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# THE OCCURRENCE AND DISTRIBUTION OF NITRIFYING BACTERIA IN THE SEA

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Of all the phases in the cycle of nitrogen in the sea perhaps that of the oxidation of ammonia has received the most attention, and yet many questions related to this process are still unsettled. Evidences of nitrifying bacteria have been found in marine bottom materials, particularly in shallower waters, but their presence in the water and plankton of the ocean has not been established.

An attempt was made, therefore, to try to ascertain (1) whether sea water itself with no added source of nitrogen could produce nitrite and nitrate, and (2) whether there were any regions of the ocean other than the ocean floor where such bacteria were present, and nitrifying processes were taking place.

## METHODS

Samples of sea water were collected from different stations and kept in the laboratory in sterile flasks for several weeks, at room temperature and in the dark. These were tested at various intervals for the presence of nitrite, nitrate, and in some cases for ammonia. Other samples of sea water were held at 80° C. for 30 minutes, and after they had absorbed sufficient oxygen again they were inoculated with different samples of fresh marine muds or with plankton. Standard sea water medium, known to be favorable for the formation of nitrites and nitrates was also inoculated with fresh marine bottom material, sea water, or plankton. This medium contained 10 g. sand, 1 g.  $CaCO_3$ , 40 cc. sea water, 5 mg.  $K_2HPO_4$ , and 5 mg.  $(NH_4)_2SO_4$  per flask. For nitrate formation, the  $(NH_4)_2SO_4$  was replaced by 5 mg.  $KNO_2$ . These cultures were also incubated at room temperature and in the dark.

Tests for nitrite were made with diethyl-alpha-naphthylamine reagent. In testing for nitrate any nitrite present was first destroyed by keeping the solution, to which sulphanic-acetic acid solution had been added in a bath of boiling water for 20 minutes, and then testing for nitrate with diphenyl amine reagent. Portions of all solutions treated in this manner were always

\* Contribution No. 188.

tested again after heating to see if all nitrite had been destroyed. The nitrate test is not as sensitive as that used for nitrite. Therefore, the former cannot be detected in as small amounts by this means. The presence of ammonia was determined by using Nessler's solution in spot plates. All test tubes and pipettes used for testing for nitrite and nitrate, were washed and rinsed several times in tap and then distilled water and steamed before using.

#### PRESENCE OF NITRIFYING BACTERIA IN BOTTOM MATERIALS AND IN SEA WATER. MUD SAMPLES IN STERILE STANDARD MEDIA

In order to determine whether organisms contained in bottom material would oxidize ammonia to nitrite and the latter to nitrate, samples of mud were taken for the first experiment, 1 mile W. by N. of Woepecket Buoy in Buzzards Bay at a depth of 14.3 meters. The fresh mud was added to sterile standard media. This experiment was repeated using sand collected off Quissett Harbor, Buzzards Bay, at a depth of 9.1 meters. The results showed that Woepecket mud produced nitrite from ammonia in 2 days, while the nitrite thus produced was oxidized to nitrate in 35 days. Similar oxidation of the nitrite occurred in the nitrite medium. The sand, however, did not give a positive test for nitrite for 11 days. Nitrite appeared in this culture after 69 days, but no nitrate was found in the medium originally containing nitrite.

To amplify this experiment and determine more definitely the locus of nitrification a series of mud cores were obtained off Nomans Land. These cores were about 5-6 inches long. Standard media were inoculated with portions of these cores. One flask of each medium received fresh sea water taken from above the core, and two other flasks both surface mud and water directly over the core. Portions of the core at a depth of 1, 5, 10 or 12.5 centimeters were also placed in these media. (Table I.) All flasks containing ammonium sulphate medium and inoculated with mud showed the production of nitrite in 9 days or less, with the exception of the sample taken at a depth of 12.5 cm. below the surface of the mud. The nitrite increased with time so that in 30 days or less all of the cultures containing the mud inocula were showing strong tests for nitrite, except the last sample. Material taken from another core at a depth of 10 cm. gave decided nitrite tests. The media inoculated with mud and water, or with water alone were much slower in giving positive tests, and when such tests occurred they were weak. Nitrate did not appear in the nitrite medium for 2 months. After that, positive tests were obtained in the cultures inoculated with mud, but not in those receiving water, or mud and water. This corroborates previous findings, that nitrites and nitrates are produced from muds in cultures when

given a supply of ammonia or nitrite respectively (Waksman, Hotchkiss, and Carey (24)).

