase hydrolysis technique of Mattson and Beck (C.A. *50,* 10156f) as a basis for a method of calculating the distribution of saturated and unsaturated fatty acids in glycerides containing predominately C_{16}–C_{18} acids (Vander Wal, *J. Am. Oil Chemists' Soc., 37,* 18). The assumption was made that all saturated and unsaturated fatty acid groups present in each of the three positions on the glyceride molecular are dispersed at random. This same technique was used to investigate the triglyceride structure of the back, visceral, blood, and milk fats of an individual cow; milk fat before and after seven days of inanition; visceral and back-fat of a pig; and the depot fat from a steer (McCarthy *et al., J. Dairy Sci., 43,* 1196). In all these samples the percentages of C_{10}, C_{12}, and C_{14} saturated acids and C_{14} and C_{16} monounsaturated acids were found to exist in higher concentrations in the monoglycerides as a result of the action of pancreatic lipase than in the intact fat. On the other hand, C_{18} saturated, diunsaturated, and triunsaturated acids were found in lower concentrations in the monoglycerides. The mixed glycerides of various animal and vegetable fats were analyzed by means of partial lipase hydrolysis and subsequent chromatographic fractionation (Desnuelle and Savary, *Fette, Seifen, und Anstrichmittel, 61,* 871). In the vegetable glycerides the unsaturated fatty acid residues occupied the middle C of glycerol while the saturated residues occupied the end C's. The same distribution was observed for animal fats, except for pork, in which the reverse was found. Palmitic acid was predominant over stearic at the central glycerol C in pork fat.

A paper chromatography method for glyceride analysis was described (Kaufmann and Schnurbusch, *ibid.,* 523). Paper that was impregnated with silicone oil and a solvent mixture of acetone and acetonitrile were employed. The spots were colored with Cu acetate and K ferrocyanide after saponification. In an extension of this study the same author employed paper that was impregnated with undecane and acetic acid as the solvent (Kaufmann and Makus, *ibid., 631*). He found that the number of zones obtained for a natural fat or oil was constant and suggested that the method be used as a proof of the purity of these fats and oils. A critical study was made of Hilditch's and Kartha's oxidation method for the determination of glyceride composition (Lakshminarayana and Rebello, *J. Am. Oil Chemists' Soc., 37,* 274). Using pure concentrates of various triglycerides, the authors found both methods high for trisaturated-glyceride content. The saturated acid content was accurate with Kartha's and Bertram's separation procedure, and slightly low with Hilditch's technique. With the Hilditch method disaturated azelao glycerides were unaffected by the Na_2CO_3 wash and the disaturated monounsaturated glycerides hydrolyzed considerably during oxidation. With Kartha's method the latter were slightly affected whereas the monosaturated diunsaturated glycerides hydrolyzed appreciably. This resulted in increased disaturated and triunsaturated contents and a decreased diunsaturated content when Kartha's method was applied to monosaturated diunsaturated glycerides. It is suggested that, because the experimental basis of Kartha's "restricted random" distribution theory is unsound, the theory should be re-examined.

Infrared absorption spectra were recorded for five polymorphic forms of trimargarin, three polymorphic forms of trielaidin and triolein; and at room temperature for 1-oleodipalmitin, 2-oleodipalmitin, 1,2- and 1,3-dilaurin, 1,2- and 1,3-dimyristin, 1,2- and 1,3-dipalmatin, 1,2- and 1-3-distearin, 1- and 2-monomyristin, 1-mono-olein, 1-monocaprylin, 1-monocaprin, 1-monolaurin, 1-monomyristin, 1-monopalmitin, and 1-monostearin (Chapman, *ibid.,* 73). These curves were discussed with regard to detecting polymorphic form, chain-length, type of unsaturation, and configuration.

A method, based on the reaction of unsaturated glycerides with mercaptoacetic acid, was described for the determination of trisaturated glycerides in fats (Eshelman *et al., Anal. Chem., 32, 844*). The mercaptoacetic glycerides formed were separated from the neutral trisaturated glycerides by extraction of the NH_4 salts, followed by ion-exchange treatment. The four-temperature test for the identification of edible oils was described in detail and its application for determining different glyceride structures and detection of adulterations was discussed (Heller, *Seifen-Öle-Fette-Wachse, 86,* 365).

An unique and intriguing technique, employing gas chromatographic separation of pyrolytic products, has been applied to the structural studies of fats and oils (Janak, *Nature, 185,* 684). The experiments were carried out with a high-temperature chromatograph with squalane as the stationary phase, N as the carrier gas, and H for maintaining the flame. The pyrolytic products spectra of some of the oils and fats and their related barbituric acid derivatives were presented. The

graphs showed that the fragments of the molecules present were always qualitatively as well as quantitatively highly specific.

Lipid Composition Analysis. A compilation of reviews covering various aspects of the properties of lipids and methods for their analysis has appeared (Sunderman and Sunderman, eds., *Lipids and Steroid Hormones in Clin. Med., Proc. Appl. Seminar, Washington, D. C.,* Lippincott Company, 1960). A complete listing of titles in the book follows: Classification of the Lipids; Extraction of Lipids from Serum, General Considerations, Extraction of Serum Lipids; Total Lipids in Serum, Determination of Total Lipids and of Triglycerides by Difference, Determination of Total Serum Lipids; Cholesterol and Cholesterol Esters in Serum, Brief Historic Review of Cholesterol Analysis; Cholesterol and Cholesterol Esters in Serum, General Considerations; Phospholipids in Serum, General Considerations; Phospholipid Separation; Estimation of Phosphorus in Serum; Method for Lipid Phosphorus in Serum; Total Fatty Acids in Serum, General Conditions, Total Fatty Acids in Serum, Stoddard-Drury Method; Esterified Fatty Acids in Serum, Stern-Shapiro Method; Free Fatty Acids in Serum, Analytical Considerations and Clinical Significance, Free Fatty Acids in Serum, Modification of Dole Method; Lipoproteins in Serum, Characterization and Methods of Analysis; Lipoproteins in Serum, Clinical Significance; Fractionation of Lipoproteins in Serum by Paper Electrophoresis; Lipids in Feces, Analytical Considerations and Clinical Interpretations; Methods for Total Lipids and Fatty Acids in Feces; Meaning of Hyperlipidemia; Clinical Significance of Lipids in Cardiovascular Disease; Fundamental Chemical Considerations of the Steroid Hormone Metabolites, Their Origins, Distribution, and Measurement; Colorimetric Reactions for the Estimation of the Steroid Hormones; Partition Chromatography as Applied to Steroids: I. Column Chromatographic Methods for the Analysis of Neutral Urinary Steroid Metabolites, II. Procedure for the Estimation of Pregnanediol and Pregnanetriol in Urine, III. Comments on Pregnanediol and Pregnanetriol; Use of Radioisotopes in Steroid Methodology; Methodology of Urinary 17-Keto Steroids, General Considerations, Determination of Urinary Neutral 17-Keto-Steroids; Methodology of Corticosteroids and Aldosterone, Measurement of Urinary Corticosteroids, Modified Porter-Silber Method; Measurement of Plasma or Serum Cortisol; Stimulation and Suppression Tests of Adrenal Cortical Function; Determination of Aldosterone in Urine; Clinical Significance of Measurements of 17-Keto-Steroids and 17-Hydroxy Corticosteroids; Estimation of Urinary Estrogens, Estrogen Effect Estimated by Vaginal Cytology, Determination of Estrogens in Urine by Bioassay, Chemical Method for the Estimation of Urinary Estriol; Clinical Significance of Measurement of Estrogens and Progesterone Metabolites; reviews with many references.

The following review articles on lipid analysis appeared in the *J. Japan Oil Chemists' Soc.*: Chromatography (Kuwada, *Yukagaku, 8,* 565); Gas Chromatography of Fats, Fatty Acids, Their Esters, and Unsaponifiable Matter (Asahara and Yamashita, *ibid.,* 590); Column and Paper Chromatography of Fats, Fatty Acids, and Unsaponifiable Matter (Noda, *ibid.,* 598); Chromatography of Edible Oils and Their Additives (Terada, *ibid.,* 604); Chromatography of Sterols (Tamura and Matsumoto, *ibid., 610*); Column Chromatography of Conjugated Lipids (Hara, *ibid., 616*); and Chromatography of Surface-Active Agents and Their Builders (Ishiwata and Nagayama, *ibid., 9,* 25). The application of mass spectrometry to lipid research (Ryhage and Stenhagen, *J. Lipid Res., 1,* 361) and infrared absorption for the analysis of tissue lipids (Schwarz, *Advances in Clin. Chem., 3,* 1) were reviewed.

Radioactive reagents were employed in the analysis of lipids by converting free acids to Me esters with $C^{14}H_2N_2$ and esterifying hydroxyl and amino groups being esterified with either $(C^{14}H_3CO)_2O$ or $(CH^3{}_3CO)_2O$ (Mangold, *Fette, Seifen, und Anstrichmittel, 61,* 877). The esters were separated into groups by adsorption on silicic acid columns, and the groups were separated into individual components by paper chromatography. The components were identified and analyzed by radioactive counts and photographic techniques. Similar techniques (thin-layer chromatography) were described in a separate paper along with micro methods or the resolution of unusual fatty acids in total acid fraction that were isolated after saponification of lipids from vegetable and animal sources. Methods for the separation of unsaponifiable fractions were also reported (Mangold and Malins, *J. Am. Oil Chemists' Soc., 37,* 383). Further application of these techniques and the use of "siliconized chromatoplates" for the fractionation of classes into their constituents by reversed-phase partition chromatography were described (Malins and Mangold, *ibid.,* 576). Essentially the same methods were used for the separation of fatty acids, diglycerides, hydroxy- and episulfido-fatty acids, fatty alcohols, and triglycerides (Kaufmann and Makus, *Fette, Seifen, und Anstrichmittel, 62,* 1014). Cormier used paper that was impregnated with silicic acid to separate neutral fats of blood serum and to detect esterified and free cholesterol in lipid extracts (Cormier *et al., Bull. soc. chim. bio., 41,* 1037). The technique described made possible a separation of the lipids into three groups: steroids, triglycerides, and mono-and diglycerides of cholesterol. An extensive study of the chromatography of lipids on silicic acid was reported (Wren, *J. Chromatography, 4,* 173).

Dieckert and Reiser's method of employing glass fiber paper impregnated with either silica gel or water-glass for the separation of lipids was investigated for the effects of various factors (Černíková and Horáček, *Arch. klin. u. exptl. Dermatol., 209, 572*). The basic requirement for satisfactory separation of lipids was the thorough saturation of the closed system and the glass fiber paper with the solvent mixture. The low-temperature chromatography of lipids on cellulose was described (Collins and Shortlander, *J. Lipid Research, 1,* 352). Experiments were reported that demonstrated the feasibility of analyzing certain lipid-containing body fluids, such as serum, by direct chromatography without solvent extraction (Marinetti and Stotz, *Biochem. et Biophys. Acta., 37,* 571). The solvents were sufficiently polar to rupture the lipid-protein bonds and cause the lipids to migrate as discrete spots. The proteins remained at the origin. The chromatograms could be washed in water without an appreciable loss of lipids but with loss of most of the nonlipid compounds present. Dialysis, in conjunction with chromatography on silica, was employed to separate phospholipids, free fatty acids, sterol esters, and glycerides in tissue lipid extracts (Böttcher, *Rec. trav. chim., 78, 794*). The dialyzate was extracted with aqueous KOH to obtain free fatty acids, and this was followed by chromatography to separate sterol esters, glycerides, and free sterols. The phospholipids dialyzing through the rubber membrane were converted to methyl esters for analysis by gas chromatography. A modified Soxhlet extraction apparatus was described, which allowed the extraction to be carried out in an atmosphere of nitrogen and afforded a more efficient removal of lipid from serum and tissue lipoproteins. Less than 2% of the lipid remained in the protein residue after 4–5 hrs. of extraction with a mixture of $CHCl_3$- Et_2O-EtOH (3:1:1) (Elmendorff, *Z. Ges. Exptl. Med., 130,* 142). A colorimetric determination of ester groups in lipid extracts was reported (Antonis, *J. Lipid Research, 1,* 485).

A phospholipid-free extract of cholesterol and triglyceride was obtained from rat plasma by shaking the plasma with chloroform in the presence of a synthetic zeolite (Cheng and Zilbersmit, *J. Lipid Research, 1,* 190). The triglyceride was determined by analysis for glycerol after saponification of the sample. Wegner reviewed recent results in the isolation and identification of phosphatides and glycolipids by use of thin-layer chromatography, paper chromatography, Craig-distribution, and IR-spectrophotometry (Wagner, *Fette, Seifen, und Anstrichmittel, 62,* 1115). The relative mobilities of monophosphoinositide and of the common choline-containing phosphatides were measured on paper chromatograms that were obtained with various papers and solvents (Renkonen, *Ann. Med. Experimentalis et Biol. Fenniae* [Helsinki], *37,* 197). In a paper that followed, these authors considered the separation between various nonpolar nonphospholipides and their differentiation from the most rapidly-running phosphatide range in the system used (*ibid.,* 203). A procedure for the quantitative hydrolysis of phospholipids and the separation and estimation by ion-exchange chromatography of the water-soluble phosphates was described with reference to the analysis of tissue phospholipids (Hübscher *et al., J. Lipid Research, 1,* 433). A similar method was also reported in an independent paper (Dawson, *Biochem. J., 75,* 45). Staining techniques for the detection of phospholipids on paper were: the tricomplex staining method with acid fuchsin and U ions at pH 2 to stain phosphatides of the acid-base variety; immersion in an acid MoO_3 solution for staining all phosphate-containing lipid spots blue; the tricomplex stain with brilliant green to stain phosphate-containing acid compounds green and acid-base type of compounds red; and the tricomplex stain with Rhodamine 6 G to render lipid substances visible without the need of ultraviolet light (Hooghwinkel *et al., Koninkl. Ned. Akad. Wetenschap., Proc.,* Ser. B *62,* 222). The saponification and hydrogenation of lecithin on paper, followed by separation of the fatty acids, was investigated (Kaufmann and Wessels, *Fette, Seifen, und Anstrichmittel, 62,* 1020).

A silica gel column chromatography method for the separation of phospholipids was reported (Sakagami *et al., J. Bio-*

chem [Tokyo], *46*, 1607). The separation of polyglycerophospholipid, phosphatidylserine, phosphatidylethanolamine, inositol-containing phospholipid, phosphatadylcholine, and combined phosphatidylcholine-sphingomyelin fractions was fairly reproducible. Carlson and Wadström used a silicic acid column to separate phospholipids from other lipids in a $CHCl_3$-MeOH extract after treatment with acid phosphate. The unesterified fatty acids with the phospholipids were separated by ion exchange from the purified extract, converted to their Me esters, and determined colorimetrically by the hydroxamic acid reaction (Carlson and Wadström, *Scand. J. Clin. and Lab. Invest., 10*, 407). The method has the advantage of accuracy ($\pm$0.02 meq./l) and specificity for acids with more than 14 C atoms. A modified hydroxamic acid method for total esterified fatty acids in plasma entailed a treatment of the acids with $FeCl_3$ to form highly-colored complexes with absorption maximum at 515 mμ (Morgan and Kingsbury, *Analyst, 84*, 409). A micro-titration method permitted duplicate determinations of total serum fatty acids, cholesterol, lipid P, and triglycerides by difference with only 1 ml. of specimen (Albrink, *J. Lipid Research, 1*, 53). Essential steps were extraction of the lipids, saponification, extraction, and micro-titration of the liberated fatty acids. Recoveries of pure fatty acids and triglycerides were 97% complete. A single extraction of blood plasma with a 2-phase heptane-iso-PrOH-H_2O system provided sufficient analytical specificity for determining long-chain nonesterified fatty acids in the presence of volatile acids (Dole and Meinertz, *J. Biol. Chem., 235*, 2595). This method was further modified to improve its specificity by first washing the fatty acid solution with 0.05% H_2SO_4; this removed interfering lactic acid and acetone-insoluble materials (Trout *et al., J. Lipid Research, 1*, 199).

A semi-micro determination of total fatty acids and unsaponifiable matter involved the saponification and recovery of the total fatty acids, removal of the fatty acids with anion exchange resins, and determination of the unsaponifiable matter by weight (Benedict, *J. Am. Oil Chemists' Soc., 37*, 415). This method showed a standard deviation from the mean of 0.21% for total fatty acids and 0.06% for the unsaponifiable content. A method was described for the separation of unsaponifiables into their major chemical classes by silic acid adsorption chromatography (Capella *et al., ibid.*, 564). The procedure was tested with synthetic mixtures of hydrocarbons, esters, alcohols, and sterols. Infrared analysis, carbon-hydrogen analysis, melting point of derivatives, and paper chromatography of the sterol fractions from olive oil, soybean, tea seed, and rapeseed oils, lard, and tallow were also reported. An excellent separation of steroids was effected by gas chromatography on a silicone rubber-Chromasorb W column (Van Heuvel *et al., ibid., 82*, 3481). When 2 to 3 parts per 100 of silicone gum on the support were used at a column temperature of 222°C, all the steroids were eluted as single components with no sign of decomposition. Cholesterol required only 35 min. for elution at a flow rate of 30 ml./min. on a 183-cm. column. The mass spectra of methyl esters of bile acids possessed features characteristic enough for their analysis (Bergström *et al., Acta Chem. Scand., 12*, 1349). Free sterols and acetylated sterols were both separated by paper chromatography (De Zotti *et al., Fette, Seifen, und Anstrichmittel, 61*, 1114). The R_f values for several sterols and their derivatives were given.

