

The fatty acids of *Euglena gracilis*

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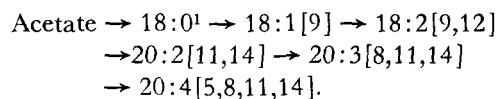
SUMMARY The complete structures of 51 fatty acids of *Euglena gracilis* have been determined. These include saturated fatty acids from C₉ to C₁₉, monounsaturated fatty acids from C₁₃ to C₁₉, diunsaturated fatty acids from C₁₅ to C₂₀, triunsaturated fatty acids from C₁₅ to C₂₀, tetraunsaturated fatty acids from C₁₅ to C₂₂, pentaunsaturated fatty acids from C₂₀ to C₂₂, and one docosahexaenoic acid. No evidence was found for branched or cyclic acids. All the double bonds are of the *cis*-configuration, and the double bonds of polyunsaturated fatty acids are all in methylene-interrupted sequences. *E. gracilis* synthesizes linoleic acid and linolenic acid, as well as longer chain polyunsaturated fatty acids structurally related to each, thus demonstrating fatty acid biosynthetic mechanisms characteristic of both plants and animals.

A SURVEY of the literature on the fatty acid composition of plants, animals, and bacteria (1) immediately suggests that the distribution of fatty acids may be a useful tool in developing taxonomic and phylogenetic relationships. Whereas, in general, the same amino acids, purines, pyrimidines, and sugars occur throughout nature, with exceptions being too restricted to serve as the basis for establishing interrelationships among species, the opposite is true of the distribution of fatty acids, especially unsaturated fatty acids. If one also considers the fact that many fatty acids may be synthesized by different pathways in different organisms (and perhaps within one organism), the possible permutations and combinations become very large indeed.

Higher plants characteristically synthesize the 18-carbon mono-, di-, and triunsaturated fatty acids, oleic, linoleic, and linolenic. Higher animals also synthesize oleic acid, but not linoleic and linolenic acids. However, animals do convert all three of these acids to polyunsaturated fatty acids of longer chain length (which plants do not (2)) by the introduction of double bonds in a divinyl methane pattern, towards the carboxyl end of the molecule, and repeated elongation of the fatty acid chain by addition of 2-carbon units. Bacteria do not generally

synthesize polyunsaturated fatty acids and, therefore, contain mainly monounsaturated fatty acids, and saturated fatty acids that are in many cases branched or contain ring systems (2). Although the fatty acids of plants, animals, and bacteria are thus clearly different no patterns have been discerned that permit subgroupings within each division.

Recently, it has been reported from this laboratory (3) that the soil amoeba, *Acanthamoeba sp.*, synthesizes arachidonic acid by the following sequence of reactions:



This combines in one organism the ability, like plants, to synthesize linoleic acid, and the ability, like animals, to convert linoleic acid to arachidonic acid. The amoebas do not, however, convert arachidonic acid to 22-carbon polyunsaturated fatty acids as do higher animals, nor do they synthesize linolenic acid, the fatty acid characteristic of plants. Another protozoan, the parasitic zooflagellate *Leishmania enriettii*, was found (4) to be "plant-like" in its fatty acid composition, and to synthesize oleic acid, linoleic acid, and linolenic acid, but no detectable unsaturated 20-carbon fatty acids. Finally, a third protozoan, *D. discoideum*, contains only unique polyunsaturated fatty acids not yet found elsewhere (5). For these reasons it was thought that the fatty acids might supply a useful biochemical taxonomic tool for classification of the protists, the unicellular (or acellular) plants and animals.

Euglena gracilis is an interesting organism thought to be closely related to the primitive protists that may have been the common origin of plants and animals (6). When grown in the light, *E. gracilis* are typical chlorophyll-

¹ In the designation of fatty acids and their methyl esters, the first number refers to the number of carbon atoms in the molecule, the second number to the number of double bonds in the molecule, and the numbers in brackets to the positions of the double bonds counting from the carboxyl end of the molecule.

containing *phytoflagellates* capable of utilizing CO_2 as their sole carbon source. *E. gracilis* will also grow in the dark, on a more complex medium, in which case they do not contain chlorophyll and might be considered to be *zooflagellates* (6). Chlorophyll-less mutants of *E. gracilis* exist that are "animal-like" whether grown in the light or dark (7). Erwin and Bloch (7) have found that the content of linolenic acid is much higher in *E. gracilis* grown in the light than in *E. gracilis* grown in the dark and in chlorophyll-less mutants. Rosenberg (8) has extended this observation by demonstrating that many of the polyunsaturated 16-, 18-, and 20-carbon fatty acids of *Euglena gracilis* are present in much higher concentration in cells grown in the light than in dark-grown cells. In neither investigation were the fatty acids of *Euglena gracilis* fully characterized. Erwin and Bloch (7) restricted their attention to the 18-carbon acids, and Rosenberg (8) attempted only preliminary identifications based largely on retention times obtained by gas-liquid chromatography (GLC).

In this paper, 51 fatty acids are identified in extracts of *Euglena gracilis*. This fantastically diverse pattern is remarkable in that it contains all the fatty acids typical of both higher plants and higher animals.²

EXPERIMENTAL PROCEDURE

Growth of Cells

Euglena gracilis strain Z was obtained from American Type Culture Collection (No. 12716). The original source was Dr. S. H. Hutner, Haskins Laboratory, New York. The cells were grown under fluorescent light on Hutner's pH 3.3 medium in which the carbon sources are CO_2 , glutamic acid, and malic acid (10). One batch of cells was grown for 5 days, and three batches were grown for 10 days.³ Gas-liquid chromatograms of the fatty acids extracted from all four batches of cells were very similar. The fatty acids from each batch of cells were isolated and analyzed separately.

Preparation of Fatty Acid Methyl Esters

At the end of the growth period, the cells were collected by centrifugation and washed several times with distilled water. The washed cells were extracted overnight in about 20 volumes of chloroform-methanol, 2:1, the residue was then removed by filtration, and the solvent was evaporated under reduced pressure at 40° . The residue was extracted with chloroform, the chloroform was evaporated under a stream of nitrogen at room temperature, and the total lipids were methanolized in 0.5 N NaOH

in absolute methanol at 40° for 1 hr under nitrogen (11). The solution was cooled to 0° , acidified, and an equal volume of water was added. Methyl esters of the fatty acids were extracted into redistilled pentane. Part of this pentane solution was saved, but most of it was evaporated under a stream of nitrogen, and saponified under nitrogen in 0.5 N NaOH in 50% methanol at 80° for 90 min. The alkaline solution was extracted with pentane. The solution was then acidified, and the fatty acids were extracted with pentane. The pentane was removed under a stream of nitrogen at room temperature, and the fatty acids were converted to their methyl esters by reaction with BF_3 -methanol. The methyl esters of the fatty acids were then extracted into pentane, and were stored at -20° .

