FOREWORD

Marine contamination by petroleum, whether by natural seepage or by spills from ships at sea, by accidents in harbour or at offshore installations or by atmospheric or terrigenous input is by no means a new or rare phenomenon. In recent years however, the problems have been highlighted not only by the increased utilisation and marine transport of oil but also by a number of spectacular accidents which have raised questions about possible effects on the ecosystem. A number of detailed studies have been carried out in an attempt to answer these questions. The demands for such knowledge have been further increased by the various questions raised as a result of expansion of offshore exploration and exploitation for oil, particularly in environments hostile to these operations, in regions as far apart as the northern North Sea and the coast of Alaska.

Consequently, diverse aspects of the problem are being studied in several parts of the world by chemists and biologists who are often asking the same questions but using different approaches and sometimes producing conflicting views. Against this background, it seemed timely therefore to bring together a group of scientists from university, industry and government, actively engaged in such work, to examine and discuss common problems relevant to petroleum hydrocarbon contamination of the marine ecosystem and so a Workshop was sponsored by the International Council for the Exploration of the Sea, and held in Scotland at Aberdeen in September 1975.

The Workshop considered methodology, occurrence and fate in the environment, and effects on the ecosystem of petroleum hydrocarbons in the sea. Most of the papers presented and updated where necessary, are brought together in the present volume together with an edited version of the recorded discussion that followed each session. Of necessity, the reportage of the discussion is very brief although the proportion of time available for discussion compared favourably with that set aside for formal presentation of the papers. In preparing the discussion reports, the editors were assisted in particular by Dr R. Hardy, Dr R. Johnston, Mr P. R. Mackie and Dr I. C. White, and by comments from several contributors.

No attempt was made to produce specific recommendations but a study of the papers in this volume does give a clear indication of several lines of research which must be followed up before an adequate understanding can be reached of the effects of petroleum in the sea and it is evident that widespread monitoring operations will be fully effective only when the basis of our knowledge has been thus extended.

A list of participants to the workshop may be found in Appendix I.

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ON THE ORIGIN OF HYDROCARBONS IN MARINE ORGANISMS

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Using radiochemical techniques a study has been made of the aliphatic hydrocarbons of certain algae and also of a mixed zooplankton culture which had been allowed to graze on ^{14}C labelled *Phaeodactylum*. In each case only a small number of specific compounds in the alkane array were labelled. The much wider range of hydrocarbons found in harvested marine mixed plankton samples suggests that some of this hydrocarbon may be of exogenous origin.

INTRODUCTION

Oro et al (1967) and Blumer et al (1971) have surveyed the hydrocarbons produced by a large number of marine and freshwater algae and discussed the findings in relation to the taxonomic position of the individual organisms. Fatty acids and hydrocarbons in algae have been analysed by a number of investigators (Schneider et al, 1970; Gelpi et al, 1970; Lee and Loeblich, 1971) and the possibility that such hydrocarbons are produced by decarboxylation of fatty acids has been examined and discussed.

In this laboratory, studies have been initiated to identify the hydrocarbons produced by the marine biomass and, in particular, because of their importance in the marine food chain, by algae. To this end it was decided to culture algae on media containing a specific carbon source, $^{14}CO_2$, and thus establish unambiguously the types and amounts of hydrocarbon, if any, synthesised by them. In so doing it was hoped to use some of the algae to feed to organisms further up the food chain to establish (a) whether the higher organisms are capable of synthesising hydrocarbons and (b) whether the algal hydrocarbons are concentrated in these organisms.

Some preliminary results of this work are now reported.

MATERIALS AND METHODS

ORGANISMS AND CULTURE CONDITIONS

The culture of *Phaeodactylum tricornutum* was a gift from Dr J. Overnell who had originally obtained it from Dr M. Droop of the Scottish Marine Biological Association.The cultures of *Tribonema aequale* and *Porphyridium aerugineum* were obtained from the Cambridge Culture Collection numbers 880/1 and 1380/2 respectively.

