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Research Article

THE ISOLATION OF POLIOMYELITIS VIRUS FROM HUMAN EXTRA-NEURAL SOURCES.[1] III. PERSISTENCE OF VIRUS IN STOOLS AFTER ACUTE INFECTION[2]

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The possibility that the occurrence of virus in stools may form a link in the chain of events operating in the spread of poliomyelitis makes it important to know how long virus is excreted. Little is known of the persistence of virus in stools beyond the first four weeks of the disease. The literature up to 1940 has been summarized by Trask, Paul, and Vignec (2, 3). These surveys indicate that virus is excreted frequently during the first three or four weeks, and rarely thereafter. The number of tests on late specimens however, was few, and insufficient to form the basis for conclusions as to the period of infectivity of stools. The outstanding example of long persistence of the virus was reported in 1939 by Lepine and his associates (4), who demonstrated it in the feces of a child 74 and 123 days after the abortive disease. Since 1940 two late isolations have been reported: one by Howitt, Buss, and Shaffrath (5), 50 days, and another by Wenner and Casey (6), 45 days after the onset of poliomyelitis. Piszczek, Shaughnessy, Zichis, and Levinson (7) recovered virus from the stools of a healthy contact who had been exposed one and two months before the specimen was collected, and Brown, Francis, and Pearson (8) found virus in the stool of a contact 19 days before the individual developed paralytic poliomyelitis.

The present study was undertaken to determine the average duration of excretion of virus in stools of patients following acute infection, and to ascertain whether a chronic carrier state similar to that occurring in typhoid fever exists in poliomyelitis.

MATERIALS AND METHODS

Sixty patients, who were admitted to the New Haven Hospital during the summer epidemic of 1943, and one who entered in February, 1944, were selected for study. In all of these, a clinical diagnosis of poliomyelitis had been established. The number of paralytic cases outnumbered the non-paralytic type, there being 46 and 15 respectively.

Collection of stools: An attempt was made to collect specimens during the first or second week of the disease, and at 4- to 6-week intervals thereafter. In some, however, acute specimens are lacking, and in others the time interval between specimens is irregular. Some of the specimens represent stools alone, others stools plus enema returns, and a few, enema returns alone. All materials were frozen immediately or within a few hours after collection, and stored on solid carbon dioxide.

Preparation of specimens for inoculation: The specimens were usually 38 to 50 grams in quantity, although some were as small as 10 grams. The technique of preparation was that developed by Melnick (9). It consisted of mixing the stool with sterile distilled water in a Waring blendor for 5 minutes, then spinning the resultant thick mixture in the Sharples supercentrifuge at approximately 25,000 RPM for 20 minutes. The sediment was discarded, and the supernatant fluid was shaken with ether and allowed to stand in the refrigerator at 4° C. overnight. On the following day, the ether was removed, and the stool suspension was cleared of gross sediment by centrifugation in the angle centrifuge at 3000 RPM for 20 minutes. Approximately 30 ml. amounts of each specimen were then spun in the ultracentrifuge at 39,000 RPM for one hour. The pellets thus thrown down were resuspended in 2 ml. of 10 per cent normal rhesus serum in distilled water. The suspension was either frozen until inoculated, or prepared for immediate inoculation by a second ultracentrifugation at low speed (18,000 RPM) to clear the solution and render it bacteria-free.

Test animals: Immature rhesus monkeys, weighing 2 to 4 kgm. were used in the testing of all specimens. Daily exercise records were kept, and temperatures were recorded daily or every other day. Since the number of specimens was so large, it was not possible to use a new animal for each test. If, therefore, a monkey showed a negative clinical course and a normal temperature record during the 4- to 5-week observation period, the result was regarded as negative and the animal was reinoculated with a different specimen. An attempt was made to use specimens for reinoculation which corresponded in time of collection to the materials which the animal had received previously. As is shown in Table I, some animals so reinoculated were proven to be susceptible to poliomyelitis after as many as 3 and 4 negative tests. Those animals which were repeatedly negative

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[2] A preliminary report of part of this work has appeared in the J. A. M. A. (1).