### MUD SAMPLES IN STERILE SEA WATER WITH NO ENRICHMENT

Top and bottom layers of other cores obtained off Nomans Land were inoculated into sterile sea water. The water, after heating to 80° C., was placed in the refrigerator to become resaturated with oxygen; it was then

TABLE I

PRODUCTION OF NITRITE AND NITRATE FROM PROFILE OF MUD COLLECTED OFF NOMANS LAND AT DEPTH OF 31.1 METERS

	NITRITE								NITRATE
	Incubation, Days								Inc. Days
	3	6	16	22	30	44	64	99	99
a) Nitrite Medium									
1. Water above core	0	0	0	0	0	0	†	†	0
2. Surface mud and water	0	0	0	0	0	0	tr.	††	0
3. Core, 1 cm. deep	0	0	††	††††	†††††	†††††	†††††	0	†
4. Core, 5 cm. deep	0	tr.	†	††††	†††††	†††††	†††††	0	††††
5. Core, 12.5 cm. deep	0	0	0	0	0	0	0	*	
6. Control	0	0	0	0	0	0	†	†	
b) Nitrate Medium									
1. Water above core	0	0	0	0	0	0	0	0	
2. Surface mud and water	0	0	0	0	0	0	0	0	
3. Core, 1 cm. deep	0	0	0	0	0	tr.	††	†††	
4. Core, 5 cm. deep	0	0	0	0	0	0	†	†	
5. Core 12.5 cm. deep	0	0	0	0	0	0	††	†	
6. Control	0	0	0	0	0	0	0	0	

\* Discarded after 64 days.

tr. = trace, † = positive test, †† = fair, ††† = good, †††† = very good, ††††† = abundant.

inoculated with the mud samples. The cultures were tested for ammonia, nitrite, and nitrate with continuously negative results up to 53 days.

Samples of mud bottom taken off Gay Head were added to 100 cc. portions of sterile sea water, as well as the water directly over the core. None of these gave any positive tests for ammonia, nitrite, or nitrate even after 53 days incubation at room temperature. This material gave positive tests for nitrite in 14 and 16 days, and for nitrate in 63 and 64 days, when inoculations were made into standard media as shown in Table I. Negative results were also obtained when "cultured" sea water, i.e., sea water which had stood for 5 days in the laboratory and had been resaturated with oxygen, was inoculated with mud and sand collected in Buzzards Bay near Woepecket Island at a depth of 9.1 meters although incubated for 59 days.

Samples of the same material gave positive tests when inoculated into standard media.

These experiments show that the presence of ammonia and nitrite-oxidizing bacteria can be demonstrated under certain experimental laboratory conditions.

To confirm this, in a second series of experiments mud from Gay Head and Buzzards Bay was added to sterile sea water and also to standard media. The depths at which these bottom samples were taken ranged from 14 to 34.7 meters. Odd numbers in the table indicate the surface layers, and even numbers the deeper layers of the core. No positive tests were obtained in the sterile water even after 52 days. In the standard medium nitrite appeared in small amounts after 5 days, and at 52 days or possibly sooner, the nitrite was being oxidized to nitrate (Table II). At the end of 90 days all the nitrite had disappeared. Complete oxidation to nitrate in the medium originally containing ammonia probably occurred before this time, but tests were not made between 52 and 90 days. Nitrate appeared in the nitrite containing medium in one case in 30 days, and in all flasks at 90 days. This again proves that enriched cultures favor production of nitrite and nitrate when inoculated with mud, whereas sterile sea water inoculated with mud does not give this reaction in the same incubation period.