Phaeodactylum and *Porphyridium* were grown in a defined marine medium S50 (Droop, 1958) at pH 8·2, 22°C. The freshwater organism *Tribonema* was cultured on the modified Chu No. 10 medium as outlined by Blumer et al (1970) except that sodium bicarbonate was omitted and the final pH brought to 7·5.

LABELLING OF CULTURES

Cultures were grown at a light intensity of approximately 2000 lux in magnetically stirred non-aerated 2 litre batches within 5 litre conical flasks plugged with cotton wool. The initial inoculum was allowed to multiply for about five days before aseptic addition of $Na_2{}^{14}CO_3$. Typically, 1 ml sterile solution containing 100 μCi active carbonate (specific activity 59·3 mCi/m mol) was added to each culture. Cells were harvested by centrifugation 5—8 days later for the *Phaeodactylum* cultures or 10—12 days later for the *Porphyridium* and *Tribonema* cultures. Cultures were tested for bacterial sterility at regular intervals (Spencer, 1952) and rejected if contaminated.

ZOOPLANKTON

A mixed zooplankton population (500 mg dry weight) the major components of which were *Acartia clausi* (17 %), *Calanus finmarchicus* (16 %), *Temora longicornis* (16 %), *Cirriped cyprids* (15 %) *and Centropages hamatus* (11 %) was obtained locally by towing using a nylon net. It was maintained for seven days in an aerated 100 litre seawater aquarium at 9°C. Aliquots (108×10^6 dpm) of living, high activity labelled *Phaeodactylum* suspension were added twice daily during

the first three days and daily over the next three days before a starvation period of 24 h to allow gut clearance. Zooplankton were harvested by filtration through a 250 μm mesh nylon net at 1·5, 4 and 7 days, in the ratio of 1:2:2 of the initial population calculated on a dry weight basis. After washing in seawater the lipids were extracted from the individual samples and subsequently bulked before fractionation on a silicic acid column.

CYANOPHYCEAE

Four species of blue-green algae, *Anabaena cylindrica, Anabaena variabilis, Anacystis nidulans* and *Nostoc endophytum* were kindly provided by Dr N. G. Carr, University of Liverpool. The organisms had been grown on mineral salts media and labelled using $Na_2{}^{14}CO_3$ in a closed circulation system. Only limited results are reported here for these organisms.

EXTRACTION

Lipids were extracted from the moist cells by a modification of the Bligh and Dyer (1959) technique. After addition of a crystal of the antioxidant, butylated hydroxy toluene (BHT), the chloroform layer was taken to dryness under reduced pressure on a rotary evaporator and the residue taken up in 5 ml hexane.

FRACTIONATION PROCEDURE

Deactivated silicic acid (~ 3 g) was dry packed into a glass tube to give a column bed of 50×12 mm. A 5 mm layer of alumina was added and the column prewashed with 30 ml hexane under nitrogen pressure. The extract was added and eluted sequentially with hexane (50 ml), benzene (50 ml) and methanol (50 ml). Each fraction was concentrated as outlined above.

FATTY ACID METHYL ESTERS

These were prepared from the methanol fraction by esterification using a methanol/sulphuric acid mixture. The extracted esters were further purified by elution from a silicic acid column using 50 ml ether in hexane (3:97) and care was taken to prevent oxidation of the unsaturated esters by addition of BHT before reducing the eluate to dryness.

SOLVENTS

All solvents used were purified by fractional distillation and the solid reagents were washed with purified ether before use (Mackie et al, 1974).