Various waxes were clearly differentiated by their infrared spectrograms (Kuhn, *Studies in Conservn. Abstr., 5*, 71). Spectrograms were presented for beeswax, Punic wax, beeswax-colophony mixture, beeswax-A.W.$_2$ mixture, carnauba wax, esparto wax, montan wax, stearin wax, pariffin, and IG wax N. Ion-exchangers in nonaqueous solution were utilized for the group separation of waxes. Wax acid components of natural or synthetic waxes in organic solvents were separated and determined quantitatively with adsorption chromatography and suitable anion exchangers (Presting and Janicke, *Fette, Seifen, und Anstrichmittel, 62*, 81). Chromatography on specially-prepared Al_2O_3 separated a known mixture of wax constituents into four groups, *viz.*, dotriacontane, octadecyl stearate, stearone, octadecanol, and stearic acid (Cole and Brown, *J. Am. Oil Chemists' Soc., 37*, 359).

COMPOSITION AND CHARACTERISTICS. Investigation of the composition and chemical and physical properties of fats and lipids of wide variety of materials was the major subject of a great number of communications. The volume of these data prohibits a detailed listing of specific characteristics. Consequently only a general description of the types of fats and lipids investigated is presented below. An asterisk (*) appearing after the reference indicates that the fatty acid composition of the particular fat or fats is given. A double asterisk (**) indicates that the glyceride composition is also reported. No asterisk indicates that only physical and chemical properties are given.

The reader is encouraged to contact this reviewer for more detailed information on data of interest that cannot be conveniently located through the reference.

In addition, much of the literature in the previous sections on analytical methods also contains information on the composition and characteristics of certain fats and lipids. Although an attempt was made to include the more important ones here, reference should be made to the other sections for a complete review.

A few articles dealt with a wide variety of oils. In a continuation of a series of studies on lesser known seed oils, the iodine number, sapon. number, and fatty acid composition were reported for the following plant sources: *Hesperis matronalis, Matthiola bicornis, Euphorbia marginata, E. heterophylla, Majorana hortensis, Monarda fistulosa, Nepeta mussinii, Ocimum basilicum, Perilla frutescens, Salvia columbariae, Satureja hortensis, Thymus vulgaris, Linum usitatissimum* (Earle, *et al., J. Am. Oil Chemists' Soc. 37*, 48);* *Ageratum houstonianum, Artemisia absinthium, Centaurea cyanus, Chrysanthemum leucanthemum, Chrysanthemum coronarium, Cosmos bipinnatus, Cynara cardunculus, Dimorphotheca aurantiaca, Helichrysum bracteatum* var. *monstrosum, Heliopsis helianthoides, Liatris spicata, Rudbeckia bicolor* var. *superba, Vernonia anthelmintica, V. baldwini, V. missurica* (Earle and Wolff, *ibid., 37*, 254)*; *Zelkova serrata, Sassafras albidum, Cuphea Havea* var. *miniata, Celastrus orbiculata, C. Havea, S. albidium, Mecurialis annual, pomegranate, catalpa, Bignoniaccae, Curcurbitaceae, Chilapsis linearis, Hibiscus moscheutos*, and *H. syiacus* (Earle *et al., ibid., 37*, 440).* The last article contains analytical data and infrared spectra for 158 species, representing 52 plant families in 23 orders. Of these 138 were previously unreported in books or in recent references. Newly developed varieties of sunflower seed and linseed were compared with varieties most abundant in the U.S.S.R. for their fat content and iodine numbers (Lebedeva and Stepanova, *Trudy Vsesoyuz. Nauch.-Issledovatel. Inst. Zhirov., 1958* [18], 185). The fatty acid composition of lipids of pasture grasses were similar to those of the leaf lipids of maize and the lipids of clover-rich pasture (Garton, *Nature, 187*, 511). The vicinal unsaturated hydroxy acid contents of seed oils *Dimorphotheca Aurantiaga, Artemisia absinthium, Calliandra eriophylla, Balanites aegyptica, Coscos bipinnatus, and Helianthus annuus* were reported (Morris *et al., J. Am. Oil Chemists' Soc., 37*, 323).* The fatty acid compositions of seed oils from *Crambe abissinica, Perilla ocynoides, Camelina sativa, Carthamos tinctoris, Euophrbia lathyris* (Grynberg *et al., Fette, Seifen, und Anstrichmittel, 61*, 908,* and the conjugated oils *Impatiens, Oiticica, and Parinarium annamense* (Kaufmann and Sud, *Fette, Seifen, und Anstrichmittel, 62*, 3, 160)* were determined by paper chromatography.

For the convenience of the reader, specific fats and oils investigated for their composition and characteristics are organized into subsections according to general types. The subsections, *Lipids, Vitamins*, and *Physical Properties* contain those articles that deal exclusively with these characteristics. The asterisks have the same meaning as explained above. An "x" appearing after the asterisk indicates that the composition and/or characteristics of the unsaponifiable matter was also reported.

Vegetable Oils (Sources): Appleseed (Wakayama *et al., J. Hokkaido Gakugei Univ., 7*, 98),* *Acacia decurrens W* (Rao, *Current Sci.* [*India*], *28*, 410), *Aglaia odoratitssima* (Baslas, *Indian J. Appl. Chem., 22*, 125),*x *Asclepias Syriaca* (Chisholm and Hopkins, *Can. J. Chem., 38*, 805),* *Caryodendron orinocense* (Seelkopf, Z. *Lebensm.-Untersuch. u. -Forsch., 112*, 499), castor leaf (Mihara, *Nippon Kagaku Zasshi, 80*, 641),* *Chlorella Pyrenoidosa* (Schlenk *et al., J. Am. Oil Chemists' Soc., 37*, 547),* cherry seed (Weckel and Lee, *Food Tech., 14*, 151),* *Chrysanthemum coronarium* (Smith *et al., Chem. and Ind.* [London], 1959, 259),* *Citrus microcarpa* (Agrawal *et al., J. Proc. Inst. Chemists* [India] *31*, 207),* Egyptian and Chinese cottonseed *(Egyptian Pharm. Bull., 40*, 235), Indian cottonseed (Achaya and Saletore, *Cottonseed and By-Prods., Proc. Symposium Hyderabad, 1958*, 275),* "gossypol-free" cottonseed (Mattson *et al., J. Am. Oil Chemists' Soc., 37*, 154),* *Cuphea llavea seed* (Wilson *et al., ibid.*, 675),* *Cyperus esculentus* (Gad and Osman, *Egypt. J. Chem., 2*, 123),* *Eruca sativa* (Popov and Mazhdrakov, *Compt. rend. acad. Bulgare sci., 11*, 279),* *Fritillaria camschatcensis* (Shibata and Takakuwa, *Phyton* [Buenos Aires], *12*, 25),* grapeseed (Morand and Silvestre, *Ann. fals. et expert. chim., 53*, 193),* *Hibiscus esculentus seed* (Kapur and Seng-Gupta, *Indian J. Appl.*

Chem., 23, 45),* horse chestnut (Alexa *et al., Studia univ. Victor Babes et Bolyai, 3,* 193), *Lawsonia alba* (Agrawal *et al., Indian Oil and Soap J., 25,* 145),* lemon seed (Franguelli and Mariani, *Olii minerali, grassi e saponi, colori e vernici, 36,* 407),* *Lepidium sativum* (Popov and Mazhdrakov, *Compt. rend. acad. Bulgare sci., 11,* 279),* *Limnanthes douglasii* seed (Smith *et al., J. Organic Chem., 10,* 1770),* *Malvaceae,* six species (Hopkins and Chisholm, *J. Am. Oil Chemists' Soc., 37,* 682),* *Matthiola incana* (Joshi and Bhakuni, *Natl. Acad. Sci. India, A28,* 190),* *Meratia praecox* (Kusunose and Adachi, *Yukagaku, 8,* 76),* morro seed (Lewy-van Séveren, *J. Am. Oil Chemists' Soc., 37,* 402),* *Oldenlandia biflora* (Bhakuni, *J. Sci. Ind. Res.* [India], *18B,* 445),*x olive (Crespo and Cattaneo, *Anales Asoc. quim. arg., 46,* 368),* Ceylon sweet orange (Weerakoon, *J. Sci. Food Agr., 11,* 273),* bitter orange seed (Zaganiaris, *Compt. rend. congr. intern. chim. ind., 31e, Liége,* 1958 [2], 658),* ongokea (Pouliquen, *Oleagineux, 14,* 453),* papaya seed (Venkatesh and Rao, *Food Sci.* (Mysore), *9,* 49), *Phyllanthus maderas-patensis* (Bhakuni, *J. Sci. Ind. Res.* [India], *18B,* 446),* pumpkin seed (Froelich, *Pharmazie 14,* 355), raisin seed (Flanzy and Flanzy, *Ann. inst. natl. recherche agron., Ser. E 8,* 107),* rice bran, a review, (Kamath, *Bombay Technologist,* 9, 12), *Secale cornutum,* (Tišler, *Fette, Seifen, und Anstrichmittel, 60,* 95),* sesame seed (Krishnamurty *et al., Food Sci.* [Mysore], 8, 1316),* Spanish sunflower seed (Casares and López-Herrera, *Anales bromatol.* [Madrid], *11,* 477), *Sporidesmium bakeri* (Hartman *et al., Biochem., J. 75,* 274),* *Strophanthus* seed (Gunstone and Morris, *J. Sci. Food Agr., 10,* 522),* *Zanthoxylum rhetsa* (Agrawal *et al., Indian Oil and Soap J., 25,* 26).*

MAMMAL FATS. Human blood lipids (Kaufmann and Schmidt, *Fette, Seifen, und Anstrichmittel, 62,* 399),*x Hanahan *et al., J. Lipid Res., 1,* 421),* human depot fat (Dickman, *Diss. Abst. XXI* [4], Mic 60-4073),* pig back fat (Hart and van der Veen, *Landbouwk Tijdschr., 69,* 343), *Phyllodromia germanica, insect* (Ono and Adachi, *Yukagaku 8,* 72),* sperm whale oil (Moldavskaya and Dmitrieva, *Masloboĭno-Zhirovaya Prom., 1955,* 30)x.

Fish Oils. Herring (Klenk and Steinbach, *Z. Physiol. Chem., 316,* 31),* *Ascidians* (Ito *et al., Nippon Kagaku Zasshi, 81,* 662)* x menhaden body oil (Stoffel and Ahrens, *J. Lipid Research, 1,* 139),* sardine and mackerel (Toyama *et al., Fette, Seifen, und Anstrichmittel, 61,* 461; ibid., 846),* sea-hare *Aplysia kurodai* sterols (Tanaka and Toyama, *Nippon Kagaku Zasshi, 80,* 1326),*x unsaponifiables other than sterols in *A. kurodai* (*ibid.,* 1329),*x egg mass of *A. kurodai* (*ibid. 81,* 831)x *Xiphias gladius,* head bone oil, (Labruto and Bruni, *Atti soc. peloritana sci. fis. mat. e nat., 5,* 513).*

Dairy Products. Bovine butter oil and fat-globule membrane (Thompson *et al., J. Dairy Sci., 42,* 1651),* Ost-Friesian (cow) milk fat (Plekhanova, *Molochnaya Prom., 20,* 38), sheep's milk fat (Prekopp, *Výživa Lidu, 12,* 46; *ibid.,* 61), cow's milk fat (Jack, *J. Agr. Food Chem., 8,* 377),** butterfat (Hansen *et al., Biochem. J., 77,* 64),* lyophilized milk fat (Jensen and Gander, *J. Dairy Sci., 43,* 1758).* Data were presented for the distribution of free fatty acids in normal and "lipase" milk and in cream, skimmilk, butter, buttermilk, cheese, and whey derived from them (Willart and Sjöström, *Svenska Mejeritidn, 52,* 443). The average total monoglyceride of fresh raw milk, pasteurized whole milk, homogenized milk, 40% cream, butter, and blue cheese were reported (Jense *et al., J. Dairy Sci., 42,* 1913).

Processed Fats. Margarine and shortenings from England, Germany, Norway, Belgium, and the United States were analyzed for their fatty acid compositions (Nakazawa *et al., Yukagaku, 9,* 200;* *ibid.* 383*). Margarines and shortenings were also investigated for their *trans* fatty acid contents (Mabrouk, *Univ. Microfilms* [Ann Arbor, Mich.] L. C. Card Mic 60–85).* Rapeseed, soybean, cottonseed, and sunflower oils were studied spectrophotometrically during hydrogenation to follow the development of structural isomers (elaidic and brassic acids) and to determine the equilibrium and rate constants of such transformations (Artamonov, *Trudy Vsesoyuz. Nauch.-Issledovatel. Insti. Zhirov, 1957,* 55).* Characteristics and chemical composition of tobacco seed oil hydrogenated under different conditions were studied (Chakrabarty and Chakrabarty, *J. Sci. Ind. Res.* [*India*], *18A,* 539).* Three grades of commercial stearic acid were analyzed for their fatty acid composition by fractional distillation (Casal, *Anales direc. nacl, quim.* [*Buenos Aires*], *8,* 22).*

Lipids. Hawthorne presented a thorough review of the chemistry of phospholipids containing inositol (Hawthorne, *J. Lipid Research, 1,* 255).* The existence of sterol glycosides in rapeseed and linseed was reported (Aylward and Nichols, *Nature, 184,* 1319). A new complex lipid, triphosphoinostide, was isolated from ox brain (Dittmer and Dawson, *Biochim. et Biophys. Acta, 40,* 379). A study was made of the phospholipids of ox spleen with special reference to the fatty acid and fatty aldehyde composition of the lecithin and kephalin fractions (Gray, *Biochemical J., 77,* 82).* The aldehydes in nonphosphatide aldehydogenic lipids in milk fat, beef tallow, and ox heart are bound as enol-ethers and located mainly in the *alpha*-position of the glycerol molecule (Schogt *et al., J. Lipid Research, 1,* 446. The composition of the total lipids of the myocardium, conducting bundle, and valves of beef heart (Kochen *et al., J. Lipid Research, 1,* 147) and of kidney and liver lipids from the American antelope (Miller, *J. Am. Oil Chemists' Soc., 37,* 247) were reported. No differences were found in the fatty acid composition of milk phosphatides derived from full-cream milk, buttermilk, and cream cheese (Badings and Koops, *Fette, Seifen, und Anstrichmittel, 62,* 302).* The lipids of wheat were fractionated on silicic acid, and their compositions were determined (Fisher and Broughton, *Chem. and Ind., 1960,* 869).* An investigation of the minor constituents of olive oil showed that the unsaponifiable matter contained about 65% hydrocarbons with squalene as the main constituent (Vitagliano, *Olii minerali, grassi e saponi, colori e vernici, 37,* 136). Tetracosanol and *beta*-sitosterol were identified in the unsaponifiable matter in the fat of silk-worm feces (Tamura *et al., Nippon Kagaku Zasshi, 77,* 1424). Cycloartenol and hexacosanol were found in the unsaponifiables of rapeseed oil (Tamura *et al., ibid, 79,* 1053). The sterol composition was determined for the molecular distillates of corn oil (Kuksis and Beveridge, *J. Lipid Research, 1,* 311), the residue from the distillation of fatty acids in the refining of peanut oil (Uzzan, *Olearia, 13,* 215), the seeds of *Psoralea corylifolia* (Khastgir *et al., Indian J. Appl. Chem., 22,* 35), and Mahua oil (Singh, *ibid.,* 61). Phospholipid compositions were reported for the lipids of soybeans (Nielson, *J. Am. Oil Chemists' Soc., 37,* 217), cabbage leaf (Wheeldon, *J. Lipid Research, 1,* 439),* and rabbit skin (Schwarz *et al., Arch. Biochem. and Biophys., 87,* 171). In variance with the literature $Me(CH_2)_{15}CH(OH)CH(OH)CH(NH_2)CH_2OH$ was proposed as the structure of the cerebrin base in yeast (Prostenik and Stancev, *Chem. Ber., 91,* 961).

Waxes. A classification of the waxes was given (Ivanovszky, *Fette, Seifen, und Anstrichmittel, 62,* 37). The chemistry of peat and montan waxes was reviewed (Howard and Hamer, *J. Am. Oil Chemists' Soc., 37,* 478). The chemical characteristics and the chromatographic separation of 24 Polish beeswax samples were reported (Curylo and Zalewski, *Pszczelarstwo, 1,* 105). The chemical characteristics of the wax of sugar cane (Torricelli, *Ind. vernice* [*Milan*], *11,* 8; *Chem. Zentr., 129,* 3468), and the composition of the wax of *Bulnesia Retama* (de Badin and Brenner, *Anales asoc. quim. arg., 46,* 318; Brenner *et al., ibid.,* 336)* were presented. Noble reported the isolation of hydroxy acids from wool wax acids by a Craig countercurrent separation of the methyl esters (Noble *et al., J. Am. Oil Chemists' Soc., 37,* 14). In a similar study on wool wax acids Barnes concluded that *delta*-hydroxy acids were not present in wool fat and that fractions reputed to be such actually consist of non-hydroxylated wool wax acid, formed by partial esterification during the acid treatment (Barnes, *Australian J. Chem., 13,* 184).

