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Diagenesis as a function of redox conditions in nature: A comparative survey of certain organic compounds in anoxic and oxic Baltic basin sediments

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INTRODUCTION

The transformations that organic materials undergo at the sediment-water interface are commonly believed to be the most important throughout the long diagenetic process (Degens et al,1964; Bordovskiy,1965a; Moore,1969).

One reason for this is the presence of bottom feeders. Their numbers can be quite significant, as in the Kieler Bight, where they average 43 g per m^2 of sediment surface (Kühlmorgen-Hille,1963). They are also quite efficient. Molluscs can process particles as fine as one micron (Strickland,1965) at the rate of 3,6oo ml of water per hour (Voskesenskiy,1946,cited in Bordovskiy,1965b), while worms can work over more than four to six times their biomass in 24 hours (Sinitsa,1941,cited in Bordovskiy,1965b). These bottom dwellers are capable of reworking from 8o - 9o% of the organic material found in the sediment, turning it into carbon dioxide and salts. The net effect is the depletion of the organic material (Harvey,1957; Moore,loc.cit.; Strakhov,196o,cited in Bordovskiy,1965b; Bordovskiy,1965b; Degens,1967).

Another reason is the considerably greater abundance of bacteria at the sediment surface than at deeper points in the sediment. Degens (1967) and Zobell (1946) estimated about $1o^8$ bacteria per gram sediment at the surface, while at a depth of about one meter this number falls to about $1o^3$ per g. These surface bacteria pro-

bably have a leading role to play in diagenesis (Oppenheimer,1960). Altogether, the estimated biomass of sediment surfaces at water depths from 0 - 200 m average 200 g per m^2 (Zenkevich et al,1965, cited in Bordovskiy,1965a).

If oxygen is gradually removed from the sediment-water interface several things begin to happen. First of all, it can be assumed that the larger bottom organisms will migrate or will die out. Ordinary marine bacteria which have a high oxygen requirement of 30 ml O_2 per g of bacteria (Zobell,1940) will also no longer be able to function.In addition Waksman and Carey (1935) have pointed out that bacterial utilization of organic material is dependent upon oxygen tension. When the oxygen has been fully used up, highly poisonous hydrogen sulfide appears, and as a result only anaerobic bacteria are able to make up the biosphere. These bacteria are relatively inefficient (Abelson,1959), and they require ten times as much organic material in order to produce the same amount of energy as their aerobic counterparts (Bordovskiy,1965b). This means that the organic material is oxidized to a lesser degree and tends to be preserved. This preservation is augmented by the fact that the Eh is also lowered, resulting in a decrease in the thermodynamic drive. According to Richards (1965), anaerobic conditions can result in a tenfold increase of preserved organic material, while under aerobic conditions 70% of the organic material in the phytoplankton is destroyed or liberated within 30 days, and even the most resistant materials are gradually oxidized. The preservation of organic material in anoxic basins like the Black Sea where itcomposes up to 35% of the sediment (Stevenson,1960; Rolfe and Brett,1969) seems to confirm this.

The biomass at the sediment-water interface can also produce qualitative changes due to resynthesis of the bottom material. Bacteria resynthesize from 30 - 40 % of the material that they incorporate, the rest being destroyed in the production of energy (Moore,loc.cit.) This resynthesis appears to be especially important under anaerobic conditions (Rodionova,1951,cited in Bordovskiy,1965a;Rolfe and Brett,loc.cit.; Stevenson,1960;Bordovskiy,1965a). Sehiber and Katanskaya (1951,cited in Bordobskiy,1965a) indicated that under oxygen-poor conditions fewer oxidized components make up bacterial cell walls than normally. An <u>in vitro</u> study of the decomposition of

zooplankton under aerobic and anaerobic conditions was made by Krauss (1959), who found that under anaerobic conditions slightly more protein was destroyed, but considerably more lipid was either preserved and/or created.

The intention of this work was to study and compare quantitatively and qualitatively some of the organic material in a fairly well aerated Baltic basin; Bornholm Basin and in a basin with chronic oxygen deficiency or anoxia: Gotland Deep, in order to observe what effect oxygen or its lack has upon diagenesis under natural conditions. The analysis of the fatty acids, hydrocarbons, and readily extractable amino acids was especially emphasized.

SAMPLES

The samples were taken by means of a small corer composed of a plexiglass tube and several weights. They were taken on the Baltic Sea "ANTON DOHRN" cruise of April 1969 at the following positions and water depths: Bornholm Basin, $55^{o}15'N$, $15^{o}58'E$, 98 m; Gotland Deep, $57^{o}04'N$, $19^{o}50'E$, 22o m. Since this was to be a preliminary study on relative oxygen concentration effects over a geological time span, an attempt was made to make each sample as broad as possible. Each sample consisted of a homogeneous mixture composed of 1o 3o cm cores taken one after another at each station. The ten separate cores were mixed to avoid any horizontal and vertical anomalies that might have been present. Therefore the values that were obtained were average ones for each geographical point sampled. The water at the surface of the cores was carefully decanted, and the samples were stored at -2o C.