CHROMATOGRAPHY

Gas liquid chromatography of the aliphatic hydrocarbons and the fatty acid methyl esters was carried out on a radio-gas chromatograph fitted with an effluent splitter such that 10 % of the total flow was

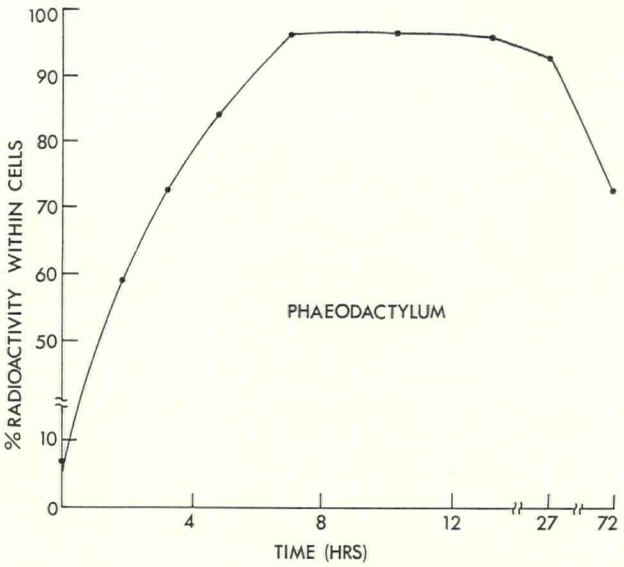

Figure 65. Uptake of radioactivity from $Na_2{}^{14}CO_3$ by *Phaeodactylum tricornutum* grown in non-aerated S50 medium at 23° C; 2000 lux. 50 μCi activity added to 100 ml cells five days after initial inoculum and 3 ml portions removed after times shown.

passed to the flame ionisation detector, the remainder being directed to a furnace tube packed with copper oxide held at 650°C. The system is essentially similar to that described by Simpson (1968). The associated gas chromatograph (Pye, 104) was interfaced to the furnace using stainless steel glass lined tubing ohmically heated to 30°C in excess of the maximum column temperature used.

Hydrocarbons were separated on glass columns (1·5 m×4 mm), fitted with glass to metal seals, packed with either Dexsil 300 GC, Apiezon L (APL), Diethylene Glycol Succinate (DEGS) or Carbowax 20 M using argon as carrier gas. Carbon dioxide at 5 % total flow was bled into the system immediately prior to the furnace to form a counting gas as was 1 % air to aid combustion. The effective chain length (ECL) of the separated hydrocarbons was obtained by reference to a graph on which the log retention time of authentic *n*-alkane standards was plotted against number of carbon atoms.

Radioactivity was monitored by ratemeter and displayed on the chart recorder simultaneously with the trace obtained from the flame ionisation detector. Counts were also accumulated on a scaler interrogated at 10 s intervals. This system allowed a two fold lowering of the detection limits as compared with the ratemeter/recorder presentation (Simpson, 1968).

Fatty acid methyl esters were similarly identified by chromatography on columns of SP 222 (Supelco), APL and DEGS using a combination of commercially avail-

able standards and indirect techniques (Ackman and Burgher, 1963; 1965). Quantisation of the separated fatty acids and hydrocarbons was made by triangulation of peak areas or by summing the counts obtained from each peak.

Capillary column techniques using a Perkin Elmer F11 model gas chromatograph were also used to aid identification of certain hydrocarbons but no simultaneous radioactivity measurements were available in these instances.

Thin layer chromatography was carried out on silica gel or alumina plates and the developed plates scanned for activity using a radiochromatogram scanning system (Panax Equipment Ltd).

Activity in solution was measured using a liquid scintillation spectrometer, whole cells being first digested in 2 N NaOH and aliquots added to NE 260 scintillant prior to counting after a dark adaptation period of 18 h.

RESULTS

UPTAKE OF ACTIVITY

Initial small scale experiments designed to test the extent and rate of labelling were done using *Phaeodactylum* cultures and a typical uptake curve is shown in Figure 65. The centrifuged cells were resuspended in 10 ml growth medium and recentrifuged before radioassay in order to eliminate possible adsorption effects. It is of interest to note that activity is lost relatively rapidly from *Phaeodactylum* cells grown under these conditions and this probably reflects the loss of low molecular weight materials.

Table 22. Activity (as % whole cell activity) in fractions from algae.

