insertion of the microelectrodes into the nucleus also influenced the magnitude of the P. D. In a normally maturating egg the P. D. disappears as the membrane breaks down. These experiments clearly indicate that the intactness of the germinal vesicle is essential for maximal voltage.

If maturation of the eggs were inhibited, according to the method described by Lillie,[1] by placing them for 70 seconds in sea water at a temperature of 32°C (controls at this time maturating 95-100%), uniform potentials varying from 15-20 mv. were obtained.

With one electrode inside the germinal vesicle, the magnitude of the potential was the same, whether the other electrode was in the cytoplasm or in the sea water. It is also interesting to note that in many experiments in which one electrode was in the cytoplasm and the other in sea water, no measurable potential could be demonstrated between the cytoplasm of this egg and the sea water during all stages from immaturity to the second cleavage stage.

Varying the hydrogen ion concentration of the sea water from pH 8.2 (normal) to pH 6.0 had no significant effect on the electrical potential across the germinal vesicle membrane as compared with the controls. However, when the KCl concentration of the sea water (maintaining isotonic conditions) was increased, the magnitude of the potential was reduced. This depression was most pronounced in a mixture of ½ sea water and ½ isotonic KCl (pH 8.0); in this medium the P. D. was only 10-20% of the control measurements.

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Purification of Poliomyelitis Virus by Adsorption and Elution.*

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The exact nature of filterable viruses is considerably obscured by their close association with substances derived from the tissues in which the virus is found. The purpose of this communication is to describe a method whereby the virus of poliomyelitis

[1] *J. Exp. Zool.*, 1908, **5**, 375.

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may be partially purified, and ultimately perhaps be obtained in its pure state. The method follows closely the procedures which enabled Willstätter and his coworkers to isolate enzymes in their purest known form. Numerous investigators[1] have reported the capacity of various suspensions to adsorb filterable viruses. The adsorbed viruses are in most instances inactivated, and in the case of poliomyelitis, Amoss[2] states that "the presence of colloidal substances with adsorptive power destroys the virulence after 1 or 2 days." Rhoads[3] described the adsorption and inactivation of poliomyelitis virus by aluminum hydroxide, type "C" of the Willstätter series, and showed that adsorption occurred at pH 5.5 and 7.0, but not at pH 8.8. Gildemeister and Herzberg[4] succeeded in adsorbing bacteriophage on kieselguhr and subsequently eluting it with dilute ammonia. Kligler and Olitzki[5] confirmed these observations on bacteriophage, and were able to do the same with fowl-pox virus, using kaolin as the adsorbing agent.

If the inactivation of the poliomyelitis virus is not irreversible, it should be possible to adsorb it at an acid pH, and elute at an alkaline pH. For adsorption of the virus I used alumina gel "C", prepared according to the method of Willstätter and Kraut[6] excepting that the centrifuge was used, instead of natural sedimentation. An effective gel was thus obtained, the process requiring only 2 days as compared with 2 weeks or more in the original procedure. The gel was standardized to contain 22-25 mg. Al_2O_3 per cc. The poliomyelitis virus† was a Seitz-filtrate of a 5% monkey cord suspension. The procedure in a typical adsorption-elution experiment was as follows: To 5 cc. of alumina gel "C", 1 cc. of M/15 KH_2PO_4 and 5 cc. of 5% virus filtrate were added. Immediate flocculation of the gel occurred. The mixture was shaken for 20-30 min. and left in the refrigerator for 3-4 hours. It was then shaken again and centrifuged for 20 min., or until the densest possible packing of the gel occurred. The supernatant liquid which had a white (lipoid-containing) "cake" at its surface was poured off. The sides of the tube and the surface of the gel were carefully washed with

[1] Levaditi, C., and Nicolau, S., *Compt. rend. Soc. biol.*, 1923, **88**, 66. Lewis, M. R., and Andervont, H. B., *Am. J. Hyg.*, 1927, **7**, 505.

[2] Amoss, H. L., in "Filterable Viruses," ed. by Thos. Rivers.

[3] Rhoads, C. P., *J. Exp. Med.*, 1931, **53**, 399.

[4] Gildemeister, E., and Herzberg, K., *Centbl. f. Bakt.*, Orig. I, 1924, **91**, 228.

[5] Kligler, I. J., and Olitzki, L., *Brit. J. Exp. Path.*, 1931, **12**, 172.