ANALYSIS

The sediment samples were extracted in a soxhlet apparatus with a mixture of benzene and methanol. The extract was then purified and saponified, and all the fatty acids were methylated with methanol and BF_3. (Metcalfe et al.,1966.) The hydrocarbons were separated from the fatty acids on a silica gel column. Fifty percent of the fatty acid ester extract and fifty percent of the hydrocarbon extract was hydrogenated, so that one could see to what extent the samples were unsaturated by comparing the values found before and after hydrogenation. The linear fatty acids and hydrocarbons were determined by means

of gas-liquid chromatography. Parker et al.(1967) have reported that a high quantity of iso and anteiso hydrocarbons indicates a high degree of bacterial contribution. Therefore it was decided to measure the amount of these compounds. It was possible to separate them by GLC from the linear hydrocarbons but not from each other. As a result anteiso and iso hydrocarbons are reported together as iso ones. The amount of isoprenoid hydrocarbon pristane was also determined.

The extracted sediment was then digested overnight with HCL saturated anhydrous methanol in order to release organic material bound within mineral matrices. These extracts were treated the same way as the ones obtained with soxhlet extractions.
Afterwards the sediment was refluxed with NaOH. Considerably more organic material was released by this but no hydrocarbons or fatty acids. In each step of the extraction, the amount of organic carbon and total nitrogen still present within the sediment was determined by means of a CHN analyzer.

The amino acids were analyzed as their TAB esters with a GLC (Palmork, 1969). The sediment was extracted with water to obtain all the amino acids that were either free or bound unto water soluble material. An attempt was also made to extract the amino acids bound unto insoluble material by hydrolyzing the sediment. However, the extract produced had the consistency of burnt sugar and could not be injected into the GLC.

CONCLUSIONS

Since the data is to be evaluated quantitatively as well as qualitatively, some sort of factor has to be used in order to take into consideration that the different Baltic basins have different rates of primary productivity in their respective waters. Sarma (197o) found that in general the Bornholm Basin has a primary productivity per unit area that is about twice as large as the one to be found in the Gotland Deep. Unfortunately, however, long term values are not available and even if they were, they would be questionable when extrapolated to 3,ooo years.

Blumer (1965) has pointed out that the hydrocarbons derived from phytol are very stable and are neither readily digested nor prone

to chemical reactions. Therefore, it was decided that a rough relative indication of the amount of initial organic material that was deposited in the sediments might be obtained by comparing the pristane, a phytol derived hydrocarbon, values. They were 60 and 40 ppb for the Bornholm Basin and the Gotland Deep respectively, which would indicate that there was 1.5 times as much organic material deposited in the Bornholm Basin as in the Gotland Deep. This assumes that no unusually large amounts of pristane were introduced from diffusion or other unexpected sources, and also that pristane was neither broken down or removed in quantity over the span of time in question. Based on this whenever the data is compared quantitatively the Gotland Deep values will be multiplied by a factor of 1.5 in order to compensate for the lower primary production in this basin. This might be considered to be specious, but unless the amount of organic material originally deposited is taken into consideration in some way, a quantitative comparison is of no value whatsoever.

Fig. 1 compares the total amount of organic carbon, soluble amino acids, fatty acids, and hydrocarbons in the two sediments. The amount of each material found in the Bornholm Basin is made equal to one, and the adjusted amount of each material found in the Gotland Deep is expressed relative to this. In all cases the Gotland Deep material shows higher values. The difference is least for the total soluble amino acids, but that is to be expected since amino acids are produced by organisms at the sediment-water interface (Degens et al, 1964). Therefore the fact that more amino acids are being produced by the more abundant life on the Bornholm Basin sediment serves to overcome the fact that amino acids are probably being broken down more efficiently with the greater amount of oxygen available.

The difference in the total organic carbon is also not very great, but that too is to be expected, since the measuring of the total organic carbon does not differentiate between organic material that is highly oxidized such as kerogen and material that still has a metabolic value such as sugars. That is to say total organic carbon by itself does not enable us to see which sample has been diagenesised to a greater degree. The values for the total fatty acids and hydrocarbons indicate a strong preservative tendency in the material deposited in the usually oxygen poor Gotland Deep.