Organism	Total lipid[a]	Hexane[b]	Benzene[b]	Methanol[b]
Phaeodactylum tricornutum	35·2	0·44	2·70	22·45
Tribonema aequale	20·0	0·12	0·72	14·40
Porphyridium aerugineum	19·0	0·31	3·60	14·51
Nostoc endophytum	19·1	0·64	0·41	6·20
Anabaena variabilis	13·2	0·36	0·23	4·97
Anabaena cylindrica	3·0	0·11	0·20	0·62
Anacystis nidulans	16·7	0·31	0·21	9·10

a Total lipid is defined as material contained in chloroform layer (see extraction section, Materials and Methods).
b Eluates from the silicic acid column fractionation of the total lipid after solution in hexane.

Table 23. Hydrocarbon composition of several algae.

Organism	Hydrocarbon tentatively identified	Percent total activity	Additional information
Phaeodactylum tricornutum	6Δ[a]-C_{21}	85	Peaks evident at ECL 23·7 and 24·6 on DEGS; one peak only at ECL 21·0 after hydrogenation.
	5Δ-C_{21}	15	
Tribonema aequale	*n*-C_{15}	100	Additional hydrocarbons if present comprise < 0·3% *n*-C_{15} activity.
Porphyridium aerugineum	*n*-C_{17}	91	5% total activity at ECL 23·0 and 4% total activity at ECL 25·0 on APL.
Nostoc endophytum	*n*-C_{17}	80	Trace of activity at *n*-C_{16} elution position
	br[b]-C_{18}	20	
Anabaena variabilis	*n*-C_{17}	85	Additional hydrocarbons if present comprise < 0·3% total activity.
	br-C_{18}	15	
Anabaena cylindrica	Δ-C_{17}	60	br-C_{18} hydrocarbon 10%. Remaining activity associated with another Δ-C_{17} hydrocarbon.
	n-C_{17}	20	
Anacystis nidulans	*n*-C_{15}	45	*n*-C_{16} and Δ-C_{17} hydrocarbons each contain 5% of total activity
	n-C_{17}	45	

a Δ unsaturated, preceding digit denotes number of double bonds.
b Br, branched chain.

Uptake curves for *Porphyridium* and *Tribonema* cultures grown under similar conditions showed a slightly slower rate of assimilation of activity and losses of total activity, not noted in *Phaeodactylum* cultures, were apparent in the *Tribonema* culture (44 % in 10 h) and to a lesser extent in the *Porphyridium* culture (42 % in 24 h). These losses were not unexpected for the *Tribonema* growth due to the lower pH of the medium used but this explanation cannot hold for the *Porphyridium* culture since the growth medium was identical with that used for *Phaeodactylum*.

DISTRIBUTION OF ACTIVITY

Fractionation of the total cell contents of the labelled algae yields the data shown in Table 22. The activities in the individual fractions from the column when summed are substantially less than that in the total chloroform extractable material (total lipid). This discrepancy is explained by the fact that the total lipid material when taken to dryness did not always redissolve in hexane. Additionally, substantial levels of activity were invariably retained on the column after elution with methanol.

The results of the various chromatographic analyses of the hexane eluates from the individual organisms are summarised in Table 23.