Separation of Fatty Acids

Fatty acid methyl esters were reacted with mercuric acetate according to Mangold (12), and the adducts were separated by chromatography on silicic acid by the method of Erwin and Bloch (13) with slight modifications. By these procedures, one obtains five classes of fatty acids: saturated, monounsaturated, diunsaturated, triunsaturated, and a mixture of tetra-, penta-, and hexaunsaturated. Under optimal conditions there is very little cross-contamination among these classes. In one experiment, however, the tetraunsaturated fatty acids were partially eluted with the triunsaturated fatty acids. These were separated by re-chromatography of the adducts. In another experiment, the monounsaturated and diunsaturated fatty acids were eluted together, and these were separated, after regeneration of the original fatty acid methyl esters, by chromatography on silicic acid impregnated with AgNO_3 (14).

The unsaturated fatty acid methyl esters were regenerated from their adducts by treatment with methanolic HCl, and analyzed by GLC. Each of the four classes were fractionated further by preparative GLC. Emerging peaks were collected on Celite coated with silicone from which the compounds were eluted with pentane. In general, from 1 to 10 mg of each fraction was available for structural studies. Each fraction was analyzed for purity by analytical GLC; the fractions used for the structural studies were each at least 90% homogeneous, although several fractions had to be subjected to two gas-liquid chromatographic separations to attain this degree of purity. Most of the apparently "pure" monounsaturated and diunsaturated fatty acids actually contained several isomeric fatty acids and, in one instance, an apparently homogeneous peak contained two fatty acids differing both in chain length and degree of unsaturation.

Structural Studies

The chain length of each fatty acid was determined by gas-liquid chromatographic identification of the satu-

² A preliminary report on some of the data reported in this paper has been published (9).

³ Dr. C. L. Greenblatt kindly provided the cultures.

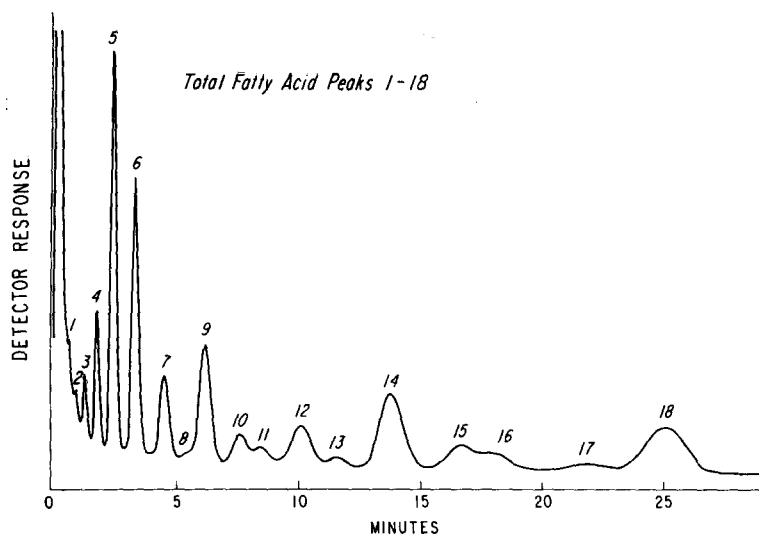


FIG. 1. Partial gas-liquid chromatogram of the methyl esters of the fatty acids of *E. gracilis*: Peaks 1 through 18. Sample was chromatographed on 17% ethylene glycol succinate polyester at 175°, and an argon pressure of 15 psi.

rated fatty acid produced by catalytic hydrogenation (15). The number of double bonds in the molecule was then deducible from the retention time of the original fatty acid methyl ester, and the chromatographic behavior of its mercuric acetate adduct.

The number of double bonds in the molecule was confirmed, and their positions were established by analysis of the products of permanganate-periodate oxidation performed by a modification (15) of the procedure of von Rudloff (16). In this procedure, each unsaturated fatty acid is degraded to a dicarboxylic acid that is derived from the carbon atoms from the carboxyl group to the first double bond, and a monocarboxylic acid that is derived from the carbon atoms from the last double bond

to the methyl terminal end. These fatty acids were identified by GLC of their methyl esters. It is important before the oxidation to remove the ethylene glycol succinate that contaminates samples obtained by preparative GLC. This was done by saponification of the fatty acid esters and extraction of the free fatty acids with pentane after acidification of the saponification mixture. Succinic acid is not extracted.

Gas-Liquid Chromatography

Analytical GLC of the methyl esters of long chain fatty acids was performed on columns (6 ft × 5 mm) of 17% ethylene glycol succinate on Gas Chrom P, 80-100 mesh, at a temperature of either 190° or 175°, and an input

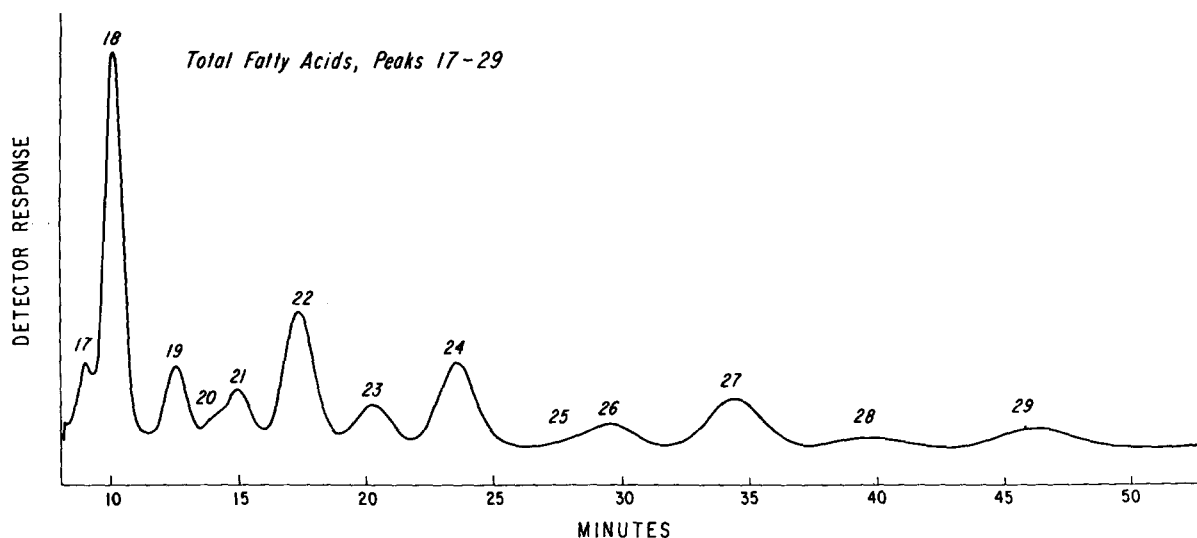


FIG. 2. Partial gas-liquid chromatogram of the methyl esters of the fatty acids of *E. gracilis*: Peaks 17 through 29. Sample was chromatographed on 17% ethylene glycol succinate polyester at 192° and an argon pressure of 15 psi.