[6] Willstätter, R., and Kraut, H., *Ber. Chem. Ges.*, 1923, **56**, 149.

† I am very much indebted to Drs. Brebbner and Weyer for supplying me with poliomyelitic monkey cords, and their interest in this work.

distilled water. The sediment was now mixed with 5 cc. of M/15 Na_2HPO_4. The gel which was formerly flocculated became homogeneous; after shaking for 20 min., it was left at room temperature or in the refrigerator over night. The following morning it was again shaken and then centrifuged. The supernatant liquid was colorless and water-clear without any "cake" at its surface. The original virus filtrate, the adsorbed supernatant liquid and the eluate were then diluted to the same volume. One cc. of the dilutions (equivalent to 0.05 cc. of the original) was injected intracerebrally into monkeys. The results are shown in Table I.

TABLE I.
Adsorption and Elution of Poliomyelitis Virus.

Monkey No.	Solution Tested	Dose cc.	Result
1	5% Virus filtrate—"593"	0.05	Typical poliomyelitis, 6 days
2	Adsorbed supernatant liquid	0.05	No symptoms
3	M/15 Na_2HPO_4 Eluate	0.05	Typical poliomyelitis, 5 days
4	5% Virus filtrate—"590"	0.05	Typical poliomyelitis, 6 days
5	Adsorbed supernatant liquid	0.05	No symptoms
6	M/150 Na_2HPO_4 Eluate	0.05	Typical poliomyelitis, 10 days

It may be seen that with the doses used, the adsorption of the virus is probably complete, and that the M/15 Na_2HPO_4 eluate, volumetrically equivalent to the original virus filtrate, produced poliomyelitis even more rapidly than the control. When M/150 Na_2HPO_4 was used for elution, the injected monkey developed poliomyelitis 4 days later than the control. Although one cannot yet definitely state that differences in the incubation period are indicative of the virulence or quantity of virus, I have the feeling that the M/15 Na_2HPO_4 is the more effective eluting agent.

Preliminary quantitative determinations indicated that 80-90% of the organic constituents of the original virus filtrate remained in the inactive adsorbed supernatant liquid, and that not more than 10% was released from the gel during elution. The eluted virus solution had no coagulable substances and failed to give a Biuret or Ninhydrin reaction, but since Seitz-filtrates of 5% monkey cord and brain suspensions, which yield an appreciable amount of heat-coagulable material, are either Biuret-negative or only faintly positive, these chemical tests are of little value here. The eluted virus solution also failed to give a precipitin reaction with the serum of 2 horses which had been under immunization for a long period of time with poliomyelitic monkey cord and brain; but this again does not exclude the presence of neuro-proteins since the

precipitin content of these sera for concentrated suspensions of normal or poliomyelitic monkey cord is extremely low. Similarly there was no complement fixation in a mixture of eluted virus and antipoliomyelitic horse serum. However, I consider the eluate as well as the original filtrate to be very dilute solutions (suspensions) of virus, and am postponing a more detailed chemical and immunological study of the virus to the time when I shall have purified and concentrated a large quantity of it.

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Vaccination of Humans against Yellow Fever with Immune Serum and Virus Fixed for Mice.

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(Introduced by T. P. Hughes.)

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The method of vaccination which we have found effective in immunizing human beings against yellow fever is based on the well known fact, reported by other investigators and frequently observed in our laboratory, that monkeys inoculated with highly virulent strains of yellow fever virus and given simultaneous or preceding protective injections of yellow fever immune serum are found to have a solid active immunity after the passive immunity has disappeared. As accidental infections of laboratory workers with the virulent strains of virus maintained in monkeys have caused many serious illnesses and 5 deaths,[1] we have substituted for such virus in the vaccine a less dangerous strain which was established in mice by Theiler,[2] through intracerebral inoculations, and has been passed successively through more than 100 of these animals. Although this virus has lost the power to kill monkeys on subcutaneous inoculation, animals so inoculated frequently show fever and we consider necessary the simultaneous injection of immune serum with the vaccine. Moreover, the 3 recorded[1] accidental infections of man with yellow fever virus fixed for mice resulted in definite though mild attacks of yellow fever.

The vaccine is prepared in 2 parts. (a) A 10% suspension of

[1] Berry, G. P., and Kitchen, S. F., *Am. J. Trop. Med.*, in press.
[2] Theiler, M., *Ann. Trop. Med. and Parasit.*, 1930, **24**, 249.