All C/N ratios given below are in atoms. If they were given by weight in the literature, they were recalculated. The C/N ratio for Bornholm and for Gotland sediment is 9.4o and 9.8o, respectively. These two values are rather close to one another, and in good agreement with the ratio found by Ehrhardt (1969) for the particulate matter in the water of the Gotland Deep. They are a bit high when compared to the average northern Baltic value of 8.9 for post glacial sediments found by Gripenberg (1934) and the average sediment value of 8.4 from Trask (1932). They are also considerably higher than the various data for the plankton, which range from 5 to 7.5 (Krey,1958; Fleming,194o; Strickland,196o) or the figure for dissolved organic material given by Degens (1968) as being about 3.

Fig. 2 divides the total organic carbon found in the sediments into three main extraction categories: easily extractable, hard to extract and unextractable. The C/N ratios are also given. The easily extractable group is that group which was obtained from methanol-benzene soxhlet extraction and anhydrous HCl digestion. It consists of organic compounds that are the least condensed and most soluble, such as free amino acids, sugars, hydrocarbons, and fats. These compounds are also the ones that are oxidized the least and therefore have the most energy. As is to be expected, the Gotland sediment has the greatest relative amount. The high C/N ratio indicates that the material is enriched in lipids.

The hard to extract fraction is the one that consists of material that was made soluble in methanol or water after strong basic hydrolysis. It probably includes a great deal of the humic acid material which is very plentiful in all sediment samples. However, since marine humic acids have a C/N value around 1o (Zobell,1964; Duursma, 1965), nitrogen rich compounds must also be present in order to account for the low C/N ratio. Humic acids from soil with a C/N around 29 (Degens,1967) are very unlikely contributors.

The unextractable group is the material that is left after the above treatment, and its insolubility makes it rather certain that it will be preserved (Vallentyne,1962). It consists of highly condensed material that is most likely well oxidized and contains very little energy. As is to be expected, the Bornholm Basin sediment has a greater percentage of this material than does the Gotland Deep sediment. The fact that this material has a C/N around 8 indicates that it is

not yet fully stable, since the organic material in rocks was found to have a C/N of about 16 (Trask and Patnode,1942). If it contains kerogen, the name given to the insoluble organic material that is the major component of geological samples, it must be highly diluted with nitrogen rich compounds, as kerogen has a C/N of about 25 to 6o (Forsman and Hunt,1958; Robinson,1969). A major contribution of lignin with a C/N of about 68 in this sample can be considered to be most unlikely. Since lignin is a very abundant product of terrestial vegetation, an argument against the influence of organic material from river runoff exists.

The determination of the amino acids in a sediment as their TAB esters by means of gas-liquid chromatography was not as simple as had been hoped. Some of the free aminoc acid derivatives were interfered with by compounds having the same retention times. It is not known for certain what these compounds were, but if the amino acid extract was hydrolized before hand, as it was done in order to determine the total soluble amino acids, the interfering compounds were not present. They could probably also be removed by more refined extraction and purification procedures, but one must keep in mind that each additional step would also result in some loss of the compounds that are to be tested. The difficulties encountered in studying the extracts obtained from hydrolized sediments has already been discussed above. The procedure as it stands now seems to be ideal for studying the total soluble amino acid compounds in the sediments, but in order to apply it to the study of the free amino acids and to the amino acids that are released when a sediment is extracted after hydrolisis a more thorough purification method must first be developed.

A qualitative picture of the total soluble amino acid distribution can be obtained from table 1 in the appendix, where the individual amino acids are given as mole percentages of the total amounts determined. Although there are certain differences such as the fact that the Gotland sample has considerable more valine, leucine, isoleucine, and tyrosine at the expense of glycine, the overall view does not indicate too great a difference. One is tempted to conclude that aerobic and anaerobic bacteria produce roughly the same amino acid pattern. One striking exception to this is the value for tyrosine. Although conclusions based on tyrosine have to be made with some degree of caution since it is partially destroyed by the acid

hydrolysis, one could conclude that it is preferentially used by the aerobic bacteria to make humic acids and other highly condensed structures since it contains a phenolic OH.

On fig. 3. the readily extractable linear fatty acids, saturated and unsaturated combined, are plotted as weight percentages of the total amount. Both patterns are remarkable similar. In addition one can see on fig. 3 the OEP values (Scalan and Smith,197o) for the fatty acids in question. OEP stands for odd-even predominance and indicates the relative predominance of odd carbon numbered compounds over even carbon numbered compounds at any one point of a sequence. Values less than one indicate a predominance of even numbered compounds. Scalan and Smith (loc.cit.) have shown that materials with similar OEP lines probably have a similar origin. The OEP lines of the sediments studied here are almost superimpossable. Based upon this and the similarity of the distributions, one must conclude that a deficiency of oxygen at the sediment water interface does not alter the results of the diagenesis of the total readily extractable fatty acids.