pressure of 15 psi of argon. An argon ionization detector was used. Analytical chromatography of methyl esters of the short chain fatty acids produced by oxidation of the long chain unsaturated acids was carried out on columns containing ethylene glycol adipate at 100°, and a pressure of 10 psi of argon. Methyl esters of short chain fatty acids were also analyzed, especially to detect methyl propionate, on 10% Carbowax 400 on Gas Chrom P at 85°, and an input pressure of 5 psi of argon. Methyl esters of dicarboxylic acids were chromatographed on 17% ethylene glycol succinate at 185°, and 15 psi of argon.

Preparative GLC was carried out on columns (6 ft × 15 mm) containing 17% ethylene glycol succinate. Temperatures and pressures were varied, depending on the fraction to be separated. The effluent stream was split; about 1% went to the detector, and the remainder to a manually operated fraction collector containing glass cartridges packed with Celite coated with silicone (17). The esters were eluted from the Celite with pentane in better than 95% over-all yield.

Calculations of Per Cent Composition

The area per cent of each of the 29 peaks in the chromatograms of the total fatty acids was calculated. Then the area per cent of each of the corresponding peaks in the chromatograms of the five fractions obtained by separation of the mercuric acetate adducts was calculated, assuming complete recovery of the adducts. In experiments with radioactive fatty acids we have always recovered essentially all the radioactivity. The ratio of isomers present in a given peak was calculated to the closest whole number from the relative concentrations of the dicarboxylic acids produced by oxidative cleavage. The data reported in the tables are, in most cases, the averages of independent analyses of the fatty acids of three batches of cells, all of which were very similar.

RESULTS

Chromatographic Analysis of the Total Fatty Acids

In order to achieve the best possible resolution of all the fatty acids GLC was carried out at two different temperatures as shown in Figs. 1 and 2. Peaks 17 and 18 are shown on both partial chromatograms to facilitate the comparison of the relative areas of the peaks. Although 29 peaks are clearly discernible in the two chromatograms, this gives little indication of the true complexity of the mixture, and chromatograms such as these are of little help in the final identification of the components. One problem is that the relative retention times of compounds of differing degrees of unsaturation vary with the conditions, and with the particular batch of ethylene glycol succinate used as support. Thus, in some chroma-

tograms peaks 12 and 13 were not separated, and peaks 15 and 16 were merged into one asymmetric peak. Even in Fig. 1, the apparently symmetrical peak 14 contained, as will be shown below, five different fatty acids, including molecules of three different chain lengths. Peak 14 superficially had the same retention time as methyl oleate, but methyl oleate accounted for only 10% of the area of the peak. This was also true, to a lesser extent, of other peaks so that the 29 peaks shown in Figs. 1 and 2 contained more than 50 different fatty acids. Some of these were separable by fractionation of the mercuric acetate adducts, while others were revealed only by analysis of the products of oxidation of mixtures that appeared to be homogeneous chromatographically.

Hydrogenation of an aliquot of the complete mixture of fatty acid methyl esters produced all the saturated normal fatty acids from C₉ to C₂₂. There was no indication of the presence of branched or cyclic fatty acids, or of fatty acids of chain length greater than 22 carbon atoms. Saturated fatty acids of less than 9 carbon atoms would not have been detected if present.

In addition to the peaks shown in Fig. 1, other peaks were present in the chromatogram of the mixture obtained by direct methanolysis of the total lipids. These extra components could be extracted from the alkaline saponification mixture and were, therefore, not fatty acids. It has also been demonstrated that they were not aldehydes or dimethylacetals, and it appears most likely that they were fatty alcohols. Some of them were unsaturated. Tetradecyl alcohol has been demonstrated by Rosenberg (8) to be present in *E. gracilis*.

Saturated Fatty Acids

The chromatogram of the saturated fatty acids isolated by silicic acid chromatography of the mercuric acetate adducts of the total fatty acids is shown in Fig. 3. The peaks are numbered to agree with the corresponding peaks in Fig. 1. Identification of the fatty acids is summarized in Table 1. The logarithms of the retention

TABLE 1 SATURATED FATTY ACIDS OF *E. Gracilis*

Peak*	Per Cent of Total	Relative Retention Time†	Fatty Acid
1	0.2	0.061	9:0
2	0.2	0.077	10:0
3	1	0.117	11:0
4	3	0.158	12:0
5	13	0.215	13:0
6	10	0.292	14:0
7	2	0.397	15:0
9	7	0.536	16:0
11	1	0.732	17:0
13	0.7	1.000	18:0

* Peaks refer to Fig. 3.

† Relative to methyl stearate under conditions as in Fig. 1.

times of the saturated fatty acids fall on a straight line when plotted against the number of carbon atoms in the molecule (Fig. 4). *E. gracilis* contains all the odd- and even-numbered, normal fatty acids from C₉ to C₁₈. Saturated fatty acids of less than 9 carbon atoms were not looked for, and saturated fatty acids of more than 18 carbon atoms were not found.

Monounsaturated Fatty Acids

The gas-liquid chromatogram of the major monounsaturated fatty acids is shown in Fig. 5, and their identification is summarized in Table 2. Peaks 5A and 6A were present in too low concentrations to appear in the chromatogram. When the material emerging from the preparative gas-liquid chromatographic column from the front to peak 8 was collected, and then analyzed at a much higher sensitivity, two peaks appeared with retention times between those of peaks 5 and 6, and peaks 6 and 7, respectively. They were then separated from each other by a second preparative chromatographic run at lower temperature. Similarly, peak 16 was observed only after rechromatography of material that emerged after peak 14 in the original chromatogram.