Vitamin Content. The tocopherol content sof sunflower oil (Omel'chenko, *Masloboĭno-Zhirovaya Prom., 25,* 10), some fodder crops and wild plants of meadows and pastures in southern Italy (Di Celso, *Ann. sper. agrar.* [Rome], *12,* 169), and Buenos Aires olive oil (Bertoni and Cattaneo, *Anales asoc. quím. arg., 47,* 52) were reported. The carotene, vitamin A, and vitamin E content of yellow beef fat were also investigated (Mirna, *Z, Lebensm.-Utersuch, und Forsch., 111,* 393). Other references on this subject appear in the foregoing sections on the effects of variety, maturity and environment.

Physical Properties. The viscosity-temperature relationship of some Indian vegetable oils were reported (Varshni, *Vijnana Parishad Anusandhan Patrika, 3,* 35). The dielectric constant and loss tangent of castor, linseed, olive, mustard, and sesame oils were measured at various ratio frequencies (Sharma, *J. Sci. Ind. Res.* [*India*], *19B,* 5). The dielectric constants of the oils, with the exception of castor oil, did not vary with frequency. Phase equilibrium data are presented in tables and graphically for methyl esters-furfural-hexane and methyl esters-nitromethane-hexane systems at 30°C. (Lakshmanan and Laddha, *J. Am. Oil Chemists' Soc., 37,* 466). The volumes of mixing fatty oils with various solvents (Kusano, *Yukagaku, 7,* 400), the heats of mixing fatty oils with various solvents (*ibid.,* 406), the vapor pressures of fatty oil solutions (Kusano, *ibid., 8,* 8), and the entropy of mixing fatty oil with solvents (*ibid.,* 15) were reported. Other physical propertites were reported in

the references lisited in the section on the Analysis of Physical Properties.

DETECTION OF ADULTERATION. Gas chromatography of the mixed methyl esters of peanut and rapeseed oil permitted the detection of 5% rapeseed oil in peanut. The addition of coconut or palm-kernel oil to butter was also detected by this technique (Wolff and Wolff, *Rev. Franc. Corps Gras, 7,* 73). A paper chromatographic separation of the sterols from fats was used to demonstrate the presence of plant fats in animal fats by the relative phytosterol and cholesterol contents (Peereboom and Roos, *Fette, Seifen, und Anstrichmittel, 62,* 91). The addition of an alkylated dihydroxydiphenylmethane was recommended as an additive to hydrogenated fats in order to render them detectable when used to adulterate oleaginous materials. As little as 10 mg./kg. developed a deep purple color when treated with potassium ferricyanide in base (Council of Scientific and Industrial Research [by B.S. Joshi] *Indian, 64,366,* May 25, 1960). The size of drops of alkaline solutions, when allowed to run into a benzene solution of vegetable oils, was related to the composition of the oil (Martinez, *Grasas y Aceites* [Seville, Spain], *10,* 14). This method could detect the presence of a small amount of an oil in a large amount of another oil.

Differences in the properties of refined and unrefined cold-pressed olive oils were studied as a means of distinguishing the two types. The cold-pressed oil showed high electrical conductivity, high acidity, intense coloration, characteristic odor and taste, and a spectrophotometric extinction maximum at 670 mμ (Lauber, *Mitt, Lebensm. und Hyg., 50,* 553). Adulteration of olive oils with other oils may be suspected if the linoleic acid content is greater than 11.6%. Spectrophotometric analysis of 46 samples of native olive oils gave values in the range 1.63 to 13.6% linoleic acid in 99.7% of the cases (Ninnis and Birbili-Ninnis, *Prakt. Akad. Athenon, 33,* 103). The K spectrophotometric values at 262, 263, and 264 mμ and the ΔK's were reported for several neutral, acid, rancid, and rectified olive oils and for samples treated with sunlight, H_2O_2, steam, decolorizers, and neutralizers (Morani and Marignoli-Colloca, *Ann. sper. agrar.* [Rome], *14,* 293). The *K's* were useful for differentiating virgin oils from rectified ones. The same information appeared in a duplicate paper (Morani and Marignoli-Colloca, *Olii minerali, grassi e saponi, colori e vernici, 36,* 327). Suggestions were presented for fixing standard maximum and minimum limits for absorption values for pure olive oils (Montefredine and LaPerta, *ibid.,* 325). Tests on more than 400 samples of olive oil showed that the pressure oils, and the rectified seed oils contained these compounds (Fabris and Vitagliano, *ibid.,* 313). Advantages of ultraviolet spectrophotometry of olive oil for detecting purity, aging, and regeneration were detailed (Doro and Sadini, *Boll. lab. chim. provinciali* [*Bologno*], *10,* 124). These authors urged setting up spectrophotometric standards for olive oil to be used in the European Common Market. Absorption coefficient limits for the classification of superfine virgin olive oil were given (Albonico, *Olii minerali, grassi e saponi, colori e vernici, 37,* 343).

Olive oil was "marked" by the addition of curcumin in order to detect adulteration. One gram of curcumin/quintal of olive oil was found sufficient for the quantitative detection of curcumin even after a 10-fold dilution prior to the colorimetric determination in the form of roseyanin (Rotini and Galoppini, *Chim. e ind.* [Milan], *42,* 605). A paper chromatographic procedure for the detection of adulteration in olive oil was described (Kaufmann and Aparicio, *Fette, Seifen, und Anstrichmittel, 61,* 768). Pure olive oil gave three triglyceride spots on the chromatogram. In the presenec of other oils or fats a greater number of spots were obtained. The lowest quantity of detectable adulteration by this method varied from 5 to 10%. Foreign oils in olive oil were detected by separation and determination of the melting-points of the isolated sterol acetates (Vitagliano and D'Ambrosio, *Olearia, 11,* 169). A new method was described for detecting virgin olive oil by a titrimetric analysis of the alkaline impurities (Romeo *et al., Atti. soc. peloritana sci, fis. mat. e nat., 5,* 533). The possibility of identifying esterified oils in olive oil by means of infrared spectroscopy was evaluated (Provvedi, *Olii minerali, grassi e saponi, colori e vernici, 36,* 375). The esterified oils showed a slope on the infrared spectrum in the zones 4,000 to 3,000 $cm.^{-1}$ and 1,000 to 950 $cm.^{-1}$ which was not given by the olive oils. Recent adulterants of olive oils consisted of fatty acid methyl esters with similar characteristics. These can be detected by saponification and testing for MeOH (Canneri, *Olearia, 13,* 179). A recommendation was made to the International Commission to standardize the procedure for the thermosulfuric index of olive oils in order to obtain more accurate results (Bigoni, *Olli minerali, grassi e saponi, colori e vernici, 37,* 49). The Fiteison reaction for the detection of tea seed oil in olive oil was found reliable only when more than 15% of tea seed oil was present (Bigoni, *Olii minerali, grassi e saponi, coli e vernici, 34,* 458).

The purity of butter was determined by gas-liquid chromatography of its Me esters (Wolff, *Ann. fals. et expert, chim., 53,* 318). Results obtained with butter of various origins, margarines, copra, and palm fat were given. A short review of the detection of adulteration of butterfat was included (Holla, *Bombay Technologist, 9,* 16). The ultraviolet absorption curves of butter-margarine and olive oil-inferior oil mixtures permitted the detection of quantities of the order of 10% of such adulterations (Strivek and Doppelmann, *Anales univ. católica Valparaíso, 2,* 29). Vitamin A fortification of vanaspati was found to have no averse effect on the Baudouin test for the detection of hydrogenated oils in butter and ghee (Sastry *et al., J. Sci. Ind. Research* [India], *18A,* 377). A semi-micro Baudouin test, positive at adulteration levels of 10%, was described (Kapur *et al., J. Proc. Inst. Chemists* [India] *31,* 177). Circular paper chromatography of ghee could detect as little as 10% vanaspatit or 5% buffalo body fat (Ramachandra and Dastur, *Indian J. Dairy Sci., 13,* 29). Direct viewing of the spots in ordinary light instead of ultraviolet was found more effective. Chromatography of the unsaponifiable material from butterfat on a glass plate coated with a mixture of plaster of Paris and silicic acid could detect adulteration at the 10% level (McGugan, *Intern. Dairy Congr., Proc., 15th, London, 1959,* 1534). At least 5% of tocopherol-rich vegetable oils in Indian butter was detected by analysis of the tocopherol (Nazir and Magar, *Indian J. Dairy Sci., 12,* 125).

Methods for detecting foreign fats in cacao products were critically reviewed, and a method based on gas chromatography of the fatty acid methyl esters was described and recommended (Woikich, *Z. Lebensm. -Untersuch und -Forsch., 112,* 184). Composition curves showed that as little as 5% coconut fat or 1% tengkawang fat could be detected. The common methods for the investigation of cocoa butter and chocolate adulterants were reviewed (Purr, *Fette, Seifen, und Anstrichmittel, 61,* 675). A comparative examination of the spectra of cocoa butter and chocolate fat between 2 and 15 μ was described with reference to the quantitative detection of *trans-olefins.* Additions of up to 5% elaidic acid could be easily detected (Purr *et al., Rev. intern. chocolat., 14,* 322). The apparatus and procedures for the separation of fatty acids by inverse partition chromatography were described as a method for the detection of cocoa butter substitutes (Purr, *ibid.,* 204). Coconut oil adulterants were also detected by saponifying the fat with hydroxylamine and chromatographing this mixture on acetylated paper in order to detect small amounts of lauric acid (Pietschmann, *Fette, Seifen, und Anstrichmittel, 61,* 682).

The Pavolini-Isidoro reaction for the detection of sesamin in the presence of furfural was modified for the identification of sesame oil in hydrogenated fats (Daghetta, *Olii minerali, grassi e saponi, colori e vernici, 34,* 423). The sesamin could be detected at a level of 0.05 to 0.075%. The extraction of coal tar dyes from sesame oil with concentrated HCl also removed a fraction which gives the color in the furfural test. Dilution of the HCl with ethyl ether eliminated this interference (Roy *et al., J. Proc. Inst. Chemists* [India], *31,* 16).

The difference in the arachidonic acid content of lard and goose fats was used to detect the degree of adulteration of the latter. Other constants for various mixtures of these fats were reported (Wurziger, *Fette, Seifen, und Anstrichmittel, 61,* 1046). A sample method for the adulteration in beeswax was based on differences in the cloud-point for various types of pure and adulterated materials (Parker, *Drug Standards,* 28, 26). A quantitative determination of beeswax adulteration by mineral waxes employed gas chromatography (Curylo and Zalewski, *Pszczelelnicze Zeszyty Nauk, 1,* 118). Trace amounts of hydrocarbons at 0.016 to 0.567% in vegetable oils were quantitatively determined by gas chromatography. Iso-octane was used as an internal standard. (Prevot and Cabeza, *Rev. Franc. Corps Gras., 7,* 34). Methods for detecting resinoids and resins in fats and oils were reviewed. (Morais, *Rev. port. quim., 1,* 363). Gossypol in cottonseeds was determined by extraction in the cold with ether, by transfer of the extracts to a borax solution, separation by acidification with HCl, and development of the color with antimony trichloride in chloroform (Sadykov *et al., U.S.S.R. 122,827,* October 10, 1959).

Deterioration of Fatty Materials

There has been a contining and accelerated interest in problems associated with fat and oil deterioration. These problems include chemical, physical, and organoleptic changes and the conditions that bring them about. Interest has been stimulated

by fears of the toxicity of heated oils and of fats added to animal feeds. Another stimulant has been the increased scrutiny by the Pure Food and Drug Administration of antioxidants and other additives.

REVIEWS. Many aspects of fat deterioration have been considered in a symposium on "Oxidation and Its Prevention" (Kuwada, *Abura Kagaku, 7,* 247). These included: reaction mechanisms of autoxidation and bond energies (Takashi, *ibid.,* 248); oxidation of edible fats and its evaluation (Mitsunaga and Shimamura, *ibid.,* 248); oxidation and flavor problems in edible oils (Yoshitomi, *ibid.,* 285); stability of fatty acids (Yamane, *ibid.,* 298); oxidation of shortening and margarine (Murata, *ibid.,* 302); vitamins and antioxidants (Katsui, *ibid.,* 308); rancidity of soap (M. Nonaka, *ibid.,* 317); rusting of fish oil products (J Nonaka, *ibid.,* 317); oxidation of foods and its prevention (Kihara, *ibid.,* 322).

The reaction of fats with oxygen, peroxide formation, the effects of spontaneous hydrolysis, thermal polymerization, lipase hydrolysis, chemical changes that take place during refining and processing production, and the stability and physical properties of oils have been examined and discussed (Wolff, *Rev. Franc. Corps Gras, 6,* 275). A "Journée d'Étude sur l'Alteration Oxydative des Corps Gras" (*Rev. Franc. Corps Gras,* special issue, April 30, 1959) comprised studies on the chemical aspects of autoxidation (Naudet, *ibid.,* 7); techniques for the determination of oxidation products (Wolff, *ibid.,* 12); evaluation of rancidity in alimentary fats (Uzzan, *ibid.,* 22); and consequences of the use of oxidized fats in soap industry (Vassal, *ibid.,* 37).

DETERIORATION BY HEAT. The nutritional significance of heated fats was discussed at a symposium on the "Influence of Physical Methods of Preservation on the Quality of Foods" (*Acta Chim. Acad. Sci. Hung., 23* [1–4]). The topics considered included: influence of feeding fractionated esters of autoxidized lard and cottonseed oil on growth, thirst, organ weights, and liver lipids (Kaunitz *et al., ibid.,* 40); some problems of the heat treatment of fats (Custot, *ibid.,* 42); nutritional and pathological effects of the nutritional use of fish oil deodorized by heat (Raulin *et al., ibid.,* 43); influence of the stereo-chemical structure of fatty acids on the nutritional efficiency of fats (Raulin and Jacquot, *ibid.,* 44). Also the influence of cold on the nutritive value, the digestibility, and the organoleptic properties of oils, fats, and lipid fractions of foods was considered (Moreno Calvo, *ibid.,* 72).

Reviews have been published on the mechanism of formation of rancid fats and their biological effects (Kaunitz, *Exp. Med. Surg., 18,* 59); the nutritive evaluation of heated fats (Rice *et al., J. Am. Oil Chemists' Soc., 37,* 607); Anon., *Nutrition Rev., 18,* 119); and the peroxidation products of lipids of biological importance (Lundberg, *Am. J. Clin. Nutrition, 6,* 60).

Laboratory data on heated oils could not be projected to predict whether an unsaturated oil will be damaged sufficiently during processing and commercial frying operations to be harmful (Perkins, *Food Technol., 14,* 508). Nevertheless these data justified the suspicion that the use of such oils containing polymeric materials may not be desirable from a nutritional point of view. Similar conclusions were reached after a short-term procedure for the detection of heat-induced changes was applied (Poling *et al., J. Nutrition, 72,* 109). The procedure involved the measurement of the biologically available energy of the fats fed and the variations in liver weights.

Several experiments were performed under laboratory conditions on chemically pure compounds. Ethyl oleate and methyl linoleate were heated in an oxygen current, the products were fractionated by distillation and urea compound formation and fed to weanling rats at an 8% level. Some of the fractions exerted harmful effects, as reflected in depressed growth, enlargement of organs, high water-intake, and, in several cases, death. The more toxic fractions led to low liver and serum cholesterol levels. One fraction of the oxidized oleate was essentially atoxic but was associated with low liver cholesterol values (Kaunitz *et al., Metabolism, 9,* 59). Cyclic esters separated from heated rapeseed oil, ethyl linoleate, β-eleostearate plus acrolein, and cuttle-fish oil were toxic to rats when given in the diet at a 10–20% level (Matsuo, *Eiyô to Shokuryô, 12,* 118, 206, 210; *Nippon Kagaku Zasshi, 81,* 469; *Yukagaku, 9,* 37). Also cyclohexene dicarboxylic acid was deleterious in these conditions.

Under conditions closer to the commercial heating of oils Andrews *et al.,* (*J. Nutrition, 70,* 199) found that the concentration of a toxic principle present in air-oxidized soybean oil corresponded closely to the peroxide concentration of the oil. Absorption studies indicated that, although the reduced products of the peroxides were absorbed, the peroxides themselves were destroyed in the intestine. The toxic action of the lipid peroxides seemed to take place at the level of the intestinal enzymes. Analogous reseults were reported (Glavind and Tryding, *Acta Physiol. Scand., 49,* 97) for lipoperoxides obtained at room temperature.

A more unsaturated oil like herring oil, deodorized by heating, was digested but not effectively utilized; this caused growth depression, modified secretion of body neutral fat, alterations in the levels of body lipids, and myocarditis in 60% of the animals (Raulin *et al., C.R., 248,* 1229).

Peanut (crude, refined, or hydrogenated), mustard, sesame, and coconut oil heated at 180°C. showed detrimental properties on the growth of rats, depending upon the length of the heating period. Oils heated for one hour had no effect, but those heated for 10 hours depressed growth (Esh *et al., Ann. Biochem. Exp. Med., 20,* 41). Similar differences were found between lard that had been heated to a high temperature for a short time and lard that had been repeatedly used for frying; the latter caused poorer growth in male rats. No significant differences were noted in female rats (Hashimoto *et al., Ann. Rep. Natl. Inst. Nutrition, Tokyo, 1959,* 90).