TABLE I

Incidence of positive tests in animals used various times

Number times used	Number monkeys	Positive	Positive	Positives in relation to week of disease					
				1 to 2	3 to 4	5 to 6	7 to 8	9 to 10	11 to 12
		number	*per cent*						
1	36	24	67	20	1	3			
2	33	8	24	3	2	1	1		1
3	11	5	44	4	1				
4	7	1	14		1				
5	5	1	20				1		
6	1	0	0						

received for the most part late specimens—from the 8th to the 24th week.

Technique of inoculation: Two routes of inoculation were employed: intracerebral, and directly into the lumbar cord (10). In 64 tests, up to 1.0 ml. of inoculum was given through a small trephine opening over the frontal lobe, and in 80 a similar amount was given in multiple injections into the lumbar cord; in 12, both the intracerebral and intralumbar routes were used. As the experiment progressed, it became apparent that the latter route was less reliable than the intracerebral approach, and it was abandoned. A few specimens which were negative when injected into the lumbar cord, were positive when retested intracerebrally. A comparison of the two techniques has been reported (10).

Criteria for the detection of poliomyelitis virus: Two requirements were deemed necessary in establishing a test as positive: (*1*) The production of the clinical picture of poliomyelitis in the rhesus monkey after a variable incubation period. In all cases this meant the presence of some or all of the usual signs such as fever, tremors, ataxia, weakness, and paralysis. (*2*) The demonstration of typical pathological lesions in the spinal cord and brain stem. The first requirement was met in all positive tests. The second was fulfilled in all but two instances, where the monkeys developed typical paralytic disease and were spared for usage as controls in another experiment.

RESULTS

In all, 157 tests were performed on 133 specimens. Thirty-six specimens were positive, 92 negative and 4 were incomplete due to premature death of the test animal. Figure 1 illustrates the time distribution of the positive tests among the 128 specimens on which complete tests were carried out.

Virus appears to be excreted in a relatively high percentage (50 to 70) of instances in the first 4 weeks of the disease. The rate then falls off steadily, although at 5 to 6 weeks, 27 per cent of specimens tested were positive and at 7 to 8 weeks, 12.5 per cent. Between the 9th and 24th weeks, only 1 of 53 specimens tested was shown to contain virus; this was the latest positive obtained, and came from a boy 8 years old with paralytic disease, in the 12th week after onset. The individual tests are summarized in Table II.

It has been emphasized previously that the stools of young children under 8 years more frequently contain virus than do those of older children; as a result the younger child has been suspected of being the more frequent and important carrier. However, we found in this series no significant difference in the results obtained with specimens from the two age groups, within the first four weeks of onset, 67 per cent being positive in the younger group, and 70 per cent in the older group. Sabin and Ward (11) have summarized the literature on this point, and our results are added to their summary in Table III.

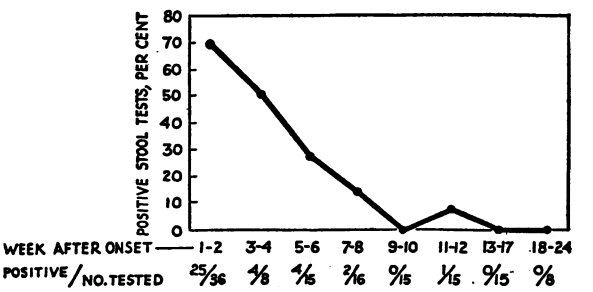

FIG. 1. EXCRETION OF VIRUS IN STOOLS OF POLIOMYELITIS PATIENTS

Although the number of paralytic cases in our series is over three times as great as the nonparalytic, the totals are sufficient to make some comparison between the two groups. Analysis of patients whose stools were tested at least once during the first six weeks of the disease (Table IV) shows no significant difference in the distribution of positives between paralytic and nonparalytic cases, either in the younger or older age groups.

