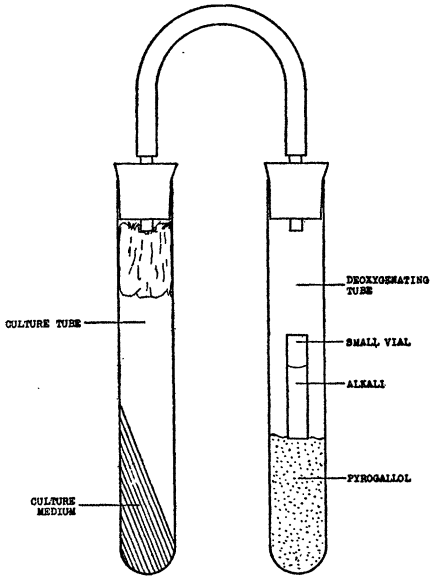

The diagram is almost self-explanatory. Deoxygenation is obtained by the use of an alkaline solution of pyrogallol. It has been our custom to use enough pyrogallol to form a solid block of about one and one-half inches in a $5 \times \frac{5}{8}$ test-tube. The alkali (sodium hydroxide or potassium carbonate) is pipetted into the small vial in the deoxygenating tube and the connections are made as shown. The deoxygenating tube is then tipped to allow the alkali to mix with the pyrogallol. In performing this procedure it is well to have the mouth of the deoxygenating tube low enough to prevent flow of the solution across to the culture tube.

It is possible to open the culture tube several times for examination without renewing the deoxygenating tube by clamping the connecting tube securely. We experimented a number of times with methylene-blue control tubes and found that this procedure could be followed from 4–6 times safely.

Unless the outfit is to be used continuously for more than a week, it is not necessary to seal the connections. When it is desirable to seal the apparatus, collodion is found to be the most suitable material. The collodion should be allowed to dry before deoxygenating the system, otherwise ether fumes may be drawn into the tube, killing the organisms.

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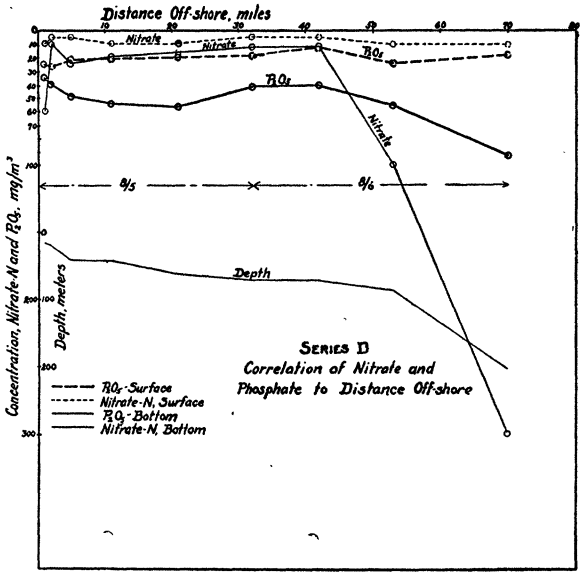
SPECIAL ARTICLES

PHOSPHATE, NITRATE AND NITRITE IN THE SEA-WATER OF REGIONS ADJACENT TO CAPE COD

THE first objective in the chemical program undertaken at the Woods Hole Oceanographic Institution, during the summer of 1931, was a general survey of the waters of the Woods Hole region with respect to their phosphorus and nitrogen content, and especially a comparison of the inshore or neritic waters with the oceanic water found at or near the Continental Slope. The broad shelf to the south of Martha's Vineyard and Nantucket offered a particularly favorable opportunity to study this latter point.

For this purpose two series of stations were established in this area: one, Series D, extending seventy miles to the south of Block Island to the edge of the Continental Shelf, and another Series A, to the south of Martha's Vineyard in a similar manner. Water samples were taken from the surface, bottom and intermediate depths at stations of varying distances from shore. Phosphate, nitrate and nitrite were determined by standard analytical methods.

The samples from Series D were taken on a single trip, covering a total time of less than two days. The results are shown in Fig. 1, correlating the phosphate and nitrate content, both surface and bottom,



with the depth of water and distance off-shore. The most significant feature is the increase in phosphate, and more particularly nitrate, found at the bottom as the Continental Slope is approached. The distinction between "oceanic" and inshore water, not only in this series of stations but in others as well, is to be found in the concentrations of phosphate, and more especially nitrate, at the bottom.

