

serum of 1 animal was invariably more potent than the others, neutralizing when diluted 1-400. Since equal parts of serum and virus suspension were used, the final dilutions were always double the original amounts.

At the end of 2½ months of immunization, a large bleeding was obtained from the animals and the pooled serum was sent to the Eli Lilly Company for concentration. Two lots of the refined material neutralized *in vitro* from 16 to 32 minimum infective doses of the potent monkey passage strain of virus obtained from Dr. M. Brodie, of the New York City Health Department, in a dilution of at least 1-1,000 to 1-2,000 but not 1-5,000, while the original unconcentrated pooled serum was potent only to a 1-500 dilution and not a 1-1,000. The strength was thus increased and not lost during the refining process. Intraperitoneal inoculation of 20 cc of the pooled concentrated serum into a monkey failed to give any reaction.

Neutralization also occurred when using a virus recently isolated during the 1934 outbreak of poliomyelitis in northern California. Cord removed at autopsy from a human case produced typical poliomyelitis in a monkey after eight days incubation and has since been carried through five passages in monkeys. The second generation virus was potent in a dilution of 1-400 of the 5 per cent. suspension.

The following experiments were performed to test the strength of the concentrated serum when used both therapeutically and prophylactically. 0.005 cc of a 5 per cent. suspension of virus (0.00125 cc M. I. D.) was inoculated intracerebrally into 4 monkeys. Three animals then received 25 cc of the concentrated serum intramuscularly 4, 5 and 6 days, respectively, after the infecting dose and during the prodromal period. The fourth received no serum. All 4 animals developed paralysis and succumbed to typical poliomyelitis. Only 1 was given a second dose of serum but without effect.

The concentrated serum was then tried prophylactically, the virus being administered intracerebrally in 1 series and intranasally in 2 others.

In Series I, 3 animals were given 10 cc and 3 others 20 cc of concentrated horse serum intramuscularly. 0.005 cc of virus were inoculated intracerebrally into 2 monkeys, respectively, 1, 2 and 9 days later. One control animal was used. Only 2 of the 7 monkeys survived, 1 receiving 10 cc of serum 24 hours previous to infection and another receiving 20 cc 2 days beforehand. Although the other 2 monkeys given 20 cc of serum succumbed to the disease, yet the incubation period was greatly prolonged, being 15 days, as compared to 8 days for the control animal. The serum apparently could be of some

effect even after the drastic method of infection directly into the central nervous system.

In Series II, the same number of animals was given similar amounts of concentrated serum intramuscularly. Ten per cent. virus was administered by intranasal instillation according to the method of Schultz and Gebhardt⁹ at 1, 2 and 7-day intervals. Two control monkeys were included. Two animals receiving the 10 cc amounts of serum died of intercurrent infections. Of the others, 1 control acquired the disease, while all the remaining animals survived.

In Series III, 3 monkeys received 20 cc of concentrated horse serum into the muscles and 3 others 25 cc each of unconcentrated immune sheep serum. Respectively, 1, 2 and 7 days later one of each group was given a 20 per cent. suspension of poliomyelitic virus by intranasal instillation, according to the aforementioned method. Three control monkeys were included. Two of the latter succumbed to the disease, as did also the treated animal receiving sheep serum 1 week previous to the virus. The incubation was prolonged to 19 days, however. All the others and 1 control survived. It seemed apparent that the serums were of some value when inoculated prior to the administration of the virus by the less severe intranasal route.

In summary—a potent hyperimmune antipoliomyelitic horse serum has been developed which may be increased in strength by concentration as demonstrated by the *in vitro* neutralization test. The concentrated serum was without effect if given therapeutically during the prodromal stage after intracerebral injection of monkeys, but was apparently of value if administered prophylactically. This was demonstrated mainly after intranasal infection and only in part after intracerebral inoculation of monkeys.

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⁹ E. W. Schultz and L. P. Gebhardt, *Proc. Soc. Exper. Biol. and Med.*, 30: 1010-1012, 1933.

BOOKS RECEIVED

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