To test the presence of nitrifying bacteria at greater distances from land 500 cc. portions of water obtained by the "Atlantis" from Station 2895 at depths of 10, 35, 90, 130 and 200 meters was used. Three series of flasks were prepared, one receiving fresh sea water alone; the second being enriched with 10 mg.  $(NH_4)_2SO_4$ , and the third containing 20 cc. of fresh sea water and 40 cc. of standard medium containing  $(NH_4)_2SO_4$ . The flasks were placed in the dark and tested at various intervals for ammonia, nitrite and nitrate. Nitrite and nitrate were negative up to 44 days, and tests for ammonia were positive only where this substance had been added to the water. Mud samples obtained from Station 2895 (lat.  $40^\circ 04'$ , long.  $70^\circ 50'$ ) and inoculated into standard media showed, in a few cases, slight traces of nitrite after 2 weeks, but no nitrate up to 86 days. These nitrite tests, however, were only faint and not sufficient to warrant the assumption that ammonia oxidizing bacteria were present in the muds.

Similar results were obtained from bottom samples taken from greater depths, 1137-4000 meters, at latitudes of  $40^\circ 04'$  to  $40^\circ 25'$ , and longitudes  $67^\circ 46'$  to  $66^\circ 00'$ .

#### NITRIFICATION IN PLANKTON MATERIAL

In the first experiments, 10 cc. of concentrated diatom tow from Vineyard Sound was added to 250 cc. of fresh sea water containing 5 mg.  $K_2HPO_4$  per liter. This was put into a sterile flask containing a little sterile sand.

TABLE II  
 PRODUCTION OF NITRITE AND NITRATE IN STANDARD MEDIA BY BOTTOM SAMPLES TAKEN OFF GAY HEAD AND IN BUZZARDS BAY

LOCATION AND DEPTH OF BOTTOM MATERIAL	NITRITE								NITRATE							
	Incubation, Days								Incubation, Days							
	5	7	14	24	30	37	52	90	90	5	7	24	30	37	52	90
1. Gay Head $5\frac{1}{4}$ mi. W. by S. $\frac{1}{4}$ S. Depth 32.9 M. Top	0	0	tr.	++++	++	++++	+++	0	+++	0	0	0	+	+	++	++
2. Gay Head $5\frac{1}{4}$ mi. W. by S. $\frac{1}{4}$ S. Depth 32.9 M. Bottom	0	ft. tr.	+	++	+++	+++	+++	0	+++	0	0	0	0	0	++	+++
3. Gay Head $\frac{1}{2}$ mi. S. $\frac{1}{2}$ E. of 1. Depth 34.7 M. Top	0	0	0	tr.	tr.	tr.	+++	0	tr.	0	0	0	0	0	tr.	++
4. Gay Head $\frac{1}{2}$ mi. S. $\frac{1}{2}$ E. of 1. Depth 34.7 M. Bottom	ft. tr.	ft. tr.	+	+	+++	++++	tr.*	0	+	0	0	0	0	0	0	0
5. Buzzards Bay 2 mi. W. N. W. Woepecket Rock. Depth 14.3 M. Top	+	++	+++	++++	++++	++++	++++	0	++	0	0	0	0	0	0	+
6. Buzzards Bay 2 mi. W. N. W. Woepecket Rock. Depth 14.3 M. Bottom	tr.	tr.	++	+++	+++	+++	0**	0	+	0	0	0	0	0	0	tr.
7. Buzzards Bay 1 mi. N. W. by W. Woepecket Rock. Depth 14.6 M. Top	+	+	++	+++	+++	+++	**v.f.tr.	0	++	0	0	0	0	0	0	tr.
8. Buzzards Bay 1 mi. N. W. by W. Woepecket Rock. Depth 14.6 M. Bottom	tr.	tr.	+	++	++++	++++	0*	0	+	0	0	0	0	0	0	0
9. Buzzards Bay $\frac{1}{2}$ mi. N. Woepecket Rock. Depth 14.0 M. Top	++	++	+++	+++	++++	++++	0**	0	++	0	0	0	0	0	0	+
11. Buzzards Bay 1 mi. N. Gong 6. Depth 14.6 M. Top	tr.	+	++	+++	++++	++++	++++	0	++	0	0	0	0	0	+	tr.
14. Buzzards Bay Buoy 42. Depth 33.8 M. Bottom	0	0	tr.	+	++	++	+++	0	++	0	0	0	0	0	0	++
15. Control	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0

\* = Nitrate +; \*\* = Nitrate +++; Ft. tr. = faint trace, tr. = trace, + = positive test, ++ = fair, +++ = good, ++++ = very good, +++++ = abundant.

