THE ISOLATION OF POLIOMYELITIS VIRUS FROM HUMAN EXTRA-NEURAL SOURCES.[1] I. COMPARISON OF VIRUS CONTENT OF PHARYNGEAL SWABS, OROPHARYNGEAL WASHINGS, AND STOOLS OF PATIENTS

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(Received for publication December 15, 1945)

This series of papers is concerned with the isolation of poliomyelitis virus in the human disease. In them the attempt has been made to compare the ease or difficulty with which the virus may be isolated from several different anatomical sites in man, during and after acquisition of the disease, or exposure to it. In papers I and II (1) virus tests on oropharyngeal swabs and washings are compared with tests on the feces. Paper III (2) is concerned with the duration of excretion of virus during the convalescent period. In number IV (3), tests for virus in the blood are reported.

There have been a number of comparable studies carried out within the past 35 years; most of them have been published recently, and most of them deal with one particular aspect of the many complex problems involved. Some of these studies have been reported from this laboratory (4 to 7). In the present series, however, we have tried to relate several different aspects one with another; methods for the detection of the virus have been more delicate, the clinical material has been more extensive, and *serial tests* have been made on the same patients or group of patients in several instances. From the results certain new conclusions can be drawn with regard to the presence of poliomyelitis virus in different anatomical sites in man. We do not believe, however, that we are yet far enough along to attempt to interpret these findings from an epidemiological standpoint. We do believe on the other hand, that when certain obvious gaps in present knowledge are filled in by other studies, the epidemiological interpretation of the findings can go forward quite rapidly.

Historical. The groundwork of investigations of this type was laid in 1910 by Kling, Wernstedt, and Pettersson (8). No subsequent study has been so revealing as to the anatomical sites and secretions or excretions from which the virus can be isolated from both patients and carriers. The Swedish workers described the isolation of poliomyelitis virus from washings from the nasopharynx and the intestinal tract. They found it not only in paralytic and nonparalytic cases, but in contacts. To them should go the credit for the first *clinical and laboratory* study of poliomyelitis. Unfortunately their criteria for determining the presence of the virus were not as definite in some of their positive results as in others. Particularly did this concern the interpretation of the histological findings in the spinal cord of monkeys which had been inoculated with *intestinal* washings from patients. This served to minimize the significance of their tests on material obtained from this source. In confirmation of their work with nasopharyngeal washings there soon followed the demonstration of the virus in the nasopharyngeal washing of a group of familial *contacts* in this country, by Flexner, Clark, and Fraser in 1913 (9), and a repetition of this finding within a year by Kling and Pettersson (10). Subsequently, during a period of approximately 25 years (1914 to 1938), relatively few successful experiments on the isolation of poliomyelitis virus from patients were reported (11, 4, 5). At that time efforts were directed almost entirely towards the isolation of virus from the nasopharynx. As far as the clinical disease was concerned, the intestinal tract was for the time being forgotten. This situation changed when Harmon called attention to his having isolated virus from the lower intestine in the acute human disease in 1937 (12). Within the next 8 years a great deal of attention was turned to the intestinal tract. Fecal material in particular was studied as a source of virus because it had become apparent that Kling's statement made in 1929 was true: that it was much easier to find the virus in intestinal contents than in the oropharynx (13). During this period the virus was found frequently in human feces obtained during the acute paralytic and nonparalytic disease, and in convalescence (14). It was also found in apparently healthy contacts. But since 1943 *both* the oropharynx and the intestinal tract have again come under consideration in view of the fact that the former site has again yielded the virus in several recent series of tests.

Vignec, Trask, and Paul (15) had found in their summary of the literature up to 1938 that virus had been detected in material from the nasopharynx, tonsils, and trachea in 15 per cent of 105 attempts made during the first 5 days of the disease and in 7 per cent of 182 at-

[1] Aided by a grant from the National Foundation for Infantile Paralysis, Incorporated.

tempts made thereafter. The great majority of these tests were accomplished with what would be today considered relatively crude forms of technique.