A similar analysis was made of the unsaturated, readily extractable linear fatty acids, and the results are shown in fig. 4. Here the abundance curves still look fairly similar but not nearly so much so as for the total fatty acids. The OEP curves are rather different, especially in the upper range, and the Gotland Deep sediment shows a greater degree of even over odd predominance throughout. This tends to show that the diagenesis of readily extractable, unsaturated linear fatty acid is somewhat different in regions with different amounts of oxygen at the interface.

Another indication of this is the fact that while only 31% of the total fatty acids from the Bornholm Basin were unsaturated, 57% of the Gotland Deep were. This seems to clearly indicate that a lack of oxygen at the interface retards the breakdown of the unsaturated linear fatty acids. The fact that there is probably less bacterial activity in oxygen poor waters can be shown by the linear/iso ratios, which were 3.7 and 4.5 for the Bornholm Basin and Gotland Deep respectively. A higher value indicates a lower percentage of iso compounds and, therefore, a lower amount of bacterial contribution.

The amount of fatty acids that were bound within a mineral matrix and only releasable with acid digestion, were only about 1o% of the readily extractable fatty acids. The results seem to indicate that

the minerally bound material, especially in the Gotland Deep, was less diagenesized. The linear/iso ration increased from 4.5 to 6.9 (cf.fig. 6), indicating a lesser bacterial contribution, and the unsaturation increased from 57% to 62%. This was not the case with the Bornholm Basin sediment where both the linear/iso ratio and the degree of unsaturation remained about the same. In addition the distributions between the two sediments also show some difference in that the Gotland Deep sample has a relatively large amount of the C_{20} acid (cf.fig. 5). There was insufficient data to draw OEP lines. All the above factors indicate a preservative effect for the minerally bound organic material in the anaerobic basin (cf. Hamilton and Greenfield,1965; Shearman and Skipwith,1965; Weiss,1969). This preservative effect however was not found in the material from the aerobic basin. Perhaps the aerobic conditions enable more active forms of metabolism to exist that permit the attacking of minerally bound organic material to a better degree.

A similar analysis of the hydrocarbons yields similar results. A view of the total, readily extractable linear hydrocarbons shows that they have similar distributions and OEP lines (cf.fig. 7). While an analysis of the readily extractable unsaturated hydrocarbons (fig. 8) again shows differences between the two sediments. Gotland Deep material shows a higher CPI value 2.5 vs. 2.0, and the OEP lines are quite different, as are the distribution curves. The conclusion is again that a defficiency in oxygen will mainly result in a difference in the decomposition of the olefinic hydrocarbons while not seriously effecting the normal qualitative diagenesis of saturated hydrocarbons.

A look at the minerally bound hydrocarbons shows that while the distribution curves (fig. 9) are not too much different, the Gotland Deep material has a very large increase in the percentage of unsaturated material (fig. 6). This again shows a preservative effect resulting from the organic material being bound within a mineral matrix.

