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Changes in the fatty acid composition of *Coscinodiscus eccentricus* with culture-age and salinity

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Abstract

The fatty acid composition of the diatom *Coscinodiscus eccentricus* Ehrenberg, has been examined at six stages in the growth cycle for cells maintained in four different salinity media (20, 25, 30 and 35 ‰) using gas-liquid chromatography. The predominant acids were those containing 16 carbon atoms (45 to 76 % of the total); the higher percentages were found at the later growth stages. Several growth/salinity changes in the individual fatty acid compositions are described, and attention is drawn to the changes in the ratio of the 16:0:16:1ω7 acids with growth in comparison with that previously published.

Introduction

Before the appearance of the papers by Ackman et al. (1968) and later by Cheucas and Riley (1969), who both studied the fatty acid composition of a wide range of marine phytoplankton species, the information on this subject was somewhat sparse and confused. This confusion mainly arose from the wide variety of techniques which were used in the preparation and analysis of the samples. The necessity for the standardisation of these techniques is now evident. The present study of the fatty acid composition of the lipids from cells of the marine diatom *Coscinodiscus eccentricus* Ehrenberg was undertaken as part of a larger project to investigate changes in the biochemical composition of this species during its growth cycle in culture, in four different salinity media, and the relevance of these changes to observed variations in the sinking rates of the cells. The analyses of the fatty acid composition were carried out according to the recommendations of Ackman (personal communication) and approximate those laid down by the American Oil Chemists' Society. They do, however, differ slightly from the standard procedure suggested later by Cheucas and Riley (1969), especially in the esterification procedure.

Ackman et al. (1968) investigated the fatty acids of 12 marine unicellular algae, including 4 species of diatoms, primarily in an attempt to detect some taxonomic differences. In some of the species which they used, they also investigated the variation of the fatty acid composition with culture-age and temperature, but none of these latter studies were applied to the diatom species. Cheucas and Riley (1969) similarly investigated 27 marine phytoplankton species, including 5 diatom species. Previous to these works, the main studies on marine diatom fatty acids were carried out by Klenk and Eberhagen (1962), Ackman et al. (1964), Kates and Volcani (1966), Kayama et al. (1963), Williams (1965) and Collier (1967). The latter three papers all reproduce rather incomplete analyses of the fatty acid composition and thus make any detailed comparisons difficult. Cheucas and Riley (1969) have, however, pointed out that the method of lipid extraction or purification, using non-polar solvents, as employed by Kayama et al. (1963) and Williams (1965), is unsatisfactory, and can lead to confusing and erroneous results. Ackman et al. (1964) studied the changes in the fatty acid composition of cells at five different stages in the growth cycle of *Skeletonema costatum* in culture in reference to their significance in the marine food chain, and it is with these results that the present work can best be compared. Kates and Volcani (1966) studied the variation of the fatty acid composition of three species of *Nitzschia*, and it is interesting to be able to compare variations in the fatty acid composition found within a single species, with the variation observed within a single genus.

Material and methods

Culturing conditions

A unialgal culture of the diatom *Coscinodiscus eccentricus* was grown in the laboratory from a single cell isolate of the species from Southampton Water. *C. eccentricus* is an important diatom in this area and is present in the phytoplankton for most of the year in relatively high proportions. The stock and experimental cultures were grown in constant temperature water baths at 20° ± 1 C° under continuous fluorescent illumination of 3500 to 4500 lux. The growth medium used was Guillard's *F* medium (1963) used at full strength. This is an enrichment medium and it was added to sea water which had been adjusted to 4 salinities, 20, 25, 30 and 35‰, by the addition of

distilled water. For these experiments, 3 l of the culture medium were sterilised in 5 l conical flasks and to these were added 100 ml of the stock cultures of the diatom for each particular salinity. 1 ml samples of the experimental cultures were removed daily for cell counts to be made and thus to determine the growth stage of the culture. Large samples, 500 ml or 1 l, of the culture were withdrawn at up to six stages during the normal growth cycle of the cells and used for the analysis of the fatty acid composition. Each stage represented a particular point in this cycle; namely (1), (2), (3) early, middle and late log phase; (4) the phase when the rate of cell division is decreasing towards zero; (5), (6) early and later stationary phase.

