cations (*i. e.*, both under and over-calcification, as judged from the staining).

These findings do not differ essentially from those reported by Erdheim and Toyofuku (who removed only parathyroids), but do contradict a statement by Gies.[4] An interpretation of the data will be attempted in our final paper.

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## Ultrafiltration Studies on the Virus of Poliomyelitis.*

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Ultrafiltration studies on so-called filterable viruses, including fowl-plague, vaccinia, herpes, foot-and-mouth disease and mosaic diseases of plants, have been reported by Andriewsky,[1] Doerr and Pick,[2] Levaditi and Nicolau,[3] Duggar and Karrer-Armstrong,[4] Levaditi, Nicolau and Galloway,[5] Olitsky and Boëz,[6] Zinsser and Tang,[7] Berger,[8] but to our knowledge no such investigations have thus far been reported on the virus of poliomyelitis. While the filterability of this virus through Berkefeld candles has been reported (Landsteiner and Levaditi,[9] Flexner and Lewis[10]) it is still not known whether the etiological agent is a small bacterium or a true ultrascopic virus. With this in mind we have undertaken a program of investigation designed to elicit more accurate information as to the magnitude of the organism concerned. The present paper deals with some of our observations thus far.

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[4] Gies, W. J., and collaborators, *J. Natl. Dent. Assn.*, 1918, v, 527.

* Aided by the Mrs. Mary Hooper Somers Medical Research Fund.

[1] Andriewsky, P., *Centralbl. f. Bakteriol.*, Abt. I., Orig., 1915, lxxv, 90.

[2] Doerr, R., and Pick, R., *Ibid.*, 1915, lxxvi, 476.

[3] Levaditi, C., and Nicolau, S., *Compt. rend. Acad. des Sci.*, 1923, clxxvi, 717.

[4] Duggar, B. M., and Karrer-Armstrong, J., *Ann. Missouri Bot. Gard.*, 1923, x, 191.

[5] Levaditi, C., Nicolau, S., and Galloway, I. A., *Compt. rend. Acad. Sci.*, 1926, clxxxii, 247.

[6] Olitsky, P. K., and Boëz, L., *J. Exp. Med.*, 1927, xlv, 685.

[7] Zinsser, H., and Tang, E. F., *Ibid.*, 1927, xlvi, 357.

[8] Berger, E., *Z. Hyg.*, 1928, cviii, 315.

[9] Landsteiner and Levaditi, *Compt. rend. Soc. Biol.*, 1909, lxvii, 592.

[10] Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1909, liii, 2095.

Our experiments were carried out with a highly virulent strain of poliomyelitis virus (Aycock strain). The virus suspensions were prepared by grinding the recently harvested cord and medulla of poliomyelitis monkeys in a mortar,† in the presence of quartz sand, for at least one hour.. The material was then made up into a 5% suspension in neutral physiological saline. Before filtration the suspensions were centrifuged at moderate speed for 5 to 15 minutes to remove the larger particles. The reaction of the suspension after centrifugation was then determined by a colorimetric method and was found to be between pH 5.80 and pH 5.90.

The membranes through which the suspensions were filtered were prepared by dipping Whatman No. 1 filter paper into glacial acetic acid containing various percentages of dried Anthony's negative cotton. After washing them in successive changes of sterile distilled water, to free them of the acetic acid, they were mounted between the ground glass surfaces of 2 opposing cylinders of a special filter device.‡ These membranes were carefully checked for defects both before and after each experiment. They were graded on the basis of their permeability to colloidal particles of known charge and particle size and on their permeability to water (data based upon Poisseuille's formula). The filtrations were all carried out under low negative pressures (0.5, 1.0 or 1.5 cm. Hg). The H-ion concentration of the filtrates ranged between pH 5.80 and 5.90. The monkeys received the respective filtrates in single doses of 2 cc. injected into the frontal lobe of the brain.

No difficulty has been experienced in producing the typical disease in monkeys inoculated with filtrates obtained by passing the suspensions through 2 superimposed 0.5% colloidin membranes, though these filtrates appeared water-clear, or at most slightly opalescent. Four monkeys inoculated with 4 different filtrates have succumbed to the disease within the usual period of time. It has not been possible, however, to get "takes" with filtrates obtained by directly passing the crude virus suspensions, even after centrifugation, through denser membranes (1.0%, 1.5%, 2.0% and 2.5%).

These results might suggest a relatively large magnitude for the virus or virus-bearing particle were it not for the fact that it is possible to put it through denser membranes after it is freed of most of the colloidal matter by a preliminary filtration through 2 superimposed 0.5% colloidin membranes. By doing this we have been able to get repeated "takes" with filtrates through 1.0%, 1.5% and

† Driven mechanically in a special machine designed by Schultz and Banham.

‡ A description of this filtering apparatus will be published in another paper.

2.0% membranes. Thus far less permeable membranes have completely retained the virus. While our experiments do not place the virus definitely in the ultravisible group, there remains a possibility that with further perfection of the experimental procedure the virus may prove capable of traversing membranes of even greater density.

The 2% membranes in question retained completely cultures of *B. prodigiosus* as well as aqueous and broth suspensions of various Streptococci. The membrane series used in these experiments has been carefully studied by one of us (A. P. K.) along the lines suggested by Hitchcock,[11] Walpole,[12] and others. The results of this particular investigation of the membranes themselves will be reported later. While we are not in a position to definitely fix the lower limits of filterability of poliomyelitis virus, because of the disturbing influence of associated colloids, the results obtained thus far indicate that the magnitude of the virus lies below 300$\mu\mu$. From our observations it appears that vaccinia (Levaditi) and herpes (Goodpasture) viruses, employed in the form of virus brain suspensions and filtered under comparable conditions, are no more filterable than the virus of poliomyelitis. There can be no question that such factors as the association of virus particles with protein aggregates and the adsorption of such aggregates, or of free virus corpuscles, at the pore surfaces deserve further study. The influence of proteins and protein derivatives on ultrafiltration is clearly indicated by the results recently reported by Krueger and Tamada,[13] in connection with similar studies on the bacteriophage. Ultrafiltration experiments carried out with virus brain or cord suspensions are even further complicated by the high lipoid content of the menstrum.

It is significant that aerobic and anaerobic cultures made from the filtrates injected failed to reveal the presence of streptococci or other microorganisms.

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[11] Hitchcock, D. I., *J. Gen. Physiol.*, 1925, ix, 755.

[12] Walpole, G. S., *Biochem. J.*, 1915, ix, 284.

[13] Krueger, A. P., and Tamada, H., PROC. SOC. EXP. BIOL. AND MED., 1929, in press.