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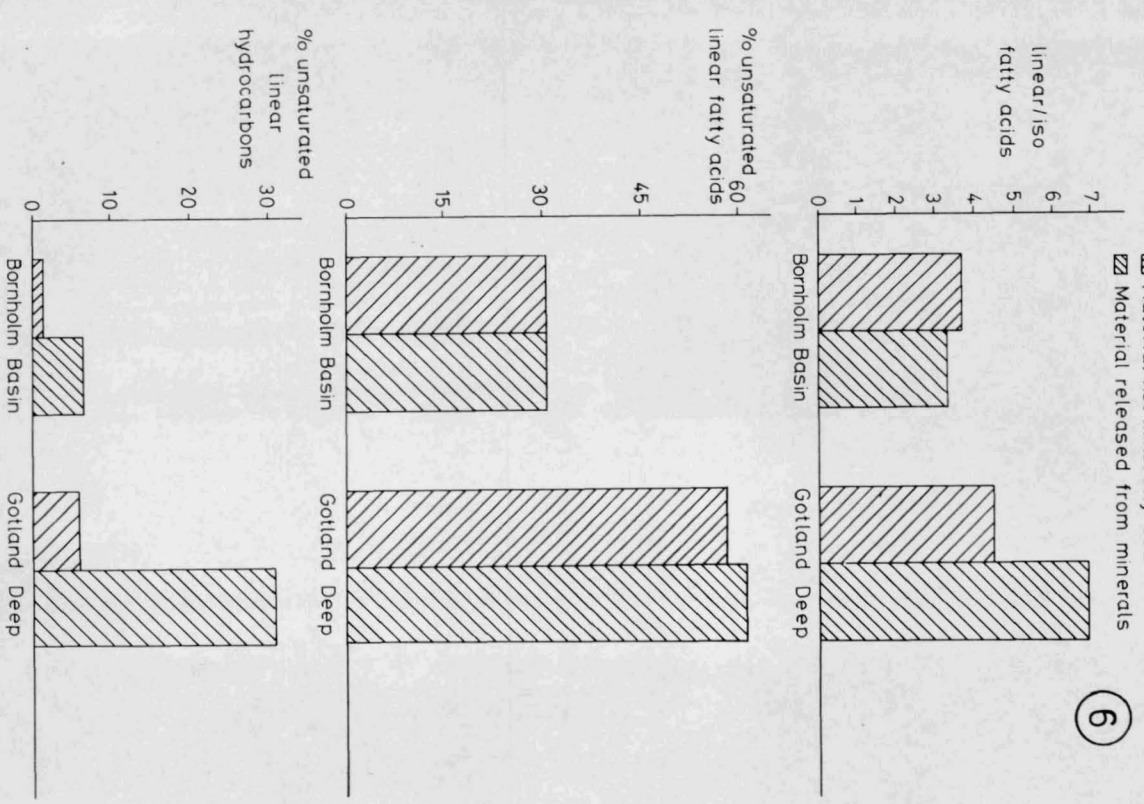
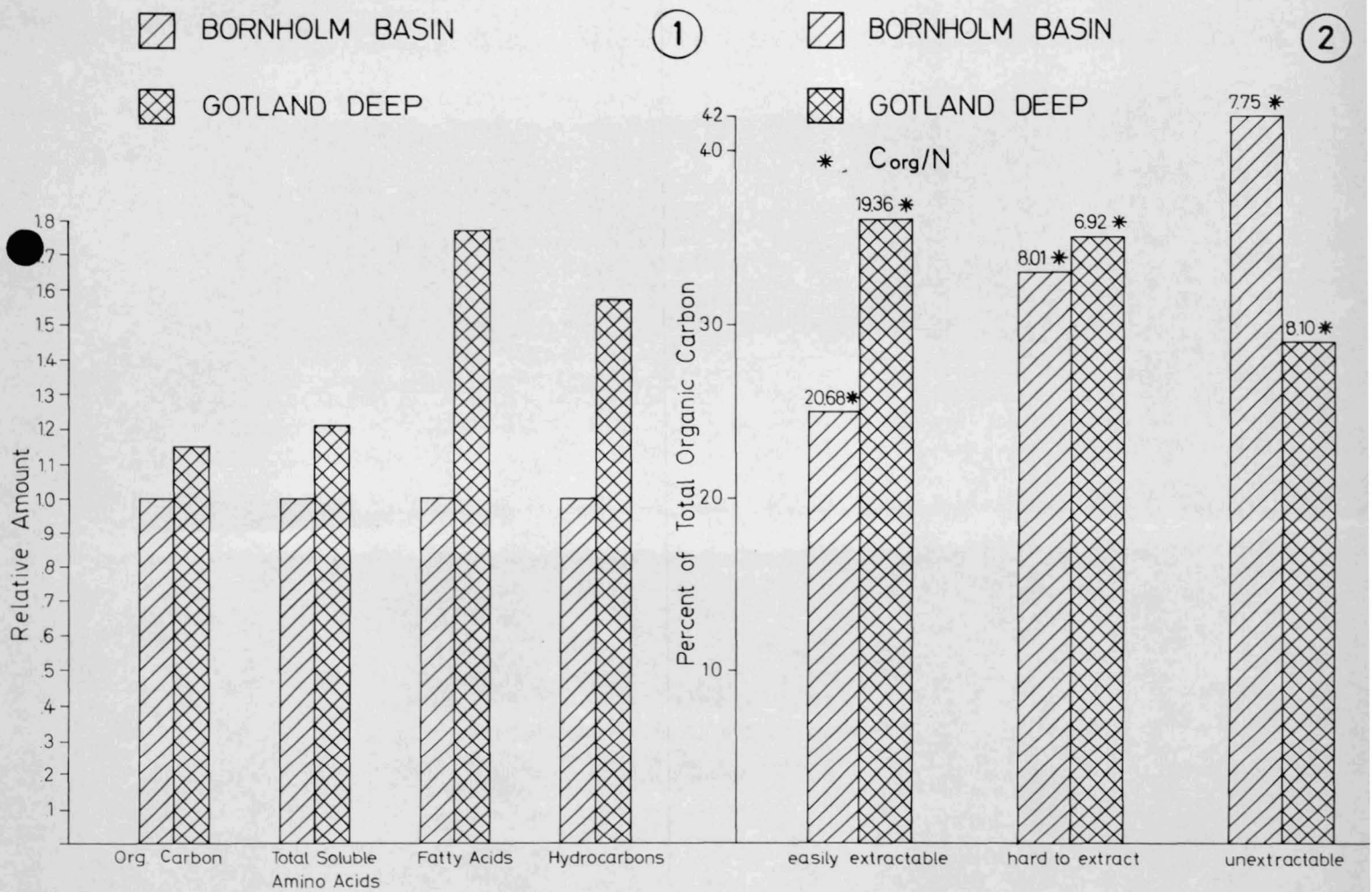
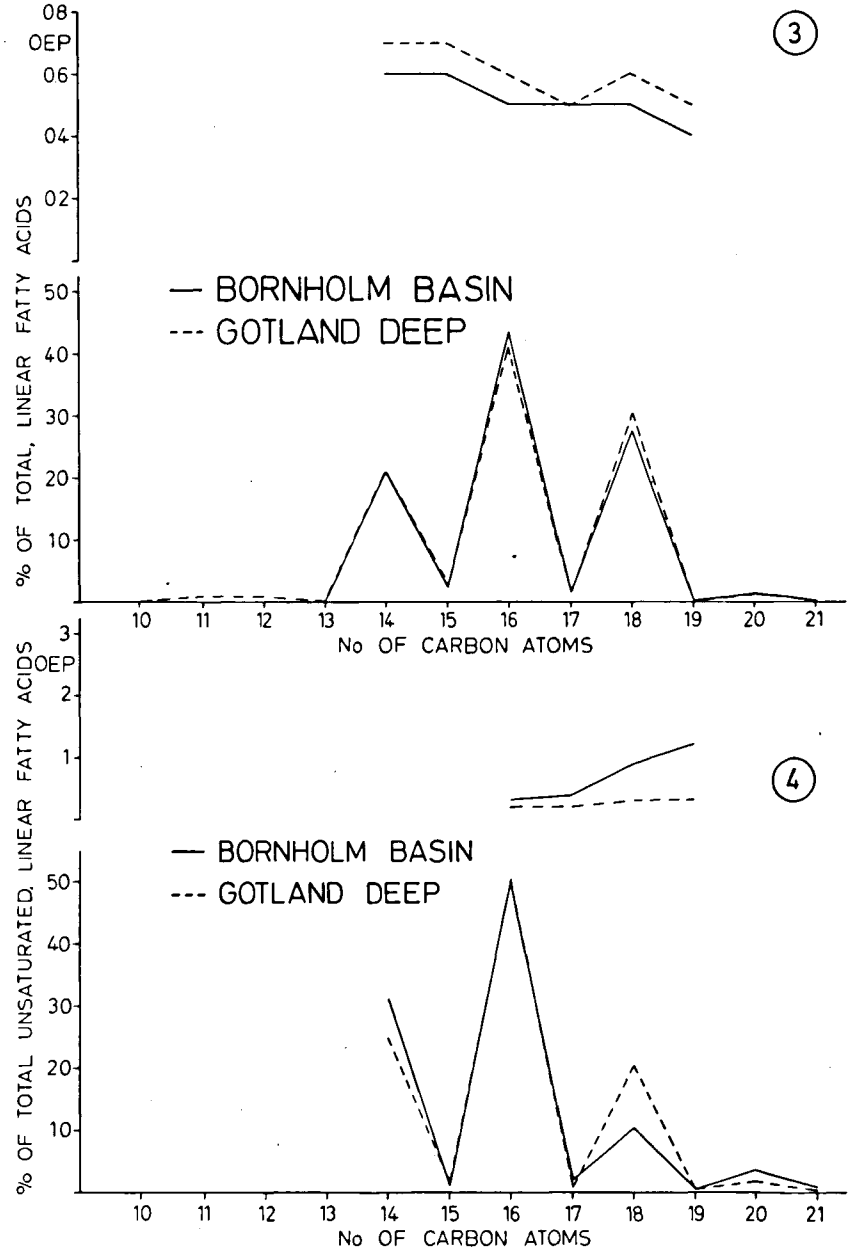
Table 1:

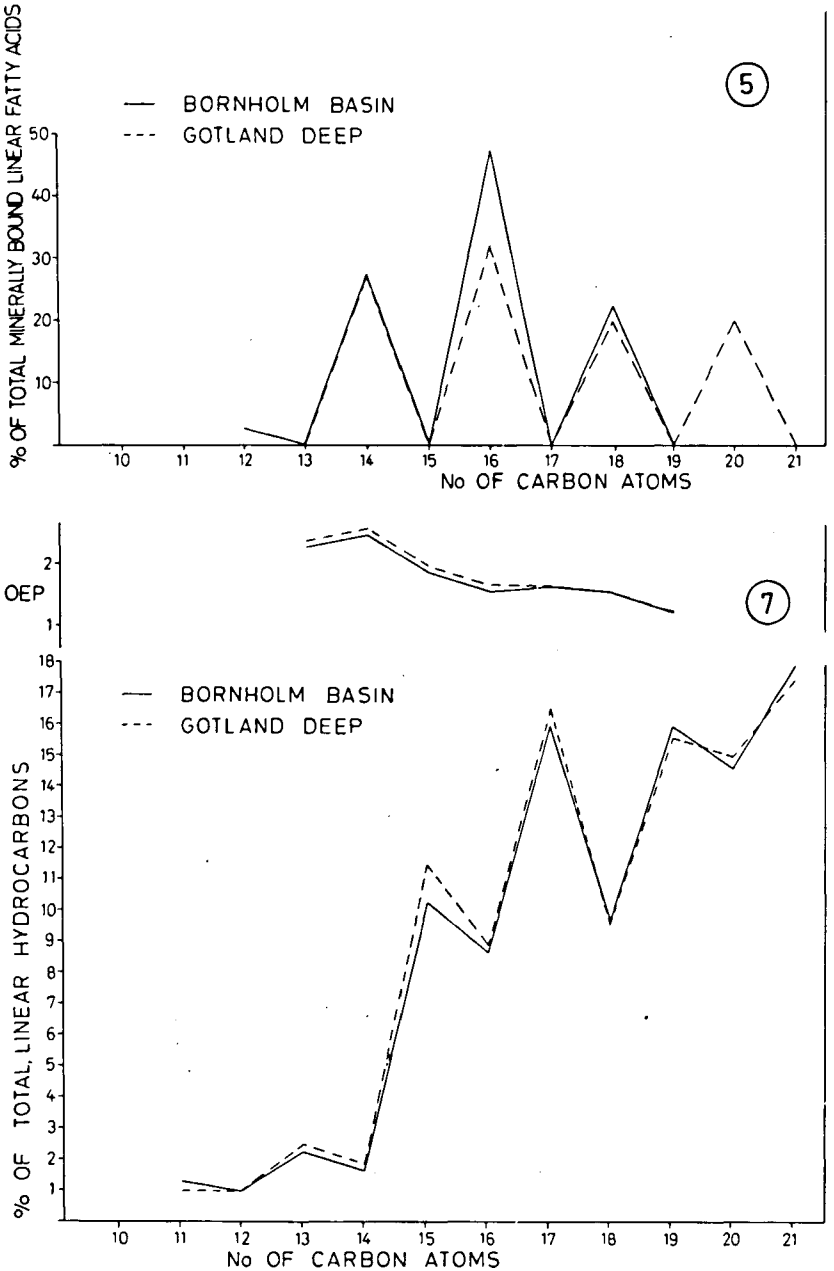
Mole Percent of Total Water Extractable Amino Acids