The high incidence of encephalomalacia in chickens that were fed diets low in vitamin E and containing heated and aerated unsaturated fats correlated with the linoleic acid but not with the linolenic acid content of the fats fed. Feeding of highly purified linoleic acid resulted in 100% incidence. When 20% cottonseed oil was the fat source, between 44 and 88 I.U./kg. of vitamin E or between 0.0125% and 0.025% of the antioxidant ethoxyquin were required for prevention of the disease (Machlin and Gordon, *Proc. Soc. Exptl. Biol. Med., 103,* 659). Experiences with nonheated cottonseed oil as dietary fat demonstrated that the presence of oxidation products of long-chain unsaturated fatty acids accelerated the development of cerebellar disorders. 12-Oxo-*cis*-9-octadecenoic was the most potent keto-acid tested, and in adequate conditions it could induce the disease at the end of seven days (Kokatnur *et al., Proc. Soc. Exptl. Biol. Med., 104,* 170). On the other hand, this compound, reduced linoleic acid hydroperoxide, or fresh methyl linoleate was innocuous when injected intravenously, as opposed to untreated linoleic acid hydroperoxide, which caused cerebellar disorders to appear 1–5 hrs. after the injection. Eight milligrams percent of vitamin E in the previous diet prevented the development of those disorders, but the absence of vitamin E in high-protein, high-fat diets increased their severity (Nishida *et al., Proc. Soc. Exptl. Biol. Med., 105,* 308).

Some fresh oils seemed to show a protective effect when fed together with altered fat. Medium-chain saturated triglycerides (MCT), cottonseed oil, corn oil, chicken fat, lard, coconut oil, butter, beef fat, long-chain saturated triglycerides (LCT), and ethyl esters derived from CSO were fed to weanling rats in combination with cottonseed oil aerated at 95° for 300 hrs. The oils with a low melting-point (MCT, cottonseed and corn oils) gave the most protection against weight loss. Those with high melting-points (beef fat and LCT) increased weight losses. Lard, chicken fat, butter, and coconut oil formed an intermediate group with respect to melting point and effect. The beneficial effect depended upon the presence of the fatty acids as triglycerides (Kaunitz *et al., J. Nutrition, 70,* 521).

The properties of fats subjected to a normal doughnut frying did not change with respect to their absorption properties if a high enough fat turn-over and a properly designed fryer were provided (Stern and Roth, *Cereal Sci. Today, 4,* 176). When four methods of prolonging the frying life of a hydrogenated vegetable fat used in deep frying were investigated, the results showed that, regardless of the method used, there was a significant correlation between the acid number of the fat and the acceptability of the fried products during frying. In general, there was little correlation between chemical and physical tests used to determine the percentage of fat in the fried product (Rust and Harrison, *Food Technol., 14,* 605).

Alterations taking place in soybean, rapeseed, and sesame oils heated in the presence of air (Toi and Ota, *Kasei-gaku Zasshi, 8,* 197, 269), and in fats used in the deep frying of fish meats and vegetables (Yamamoto *et al., Kasei-gaku Zasshi, 10,* 57) have been established. No differences were encountered between raw soybean oil and that used in sautéing or in roux preparation (Hashimoto, *Ann. Rep. Natl. Inst. Nutrition, Tokyo, 1958,* 86).

The presence of vitamin C retarded the oxidation of fried food materials. NaCl played the same role, but a high water content accelerated the oxidation process. When different oils were compared, cottonseed oil proved more stable than soybean oil, and sesame oil more than rapeseed oil (Bito and Yamamoto, *Yukagaku, 9,* 41).

Frying markedly reduced the nutritional utilization of carotene subsequently added to fried oils (Rangnekar, *Sci. Culture*

Calcutta, 24, 530). Important factors in this reduction were the vitamin E content of the oil and the degree of hydrogenation. Coconut oil, which is devoid of vitamin E, provided the best carotene utilization.

Thiobarbituric acid tests performed on triglycerides and proteolipids from cooked pork tissue demonstrated that the latter were responsible for the intensive rancidity induced by heat (Younathan and Watts, *Food Research, 25,* 538).

Patents have been issued for the preparation of a shortening containing thermally oxidized fat (Bremer and Hifman, *U.S. 2,865,760*); polymerization of unsaturated fatty acids (Myers and others, *U.S. 2,955,490*); and thermal degradation of ricinoleates (Stein, *U.S. 2,913,490*).

DETERIORATION DURING STORAGE. Kapur and Srivatan (*Food Sci., 8,* 257) reviewed the subject on storage and preservation of fatty materials, Rao (*Lit. Rev. Oils Fats, 1956,* 56) that of spoilage, and Smith (*Soybean Dig., 18* [12], 14) the relationship between the presence of peroxides, trace metals, and tocopherols and soybean oil stability.

The addition of 0.01% of vitamin A, B, C, or K to safflower oil retarded the formation of peroxides. The effect was greater when the oil was kept in the dark. The best effect was obtained with vitamin B_1 and with a mixture of A and B_1, both in the dark and in daylight (Plissov *et al., Trudy Odessk. Gosudarst Univ. im I.I. Mechnikova, Ser. Khim. Nauk., 146* [5], 97; *c.f., Trudy Odessk. Gosudarst Univ., 3,* 5). Spans prolonged the oxidation induction period of lard and olive oil, but Tweens, Myrj, and Brij had the opposite effect (Finholt and Hopp, *Medd. Norsk. Farm. Selskap., 20,* 23). In general, the addition of 2 mg.% of carotene increased the storage stability and improved the organoleptic properties of fats (Arisheva, *Izvest. Vysshikh. Ucheb. Zavendenii, Pishchevaya Tekhnol., 1959* [1], 104). The presence of Cu and Fe accelerated the oxygen absorption by cow ghee (Vachha *et al., Indian J. Dairy Sci., 11,* 91) and increased the peroxide value of fat containing baked products when stored at 18–20° but not at 35–37°C. (Täufel and Sergisko, *Ernährung-forschung, 3,* 100). Phospholipids protected dehydrated fatty emulsions stored at elevated temperatures, especially in the presence of protein. Hemoglobin accelerated catalytically the oxidation reaction (Bishov *et al., Food Research, 25,* 174).

The stability of lard in chilled doughs was not affected by baking after low-temperature storage, the percentage of fat in the pastry, or the storage temperature between −10° and 20°F. The addition of 0.01% of butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT) or a combination of both markedly extended the fat stability of the pastry. Peroxide values increased slightly during the first eight weeks of cold storage and thereafter remained at a low level (Weir *et al., Food Research, 25,* 120).

The influence of the quality of the original seeds (Rutkowski and Markus, *Fette, Seifen, und Anstrichmittel, 61,* 532), of the storage conditions (Harwalkar and Saletore, *Indian J. Appl. Chem., 21,* 61; Byonisz *et al., Roczniki Pánstwowego Zakładu Hig., 9,* 255), of the storage and frying conditions (Wurziger and Lindemann, *Fleischwirtschaft, 10,* 599), and of the refining process (Grau and Mirna, *ibid.,* 694; *Fette, Seifen, und Anstrichmittel, 60,* 533) on the stability properties of the final oil have been studied. The effects of storage on the lipids constituents of rye bread and crispbread and their nutritional properties have also been considered (Halden and Karp, *Brot u. Gebäck, 14,* 104).

Autoxidation and rancidification of essential fatty acids could be prevented by their incorporation into foods as urea inclusion compounds (Holman, *U.S. 2,875,060*).

Work done on fish oils indicated that their oxidation was accelerated by the presence of chloroprene. The action of this compound increased in the presence of Cu and decreased in the presence of carotenoids and vitamin A (Mkhitaryan, *Izvest. Akad. Nauk. Armyan. U.S.S.R. Khim Nauk., 11,* 109). Mackerel fat suffered hydrolysis and oxidation when stored at −8° to −10°C. but not at −40° to −50°C. Sterilization decomposed unstable peroxides to hydroxyacids and aldehydes (Mel'nikova and Khalina, *Izvest. Tikhookeans Nauch. Isledovatel Inst. Ribnogo Khoz. i Okeanog., 42,* 299; *c.f., Zhur. Khim., 1956,* Abstr. No. 31,180). The rate of phospholipid hydrolysis in cod stored at 20, 0, −14, −22, and −29°C. was compared (Olley and Lovern, *J. Sci. Food Agr., 11,* 644). All phospholipid breakdown in iced cod could be attributed to autolysis, with formation of free fatty acids and water-soluble phosphorus derivatives.

Severe loss of essential fatty acids was experienced when flour that was heavily treated with chlorine dioxide was stored for 12 days in air. Storage under nitrogen considerably reduced the loss (Daniels *et al., J. Sci. Food Agr., 11,* 658). The fatty acid composition of the oils in the flour, treated with more than 120 p.p.m. of chlorine dioxide, showed oxidative changes after several weeks of storage. The linoleic acid content of the oils decreased as a result of the treatment (Daniels *et al., J. Sci. Food Agr., 11,* 664).

Flavor alteration in milk and related products has been studied. It was found that an enzyme mechanism is involved in spontaneously produced oxidized flavor in milk, and a chemical one in induced oxidized flavor. The former is prevented by heat or an enzyme-inhibitor (p-chloromercuribenzoate). Copper-induced flavor is not prevented by the foregoing treatment but by a copper-chelating agent and by cysteine (Aurand *et al., J. Dairy Sci., 42,* 961). The site of the oxidative reactions that cause the oxidized flavor in milk is the surface of the fat globules, and the reactants are phospholipids and oxygen, catalyzed by a Cu-protein complex (Tarassuk and Koops, *J. Dairy Sci., 43,* 93).

The effect of deodorized milk fat on the flavor deterioration of dry whole milk was also investigated (Tharp, *Univ. Microfilms* [Ann Arbor, Mich.], L.C. Card Mic. 59-5132; *Dissertation Abstr., 20,* 1505.

Of 25 lipolytic micro-organisms tested, 17 produced a rancid flavor when inoculated into milk samples. Eighteen brought about increases in free fatty acids (Overcast and Skean, *J. Dairy Sci., 42,* 1479). A distinct fish oil or codliver oil flavor appeared after storage in commercial butterfat containing nordihydroguaiaretic acid (NDGA) and citric acid dissolved in propylene glycol (Pont *et al., J. Dairy Research, 27,* 205). Two fractions were obtained from a flavor concentrate from fishy butterfat: the one of oily flavor contained n-hexanal, n-heptanal, hex-2-enal, and heptan-2-one; the other of metallic flavor contained a single carbonyl compound present in a relatively small amount. These compounds, with the exception of heptan-2-one, were considered responsible for the characteristic fish oil flavor (Forss *et al., J. Dairy Research, 27,* 211). The same range of carbonyl compounds was found in tallowy and painty-flavored butterfat and in fishy-flavored washed cream (*ibid.,* 373). Nevertheless the carbonyl compounds present were not the same. Carbonyl compounds also developed in autoxidized milk fat (Lillard, *J. Dairy Sci., 43,* 585) and in oxidized soybean oil (Evans *et al., J. Am. Oil Chemists' Soc., 37,* 452). The extent of carbonyl development was considered related to the state of loss of flavor and oxidative stability. On the other hand, cheddar cheese flavor was more related to free fatty acids and H_2S concentrations than to NH_3, free amino acids, and acidic and neutral carbonyl compounds (Kristoffersen and Gould, *J. Dairy Sci., 43,* 1202). No accumulation of carbonyl compounds took place in rancid wheat flour; rather oxidation and hydrolytic processes and increase in acid value were verified (Vakar *et al., Biokhim. Zerna, Sbornik, 1958* [4], 206).

The problem of "color reversion," *i.e.*, darkening of refined vegetable oils during keeping, has been attacked. In deodorized soybean oil the speed of color reversion paralleled the initial velocity of autoxidation (Harada *et al., Nippon Nogeikagaku Kaishi, 34,* 545). Comparative studies with several oils demonstrated that the substrate for color reversion was not in the glyceride portion but probably in the unsaponifiable matter. Experiments on its physical and chemical properties led to the conclusion that it was tocopherol (*ibid.,* 551). Iron (Fe) accelerated the color reversion of soybean oil heated in its presence. Primary antioxidants (propyl gallate, BHA, NDGA, and isoamyl gallate) did not prevent color reversion, but synergists (tartaric and oxalic acids, NH_4 citrate, and H_3PO_4) caused strong inhibition (*ibid.,* 558).

Several methods for the determination of rancidity in fatty materials (Sedláček, *Nahrung, 2,* 655; Vargas Romero and Gutierrez Conzalez-Quijano, *Grasa y Aceites, 9,* 14; Ishikawa and Horikawa, *Jissen Joshi Daigaku Kiyo Shizen Kagaku, Kasei-gaku, 3,* 1; Kartha, *J. Sci. Ind. Research, 17B,* 135), as well as colorimetric iodometric procedures for the determination of the peroxide value of edible fats (Swoboda and Lea, *Chem. and Ind., 1958,* 1090) have been compared.

The Swift test was used as a source of information for the alteration of various peanut oils (Debruyne, *Rev. Franc. Corps Gras, 7,* 3); polarography for the study of autoxidized lard (Kuta and Quackenbush, *J. Am. Oil Chemists' Soc., 37,* 148); the dielectric and refractodensimetric constants for the evaluation of aging (Ludde, *Fette, Seifen, und Anstrichmittel, 61,* 1157); and spectrophotometry for the estimation of rancidity in linseed oil (Machulis, *Uchenye Zapiski Vil'nyus Univ. Ser. Mat. Fiz. i Khim. Nauk., 1956* [6], 83; *c.f., Zhur. Khim., 1958,* Abstr. No. 2715).

Methods for the determination of malonal aldehyde in rancid foods (Tarladgis *et al., J. Am. Oil Chemists' Soc., 37,* 44) and

for testing the oxidative deterioration of milk (Vleeschauwer *et al., Mededeel Landbouwhogeschool en Opzoekingsstations Staat Ghent, 23* [1], 121) have been described.

Analytical methods for the characterization of polymerized fats were compared by Täufell *et al.*, (*Deut. Lebens. Rundschau, 54,* 245), and a procedure for the evaluation of the heat treatment of soybean meal was reported (Pomeranz and Lindner, *J. Am. Oil Chemists' Soc., 37,* 124).

DETERIORATION BY IRRADIATION. A review article on the presence of carbonyl compounds in irradiated foods and their significance as inhibitors of lipid metabolism was presented before a symposium on "New Aspects of Nutrition Uncovered in Studies with Irradiated Foods" (Monty, *Federation Proc., 19,* 1034). Techniques used in ionizing radiations and radioisotopes in fats and their derivatives have been reviewed and discussed (Gatineau and Uzzan, *Rev. Franc. Corps Gras, 6,* 228).

The chemical changes induced in foods by irradiation have been considered with regard to their effects on the quality and on the nutritive value and possible toxicity (Ley and Hickman, *Research, London, 13,* 193). The peroxide number of fats increased on irradiation but rarely exceeded 100, which is a nontoxic level. Large losses of carotenoids and preformed vitamin A took place. Poor reproductive performances in rats fed on irradiated semi-synthetic diets might be attributed to vitamin E destruction.

When chicks were fed γ-irradiated soybean oil, corn oil, beef and pork fatty tissues, essentially equal nutritive values were found as compared to nontreated fats (Ritchey and Richardson, *Poultry Sci., 30,* 404).

The autoxidation of linseed oil under ultraviolet light probably took place in three steps: hydroperoxide formation, decomposition of the formed hydroperoxides and formation of secondary oxidation products, and decomposition of hydroperoxides and secondary oxidation products, formation of scission products, and polymerization (Helme *et al., Rev. Franc. Corps Gras, 6,* 609). Irradiation with ultraviolet light increased the *trans*-fatty acids content of lard and olive oil. A slight increase took place in beef fat and a decrease in hydrogenated peanut oil (Kühn and Lück, *Z. Lebensm.-Untersuch. und Forsch., 109,* 306). In similar studies performed on ethyl oleate, olive oil, and lard (Kühn and Lück, *Fette, Seifen, und Anstrichmittel, 61,* 680) peroxide, acid, iodine, and hydroxyl values as well as spectroscopic determination of *trans*-isomerization were employed to follow the course of the reaction.

Linoleic acid and a group of acids containing the vinyl group and branched chains were responsible for the fishy and candlelike flavor, respectively, of irradiated milk fat. These two flavors blended upon mixing to give the typical tallowy oxidation flavor of that product (Hoff *et al., J. Dairy Sci., 42,* 468).

Hexanal and heptanal were identified with the aid of paper chromatography and infrared spectroscopy as products of linseed oil autoxidation under light radiation (Kaufmann and Vogelmann, *Fette, Seifen, und Anstrichmittel, 61,* 561).

Mice have been exposed to multiple sublethal doses of total body x-irradiation, and several antioxidants have been added to the diet in order to determine their effect on the survival time (Ershoff and Steers, *Proc. Soc. Exptl. Biol. Med., 104,* 274). Propyl gallate, 2,5-di-*tert*-butyl-hydroquinone, and BHT at levels of 0.25 or 0.5% in the diet increased the survival over that on basal unsupplemented rations. Mixed tocopherols and Santoquin at the former level and DPPD at both levels had little, if any, protective effect.

LIPOXIDASE OXIDATION. A mechanism that accounted for the lipoxidase-catalyzed oxidation of linoleic acid was proposed. Sodium linoleate was oxidized with oxygen at 0°C. in the presence of lipoxidase, and the variation in the content of *cis*- and *trans*-isomers was estimated by infrared analysis (Khan, *Oléagineux, 15,* 759).