It can be seen from Table 2 that monounsaturated fatty acids from C₁₃ through C₁₉ were present and, in most instances, more than one isomer of each chain length. As with the saturated fatty acids, the logarithms of the retention times of the methyl esters of the monounsaturated fatty acids fall on a straight line when plotted against the

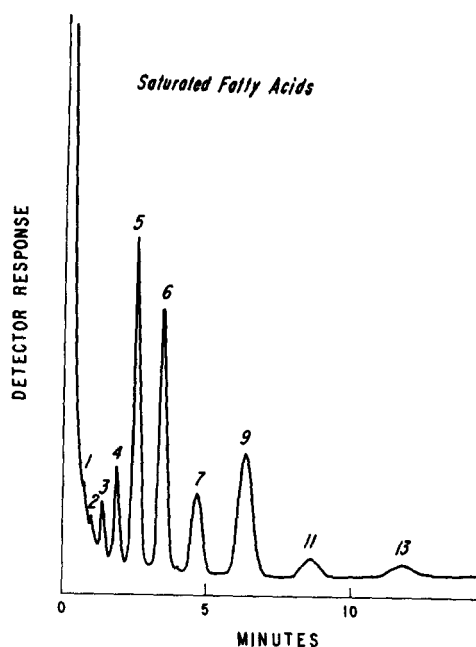


FIG. 3. Gas-liquid chromatogram of the methyl esters of the saturated fatty acids of *E. gracilis*. Conditions were the same as in Fig. 1. The peaks are numbered as the peaks with the same retention times in Fig. 1. The components of each peak are identified in Table 1.

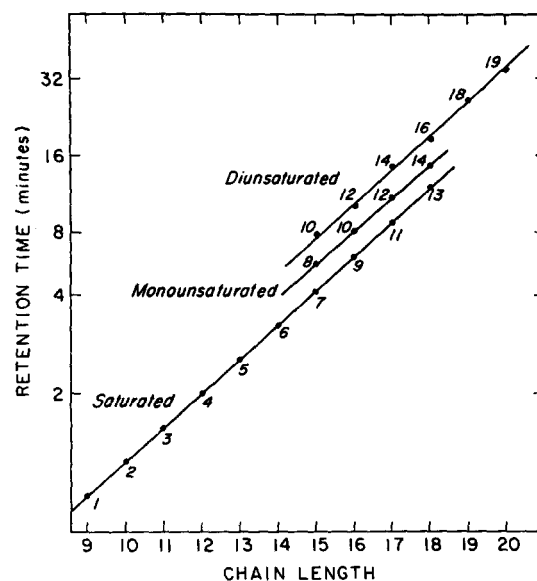


FIG. 4. Semilogarithmic plot of the retention times of the methyl esters of the saturated, monounsaturated, and diunsaturated fatty acids vs. the number of carbon atoms in the molecule. The retention times were taken from Figs. 3, 5, and 6. The numbers identifying each point are the numbers of the peaks on those figures.

chain length of the molecules (Fig. 4). Differences in location of the double bond do not appear to have had a measurable effect on retention times.

Diunsaturated Fatty Acids

The chromatographic pattern of this fraction is shown in Fig. 6, and the identification of the components is sum-

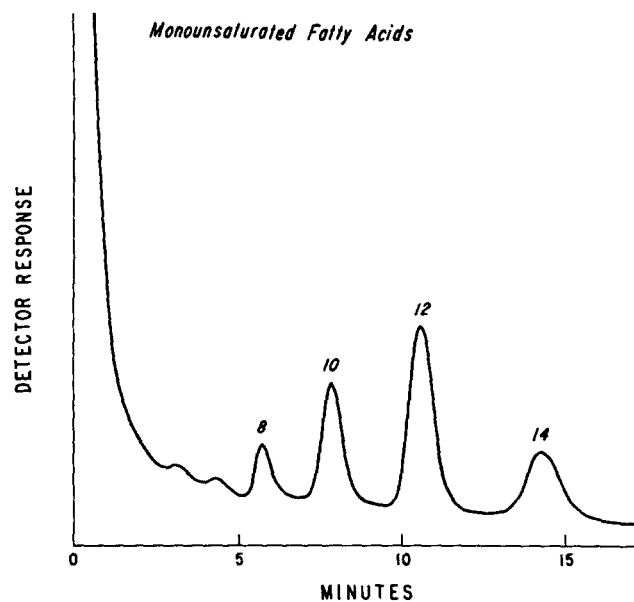


FIG. 5. Gas-liquid chromatogram of the methyl esters of the monounsaturated fatty acids of *E. gracilis*. Conditions were the same as in Fig. 1. The peaks are numbered as the peaks with the same retention times in Fig. 1. The components of each peak are identified in Table 2.

TABLE 2 MONOUNSATURATED FATTY ACIDS OF *E. Gracilis*

Peak*	% of Total	Relative Retention Time†	Hydrogenation Product	Oxidation Products		Structure of Fatty Acid	Proportion of Isomers
				Dicarboxylic	Monocarboxylic		
5A	—	0.266	13:0	C ₅ , C ₇ , C ₉	C ₈ , C ₆ , C ₄	13:1[5] 13:1[7] 13:1[9]	3 3 1
6A	—	0.358	14:0	C ₅ , C ₇ , C ₉	C ₉ , C ₇ , C ₅	14:1[5] 14:1[7] 14:1[9]	8 2 1
8	0.3	0.480	15:0	C ₇ , C ₉	C ₈ , C ₆	15:1[7] 15:1[9]	2.5 1
10	2	0.658	16:0	C ₇ , C ₉ , C ₁₁	C ₉ , C ₇ , C ₅	16:1[7] 16:1[9] 16:1[11]	5 5 1
12	2.0	0.888	17:0	C ₉	C ₈	17:1[9]	
14	0.9	1.196	18:0	C ₉ , C ₁₁	C ₉ , C ₇	18:1[9] 18:1[11]	9 1
16	—	1.642	19:0	C ₁₁	C ₈	19:1[11]	

* Peaks refer to Fig. 5. Peaks 5A, 6A, and 16 were not detectable in the original chromatogram (see text).

† Relative to methyl stearate under conditions as in Fig. 1.

marized in Table 3. Diunsaturated fatty acids of chain length C₁₆ through C₂₀ were present with two isomers of each of the odd-numbered fatty acids. The plot of the logarithms of their retention times vs. chain length (Fig. 4) is again linear, although the points do not all lie perfectly on the line. This may be due to some influence of the positions of the double bonds within the molecule. This effect will be seen to be very pronounced in fatty acids with three and more double bonds.