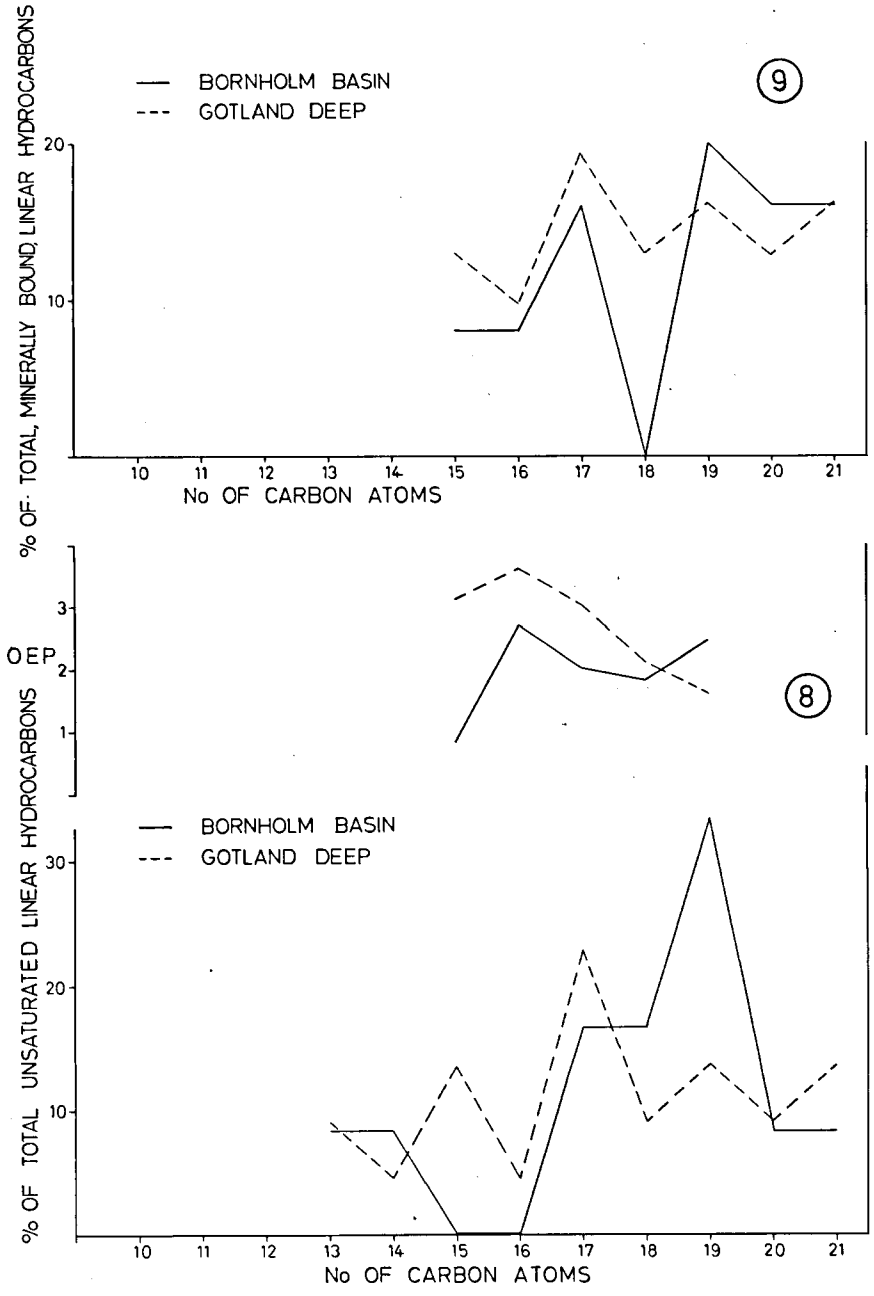
	Bornholm Basin	Gotland Deep
glycine	26.1	14.7
alanine	13.1	12.o
valine	6.5	1o.3
leucine	4.4	9.4
isoleucine	3.8	6.7
proline	3.5	3.5
hydroxyproline	o.7	o.5
serine	16.5	13.3
threonine	9.9	12.8
phenylalanine	1.9	1.2
tyrosine	o.o	6.6
aspartic acid	8.o	5.7
glutamic acid	2.8	3.o
methionine	o.7	o.1