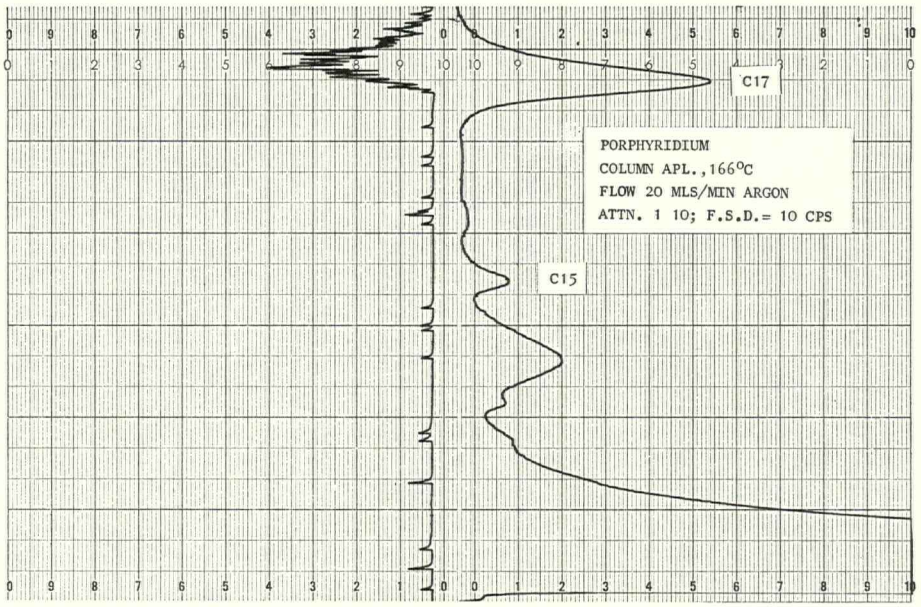

Figure 66. Mass and radioactivity traces from aliphatic hydrocarbon fraction of ^{14}C labelled *Porphyridium aerugineum* culture. The radioactivity trace is displaced slightly from the mass trace due to the hold up volume of the furnace and counter tube.

In some gas chromatographic profiles non-radioactive hydrocarbon peaks were evident. An example of this phenomenon is shown in Figure 66 in which it is obvious that the activity is associated with the heptadecane peak only.

The fatty acid analyses of three algal species are shown in Table 24. Trace components were not studied in detail since the object of this analysis was to determine whether the major fatty acids present could be correlated with the hydrocarbons found in the same samples. The results obtained for the *Phaeodactylum* sample are in good agreement with those found for this organism (Hinchcliffe and Riley, 1972) but marked differences are apparent in the values of the *Tribonema aequale* sample from those obtained by Lee and Loeblich (1971) for the same species.

Table 24. Principal fatty acids (as weight per cent of total acids) in three algal species.

Fatty acid[a]	*Tribonema*[b]	*Porphyridium*[c]	*Phaeodactylum*[d]
14:0	10·5	1·5	10.1
16:0	17·7	32·2	15·5
16:1 (9)	31·8	Trace	26·0
16:3 (6, 9, 12)	NIL	NIL	19·1
18:1 (9)	9·3	Trace	Trace
18:2 (9, 12)	5·7	27·4	Trace
20:4 (5, 8, 11, 14)	10·4	27·8	Trace
20:5 (5, 8, 11, 14, 17)	14·6	11·1	24·3
22:6 (4, 7, 10, 13, 16, 19)	NIL	NIL	4·9

[a] Only those acids present at the level of 1% or greater are included.

[b] Trace amounts of 18:0, 18:3 (9, 12, 15) and 18:4 (6, 9, 12, 15) were also provisionally identified.

[c] A trace amount of 18:0 was found and similar quantities of 18:3 (9, 12, 15) and 18:4 (6, 9, 12, 15) were provisionally identified.

[d] A trace amount of 18:0 was present and similar quantities of 18:3 (9, 12, 15) and 20:1 (11) were provisionally identified.

ZOOPLANKTON STUDIES

A total of 14·9 % of the activity added as *Phaeodactylum* was recovered in the three samples of zooplankton retained by filtration through a 250 μm nylon net. Total lipid recovered from this zooplankton accounted for 28·0 % (activity basis) but the individual lipid extracts showed a wide variation (×14) in the extent of incorporation of activity, the highest level being obtained at the final harvest. Fractionation of the bulked lipid indicated that 1·3 % of the lipid activity (5×10^5 dpm) was recovered in the hexane eluate a further 60 % being removed by benzene and methanol.

Figure 67 shows the scan of a thin layer chromatogram of a sample of the hexane eluate, after development in hexane. The major activity peak (R_f 0·59) is almost exactly co-incident with hexadecane and a minor activity peak is also evident adjacent to the squalene marker.

