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# Serum Protection Tests with the Lansing Strain of Murine Poliomyelitis Virus.

C. G. HARFORD AND J. BRONFENBRENNER.

*From the Department of Bacteriology and Immunology, Washington University School of Medicine, St. Louis, Mo.*

Armstrong's[1, 2] method for the adaptation of the Lansing strain of human poliomyelitis virus has consisted of preliminary passage in monkeys and cotton rats. Because previous attempts by many workers[3] to adapt the virus of poliomyelitis by direct passage to mice have failed, it has been suggested that the Lansing virus in mice may possibly represent not the original human virus but some other virus picked up during that passage. Although Armstrong[2] showed that the sera of some monkeys convalescent from the disease protected against the virus, we thought that further study of the identity of the virus by means of serum protection tests was desirable.

In our experiments protection tests were carried out in such a way as to allow direct statistical analysis of the findings and have found that serum tests carried out and evaluated as described give significant results. The protection tests were carried out mainly with human and monkey sera taken early and late after onset of paralysis in order to see whether antibodies capable of protecting against the Lansing strain developed following the paralytic disease. We have included also a sample of pooled human convalescent serum as well as the serum of rabbits immunized by injections of mouse passage virus.

Fresh virus suspensions were prepared for each individual experiment by grinding the base of the brain and the spinal cord of one or two paralyzed mice with enough saline to make about a 1:4 suspension. Only mice paralyzed within 8 days after inoculation and those in which paralysis had existed for not longer than 2 days were used. After centrifugation, 0.75 cc of the supernatant was added to an equal amount of serum, mixed thoroughly and without further incubation 0.03 cc of the mixture was injected intracerebrally into each of 20 Swiss mice. Care was taken to make the control normal

1 Armstrong, C., *Pub. Health Rep.*, 1939, **54**, 1719.

2 Armstrong, C., *Pub. Health Rep.*, 1939, **54**, 2303.

3 Harmon, P. J., Shaughnessy, J. H., and Gordon, F. B., *J. Prev. Med.*, 1930, **4**, 59, 89.

TABLE I.

| Experiment No. | Age of mice (days) | Serum | Day of death or onset of paralysis* | Day survivor discarded | No. infected§ | Result | $X^2$ value |
|---|---|---|---|---|---|---|---|
| 1 | † | Normal infant‡ | *2* *2* *2* 2 2 2 2 2 2 3 5 10 12 12 *12* *15* D 19 *45* | 57+ | 18/19 | | |
| | | Pooled human convalescent | *28* 29 D | 57+ | 2/19 | + | 27.02 |
| 2 | 30 | R.T. ♂ 3, 2 days after onset of paralysis‡ | D 2 *3* 3 3 3 3 *4* *4* *7* D *16* *16* *18* *18* *21* *23* 25 *28* | 84 | 17/18 | | |
| | | R.T. ♂ 3, 78 " " " " " | *3* *3* 3 3 3 *4* 4 *5* *18* 19 19 19 20 D *32* 32 32 24 | 84 | 17/19 | — | |
| 3 | 30 | Normal infant‡ | *2* *2* 2 2 2 *3* *3* 3 3 3 4 *11* *11* 11 17 *17* 21 21 21 22 | 62 | 20/20 | | |
| | | J.M. ♀ 16, 17 days after onset of paralysis | 27 30 33 34 *36* 40 | 62 | 6/20 | + | 21.53 |
| | | J.M. ♀ 16, 111 " " " " " | 7 *11* 19 39 | 62 | 4/20 | + | 26.66 |
| 4 | 27 | Normal infant‡ | *3* *3* 3 3 4 4 *6* 8 9 10 12 12 14 18 19 22 28 28 30 | 67 | 19/20 | | |
| | | E.H. ♂ 20, 11 days after onset of paralysis | 7 *12* *17* D | 67 | 3/19 | + | 24.86 |
| | | E.H. ♂ 20, 90 " " " " " | *3* 3 4 4 4 5 *5* 5 *8* *16* 24 *26* 32 33 53 | 67 | 15/20 | — | 3.14 |
| 5 | † | Monkey, normal | 4 *5* 20 20 21 23 *24* 28 32 32 36 | 36 | 11/20 | | |
| | | Monkey R.H. 42, 1 month after paralysis‡ | 2 2 2 2 *3* 3 3 3 5 5 *7* 10 10 12 *14* 15 17 *21* 25 | 36 | 19/20 | | |
| | | Monkey R.H. 42, 3 " " " | 15 *21* 22 30 *34* 36 | 36 | 6/20 | + | 18.02 |
| 6 | 24 | Monkey R.H. 10 (normal) | D *3* *3* *3* *3* 3 3 3 4 *9* *10* 12 *12* 17 27 33 51 D | 60 | 17/18 | | |
| | | Monkey R.H. 135 " ‡ | *3* *3* 3 3 3 3 *5* *8* 8 9 11 *12* 16 22 27 *28* *32* 38 | 60 | 18/20 | | |
| | | Monkey R.H. 42, 1 month after paralysis | *3* 3 3 4 5 *6* 8 10 14 15 *19* 19 22 23 33 37 | 60 | 16/20 | — | 0.78 |
| | | Monkey R.H. 42, 3 " " " | 4 *5* 5 *14* 20 23 33 *60* | 60 | 8/20 | + | 10.98 |
| 7 | 28 | Monkey R.H. 10 (normal)‡ | *2* 2 2 2 3 4 6 10 12 13 *15* *18* 19 20 *26* 38 49 | 64 | 17/20 | | |
| | | Monkey R.H. 135 " | *2* *2* *2* 2 2 4 5 *6* 6 10 11 *15* 15 16 *17* *23* 34 49 *53* | 64 | 19/20 | | |
| | | Monkey R.H. 42, 1 month after polio | *2* *2* 2 *3* 3 4 4 8 15 15 17 *18* D 22 26 D 40 57 59 | 64 | 17/18 | — | |
| | | Monkey R.H. 42, 3 " " " | D 5 *10* D D 31 *38* *47* | 64 | 5/17 | + | 11.78 |
| 8 | † | Rabbit (normal)‡ | 4 *5* 5 8 9 10 12 13 16 *17* D 18 D *21* 25 | 31 | 13/18 | | |
| | | Rabbit (immunized)‖ | *9* 10 11 13 18 18 *19* 24 31 | 31 | 9/20 | — | 2.88 |

