

# THE ISOLATION OF POLIOMYELITIS VIRUS FROM HUMAN EXTRA-NEURAL SOURCES.<sup>1</sup> II. COMPARISON OF VIRUS CONTENT OF BLOOD, OROPHARYNGEAL WASHINGS, AND STOOLS OF CONTACTS

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The importance of healthy contacts as potential carriers of poliomyelitis virus has long been recognized, and efforts to demonstrate the infective agent in such individuals have been reported.

The first successful isolation from the nasopharynx of an asymptomatic contact was that reported by Flexner, Clark, and Fraser, in 1913 (1). Kling and Pettersson in 1914 (2) obtained positive results in one of three pools of nasopharyngeal washings collected from the immediate families of three patients. In 1932 and in 1935, Paul, Trask, and Webster (3, 4) were unable to demonstrate virus in nasopharyngeal washings of 24 contacts. No attempt to recover virus from the blood of contacts is known to have been reported heretofore.

In contrast to these few positive results from the nasopharynx, stools of individuals exposed to the disease have yielded virus relatively frequently. Thus Kramer, Gilliam, and Molner in 1939 (5) reported isolation of virus from 4 of 20 well contacts and from 2 of 3 individuals with transient symptoms. Piszczek and his associates (6) found positive stools from 8 of 38 individuals exposed to poliomyelitis, and McClure and Langmuir (7) claim to have demonstrated virus in the feces of 10 of 14 well contacts, and 10 of 13 contacts with minor illnesses. This latter is an extraordinarily high score in contrast with the results of other workers. In the study by Wenner and Casey (8) stools of virtually the entire population of a small community were tested 7 to 12 weeks after an outbreak of poliomyelitis occurred. Specimens from 62 individuals with minor illnesses and 108 with no symptoms were negative; one child with a vague antecedent illness had a positive stool on two occasions. Brown, Francis, and Pearson (9) detected virus in the stools of 5 of 6 close contacts of a patient with paralytic poliomyelitis 7 days after the onset of the patient's illness, while nasopharyngeal washings from 4 of the 5 positive contacts were negative. One of the positive contacts developed paralytic poliomyelitis 19 days after the positive specimen was obtained; nasopharyngeal washings of the same date were not tested. Pearson and his associates (10) in a community study during an epidemic found that 6 of 8 households representing 27 familial contacts and 8 of 45 households containing 80 non-familial contacts were positive for virus. In contrast, from only

2 of 127 households representing 374 non-contacts was virus recovered.

The present study was undertaken to compare three materials—blood, oropharyngeal washings, and stools—in a single group of 75 contacts who had been exposed to poliomyelitis in a small epidemic in which an extraordinarily high attack rate prevailed. The character of the group makes it possible to determine the "carrier rate."

## MATERIALS AND METHODS

During the 1943 poliomyelitis epidemic in Chicago, four boys who were members of a football squad of a Chicago high school developed the paralytic form of the disease between September 3rd and 13th. These 4 boys, with 71 other members of the squad and 4 younger assistants, had been engaged in fall practice when the outbreak occurred. The group, who had been living in their respective family homes, left Chicago on August 28, 1943 for a training camp in Hudson Lake, Indiana, 75 miles from the city, where they remained for 10 days. No poliomyelitis had been reported in this area. Except for 4 boys aged 10 to 12 years who served as water boys and kitchen helpers, the ages of the group ranged between 15 and 19 years. None of the 75 contacts had been ill enough to prevent attendance at daily vigorous practice sessions; yet when questionnaires were filled out on September 16th, 28 boys admitted having had transient symptoms such as headache, sore throat, diarrhea, or feverishness, and 6 had had a combination of at least two of these complaints since arriving at camp. Consequently all these boys cannot be considered as healthy contacts in the true sense of the word. Many of those who admitted minor transient illnesses may well have had abortive attacks of poliomyelitis, although it should be emphasized that none complained of any illness during the time of alleged symptoms nor were any sick enough to be absent from any of the daily strenuous bouts of exercise.

Samples of blood, oropharyngeal washings, and stools were collected from each of the 75 contacts<sup>2</sup> on Sep-

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TABLE I

*Search for virus in pooled specimens of blood, oropharyngeal washings, and stools of poliomyelitis contacts*

Pool no.	Number of specimens	Ages	Symptoms	Results		
				Blood	Oropharyngeal washings	Stools
1	5	15 to 18	Headache	—	—	—
2	5	15 to 18	Headache	—	—	—
3	5	15 to 18	Diarrhea	—	—	—
4	6	15 to 18	Diarrhea	—	—	—
5	5	15 to 18	Sore throat	—	—	+
6	6	15 to 18	{ Headache*	—	+	—
			{ Sore throat			
			{ Fever			
			{ Diarrhea or constipation			
7	8	15 to 18	Asymptomatic	—	—	inc.
8	8	15 to 19	Asymptomatic	—	—	—
9	9	15 to 18	Asymptomatic	—	—	+
10	2	10 to 12	Sore throat or fever	—	—	—
11	2	10 to 12	Asymptomatic	—	—	—
12	7	15 to 18	Asymptomatic	—	—	—
13	7	15 to 18	Asymptomatic	—	—	+
Totals	75			0 in 12	1 in 13	3 in 12

+ Monkey developed poliomyelitis.