Oral administration of lipoperoxidase (1 mg./ml.) in drinking water for 40 days to mice caused profuse loss of hair with alopecia zones all over the body. Microscopic examination showed atrophied hair follicles. Topical application of a mixture of lipoperoxidase and linoleic acid caused alopecia and ulcerative lesions (Puig Muset *et al., Arzneimittel Forsch., 10,* 234). Administration of lipoxidase (250 mg./kg.), x-irradiation (5.5 r/min.) or a combination of both caused weight loss in rats; the effect was more severe with the combined treatment. Produced peroxides were considered responsible for these effects (Puig Muset *et al., Nature, 184,* Suppl. No. 9, 1506).

DETERIORATION MECHANISM. General discussions on the chemical mechanism involved in auto- and induced-oxidation have been published. Privett (*J. Am. Oil Chemists' Soc., 36,* 507) discussed oxidation and autoxidative polymerization. Lathlean (*Paint J. Australia-New Zealand, 3* [1], 12, 15) reviewed the mechanism of hydroperoxide formation and decomposition, also subsequent polymerization during thermal oxidation of oils. Also described were paper chromatography methods for studying scission products. Other subjects reviewed have been: mechanism of linoleic acid oxidation, especially in the presence of metal catalysts (Uri, *Proc. 4th Intern. Conf. Biochem. Problems of Lipids, Oxford, 1958,* pp. 30); natural hydroxy and epoxy acids (Gunstone, *ibid.,* 36); kinetics of oxidation (Knorre *et al., Izvest. Akad. Nauk. U.S.S.R., Otdel. Khim. Nauk., 1957,* 693; chemistry of thermally oxidized oils (Perkins, *Univ. Microfilms* [Ann Arbor, Mich.], L.C. Card Mic 58-5475; *Dissertations Abstr., 19,* 959); formation, reaction, and analysis of fat peroxides (Rieche, *Fette, Seifen, und Anstrichmittel, 60,* 637); oxidation of fats and oils (Ishii, *Kagaku, Kyoto, 11,* 662; Radzitzky, *Ind. Chim. Belge, 23,* 233).

After investigating the thermal oxidation of methyl laurate, stearate, and oleate, Ramathan (*J. Am. Oil Chemists' Soc., 36,* 344) proposed the following possible mechanism for the thermal oxidation of fatty acid esters: desaturation; hydroperoxide formation; instantaneous decomposition of the hydroperoxides into various hydroxy, carbonyl, and carboxyl compounds; decomposition of these compounds to release CO_2, CO, and H_2O; and polymerization through either carbon-carbon linkage or ester linkages.

The development of various types of intermediates and end-products of oxidation and the formation of free radicals on the initiation of the oxidation reactions were considered responsible for the different behavior of various fatty acids upon catalytic oxidation (Schuler *et al., Fette, Seifen, und Anstrichmittel, 62,* 389). The fatty acids tested were linoleic, α-eleostearic, arachidonic, clupanodonic, and erucic; and the catalysts were cortisone, and Cu and Co ions.

The initial stages of fatty acid oxidation have been studied by means of infrared spectroscopy (Mironova, *Primenenie Metodov Spektroskopii v. Prom. Prodovol'stven. Tovarov i Sel'sk. Khoz. Leningrad Gosudarst Univ. im A. A. Zhdanova, Leningrad, 1955,* 94).

Four hydroperoxide isomers have been demonstrated to be formed during autoxidation of methyl oleate. The hydroperoxide group was located in the α-position relative to the double bond (Privett and Nickell, *Fette, Seifen, und Anstrichmittel, 61,* 842). Carbonyl compounds present in the same autoxidized material were propionic, enanthic, and pelargonic aldehydes, azelaic half-aldehyde, and dioxystearic acid (Nonaka, *J. Tokyo Univ. Fisheries, 43,* 127). The same, plus acetic, capric, and crotonic aldehydes, were found in autoxidized linoleic acid. Carbonyl compounds also occurred in oxidized lard, probably as a consequence of breakdown of precursors by reaction conditions (Gaddis *et al., Food Research, 25,* 495).

The rate of autoxidation of methyl linoleate and linoleic acid emulsions in aqueous buffer solutions increased with an increase in the pH of the buffer. The rate decreased with increased concentrations of NaCl in the system. The activation energy for the monomolecular and bimolecular reactions of both the acid and ester was found to be independent of the pH value and NaCl concentration (Mabrouk and Dugan, *J. Am. Oil Chemists' Soc., 37,* 486). Methyl linoleate hydroperoxide acted as a catalyst in the same reaction (Kern *et al., Makromol. Chem., 32,* 184, 191; see also Dulog and Kern, *Deut. Farber-Z., 14,* 10). The catalytic action of sorbic acid in the oxidation of this and other methyl esters and fats were also investigated (Täufel *et al., Nahrung, 3,* 134).

Different polymeric and peroxidic materials formed during methyl linoleate and methyl linolenate oxidation have been examined (Khan, *J. Sci. Ind. Research, 1,* 12). n-Deca-2,4-dienal was shown to result from the deodorization and heat decomposition of the former (Patton *et al., J. Am. Oil Chemists' Soc., 36,* 280).

When autoxidized fatty methyl esters and fatty hydroperoxides from linoleate- and linolenate-rich oils were heated in the absence of oxygen, the principal reaction was dimerization with elimination of the hydroperoxide groups. Dimers were characterized as monounsaturated compounds with a structure different from a six-membered ring (Frankel *et al., ibid., 37,* 418).

Analysis of the products of catalytic autoxidation of ricinoleic acid showed that there was considerable dehydration and self-esterification, followed by oxidative rupture of the molecule and recombination of the scission products to form polymers. In the absence of oxygen, esterification occurred but not dehydration (Gulbekian and Skellon, *J. Appl. Chem., 9,* 224). When the same acid was heated or kept at room temperature

in an inert atmosphere, the main reaction was the formation of estolides. The autoreactions of ricinoleic acid were intramolecular and intermolecular dehydration (Hawke and Kohll, *J. S. African Chem. Inst., 12,* 1).

Heated in the absence of oxygen, linseed oil fatty acid ethyl esters formed predominantly dimers and trimers. The cleavage products that were originated were volatile and rich in double bonds and contained acids, alcohols, aldehydes, and ketones. The gaseous cleavage products consisted of C_2H_4 and CO_2 and small quantities of CO, H_2, CH_4, C_3H_8, propylene, butylene, and butadiene. The reaction affected mostly unsaturated fatty acids, especially linoleic. The newly formed substances contained only isolated double bonds. The *cis* olefins change into the *trans* forms during heating. Infrared data suggested the presence of a cyclic monomer. The addition of iron inhibited the loss of double bonds and promoted polymerization processes, the formation of the constituent parts of the pyrolysis fraction that cannot be distilled, the cleavage of CO_2, and thus ketone formation (Axt, *Nahrung, 3,* 18). The oxidation kinetics of linseed oil in the presence of various catalysts (Pb and Co stearate, also dispersed metals) was investigated (Lunina, *Nauch. Doklady Vysshei Shkoly, Khim. i Khim. Tekhnol., 1958* [2], 275). A possible mechanism for its SO_2 catalyzed oxidative polymerization was proposed (Boelhouwer *et al., C.R. 31e Congr. Intern. Chim. Ind., Liège, 1958;* publ. as *Ind. Chim. Belge,* Suppl. 2, 556, *1959*). Small differences were found in the X value (X = 1.58 B − A, where A = iodine number, and B = bromine number) of the same oil polymerized by heat alone and by heat and air (Taniewki and Bulczyńska *Przemysł. Chem., 35,* 324).

Autoxidation products of cetoleic acid formed during storage for 18 years were isolated by means of solvent extraction, urea-adduct separation, and inverse-phase chromatography. The presence of the *erythro* and *threo* forms of 11,12-dihydroxybehenic acid was demonstrated (Matsuura *et al., Nippon Kagaku Zasshi, 81,* 825).

ANTIOXIDANTS. Two review articles on the relationship between vitamin E and lipid metabolism (Alfin-Slater, *Am. J. Clin. Nutrition, 8,* 445; Horwitt, *ibid.,* 451) have been published. The antioxidant action of phenolic compounds on animal fats increased with increasing amounts until a maximum effect was obtained. After this, additional amounts lowered the antioxidant effect. The addition of phenolic antioxidants to vegetable fats had practically no effect. When the total amount of antioxidants (natural plus added) was taken into account, no difference in the behavior of animal and vegetable fats existed (Heimann and von Pezold, *Z. Lebensm.-Untersuch. und -Forsch., 108,* 317).

Several properties of antioxidants, synergists, and other agents in autoxidation reactions have been tested in a methyl linoleate-water model system. The efficiency of α- and β-naphthol, diphenylamine, phenyl α-naphthylamine, and DPPD as antioxidants was pH-dependent and decreased slowly with increasing alkalinity. The stabilization afforded at pH 9.2 made them suitable for fats products of slightly alkaline reaction. All the antioxidants that were studied showed higher apparent activities in the presence of aqueous phases of low pH values than in dry linoleate (Spetsig, *Arkiv. för Kemi, 15,* 1). Oxidized polyphenolic antioxidants could be restored to their original form by direct hydrogen transfer from suitable synergistic antioxidants. A possible mechanism was the addition of the synergistic antioxidant to the quinone, representing the spent primary antioxidant, to give a substituted polyphenol with primary as well as secondary antioxidant properties (*ibid.,* 5). The artificial two-phase system was also used to evaluate synergistic antioxidants in combination with both polyphenols and diarylamines. Among synergists which act by direct hydrogen transfer and autoxidize in aqueous solution, ascorbyl palmitate reinforced the action of hydroquinone. Ascorbic acid diminished the induction period, owing to coupled autoxidation with the hydroquinone leading to accelerated destruction of both antioxidants. Autoxidation was also responsible for the detrimental effect of thioglycolic acid and $NaHSO_3$, which were tested as representatives of synergists that react with the quinones, representing the spent polyphenolic antioxidants, to give new substituted polyphenols. Two nonautoxidizing synergists of this type, glycine and alanine, had only adverse effects on hydroquinone, BHA, and NDGA. Phosphoric, maleic, malonic, citric, tartaric, and malic acids, which are synergists with an unknown mode of action, also had small positive or negative effects on the same primary antioxidants. In contrast, they had strong synergistic effects on phenyl-α-naphtylamine and DPPD in slightly acidic solution, as had also malonic acid, ascorbic acid, and ascorbyl palmitate. Thioglycolic acid and $NaHSO_3$ had a detrimental influence. In alkaline solution only glycine and citric acid showed any influence on diarylamine antioxidants (*ibid.,* 23). When dilute solutions of proteins, carbohydrates, and surface-active agents were added to methyl linoleate that contained hydroquinone, BHA, or DPPD, the induction period was usually increased (*ibid.,* 31). Some ingredients of foods and commercial emulsions played an important role in maintaining the stability of the methyl linoleate, whether protected with an antioxidant or not. A pronounced detrimental effect was exerted by ingredients containing autoxidizable substances, such as essential oils and phosphatides, which had already formed peroxides during storage. An appreciable catalytic activity was also shown by ingredients containing traces of heavy metals in the form of heme compounds or contaminants incorporated during handling and storage (*ibid.,* 39).

Praseodymium gallate, dodecyl gallate, BHA, BHT, *tert*-butylquinol, 2,5-*tert*-butylquinol, 2,2'-methylenebis (*tert*-butylquinol), and 4,4'-methylenebis (2,6-di-*tert*-butylphenol) have been compared at 37° and 50°C. in purified, distilled methyl esters of cottonseed, linseed, codliver oil fatty acids to which a small amount of pre-oxidized ester had been added to initiate oxidation. The relative activity of the antioxidants varied considerably with the fatty acid composition of the substrate and with the temperature and level of oxidation at which the measurements were made (Lea, *J. Sci. Food Agr., 11,* 143). Comparison of the antioxidant activities of tocopherols in similar systems at 37° and 60°C. indicate the following: in the linoleate (cottonseed) system the α- and δ-compounds were the most effective and the α-, β-, and ε-compounds the least effective in extending the induction period. In the polyunsaturated (linseed and codliver oil) systems the γ-maintained its position, but the δ- was at the bottom of the series and the α- and β-compounds near the top. The relationship between antioxidant activity and structure in tocopherols was discussed (Lea, *J. Sci. Food Agr., 11,* 212).

When the destruction of tocopherols during the autoxidation of fats was investigated, it was found that cottonseed oil and lard caused higher losses of tocopherol than highly unsaturated vegetable oils, probably because the hydroperoxides formed in the highly unsaturated vegetable oil decomposed rapidly before they reacted with tocopherol. Antioxidants that react more rapidly than tocopherol with polyunsaturated fat hydroperoxides are needed to stabilize highly unsaturated vegetable oils (Frankel *et al., J. Agr. Food Chem., 7,* 438; Khan, *Pakistan J. Biol. Agr. Sci., 2,* 24). The stability of carotene and vitamin E solutions in fats decreased with the increasing peroxide number (Savinov and Mikhailovnina, *Vitaminy, Akad. Nauk. Ukr. U.S.S.R. Inst. Biokhim., 3,* 66). The lower the concentration of carotene and vitamin A, the longer the induction periods of their autoxidation reactions. The induction periods could be shortened by the addition of fat; the more unsaturated ones have the more marked pro-oxidant effect. DPPD was the most effective of several antioxidants tested (Budowski and Bondi, *Arch. Biochem. Biophys., 89,* 66).

The most effective lard stabilizers were NDGA, α-tocopherol, and a mixture of praseodymium gallate, propylene glycol, and citric acid (Zangucchi and Delindati, *Ind. Conserve, 33,* 11). Also effective were tannin, isobutyl gallate, vitamin C, and mixtures of vitamins C and E. Traces of metals catalyzed the oxidation of fats and the decomposition of vitamin E (Prszniak, *Prace Inst. Przemysłu Miesnego, 1,* 163). The oxidation of codliver oil could be prevented by the addition of 0.05% ethyl gallate, BHT, BHA, and octyl gallate (Davydova and Treshcheva, *Rybnoe Khozy., 34* [10], 70). Gallates were also suitable antioxidants in hog fat (Pokorný, *Sbornik Ceskoslov. Akad. Zeniěděl Ved., Vet. Med., 4,* 235), salt herring (Marcusse, *Fette, Seifen, und Anstrichmittel, 60,* 482), and peanut oil (Roy and Guha, *Invest. Composition Nutritive Value, Vanaspati, 2,* 1), but neither they nor BHA prevented tocopherol destruction. Gallates seemed to be toxic beyond the level of 0.01% (van Esch, *Voeding, 16,* 683).

The most effective antioxidants in ethyl oleate were NDGA, propyl gallate, and dodecyl gallate; moderately effective was BHA (Bottoni and Fogliani, *Farmaco, Ed. Prat., 11,* 446).

Metabolic studies demonstrated that, given at low doses to rats, BHA and its accompanying isomers (2- and 3-*tert*-butyl-4-hydroxyanisole) were absorbed, readily metabolized, and excreted in amounts ranging from 82 to 100% of that ingested (Astill *et al., Biochem. J., 75,* 543). Normal testing doses of BHA produced no apparent changes in growth, food consumption, reproduction, mortality, organ weights, and post-mortem pathology of rats. On the other hand, normal doses of BHT significantly reduced the initial growth rate and mature weight of male but not female rats. BHT also produced a significant increase in liver weight and loss of hair. Neither affected the reproductive cycle or the histology of the spleen, kidneys, liver,

testes, or skin (Brown, *Australian J. Exp. Biol. Med. Sci., 37,* 533). BHA could also be ingested by dogs without harm, as indicated by 15-month feeding experiments in which the antioxidant was given at a level at least 220 times its maximum allowable level in lard (Wilder *et al., J. Agr. Food Chem., 8,* 504). DPPD accumulated mainly in the liver and body fat of hens fed different levels of it. The maximum level in eggs and organs was reached after two weeks of oral administration, and practically all stored DPPD had disappeared two weeks after its withdrawal from the diet (Ascarelli *et al., J. Sci. Food Agr., 11,* 509).

Thirteen antioxidants have been evaluated at various dose levels for effectiveness in protecting rats against necrotic liver degeneration. Di-*tert*-amylhydroquinone, Santoquin, and DPPD were effective, but ascorbic acid and methylene blue afforded no more than 30–40% protection (Schwarz, *Proc. Soc. Exptl. Biol. Med., 99,* 20).

The microsome fraction from rat livers deteriorated by lipid peroxidation at a rate of 0.1 ml. of oxygen reacted/mg. nitrogen/hr. at 37°C. Added tocopherol inhibited the reaction. Cytochrome b_5 and other hemochromes that were present appeared to act as catalysts (Tappel and Zalkin, *Nature, 185,* 35). Peroxidation in various tissue homogenates was affected by the presence of vitamin E in the diet. When dietary vitamin E was adequate, only the brain formed peroxides. When depleted, all tissues rapidly formed lipid peroxides (Bieri and Anderson, *Arch. Biochem. Biophys., 90,* 105). Lipid peroxidation was also found in mitochondria isolated from vitamin E-deficient rabbit livers (Tappel and Zalkin, *Arch. Biochem. Biophys., 88,* 113), but the presence of vitamin E or selenite in the diet inhibited the reaction (Zalkin *et al., Arch. Biochem. Biophys., 91,* 117). From these and other experiments it was concluded that vitamin E functions solely to stabilize cellular unsaturated lipids against oxidative deterioration, thus maintaining structural and functional integrity at the subcellular level. It was also suggested that dietary selenite functions by forming lipid antioxidants in the animal. Selenite and other antioxidants also had an influence on exudative diathesis in chicks (Sondergaard *et al., Experientia, 16,* 554).