Triunsaturated Fatty Acids

The triunsaturated fatty acids are shown in Fig. 7, and identified in Table 4. Peak 17 was identified as 17:3 [9,12,15] because the product of hydrogenation was

17:0, and the only dicarboxylic acid produced by oxidation was azelaic. The absence of a monocarboxylic acid in the oxidation products is not unexpected, since methyl acetate, the expected product, would not have been recovered by the methods used.

Peak 19 has the retention time of 19:3, but it was not identified. Not apparent in Fig. 7 is another component with a retention time corresponding to peak 12 of Fig. 1. This peak appeared when a larger aliquot of the fraction was chromatographed at a lower temperature. It has been identified only as a 15-carbon acid.

The logarithms of the retention times of 16:3[7,10,13], 18:3[9,12,15], and 20:3[11,14,17] form a straight line when plotted against their chain lengths (Fig. 8) as pre-

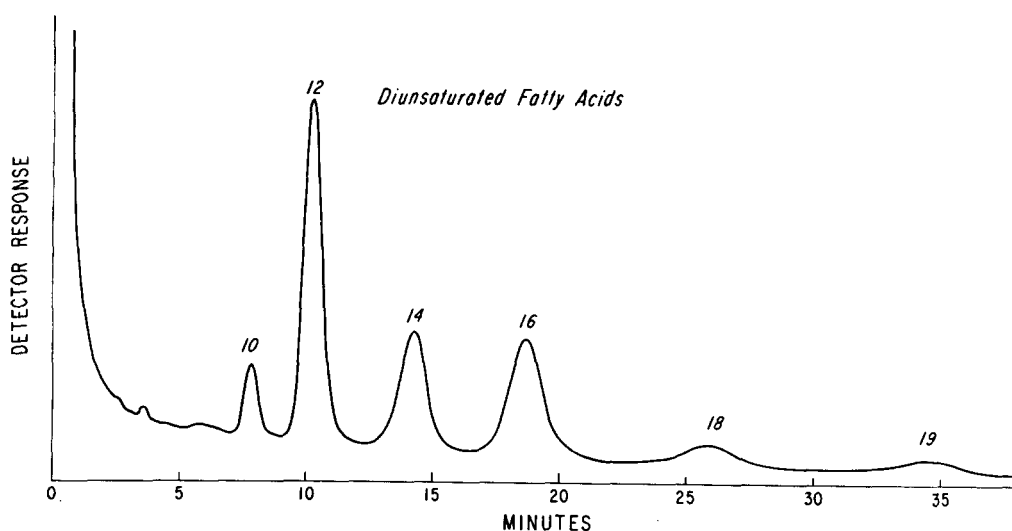


FIG. 6. Gas-liquid chromatogram of the methyl esters of the diunsaturated fatty acids of *E. gracilis*. Conditions were the same as in Fig. 1. The peaks are numbered as the peaks with the same retention times in Fig. 1. The components of each peak are identified in Table 3.

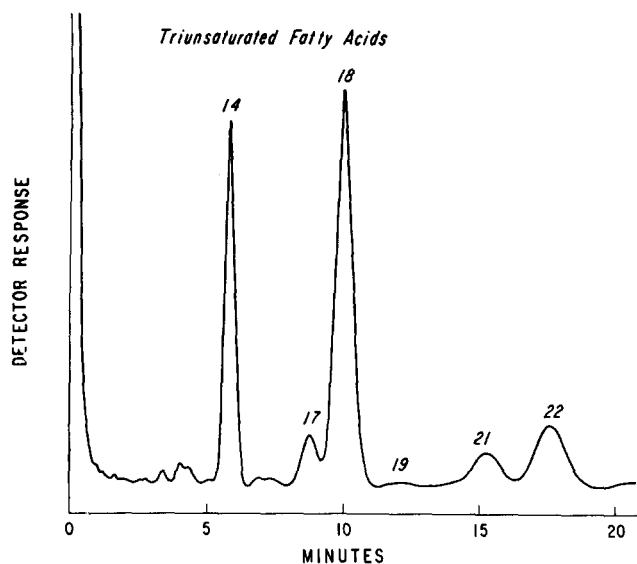


FIG. 7. Gas-liquid chromatogram of the methyl esters of the triunsaturated fatty acids of *E. gracilis*. Conditions were the same as in Fig. 2. Peaks are numbered as the peaks with the same retention times in Fig. 2. Peak 14 is not shown on Fig. 2. The components of each peak are identified in Table 4 and in the text.

dicted (18) for fatty acids homologous with respect to the distance of the last double bond from the methyl end of the molecule. The retention time of 20:3[8,11,14] falls below this line, and the retention time of 17:3[9,12,15] falls above this line, as expected.

Tetra-, Penta-, and Hexaunsaturated Fatty Acids

Most of the fatty acids in Fig. 9 are characterized in Table 5. Peak 24 was found to contain two fatty acids, 21:4[7,10,13,16] and 20:5[5,8,11,14,17]. Fatty acids 16:4[4,7,10,13] and 20:4[8,11,14,17] are homologous with respect to the positions of the double bonds from the methyl end of the molecules. The plot of the logarithms of their retention times against chain length is shown in Fig. 8. Peak 19 was not identified, but the fact that the logarithm of its retention time falls on that line (Fig. 8)

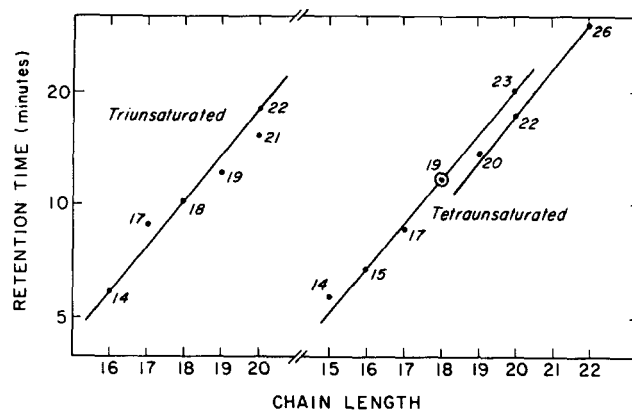


FIG. 8. Semilogarithmic plot of the retention times of the methyl esters of the triunsaturated and tetraunsaturated fatty acids vs. the number of carbon atoms in the molecule. The retention times were taken from Figs. 7 and 9. The numbers identifying each point are the numbers of the peaks in those figures. Peak 19 was not identified but its retention time indicates it may be 18:4[6,9,12,15] (see text).

precisely at the position of an 18-carbon acid suggests that it may be 18:4[6,9,12,15].

