

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 nitrite 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 non-protein inert particles I injected 2 rabbits, in 1930, with collodion particles treated with purified type-specific substance of pneumococcus type I.<sup>1</sup> The results were negative. In May, 1931, I started similar experiments with a carbohydrate solution prepared

<sup>1</sup> The preparation was obtained through the kindness of Dr. M. Heidelberg.

from the anthrax bacillus.<sup>2</sup> 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.<sup>3</sup> 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<sup>4</sup> with the combination of collodion and haptens. Later the work was continued independently.<sup>5</sup>

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<sup>6</sup> did not produce agglutinins or precipitins. Work with other haptens and adsorbents is in progress.

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<sup>2</sup> The hapten preparation was given to me at my request by Dr. J. Zozaya, of the H. K. Mulford Co.

<sup>3</sup> 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.

<sup>4</sup> J. Zozaya, *SCIENCE*, 74, No. 1915, p. 270, Sept. 11, 1931.

<sup>5</sup> J. Zozaya, *J. Exper. Med.*, 55, 325, 353, 1931.

<sup>6</sup> The hapten preparation was obtained through the kindness of Drs. Avery and Goebel.