The thirteen stations in Series A were visited at various times during the summer, and consequently the results do not represent a continuous profile. They indicate that water movements of a very fundamental sort take place. This is especially noticeable in the vicinity of the slope, where the influence of oceanic water varies over many miles, extending considerably further inshore at some times than at others.

Phosphate and nitrate concentrations at inshore stations, not only in these two series but in others in Vineyard Sound and Buzzards Bay, were found to be variable, even over comparatively short periods of time. This is to be expected from the continuous movements of water due to tidal currents, etc., and is in accord with similar results obtained by the author in the neritic waters of the Gulf of Maine. It is evident that inshore waters can not be distinguished or characterized in terms of their phosphate or nitrate content.

The interpretation of the variations in nitrite is a problem in itself. With a few significant exceptions the following general principles seem to be true: Nitrite is seldom, if ever, found at the surface, and almost always found at the bottom, although there is usually not a very high concentration at the bottom of oceanic stations. It is exceedingly variable from time to time. It is apparently used up by some process at the surface and produced by some process at or near the bottom. This last conclusion is supported by the fact that nitrite-free sea-water inoculated with nitrite-free bottom mud develops nirite on standing.

There were a few cases of exceptionally high nitrite values for which no good reason was apparent, although it may be significant that these came from stations visited earlier in the season than any others.

The source, variations and fate of nitrite will receive intensive study in a future investigation.

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A METHOD OF IMMUNIZATION WITH CARBOHYDRATE HAPTENS ADSORBED ON COLLODION PARTICLES

To ascertain whether collodion particles combined with haptens induce antibody formation, *i.e.*, whether haptens can be made antigenic by adsorption on nonprotein inert particles I injected 2 rabbits, in 1930, with collodion particles treated with purified type-specific substance of pneumococcus type I.[1] The results were negative. In May, 1931, I started similar experiments with a carbohydrate solution prepared from the anthrax bacillus.[2] Before the immunization experiments were begun I demonstrated that the hapten was adsorbed by collodion particles and that the hapten adsorbed on collodion could not be removed by washing, and was able to react in vitro with its antibody. 0.5 per cent. solution of the hapten was mixed with collodion particles, which, after washing four times, were agglutinated by anthrax-immune serum in dilutions from 1:5 to 1:50. The agglutination was observed best in hanging-drop preparations.

In immunization experiments it was found that when collodion particles are injected into the ear veins of rabbits the Kupffer cells contain collodion particles demonstrable with Ziehl-Neelsen method.[3] These results made it highly probable that carbohydrate haptens adsorbed on collodion particles would induce antibody formation in vivo.

Subsequently and after personal communication Dr. Zozaya reproduced my results. Then we immunized rabbits[4] with the combination of collodion and haptens. Later the work was continued independently.[5]

In one of three rabbits injected with collodion coated with anthrax-carbohydrate the serum gave a faintly positive precipitin reaction with the solution of anthrax-carbohydrate. Rabbits injected with collodion particles coated with the specific substance from pneumococcus type III[6] did not produce agglutinins or precipitins. Work with other haptens and adsorbents is in progress.

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[1] The preparation was obtained through the kindness of Dr. M. Heidelberger.

[2] The hapten preparation was given to me at my request by Dr. J. Zozaya, of the H. K. Mulford Co.

[3] J. Freund, *Amer. Rev. Tuberc.*, 12, 124, 1925; J. Freund, *Proc. Soc. Exp. Biol. and Med.*, 28, 65 and 1010, 1930; J. Freund, *J. Exper. Med.*, 55, 18, 1931; S. Mudd, B. Lucke, M. McCutcheon and M. Strumia, *J. Exper. Med.*, 52, 313, 1930.

[4] J. Zozaya, SCIENCE, 74, No. 1915, p. 270, Sept. 11, 1931.

[5] J. Zozaya, *J. Exper. Med.*, 55, 325, 353, 1931.

[6] The hapten preparation was obtained through the kindness of Drs. Avery and Goebel.