Peak 14 was identified by the product of hydrogenation, and the dicarboxylic acid produced by oxidative degradation. No monocarboxylic acid was found, but that is to be expected if the product were acetate. The retention time of peak 14 does fall above the line connecting 16:4[4,7,10,13] and 20:4[8,11,14,17] (Fig. 8), as is predicted for the indicated structure.

Peak 17 gave 17:0 as the only product of hydrogenation, but was not further identified. Peak 18 is probably due to contaminating 18:3[9,12,15]; it formed 18:0 upon hydrogenation.

As might be expected, the retention time of 19:4[5,8,11,14] falls just above the line drawn through the retention times of 20:4[5,8,11,14] and 22:4[7,10,13,16] (Fig. 8). The retention time of peak 21:4[7,10,13,16] probably also falls above this line, but the precise retention time of this acid is not known because it was never separated from 20:5[5,8,11,14,17].

TABLE 3 DIUNSATURATED FATTY ACIDS OF *E. Gracilis*

Peak*	% of Total	Relative Retention Time†	Hydrogenation Product	Oxidation Products		Structure of Fatty Acid	Ratio
				Dicarboxylic	Monocarboxylic		
10	0.5	0.651	15:0	C ₄ , C ₇	C ₈ , C ₅	15:2[4,7] 15:2[7,10]	1 5
12	3	0.855	16:0	C ₇	C ₆	16:2[7,10]	
14	2	1.19	17:0	C ₇ , C ₉	C ₇ , C ₅	17:2[7,10] 17:2[9,12]	1 3
16	3	1.55	18:0	C ₉	C ₆	18:2[9,12]	
18	1	2.17	19:0	C ₉ , C ₁₁	C ₇ , C ₅	19:2[9,12] 19:2[11,14]	1 2
19	2	2.87	20:0	C ₁₁	C ₆	20:2[11,14]	

* Peaks refer to Fig. 6.

† Relative to methyl stearate under conditions as in Fig. 1.

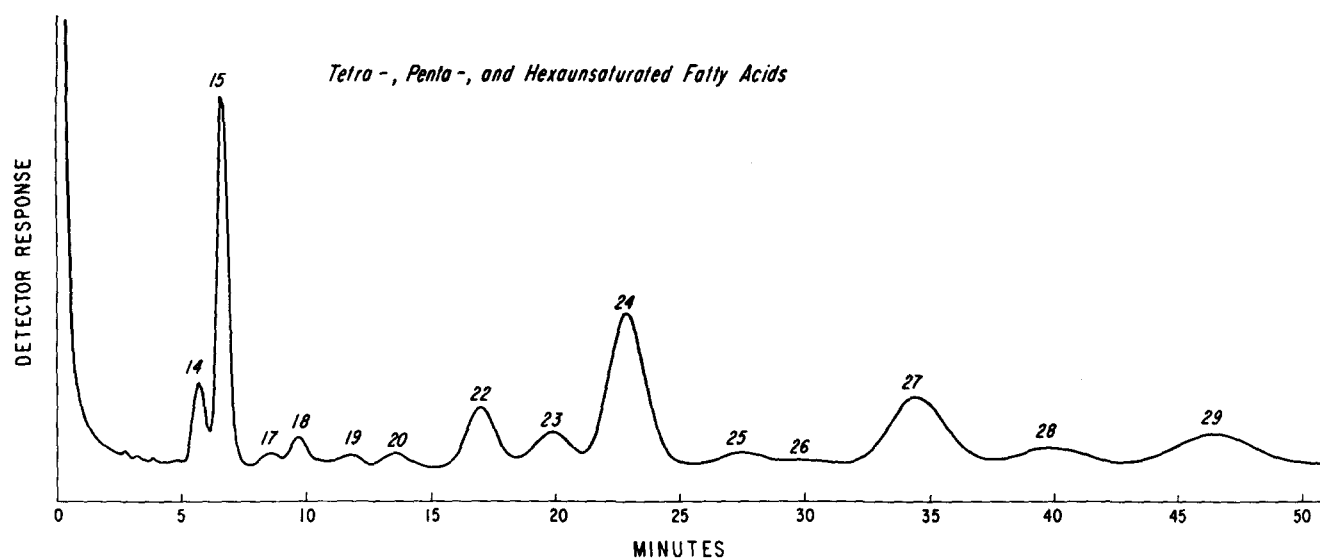


FIG. 9. Gas-liquid chromatogram of the methyl esters of the tetra-, penta-, and hexaunsaturated fatty acids of *E. gracilis*. Conditions were the same as in Fig. 2. Peaks are numbered as the peaks with the same retention times in Fig. 2. Peaks 14 and 15 are not shown in Fig. 2. The components of each peak are identified in Table 5.

For unexplained reasons, the relative area of peak 26 in Fig. 9 is less than the area of peak 26 in Fig. 2. No component with the retention time of peak 26 was found in any other adduct fraction.

Other Structural Studies

The ultraviolet absorption of all the polyunsaturated fatty acids was determined and in no case was there any indication of the presence of conjugated double bonds (19). Infrared spectra were obtained for the following fractions: 16:1, 18:1, 16:2[7,10], 18:2[9,12], 16:3[7,10,13], 18:3[9,12,15], 20:3[8,11,14], and all the tetra-, penta-, and hexaunsaturated acids except 15:4[4,7,10,13]. In no case was there any significant absorption at 965 cm^{-1} indicative of the *trans*-configuration (20). Thus, all double bonds are apparently of the *cis*-configuration.

The retention times of the following fatty acids were identical with those of standards on ethylene glycol succinate: all the saturated fatty acids, 16:1, 18:1, 18:2[9,12], 20:2[11,14], 18:3[9,12,15], 20:3[8,11,14], 20:4[5,8,11,14], 20:5[5,8,11,14,17], and 22:6[4,7,10,13,

16,19]. Standard fatty acids 20:5[5,8,11,14,17] and 22:6[4,7,10,13,16,19] were obtained from the Lipid Standards Program, National Heart Institute; 20:2[11,14] and 20:3[8,11,14] were isolated from *Acanthamoeba* (3); the other standards were purchased from Applied Science Laboratories, Inc., State College, Pa.

