SCIENTIFIC APPARATUS AND LABORATORY METHODS

A METHOD OF FREEING SEA WATER OF PHOSPHATE

IN the analysis of sea water for phosphate by the Deniges colorimetric method,[1] the effect of the dissolved salts upon the color development is appreciable and should not be disregarded.[2] Although the standards may be prepared in distilled water and the apparent results multiplied by a correction factor, it is better to make the standards in a salt-water medium and eliminate this correction. Inasmuch as sodium chloride is the main constituent of the dissolved salts in sea water, a sodium chloride solution is sometimes utilized as a synthetic sea water. There are a number of disadvantages involved in so doing. The commercial C. P. sodium chloride invariably contains phosphate as an impurity, different lots of sodium chloride even from the same company seldom contain the same amounts of phosphate and usually it is present in an amount which makes necessary the application of an undesirably large blank correction. Also such standards become very expensive when used for routine work. Furthermore, it has even been asserted that the "salt effect" of sea water is not due to the main dissolved constituents but to small amounts of copper.[3] Although this has been questioned,[4] it is obvious that the best medium would be phosphate-free sea water itself.

Certain pelagic forms, mainly diatoms and algae, extract phosphate, nitrate and other nutrient substances from the sea. When conditions are suitable the growth of these forms may be sufficient to remove completely these nutrients unless the supply is replenished from the rich lower waters through turbulence or upwelling. Because of constant replenishment the waters of Puget Sound region as well as those along the Washington coast are very rich in nutrients at all seasons of the year. It is only in isolated inlets and estuaries that the phosphate is diminished appreciably by the diatom "flowering" and then seldom entirely removed. Consequently, the supply of phosphate-free water from this source is uncertain.

A far more certain and convenient method of acquiring phosphate-free sea water has been to grow these pelagic forms in glass bottles or carboys containing sea water. In a number of cases the growth of the normal plankton population has been sufficient to effect the phosphate removal. A better procedure has been to enrich the water with added plankton and in such cases the phosphate has been quickly removed.

Table 1 contains the records of a series of samples

TABLE 1

REMOVAL OF PHOSPHATE FROM SEA WATER BY PLANKTON

Mg. at. NO_3–N per liter	uga. PO_4–P per liter				
	0 days	1 day	2 days	6 days	9 days
0.04 + 0.00	1.45	0.93	0.32	0.24	0.00
+ 0.88	1.45	1.09	1.13	0.34	0.00
+ 1.47	1.45	1.00	1.20	0.37	0.00
+ 2.06	1.45	1.35	2.05	1.70	0.00
+ 2.94	1.45	0.75	0.55	0.30	0.00
+ 4.40	1.45	0.97	1.63	0.37	0.00
+ 5.88	1.45	0.57	0.93	0.24	0.00
+ 8.82	1.45	0.97	0.85	0.00	

Note: Mg. at. or milligram atom is equivalent to gram atom $\times 10^3$.
uga. or microgram atom is equivalent to gram atom $\times 10^6$.

treated in such a manner. Eight two-liter samples of water which had been collected from the bay at Friday Harbor on an incoming tide were placed in glass bottles. Plankton, mainly diatoms, was collected with a hand net from the bay and aliquot portions were added to each of the samples. Additional nitrate was added to insure that it would not be a limiting factor in the plankton growth. As it was possible that there might be some optimum nitrate concentration other than that naturally occurring in sea water, nitrate was added to the samples in varying amount to test this effect. The temperature and light conditions were not controlled except that the samples were kept in the shade and exposed only to diffuse light. Every few days the bottles were shaken vigorously to eliminate stratification and a fifty milliliter sample of water was taken from each bottle for analysis. Suspended plankton was removed by filtering through filter paper. The analysis was made in the normal manner using the Zeiss-Pulfrich Gradation photometer for the colorimetric estimations.