The control flask contained no plankton. These flasks were incubated in the dark. Nitrite appeared after 20 days, and became very strongly positive, while ammonia decreased and finally gave no test. No nitrate was produced up to 64 days. The controls all remained negative.

Another series of flasks were set up using unfiltered fresh sea water from Woods Hole, a little sand, and 5 mg.  $K_2HPO_4$  per liter. Two hundred and fifty cc. portions of this water were put into each flask. Two flasks were inoculated with 10 cc. of concentrated diatom tow collected from Woods Hole or Vineyard Sound. The plankton consisted largely of *Rhizosolenia* and some *Guinardia*. In about 4 days ammonia was found in the flask inoculated with the sample of tow from Woods Hole. The ammonia in the flask increased for a time, and in 2 weeks nitrite appeared and became very strong after 29 days, when the ammonia test became negative. The flask inoculated with tow from Vineyard Sound, gave the same results, but produced ammonia more slowly. Nitrate was not present in either flask up to 29 days.

A third series of flasks was set up using, in one case, standard medium for the production of nitrite, and in the other case the same medium without ammonium sulphate. These were inoculated with 0.5 cc. of concentrated diatom tow. Ammonia was soon found in the flask containing tow and no ammonium sulphate. Nitrite appeared in both flasks, more rapidly, however, in the flask containing only plankton as a nitrogen source, but after 13 days they each gave equally positive tests. With no added ammonia the nitrite test did not finally become as strong, and after 27 days it disappeared; this was probably due to its utilization by other organisms, as no tests for nitrate were obtained up to 43 days. The flask with 5 mg.  $(NH_4)_2SO_4$  showed decidedly positive tests for nitrite (3+) even up to 43 days. No nitrate was produced in this flask either. The controls were negative throughout.

Following these experiments plankton tow was collected from three different stations and added to sterile sea water. Tests were made at different intervals for nitrite, nitrate and ammonia. The first sample was taken from Vineyard Sound between Woods Hole and Tarpaulin Cove, and consisted almost entirely of copepods, a very few diatoms being present. Two tenths, 0.5, 1.0, 2.0, and 5.0 cc. of tow were added to 100 cc. portions of water. When tested 2 days after inoculation by the spot plate test ammonia was present increasing in concentration with the increase in the amount of plankton added. Nitrite first appeared in the flask containing the smallest amount of tow after three weeks. After 27 days the flasks receiving 0.5 and 1.0 cc. portions of the tow showed nitrite, and after 44 days a trace was present in the 2 cc. amount (Table III). Up to 94 days no nitrite had appeared in the flask containing 5 cc. of tow. No nitrate appeared in any culture up to 94 days. As the nitrite increased in the cultures

TABLE III

PRODUCTION OF AMMONIA AND NITRITE IN STERILE SEA WATER ON ADDITION OF COPEPOD TOW FROM VINEYARD SOUND

Flask No.	Plankton cc.	AMMONIA										NITRITE										NITRATE			
		Incubation, Days										Incubation, Days										Inc. Days			
		2	11	15	20	23	27	32	44	52	74	94	2	11	15	20	23	27	32	44	52	74	94	2	74
1	0.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0.2	†	††	†	†	†	†	0	0	0	0	0	v.f.	tr	†	†††	††††	†††††	†††††	†††††	†††††	†††††	0	0	0
3	0.5	††	†††	††	††	††	††	††	†	†	†	0	0	0	0	0	†	††	††††	††††	††††	††††	0	0	0
4	1.0	†††	†††	††	††	††	††	††	††	†	††	0	0	0	0	†	†††	†††††	†††††	†††††	†††††	†††††	0	0	0
5	2.0	††††	††††	††††	††††	††††	††††	†††	†††	†††	††	0	0	0	0	0	0	0	tr.	tr.	†	††	0	0	0
6	5.0	†††††	†††††	†††††	†††††	†††††	†††††	†††††	†††††	†††††	†††	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Medium contained 100 cc. sterile sea water with varying amounts of copepod tow. v.f.tr. = very faint trace, tr. = trace, † = positive test, †† = fair, ††† = good, †††† = very good, ††††† = abundant.



