ORIGINAL ARTICLES

ON THE PROBLEM OF IMMUNIZATION AGAINST POLIOMYELITIS*†

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WE wish to present briefly our observations on *passive,* and also on *active* immunization against experimental poliomyelitis, and to offer an interpretation of the results.

EXPERIMENTS TO DETERMINE THE PROTECTIVE VALUE OF IMMUNE SERUM

Fourteen experiments were carried out in an effort to determine the protective value of immune serum. In all of the experiments the serum was administered at least twenty-four hours preceding the inoculation of animals with virus. An immune *horse* serum of high virucidal titer was used in most of the experiments. Pooled monkey convalescent serum, and pooled normal adult human serum, were included in several experiments for comparison. These serums were administered by different routes and for the most part in very large doses, most of the animals receiving a total of about 5 cubic centimeters per kilo, or an amount equivalent to about 350 cubic centimeters for the average human adult. The virus was inoculated intracranially in half of the experiments, and in half intranasally. The doses used ranged from about 4 to more than 200 M. I. D.

The results obtained were, briefly, as follows: Out of a total of ninety-one monkeys which received immune serum, only twenty-eight, or 30 per cent, escaped infection. Since 7 per cent of the twenty-seven controls used also failed to develop the disease, the net protection for the entire series was not over 23 per cent. The net protection of serum-treated animals inoculated with virus by the intranasal route was 29 per cent.

The incidence of serum-treated animals which escaped infection bore some relationship to the dose of virus with which the animals were inoculated. Of the animals which received more than 100 M. I. D.'s of virus, only 20 per cent escaped infection; of those which received less than this amount, 34 per cent escaped the disease. In certain experiments in which large doses of immune horse serum were administered, and the dose of virus was reduced to less than 10 M. I. D., the net protection rose to about 70 per cent. Convalescent monkey serum proved less effective than immune horse serum, and pooled "normal" adult human serum was the least effective. In short, three-fourths of the animals which were injected with immune horse serum sufficient to neutralize at least 200,000 M. I. D. of virus, failed to resist 100 M. I. D.'s, or more of virus; a fourth failed to resist less than 10 M. I. D. It is apparent, therefore, that for protection against a given dose of virus a disproportionately high concentration of antibodies is necessary.

OTHER FEATURES OF EXPERIMENTAL RESULTS

A noteworthy feature of our results was that while immune serum sometimes seemed to have the effect of considerably prolonging the incubation period, especially in animals which had been inoculated with small doses of virus, when these animals finally developed the disease, they came down with as extensive paralysis as the controls, suggesting that immune serum tends to effect a reduction in the quantity of virus *free to initiate infection,* but that infection once established is not appreciably altered by immune serum. This is in harmony with recent observations on the pathogenesis of poliomyelitis, which indicate that from the beginning of infection to the end of the disease, the virus is in close, probably largely intracellular relationship with neurons, and in harmony, also, with observations we have reported elsewhere; which indicate that serum administered two or more days after the inoculation of animals with virus is without power to modify the course of an infection.

However, while there is evidence that immune serum is without value once the virus has become established in a given neuron, it is not quite so apparent why animals which have been injected with large doses of a high titer immune serum should not be better protected. The explanation probably rests on the fact that the olfactory nerve, the usual portal of entrance, is really very accessible to the virus. Should the specialized endings of this nerve—the so-called olfactory hairs—represent the true portal, then it is easy to understand why the injection of an immune serum does not effectively "block" the entrance of the virus; for it seems quite unlikely that the immune substances injected come into anything like intimate contact with these free endings. While recent measurements on the size of the virus (8 to 12 mu*) are not against this possibility, we are inclined to believe that the true portal may be the "olfactory cells"—in other words, the nuclei of the first group of neurons of the olfactory nerve. This appears the more likely to us in view of the fact that immune serum *does* seem to have the effect of reducing *somewhat* the dose of the virus *free to initiate infection,* a conclusion which may be drawn from the longer incubation periods sometimes seen in serum-protected animals. But whether the exact portal of entry is the olfactory "hairs" or the olfactory cells, or both, the fact remains that immune serum does not seem to provide a very effective barrier at the portal of entrance, a very high humoral immunity being required for uniform protection against even a small dose of virus.

ATTEMPTS AT ACTIVE IMMUNIZATION

Since serum cannot be depended on to prevent the implantation of virus, and since infection,

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† See also pages 108 and 123.