Preparation of the samples for gas-liquid chromatography

The 500 ml or 1 l samples from each experimental culture were filtered through 9.0 cm Whatman GF/C filter papers, which had previously been ashed at 500 °C, and the cells rinsed with isotonic ammonium formate. The filter paper was carefully rolled up and placed in a 4 ml siphoning volume Quickfit Soxhlet extractor and refluxed with chloroform:methanol (2:1 for up to 8 h, i.e., until the extracting volume was devoid of any coloration. Water was then added to the extract according to the procedure of FOLCH et al. (1957), and the upper layer, containing non-lipid material, discarded. After rinsing the interphase with equilibrated upper phase wash solution, the lower phase was evaporated to dryness under a stream of nitrogen at room temperature. It was hoped to be able to make weighings of these samples, but the yields were so small that no accurate measurements could be made with the apparatus available.

The lipid residue was saponified using 5 ml of 10% KOH in a 50% methanol solution containing quinol (GETZ and BARTLEY, 1961). The solution was refluxed for 2 h at 100 °C under an atmosphere of nitrogen, and then distilled water was added to the cooled solution. The non-saponifiable material was removed by shaking with 3 × 15 ml of petroleum ether (40° to 60 °C B.P.). After acidification with 5% sulphuric acid in methanol, the free fatty acids were extracted with a further 3 × 15 ml of petroleum ether. The combined extracts were washed with distilled water and evaporated to dryness under a stream of nitrogen.

Two ml of Boron trifluoride:methanol reagent ($\frac{1}{5}$ the strength of the BF_3:methanol reagent obtained from BDH Chemicals Ltd.) were added to the residue and the solution was refluxed for 5 min over a boiling water bath (METCALFE and SCHMITZ, 1961). The commercial reagent was diluted in this way, as LINFORD (personal communication) reported that the full strength reagent can cause some alterations in the fatty acid structures during the methylation procedure. The diluted reagent does not have this effect and is still as efficient a methylating agent.

The fatty acid methyl esters produced were extracted into petroleum ether, washed with distilled water to remove any traces of the methylating reagent, and then dried in an atmosphere of nitrogen, before being evaporated to a small volume in readiness for gas chromatography. The samples were analysed as soon as possible to avoid any oxidative changes in the acids during storage.

Gas-liquid chromatography

A Pye Series 104 Model 24 Dual Flame Hydrogen Ionisation Gas Chromatograph, connected to a 1 mv Honeywell recorder, was used. 5 feet (152 cm) glass columns (internal diameter 4 mm) were packed with 20% diethylene glycol succinate (DEGS) on 80 to 100 mesh HMDS Chromosorb W (obtained from Perkin Elmer Co. Ltd.). The columns, after preconditioning by heating at 185 °C with normal carrier-gas flow for 2 days, were maintained isothermally at 160 °C with 50 ml/min argon (inlet pressure 2100 g/cm²) passing through them. Samples (usually 10 µl) of a solution of the fatty acid methyl esters dissolved in petroleum ether were injected on to the column using a Hamilton or S.G.E. 10 µl microsyringe.

Various methods were used in the identification of the peaks obtained from the samples. The following fatty acids were commercially available as standards: 12:0, 14:0, 16:0, 18:0, 18:1ω9, 18:2ω6, 18:3ω3 and 22:0[1]. Using these methylated acids, the retention times of each could be calculated, and thence, using the 18:0 acid as a reference point, the relative retention values of the other acids could be calculated and compared with those in the literature. Values for σ (the standard deviation) could also be obtained for determination of the peak areas, according to the procedure of BARTLET and SMITH (1960). An analysis of cod-liver oil methyl esters was carried out and compared with known results (ACKMAN and BURGHER, 1964, 1965) as a further aid to identification, and then a comparison of relative retention times and separation factors from these two sets of results and from the samples was made (see ACKMAN, 1963a, b; ACKMAN and BURGHER, 1963; ACKMAN and JANGAARD, 1963; ACKMAN et al., 1963).