Inhibitors of free radical reactions, like propyl gallate, have been found to inhibit mitosis in human cancer cells (Lipchina *et al., Doklady Biol. Sci. Sect., 131,* 204).

Several compounds have been tested for antioxidant activity, namely, ascorbyl palmitate (Cerutti, *Olearia, 10,* 39, 130); alkaline whey, extract of oats, quercetin, dehydroquercetin, and a combination of quercetin and citric acid (Masĕk and Holá, *Olearia, 10,* 100); tioxans (Paquot and Mercier, *Oléagineux, 14,* 591); lecithin and its products of hydrolysis (Urakami and Kameyama, *Bull. Chem. Soc. Japan, 33,* 29); and two alkyl phenylenediamines (Draper and Johnson, *J. Agr. Food Chem., 6,* 920). Chlorophyll acted as a pro-oxidant in the daylight but was ineffective in the dark. In combination with antioxidants it acted as a synergist in the dark (Täufel *et al., Fette, Seifen, und Anstrichmittel, 61,* 1225). Antioxidants for pitted fruit oils were also described (Shevlyagina *et al., Trudy Vsesoyuz Nauch. Issledovatel Inst. Sintet. i Natural Dushistykh Veshchestv., 1958* [4], 119).

Peredi (*Élelmezési Ipar, 12,* 97) reviewed the antioxidants used in industry.

The addition of antioxidants to the paper wrapper of bakery products inhibited rancidity. Trace metals had the opposite effect (de la Barbolla y Alcala and Vargas Romero, *Grasas y Aceites, 9,* 55).

Patents have been issued for the following antioxidants: dialkyl-p-phenylenediamine (Chenicek, *U.S. 2,856,293*); polyamines (Rachinskiĭ *et al., U.S.S.R., 118,935*); unsaponifiable residue of natural fats (Stein and others, *Ger. 936,646*); 1,4-dihydroxy-5,8-ethane-hydronaphtalene (Thompson, *U.S. 2,903,368*); antioxidants from rootlets (Baker and Dockstader, *U.S. 2,925,345*). See also Dugan and Kraybill *(U.S. 2,950,975)*. A solvent for fat and oil antioxidants was also patented (Knowles and Pridgen, *U.S. 2,944,908*).

Methods have been described for the determination of gallates in edible fats (Cassidy and Fisher, *Analyst, 85,* 295), and DPPD in biological materials (Budowski *et al., J. Sci. Food Agr., 11,* 503; Csallany and Draper, *Proc. Soc. Exptl. Biol. Med., 104,* 739). Also a method for the evaluation of antioxidants, based on vitamin A protection, has been developed (Morgan and Greb, *Poultry Sci., 38,* 1563).

Paper chromatography permitted the identification of *tert*-BHA and *tert*-BHT, and octyl, decyl, and dodecyl gallate (ter Heide, *Fette, Seifen, und Anstrichmittel, 60,* 360).

Production Processes

EXTRACTION. New extraction apparatus reported during the year include a stationary basket extractor (R. P. Hutchins, *J. Amer. Oil Chem. Soc. 37,* 674); a continuous bucket chain extractor (W. Depner, *Ger. 890, 940*); a countercurrent flow apparatus (H. Stoltenberg *Ger. 1,004,310*); and a high pressure extractor (L. A. Yutkin and L. I. Gol'tsova, *U.S.S.R. 120,* 884). Hexane was recommended for filtration-extraction process to Jojoba seed (J. J. Spadaro, *et al., J. Amer. Oil Chem. Soc. 37,* 121). High grade oil was obtained by direct solvent-extraction of castor beans (E. L. D'Aquin, *et al. J. Amer. Oil Chem. Soc. 37,* 93). Sunflower seed and soybeans were processed by a single stage pressing and uninterrupted extraction (M. A. Minasyan and E. Z. Plyushkin, *Masloboino Zhirovaya Prom. 1954*(*5*), 29). Lipid fractions with increased iodine number and free of phosphatides were obtained by extracting the crude products at −30° to −60° with hexane, ethyl chloride, or liquefied propane containing 30% butane (H. P. Kaufmann, *Ger. 1,000,255*). The prepressed oil cake was ground to a flour and then sprayed with atomized water until it contained 7-13% before extraction (A. I. Skipin, *U.S.S.R. 118,933*).

The quality of vegetable marrow oil was improved and the extraction facilitated by the use of higher temperature of roasting (G. Gorbach and P. Stranger-Johannessen, *Fette, Seifen und Anstrichmittel 61,* 1001). The mechanism of extraction of soybean by different solvent was reported (D. F. Othmer and W. A. Jactinem, *Rev. Franc Corps Gras 6,* 408). The effect of temperature on critical miscella concentration was studied (M. E. Ginn *et al., J. Amer. Oil Chem. Soc. 37,* 183).

REFINING. A number of new or improved refining processes were reported. The rotating contactor offers a most versatile refining plant (H. R. Kaiser and C. M. Doyle, *J. Amer. Oil Chem. Soc. 37,* 4). Urea was used to remove fatty acids and mono-acid diglycerides formed during storage of raw oils (T. N. Metha and M. S. Murty, *Grasas y Aceites, 9,* 316). Fats were stabilized with oxygen acceptors before refining (A. Nichterlein, *Ger. 1,001,013*). The use of soda ash (B. H. Thurman, *U. S. 2,917,525*) controlled amount of alkali (Noblee & Thoerl G. m. b. H., *Brit., 804,022*) and aqueous solution of ammonia (B. Clayton, *U. S. 2,939,790*) for refining were patented.

Various solvents were used in the refining process. Neutralization was made in a lipophilic and hydrophilic solvent (C. Vaccarino and G. Vaccarino, *Ital. 550,940*). Ethanol was used as a selective solvent of refining (M. Jaky and J. Peredi, *Fette, Seifen, und Anstrichmittel 61,* 651). Refining was done in a solvent miscible in all proportions with both water and the oil (C. Vaccarino and G. Vaccarino, *U. S. 2,944,072*). Solvent refining of oils and fats was reviewed (A. D. Shitole, *Bombay Technologists 7,* 51).

Purification of fats was accomplished by the use of ion-exchange resins (A. O. G'omez and A. S. Cartaya, *Grassas y Aceites 9, 296*) and in miscella (W. Depner, *Ger. 1,015,560*). Theories for the reactions which contribute to the overall losses in the processing of oils (M. Naudet, *Rev. fermentations et inds. aliment. 14, 51*) and for the washing process (W. King *et al., J. Amer. Oil Chem. Soc. 37,* 30) were reported. A new laboratory apparatus for the refining of oils and fats was described (G. B. Martinenghi, *Olearia 13,* 114). The proceedings of the European Conference on oils including degumming, refining, decoloration, and deodorization of oils were published (*Spec. Number, Rev. Franc. Corp. Gras., 1959*). Some recent developments in the refining of fatty oils were reviewed (M. Mattikow, *J. Amer. Oil. Chem. Soc. 37,* 211).

Refining of crude coconut oil was studied in a pressure system with a continuous process (F. E. Sullivan, *J. Amer. Oil Chem. Soc. 37,* 195). Refining of olive oil was studied for the removal of parathion and its residues (M. E. Alessandrini and F. Palazzo, *U. S. 2,937,193*). Refining of safflowerseed oil was studied for the best over-all results (E. V. A. Chari and B. S. Kulkarni, *J. Proc. Tech. Assoc. India, Kanpur 13,* Pt. 1-2, 1). Peanut oil was neutralized wih a two-step process (M. Naudet, *et. al., Grasas y aceites 9,* 292). The effect of the methods of degumming on neutral triglyceride and phosphatide yields was studied (R. Carlotti, *Rev. Franc. Corp. Gras., 6,* 341). The refining of cottonseed for use in Vanaspati Manufacture was reported (C. R. Das and S. Kumar, *Papers Symposium Cottonseed and By-products, Hyderabad, India 1958,* Sect. IV, 30; P. V. Krishna, *Bombay Technologists 8,* 67).

BLEACHING. A highly viscous solution of an alkali metal pyrophosphate in hydrogen peroxide (K. Kühn, *Ger. 1,012,014*), and chromium trioxide plus sulfuric acid in the presence of foam destroyers (R. Schirmer *et al., Ger. 1,007,918*) were used for the bleaching of fats and oils. Nigerian Palm oil of excellent bleachability can be obtained if sufficient care is taken in its processing (G. R. Ames, *et al., J. Sci. Food Agr. 11,* 194). Gossypol can be removed from cottonseed oil by the use of anthranilic or some aromatic acid or amine to form oil insoluble

derivatives (V. P. Rzhekhin and A. B. Petushina, *U. S. S. R. 119*, 642). Color of cottonseed oil was improved by the use of hydrogen peroxide (F. A. Norris, *U. S. 2,915,538*). Crude tall oil was decolorized by heating with 0.3% zinc chloride followed by distillation (T. Waida and M. Terauchi, *Kogyo Kagaku Zasshi, 62*, 1734).

Periodic clarification of bleached oily suspension was accomplished by a centrifugal apparatus (W. Buddeberg and H. V. Posern, *Ger. 1,007,919*). An inverse relationship was demonstrated between the apparent bulk density of a bleaching clay and its oil retention (A. D. Rich, *J. Amer. Oil Chem. Soc. 37*, 305).

DEODORIZATION, WINTERIZATION, AND FRACTIONATION. The energy consumption for deodorizing oils in a pilot plant was measured (F. R. Ayerbes, *Grasas y Aceites, 9*, 318). Acid fats were partially esterified and the residual unreacted acids were 5–10% steam distilled without transfer to another apparatus (C. Berti, *Ital. 559,292*). The disagreeable odor of Neem oil was removed by heating at 200° for approximately half an hour and then boiling with alkaline potassium permanganate (O. Prakhash *et al., J. Oil Technologists Assoc. India, Kanpur 13*, Pt. 1–2, 14).

Cottonseed oil was winterized by mixing the oil with 15% or less of a low molecular weight ketone, acetate, or ether, rapidly chilling the solution to 28 - 39° F., holding at that temperature range for not more than 8 hours, and finally separating the crystallized from the liquid fraction (L. J. Rubin, *U. S. 2,910,363*).

Fractionation of coconut oil and hydrogenated coconut oil was accomplished by molecular fractional distillation (N. Naudet *et al., Bull. Soc. Chim. France, 1959*, 718). Fractionation of dehydrated castor oil was studied with isopropyl alcohol as the solvent (V. R. Ambekar and K. K. Dole, *Indian J. Appl. Chem. 21*, 193).

HARDENING. Catalyst for hydrogenation was studied in various aspects. Patents on the hydrogenation catalysts were reviewed (Shizuo Takumi, *Yukagaka 8*, 262). Formula for estimation of catalyst activity was reported (I. Kaganowicz, *Prace Inst. i Lab. Badawczych Przemystu Spozywcaego 8* (4), 33). The selectivity in the hydrogenation of oleic-linoleic acid oils with commercial nickel catalysts was studied (K. Nielsen, *et al. J. Amer. Oil Chem. Soc. 37*, 271). The process on regeneration of nickel and copper from used catalysts was simplified by the use of hydrogen peroxide (A. G. Koblyanskii and N. Kh. Kameneva, *Trudy Krasnodar. Inst. Pish Chevoi Prom. 1956, No. 13*, 3). Studies on copper-nickel alloy catalysts were reviewed (T. Takeuchi, *Yukagaku 8*, 231). The development of the theory of catalytic hydrogenation (T. Keii) and hardening of fats and oils with metallic sodium (M. Fukushima, *Yukagaku 8*, 240) were also reviewed. A new process for treating oils with hydrogen consists of the following steps: moving oil through a catalyst containing filter into a reaction chamber while dislodging the catalyst from the filter; introducing hydrogen under pressure into the reaction chamber through the filter; and finally releasing the pressure of the reacting gas to move the finished product back through the filter (J. E. Thompson, *U. S. 2,932,658*).

The rate of hydrogenation of free fatty acid was compared with that of ester (T. Tsuchiya, *Uykagaku 9*, 83). The hydrogenation of fats at low temperature was studied (B. N. Tyntyunnikov and I. I. Novitskaya, *Trudy Khai Kov Politeka, Inst. in V. I. Lenina., Ser Khim-Tekhnol. 13* (4), 97). Marine oils were hydrogenated with palladium catalyst (M. Zajcew, *U. S. 2,948,742*). Cottonseed oil was hydrogenated with nickel-copper catalyst (A. L. Markman and A. A. Abdurakhimov, *Uzbek, Khim, Thur. Akad, Nauk, Uzbek. U.S.S.R., 1958*, No. 4, 45). Methyl linolenate was hydrogenated at 140° C. with 0.5% Nickel catalyst and the products fractionated and characterized (L. R. Scholfield, *et al., J. Amer. Oil Chem. Soc. 37*, 579).

Palladium catalyst was used for the preparation of shortening stocks in the laboratory (M. Zajcew, *J. Amer. Oil Chem. Soc. 37*, 11) and in the pilot plant (M. Zajcew, *J. Amer. Oil Chem. Soc. 37*, 130). Methyl oleate (E. R. Cousins and R. O. Feuge, *J. Amer. Oil Chem. Soc., 37*, 435) and cottonseed oil (L. F. Albright *et al., J. Amer. Oil Chem. Soc. 37*, 315) were hydrogenated in solvent.

INTERESTERIFICATION. Acid chlorides were used as a means of obtaining a reproducible and predetermined degree of interesterification or glycerolysis of fats (K. Taufel, *Fette, Seifen, und Anstrichmittel 62*, 591). The changes in drop point of a fat mixture during a single phase interesterification are related to the activity of the catalysts used (H. P. Kaufmann and B. Grothues, *Fette, Seifen, und Anstrichmittel 62, 489*). Coconut oil was reacted with capric-caprylic triglyceride in the presence of sodium methylate as a catalyst to produce excellent margarine oils and medicinal oils (G. Barsky and V. K. Babayan, *U. S. 2,914,546*). The interesterification of fats promoted the growth of smaller crystal. The interesterified fat showed changed crystallization habits as compared to the original mixtures (E. Becker, *Fette, Seifen, und Anstrichmittel 61*, 1040). Soybean oil and interesterified soybean oil were extracted at 0° with nitromethane. The extract phase and the raffinate phase showed no difference in the distribution of linoleic and linolenic acids (C. Kimura *et al., Kogyo Kagaku Zasshi, 62*, 1381).

PARTIAL ESTERS AND SPLITTING. High yields of monoglycerides were obtained by cooling the reaction mixture rapidly by adding glycerol at the end of the reaction and distilling glycerol off without adding heat (G. E. Woods, *U. S. 2,905,540*). Laboratory methods for the preparation of monoglycerides from marine oils and for the preparation of acetylated alpha-monoglycerides were described (E. H. Gruger, Jr., *J. Amer. Oil Chem. Soc. 37*, 214). The yield of monoglyceride was shown not to depend upon the fatty acid composition of the oil but on the solubility of the glycerol in oil (R. B. R. Choudhury, *J. Amer. Oil Chem. Soc. 37*, 483). The equilibrium constant favoring esterification of primary hydroxyl over secondary of glycerol is ca. 2.3 at 200° C. and between 6 and 10 at room temperature. Therefore, monoglycerides as customarily prepared are not at equilibrium at room temperature and undergo intramolecular migration of acyl group from beta to alpha positions (I. D. Brandner and R. L. Birkmeier, *J. Amer. Oil Chem. Soc. 37*, 390). The reaction of glyceryl esters with fatty acids has been demonstrated to form an equilibrium mixture containing the 1, 2-isomers as well as the 1, 3-isomers. This procedure offers a method of synthesizing simple or mixed 1, 2-diglycerides (S. B. Radlove, *et al., J. Amer. Oil Chem. Soc., 37*, 570). An unstable form of a solid polymorphic fatty monoglyceride can be transformed into the stable polymorphic form by mechanical working to bring about a solid-solid transition (J. G. Baldinus, *U. S. 2,910,491*).

A glycol monoester of a fatty acid was prepared by the reaction of a carboxylic acid and an alkalene oxide in the presence of an ammonium halide catalyst (J. D. Malkemus, *U. S. 2,910,490*). Alkoxyglycerolesters were isolated from natural source lipid-containing starter materials and from triglycerides by subjecting the starting material to the hydrolytic triglyceride-splitting action of a lipolytic enzyme inert to alkoxyglycerolesters (J. K. O. H. Holmberg and A. G. Sellman, *U. S. 2,916,419*).

A water-in-oil emulsion obtained from splitting of soapstock was converted into an oil-in-water emulsion which could be separated in a centrifuge by exposing to rapidly rotating beating blades (H. Gates, *Dutch 90,078*).

VEGETABLE OILS AND ANIMAL FATS. The processing of rice-bran oil was reviewed (K. M. Kamath, *Bombay Technologists 9*, 12). 2-Propanol is the most suitable solvent for the rice-bran oil extraction (S. I. El Hinnawy, *A'in Shams Univ. Fac. Agr. Bull. No. 1, 36 pp., 1958*). A process for the refining of rice-bran oil was patented (G. B. Martinenghi, *Ital. 554,502*). Current problems in the production of olive oil were discussed (M. Th. Francois, *Bull. Soc. Pharm. Nancy 38*, 5). The proceedings of a symposium on cottonseed and by-products were published in 13 papers (*Papers Symposium Cottonseed and By-products, Hyderabad, India, 1958*). Detoxification and deallergenization of castor beans were studied (H. K. Gardner, *et al., J. Amer. Oil Chem. Soc. 37*, 142).