Gas liquid chromatography of the eluate on a SCOT column (OV 101, 15 m×0·5 mm, helium carrier gas) showed a low background of *n*-alkanes with a major

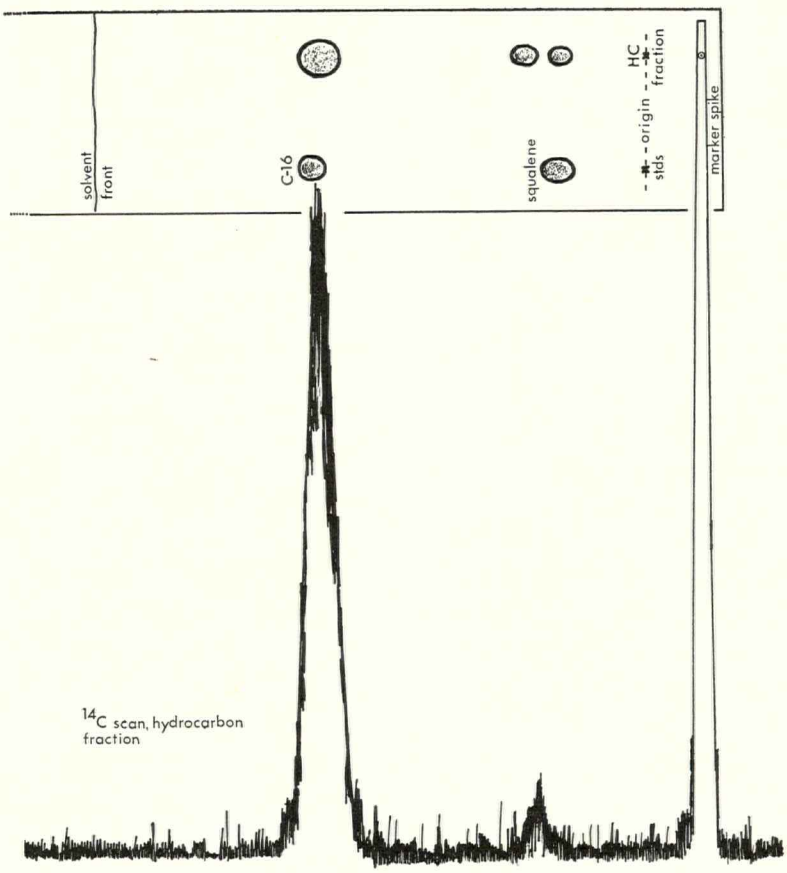

Figure 67. Scan of activity after thin layer chromatography in hexane of the hydrocarbon fraction obtained from zooplankton fed with *Phaeodactylum*. stds are authentic reference compounds.

component (concentration factor 10^3 compared with the average *n*-alkane value) eluting near heptadecane. Co-injection with authentic standards demonstrated the major peak to be pristane which can be resolved from *n*-C_{17} on this phase. This identification was supported by radio-gas chromatography on DEGS which showed activity to be associated with the pristane peak. Additional small activity peaks (ECL 23·6 and 24·5), comprising together 2·8 % of the activity noted in pristane, were also evident and it is highly likely that these correspond to the two polyenes found in the *Phaeodactylum* sample (Table 23) fed to the zooplankton.

Unfortunately the background of *n*-alkanes was at such a low concentration that, assuming the specific activities of *n*-alkanes and pristane to be equal, any activity present in the background components was below the level necessary for positive identification.

DISCUSSION

Clark and Blumer (1967) have stressed the vital importance of solvent purity and the use of adequate blank runs during the analysis of the hydrocarbon fraction of biological materials by gas chromatographic techniques. In supporting these authors in this view we would draw attention to another possible source of error which we have occasionally noted. Injection of relatively concentrated hydrocarbon standards followed, after separation, by a second injection of an air sample from a different syringe has shown essentially the same mass profile from the second injection as from the first although at a much smaller concentration. We believe this artefact may be due to entrainment of material from the septum of the gas chromatograph. The identification of radioactivity rather than mass removes this potential source of error when relative amounts of hydrocarbons are being assessed and provides part of the rationale for the present approach to the identification of hydrocarbons in cultured organisms.