Finally, a mercuric acetate adduct separation of the fatty acids according to their degree of unsaturation was made (KORN et al., 1965). By all these methods it was possible to make tentative identifications of the fatty acids represented by each of the peaks obtained.

[1] This is part of a shorthand notation to identify each fatty acid. It gives details of the number of carbon atoms present in the molecule (1st number); the number of double bonds present (2nd number); and the number of carbon atoms from the centre of the ultimate double bond furthest from the carboxyl end group to the terminal methyl group of the fatty acid (3rd number).

Table 1. *Coscinodiscus eccentricus. Variation in the proportions of the individual fatty acids of cells with culture-age and salinity. t: trace amount. Sh: definite, but indeterminate shoulder on preceding or succeeding peak*

Tentative peak identification		20 ‰ S Growth stage					25 ‰ S Growth stage						30 ‰ S Growth stage						35 ‰ S Growth stage				
		1	2	3	4	5	1	2	3	4	5	6	1	2	3	4	5	6	1	3	4	5	6
Saturated																							
12:0		3.7	1.4	1.1	0.9	0.7	3.9	2.0	1.1	1.1	0.8	0.4	7.3	1.8	1.1	1.4	0.8	0.3	0.9	1.5	0.8	0.4	0.3
13:0		2.2	4.6	0.3	2.4	1.8	14.2	4.2	2.5	0.5	1.2	1.5	4.2	1.5	0.7	1.0	2.0	t	1.0	10.5	0.9	0.4	0.8
14:0		4.2	9.6	9.4	12.0	10.8	9.2	9.5	6.6	7.0	7.2	7.0	9.1	8.6	7.4	7.9	7.4	4.0	12.6	12.9	15.3	14.1	14.9
15:0		3.4	1.7	1.8	1.6	1.6	1.8	2.0	1.4	1.4	1.5	1.0	2.0	2.0	1.9	1.7	1.5	1.4	2.3	1.6	1.4	0.5	1.3
16:0		20.1	22.7	25.0	24.8	19.2	14.9	18.0	12.6	13.3	14.8	15.0	17.0	16.0	13.9	16.8	16.1	23.1	18.3	15.9	14.5	13.8	16.1
18:0		4.1	1.5	1.4	Sh	t	1.8	2.3	1.0	1.1	0.6	0.4	3.1	4.0	1.5	1.3	1.3	1.2	2.8	1.5	1.1	0.9	1.5
Mono-unsaturated																							
14:1ω5 ?	9	3.3	2.1	0.8	1.2	1.2	3.2	2.0	1.3	1.1	1.0	0.5	2.8	1.7	0.9	1.7	1.5	0.6	1.1	1.9	1.7	0.5	1.0
15:1ω ?[a]	?	2.9	0.0	0.0	1.2	Sh	1.4	0.9	1.7	1.1	0.7	1.3	1.0	1.2	1.0	0.6	1.2	0.3	0.9	0.8	1.3	0.1	0.3
16:1ω7	9	19.3	25.1	24.8	25.9	26.8	16.5	19.1	15.2	19.8	29.1	34.3	15.5	17.4	15.0	16.4	15.9	40.9	13.4	15.1	16.7	22.9	24.7
16:1ω4	12	Sh	—	—	t	t	Sh	t	—	Sh	—	—	—	—	—	—	—	—	Sh	—	—	—	—
18:1ω9	9	10.3	4.7	3.1	3.0	1.5	4.4	5.2	3.6	4.1	2.6	1.5	7.8	6.0	5.1	4.4	3.8	2.6	6.5	3.3	2.9	2.8	1.8