% unsaturated linear hydrocarbons
0
10
20
30
Bornholm Basin
Gotland Deep
% unsaturated linear fatty acids
0
15
30
45
60
Bornholm Basin
Gotland Deep
linear/iso fatty acids
0
1
2
3
4
5
6
7
Bornholm Basin
Gotland Deep
Material not minerally bound
Material released from minerals
6


BORNHOLM BASIN
GOTLAND DEEP
1
Relative Amount
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
Org. Carbon
Total Soluble Amino Acids
Fatty Acids
Hydrocarbons
BORNHOLM BASIN
GOTLAND DEEP
* Corg/N
2
Percent of Total Organic Carbon
42
40
30
20
10
20.68*
19.36*
8.01*
6.92*
7.75*
8.10*
easily extractable
hard to extract
unextractable


3
OEP
08
06
04
02
— BORNHOLM BASIN
--- GOTLAND DEEP
% OF TOTAL, LINEAR FATTY ACIDS
50
40
30
20
10
10 11 12 13 14 15 16 17 18 19 20 21
No OF CARBON ATOMS
OEP
3
2
1
4
— BORNHOLM BASIN
--- GOTLAND DEEP
% OF TOTAL UNSATURATED, LINEAR FATTY ACIDS
50
40
30
20
10
10 11 12 13 14 15 16 17 18 19 20 21
No OF CARBON ATOMS


5
— BORNHOLM BASIN
--- GOTLAND DEEP
% OF TOTAL MINERALLY BOUND LINEAR FATTY ACIDS
50
40
30
20
10
10 11 12 13 14 15 16 17 18 19 20 21
No OF CARBON ATOMS
OEP
2
1
7
— BORNHOLM BASIN
--- GOTLAND DEEP
% OF TOTAL, LINEAR HYDROCARBONS
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
10 11 12 13 14 15 16 17 18 19 20 21
No OF CARBON ATOMS


BORNHOLM BASIN
GOTLAND DEEP
9
% OF TOTAL, MINERALLY BOUND, LINEAR HYDROCARBONS
20
10
10 11 12 13 14 15 16 17 18 19 20 21
No OF CARBON ATOMS
OEP
3
2
1
8
BORNHOLM BASIN
GOTLAND DEEP
% OF TOTAL UNSATURATED LINEAR HYDROCARBONS
30
20
10
10 11 12 13 14 15 16 17 18 19 20 21
No OF CARBON ATOMS