Of all the tests performed, in only one patient was a negative test followed by a positive one. M. L., a 5-year-old, passed a stool during the first week of her disease which gave a negative test. Five weeks later a second stool specimen was obtained and this yielded a positive test. It should be pointed out that with a few exceptions only one monkey was used to test each specimen; the bio-

TABLE II

Excretion of poliomyelitis virus in stools of patients

Name	Age	Type disease	Week after onset																					
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1 J. Pa.	1½	P		+			−																			
2 R. Z.	1½	P			−									−, −				−								
3 S. McK.	1½	P																								
4 L. W.	2	P					−					−														
5 L. P.	2	P	±, +									−, −														
6 J. D.	2	P		+, +			−															−				
7 P. S.	3	P					−																			
8 F. C.	3	P								−, −					−											
9 M. F.	3	P										−, −					−									
10 R. G.	3	P	+						inc.					−												
11 T. R.	4	P	+, +					−, −																		
12 T. C.	4	P						−				−														
13 B. D.	4	P	+, +										−				−, −									
14 A. Ba.	4	P				−										−, inc.										
15 V. W.	5	P							inc., −					−												
16 N. G.	5	P								−																
17 M. L.	5	P	−				+		−							−										
18 M. S.	5	P						−																		
19 B. S.	6	P													−											
20 B. F.	6	P	−										inc.													
21 P. R.	7	P				+					−															
22 B. DiC.	7	P		+					−									−								
23 D. S.	8	P	+								−, −					−										
24 J. N.	8	P	+											+												
25 R. L.	8	P	+															−					−			
26 R. H.	9	P			+							−														
27 J. B.	10	P			−, −						−															
28 P. C.	10	P	−										−													
29 E. K.	11	P						+												−						
30 R. S.	12	P	−						−																	
31 J. F.	12	P	+										−									−				
32 J. Pr.	13	P	+											−												
33 E. V.	13	P						−																		

TABLE II—*Continued*

Name	Age	Type disease	Week after onset																						
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
34 K. B.	14	P										−													
35 K. K.	14	P	−					−																	
36 K. Kl.	14	P											−												
37 E. M.	15	P	+				−					−, −													
38 J. S.	15	P	+										−, −												
39 M. G.	15	P					+					−								−					
40 A. L.	15	P	+							−, −				−, −											
41 W. W.	16	P		+				+														−			
42 R. C.	16	P							+										−						
43 W. M.	17	P		−						−, −															
44 R. K.	17	P					−, −							−											
45 M. M.	23	P				−, +				−															
46 D. C.	33	P		−	−, −					−															
47 H. B.	2	NP	+						−																
48 T. F.	3	NP	+									−, −													
49 R. W.	4	NP											−												−
50 E. W.	5	NP	−									−													
51 E. S.	8	NP	−														inc.								
52 F. O'C.	8	NP	+												−										
53 J. McD.	9	NP	+							−					−										
54 E. B.	9	NP	−, +								−														
55 L. S.	11	NP		+										−											
56 A. Br.	11	NP	+	−					−																
57 J. A.	12	NP	−								−, −														
58 F. G.	12	NP		+											−										
59 G. M.	13	NP	+							+									−						
60 M. C.	15	NP			+									−						−					
61 F. J.	15	NP	inc.							−															

P = paralytic poliomyelitis
NP = non-paralytic poliomyelitis
\+ = positive test for poliomyelitis in a rhesus monkey
− = negative test for poliomyelitis in a rhesus monkey
inc. = incomplete test; monkey died from other causes within 28 days of inoculation
Each symbol represents the result of a test in one monkey.

TABLE III

Relation between age of patient and incidence of positive isolations of poliomyelitis virus from stools in the first four weeks

Source of data	Virus isolated from stools of patients: Under 8 years	8 years and over
Kramer, Hoskwith, and Grossman	2 in 4 (50 per cent)	1 in 5 (20 per cent)
Kramer, Gilliam, and Molner	5 in 11 (45 per cent)	1 in 8 (12 per cent)
Howe and Bodian	8 in 11 (73 per cent)	2 in 3 (67 per cent)
Trask, Paul, and Vignec	7 in 21 (33 per cent)	1 in 32 (3 per cent)
Sabin and Ward	7 in 11 (64 per cent)	2 in 12 (17 per cent)
Total	29 in 58 (50 per cent)	7 in 60 (12 per cent)
Horstmann, Ward, and Melnick	10 in 15 (67 per cent)	19 in 27 (70 per cent)

logical variations in the susceptibility of this host may at times result in false negatives, especially when only one animal is used per test.