19:1ω ?	?	—	—	—	—	—	Sh	—	0.2	0.1	0.1	—	—	—	—	0.2	—	0.1	0.2	0.7	0.3	0.2	0.1
20:1ω9[a]	11	1.0	0.6	1.0	1.4	1.0	0.4	0.8	0.2	0.5	0.3	0.4	0.8	Sh	1.6	0.5	0.4	0.2	t	0.4	0.3	0.6	0.4
22:1ω9[a]	13	—	t	Sh	t	t	Sh	Sh	0.3	Sh	Sh	Sh	Sh	Sh	Sh	Sh	Sh	Sh	t	0.2	0.2	0.2	0.1
Polyunsaturated																							
16:2ω7	6,9	t	—	—	Sh	Sh	1.2	2.0	2.0	2.2	3.0	3.1	1.3	2.1	1.8	2.5	2.4	2.6	0.8	1.0	1.8	1.8	1.9
16:2ω4	9,12	3.7	3.2	3.0	3.5	1.8	4.0	5.2	4.6	5.5	6.4	5.8	4.1	5.5	4.4	5.8	5.3	4.6	4.5	4.0	5.5	4.5	4.5
16:3ω4	6,9,12	5.6	5.1	6.0	7.0	3.4	7.9	9.8	9.9	12.8	10.6	8.2	6.5	11.3	9.6	12.8	18.2	4.9	6.0	8.8	13.8	12.9	10.3
16:4ω1[b]	6,9,12,15	2.8	2.3	2.2	2.5	4.2	1.4	2.8	2.4	5.1	3.0	2.8	2.9	2.8	2.2	3.0	3.7	1.0	2.9	2.7	3.3	2.5	2.4
18:2ω6[b]	9,12	2.8	2.3	2.2	2.5	4.2	1.4	2.8	2.4	5.1	3.0	2.8	2.9	2.8	2.2	3.0	3.7	1.0	2.8	2.7	3.3	2.5	2.6
18:3ω6	6,9,12	1.1	0.4	0.1	Sh	1.0	0.7	0.8	0.4	0.6	1.0	0.5	0.7	0.5	t	0.5	0.8	0.4	1.6	1.3	0.3	0.5	0.5
18:3ω3	9,12,15	0.9	0.6	0.5	0.4	3.8	0.5	0.5	0.3	0.6	0.3	0.2	0.6	0.5	0.7	0.5	0.4	0.1	1.0	0.5	0.4	0.3	0.2
18:4ω3	6,9,12,15	1.9	1.7	1.7	1.4	0.2	1.1	1.0	0.6	0.7	0.5	0.6	1.0	1.0	1.5	0.9	0.7	0.5	1.4	0.8	0.7	0.8	0.8
20:2ω6	11,14	t	0.4	0.2	Sh	0.3	0.6	0.5	0.4	0.3	0.2	0.2	0.6	0.3	0.5	0.3	0.3	0.2	0.6	0.3	0.2	0.1	0.1
20:3ω6	8,11,14	0.7	0.4	0.5	0.4	0.4	0.5	0.3	0.6	0.4	0.3	0.1	0.6	Sh	0.4	0.2	0.2	0.1	0.5	0.3	0.3	0.2	0.2
20:4ω6	5,8,11,14	0.9	0.6	0.6	0.5	0.4	0.4	0.5	0.5	0.3	0.2	0.1	Sh	0.9	0.5	0.2	0.1	0.1	0.7	0.3	0.5	0.2	0.1
20:4ω3	8,11,14,17	0.9	0.5	0.4	0.4	0.4	0.5	0.6	0.6	0.3	0.2	0.2	0.9	0.6	0.6	0.4	0.4	0.2	1.0	0.3	0.3	0.3	0.2
20:5ω3	5,8,11,14,17	5.5	6.6	10.3	5.5	10.0	6.4	6.4	19.0	9.1	5.6	7.3	5.1	8.5	13.6	11.6	8.2	5.8	7.8	8.2	10.1	12.1	9.7
22:2ω6	13,16	—	t	Sh	Sh	0.4	t	0.7	0.7	0.4	0.3	0.2	Sh	0.4	4.4	Sh	0.4	0.3	1.4	0.4	0.2	0.2	0.2
22:3ω6	10,13,16	1.7	0.7	0.9	0.8	0.6	0.8	1.2	1.9	0.9	0.7	0.3	1.8	1.1	1.6	1.0	0.6	0.4	2.6	0.7	0.5	0.5	0.3
22:4ω6	7,10,13,16	—	0.5	1.5	0.9	2.1	1.0	t	2.8	2.0	2.3	1.5	0.8	1.9	1.2	1.5	2.1	1.7	0.9	1.2	1.7	1.1	1.7
22:5ω6	4,7,10,13,16	—	0.7	1.0	0.9	1.5	—	t	1.5	1.3	1.1	1.1	0.8	0.5	0.5	1.1	1.0	0.7	0.7	0.5	0.9	2.0	1.0
22:5ω3	7,10,13,16,19	—	t	0.7	0.6	0.9	—	t	1.9	1.5	0.8	1.3	0.8	0.9	0.9	0.8	0.6	0.6	0.7	0.6	0.9	0.8	1.1
22:6ω3	4,7,10,13,16,19	t	1.4	1.0	1.0	3.9	1.1	1.7	2.2	4.9	3.6	3.2	0.8	1.3	5.1	2.8	3.1	1.1	6.1	1.7	1.7	1.7	1.3