Subsequently, Sabin and Ward (15), using autopsy material, found virus in the pharyngeal wall either with or without the tonsils in 4 of 7 patients who had died within 6 days of onset of poliomyelitis. The colon contents were tested in 6 of these patients and virus was demonstrated in all of them. Sabin and Ward also tested the stools and the nasal secretions collected from 22 living patients, and in addition saliva and oral secretions from 20 of these (17). The materials were collected during the first and second weeks of the disease. No virus was found in the nasal or oral secretions, while 9 of the 23 stool specimens were positive for virus. Also using autopsy material, Kessel and his associates (18) found virus in the tonsils of 3 of 6 and in the colon contents of 5 of 19 patients. From living patients, the same authors recovered virus from the stools of 11 of 53 individuals, but from the *nasal washings* of none of 139 patients. More recently, Howe, Wenner, Bodian, and Maxcy (19) found virus to be present in 43 per cent of *oropharyngeal* swabs taken from 23 cases during the first 3 days of illness whereas it was not detected in 13 swabs taken the 4th day to the 9th day of the disease. In no instances however have serial tests for virus been repeated in the same patient both in the stools and in the oropharynx.

It was believed therefore that a systematic series of tests for the presence of virus in the pharynx and a record of its persistence there as compared with its elimination in the stools in the same patients might supply information concerning the relative importance of the two sites as sources of recoverable virus. This is the purpose of the study reported in the first paper of the series.

MATERIALS AND METHODS

Throat swabs, oropharyngeal washings, and stools were collected at weekly intervals from 20 patients during the 1944 North Carolina epidemic. Both paralytic and nonparalytic cases were represented; the ages of the patients varied from 5 to 16 years. An attempt was made to collect the first specimen as early as possible in the course of the disease (between the second and eighth day after onset). Throat swabs dipped in 10 per cent sterile horse serum [2] in distilled water were used, and the posterior pharynx, tonsillar, and peritonsillar areas were swabbed vigorously. The patients then gargled over and over 50 to 75 ml. of 10 per cent horse serum for 5 to 10 minutes. In small or acutely ill children, a nasopharyngeal irrigation with 10 per cent horse serum was obtained instead of gargle washings. Stool or enema returns were collected on the same or the day following the collection of materials from the throat. Specimens were obtained in this fashion at weekly intervals for 6 weeks. All materials were frozen on dry ice immediately after collection and maintained in the frozen state until tested.

The throat swabs were prepared for intracerebral inoculation by the method of Howe, Wenner, Bodian, and Maxcy (19). Oropharyngeal washings and stools were prepared for intracerebral inoculation by ultracentrifugation as described elsewhere (20, 1, 2).

Rhesus (*Macaca mulatta*) monkeys were used as test animals, and all specimens were inoculated intracerebrally. Throat swabs were tested first, and if the monkey remained asymptomatic for 5 to 6 weeks, it was used as the test animal for oropharyngeal washings taken in the same week from the same patient. If the monkey showed any signs of experimental poliomyelitis or symptoms remotely suggestive of the disease, it was sacrificed at an appropriate time. After receiving oropharyngeal washings, and being observed for 4 to 5 weeks, all monkeys were sacrificed. All monkeys inoculated with stools were sacrificed when symptoms of poliomyelitis were present or if clinically normal, at the end of 4 or 5 weeks. Histological examination was carried out in all test animals and typical poliomyelitis lesions in the spinal cord were required to be present for a test to be recorded as positive.

RESULTS

Information about the patients and the results of the tests for virus are described in Table I. All 20 patients were examined for the presence of virus in the *oropharynx*. Of 19 tests on pharyngeal swabs taken during the first week of illness, one from an 8-year-old nonparalytic patient was positive. Of 16 tests on first week oropharyngeal washings, one was positive and one was incomplete. Unfortunately the specimens of washings from two patients became mixed and the positive result was obtained from material pooled from these 2 cases. One should note that neither one of these two patients had detectable virus in his pharyngeal swab, illustrating perhaps the inadequacy of present methods.

Twelve *stool* specimens were selected at random from the first week specimens and were tested for virus. Of these, 10 gave satisfactory tests, 7 being positive and 3 negative. Of the remaining two, one (rhesus 2778) died of a brain abscess on the fourth day following inoculation, and this is recorded as an incomplete test. Another monkey (rhesus 2789) had a clinically negative course. When sacrificed at the end of 28 days this monkey

[2] Horse serum was used in these collections because of the protective action of serum proteins on the stability of viruses. That used for the collection of first week specimens—but not the later ones—had been heated at 56° for 45 minutes.

