THE POLYUNSATURATED 20-CARBON AND 22-CARBON FATTY ACIDS OF EUGLENA

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Higher plants synthesize linoleic acid and α-linolenic acid while higher animals synthesize neither of these two fatty acids but do convert both of them to polyunsaturated 20-carbon and 22-carbon fatty acids. The essential difference is that plants, starting with oleic acid, produce a system of methylene-interupted double bonds that approaches the methyl terminal end of the fatty acid whereas animals introduce new double bonds so as to progress towards the carboxyl end of the molecule.

Recently, I have found (Korn, in press) that Acanthamoeba synthesize arachidonic acid by the pathway:

18:0[1] ⟶ 18:1[9] ⟶ 18:2[9,12] ⟶ 20:2[11,14] ⟶ 20:3[8,11,14] ⟶ 20:4[5,8,11,14].

This observation, together with the recent finding (Haines et. al., 1962) that Ochromonas danica (a photosynthetic protist) contains, among other fatty acids, 18:2[9,12], 18:3[9,12,15], 20:3[8,11,14] and 20:4[5,8,11,14] lead to the hypothesis (Korn, in press) that the primitive protists, thought to be the common precursor of plants and animals, might have been able to synthesize both linoleic and α-linolenic acids and the polyunsaturated 20- and 22-carbon fatty acids. Evolution of plants might then have been accompanied by loss of ability to synthesize the polyunsaturated 20- and 22-carbon acids while animals lost the ability to

[1] The first number refers to the number of carbon atoms in the chain, the second to the number of double bonds, and the numbers in brackets to the positions of the double bonds counting from the carboxyl group.

synthesize α-linolenic acid and, in some instances, linoleic acid. With this in mind, I have investigated the fatty acid composition of Euglena, a phytoflagellate thought to be closely related to the primitive protists.

Euglena gracilis strain Z (American Type Culture #12716) was grown[2] in light on Hutner's pH 3.3 medium in which the only carbon sources are glutamic acid, malic acid, and CO_2 (Greenblatt and Schiff, 1959). After 5 to 10 days growth the cells were harvested, washed, and extracted with chloroform-methanol(2:1). The lipid extract was evaporated to dryness, the lipids were extracted from the residue into chloroform and were then transesterified in 0.5 N methanolic NaOH (Morgan et. al., 1963). The methyl esters of the fatty acids were extracted into petroleum ether and analyzed by gas-liquid chromatography on ethylene glycol succinate polyester.

At least 35 fatty acids were found with chain length greater than 9 carbons. Not all of these are separable upon gas-liquid chromatography of the total mixture, but appear after separation of the fatty acids into classes of different degrees of unsaturation. All the odd and even numbered saturated fatty acids from C9 to C19 are present as also are 15:1[9], 16:1[9], 17:1[9], 18:1[9], 18:1[11], 18:2[9,12] and 18:3[9,12,15][3]. Preliminary evidence has also been obtained for the presence of 16:2[7,10], 16:3[7,10,13] and 16:4[4,7,10,13]. The details of the complete identification of all of these fatty acids will be published elsewhere. In this paper I will discuss only the polyunsaturated 20- and 22-carbon fatty acids shown in the partial chromatogram in Fig. 1.

The mixture of fatty acid methyl esters was separated by silicic acid chromatography of the mercuric acetate adducts into five classes (Erwin and Bloch, 1963). Each fatty acid methyl ester was then isolated

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[2] I am indebted to Dr. Greenblatt for the Euglena.

[3] The presence in E. gracilis Z of 14:0, 16:0, 16:1[9], 18:0, 18:1[9], 18:2[9,12] and 18:3[9,12,15] has been previously reported by Erwin and Bloch (1962).

in at least 90% purity, and in amounts varying from 1 to 6 mg, by preparative chromatography on ethylene glycol succinate polyester. Samples were hydrogenated and the saturated fatty acid methyl esters identified by comparison of their retention times to standards. The number of double bonds was deduced from the chromatographic behavior of the mercuric acetate adducts and the relative retention times of the original acids knowing their chain lengths. In addition, 20:2[11,14], 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] were shown to have retention times identical to standards. The retention times of the other fatty acids fell on the appropriate straight lines when plotted according to Ackman (1962). The positions of the double bonds in each fatty acid were confirmed by identification of the monocarboxylic and dicarboxylic acids produced by permanganate-periodate oxidation (von Rudloff, 1956). The absence of any conjugated double bonds was demonstrated by the lack of absorption in the ultraviolet region typical of such configuration (Herb and Riemenschneider, 1953). All double bonds in each acid were of the cis-configuration as shown by the absence of the C-H deformation at 965 cm^{-1} (Ahlers et. al., 1953).

