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.

mouse-brain tissue containing yellow fever virus (French strain, more than 100 passages through mice) in fresh, sterile, human immune serum was prepared, tested for sterility by making cultures, and centrifuged. The supernatant fluid was passed through Seitz or Berkefeld N. filters, tubed in 1 cc. portions, dried in the frozen state by a method previously published,[3] stored in sealed tubes in the refrigerator, and later tested in animals. This part of the vaccine, redissolved in distilled water, had no effect on mice when injected intraperitoneally but caused fatal yellow fever encephalitis when injected intracerebrally. Monkeys vaccinated with this material together with supplementary immune serum remained well and developed immunity. (b) Immune serum for supplementary injections was secured from persons who had recently had yellow fever. To the pooled serum was added 0.2% tricresol. This serum was considered suitable for use if 0.3 cc. per kilogram of body weight given subcutaneously protected monkeys against a simultaneous subcutaneous injection of virulent virus from monkey source.

The dried vaccine was dissolved and brought to the original volume with distilled water. The amount of this fluid injected was 0.03 cc. per kilogram of body weight, and the amount of immune serum 0.3 cc. per kilogram. A small part of the total amount of this serum was in the dried vaccine and the larger part was given in supplementary injections. The supplementary serum was injected subcutaneously in 2 different places in the abdominal wall, and immediately afterwards the redissolved vaccine was injected in the same way.

Ten persons were vaccinated between May 13 and June 29, 1931. The first 4 received a vaccine which was prepared and administered by methods differing somewhat in detail from the description given above. Unlike the later preparations, it had not been centrifuged or filtered and consequently contained much more mouse-brain tissue. As a result there was much soreness of the abdominal muscles at the sites of injection of the vaccine and some local redness and swelling. These manifestations disappeared almost completely during the day following vaccination. The persons later vaccinated received filtered vaccine and had, as a rule, less local reaction. No other symptoms of importance were observed except slight elevations of temperature in some of the cases. At-

[3] Sawyer, W. A., Lloyd, W. D. M., and Kitchen, S. F., *J. Exp. Med.*, 1929, **50**, 1.

tempts to recover the virus from the circulating blood of 3 persons during the first 24 hours after vaccination were unsuccessful.

The production of immunity. Sera were obtained from the vaccinated persons before vaccination and at approximately weekly intervals thereafter, and were tested by the intraperitoneal protection test in mice (Sawyer and Lloyd[4]). All sera were without protective power before vaccination, but in every case protective power against yellow fever virus was demonstrated later. In one case protection appeared for the first time 7 days after vaccination; in two cases 9 days after; in 6 cases 12 to 14 days after; and in one case 21 days after. Sera from 7 persons were tested also in monkeys and gave protection.

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Cultivation of the Virus of the Common Cold in Tissue Medium.

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In a previous report[1] we presented evidence of the cultivation of the virus of the common cold in tissue medium *in vitro*. A single culture was maintained for 15 generations representing duration of life outside the human body of 74 days.

We now report a second cultivation of the virus of the common cold by a technic similar to the one previously described. Naso pharyngeal washings were obtained from a patient within the first 24 hours of a typical acute cold. These were passed through a Seitz filter, the activity of the filtrate ascertained by the intranasal inoculation of apes, and it was planted without concentration in the medium previously described. In this series bouillon made from the special peptone of Dubos was used as diluent instead of Tyrode's solution. The culture was carried under vaseline seal for 17 generations. The total duration of life outside the human body was 73 days. The final dilution of the original material was 1-100 quadrillion.

The average time of transfer varied from 3 days to 6 days. The

[4] Sawyer, W. A., and Lloyd, W., *J. Exp. Med.*, 1931, **54**, 533.

[1] Dochez, A. R., Mills, Katherine C., and Kneeland, Yale, Jr., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 513.