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Poliomyelitis Mouse Neutralization Test, Applied to Acute and Convalescent Sera.*

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Through the adaptation of a strain of poliomyelitis virus to the mouse by Armstrong,[1] a mouse neutralization test has been made possible. Haas and Armstrong[2] have already applied this test to post-epidemic surveys, but with results which are difficult to interpret. Thus, we felt it wise to investigate its significance somewhat further by testing a series of acute and convalescent sera from poliomyelitis patients against this same virus. Twenty-three pairs of such sera have now been tested. They were collected from 3 different outbreaks over a period of 2 years. Sera from 1940 and 1941 from the Los Angeles County Hospital were supplied by Dr. John F. Kessel. Another group, collected during the Tacoma, Washington, epidemic in 1940, were obtained from Miss Beatrice Howitt. None of the patients were seen by us, but the clinical diagnoses were made and checked by clinicians well trained in infectious diseases. The diagnosis of the non-paralytic case, in particular, is liable to error, but spinal fluid examination in all instances, together with serum neutralization tests to eliminate the virus encephalitides in several instances, reduced the possibility of error. From one non-paralytic case poliomyelitis virus was isolated from the stool by Miss Howitt.

Method. Both the admission (acute) and the discharge (convalescent) serum from each patient were tested simultaneously to insure comparable results. Positive and negative controls were always tested at the same time. A minimum of 8 mice was used for each serum, and tests on many pairs were repeated to check results. The first series was run by the method described by Armstrong,[2] employing 3 twofold dilutions of virus, but later a technic was adopted which is more comparable to that used for the yellow fever virus, using a large number of mice and only one dilution. A stock, frozen (–76°C), virus was used in all tests. The 50% end point of this frozen virus was calculated by the method of Reed

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1 Armstrong, Charles, *Pub. Health Rep.*, 1939, **54**, 2302.

2 Haas, V. H., and Armstrong, C., *Pub. Health Rep.*, 1940, **55**, 1061.

and Muench after titration under the exact conditions of the neutralization test, each dilution of virus mixed in equal parts with a known negative serum. A dilution containing 10 times the virus of the 50% end point was used for the neutralization test. We found that this selected dilution contained approximately 100 times the greatest quantity of virus which would just permit survival of all mice when mixed with a known negative serum. A few human sera have been found capable of protecting all mice in the presence of a greater amount of virus than that selected, but this dilution was found to represent the approximate limit of protection of the average adult serum. A serum able to protect half the mice was interpreted as a weak positive (+), and one protecting all or all but one mouse as a strong positive (++). All mice were observed for 21 days for paralysis or death. Further details regarding the method and our reasons for adopting it have been discussed elsewhere.[3] Over 200 sera have now been tested against the mouse-adapted poliomyelitis virus by this technic, and we are satisfied as to the constancy of results in our hands.

TABLE I.

Neutralization Tests on Acute and Convalescent Sera of Poliomyelitis Patients.

Case	City	Year	Age	Days interval between bleedings	Days after onset final bleeding	Type of disease	Acute serum	Convalescent serum
1	Los Angeles	'40	4	16	22	Spinal paralytic	0	0
2	" "	"	9	8	24	" "	0	0
3	" "	"	7	19	21	" "	0	+
4	" "	"	14	18	20	Non-paralytic	0	+
5	Tacoma	"	7	10	16	Bulbar paralytic	0	++
6	"	"	9	20	21	" "	0	++
7	Los Angeles	'41	11	—	21	Spinal "	0	++
8	" "	'40	15	13	16	Non-paralytic	0	++
9	Tacoma	"	15	27	29	Bulbar paralytic	0	++
10	Los Angeles	"	16	14	17	Spinal "	0	++
11	" "	'41	30	—	22	" "	0	++
12	" "	"	12	—	21	Non-paralytic	+	++
13	Tacoma	'40	9	14	19	" "	++	++
*14	"	"	12	17	22	Bulbar paralytic	++	++
15	Los Angeles	'41	13	—	20	" "	++	++
16	Tacoma	'40	15	15	17	Non-paralytic	++	++
17	"	"	18	14	17	" "	++	++
18	Los Angeles	'41	18	—	31	Spinal paralytic	++	++
19	Tacoma	'40	25	16	20	Non-paralytic	++	++
20	Los Angeles	"	28	16	21	Spinal paralytic	++	++
21	" "	"	28	18	21	Bulbar and spinal paralytic	++	++
22	" "	"	30	19	21	Non-paralytic	++	++
23	" "	"	38	20	21	" "	++	++

*Poliomyelitis virus isolated from feces.