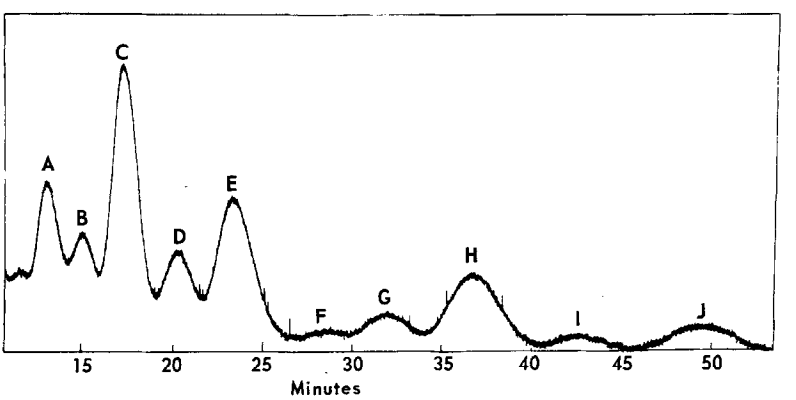
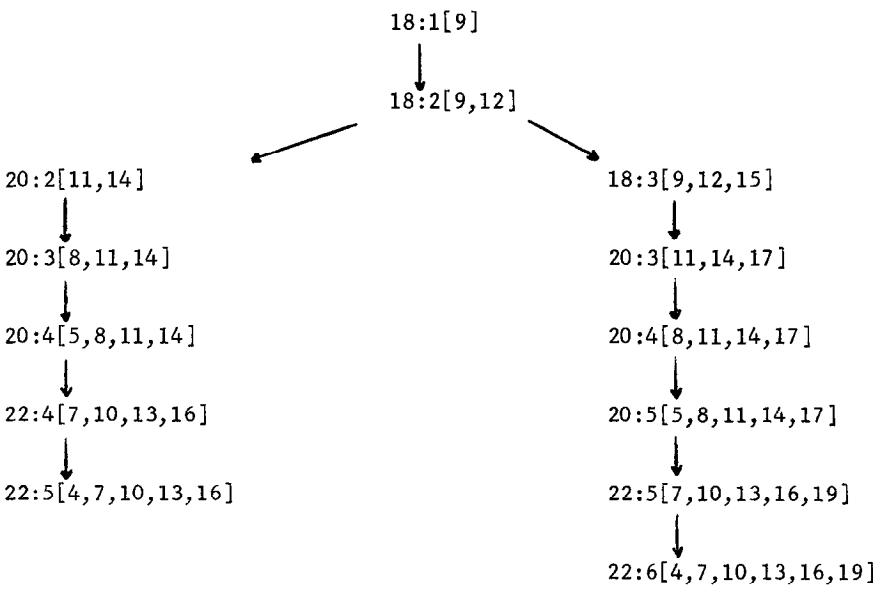


Fig. 1 - Partial gas-liquid chromatogram of the methyl esters of the fatty acids of E. gracilis Z to show the polyunsaturated 20 and 22 carbon fatty acids. The liquid phase was 17% ethylene glycol succinate on Gaschrom P; temperature was 197°; inlet pressure 14 p.s.i. The fatty acids are identified in Table I.

It is apparent from the data in Table I that Euglena synthesize polyunsaturated acids of both the "linoleate series" and the "linolenate series"[4]. The presence of 20:2[11,14] and 20:3[8,11,14] but no detectable 18:3[6,9,12][5] is presumptive evidence, but not proof, that arachidonic acid is synthesized in Euglena by the pathway shown to occur in Acanthamoeba rather than the partially different pathway found in the rat by Mead (1961). The following diagram summarizes the structural relationships (and possibly, but not necessarily , the biosynthetic pathways) of the fatty acids identified in Table 1:



That Euglena synthesize all of the common fatty acids of higher plants and animals supports the initial hypothesis. It is well to be cautious, however, in relating such analyses to phylogeny. Two organisms may contain the same fatty acid but synthesize it by different paths. For example, there are now two known pathways to arachidonic acid, as

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[4] Erwin and Bloch (1962) reported the presence of fatty acids 20:2, 20:3, and 20:4 in E. gracilis Z. These were not further characterized but in a later paper (Erwin and Bloch, 1963), they imply that arachidonic acid is not synthesized by Euglena.

[5] Unpublished observation and Erwin and Bloch (1962).

TABLE I

IDENTIFICATION OF POLYUNSATURATED 20-CARBON AND 22-CARBON FATTY ACIDS OF EUGLENA GRACILIS Z

Peak	Adduct Fraction	Hydrogenation Product	Oxidation Products Dicarb	Oxidation Products Monocarb	Proposed Structure	Approximate % of Total Fatty Acids
A	Diunsat.	20:0	C11	C6	20:2[11,14]	2
B	Triunsat.	20:0	C8	C6	20:3[8,11,14]	1
C 1.	Triunsat.	20:0	C11	C3	20:3[11,14,17]	2
2.	Tetraunsat. and more	20:0	C5	C6	20:4[5,8,11,14]	4
D	"	20:0	C8	C3	20:4[8,11,14,17]	2
E	"	20:0	C5	C3	20:5[5,8,11,14,17]	6
F	"	21:0	C4	C5	21:5[4,7,10,13,16]	0.5
G	"	22:0	C7	C6	22:4[7,10,13,16]	1
H	"	22:0	C4	C6	22:5[4,7,10,13,16]	4
I	"	22:0	C7	C3	22:5[7,10,13,16,19]	1
J	"	22:0	C4	C3	22:6[4,7,10,13,16,19]	2

mentioned above, either or both of which might exist in a given species. On the other hand, the inability to detect a particular fatty acid in a given organism may only mean that one is dealing with a culture of a mutant strain deficient in a single enzyme present in the "wild type". When all this is said, however, there remains the intriguing possibility that knowledge of the patterns of polyunsaturated fatty acids, and the pathways by which they are synthesized, may be useful in establishing taxonomic and phylogenetic relationships, at least among the protists.

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