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An Effective Method of Intraneural Inoculation of Poliomyelitis Virus.*

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The uncertainty of results obtained by most investigators with intraneural injections of poliomyelitis virus has limited the use of this method of inoculation for inducing experimental poliomyelitis. Nevertheless, if the virus of poliomyelitis is truly neurotropic, one would expect that intraneural inoculation would be as effective as intracerebral inoculation, and simpler, if the virus could actually be made to come into contact with numerous nerve fibers, rather than being forced along and between connective tissue sheaths within the peripheral nerve. This of course is strongly suggested by the work of Fairbrother and Hurst,[1] who showed that trauma during intraneural injection facilitates "takes" by this method of inoculation.

Going one step farther, and with the knowledge that during the first few days after nerve section the nerve cells with axons cut are more susceptible to the virus than normal cells,[2] it was decided to determine whether simple section of a peripheral nerve and immersion of the central stump in virus suspension for a few minutes was sufficient to produce poliomyelitis. This method, which involves no mechanical injection pressure, and which places the virus in contact with the protoplasm of every axon in the nerve, was found to be highly successful in producing poliomyelitis. When a large nerve, such as the sciatic nerve, was used, this method of inoculation was invariably successful with two strains of known potency, the MV and Wfd[3] strains.

In 9 Rhesus monkeys the sciatic nerve was sectioned with sharp scissors peripheral to the sciatic notch or at the mid-thigh level, and the central cut end then soaked in as little as 0.1 cc of 20% virus suspension for several minutes. Poliomyelitis resulted after an incubation period of 4-6 days. In 5 cases the leg on the side of inoculation was completely paralyzed on the fifth day; the opposite leg was usually paralyzed completely also during the course of the same day.

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1 Fairbrother, R. W., and Hurst, E. W., *J. Path. and Bact.*, 1930, **33**, 17.

2 Howe, H. A., and Bodian, D., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 346.

3 Trask, J. D., and Paul, J. R., *J. Bact.*, 1936, **31**, 527.

A definite quantitative factor was observed with respect to the number of nerve fibers exposed to the virus. In 3 cases in which only the nerve to the hamstring muscles was cut and the cut end then soaked with virus, no paralysis resulted. Inoculation of the cervical sympathetic trunk by the same method in 2 animals failed also. In one case in which the central cut end of the vagus nerve was immersed in virus for 3 minutes, paralysis did not result, whereas in another animal in which the vagus nerve was similarly soaked for 15 minutes, neck paralysis and hoarseness resulted after an incubation period of 17 days. Inoculation of the hypoglossal nerve by this method was also successful in one case, the incubation period being 4 days. Apparently then, the numbers of nerve fibers exposed, and perhaps the size of the fibers and the length of time immersed are significant quantitative factors. In any case, only a small quantity of virus suspension is necessary.

The significance and advantages of the above described method of inoculation of peripheral nerves are obvious. When sufficient numbers of axonal processes of nerve cells are exposed to small amounts of virus at a definite point in the peripheral nerve, paralysis results. Because of the definitely known location at which the virus first comes into contact with the nerve fibers, because of the assurance that all of the fibers of the nerve are placed in contact with the virus, and because in any particular nerve the numbers and sizes of fibers thus exposed can be determined, this method offers possibilities for quantitative determinations of virus potency, of speeds of transmission of viruses along nerves, and of other important but not easily obtained data.