[a] Other isomers are probably present.
[b] Peaks 16:4ω 1 and 18:2ω 6 are coincident and have not been resolved into separate fractions.

Results and discussion

The results for the gas-liquid chromatographic analysis of the fatty acids derived from the chloroform:methanol extraction of cells of *Coscinodiscus eccentricus* are shown in Tables 1—3. The diatom cultures were grown at four salinities, namely 20, 25, 30 and 35‰, and the stages of the growth cycle at which the samples for analysis were taken, as indicated by the number of cells per millilitre, are shown in Table 4.

Table 2. *Coscinodiscus eccentricus. Percentage fatty acid composition for each chain length at different salinities*

Chain length	Growth stage 1	2	3	4	5	6
	20 ‰ S					
12	3.7	1.4	1.1	0.9	0.7	—
13	2.2	4.6	0.3	2.4	1.8	—
14	7.5	11.7	10.2	13.2	12.0	—
15	6.3	2.6	2.6	2.8	1.6	—
16	49.6	56.9	59.5	62.0	52.7	—
18	20.0	10.4	8.2	6.4	9.3	—
19	—	—	—	—	—	—
20	9.0	9.2	12.0	8.1	12.5	—
22	1.7	3.2	5.1	4.2	9.4	—
	25 ‰ S					
12	3.9	2.0	1.1	1.1	0.8	0.4
13	14.2	4.2	2.5	0.5	1.2	1.5
14	12.4	11.5	7.9	8.1	8.2	7.5
15	3.2	2.9	3.1	2.6	2.2	2.3
16	45.0	55.0	45.1	55.2	64.9	67.3
18	9.6	11.7	7.5	10.5	7.0	5.1
19	—	—	0.2	0.1	0.1	—
20	8.8	9.1	21.3	10.9	6.8	8.3
22	2.9	3.6	11.3	11.0	8.8	7.6
	30 ‰ S					
12	7.3	1.8	1.1	1.4	0.8	0.3
13	4.2	1.5	0.7	1.0	2.0	—
14	12.0	10.3	8.3	9.6	8.7	4.6
15	3.0	3.2	2.9	2.3	2.7	1.7
16	45.4	53.2	45.5	55.3	59.1	76.4
18	15.1	13.8	10.3	9.7	9.3	6.6
19	—	—	—	0.2	—	0.1
20	8.0	10.3	17.2	13.3	9.6	6.6
22	5.0	5.7	14.0	7.2	7.8	4.8
	35 ‰ S					
12	0.9	—	1.5	0.8	0.4	0.3
13	1.1	—	10.5	0.9	0.4	0.8
14	13.7	—	14.8	16.9	14.7	15.9
15	3.2	—	2.4	2.7	0.6	1.6
16	44.0	—	45.7	53.4	56.8	58.3
18	15.1	—	9.3	7.5	6.9	6.6
19	0.2	—	0.7	0.3	0.2	0.1
20	10.6	—	9.8	11.4	13.5	10.7
22	11.3	—	5.3	6.1	6.5	5.7