TABLE I

Tests for poliomyelitis virus in throat swabs, oropharyngeal washings, and stools of patients

Patients	Age	Type of disease	Day after onset 1st spec. collected	1st week						2nd week					
				Throat swabs		Oropharyngeal washings		Stools		Throat swabs		Oropharyngeal washings		Stools	
				Monkey no.	Test	Monkey no.	Test	Monkey no.	Test	Monkey no.	Test	Monkey no.	Test	Monkey no.	Test
JB	8	Bulbar	2	2749	−	2749	−								
DCR	16	P.	2	2767	−	2767	−	2824	+	2899	−	2899	−	2977	+
CP	5	P.	2	2770	−	2770	−	2829	+	2905	−	2905	−	2987	+
AW	5	P.	2	2752	−	2752	−	2685	+	2903	−	2903	−	2986	+
RC	16	P.	3	2775	−	2775	−	2789	?						
BH	10	P.	3	2774	−	2774	−								
SJ	6	P.	3	2788	−			2810	+						
DL	14	P.	3	2787	−	2787	−								
PP	9	P.	3	2745	−	2745	−	2808	−						
MW	9	P.	3	2753	−	2753	−	2701	+	2904	inc.	2969	inc.	2976	−
JOB	6	N.P.	4	2776	−	2776	−	2785	−						
MS	16	P.	4	2744	−	2744	+	2971	−	2901	−	2901*	−*	2985	−
MH	5	P.	6	2747	−			2907	+	2900	inc.	2968	−	2988	+
DB	15	P.	5	2769	−										
ML	15	P.	5	2748	−	2748	−	2778	inc.						
BP	8	P.	5	2786	+	2908	inc.	2970	+	2898	−	2898	−	2981	−
DCL	5	N.P.	6	2750	−	2750	−								
EE	8	P.	6	2768	−	2768	−								
NP	7	P.	6	2746	−	2746	−								
JAB	9	P.	8							2773	−	2773	−		
Positive per no. tested →					1/19		1/15		7/10		0/6		0/7		4/7
Percentage positive for each type of material					5 per cent		7 per cent		70 per cent		0 per cent		0 per cent		57 per cent
Percentage of positive patients				Oropharynx 11 per cent (2/19)				Stools 70 per cent (7/10)		Oropharynx 0 (0/7)				Stools 57 per cent (4/7)	

P. = paralytic poliomyelitis.
NP. = non-paralytic poliomyelitis.
− = negative test for poliomyelitis virus in a rhesus monkey.
+ = positive test for poliomyelitis virus in a rhesus monkey.
* = Histological sections of monkey no. 2901 revealed a few areas of cellular infiltration in the spinal cord. However no neuronal damage was present. Passage of the spinal cord and medulla of this monkey both as a 20 per cent suspension and as an ultracentrifuged concentrate, each to a separate monkey, was negative. Third week material from patient MS was inoculated as follows: throat swab into no. 3010, oropharyngeal washings into no. 3009, and stools into no. 3008. All 3 monkeys gave negative tests.
? = questionable result. Although rhesus 2789 had a negative clinical course, histological examination revealed several foci of interstitial cellular infiltration and perivascular cuffing in the medulla and upper cervical level of the cord.
inc. = incomplete test. Monkey died from causes other than poliomyelitis within 28 days of inoculation.

had mild lesions (several foci of interstitial cellular infiltration and perivascular cuffing) limited to the medulla and upper cervical level of the cord. The test is recorded as questionable, for in the absence of neuronal lesions in distal levels of the spinal cord, an important criterion for the identification of poliomyelitis virus remains unfulfilled. It has not been included either as positive or negative in evaluating the results.

Thus in the first week of the disease, virus was found in the oropharynx (including both swabs and washings) in 11 per cent (*2* out of *19*) of the patients, whereas 70 per cent (*7* out of *10*) of these patients were shown to be excreting virus in their stools at this time.

Samples from those patients (B. J. P. and pool of M. H. and M. S.) whose oropharyngeal specimens of the first week were positive for virus as well as 5 other patients, 4 of whose first week stool specimens gave positive tests were examined in the second week. Of the 8 pharyngeal swabs tested, 6 gave negative tests, while 2 were incomplete because of premature death by brain abscess. Of the 8 oropharyngeal washings,

7 were negative and 1 was incomplete. Of the negatives, 1 was questionably so. Clinically negative, this monkey (Rhesus 2901) showed a few glial nodules in the anterior horn at the lumbar level, in the medulla and pons. However, no evidences of neuronophagia, neuronal necrosis, or nerve root degeneration were present. Passage of the spinal cord failed both as a 20 per cent suspension and after concentration of the macromolecular material in the ultracentrifuge, a technique which has at times provided successful passage when passage of 10 or 20 per cent spinal cord has been negative (see footnote Table I) (21). Specimens from this patient (M. S.) were also tested in the third week, all being negative.

