to that in land soils, while others were convinced that nitrates are not formed in the sea itself but are brought into the sea from the atmosphere or by land drainage; still others denied altogether the bacterial nature of the process and suggested its origin by electric discharges or by photochemical processes.

The work of Brandt, Thomsen, Issatchenko and Lipman led, however, to a definite conclusion that, while nitrifying bacteria may be absent in sea water, they are present abundantly in the sea bottom. Issatchenko[2] demonstrated the presence of these organisms in the North Arctic Ocean and in the Sea of Azov; he found them also in the Black Sea, but only in the bottom material close to shore. Similar results were obtained at the Woods Hole Oceanographic Institution,[3] for samples taken from the Gulf of Maine and from George's Bank; the method used for demonstrating the presence of these organisms was found to be of primary importance.

One of the major criticisms[4] directed against the findings of the nitrifying bacteria in the sea only along the littoral zone was that they are not normal sea inhabitants, but that they are rather a result of introduction from land soils by means of drainage waters. In order to meet this criticism, samples of sea-bottom material were obtained on the oceanographic vessel, *Atlantis,* from deep seas, north of Bermuda. The greatest depth of the water was 4,742 meters. The methods used for obtaining the samples of sea bottom under sterile conditions as well as for determining the presence of the specific organisms are described in detail elsewhere.[5] Active formation of nitrite, in an ammonium sulfate medium, was obtained at room temperature in 7 days, in the case of some samples, and in 27 days in all samples, as shown in Table I. It may be of interest to add that when cultures from stations 1730 and 1736 were incubated at 8° C., active nitrite formation from ammonium salt was obtained in 46 days.

TABLE I

| Station No. | Depth of sea bottom, meters | Location | | Nitrite-formation in days* | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Lat. | Long. | 7 | 18 | 27 | 36 | 90 |
| 1725 | 165 | 40°01′N | 70°04′ | 0 | 0 | tr | 0 | ++ |
| 1726 | 643 | 39°51′ | 69°59′ | tr | tr | + | + | ++ |
| 1730 | 3,220 | 38°47′ | 69°11′ | tr | + | + | + | + |
| 1736 | 4,742 | 36°50′ | 68°52′ | tr | + | + | ++ | ++ |

* 0 = negative; tr = trace of nitrite; + = positive nitrite test; ++ = abundant nitrite formation.

[2] B. Issatchenko, "Sur la nitrification dans les mers." *Compt. Rend. Acad. Sci.,* 182: 185–186, 1926.

[3] S. A. Waksman, M. Hotchkiss and C. L. Carey. "Marine Bacteria and Their Rôle in the Cycle of Life in the Sea. II. Bacteria Concerned in the Cycle of Nitrogen in the Sea." *Biol. Bull.,* 65: 137–167, 1933.

[4] H. H. Gran, "Bacteria of the Ocean and Their Nutrition." *Naturen* (Bergen), 27: 33, 1903.

[5] Waksman, Hotchkiss and Carey, *loc. cit.*

Although the presence of nitrite-forming bacteria was demonstrated in practically all the samples of marine-bottom material, no matter at what depth and at what distance from shore, the formation of nitrate could not be demonstrated in the cultures. This is due to the fact that the method of testing for nitrate is much less sensitive than that for nitrite, and also because the nitrate-forming organisms develop much later. In the case of the cultures of nitrifying bacteria obtained in 1932 from the bottom deposits taken off Gay Head, it took from 42 days to nearly three months' incubation of the cultures before the nitrate-forming bacteria could be demonstrated. However, once they began to develop in the medium, further transfers resulted in active production of nitrate within a week or two.

These results prove beyond any doubt that the process of nitrification in the sea is brought about by bacteria which are native inhabitants of the sea bottom and are not merely contaminations from land.

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