Table 1 shows the complete gas chromatographic analysis of the fatty acids at each growth stage and in each salinity. The amount of each acid is expressed as a percentage of the total as calculated from the individual peak areas of each acid on the chromatogram without any correction being made for retention time differences. The identification of each acid with its degree of unsaturation is given where sufficient evidence seemed to warrant it. Some doubt did arise as to the identification of some peaks in the C_{22} region of the chromatogram, and it was suspected that artifacts might be present. However, even though any peak identification without a mass spectrum is only tentative, the mercuric acetate separation of the acids showed that these peaks still appeared in the groupings within which they would be expected according to their degree of unsaturation, and consequently fatty acid structures were identified with them.

Table 3. *Coscinodiscus eccentricus. Ratio of the 16:0 to 16:1ω7 fatty acids in different salinities*

Salinity (‰)	Growth stage 1	2	3	4	5	6
20	1.04	0.90	1.01	0.96	0.72	—
25	0.90	0.94	0.83	0.67	0.51	0.44
30	1.09	0.92	0.93	1.02	1.01	0.56
35	1.36	—	1.05	0.87	0.60	0.65

An indication of the distribution of the different fatty-acid chain lengths in the diatom extracts is given in Table 2, where the total percentage of the fatty acids identified as having a given number of carbon atoms in their chain are shown for the different growth stages and salinities.

From the results shown in Tables 1 and 2, it is evident that the C_{16} group of acids are the predominant ones, representing from 44 to 76% of the total. Of all the acids the commonest ones are 14:0, 16:0, 16:1ω7, 16:2ω4, 16:3ω7, 16:4ω1, 20:5ω3 and 22:6ω3. The predominance of these acids, especially those with 16 carbon atoms, has been shown to be characteristic of diatoms, and the present results compare favourably with those previously published. There appear to be no significant differences between the fatty acid compositions of the cells grown in each salinity, although a few minor features of variation do appear, e.g. the presence or absence of the 19:1ω? acid. However, there are several changes in the relative composition of each acid during the growth cycle, some of which appear to show a salinity dependence.

The 12:0 (lauric) acid declines in its proportion throughout the growth cycle in all salinities. This is most marked in the 30‰ culture, where it decreases

from 7.1% at Stage 1 to 0.3% at Stage 6. This acid was not recorded by ACKMAN et al. (1964).

The 13:0 acid shows some interesting variations. This acid was not recorded by ACKMAN et al. (1964) or ACKMAN et al. (1968), but KATES and VOLCANI (1966) and CHEUCAS and RILEY (1969) found it to be present in very small amounts (< 1%). In *Coscinodiscus eccentricus* it normally occurs in the 0 to 2% range, but occasionally shows a marked increase to reach values of ca. 4% and on two occasions, in the earlier growth stages of the 25 and 35‰ cultures, rose to 14 and 10% of the total. An explanation for these high values is not obvious, but may be connected with the blockings and unblockings of the biosynthetic pathways leading to longer chain fatty acids as discussed by ACKMAN et al. (1964), although odd numbered carbon-chain fatty acids are not so important in this process and their position is somewhat obscure.

The 14:0 (myristic) acid is more variable in its percentage composition and shows some salinity/growth interrelationships. In the 20‰ culture there is a marked rise in the proportion of this acid as cell division commences, from 4.2 to 9.6%, whence it remains fairly constant. In the other salinities, the percentage remains fairly constant throughout the growth cycle, except in the later stages of the 30‰ one. The 35‰ culture has a higher percentage composition of this acid (12 to 15%) than the other cultures (7 to 12%). ACKMAN et al. (1964) observed a marked rise in the percentage of this acid as growth progressed (9.6 to 32.9%), which they connected with a possible blockage in the biosynthetic pathway of longer chain fatty acids. This apparently does not occur in *Coscinodiscus eccentricus*. A high level of 14:0 was again found for *Skeletonema costatum* by ACKMAN et al. (1968), but for the other diatoms they studied, and for those analysed by KATES and VOLCANI (1966) and by CHEUCAS and RILEY (1969) (including *S. costatum*), values ranging from 2.2 to 11.4% were found, and are thus quite comparable with *C. eccentricus*.

