The problem of artificial immunization in poliomyelitis continues to hold attention because of the reappearance of epidemics of poliomyelitis in different parts of the world and the increasing knowledge of immunity to the class of so-called virus diseases, of which poliomyelitis is one example. That it is possible to produce an artificial active immunity in monkeys to the poliomyelitis virus by repeated sub-clinical, subcutaneous, and intradermal injections of the virus was shown originally by Flexner and Lewis in 1910 (2), later by Aycock and Kagan (3), and recently by Stewart and Rhoads (1). The general literature on the subject has been reviewed by the last named authors in their paper.

More recently still we have carried out a test on four Macacus rhesus monkeys in which repeated intradermal injections of a highly potent virus replaced the injections of the weaker M.A. and Aycock strains. When the less potent strains are employed, immunization usually takes place without clinical manifestations, but when the highly potent strain is used, the result is different:—The monkeys of our test became ataxic, tremulous, and excited about a week after the third intracutaneous injection, or, in other works, presented the appearances manifested by ordinarily inoculated monkeys a day or two before the onset of paralysis. Of the four monkeys showing the preparalytic symptoms, three recovered completely—that is, the symptoms aborted—while the fourth became typically paralyzed and succumbed to experimental poliomyelitis, the spinal cord and medulla revealing characteristic lesions. Flexner and Lewis described the occurrence of isolated instances of paralytic poliomyelitis in monkeys undergoing sub-clinical immunization, and Aycock and Kagan and Stewart and Rhoads...
noted the same occurrence. The greater number of monkeys, when injected repeatedly with weak virus strains, become actively immune without exhibiting abortive symptoms.

These observations raise the question whether repeated minute inoculations of poliomyelitis virus do not act in certain instances to render the treated monkeys, not more resistant but more susceptible to subsequent injections into skin and subcutaneous tissue, such as would usually be ineffective. It seemed possible that a larger quantity of potent virus given intradermally at one injection might accomplish active immunization without inducing any symptoms whatever. The experiment now to be recorded constitutes a test of this second point.

EXPERIMENTAL

Four *Macacus rhesus* monkeys were prepared by shaving the ventral surface from the clavicle to the pubis, and cleaning the skin carefully with alcohol. The material used was a 5 per cent physiological saline suspension of recently glycero-lated spinal cord of the pooled, mixed virus strain. This strain is one of known constant activity; doses as small as 0.005 cc. of Berkefeld filtrate are uniformly infective when inoculated intracerebrally, and repeated small doses of the same strain given intradermally produce poliomyelitis. Inoculation was performed with a fine needle on a tuberculin syringe, and evenly spaced blebs of the material, about 0.5 cm. in diameter, were introduced into the skin, until the entire amount, 16 cc., had been given. Great care was observed to prevent the material from entering the subcutis, to obviate the possibility of too rapid diffusion of the virus. The animals were examined daily both for symptoms of poliomyelitis and for local reaction in the skin area treated. At no time were any abnormalities observed.

A month was allowed to elapse and the animals were bled for neutralization tests. This procedure was deemed advisable since Stewart and Rhoads (1) have shown that the power of serum to neutralize virus is a more delicate test of immunity than is the resistance of an animal to actual inoculation of the virus itself. Each serum obtained was set up in a volume of 0.99 cc. against 0.01 cc. of a Berkefeld N filtrate, prepared from a 5 per cent physiological saline solution suspension of fresh poliomyelitis spinal cord. The mixtures were placed in the incubator for two hours and in the icebox overnight. The following morning each was inoculated intracerebrally in a normal monkey.

The power of the serum to neutralize virus was compared with the resistance of the animals to actual introduction of infectious material into the central nervous system. The treated monkeys with a normal control were inoculated in the cisterna magna with 0.01 cc. of virus filtrate—about five minimal lethal doses of the same strain. Since intracerebral inoculation involves considerable trauma the cisternal route was selected as providing conditions more nearly resembling those in man when infection occurs (4).
Results

The results of the experiments are summarized in the tables. The neutralizing power of the sera tested is shown in Table I. Neutralization was complete in every case, whereas in the control instance an equal amount of virus treated with normal serum led to typical poliomyelitis after a seven day incubation period. It therefore appears clear that the intradermal inoculation described produced a definite degree of immunity.

Table II shows the results of intracisternal inoculation of virus into
the monkeys that had received the immunizing treatments. As one animal of the series died of intercurrent disease, only three treated animals could be tested. Two remained free of all symptoms, while the third developed typical symptoms of poliomyelitis, became paralyzed in extremities and back, was prostrate for some time, but recovered, although marked residual paralysis persisted. The inoculated control animal, on the other hand, became paralyzed on the fourth day, prostrate on the fifth, and died on the sixth. The difference in the results of the tests in the treated and control animals is rendered more striking by the fact that the strain of virus used, when injected into normal monkeys, was almost invariably fatal within eight days.

CONCLUSIONS

1. By means of a single large dose of poliomyelitis virus, distributed at a number of intradermal sites, active immunity has been produced in Macacus rhesus monkeys, as shown by skin neutralization and intracisternal tests.

2. In the small series (four animals) of monkeys so treated, neither abortive nor paralytic signs of experimental poliomyelitis appeared.

BIBLIOGRAPHY