* Mu = a millimicron, or 1/1,000 of a micron.

once established, cannot be appreciably modified by serum, it seems plain that the only hope of controlling this disease lies in the possibility of active immunization. It should be remembered, however, that the term "active immunization" has a somewhat restricted meaning in the case of poliomyelitis, for a true, acquired active immunity to this disease is basically a cellular and not a humoral phenomenon.

We have during the past several years carried out a sufficient number of experiments on active immunization by different procedures to satisfy ourselves that it is much easier to produce an antiserum in monkeys than it is to render them significantly resistant to experimental inoculation with virus. However, feeling that we had used too large a dose of virus in testing the resistance of monkeys "immunized" earlier, we recently undertook to immunize fifteen monkeys with a 10 per cent virus suspension inactivated with 0.1 per cent formaldehyde (Brodie vaccine). Three animals received this vaccine by the subcutaneous route; four by the intramuscular route; and four by the intradermal route, a total of five injections of 0.1 cubic centimeter per kilo being administered to each animal at intervals of one week. Four animals received 1 cubic centimeter of the vaccine per kilo by the intravenous route for five injections. Twenty-four days after the final injection all of the animals were inoculated intracerebrally with about 3 M. I. D. of virus. All developed the disease in about the same length of time, and with about as extensive paralysis as the controls, despite the fact that their serums seem to have acquired slight, but definite virucidal properties. The serums of another series of animals "immunized" earlier with *living* virus (Kolmer vaccine) neutralized 30 M. I. D. doses of virus per cubic centimeter, but when these animals were subjected to intranasal instillation with active virus they all developed typical poliomyelitis. In still another experiment we repeatedly injected four monkeys with 1 cubic centimeter of formaldehyde inactivated virus by the intracerebral route. Two months after beginning the immunization they were all given an intracerebral injection of 1 cubic centimeter of a virus suspension treated with 0.005 per cent formaldehyde for twenty-four hours at 37 degrees centigrade. All developed extensive paralysis in the usual length of time.

Finally, we instilled formaldehyde inactivated virus intranasally into four monkeys, giving five instillations at intervals of a week, each instillation being preceded by an intranasal lavage. All of these animals came down following an intranasal instillation with *active* virus.

DIFFICULTIES ASSOCIATED WITH ACTIVE IMMUNIZATION

The difficulty associated with active immunization apparently rests on the fact that normally susceptible neurons are not readily modified except by intracellular contact with active virus. In other words, true acquired active immunity to this disease seems to depend on some *modifying action* which rests on *active neural infection.* As we see it, this modification need *not necessarily* be associated with demonstrable antibodies in the blood. It seems possible that such humoral antibodies as may make their appearance in *naturally acquired active immunity* to this disease may rest largely, if not entirely, on chance contact of virus with extraneural tissue and may, therefore, be entirely adventitious so far as the true immunity is concerned. It is conceivable that if the virus remained confined strictly to the neurons during the course of a natural infection, an acquired active immunity might result which would *not* be associated with antibodies in the blood plasma. That the virus, however, does not remain so confined is evidenced by round cell infiltration, not only where neurons have been damaged, but in regions where no actual damage is recognizable. Contact with extraneural tissue is all that is necessary to account for antibody production. It should be borne in mind, however, that antibody formation which results from virus which has emanated from infected neurons must be distinguished from antibody formation, which is the result of artificial extra neural injections of virus. In the former, the antibodies produced are an incidental and adventitious part of a *neuron modifying infection,* in the latter they may represent the *sum total of the protection afforded.* Such partial immunity, as may at times be demonstrated following the administration of poliomyelitis vaccines, is probably humoral rather than cellular in nature, and since such humoral immunity may largely or entirely die out with the progress of time, the *durability* as well as the *degree* of such artificially induced active immunity seems open to question.

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BLOOD CULTURES IN BRUCELLA INFECTIONS (A NEW METHOD) *

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DURING an epidemiological investigation it is very important that the nature of the Brucella infection of the patients be conclusively established. A correct differential diagnosis between the three types of infections, which may be due to Brucella melitensis, abortus and suis, without the isolation of the causative organism, is, as a rule, impossible. Some indication may be obtained with the aid of the agglutinin absorption test, but reliable information can be secured only by a detailed study of the bacteria isolated either from the blood, the urine, the spinal fluid or the suppurative lesions of the patients. It is quite generally believed that blood cultures, particularly in the abortus infections, are of little value, although the data in the literature do not entirely support this contention.

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