The C_{16} acids, which are by far the most important in diatoms, show several variations with culture age. The 16:0 (palmitic) acid remains fairly constant in its proportion throughout the growth cycle, with values of 12 to 18% of the total in all but the 20‰ culture, which had slightly higher values (19 to 25%). The 16:1ω7 acid is more variable, increasing its proportion throughout the growth cycle, starting from a level of 13 to 19% of the total, and reaching, in the stationary growth phase, values of 24 to 41%. The ratio of the two acids 16:0:16:1ω7 is shown in Table 3, and enables some comparisons to be made with other work. It can be seen clearly that this ratio drops from a value of ca. 1.0 (1.36 in the 35‰ culture) to ca. 0.5 in the senescent cultures. This conflicts with ACKMAN et al. (1964), who found a relative consistency in both these acids throughout the growth cycle, in the ratio of 0.5 with one another. This value can also be calculated from the results of KLENK and EBERHAGEN (1962) and KAYAMA et al. (1963). CHEUCAS and RILEY (1969) obtained values ranging from 0.44 to 0.57 for cells of various diatom species which were reaching the end of their log growth phase, represented by Stages 4 and 5 in *Coscinodiscus eccentricus*, and thus, these results are quite comparable with three of the present cultures, excepting the 30‰ one. The results of KATES and VOLCANI (1966), however, seem to indicate that there is no fixed relationship between

Table 4. *Coscinodiscus eccentricus. Increase in cell numbers of cultures in different salinities*

Day	20 ‰ S		25 ‰ S		30 ‰ S		35 ‰ S	
	Cells/ml	Sample	Cells/ml	Sample	Cells/ml	Sample	Cells/ml	Sample
0	127		298		298		175	
1	175		288		288		—	
2	200		545		800	1	215	
3	170		1040	1	945		243	
4	355	1	1835	2	1755	2	—	
5	425		3145		2900		920	1
6	440		4310	3	3870	3	1715	2
8	855		7555	4	6530	4	5235	3
10	1515	2	8145		7925	5	8765	4
11	1750		8330	5	8255		10965	
13	2720		7450		9075		11805	5
14	3470		—	6	—		11160	
15	4110	3			9950		11220	6
17	4890				8225	6		
19	5970	4						
24	7010	5						

these two acids, since the ratios which can be calculated for the 16:0:16:1 (double-bond position unidentified) acids vary between 0.3 for *Navicula pelliculosa* and 1.0 in *Nitzschia closterium*, for cells which were apparently in their log growth phase.

The 16:2 acids are quite constant in their percentages throughout the growth phase and are present in similar proportions, in similarity with previous reports. The 16:3ω4 acid, however, increases rapidly in its proportion during the division stages of the growth cycle, and then falls, sharply in the higher salinities, during the stationary phase. A similar feature was observed by ACKMAN et al. (1964), if one assumes that their last sample was taken just as the culture was becoming senescent. The variations in the 16:4ω1 acid are more difficult to assess due to the coincidence of this peak on the chromatogram with the 18:2ω6 acid.

The presence of high proportions of C_{16} unsaturated fatty acids is characteristic of diatoms and, although 16:0 often occurs in higher proportions in other algal classes, these other algae are more easily characterised by their unsaturated C_{18} acids, as indeed are most other plants. Thus, because of this high C_{16} percentage, there is a corresponding reduction in the C_{18} acid contribution. The major C_{18} acid in *Coscinodiscus eccentricus* is 18:1ω9, but this acid, like most of the other acids of this chain length, shows a decline from a moderately high value in the early growth stages to quite a small proportion at the end of the experiment. ACKMAN et al. (1964) also found a high level of 18:1ω9 (5%) at the first growth stage and then a decline, paralleled by all the C_{18} acids, to a much lower level as growth continued. These authors, however, found relatively high values of the 18:4ω3 acid (2 to 4%), while this acid appears to be only present in the 0.2 to 2% range in *C. eccentricus*. These latter values are more comparable with those given by ACKMAN et al. (1968) and by CHEUCAS and RILEY (1969).

