16682

Serum Neutralizing Antibodies to the Infecting Strain of Virus in Poliomyelitis Patients.*†

W. McD. Hammon and E. C. Roberts.

From the George Williams Hooper Foundation for Medical Research, University of California, San Francisco, Calif.

There has been very little satisfactory data collected regarding the development of specific neutralizing antibodies in human patients during an infection with one of the poliomyelitis viruses. Tests applied to convalescent or acute and convalescent sera, have usually been made with any convenient laboratory-adapted strain of virus. In most instances, workers have failed to demonstrate rising antibody titers, such as those encountered in many other virus infections, and frequently antibody has been found in high titer during the acute phase of the disease. These unusual and unexpected findings have been attributed usually to: (1) suspected changes in the virus strain, which might have occurred during adaptation to a laboratory animal, (2) the possibility that infection was due to a virus antigenically distinct from that used for the test, and (3) the possibility that the substance or substances responsible for neutralization are of a non-specific nature.

To our knowledge, only two instances have been reported in which a virus has been isolated from a patient and the same virus used to test the acute and convalescent phase sera of that patient for antibodies.[1,2] In both instances the test was qualitative and served only to indicate roughly the presence or absence of antibody. The results were interpreted as indicating that in the *acute stage*, antibody was absent. Antibody was definitely present in the later specimen in the one instance,[2] but the results of the test on the convalescent specimen were considered equivocal in the other.[1]

Beginning 4 years ago, serum specimens and stool specimens were collected from a series of paralytic patients from various parts of California. A large series of viruses was isolated and monkey adaptation attempted, but unfortunately, most of the viruses had a low degree of pathogenicity for monkeys and could not be satisfactorily adapted to give clear-cut results in neutralization tests. At the present time, after using over 200 monkeys, tests of a reasonably satisfactory nature have been completed on the sera from 7 patients. Since the results are fairly consistent they are being reported at this time. Tests are now in progress on sera from other patients.

Methods. After isolation of a virus from feces, it was passed in monkeys as a 20% cord suspension, or 10-fold concentrate thereof, for not less than 3 passages, or until most of the inoculated monkeys developed paralysis. At this time, except in the earliest experiments, a pool of 20% cord suspension from several monkeys was concentrated in the ultracentrifuge to approximately one-tenth of its original volume and frozen in dry ice in a series of ampoules. Two or 3 monkeys were then inoculated with a mixture of an equal quantity of normal monkey serum and this frozen virus suspension. This mixture had been incubated one hour at 37°C, then overnight at 5°C; the predetermined conditions of the neutralization test to be performed later. If all monkeys became paralyzed, the virus was considered to be adequately adapted for the neutralization test. A number of viruses had to be discarded, after numerous tests showed that paralysis did not develop consistently.

Following the preliminary assay of pathogenicity, the virus was tested with each of 2

* Aided by a grant from the National Foundation for Infantile Paralysis.

† We wish to acknowledge the assistance of W. N. Mack, R. G. Givens, and C. R. Harms in performing these experiments.

1 Trask, J. D., and Paul, J. R., *J. Exp. Med.*, 1933, **58**, 531.

2 Sabin, A. B., *J.A.M.A.*, (June 28), 1933, 749.

or 3 sera from the patient, together with other controls of normal monkey serum. The serum-virus mixtures were incubated as described above, and finally inoculated intracerebrally in monkeys using 2, 3, or 4 monkeys for each serum-virus mixture. The total volume inoculated was usually 1.0 cc, but in a few instances 1.5 cc was used. Development of clinically observable paralysis and of typical pathologic lesions was used as a criterion of failure to neutralize. Monkeys not observed to have paralysis were considered negative and were not sacrificed for sections. Such monkeys were considered to have been "protected". Acute phase sera from the first 3 patients appeared to neutralize the virus. The tests were repeated and the results confirmed. However, in doing so one serum from each series was either used completely, or was lost through an accident. Thus serial dilutions of sera could not be performed, although it became obvious at this time that with antibodies present at the onset, such a method must be employed, if possible differences in titers were to be demonstrated. In the last 4 cases, serial 5-fold dilutions of serum were tested against the concentrated virus suspensions. In order to save monkeys, the sera were usually tested first in a 1:3 or a 1:5 dilution, since undiluted sera in earlier tests had always protected. Normal monkey serum controls were made at the lowest serum dilution used for the human sera.