DISCUSSION

Structural and Biosynthetic Aspects

The fatty acids that have been characterized are summarized in Table 6, in which the percentage composition by chain length and unsaturation is also tabulated. By reference to Tables 1–5, it can be seen that the fatty acids in highest concentration are 13:0 and 14:0 followed by 16:3[7,10,13] and 18:3[9,12,15]. Most of the other fatty acids account for 1 to 3% of the total. Although many of the acids have not been previously observed in nature, all are of the usual type; the double bonds are of the *cis*-configuration, and the double bonds of the polyunsaturated acids are in methylene-interrupted sequences. The fatty acids of *Euglena gracilis* are unusual only in the

TABLE 4 TRIUNSATURATED FATTY ACIDS OF *E. Gracilis*

Peak*	% of Total	Relative Retention Time †	Hydrogenation Product	Oxidation Products		Structure of Fatty Acid
				Dicarboxylic	Monocarboxylic	
14	7	1.19	16:0	C ₇	C ₈	16:3[7,10,13]
17	1.5	1.76	17:0	C ₉	—	17:3[9,12,15]
18	8.5	2.07	18:0	C ₉	C ₃	18:3[9,12,15]
21	1.5	3.15	20:0	C ₈	C ₆	20:3[8,11,14]
22	2	3.58	20:0	C ₁₁	C ₃	20:3[11,14,17]

* Peaks refer to Fig. 7.

† Relative to methyl stearate under conditions as in Fig. 2.

TABLE 5 TETRA-, PENTA-, AND HEXAUNSATURATED FATTY ACIDS OF *E. Gracilis*

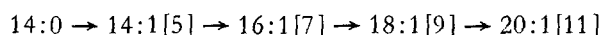
Peak*	% of Total	Relative Retention Time†	Hydrogenation Product	Oxidation Products		Structure of Fatty Acid
				Dicarboxylic	Monocarboxylic	
14	0.6	1.19	15:0	C ₄	—	15:4[4,7,10,13]
15	3	1.38	16:0	C ₄	C ₃	16:4[4,7,10,13]
20	6	2.76	19:0	C ₅	C ₅	19:4[5,8,11,14]
22	3	3.46	20:0	C ₅	C ₆	20:4[5,8,11,14]
23	2	4.06	20:0	C ₈	C ₃	20:4[8,11,14,17]
24	1.0 } 4.0 }	4.66	21:0 } 20:0 }	C ₇	C ₅	21:4[7,10,13,16]
				C ₅	C ₃	20:5[5,8,11,14,17]
25	1.5	5.60	21:0	C ₄	C ₆	21:5[4,7,10,13,16]
26	0.7	6.08	22:0	C ₇	C ₆	22:4[7,10,13,16]
27	4	7.04	22:0	C ₄	C ₆	22:5[4,7,10,13,16]
28	1	8.17	22:0	C ₇	C ₃	22:5[7,10,13,16,19]
29	2	9.48	22:0	C ₄	C ₃	22:6[4,7,10,13,16,19]

* Peaks refer to Fig. 9.

† Relative to methyl stearate under conditions as in Fig. 2.

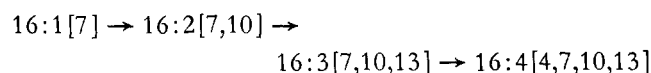
large variety present in one organism, and in the fact that they include fatty acids that in higher species are restricted either to plants or animals.

The conceivable biosynthetic relationships among these fatty acids are many. The monounsaturated acids might all be formed by direct desaturation of the corresponding saturated fatty acids. Alternatively, one can arrange many of the monounsaturated fatty acids into homologous series in which the first monounsaturated fatty acid might be formed by desaturation and the others by elongation of it. For example:

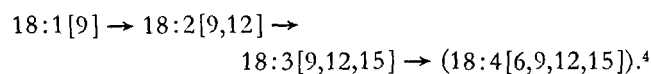


A third possibility is the mechanism proposed by Goldfine and Bloch (21) for the synthesis of bacterial fatty acids. In this scheme, the double bond is introduced initially at the β,γ -position, and retained during the process of chain elongation so that the homologous series represents intermediates of one reaction pathway.

Similarly, several alternative biosynthetic schemes are conceivable for the polyunsaturated fatty acids. For example, an obvious possibility is:



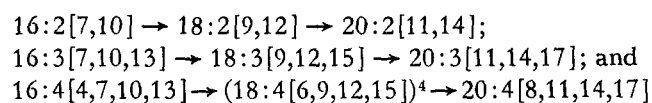
and the parallel:



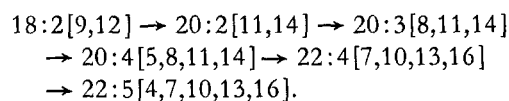
The same biosynthetic sequence can be envisaged for the 15-carbon, 17-carbon, and 19-carbon fatty acids. On the other hand, biosynthesis might proceed in the antithetical

⁴ The presence of this acid in *E. gracilis* has not been proved. There is an unidentified tetraenoic fatty acid with the retention time of 18:4[6,9,12,15].

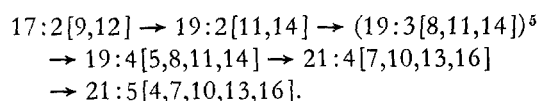
fashion:



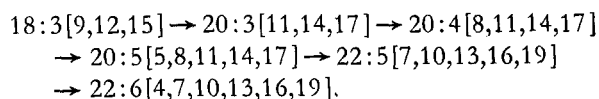
The 20-carbon and 22-carbon fatty acids can be arranged into biosynthetic patterns that have been described for other organisms (3,22,23). In these pathways new double bonds are always inserted on the carboxyl side of the existing double bonds. One such homologous series is:



This pathway to 20:4[5,8,11,14] has been proven in *Acanthamoeba sp.* (3), and the further reactions probably occur in higher animals. A strictly analogous pathway can be written for the 19-carbon and 21-carbon fatty acids:



The remainder of the 20-carbon and 22-carbon fatty acids constitute another homologous series whose synthesis might be as follows:—

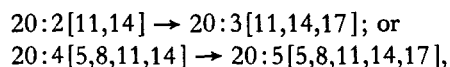


Most of these reactions have been indicated to occur in higher animals (24).

Although the above pathways seem reasonable, it should be emphasized that there is no cause for eliminat-

⁵ The presence of this acid in *E. gracilis* has not been proved. There is a trienoic acid with the appropriate retention time.

ing the possibility of other conversions:



for example, which involve desaturation on the methyl terminal side of existing double bonds.