The amounts of chloroform extractable lipid in the organisms examined vary widely (Table 22) and to a greater extent than the aliphatic hydrocarbon contained within the hexane eluate. Variations in these components have also been noted by other workers. Thus Han et al (1968a) found the hexane fraction to comprise 0·035 % and 0·032 % of the dry cell weight of

Nostoc and *Anacystis* cultures respectively, similarly Oro et al (1967) obtained 0·006 % hydrocarbons from *Anacystis nidulans*. Lee and Loeblich (1971) indicated that *Porphyridium cruentum* hydrocarbons accounted for only 0·0034 % dry cell weight while a *Tribonema aequale* culture contained 2·18 % calculated on the same basis. The percentages of total activity found within the hexane eluates from the individual organisms tend to be greater than the figures quoted above but, since ^{14}C activity has been measured rather than mass, the figures are not directly comparable. Differences in cultural conditions during growth will also presumably influence the relative amounts of material produced but the high total lipid content present in *Phaeodactylum* cultures is noteworthy since it falls outside the range quoted for the large number of samples examined by Lee and Loeblich (1971).

Comparison of the hydrocarbons found in the *Tribonema* and *Porphyridium* samples can be made with the published findings of others using similar organisms. Blumer et al (1971) indicated that pentadecane was present at a level of 10 % that of the major hydrocarbon, heptadecane, in a *Porphyridium* culture and these hydrocarbons were also found to be the major components in *Porphyridium cruentum* (Lee and Loeblich, 1971). Our findings differ from these in that, although the C_{17} hydrocarbon was the major peak found in the *Porphyridium* sample, no activity was noted at the elution position corresponding to pentadecane.

Similarly the single peak of activity found in the *Tribonema* culture in the present work differs considerably from the results obtained by Blumer et al (1971) who found the major peak in this species to be unsaturated C_{15} material accompanied by substantial amounts of another C_{15} alkene, tetradecane and pentadecane. Lee and Loeblich (1971), however, found the major hydrocarbon of this species to be a C_{22} monoene and it may be of interest to note in relation to this finding that Patterson (1967) showed that *Chlorella vulgaris* produced relatively large quantities of C_{25} and C_{27} monoenes when grown heterotrophically. These compounds were absent during autotrophic growth although a wide spectrum of saturated hydrocarbons (C_{17}—C_{36}) was obtained under both cultural conditions.

The production of the C_{21} polyenes in *Phaeodactylum* cultures is not unexpected since Lee et al (1970) found the 21:6 polyene to be the predominant hydrocarbon produced by *Skeletonema costatum* an organism which is also a member of the Bacillariophyceae. Less unsaturated forms of this compound have also been noted in related species (Blumer et al, 1971; Lee and Loeblich, 1971).

Preliminary results for the blue-green algae indicate that these organisms produce specific hydrocarbons in the C_{15}—C_{18} range only. Radioactive saturated and unsaturated C_{17} hydrocarbon has been provisionally identified in *Anacystis nidulans* and *Anabaena cylindrica* the latter producing unsaturated C_{17} hydrocarbon as the major component. Branched chain C_{18} hydrocarbons were tentatively identified in *Anabaena variabilis* and *Nostoc endophytum* similar material having already been noted as occurring in other blue-green species (Han et al, 1968b).

Fatty acid analysis indicates that the amounts of individual acids vary widely in the species examined. No obvious correlation exists between the hydrocarbons and the corresponding fatty acids in these samples. This supports the conclusion of others that hydrocarbons are not produced in general by a fatty acid decarboxylation mechanism (Oro et al, 1967; Schneider et al, 1970). Kolattukudy (1968), on the other hand, has indicated that there is no reason to expect a correlation between the quantities of a precursor and its product in a biochemical system since such a correlation is very dependent on the activity of these components in the metabolic pool. We are not able in this work to differentiate between these conflicting views but mention should be made that hydrocarbon synthesis from stearic acid has been shown to occur in blue-green algae (Han et al, 1969). Thus, it is interesting to note that *Phaeodactylum,* the only organism containing the C_{21} polyenes, is similarly the only organism examined in this work which produces the $C_{22:6}$ fatty acid. This is true of a much wider range of algal species (Lee and Loeblich, 1971) and it has been suggested that the hexaene is directly formed from the acid by a highly specific decarboxylase which is unable to act on the broad spectrum of fatty acids also present in these organisms.