Animal fat-containing proteinaceous tissue was finely ground and then heated at reduced pressure at a temperature sufficient to melt at least a portion of the fat but below the coagulation temperature of the protein. The water was substantially removed as vapor and the liquid fat could then be easily separated from the solids in the resultant slurry (C. Greenfield, *U. S. 2,911,421*).

BY-PRODUCTS. Wax was recovered from the waste material left over after separating the fiber from the leaves of the indigenous plants of the Agave family, commonly known as sisal. This wax could be used to replace carnauba wax (V. V. Mhaskar and S. M. Shah, *Res. Ind., New Delhi, 4*, 219). A process for recovery of wool wax was patented (L. F. Evans *et al., Ger. 962,630*). The treatment of the first aqueous vegetable oil refinery waste containing a suspended floc was improved (P. Bradford, *U. S. 2,925,383*).

A dry pigmentation agent was produced by drying a soybean oil soapstock (N. H. Wittee and E. Sipos, *U. S. 2,929,715*). Sebacic acid 2-octanol, and 2-octanone were produced by reacting ricinoleic stock with caustic alkali in the presence of sodium or potassium nitrate (Y. Bourgeois, *U. S. 2,935,530*). A process for the direct production of methyl esters of cotton-

seed, soybean, and corn oil directly from the respective acidulated soapstocks of these oils was described (K. M. Decossas, *et al., J. Amer. Oil Chem. Soc. 37,* 574). Hydrogenated lecithin was prepared by hydrogenation with palladium or platinum catalyst in the presence of chlorinated solvent (R. D. Cole, *U. S. 2,907,777*).

Nutrition and Biochemistry

FAT DIGESTION AND ABSORPTION. The concurrent measurement of intestinal transport and fat absorption in the unanesthesized rat has been described (Aberdeen and Simmonds, *Quart. J. Exptl. Biol. 45,* 265). A newer approach to the study of fat absorption involves the feeding to rats of a tracer amount of radioactive fat mixed with a stock ration to form a "meal" (Turner *et al., Fed. Proc. 19,* 876). *In vitro* studies utilizing everted sacs of small intestine of the hamster indicate that the site of absorption of fatty acids is the upper portion of the small intestine (Johnson, *Proc. Soc. Exptl. Biol. Med. 100,* 668; *Nutr. Rev. 18,* 319) while results from the *in vitro* incubation of palmitic acid $-C^{14}$ or $P^{32}O_4^{=}$ with intestinal segments from hamsters indicate that the phosphatidic acids are intermediates in the absorption of fatty acids from the intestine (Johnston and Bearden, *Arch. Biochem. 90,* 57). Determinations of the radioactivity of glyceride-glycerol and fatty acids after the feeding of a synthetic glycerol and palmitic acid labeled triglyceride indicate that only about half of the glyceride is completely hydrolyzed during digestion and absorption (Reiser *et al., J. Lipid Res., 1,* 241).

Other studies of lipid absorption and transport have utilized I^{131}-labeled fats. Thus, serum lipoprotein metabolism in man has been studied with I^{131}-labeled triolein (Kruger, *Am. J. Clin. Nutr. 8,* 44), and the absorption of I^{131}-labeled fat has been studied in the dog. (Michaelson, *Am. J. Vet. Res. 21,* 364). Analysis of a commercial sample of I^{131}-triolein revealed the presence of appreciable amounts of methyl esters, di- and monoglycerides of oleic acid; when I^{131}-triolein was fed to humans, 90% of the radioactivity of the serum lipids was found in the triglyceride fraction while the remainder was found in the diglyceride and free fatty acid fraction (Lakshminarayana, *Arch. Biochem. Biophys. 88,* 318).

A third area of investigation in the study of lipid transport was focused on lipoproteins. The proportion of triglyceride, phospholipid, and sterol esters and, in some cases, the fatty acid composition of these fractions were determined in α-lipoproteins and two classes of β-lipoproteins (Green *et al., J. Biol. Chem. 235,* 2884). The influence of dietary fats on blood lipids and lipoproteins was studied (Salamin *et al., Nutritio et Dieta 2,* 132), and it was also found that in the rat, the type of dietary protein markedly altered the fractions of lipoproteins (Erwin, *Proc. Soc. Exp. Biol. Med. 103,* 396). The recombining capacity towards lipids of the protein moiety of human serum α-lipoprotein was studied (Scanu and Hughes, *J. Biol. Chem. 235,* 2876). The fatty acid spectrum of chylomicrons of the serum and chyle of rats and from the serum of man following the digestion of different dietary oils was markedly similar to the fatty acid spectrum of these oils (Bragdon and Karmen, *J. Lipid Res. 1,* 167).

The role of mass cells in fat-transport (Jennings *et al., Quart. J. Exptl. Physiol. 45,* 298) and nutrition-endocrine interrelationships in the control of fat transport (Olson and Vester, *Physiol. Rev. 40,* 677) have been reviewed. Di Luzio (*J. Am. Oil Chem. Soc., 37,* 163) reported that both hepatic parenchymal and Kupffer cells participate in the removal of chylomicra with the parenchymal cells having the biggest role; the Kupffer cells probably play a metabolic or excretory function in cholesterol metabolism.

In lymph fistula rats, Swell *et al.* (*Proc. Soc Exptl. Biol. Med. 103,* 263) found that free cholesterol was absorbed to a greater extent than esterified cholesterol; also, the concept that preliminary hydrolysis is obligatory for absorption of cholesterol esters and that only free cholesterol can enter the intestinal wall is supported.

Fatty Acid Oxidation and Utilization. Interrelationships between insulin and the metabolism of fatty acids have been reviewed (Folley and Greenbaum, *Brit. Med. Bull. 16,* 228). The factors influencing the uptake and oxidation of fatty acids by various tissues have also been investigated. Thus, Spitzer and McElroy (*Am. J. Physiol. 199,* 876) studied hormonal effects on the uptake of free fatty acids by the liver; Issekutz and Spitzer (*Proc. Soc. Exp. Biol. Med. 105,* 21) studied the uptake of free fatty acids by skeletal muscle during stimulation, and Fritz and Kaplan (*Am. J. Physiol. 198,* 39) studied the effect of glucose on fatty acid oxidation in diaphragm of normal, alloxan-diabetic and starved rats. Michajlik and Bragdon (*J. Lipid Res. 1,* 164) suggest that heparin causes an obligatory oxidation of fat, presumably through the release of free fatty acid from triglyceride.

Fatty Acid Synthesis. Further studies on the mechanism of fatty acid synthesis indicate that malonyl CoA can serve as a starting point for the synthesis of long chain fatty acids in animal tissues and that the rate of formation of malonyl CoA from acetyl CoA appears to be the rate limiting step in fatty acid synthesis from acetyl CoA (Ganguly, *Biochem. et Biophys. Acta 40,* 110). Observations on the inhibition of fatty acid synthesis in a purified enzyme system by avidin and the prevention of this inhibition by the addition of free biotin, indicates that a biotin-containing enzyme participates directly in fatty acid biosynthesis (Wakil and Gibson, *Biochem. et Biophys. Acta 41,* 122).

In the synthesis of fatty acid in liver homogenates of normal and diabetic rats, growth factor requirements have been compared (Abraham *et al., J. Biol. Chem. 235,* 2551), and the effects of the addition of microsomes and oxidation of substrates have been studied (Mattes *et al., J. Biol. Chem. 235,* 2560).

Fatty acid synthesis has also been studied in brain (Brady, *J. Biol. Chem. 235,* 3099).

The *in vivo* conversion of glucose to fatty acids in mice (Paunier and Favarger, *Helv. Chem. Acta 43,* 118) and the conversion of propionate to dicarboxylic acids (Favarger and Gerlack, *Helv. Physiol. et Pharmacol. Acta 18,* 328) have been reported.

Polyunsaturated Fatty Acids. Factors influencing the requirement for polyunsaturated fatty acids (Holman, *Am. J. Clin. Nutr. 8,* 403) as well as the metabolism of the polyunsaturated fatty acids, including their interconversions, have recently been reviewed (Mead, *Am. J. Clin. Nutr. 8,* 55).

Dietary cholesterol has a profound effect on essential fatty acid deficiency. Thus, the inclusion of cholesterol in a fat-free diet accelerates the appearance of essential fatty acid deficiency in rats as judged by lowered food efficiency, lower weight gain, retarded testicular development and the polyunsaturated acid pattern of heart lipids (Holman and Peifer, *J. Nutr. 70,* 411; *Nutr. Rev. 18,* 345). Both cholesterol and sodium glycocholate accelerate the appearance of EFA-deficiency symptoms in rats, while cholic acid depresses growth alone; cholesterol, fed with either supplement, induces a marked elevation of liver lipids (Gambal and Quackenbush, *J. Nutr. 70,* 497).

The interrelationships between nonessential and the essential fatty acids have also been extensively investigated. Thus, it has been found that high ratios of saturated fats may promote essential fatty acid deficiency symptoms (Peifer and Holman, *J. Nutr. 68,* 155; *Nutr. Rev. 18,* 56); studies of the effect of increasing dietary levels of partly or completely hydrogenated coconut oil, fed alone or with cottonseed oil, on the growth and tissue pathology of rats, support the view that the biological action of hardened fats can be explained by the assumption that saturated fats containing long-chain fatty acids increase the requirement for essential fatty acids more than do unsaturated or medium chain acids (Funch *et al., Brit. J. Nutr. 14,* 171). In accord with this view, triglycerides of medium chain fatty acids lower the essential fatty acid requirement of rats as compared to triglycerides of long-chain fatty acids (Kaunitz *et al., J. Nutr. 41,* 400).

An attempt was made to settle the controversy about the activity of the *trans* fatty acids, which had been reported by some investigators, but not by others, to act as antagonists of the essential fatty acids. It was found that of the acids tested only *cis,cis*-linoleate had essential fatty acid activity in the male rat, and that the other acids tested (*cis,trans*- and *trans,trans*-linoleate, oleate, and elaidate) neither exhibited essential fatty acid activity, nor did they interfere with the utilization of *cis,cis*-linoleate (Mattson, *J. Nutr. 71,* 366). Raulin (*Ann. Nutr. et Aliment. 14,* 201) also investigated the nutritive value of lipids in relation to the stereochemical configuration of the fatty acids.

A possible mechanism of action of the essential fatty acids may be indicated by the swelling of liver mitochondria observed in rats fed diets deficient in essential fatty acids (Hayashida and Portman, *Proc. Soc. Exptl. Med. 103,* 656).

Dietary Fat and Tissue Lipids. The relationship between dietary unsaturated fatty acids and plasma lipids has been reviewed (Kummerow *et al., Am. J. Clin. Nut. 8,* 62); recent emphasis in this area has been of more comprehensive investigation into the composition of serum lipids as influenced by dietary fat. Thus, studies have been made of the fatty acids of human blood (Patil and Magar, *Biochem. J. 74,* 427), the effects of dietary intake of fat and age on the polyunsaturated

fatty acids of human serum (Patil and Magar, *Biochem. J. 76,* 417), the effect of polyunsaturated fat intake on the serum lipids of aged subjects (Nikkila and Jokipii, *Acta Med. Scand. 166,* 269) and of the fatty acid spectrum of the lipid fractions of human blood (Schrade *et al., Fette u. Seifen, Anstrichmittel 62,* 673). The levels of the free fatty acids of blood in fetal and neo-natal life were also compared (Van Duyne, *Proc. Soc. Exp. Biol. Med. 102,* 599).

Related investigations have been carried out in experimental animals. Thus, Patil and Magar (*Biochem. J. 74,* 441) studied the effect of dietary fat and cholesterol on the polyunsaturated fatty acids of various lipid fractions of rat liver, while Holmer *et al.* (*Brit. J. Nutr. 14,* 247) investigated the effects of hydrogenated and unhydrogenated peanut oil on polyunsaturated fatty acid levels of blood, heart, and liver of chicks.

Nutritive Value of Fats. Current concepts of parenteral fat nutrition (*Chicago Med. Sch. Quart. 20,* 49) and the role of fats in human nutrition (Kummerow, *J. Am. Oil Chem. Soc. 37,* 503) have recently been reviewed. The common edible fats exhibit little difference in nutritive value in prematurely weaned animals (Crampton *et al., J. Nutr. 70,* 81; *Nutr. Rev. 18,* 251), however strain differences do appear in some of the criteria used to access the nutritive value of fats. Thus, the coeficients of digestibility were lower for rapeseed oil than for corn oil in Sprague-Dawley, but not in Wistar, rats; but there was no significant effect of strain or type of oil on the liver storage of vitamin A (Beare *et al., Can. J. Physiol. 38,* 197). In confirmation of this, it was found that corn oil, olive oil and rapeseed oil had no influence on the rate at which liver stores of vitamin A were depleted over a 2-month period (Murray *et al. Can. J. Physiol. 38,* 663).

Nutritive Value of Heated and Oxidized Oils. Changes in the physical properties of oils induced by aging, oxidation, polymerization, and oxidation-polymerization have been reviewed (Ludde, *Fette u. Seifen, Anstrichmittel 61,* 1156). More recent developments in this area include the application of infrared spectrophotometry for the study of autoxidative changes in synthetic triglyceride (Kaufmann and Thomas, *Fette u. Seifen, Anstrichmittel 62,* 315) studies of the influence of the water of dried foods on the oxidation of oils (Bito and Yamamoto, *Yukagaku 9,* 41, abs. in *J. Am. Oil Chemists' Soc. 37,* 493) and studies of heat transfer in hot fat cooking (Smith, *Food Tech. 14,* 84).

Various studies on the nutritive values of heated and oxidized oils have also been carried out. Commercial deep-fat frying as contrasted to laboratory-heating does not impair the nutritional value of a majority of fats as determined by measurement of biologically-available energy of the fats fed as well as of the liver weights of rats (Poling, *J. Nutrition 72,* 109). Other studies have also been carried out on the nutritional and physiological properties of heat-treated oils (Wurziger and Ostertag, *Fette u. Seifen, Anstrichmittel 62,* 895 and Esh *et al., Ann. Bioch. and Exptl. Med. 20,* 41 (Calcutta). Matsui (cited in abs., *J. Am. Oil Chem. Soc. 37,* 495) studied the toxicity of heat-polymerized oil, while Krondl *et al.* (*Deut. Z, Verdaurings -u. Stoffwechselkrankh 20,* 47,) studied the emulsification and hydrolysis of fresh and heated fats *in vitro.*

The toxicity of air-oxidized soybean oil parallels the peroxide content of the oil; the inhibition of intestinal xanthine oxidase by this oil and the reversal of this inhibition by exogenous flavin adenine dinucleotide suggests that the toxicity of the lipid peroxides may be at the level of the intestinal enzymes (Andrews *et al., J. Nutr. 70,* 199).

The addition of fresh cottonseed oil to diets containing autoxidized cottonseed oil has a beneficial effect (Kaunitz *et al., J. Nutr. 70,* 521). In an attempt to determine the components in autoxidized fats that exert the toxic effects, fractions of oxidized oleate and linoleate were fed to weanling rats; while the urea-adduct-forming fraction of oleate was atoxic, it produced marked diuresis and reduced serum and liver cholesterol levels as compared to lard (Kaunitz *et al., Metabolism 9,* 59). Kaunitz (*Exp. Med. Surg. 18.* 56) has also discussed the biological effects of rancid fats and their fractions.

Irradiated soybean and corn oils have the same nutritive value in chicks as the unirradiated oils; storage did not decrease the nutritive value of these oils. However, irradiation of beef fat results in a lower nutritive value. Studies with vitamin supplements indicate that in pork most of the vitamin A and some of the vitamin D were lost on storage of the irradiated fat (Ritchey and Richardson, *Poultry Sci. 39,* 404).

Cholesterol Metabolism

CHOLESTEROLGENESIS AND LIPOGENESIS. A study of the effect of diet on incorporation of acetate-1-C^{14} into cholesterol by rat liver slices revealed that simple transfer from a commercial chow diet to a semi-synthetic diet decreased incorporation especially when the synthetic diet contained oleic acid or olive oil (Carroll, *Can. J. Biochem. Physiol. 38,* 649). Kritchevsky also studied synthesis as well as degradation of cholesterol and found that liver fat from rats fed either an unsaturated fat diet or a diet containing hydrogenated shortening when added to normal liver mitochondrial preparations inhibited oxidation of cholesterol 26-C^{14} (*Am. J. Clin. Nut. 8,* 53, 411). Inhibition of cholesterol biosynthesis was obtained by extracts of liver mitochondria disrupted with sonic oscillations from normal or starved rats; the inhibition appears to be between acetoacetate and mevalonate (Migicovsky, *Can. J. Biochem. Physiol.* 38, 339).

An organic compound, benzmalecane [N-(1-methyl-2, 3-di-p-chlorophenylpropyl)-maleamic acid] was shown to inhibit the *in vitro* incorporation of 2-C^{14}-mevalonic acid into cholesterol by rat liver homogenates (Huff and Gilfillan, *Proc. Soc. Exptl. Biol. Med. 103,* 41). When this compound was given to hyperlipemic and normalipemic subjects, serum cholesterol was decreased but serum neutral fat was elevated. It is postulated that these changes were caused by the diversion of acetate or acetoacetate from cholesterol synthesis to triglyceride formation (Sachs *et al., Metabolism 9,* 783).