Results. In the first 3 cases, children with spinal paralytic types of infection, ages 3 to 9, the undiluted acute phase sera taken on the 2nd, 4th and 6th days after onset neutralized the virus as did the convalescent sera. Not less than 4 monkeys were employed for each acute phase serum. Table I presents the results of the last 4 cases, showing age of patient, type of paralysis, number of days after onset of illness when each serum was drawn, and the paralysis ratio of monkeys inoculated. The first serum was frequently drawn before, or at the time of onset of paralysis, usually on the same day as the stool specimen from which the virus was isolated. This generally corresponded to the first or second day of hospitalization.

TABLE I. Monkey Neutralization Tests with Acute and Convalescent Phase Sera from Patients, Tested Against Homologous Viruses Isolated from the Same Patients.

Patient and virus	Age of patient yr.	Type of paralysis	Results* monkey serum controls	Patient's sera					
				Acute			Convalescent		
				Days after onset	Serum dilution	Results*	Days after onset	Serum dilution	Results*
A.K.	6	Spinal	4/4	5	Undil.	0/2	74	Undil.	0/2
					1:5	0/2		1:5	0/2
					1:25	2/2		1:25	0/2
					1:125	—		1:125	2/2
					1:625	—		1:625	2/2
G.P.	16	"	3/3	4	1:5	1/2	24	1:5	0/4
					1:25	2/3		1:25	3/4
Camp.	2	"	4/4	4	1:3	0/3	80	1:3	0/3
					1:15	3/3		1:15	0/3
					1:75	1/2		1:75	0/3
Mo	15	bulbar	4/4	4	1:3	0/3	45	1:3	0/3
					1:15	2/3		1:15	0/3
					1:75	3/3		1:75	0/3

* Numerator represents number of monkeys developing paralysis and the denominator the number inoculated.

Discussion and conclusions. In every instance the acute phase serum gave practically complete protection to all monkeys against a 20% monkey cord suspension which had usually been concentrated 10-fold by ultracentrifugation. A larger series of patients may show some without this titer of antibodies at, or before the onset of paralysis as did a group we reported who appeared to have been infected with a Lansing-like strain,[3] but among this group of 7 patients, ranging from 2 to 16 years of age, antibodies were definitely present either before infection or had already developed by the time of onset of paralysis.

The method of using serial 5-fold dilutions against a constant amount of virus, employed in the last 4 cases, appears to be satisfactory for this type of study and continued tests of this nature should afford important immunologic data on poliomyelitis. We realize that it would have been desirable to have known the titer of virus used in each instance, but we were influenced by consideration of the number of monkeys involved. It is obvious, however, from the results with serial serum dilutions, that the titer of virus used was neither in excess nor insufficient for the information sought.

Among the 4 cases in which serum titrations were made there is an apparent increase in antibody titer by the 45th, 74th and 80th days, but no definite rise in titer is apparent in the one case tested on the 24th day. In one instance the demonstrated increase in titer was only 5-fold, but in the others the increase was 25-fold or greater. It becomes apparent, therefore, that in poliomyelitis, as in most other virus infections, an increase in neutralizing antibodies usually develops as a result of infection. This finding encourages one to believe that a *type specific* serological test for this disease would have practical application. The frequent presence of specific antibody at readily detectable levels at the onset of paralysis or at the time of hospitalization lends further evidence against the possible usefulness of serum therapy, and possibly even serum prophylaxis. Obviously the results of these tests on man carry far more weight than tests on monkeys and chimpanzees following artificially induced infections.

[3] Hammon, W. McD., and Izumi, E. M., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 424.

16683

Hyaluronidase Inhibitors in Body Fluids in Normal and Disease States.

JOHN K. FULTON, STANLEY MARCUS, AND WILLIAM D. ROBINSON.
(Introduced by W. J. Nungester.)

From the Department of Internal Medicine and the Rackham Arthritis Research Unit, Medical School, University of Michigan, Ann Arbor.*

Because of the probable relationship of hyaluronic acid to the formation and integrity of connective tissues, a relationship between hyaluronic acid metabolism and a large group of connective tissue diseases has been suspected, particularly in the case of rheumatic fever, rheumatoid arthritis, and disseminated vascular disease. The technical problems involved and the conflicting evidence which has been forthcoming have been extensively reviewed by Meyer.[1]

This report deals with attempts to relate this group of diseases to hyaluronidase activity, and, more significantly, with the apparent association of many malignant states in humans with disturbances of hyaluronidase inhibitors in the sera of such patients.

* The Rackham Arthritis Research Unit is supported by a grant from the Horace H. Rackham School of Graduate Studies of the University of Michigan.

[1] Meyer, K., *Physiol. Rev.*, 1947, **27**, 335.