The present results where only one or two major hydrocarbons are apparent in the species examined, although a variety of possible precursor fatty acids exist, suggest that a similarly specific mechanism may be operative and that the individual hydrocarbons, if essential to the metabolism of the organism, are part of a very active metabolic pool or are of high structural specificity.

Avigan and Blumer (1968) reported the conversion of U ^{14}C phytol to pristane by *Calanus* species. The experiments here have demonstrated in zooplankton the conversion to pristane of a component of the grazed *Phaeodactylum,* presumably the phytyl moiety of chlorophyll, as well as the probable incorporation of some dietary olefins. The mixed zooplankton when caught contained the C_{21} polyunsaturated olefins and pristane in the ratio 1:92 but, after feeding on the labelled diet, the ratio changed to 1:36 on an activity basis suggesting a relative enrichment of the former. The proportion of activity found in the zooplankton lipids (28 %) compares reasonably well with that found in *Phaeodactylum* (35 %) and the total active hydrocarbons comprise 1·3 % of the lipid activity in the zooplankton

compared with 1·25 % (all polyunsaturated olefin) in *Phaeodactylum*. However, the active olefins only account for 0·04 % of the activity in the zooplankton lipid since the major component is pristane. It is not possible to define at this stage whether these olefins arise by uptake and assimilation, by intrinsic metabolism or by both but, if it is assumed that they are entirely dietary in origin, then assimilation by the zooplankton did not exceed 3 % of their level in the diet. Thus these compounds were assimilated only to a small extent over the time scale of the experiment. The apparent enrichment of the zooplankton lipid with polyunsaturated olefins may be explained on the grounds that the *Phaeodactylum* diet provided a richer source than that on which the zooplankton had grazed before catching. The pristane levels found in this experiment fall within the usual range of values found in zooplankton but it is not known whether pristane is formed as the result of intrinsic zooplankton metabolism or by microflora present in the gut.

In general the existing literature on the presence of hydrocarbons in algae suggests that blue-green species produce these in a restricted (C_{14}—C_{19}) range only whereas other algae can synthesise them, at low concentrations, over a wider spectrum (C_{14}—C_{36}). In the present work the flame ionisation traces indicated that a few samples contained a wide array of hydrocarbons of which only a limited number were radioactive and thus of biosynthetic origin. Figure 66 provides an example of this where a relatively large inactive pentadecane peak is observed and it is interesting to note this finding in relation to the reported presence of this hydrocarbon as a minor constituent of *Porphyridium* sp. (Blumer et al, 1971). It is of course possible that the inactive hydrocarbons found were derived from contaminants in the growth media or, less likely, from the work up procedures (Mackie et al, 1974). Some confirmation for this has been obtained by showing that *Phaeodactylum* can concentrate C_{14} hexadecane from growth medium containing the hydrocarbon; this mechanism might explain the presence of the background of *n*-alkanes found by other workers in some cultured algae.

The gas chromatographic profiles of plankton hydrocarbons (Mackie et al, 1974) usually show an *n*-alkane envelope peaking at or near the C_{28} component although occasionally a bicuspid curve is obtained with an earlier peak present in the C_{18} region. Little evidence has been obtained in the current work for the production of longer chain hydrocarbons (C_{22}—C_{33}) either by the individual algae or by the mixed zooplankton when fed a *Phaeodactylum* diet. One possible interpretation of these facts is that the longer chain length hydrocarbons found in natural plankton samples are of exogenous origin whereas the lower chain length members may have an origin endogenous to the species.

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