the ammonia decreased. The nitrite test became very strong in the cultures as they became older. Apparently nitrite producing organisms must have been present in the tow, and the decomposition of the copepods furnished the ammonia necessary for the oxidation to nitrite. If this ammonia is produced in too large quantities it retards the production of nitrite. Five drops of this tow were added to flasks of standard media for the production of nitrite and nitrate. Decided tests for nitrite were obtained in 27 days, but no nitrate was produced up to 95 days.

Tow was collected by the "Asterias" (Table IV) by towing 5 minutes with a No. 20 mesh net 10 miles northeast of Chatham. This tow consisted largely of Dinoflagellates, *Ceratium tripos*, and *Ceratium fuscus* as well as a

TABLE IV  
PRODUCTION OF NITRITE IN STANDARD MEDIUM WITHOUT AMMONIUM SULPHATE BY  
DINOFLLAGELLATE TOW

Flask No.	Plankton	AMMONIA							NITRITE							NITRATE				
		Incubation, Days							Incubation, Days							Inc., Days				
		4	6	9	16	28	57	79	4	6	9	16	28	57	79	4	28	57	79	
1	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	2 drops	†	†	†	0	0	0	0	0	tr.	†	†	†	†	††	0	0	0	0	0
3	5 drops	†	†	†	†	tr.	0	0	0	0	0	0	0	†	††	0	0	0	0	0
4	10 drops	††	††	††	††	††	tr.	0	0	0	0	0	0	†	††	0	0	0	0	0
5	1 cc.	††	††	††	††	††	†	tr.	0	0	0	0	0	0	††	0	0	0	0	0

Tr. = trace, † = positive test, †† = fair, ††† = good, †††† = very good, ††††† = abundant.

few copepods. The tow was concentrated by filtering, and added to standard medium not containing any ammonium sulphate in the following amounts: 2, 5, 10 drops and 1.0 cc. Ammonia was present when the tow was first tested, namely 4 days after it was obtained, and nitrite appeared in the flask containing 2 drops of tow after 6 days, while after 8 weeks it was present in all flasks in small amounts, except in the flask containing 1.0 cc. of tow. At this time the ammonia had largely disappeared. The nitrite continued to increase up to 79 days. However, no nitrate was formed.

Tow collected by the "Atlantis" at 100 meters depth, Station 2895 lat. 40° 04', long. 70° 50', off the Continental Shelf contained large amounts of copepods and other animal forms, very few diatoms and a considerable bacterial population, by the time it was received in the laboratory. This concentrated tow was also added to 100 cc. portions of sterile sea water in the following amounts: 2, 5, and 10 drops, and 1.0 cc. Ammonia was present from the beginning of the experiment, decreasing with time, but no nitrite appeared up to 70 days. Neither was there any nitrate produced up to 100

days, when this tow was inoculated into standard media without ammonium sulphate, although plenty of ammonia was produced from the decomposition of the copepods. The ammonia gradually disappeared without the production of nitrate, but very small amounts of nitrite were formed after 100 days.

The lack of production of nitrite in the copepod tow from Station 2895 is difficult to account for. This tow was collected off the Continental Shelf and at a depth of 100 meters. It may be that the reason that nitrification takes place in the tow from Vineyard Sound and off Chatham is that there is more mixing of the water, so that nitrifying organisms from the bottom get into the plankton layer and the presence of ammonia in sufficient amounts from the decomposition of the plankton permits of nitrification. Since it is difficult to demonstrate the presence of these organisms in the water mass as a whole, it is possible that they may come to inhabit this particular layer because of some peculiar fitness of the environment there. There must be some ammonia produced here due to the decomposition of the dead cells in the plankton, and this may be the reason for their presence. It would seem that they must multiply in this layer, or at least be more plentiful there than in the rest of the water. If they are merely the result of the mixing of the water, or of washing in from land, one would think that on enriching and incubating other parts of the water mass, it would be possible to demonstrate their presence. It is, of course, also possible that still longer incubation periods are necessary to show the presence of these organisms.