In all cases the removal of phosphate was completed in six to nine days. Apparently the addition of nitrate was without effect. As indicated in Table 1 there was a general decrease in the phosphate concentration with time though there was an occasional increase. Increasing phosphate concentration is attributed to bacterial action. In the transference of plankton from their natural habitat to the bottled sea water many naturally died. These were acted upon by the bacteria present resulting in liberation of phosphate. When phosphate liberation by bacteria was more pronounced than phosphate utilization by the plankton the phosphate content of the water naturally increased. This was only a temporary effect as these samples likewise became free of phosphate. However this does emphasize the necessity of the removal of the plankton by filtration

[1] G. Deniges, *Comptes rendus hebdomadaires des seances de l'academie des sciences*, 171: 802–4, 1920.
[2] Rex J. Robinson and H. E. Wirth, *Indust. and Eng. Chem., Anal. Ed.*, 7: 147–150, 1935.
[3] K. Kalle, *Annalen der hydrographie und maritimen meteorologie*, 63: 58–65, 1935.
[4] L. H. N. Cooper, *Jour. Marine Biol. Asn. of the United Kingdom*, 23: 171–8, 1938.

immediately after the samples become phosphate free as the plankton naturally died by starvation and regeneration of phosphate through bacterial action is likely to occur.

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AN IMPROVED METHOD FOR DETERMINING THE PRESENCE OF THE VIRUS OF ANTERIOR POLIOMYELITIS IN STOOL SPECIMENS

IN a previous paper[1] a modification of the current technic[2,3] for destroying the pathogenic bacteria in stool specimens from patients with anterior poliomyelitis was described. Also the fact was noted that virus in stool had remained infectious after 124 days at a temperature of approximately −5° C.

With the previous procedures as a basis, a new technic has been developed which seems to be a marked improvement. Stools are collected in the field in collapsible stiff paper containers and transferred to sterile bacteriologic water bottles with ground-glass stoppers. An attempt is made to collect two or three specimens from each patient, taken on alternate days. No specimens are accepted if a cathartic has been given recently. On receipt at the laboratory they are stored at −5° C.

In the laboratory, samples of all the specimens from each patient, totaling from 15 to 20 ml of solid stool are diluted in from 150 to 200 ml of water and are thoroughly broken up. To this suspension enough "Duponol" WA (flakes)[4] is added to make a 0.50-per cent. solution. The stool-Duponol suspension is then shaken three minutes by hand with from 15 to 20 ml of ethyl ether and is stored in the refrigerator at 8° C for twenty-four hours. At the end of this time, the usual bacterial flora (coliform bacilli, cocci and most of the spore-formers) fail to grow on nutrient agar. The ether is then boiled off under the vacuum from an "air-jector" aspirator and the material is ready for inoculation. The sediment in the bottom of the bottle is shaken into suspension and from 12 to 15 ml are injected intraperitoneally into a rhesus monkey. If, on the second day after the initial inoculation, the monkey's temperature is normal, the same quantity is again injected.

Five separate experiments have been made, with as many different stool specimens, all from cases of clinical poliomyelitis. In the first, only 0.25-per cent. Duponol was used, and the stool was not completely free of viable bacteria after forty-eight hours and two treatments with ether. The specimen, however, did infect a monkey, with resulting paralytic poliomyelitis and typical pathologic changes in the central nervous system. The virus was passed a second time.

The second two specimens were also treated with 0.25-per cent. Duponol and ether, and re-treated with ether over a period of ten days. Not only were they not free of viable bacteria at the end of this prolonged treatment, but they failed to infect monkeys with clinical anterior poliomyelitis.

In the last two cases, 0.50-per cent. Duponol was used. After eight hours' treatment with ether, samples of the stools incubated aerobically on nutrient agar showed only a few colonies; after twenty-four hours' treatment with ether, no colonies developed. Both monkeys inoculated with these treated specimens contracted paralytic anterior poliomyelities. In each case, the diagnosis of anterior poliomyelitis was substantiated by the microscopic examination of tissue removed at autopsy.

It is of interest that the pH in infective stools, as determined by colorimetric methods and checked with a glass electrode, ranged from pH 6.6 to pH 7.4. The final surface tensions before inoculation as measured on the DuNuoy tensiometer ranged between 32.7 and 37.8 dynes per centimeter.

It is suggested that, since this method involves such a simplified technic and permits large doses of stool to be given, it may prove useful in laboratory investigations in connection with epidemiologic studies. Such work is in progress in this laboratory.

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2 J. D. Trask, A. J. Vignec and J. R. Paul, *Jour. Am. Med. Asn.*, 111: 6–11, 1938.

3 S. D. Kramer, B. Hoskwith and L. H. Grossman, *Jour. Exper. Med.*, 69: 49–67, 1939.

4 A sodium laseryl sulfate manufactured by E. I. du Pont de Nemours and Company, Inc.

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