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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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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