## DISCUSSION

The presence of nitrifying bacteria in sea water, their origin, the particular part of the sea they inhabit, and the role they play, has long been a matter of uncertainty. In 1899, Vernon (22), while studying the marine organisms of the Gulf of Naples, suggested that nitrification in the sea might be due to bacteria. Brandt (2, 3, 4, 5, 6 and 7) believed that he had proved the presence of these organisms when he obtained nitrification in marine muds in the presence of ammonium salts. Neither Gran (9, 10) nor Nathansohn (17) were able to obtain similar results in either the Gulf of Naples or Norwegian waters, but Gran obtained positive results from bottom material near shore. These two investigators concluded that the nitrifying organisms were brought in from land. Thomsen (20, 21) suggested that the absence of organisms in the sea water was due to the fact that sea water contained only traces of ammonia, while bottom material, due to the decomposition of plant and animal remains, contained greater concentrations of this compound. Brandt, on the other hand, argued that if nitrate came into the sea from the atmosphere or from land by way of the rivers and streams, etc., one would expect to find the highest nitrate content in the upper layer

of the water rather than in the deeper. This is contrary to actual findings, and therefore, he concluded that nitrates must be made in the sea bottom. Thomsen was able to demonstrate the presence of nitrifying bacteria in the sea bottom, but failed to find them in the sea water, plankton, or on fixed algae. He obtained nitrification in Kiel Fjord and in the Bay of Naples near land. Nitrates were only produced from samples taken near land. Issatchenko (14, 15) found nitrifying bacteria in bottom material from the Arctic Ocean and Black Sea, but could not find them in surface waters. Neither could Berkeley (1) nor Lipman (16) find nitrifying forms in sea water, even after several months incubation. Lipman considered calcareous sand favorable for this oxidation process. Issatchenko also believed that sandy bottoms were more favorable for it.

Harvey (11, 12, 13) found that the quantity of nitrate in the English Channel was closely related to the production of phytoplankton, so that when the phytoplankton was at its maximum, nitrate was very low. In certain cases he obtained an increase of nitrate on storing. He concluded that sea water near the bottom contained nitrate producing bacteria. Rakestraw (18, 19) found a maximum of nitrate in August in the Gulf of Maine at about 40 meters depth. Nitrite was found to increase immediately after the summer growth period. Variations of nitrite in amount and distribution apparently depend on the season and the mixing of the water.

The findings in the present paper substantiate the previous results obtained by Carey and Waksman, and Waksman *et al* (8, 22, 24), where nitrite and nitrate were found to be produced from bottom samples, in the presence of an adequate nitrogen supply, and not in water under the same conditions. Nitrate was also produced from the same material but more slowly than nitrite. No attempt was made at that time to determine whether the plankton layer might contain bacteria responsible for nitrite and nitrate production.

Nitrite is an intermediate stage in the oxidation of ammonia to nitrate. Diatoms are known (ZoBell (25)) to reduce nitrate to nitrite, whereas the nitrifying bacteria build up nitrite and nitrate from ammonia. Various marine bacteria are able to reduce nitrate to nitrite, or ammonia or even to atmospheric nitrogen. Therefore, the mere finding of nitrite in sea water does not tell the manner of its formation.

Ammonia is the product of decomposition of the dead plankton cells. It is produced in sufficient amounts to provide a menstruum for considerable oxidation by the nitrite organisms. The amount of ammonia present in the culture determines the rapidity with which it is oxidized. With only a small amount of plankton added to each flask, only a small amount of ammonia was produced and the oxidation occurred quickly, but with larger amounts of plankton the production of nitrite was considerably retarded.