Taxonomic and Phylogenetic Aspects

Too few protists have yet been examined to know whether the polyunsaturated fatty acids will provide a useful phylogenetic tool. One conclusion is clear, however. The ability to synthesize all the known polyunsaturated fatty acids is present in some of the most primitive organisms, whereas higher species are much more limited. *Euglena gracilis* synthesizes the widest variety of fatty acids yet observed, and the closely related organism, *Ochromonas danica* (25) is also very versatile. The very few other protists that have been studied are much more restricted in their biosynthetic capabilities. *Acanthamoeba* (3, 15) cannot synthesize 18:3[9,12,15] or any of the 20-carbon and 22-carbon fatty acids derived from it. The amebas do convert 18:2[9,12] to 20:4[5,8,11,14], but there is no evidence for further elongation and desaturation. *Leishmania*⁶ have the ability to synthesize 18:3[9,12,15]

⁶ This is true for *L. enriettii* (3) and *L. terantolae* (E. D. Korn and C. L. Greenblatt, unpublished observation).

but do not convert either it, or 18:2[9,12], to 20-carbon acids. It is conceivable that this inability to synthesize polyunsaturated 20- and 22-carbon fatty acids is related to the parasitic mode of life of *Leishmania*. Finally, it is perhaps meaningful that the only organism other than *E. gracilis* known to contain 16:4[4,7,10,13] is the alga, *Chlorella* (26); the green algae are probably closely related to the phytoflagellates (6).

One must recognize, of course, that knowledge of the fatty acid composition of organisms may be insufficient in itself to develop unequivocal phylogenetic relationships. One may also need to know the pathways by which the fatty acids are synthesized. Enzymes are the direct genetic products, and analyses of the products of enzymatic action are of phylogenetic significance only in so far as they define enzymatic composition. Particular caution is necessary with regard to unsaturated fatty acids for which alternate biosynthetic routes are not only a theoretical possibility, but a reality. Monounsaturated fatty acids, for example, are synthesized in yeast by direct desaturation of the appropriate saturated fatty acid (27), but in certain bacteria by insertion of the double bond at an earlier stage during the process of chain elongation (21). The observation that two species contain similar or identical fatty acids does not, therefore, necessarily imply that their enzymatic and hence, genetic contents are similar.

TABLE 6 THE FATTY ACIDS OF *E. Gracilis* ARRANGED BY CHAIN LENGTH AND UNSATURATION

					%	
9:0					0.2	
10:0					0.2	
11:0					1	
12:0					3	
13:0	13:1[5]					
	13:1[7]				13	
	13:1[9]					
14:0	14:1[5]					
	14:1[7]				10	
	14:1[9]					
15:0	15:1[7]	15:2[4,7]				
	15:1[9]	15:2[7,10]	15:3*	15:4[4,7,10,13]	3	
16:0	16:1[7]	16:2[7,10]	16:3[7,10,13]	16:4[4,7,10,13]		
	16:1[9]				22	
	16:1[11]					
17:0	17:1[9]	17:2[7,10]				
		17:2[9,12]	17:3[9,12,15]	(17:4)†	7	
18:0	18:1[9]	18:2[9,12]	18:3[9,12,15]	(18:4)†	13	
	18:1[11]					
	19:1[11]	19:2[9,12]			2	
		19:2[11,14]	(19:3)†	19:4[5,8,11,14]		
		20:2[11,14]	20:3[8,11,14]	20:4[5,8,11,14]	15	
			20:3[11,14,17]	20:4[8,11,14,17]		
				20:5[5,8,11,14,17]		
				21:4[7,10,13,16]	3	
				21:5[4,7,10,13,16]		
				22:4[7,10,13,16]		
				22:5[4,7,10,13,16]		
				22:5[7,10,13,16,19]	8	
%	38	5	12	21	11	2

* Positions of double bonds not known.

† Only tentatively identified.

The converse of this consideration is that very great differences in fatty acid composition might result from the loss of only one enzyme if it catalyzed an early reaction in a synthetic pathway. This would lead to great taxonomic difficulties if one were, for example, unknowingly determining the fatty acid composition of a mutant organism.

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REFERENCES

1. Hilditch, T. P. *The Chemical Constitution of Natural Fats*. John Wiley and Sons, Inc., New York, 1956.
2. Shorland, F. B. In *Comparative Biochemistry*, edited by M. Florkin and H. S. Mason. Academic Press, Inc., New York, 1962, Vol. 3, p. 1.
3. Korn, E. D. *J. Biol. Chem.* **239**, 1964, in press.
4. Korn, E. D., and C. L. Greenblatt. *Science* **142**: 1301, 1963.
5. Davidoff, F., and E. D. Korn. *J. Biol. Chem.* **238**: 3199, 1963.
6. Wolken, J. J. *Euglena: An Experimental Organism for Biochemical and Biophysical Studies*. Rutgers University Press, New Brunswick, 1961, p. 5.
7. Erwin, J., and K. Bloch. *Biochem. Biophys. Res. Commun.* **9**: 103, 1962.
8. Rosenberg, A. *Biochemistry* **2**: 1148, 1963.
9. Korn, E. D. *Biochem. Biophys. Res. Commun.* **14**: 1, 1963.
10. Greenblatt, C. L., and J. A. Schiff. *J. Protozool.* **6**: 23, 1959.
11. Morgan, T. E., D. J. Hanahan, and J. Ekholm. *Federation Proc.*, **22**: 414, 1963.
12. Mangold, H. K. *J. Am. Oil Chemists' Soc.* **38**: 708, 1961.
13. Erwin, J. and K. Bloch. *J. Biol. Chem.* **238**: 1618, 1963.
14. De Vries, B. *J. Am. Oil Chemists' Soc.* **40**: 184, 1963.
15. Korn, E. D. *J. Biol. Chem.* **238**: 3584, 1963.
16. von Rudloff, E. *Can. J. Chem.* **34**: 1413, 1956.
17. Karmen, A., L. Giuffrida, and R. L. Bowman. *J. Lipid Res.* **3**: 44, 1962.
18. Ackman, R. G. *Nature* **194**: 970, 1962.
19. Herb, S. F., and R. W. Riemenschneider. *Anal. Chem.* **25**: 953, 1953.
20. Ahlers, N. H. E., R. A. Brett, and N. G. McTaggart. *J. Appl. Chem. (London)* **3**: 433, 1953.
21. Goldfine, H., and K. Bloch. *J. Biol. Chem.* **236**: 2596, 1961.
22. Stoffel, W. *Z. Physiol. Chem.* **333**: 71, 1963.
23. Mead, J. F. *Federation Proc.* **20**: 952, 1961.
24. Klenk, E., and H. Mohrhauer. *Z. Physiol. Chem.* **320**: 218, 1960.
25. Haines, T. H., S. Aaronson, J. C. Gellerman, and H. Schlenk. *Nature* **194**: 1282, 1962.
26. Paschke, R. F., and D. H. Wheeler. *J. Am. Oil Chemists' Soc.* **31**: 81, 1954.
27. Bloomfield, D. K., and K. Bloch. *J. Biol. Chem.* **235**: 337, 1960.