

number of patients had not yet missed a menstrual period at the time the test was made and a large group was represented by those who were in the first 4 to 6 weeks of pregnancy. The clinical material embraced all the usual problems in the differential diagnosis of pregnancy. These were represented by glandular insufficiency, functional amenorrheas, menopausal symptoms, uterine fibroid with or without pregnancy, complete or incomplete abortion, miscarriage, ectopic gestation, hysteria, dead fetus, and the like.

The test has proved 100% accurate in this study despite the rapid method and it differentiates pregnancy from conditions that simulate it. In medicolegal cases the test has been found valuable and equally important in cases demanding therapeutic abortion or in circumstances requiring prompt diagnosis or the exclusion of pregnancy.

No mortality has occurred among the rats in the course of injections.

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Incidence of "Normal" Persons Possessing Demonstrable Antibodies for Poliomyelitis Virus in Their Serum.*

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Recent observations by Aycock and Kramer¹ and by Shaughnessy, Harmon and Gordon² on the incidence of normal persons possessing poliomyelitis antibodies in their blood serum are of considerable interest not only from the standpoint of the epidemiology of this disease, but also from that of its serum therapy. The observations of Shaughnessy and his associates indicate that the titer of these antibodies in the serum of certain "normal" adults may not only equal, but appreciably exceed that of the average poliomyelitis convalescent. These important observations have prompted us to make a similar survey in this region.

The Aycock strain of the virus is being used in our studies. This strain produces poliomyelitis in rhesus monkeys with great regularity, the first symptoms of the disease appearing generally between

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¹ Aycock and Kramer, *J. Prev. Med.*, 1930, 4, 189, 201.

² Shaughnessy, Harmon and Gordon, *J. Prev. Med.*, 1930, 4, 463.

the 5th and 8th day. Pieces of cord and medulla were weighed and very finely ground in a motorized mortar³ in the presence of sterile ground pyrex glass. Enough neutral physiological saline solution was then added to this ground material to make a 5% suspension of the virus-tissue. After light centrifugation for 10 minutes and filtration through sterile filter paper this suspension was distributed in 1 cc. quantities to a series of serological tubes containing equivalent volumes of the various sera (or serum dilutions) to be tested. In the first series of tests these serum-virus mixtures were incubated at 37°C. for 2 hours. In the second series no incubation preceded the injection of the serum-virus mixtures. The various serum-virus mixtures were injected into the frontal lobe of the brain of *M. rhesus* monkeys in doses of 1.5 cc. Monkeys which were protected against injection by a given serum dilution were again used in testing the same serum in the next higher dilution 15 to 20 days later. This procedure has been tentatively adopted by us to economize on the number of monkeys which would otherwise be required in estimating the actual antibody content of these sera. Thus far no immunizing action of such serum virus mixtures has been detected. Should this later prove to be the case, it will not be without interest. Each series was adequately controlled not only with normal monkey, but also monkey poliomyelitis convalescent serum-virus mixtures. To insure more uniform results 10 or more human sera were tested at one time.

Tests were run on the sera of a total of 32 "normal" adults, persons who to their knowledge had never suffered from recognizable poliomyelitis. Thirteen of these are included in the first series, in which the serum-virus mixtures were incubated at 37°C. for 2 hours before injection into monkeys. Of this number 9 inactivated the virus in serum dilutions of 1-2.† Only 2 of the sera in this first series were tested in the next higher dilution (1-30). Both failed to protect in this dilution. The ages of the persons tested in this series ranged from 16 to 48 years; most of them were in the early twenties. Of those which neutralized the virus, a boy and his father represent the 2 age extremes, a third member of the family (25 years of age) tested in the following series, also neutralized the virus. Nineteen sera were tested in the second series, in which the mixtures were injected without preliminary incubation. Of these 9 inactivated the virus when the serum was diluted 1-2. It is not clear whether this lower incidence is to be attributed to the lack of

³ Schultz and Banham, *Am. J. Pub. Health*, 1930, 20, 771.

† Virus suspension 1 part, undiluted serum 1 part.

incubation, or to a lower incidence of neutralizing sera in this series. The ages of the persons in this series ranged from 20 to 52 years, the majority being under 25 years. Of the 9 which inactivated the virus, only 5 have thus far been retested in the next higher serum dilution (1-30). Two of these failed to neutralize the virus in this serum dilution and 3 not only protected in this dilution, but also in a dilution of 1-60. Tests have not yet been carried out to determine whether these monkeys have become refractory.

These studies are not sufficiently advanced to permit a generalization as to the actual incidence of persons possessing poliomyelitis immune substances in their blood nor to throw any further light on the concentration of antibodies represented in these sera. Nevertheless, the results tend to confirm the observations of previous investigators. There is little doubt that were tests carried out in the classical manner inaugurated by earlier investigators, such as Flexner, in which the ratio of serum to virus is much greater, a larger incidence of poliomyelitis immune persons could be detected in the normal adult population. We plan to test this hypothesis. As to the incidence of persons whose serum will titer as high, or higher than the average human convalescent serum, much more work is necessary to reveal this relationship. There is sufficient evidence to suggest that the incidence may be of fair magnitude. That not all human convalescent serum is capable of inactivating the virus even when favorable relationships obtain is well recognized. That the antibody level of the average convalescent serum may indeed be relatively low is suggested by the recent work of Shaughnessy and his associates. Of 4 human poliomyelitis convalescent sera, run as auxiliaries in our series, 2 neutralized in a serum dilution of 1-2, while 2 did not. The 2 which protected in this dilution, failed to do so in a serum dilution of 1-30. How the viricidal properties have been acquired by persons who have no knowledge of having had the disease is not entirely clear. Aycock and Kramer⁴ have adduced evidence that the immunity is a gradual acquisition, increasing with the age of the individual. They found that new born children of mothers possessing viricidal antibodies in their serum, present the same properties in their blood for a time. Thereafter the sera of younger children apparently quite uniformly fail to show this property. The natural inference is that the immune principle is acquired as a result of subclinical infections with poliomyelitis virus, though it is possible that it may not rest altogether on this basis.

⁴ Aycock and Kramer, *J. Exp. Med.*, 1930, **52**, 457.