It is difficult to explain why nitrite-forming bacteria are found in some plankton masses and not in others. The plankton which gave positive tests for nitrite was collected near shore in relatively shallow places. Collection from deeper water, off the Continental Shelf, given only slight nitrite tests. The occurrence of nitrifying organism in the plankton layer may be due to vertical mixing in the shallow regions, whereby such organisms might be brought into the upper layers of the water; or it is possible that they may multiply in the layer being very sparsely distributed throughout the sea. It is, of course, possible that such organisms may be washed in from land, and thus get into the plankton near shore. The fact that they do occur in certain plankton masses and not in others, together with the fact that this may be another locus for these organisms is of interest and warrants further study.

In the experiments reported in this paper, it was found that nitrite and nitrate were produced from samples of marine bottom in considerable amounts, when these samples were taken near land. Less nitrite was produced by similar samples taken from deep water. Nitrate was not formed by the latter samples, and its production in all cases was slower than that of nitrite.

Sea water alone apparently cannot produce nitrite or nitrate, at least in the time allowed in these experiments (52 days). It is possible, however, that nitrifying organisms are present throughout the mass of sea water, but in much fewer numbers than in the sea bottom or plankton. If this is the case, it would require a much longer period of time in the laboratory, using sea water only, for such organisms to build up sufficient amounts of nitrite and nitrate from the small amounts of ammonia present in sea water, but under natural conditions and over long periods this would add a considerable amount of nitrate to the sea.

The addition of diatom or copepod plankton to sterile sea water caused the production of nitrite, when such samples were collected near land. Plankton collected farther out, off the Continental Shelf, was apparently less active, perhaps due to the lack of mixing of the water. No nitrate was produced in plankton under any conditions.

These observations lead one to conclude that nitrifying organisms are found in marine bottoms, and that by mixing of the water they may be brought into the plankton layer, where by virtue of the death and decomposition of the plankton, ammonia is liberated which these organisms may then oxidize. The lack of rapid nitrite formation in plankton off the Continental Shelf would lead to the conclusion that fewer nitrifying organisms are present there, due perhaps to lack of vertical mixing of the water. If the organisms in such a plankton are exceedingly few, a longer time of incubation would be required to determine their presence.

## SUMMARY

1. Bottom material produced nitrite when inoculated into standard media. This was later oxidized to nitrate. The time required to accomplish these oxidations varied with the sample. Nitrate production was in all cases slower than that of nitrite.

2. Bottom material produced nitrate from nitrite in standard sea water media.

3. Water directly above the mud produced nitrite, but much more slowly than the mud itself, only traces being found after two months. No nitrate was produced from water over cores after three months incubation.

4. Bottom samples which produced nitrite and nitrate in standard media failed to do so in sterile sea water containing no added ammonia or nitrite salts, even after an incubation of 52 days.

5. Water samples collected away from land at depths of 10, 35, 90, 130, and 200 meters failed to produce nitrite after 44 days even when inoculated into standard media, or when enriched with ammonium sulphate.

6. Samples of mud from great depths produced nitrite very slowly and in exceedingly small amounts, and no nitrate in the time allotted to this experiment (i.e., 86 days).

7. Concentrated diatom tow collected from Woods Hole and Vineyard Sound and inoculated into sea water containing phosphate and sterile sand produced ammonia which was oxidized to nitrite, but no nitrate in 29 days.

8. Concentrated diatom tow added to sterile standard media with and without ammonium salts also caused the production of nitrite but no nitrate in 43 days.

9. Concentrated copepod tow from Vineyard Sound inoculated into sterile sea water in varying amounts gave decided production of nitrite. The greater the amount of tow added to the flasks, the longer was the time of appearance of the nitrite. The latter was produced at the expense of the ammonia, which decreased in amount as the nitrite increased.

10. Nitrite was also produced in standard media by copepod tow from Vineyard Sound. No nitrate was formed after three months incubation.

11. Dinoflagellate plankton, when inoculated into sterile standard media without ammonium sulphate, produced ammonia and nitrite, but neither substance was produced in as large amounts as in the copepod tow, probably due to the higher nitrogen content of the latter. No nitrate was produced from this plankton.

12. Copepod tow collected off the Continental Shelf at a depth of 100 meters and inoculated in varying amounts into sterile sea water and standard media without ammonium sulphate produced considerable ammonia and only very slight traces of nitrite after a long period of incubation.

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