TABLE IV

Type of disease and age of patients whose stools were examined for virus within 6 weeks after onset of poliomyelitis

		Total	Under 8 years	8 years and over
Paralytic	Number of cases	37	16	21
	Number positive	23	9	14
	Per cent positive	62	56	67
Non-paralytic	Number of cases	13	3	10
	Number positive	10	2	8
	Per cent positive	77	67	80

DISCUSSION

A prevalent concept of the duration of excretion of virus after poliomyelitis is that stools commonly contain virus for three to four weeks following the disease, but rarely thereafter. In view of the results of the present study, this concept must be modified, and the period of persistence of virus extended. There are obvious imperfections in the study such as the limited number of specimens, the irregularity of collection times in individual patients, and the necessity of using the same monkeys for several tests. Nevertheless, the trend here is toward a longer period of virus excretion than was formerly believed to be the case. Furthermore, the imperfections mentioned might operate to give false negative results, but they could not cloud the issue by falsely increasing the number of positives.

The reason for the greater number of positive results than has usually been reported in the past is probably associated with differences in technique. The studies in the literature all employed crude stool suspensions which were inoculated intraperitoneally and/or intranasally. Melnick (9) demonstrated that the ultracentrifuge method, which was used in the preparation of all of our specimens, greatly increases the number of isolations of virus from stools. This is due in part to the fact that larger amounts of material may be used and concentrated to amounts suitable for intracerebral inoculation, which is believed to be a more sensitive route than peripheral inoculation.

The discrepancy between our results and those of most other workers with respect to the isolation of virus from children under 8 as compared to those over 8 is not easy to explain. One hypothetical possibility is that virus is present in smaller quantities in the stools of older patients. When cruder techniques were employed, the amount may be too small for detection, whereas with greater concentration of virus such as is achieved with ultracentrifugation, more positive results are brought out.

The similarity between the paralytic and non-paralytic groups in the excretion of virus again does not bear out the impression suggested by previous work (2) that non-paralytic patients are more apt to have positive stools. This point has not been adequately studied, however, and no conclusions can be drawn.

A comparison of typhoid fever and poliomyelitis as to the excretion of the infective agent in stools reveals a striking similarity during the first two months following the onset of each disease. Topley and Wilson (12) in a review of the literature found that about 54 per cent of patients yield positive cultures from feces during the first and second weeks after an attack of typhoid fever, 62 per cent during the third and fourth weeks, 18 per cent during the fifth and sixth weeks and 3 per

cent during the seventh and eighth weeks. However, in typhoid fever a small percentage of convalescents, about 2 per cent, continue to excrete infective bacteria for many months. Such was not the case for the poliomyelitis convalescents in this study, but the number of patients was relatively small, and healthy contacts were omitted entirely. It is conceivable that a prolonged carrier state exists in poliomyelitis, with a rate comparable to that in typhoid, and that our failure to demonstrate it is due to an insufficient number of subjects studied.

SUMMARY

1. The excretion of virus in the stools of 61 patients following acute poliomyelitis was studied. It was found that virus is present in 70 per cent of cases in the first two weeks, 50 per cent during the third and fourth weeks, 27 per cent at the fifth and sixth week period, and 13 per cent at the seventh and eighth weeks. In a single instance, it was demonstrated at the 12th week after the onset of the disease. Twenty-three specimens collected from the 13th to the 24th weeks were negative.

2. No significant difference was detected in the percentage of positive stools from children under 8 as compared to those from children 8 years and over.

3. There appeared to be no difference between paralytic and non-paralytic groups of patients as to the presence of virus in stools in the acute stage of the disease, nor its persistence into late convalescence.

4. Not one of the 61 patients followed was demonstrated to become a prolonged carrier of the infective agent, such as exists among typhoid fever convalescent patients.

5. In only one patient of this series was a stool passed early in the disease found negative where a later stool was found positive for virus.

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