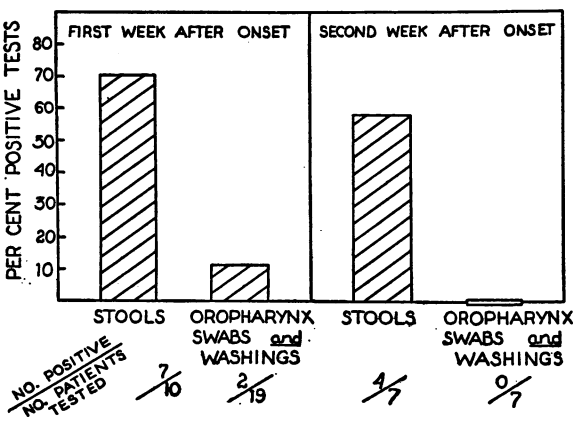

FIG. 1. POLIOMYELITIS VIRUS IN STOOLS AND IN OROPHARYNX OF PATIENTS

Of the 7 stools representing second week specimens, 4 proved to be positive. Thus in the second week of disease for these selected patients in whom virus was found in either pharynx or in stools or in both during the first week of their disease, none had detectable virus in the oropharynx, whereas 57 per cent continued to excrete virus in the stools. The results are graphically presented in Figure 1.

DISCUSSION

The tests for poliomyelitis virus described in this paper have been carried out with methods which are the most sensitive that we have available in this laboratory for the detection of the virus. The results support the observations obtained with cruder tests that virus is eliminated with greater frequency and for a longer period of time in stools than in material from the oropharynx. In making this comparison it should be pointed out that materials from the throat, both swabs and washings, represent entirely different types of specimens than does fecal material, and there is no practical way of comparing the two as to quantitative virus content. In the case of throat washings, one deals with fluid which has been in contact with pharyngeal tissues for a brief period only. Stools, on the other hand, consist of excretory waste which has been in contact with the intestinal wall for a matter of hours at least.

In the present study where the same patient was studied for two weeks after the onset of his illness, 7 out of 10 patients (70 per cent) were excreting virus in their stools in the first week, and 4 out of 7 (57 per cent) during the second week. Only 2 out of 19 (11 per cent) were found to have virus in the pharynx during the first week and none in the second week. The results of the stool tests are in complete agreement with another series from this laboratory (2) in which 70 per cent of patients (25 positives of 36 samples tested) were found to excrete virus during the first two weeks of the disease. In the present study 11 positives were detected among 17 samples (65 per cent) obtained within two weeks of the onset.

In a summary of the early literature through 1938, Vignec, Trask, and Paul (15) tabulated the isolation of virus from the upper respiratory tract in 15 per cent of cases during the first 5 days of the disease. This figure is in line with the results of our present tests, 11 per cent positive during the first week of the disease.

In the recent series of 36 tests carried out in Baltimore (19), virus was recovered only from those cases in which pharyngeal swabs were collected within the first 3 days of illness. On the other hand, in the present study the 2 positive tests were obtained on material from the oropharynx collected on the 4th to 6th day of illness. Similar material obtained from 10 patients in the first 3 days of illness gave negative tests. It would appear that the true estimate of the incidence of virus in this site will be reached as more cases are studied.

SUMMARY

An attempt has been made to compare the incidence and persistence of poliomyelitis virus in

material from the human oropharynx with that from the intestine (stool) of 20 patients during an epidemic. Material from the oropharynx was obtained from each of these patients by two methods: (*a*) pharyngeal swabs and (*b*) oropharyngeal washings.

From specimens collected during the first week, 1 of 19 pharyngeal swabs, 1 of 15 oropharyngeal washings, and 7 of 10 stools yielded virus; or 11 per cent of the samples from the pharynx proved to be positive in contrast to 70 per cent of the stool specimens.

From specimens collected during the second week, none of 6 pharyngeal swabs, none of 7 oropharyngeal washings, and 4 of 7 (57 per cent) stools yielded virus. All patients with positive material (pharyngeal or intestinal) during the first week, were studied in the second week.

This study, carried out with newer and more sensitive techniques for virus isolation, supports earlier observations that virus may be isolated with greater frequency and for a longer period of time in stools than in material from the oropharynx. These results may be due in part to the difficulties of comparing virus isolations from these two different types of materials.

We are grateful to Dr. J. S. Gaul of Charlotte, N. C., Dr. H. C. Whims, Lincoln-Catawba County Health Officer, and Miss Frances Allen, R.N., who aided greatly in the collection of the specimens.

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