*The figures indicate the day after inoculation that paralysis was first observed. Italicized figures indicate the day of death, during the night, without parálysis having been observed previously.

†In most instances all animals were of exactly the same age (indicated in days). Where the exact age is not indicated, animals of the same weight were used.

‡These data were used as control in calculations.

§Numerator indicates the number infected; denominator the total number of animals.

‖The immune rabbit serum was obtained as follows: Each of 2 rabbits was given a total of 12 injections of half of a suspension prepared by grinding a glycerinated spinal cord and base of the brain with 2 cc of saline. Rabbits were bled 2 weeks later.

Sign "D" indicates that the mouse died of a cause known not to be poliomyelitis; such ones are not included in the evaluation of the result.

A chi square value over 3.841 is considered "significant," and one over 6.635 is considered "highly significant."

serum-virus mixtures first and to inject the immune serum-virus mixtures first so that variation in time of contact with serum or time before injection could not influence the result.

The incubation period with this infection has varied within wide limits (2 days to 59 days) and, in spite of about 30 passages, this variability still persists. For this reason, in each case we have recorded the number of days elapsed between the injection and the first manifestation of infection. Calculation from the figures in Table I shows that, of those animals eventually dying or becoming paralyzed, 24% did so after an incubation period of 21 days. Furthermore, it will be noted that a greater proportion of long incubation periods tended to occur in mice receiving injections of immune serum-virus mixtures.

Schaeffer and Muckenfuss[4] have emphasized the irregularities in the results of protection tests encountered when the monkey is used as a test animal. This appears to be true also with respect to results obtained with tests in mice. However, calculation of the value of chi square, as described by Mainland,[5] indicates that our results represent data which are statistically valid. We believe that, following the procedure described above, we have avoided the use of more animals than necessary and also have obtained results of maximum significance.

The sample of pooled human convalescent serum used in Experiment 1 was part of that which was subjected to repeated testing in monkeys by Schaeffer and Muckenfuss. In our single test with the Lansing strain in mice, it also gave protection.

Neither of the samples of serum of the patient R.T. neutralized the virus (Experiment 2). This boy had definite clinical paralytic disease and may have developed protective antibody, but the failure of his serum to neutralize the Lansing virus might have been due to the fact that his infection was caused by an antigenically heterogeneous strain.

Both of the samples of J. M. showed the presence of antiviral property and this is thought to be due to the fact that the first sample was taken so late after the acute stage of the infection (17 days) that the development of antibody had already taken place (Experiment 3).