The C_{20} acids are present as 6 to 21% of the total, and average about 11%. There is only one major acid, 20:5ω3, which represents almost all the total for the C_{20} acids. The proportion of these acids, or more especially the 20:5ω3 acid, is quite variable, but appears to reach a maximum during the division stages, ACKMAN et al. (1964) observed that the 20:5ω3 acid showed some reduction during the growth stages, while the 20:4ω6 and 22:6ω3 acids virtually disappeared; this is similar to the later part of the growth cycle of *Coscinodiscus eccentricus*. CHEUCAS and RILEY (1969) found values ranging from 13.4 to 30.3% for the 20:5ω3 acid in diatom species. The only major C_{22} acid is 22:6ω3, but, as ACKMAN et al. (1964) pointed out for *Skeletonema costatum*, this acid is present in surprisingly small amounts in comparison with the 20:5ω3 acid with which it is biosynthetically related.

There are several papers concerned with the effects of the nutritive condition of cultures of other algal classes on the fatty acid composition of the cells. Thus, PASCHKE and WHEELER (1954), MANGOLD and SCHLENK (1957) and SCHLENK et al. (1960) have observed that a high nitrogen level in the culture medium of the fresh-water chlorophyte *Chlorella pyrenoidosa* is associated with high levels of C_{16} and C_{18} tri- and tetraenoic acids. ACKMAN et al. (1968), however, found that a marine chlorophyte, *Dunaliella tertiolecta*, shows high C_{16} and low C_{18} polyunsaturated components in the early growth stages, presumably when nitrogen is more available, than at later stages, when the reverse is true, i.e., lower C_{16} and higher C_{18} polyunsaturated acids. These authors also measured the fatty acid composition at two growth stages of algae from two other classes, namely *Monochrysis lutheri* (Chrysophyceae) and *Porphyidium* sp. (Rhodophyceae) and found only very low and constant levels of these acids at both growth stages. In *Coscinodiscus eccentricus*, the major polyunsaturated C_{16} acid is 16:4ω3, and this is seen to increase its proportion as the culture ages, while the C_{18} polyunsaturated acids remain at fairly constant low proportions throughout. The observed decrease in the total C_{18} fatty acids is mainly due to a decrease in the 18:1ω9 acid. Thus, the effect of the nutritive state of the culture medium on the fatty acid composition of the cells seems to vary from one algal class to another. However, it is really necessary to consider the fatty acids of each individual group of lipids and their changes, rather than the fatty acids *in toto* before a proper interpretation of nutrient-linked changes in the fatty acid composition of a cell can be made (see BROCKERHOFF et al., 1964).

Summary

1. The diatom *Coscinodiscus eccentricus* EHRENBERG was grown in four different salinity media, namely 20, 25, 30 and 35‰.

2. The fatty acid composition of the cells at 6 growth stages in these media was studied using gas-liquid chromatographic techniques.

3. The predominant acids are the 16 carbon chain ones, which represent from 45%, at the beginning of the growth cycle, up to 76%, at the end of the growth cycle, of the total acids present.

4. The other major acids are: 14:0, 20:5ω3 and 22:6ω3, the 18 carbon atoms acids being present in characteristically low proportions.

5. Several growth/salinity changes in the individual fatty acids are noted.

6. The ratio of the 16:0 to the 16:1ω7 acids drops from ca. 1.0 at the first growth stage to 0.5 in the senescent cultures. This differs from the results for *Skeletonema costatum* (ACKMAN et al., 1964) who found the ratio to remain constant at 0.5.

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