Investigations on the effect of nicotinic acid on sterol synthesis has yielded contradictory reports. Whereas Hardy *et al.* (*J. Nutrition 71,* 159) reported an increased incorporation of acetate into sterols—and a decreased incorporation of acetate into fatty acids—in liver slices of rats and chicks fed nicotinic acid and related compounds (isonicotinic acid and nicotinamide), Duncan and Best (*J. Lipid Res. 1,* 159) found no effect of the addition of 1% nicotinic acid on the incorporation of acetate $-1-C^{14}$ into serum and liver cholesterol by the intact animal. Nor did nicotinic acid affect serum, liver or total carcass cholesterol or the absorption and disappearance from serum and liver of labeled cholesterol. However, Kritchevsky *et al.* (*J. Lipid Res. 1,* 154) found that nicotinic acid added to normal rat liver mitochondria enhanced the oxidation of cholesterol $-26-C^{14}$, although in confirmation of Duncan and Best, no effects on serum level cholesterols in rats could be observed.

On the other hand, Gaylor *et al.* (*J. Nutrition 70,* 293) found that high levels of nicotinic acid, comparable to those used in human therapy, and its derivatives yielded consistent lowering of blood cholesterol in both the rat and the chick.

Pyridoxine deficiency enhances the *in vivo* incorporation of labeled acetate into liver cholesterol in rats (Shah *et al., J. Nutrition 72,* 81). The injection of pyridoxine hydrochloride reverses this enhanced synthesis. Lipotropic agents such as choline chloride, methionine, or vitamin B_{12} did not significantly influence the net biosynthesis of cholesterol in rats (Mookerjea and Lucas, *Can. J. Biochem. Physiol. 38,* 757) although they did affect the distribution of cholesterol. Hepatic lipogenesis and cholesterogenesis seem to be related to time after administering certain fats. After corn oil and lard were fed to rats, a pronounced decrease in the capacity of the liver to convert acetate to fatty acids was observed as early as one hour thereafter; however, the increase in the capacity of the liver to incorporate acetate into cholesterol developed slowly, the earliest change was observed after 12 hours (Hill *et al., J. Lipid Res. 1,* 150).

Cholesterol Turnover and Degradation. Absorption of cholesterol 4-C^{14} recovered in thoracic duct lymph revealed a dependence on the carbohydrate in the diet—sucrose fed animals absorbed approximately ⅓ less of the isotope than animals fed a 40% lactose-containing diet (Wells *et al., J. Nutrition 71,* 405). Coprostanol in appreciable amounts was found in the cecum and in the third and fourth quarters of the small intestine; smaller amounts were found in the first half of the small intestine. In rats fed cerebroside preparations and fatty acid esters obtained after hydrolysis of cerebrosides, a marked increase in sterol excretion was observed with cholesterol as the main chromogenic sterol in th feces (Carroll, *J. Lipid Res. 1,* 171). Cholesterol turnover studied in normal, alloxan-diabetic, and pancreatectomized animals revealed a decreased rate of cholesterol turnover in liver of diabetic rats. The authors (Wong and Van Bruggen, *J. Biol. Chem. 235,* 30), concluded that there may be a defect in the mechanism of cholesterol degradation in the diabetic animals. The formation of acetone and acetoacetate from cholesterol by rat and mouse liver mitochondria was reported by Whitehouse *et al.* (*Biochem. and Biophys. Acta 37,* 382).

Serum Cholesterol. Kritchevsky (*Am. J. Clin. Nut. 8,* 72) has reviewed the functions of serum cholesterol. The ingestion of cholesterol-rich diets, in which the cholesterol was labeled with C^{14} cholesterol, by healthy adults led to the presumption that

¾ of the serum cholesterol was derived from cholesterol synthesis in extrahepatic tissues (Taylor *et al., Proc. Soc. Exp. Biol. Med. 103,* 768). The addition of cholesterol supplements to a butterfat fraction ration given to University students caused a sharp increase in serum cholesterol when the intake was between 18 and 634 mg. cholesterol/day. No further significant increases were obtained at daily intakes of 1300 to 4500 mg. cholesterol (Beveridge *et al. J. Nut. 71,* 61).

Further attempts have been made to understand changes in cholesterol content resulting from a variety of dietary constituents. Thus Turpeinen *et al.* (*Lancet I,* 196) have reported a reduction in serum cholesterol levels in Finnish mental patients when the butterfat in their diet was replaced by an emulsified soya bean oil and an unsaturated margarine. It is hoped to relate this serum cholesterol reduction with frequency of coronary artery disease and other atherosclerotic manifestations. Funch *et al.* (*Brit. J. Nutrition 14,* 1) reported that butter diet consistently caused significantly higher blood cholesterol levels than did diets with margarine containing large amounts of hydrogenated whale oil and suggests that the difference in chain length of the fatty acids present in these products may be partly responsible for the differences in effect of the two kinds of fat on serum cholesterol levels. The observation that frequent taking of blood samples results in a highly significant increase in plasma cholesterol in rats fed rations containing fat (Coleman and Beveridge, *J. Nutrition 71,* 303) may explain some of the divergent results which have appeared in the past.

In studies with rabbits given diets containing fat with 20, 39, 60 and 80% saturated fatty acids, the regimen containing 20% saturated fatty acids yielded the highest weight gain, and the concentration of liver cholesterol varied inversely with the saturation of the fat (Beare *et al., J. Nutrition 73,* 17). Hegsted *et al.* (*J. Nutrition 70,* 119) in studies with cholesterol-fed chicks found that serum cholesterol levels were raised by feeding saturated fatty acids and lowered by polyunsaturated fatty acids. In hypercholesterolemic rats, the effect of feeding highly unsaturated fatty acids, or precursors of such polyenoic acids was a marked and rapid depression of plasma cholesterol accompanied by an accumulation of the higher polyenoic fatty acids in heart lipids and in liver phospholipides (Peifer *et al., Arch. Biochem. Biophys. 86,* 302). Similarly, linoleic ester was shown to lower markedly the plasma cholesterol of hypercholesteremic cholesterol-fed rats and also the liver cholesterol, provided that the fat content of the diet was low (2%) (Quackenbush *et al., J. Nutrition 72,* 196). Korenyi and Jaky reported on the ability of sunflower oil to decrease blood serum lipids (*Nahrung 4,* 225). Klein (*Am. J. Clin. Nut. 8,* 104) has reviewed the relation between polyunsaturated fatty acids and plasma cholesterol. Riley and Humor (*Biochem. J. 74,* 56) have also discussed the relation between dietary fatty acids and plasma cholesterol fatty acids. Cholesterol ester fatty acids in serum and liver of normal and lymph-fistula rats have been described by Swell *et al.* (*J. Biol. Chem. 235,* 1960).

The administration of safflower oil to hypercholesterolemic men lowered the plasma cholesterol level of half of the 8 patients tested but increased the iodine value of the plasma cholesterol ester fatty acids in all cases (Alfin-Slater and Jordan, *Am. J. Clin. Nut. 8,* 325). Safflower oil supplementation increased the linoleic acid content of plasma cholesterol esters while the percentages of total and free plasma cholesterol decreased (Okey *et al., Metabolism 9,* 791). A formula type feeding, using one half of the usual quantity of safflower oil and safflower-coconut oil mixtures, administered to 10 hypercholesterolemic patients yielded reductions in serum cholesterol levels (Davis *et al., Am. J. Clin. Nut. 8,* 808).

Fish Oils and Serum Cholesterol. The effect of fish oils, which contain fatty acids with 4, 5 and 6 double bonds, in depressing blood cholesterol levels is largely a function of their total unsaturation rather than the presence of linoleic acid in the fish oils (Stansby, *U. S. Fish Wildlife Serv., Tech. Leaflet* 28). However, although lingcod liver oil and halibut liver oil prevented the hypercholesterolemic effect of supplementary cholesterol, crude herring oil increased the hypercholesterolemia. No clear explanation could be given but that there seems to be a difference in the effects on serum cholesterol of liver oils from teleostei fish and from selachii fish (Wood and Biely, *Can. J. Biochem. Physiol. 38,* 19). When lingcod liver oil unsaponifiable material was fractionated, it was found that the fraction containing Vitamin A prevented the hypercholesterolemia (Wood, *Can. J. Biochem. Physiol. 38,* 879). A hypocholesterolemic factor in a sterol mixture obtained from the butter clam (*Saxidomus giganteus*) may be 24-methylenecholesterol (Reiner *et al., Can. J. Biochem. Physiol. 38,* 1499). Saturated and unsaturated fatty acid fractions from vegetable and fish oils were investigated for their anti-cholesterolemic properties using a 3 day fasting method on rabbits (Sokoloff *et al., J. Gerontol. 15,* 19). The effect of marine products on blood cholesterol levels in man has been revived by Wood (*J. Fish Res. Brd. Can. 17,* 903).

Proteins and Serum Cholesterol. In studies on 12 young women, after two weeks the serum cholesterol levels of subjects receiving a vegetable protein diet were significantly lower than those after eating an animal protein diet (Walker *et al., J. Nutrition 72,* 317). In rats, serum cholesterol levels were found to increase between 29 and 41 weeks and to decrease between weeks 41 and 50 regardless of diet. Serum cholesterol levels increased slightly with increasing levels of casein in the diet when increased levels of fat were fed (Lushbough, *J. Am. Oil Chemists' Soc. 37,* 98). The feeding of a low protein diet to chicks resulted in an elevated plasma cholesterol level and decreased total serum protein and serum albumin levels. Chicks fed coconut oil had higher plasma cholesterol levels than those receiving similar diets supplemented with corn oil (Leveille *et al., Arch. Biochem. Biophys. 86,* 67). The sulfur amino acids, cystine, cysteine and methionine, lowered serum cholesterol in hypercholesterolemic rats. It is suggested that the serum cholesterol-lowering effect of protein supplements is due largely to the S-containing amino acid content (Seidel *et al., J. Lipid Res. 1,* 474). The pancreas may be involved in the reduction of fecal cholesterol by dietary protein (Fragola and Magee, *Am. J. Physiol. 198,* 354). The effect of protein and other nutrients on the cholesterol level of blood has been reviewed by De Groot (*Voeding 21,* 374).

Carbohydrates and Cholesterol Levels. Sucrose and milk sugar tend to produce higher serum cholesterol values than equal calories of carbohydrates contained in fruits, leafy vegetables and legumes (Keys *et al., J. Nutrition 70,* 257). Starch in the diet led to higher levels of liver fat and cholesterol than sucrose or dextrose; sucrose lowered liver cholesterol (Guggenheim *et. al., J. Nutrition 72,* 93). Dailey (*Am. J. Clin. Nut. 8,* 34) reported that whereas a high lipid diet shortened blood coagulation time, an isocalorie carbohydrate diet produced no change.

Atherosclerotic Lesions. The composition of the cholesterol ester fatty acids of the media of human aorta and of serum was very similar and contained chiefly linoleic acid, whereas the major fatty acid of atherosclerotic plaques and of liver was oleic acid (Swell *et al., Proc. Soc. Exptl. Biol. Med. 103,* 651). A butter diet fed to rabbits induced hypercholesterolemia, hyper-β-lipoproteinemia and marked development of atheromatous lesions as contrasted to animals fed margarines or arachis oil (Funch *et al.*, Brit. *J. Nutrition 14,* 355). Heart valve-aorta sudanophilia can be reduced in the rat by feeding high dietary magnesium and/or thyroxine (Nakamura *et al., J. Nutrition 71,* 347). High dietary magnesium appeared to be "anti-sudanophilic" only when the serum cholesterol was elevated (Hellerstein *et al., J. Nutrition 71,* 339).

A ninhydrin positive, lipid bound factor, chromatographically identified as glutamic acid, has been found in an extract of human plasma α- and β-lipoproteins. This factor is deficient in plasma of the aging and in the arteriosclerotic subject (Hamilton and Pilgeram, *Proc. Soc. Exptl. Biol. Med. 103,* 574). A review concerned with the relationship of lipids to atherosclerosis has been prepared by Holman (*Am. J. Clin. Nut. 8,* 95). Michaels *et al.* (*Am. J. Clin. Nut. 8,* 38) have reported elevations in a "freely extractable lipid" fraction in blood of hyperlipemic and some atherosclerotic patients.

Food Restriction, Obesity. In rats on restricted food, total liver lipids and cholesterol were disproportionately decreased especially in older animals fed cholesterol; serum lipids and cholesterol were not greatly lowered (Okey *et al., J. Am. Diet. Assoc. 36,* 441). However, in medical students, no significant correlation was found between the serum cholesterol level and weight, weight corrected for frame size, or thickness of the fat shadow (Thomas and Garn, *Science 131,* 42).

Age. Since the possibility exists that atherosclerosis may have its genesis in infancy, studies on cholesterol levels in infants, either breast-fed or fed whole cows milk, evaporated milk and water, or formulas with soybean oil or with corn oil as the chief source of fat have been conducted (Foman and Bartels, *A. M. A. J. Dis. Child. 99,* 27). In another study, serum α-lipoprotein was measured in infants on various dietary regimens, with the patterns the same for all groups—a slight increase initially and then an almost constant level (Lindquist and Malmerana, *A. M. A. J. Dis. Child. 99,* 39). However, Pomeranze found that serum cholesterol levels of infants fed an evaporated milk formula were higher than those in infants fed a prepared milk formula containing a higher level of unsaturated fatty acids (*Am. J. Clin. Nut 8,* 340). Children (6-10 years old) of both Negro and white races had significantly lower total blood cholesterol level than older individuals. Also, children had significantly less oleic acid and significantly more

linoleic acid in their serum cholesterol ester fatty acid fractions than did older subjects (Swell *et al., Proc. Soc. Exptl. Biol. Med. 105,* 129). On the other hand, Chow (*Am. J. Clin. Nut. 8,* 321) found no decrease in plasma cholesterol after administration of Vitamin B_{12} to elderly people; when B_{12} and sorbitol (which enhances B_{12} absorption) was given, plasma cholesterol was decreased.

Hormones. Chickens fed a high cholesterol diet and chickens injected with diethyl-stilbestrol exhibited a similar rise in blood serum phosphorus, cholesterol and in protein. Electrophoresis could differentiate between protein components that increased during cholesterol feeding and the estrogen administration (Clegg *et al., Poultry Sci. 39,* 35). In pigs, testosterone administration caused a marked reduction in serum cholesterol, stilbestrol had no apparent effect, and castration resulted in hypercholesteremia (Cox and Hale, *J. Nut. 72,* 77). In young cockerels, hydrocortisone and corticosterone administration inhibited growth but increased carcass fat and blood and liver lipid; cortisone had no effect on these indices; diethylstilbestrol increased blood and liver lipids and stimulated fat deposition without inhibiting growth (Baum and Meyer, *Am. J. Physiol. 198,* 1263). Filios *et al.* have compared the potencies of uracil, thiouracil and related compounds in producing thyroid changes, hypercholesteremia and atherosclerosis in rats fed cholesterol and cholic acid (*Circulation Res. 8,* 71).

Book Review

Several reviews have appeared during the past year which may be of interest to researchers in the field of fats and oils. Law prepared a review on glycolipids (*Ann. Rev. of Biochem. 29,* 131). Lipsky, *et al.,* reviewed the field of gas chromatography of fatty acids (*Ann. Rev. of Biochem. 29,* 649).

A panel discussion on "Lipids in Health and Disease" was held at the Fifth International Congress on Nutrition, Washington, D. C., September, 1960. The various topics dealing with the role of fat in normal metabolism and atherosclerosis, metabolism role of endocrines in lipid metabolism were presented and written by outstanding authorities in their fields. New books of interest to chemists in fats, oils and detergent research are as follows:

Achaya, H. A., Ed. "Cottonseed and Its By-Products", The India Central Oilseed Committee, Hyderabad, India, 1959.

American Society for Testing Materials, "1959 Supplement to book of ASTM Standards Including Tentatives. Part 8. Paint, Naval Stores, Aromatic Hydrocarbons, Gaseous Fuels, Engine Antifreezes," Philadelphia, Pa., 1959.

American Society for Testing Materials, "1959 Supplement to Book of ASTM Standards Including Tentatives. Part 10. Textiles, Soap, Water, Atmospheric Analysis, Wax Polishers," Philadelphia, Pa., 1959.

Centre National de la Recherche Scientifique. "Alimentary Fats," 2nd. Series. Paris, 1959.

Hanahan, D. J. "Lipide Chemistry," John Wiley and Sons, New York, 1960.

Kirk, R. E. and Othmer, D.F. Ed., "Encyclopedia of Chemical Technology." First Supplement Volume. The Interscience Encyclopedia Inc., 1957.

Kirschbaum, Emil "Destiller-und Rektifiziertechnik," Springer-Verlag, Berlin/Goettingen/Heidelberg, 1960.

Kirschenbauer, H. G. "Fats and Oils: An Outline of Their Chemistry and Technology", 2nd. Ed. Reinhold Publishing Corporation, New York, 1960.

Kline, G. M., Ed. "Analytical Chemistry of Polymers," Vol. XII, Part I. Analysis of Monomers and Polymeric Materials: Plastics, Resins, Rubbers, Fibers. Interstate Publishers Inc., 1959.

Martin, J. H. and Morgans, W. M. "A Glossary of Pigment, Varnish, and Lacquer Constituents," Chemical Publishing Company, New York, 1959.

Mehlenbacher, V. C. "The Analysis of Fats and Oils," Garrard Press, Champaign, Ill., 1960.

Whistler, R. E. and BeMiller, J. N. Ed. "Industrial Gums: Polysaccharides and Their Derivatives," Academic Press, 1959.

Zuidema, H. H. "Performance of Lubricating Oils," Reinhold Publishing Corporation, New York, 1959.