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[4] Schaeffer, M., and Muckenfuss, R. S., *Experimental Poliomyelitis,* published under the auspices of the National Foundation for Infantile Paralysis, 1940.

[5] Mainland, D., *The Treatment of Clinical and Laboratory Data. An Introduction to Statistical Ideas and Methods for Medical and Dental Workers,* Oliver and Boyd, 1938.

We have no explanation as to why the serum of E.H. showed antibody shortly after onset of paralysis but failed to protect mice later on (Experiment 4).

Sera of Monkey R.H. 42 were obtained from Dr. A. B. Sabin. This animal had been paralyzed by the MV strain of poliomyelitis virus and protection tests carried out in monkeys by him had yielded the same results as those we have secured by protection tests in mice (Experiments 5, 6, and 7). The main reason for repeating the experiments with monkey sera is the fact that one presumably normal monkey serum (Experiment 5) appeared to have exhibited a considerable amount of protection. These experiments with the monkey sera indicate that antibody appeared in this animal between one and 3 months after paralysis.

Although the injection with the serum of immunized rabbits resulted in fewer animals developing the disease, calculation showed that results of this experiment were not statistically significant (Experiment 8).

Thus, while some of the human sera neutralized with Lansing virus, we have been unable so far to secure evidence that such neutralizing antibodies appear shortly after the clinical signs of infection. Similar findings with monkey tests have been obtained by other workers.[6, 7] However, the experiments with the sera of the monkey definitely indicate the development of neutralizing antibody after paralysis. These limited experiments cannot be said to have proved the identity of the Lansing virus. However, both the results with human and with monkey sera are consistent with the supposition of the existence of a serological relationship between the Lansing strain and the virus causing some cases of human disease.

Since we began these experiments, the reports of serum protection tests by Haas and Armstrong,[8] and with similar strains by Jungeblut and Sanders[9] and Toomey and Takacs[10] have been published. Nevertheless, we have considered our results worthy of recording not only because they strengthen the evidence in favor of considering the virus of Armstrong as immunologically related to poliomyelitis, but

[6] Brodie, M., Fischer, A. E., and Stillerman, M., *J. Clin. Invest.*, 1937, **16**, 447.

[7] Burnet, F. M., and Jackson, A. V., *Australian J. Exp. Biol. and Med. Sci.*, 1939, **17**, 261.

[8] Haas, V. H., and Armstrong, C., *Pub. Health Rep.*, 1940, **55**, 1061.

[9] Jungeblut, C. W., and Sanders, M., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 375; *J. Exp. Med.*, 1940, **72**, 407.

[10] Toomey, J. A., and Takacs, W. S., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 319.

also because they have been considered in connection with the time of onset of paralysis. This has seemed particularly desirable since Haas and Armstrong found that the occurrence of antibody could be correlated more closely with age than with the clinical disease.

*Summary.* Serum protection tests in mice have been done and the results are significant as tested by a simple statistical method. The experiments are in favor of the view that the Lansing strain is immunologically related to human poliomyelitis.

We acknowledge, with thanks, assistance from the following: Mr. Louis Mihelich and Miss Frances Love (technical assistance), and Doctor Martin Bronfenbrenner (advice on statistical method).

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## A Human Serum Containing Four Distinct Isoagglutinins.

ALEXANDER S. WIENER* AND SAMUEL FORER. (With the technical assistance of Eve Sonn.)

*From the Serological Laboratory, Office of the Chief Medical Examiner, New York City.*

The serum to be described was studied because the patient from whom it was derived had had hemolytic reactions following transfusions of apparently compatible blood. In this report we shall present the serologic findings; the clinical data will be described elsewhere.[1]

The patient belonged to group 0, type N. The patient's serum agglutinated every one of 28 consecutive group 0 bloods. That we were not dealing with an autoagglutinin was proved by the absence of agglutination in mixtures of the patient's serum with her own cells. Evidently the serum contained isoagglutinins in addition to anti-A and anti-B.

Since the patient was Rh negative,[2, 3] it seemed possible that anti-Rh isoagglutinins might be present in her serum. Accordingly the serum was absorbed with pooled, washed cells $A_1$MNRh- and

* Aided by a grant from the Committee on Human Heredity of the National Research Council.

1 Wiener, A. S., Forer, S., and Crooks, P. E., in preparation.

2 Landsteiner, K., and Wiener, A. S., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 223.

3 Wiener, A. S., and Peters, H. R., *Ann. Int. Med.*, 1940, **13**, 2306.