

minimal infective dose, it is impossible to detect small amounts of immune substances in fluids or tissues.

Summary. 1. The parotid glands of monkeys were injected with various mixtures of mumps-virus-containing gland suspensions and of saliva or serum of mumps-immune and normal monkeys, and saliva from immune and normal human beings. 2. The results of these injections give no evidence for the presence of any considerable amount of antiviral substance in either serum or saliva. 3. More delicate and accurate techniques are needed to demonstrate the nature of antiviral immunity to mumps.

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Attempted Chemical Isolation of the Virus of Poliomyelitis.

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The demonstration of the chemical nature of the tobacco mosaic virus by Vinson and Petre,¹ its recent isolation as a crystalline protein by Stanley,² and the experiments of Howitt³ indicating that the virus of poliomyelitis may be also a chemical entity, have led us to employ Stanley's procedure in an attempted isolation of the virus of poliomyelitis in the form of a crystalline chemical substance.

This attempt was not successful but, because the results are of interest in that they afford added evidence for the theory of the chemical nature of viruses, they are briefly reported herewith.

One hundred grams of spinal cords from monkeys infected with the M.V. strain and developing typical paralysis in from 7 to 11 days after intracerebral inoculation, were finely ground with sand and extracted overnight in the refrigerator with 500 cc. of a solution of disodium hydrogen phosphate at pH 8. The thoroughly mixed suspension was poured onto a fine wire screen and the retained precipitate again extracted for 24 hours with 500 cc. of the phosphate solution, poured through the screen and the combined milky suspensions filtered by gravity through coarse fluted filter paper. A monkey inoculated with 0.5 cc. of this filtrate developed typical paralysis in 9 days.

¹ Vinson, C. G., and Petre, A. W., *Bot. Gaz.*, 1929, **87**, 14.

² Stanley, W. M., *Science News Serv.*, 1935, **81**, 644.

³ Howitt, B., *Proc. Soc. Exp. Biol. and Med.*, 1930, **28**, 158.

The filtrate was weighed and sufficient solid ammonium sulfate added to bring the final concentration to 30% by weight. The precipitate of crude globulin was separated by gravity filtration and extracted twice with 500 cc. portions of phosphate solution at pH 8. The combined filtered extracts were again precipitated with ammonium sulfate at 30% concentration and again extracted with two 500 cc. portions of phosphate solution.

The combined filtered extracts were now precipitated with ammonium sulfate at 20% concentration and the globulin dialyzed against distilled water until free from sulfate.

This purified globulin was then extracted for 24 hours with 500 cc. of phosphate solution at pH 8, filtered with suction through a layer of celite, and dilute acetic acid added to the slightly opalescent filtrate to the point of apparent maximum precipitation (about pH 4). Since microscopic examination of the suspension after standing overnight in the refrigerator gave no evidence of crystal formation, the precipitate was filtered off, mixed with a small amount of celite and again filtered through a pad of celite. The celite filter cake was extracted 3 times with 100 cc. portions of phosphate solution and the combined extracts filtered through a layer of celite. The clear filtrate was again treated with dilute acetic acid to the isoelectric point but despite the apparent purity of this filtrate no crystals were observed in the precipitate. The latter was therefore filtered off, dissolved in phosphate solution at pH 7.5 and a monkey inoculated with 0.5 cc. of the clear solution. Typical flaccid paralysis developed in 11 days.

The final globulin solution appeared quite pure, had a protein content of 0.01%, was free from salts present in the original cord, and precipitated fairly sharply at pH 4.0. To assume an organism capable of withstanding the foregoing treatment presupposes one of unusual vitality, possibly encapsulated. On the other hand, the reactions involved are not inconsistent with what may be expected of a pure globulin and, though very intimate association of an organism with this protein has not been excluded, the above series of reactions point strongly to the etiologic agent in poliomyelitis being a chemical (protein) agent or closely associated with it.