— Monkey failed to develop poliomyelitis.

inc. Incomplete due to premature death of monkey.

\* Boys in this pool had 2 or more of the symptoms listed.

1 in 13 Of 13 pools tested, 1 yielded a positive result.

tember 16th, 3 days after the last frank case occurred; at this time none of the boys had any symptoms. All three types of specimens were collected on the same day with the exception of 15 stools which were brought in by the boys during the following 2 days. The blood samples were 20 to 50 ml. in amount. Oropharyngeal washings consisted of 50 ml. of 10 per cent normal horse serum gargled again and again for 20 minutes; about 35 ml. were returned at the end of this period. Stools weighed 10 to 40 grams, averaging 25 grams. All specimens were frozen immediately on solid carbon dioxide and maintained so, until prepared for inoculation. Thirteen pools of each of the three types of materials were made on the basis of symptoms, and in the case of the four younger boys, on the basis of age (see Table I).

*Preparation of materials:* All specimens were prepared by ultracentrifugation (11). The details of the methods for the preparation of bloods and stools are reported in two other papers of this series (12, 13). The oropharyngeal washings were spun for 20 minutes in an angle centrifuge to remove gross particles. One hundred ml. were then ultracentrifuged at 39,000 RPM for 1 hour, and the pellets were resuspended in 2 ml. of 10 per cent normal rhesus serum in distilled water. This suspension and any excess material left after the first angle centrifugation were each shaken with ether and allowed to stand in the refrigerator at 4° C. over night. On the following day, after removal of the ether, the materials were either frozen on carbon dioxide ice for future use, or were cleared at once by a short centrifugation (angle) before being inoculated.

*Technique of inoculation:* The bloods were inoculated intracerebrally in amounts varying from 0.7 to 1.2 ml.; 11 animals were given "reinforcements" of 1 ml. amounts intracerebrally 1 to 2 weeks after the first inoculation; 4 received in addition, intraperitoneally, the pellet resulting from the final angle centrifugation suspended in distilled water. One blood pool (No. 9) received instead of ultracentrifugation, two runs in the Sharples supercentrifuge, first at 18,000 RPM for 20 minutes, and the resulting supernatant material at 48,000 RPM for 2 hours. The inoculum was given intracerebrally and intraperitoneally.

The oropharyngeal washings were given similarly by two routes in 11 instances: intracerebrally for the ultracentrifuged portion, and intraperitoneally for the unconcentrated excess. In one test (pool 2) the intracerebral route only was used, and in another (pool 7) the intraperitoneal route only was employed, unconcentrated washings alone being inoculated.

The stools were inoculated intracerebrally in 0.5 to 1 ml. amounts.

*Test animals:* Immature rhesus (*Macaca mulatta*) monkeys were used for the testing of all pools. Four of the animals had been used previously for other specimens, with negative results. All of the monkeys receiving blood were reinoculated with oropharyngeal washings from the same patients; 3 of these same monkeys then received stools of the corresponding pool. The criteria for the detection of poliomyelitis virus were those already noted (10).

## RESULTS

Virus was recovered from one pool of oropharyngeal washings, and from three pools of stool specimens. None was obtained from any of the blood pools. The positive pool of oropharyngeal washings was derived from 6 boys who had complained in the previous two weeks of at least two symptoms common in abortive poliomyelitis. One of the positive stool pools represented 5 boys who had had sore throats within a similar period, but in the case of the other two positives, representing 9 and 7 boys, no symptoms had been present at any time. The stool pool corresponding to the positive pool of oropharyngeal washings was negative. Both pools made up of materials from the four younger boys were negative. The findings are summarized in Table I.

## DISCUSSION

The results of this study are in line with those of other reports. Virus was found to be present in stools of contacts more often than in their oropharyngeal washings.

It may be possible to differentiate a healthy contact from one having minor symptoms, but the borderline must be extremely vague. In this series, virus was found in approximately the same ratio among the boys who had admitted to no symptoms (2 positive out of 7 pools tested) and among those who had complained of minor illnesses (2 positive out of 6 pools tested).

The clinical observation that strenuous exercise frequently precedes the onset of poliomyelitis has been made many times in the past. The group of boys in the present study were all engaged in vigorous physical exertion, and the attack rate was extremely high (4 per 79, or 53 per 1000). Practice sessions and games were cancelled for the remainder of the year after the fourth case of poliomyelitis appeared. Since the number of positive samples in each of the positive pools is unknown, all that can be said is that virus was present either in the throat or stools in *at least* four boys besides the four who developed paralytic poliomyelitis. Whether further cases would have developed if practice had not been abandoned is an open question.

## SUMMARY

Samples of blood, oropharyngeal washings, and stools from 75 contacts of poliomyelitis patients at

one epidemic focus were divided into 13 pools of each type of specimen and examined for virus. None was detected in the blood pools; one pool of oropharyngeal washings and three of stools yielded virus. The stool pool corresponding to the positive oropharyngeal material was negative.

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