[3] Hammon, W. McD., and Izumi, E. M., *J. Immunol.*, in press.

Findings. In Table I are presented the results of the tests on the 23 pairs of acute and convalescent sera. The patients' ages ranged from 4 to 38 years with a mean of 17.1 and a median of 15. This group was obviously above the average in age for this disease.

Acute bloods from 11 patients showed no protection. Two of these failed to develop definitely demonstrable antibody 22 and 24 days after onset. These were both spinal paralytic cases. Nine of the 11 negatives changed to positive in from 16 to 29 days after onset. Two of these were non-paralytic, 2 were bulbar and 5 spinal paralytic. One non-paralytic patient's serum changed from weakly positive to strongly positive 21 days after the onset.

A group of 11, composed principally of non-paralytic cases (6 non-paralytic, 1 bulbar and 3 spinal paralytic), showed strong protection at the time of both bleedings.

Discussion. Considering the limited time of hospital observation, 16 to 29 days, it appears quite significant that 9 of 11 patients whose serum was originally without antibody showed a definite increase during the course of the disease, a large increase in most instances. Another, changing from a weak to a strong positive increases the series responding with a definite increase of titer to 10 out of 12.

The series of 11 possessing a high titer of antibody at the time of first bleeding raises a question not possible to answer at this time. It will be noted, however, that this group has a higher mean and median age than does the preceding series; also contains more non-paralytic cases. This antibody, associated with relative immunity, was most likely due to previous inapparent infection and accounts in part, at least, for the infrequent attacks of recognized infection in this older age group. This resistance is probably, however, only relative and may be broken down by some factor such as overwhelming dosage. As suggested by recently published data, immunological differences between strains of poliomyelitis viruses might also be considered in attempting to explain clinical poliomyelitis in persons already endowed with serum neutralizing antibody to one strain of virus.

Larger series of paired sera from many geographical areas should help to answer some of the problems. However, unless a standardized method is employed for both the test and its interpretation, a comparison of various series may very well result in confusion rather than clarification.

The practical application of the mouse neutralization test as a diagnostic laboratory method is obviously limited. In the first place, an acute stage serum must be available to be tested with the con-

valescent specimen. Also, if the first specimen is strongly positive (in this very limited series about 50% of the cases) the test would appear to offer no help in diagnosis. Case 14, in which the diagnosis for this reason could not be confirmed by the neutralization test, yielded virus by inoculation of a stool specimen.

As to the application of this test to population surveys, it seems probable that it will serve in the same way as has the monkey test, and possesses no advantages other than those resulting from economy.

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Physiological Observations Upon Larval Eustrongylides. III. Culture Attempts *in vitro* Under Sterile Conditions.

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Many phases of the metabolism of internal parasites can be studied only with animals kept *in vitro*. Most helminths, however, live only a short time outside their host. A serious objection[1, 2] against such investigations is that the data may be derived from dying animals and may not be representative for healthy specimens. Preliminary experiments mentioned in the first paper of this series[3] showed that a larval Eustrongylides could be kept alive for long periods in sterile surroundings. The goal of the present investigation was to find a medium that would favor the survival *in vitro* of this worm and be simple enough to promise success for future chemical investigations.

Material and Methods. The worms used were larval Eustrongylides (probably *Eustrongylides ignotus*) from *Fundulus heteroclitus*. The sterile extraction of the nematodes required 2 persons. The fish was killed by pithing. It was pinned down on its back and its entire ventral surface was painted with a strong alcoholic iodine solution. The abdominal cavity was opened with sterile instruments and the worm cysts located. Their surface was sterilized by application of iodine solution, and the cyst was then torn open with two

[1] Lapage, G., *Nematodes Parasitic in Animals*, Chemical Publishing Co. of N. Y., Inc., 1938, 30.

[2] Stunkard, H. W., *J. Parasitol.*, 1940, **26**, 1.

[3] von Brand, Th., *J. Parasitol.*, 1938, **24**, 445.