

# THE ISOLATION OF POLIOMYELITIS VIRUS FROM HUMAN EXTRA-NEURAL SOURCES.<sup>1</sup> IV. SEARCH FOR VIRUS IN THE BLOOD OF PATIENTS

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The search for poliomyelitis virus in the blood of human beings has yielded only negative results.

Thus Leiner and von Wiesner state that their tests for virus by using intracranial, intravenous, and intraperitoneal inoculations of human blood were negative (1). Straus and Huntoon (2) tested blood samples from 2 patients on the third and fifth days of illness, respectively; 2 ml. from the earlier case were given intraperitoneally to a monkey, and 5 ml. from the later case were inoculated by the same route (3 ml.) and intradurally (2 ml.) into another monkey. Both animals were negative although neither was subjected to histological examination of the central nervous system. Flexner and Clark obtained negative results following the injection of monkeys with 10 to 20 ml. of blood (from an unspecified number of patients) partly intracerebrally and partly intraperitoneally (3). Clark, Fraser, and Amoss studied 12 patients on various days of illness ranging from the second to the thirteenth, using different routes for monkey inoculation; for example, intracerebral (2 to 4 ml.), intrasciatic (2 ml.), and intraperitoneal (1 to 30 ml.)—all with negative results (4).

These negative findings, based on fairly limited investigations carried out over thirty years ago, were considered worth reinvestigating in the light of certain new facts and recently confirmed older observations, namely, poliomyelitis virus has been recovered from the blood of monkeys experimentally infected by various routes (4 to 6) including the oral (7) and intracutaneous (8). Virus has been found, moreover, in a pool of human viscera (lung, liver, spleen, and kidney) (9). Furthermore, a new method of concentrating large volumes of material for intracerebral inoculation seemed to offer a more delicate test than has been used for blood heretofore (8).

The purpose of the present report is to record the results of tests for virus in the blood of 111 poliomyelitis patients, only one of which was positive.

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## MATERIALS AND METHODS

Samples of blood were drawn from 111 patients who were seen during five different outbreaks, which occurred in New Haven, Conn., Chicago, Ill., and Bakersfield, Calif., in 1943, and in and around Hickory, N. C. and New York City in 1944. The distribution of cases according to the type of illness (paralytic or non-paralytic) and the day of the disease on which blood was taken is shown in Table I. The numbers of paralytic and non-

TABLE I

*Type of illness and day of disease on which patient was bled*

Day of disease*	Type of illness		
	Non-paralytic†	Paralytic	Total
1	18	2	20
2	16	2	18
3	8	17	25
4	5	6	11
5	1	6	7
6 to 13	9	13	22
undetermined	3	5	8
Total	60	51	111

\* Day of disease: day of onset of symptoms = 1st day of disease.

† Non-paralytic poliomyelitis arbitrarily includes so-called abortive and questionably abortive types of illness occurring in the midst of epidemics.

paralytic cases were 51 and 60, respectively. Sixty-three patients were bled on or before the third day of illness and 20 of these were bled within 24 hours of onset. The age distribution of 90 patients is shown in Table II. Sixty patients, or two-thirds of the group, were between 5 and 15 years old. This reflects the recently observed trend of poliomyelitis to attack somewhat older age groups than formerly (10). The sex distribution showed a slight pre-

TABLE II

*Age distribution of 90 patients*

Patients	Age group (years)			
	0 to 4	5 to 9	10 to 14	15 and over
Number	15	33	27	15
Percent.	16.6	36.6	30	16.6

dominance of males (60 per cent) in accord with poliomyelitis in general.

The volume of individual blood samples varied from 5 to 175 ml., the average being 45 ml. In most instances the blood was allowed to clot immediately in sterile lusteroid tubes and frozen within a few minutes in a dry-ice storage box where it remained until preparation for inoculation. A few samples consisted of whole citrated blood and in one instance the separated blood cells of 11 patients (N. Y. C.) were pooled. The specimens of blood were prepared for inoculation according to the following general procedure: the specimen was thawed rapidly, and to it sufficient sterile distilled water was added to bring the total volume to approximately 100 ml. The mixture was blended in a Waring blender, followed by centrifugation at 3,000 RPM for 15 to 20 minutes in an angle centrifuge. The supernatant fluid was thereupon subjected to high speed centrifugation (39,000 RPM). The pellet was resuspended in 3 to 4 ml. of a serum-saline solution prepared by adding 10 ml. of normal rhesus monkey serum (inactivated at 56° C. for one half hour) to 90 ml. of normal saline. As a rule 2 ml. of pellet suspension were inoculated intracerebrally into a rhesus monkey. The remainder of the suspension was stored in the frozen state and used for re-inoculation a week or so later. In 6 instances 1 ml. of blood similarly concentrated was inoculated into the lumbar region of the spinal cord. This procedure was attempted because ultracentrifuged preparations of stool inoculated by this route had proven not only to be infective but less conducive to untoward effects which often have followed intracerebral inoculations of such material (15).

Fifty-five blood samples were pooled in appropriate groups of 2 to 4 specimens each, making 19 pools. These were prepared according to the same method and each pool was inoculated into 1 monkey. The remaining 56 blood samples were tested individually.

Young rhesus monkeys weighing about 2 to 4 kgm. were used. They were observed daily for 5 weeks and their temperatures were recorded either daily or every other day for 3 weeks. At the end of the 5-week observation period a number of monkeys were inoculated with other blood samples. All animals were sacrificed ultimately and sections of the spinal cord and medulla were examined histologically. During the 5-week observation period 9 inoculated animals died of causes other than poliomyelitis. Sufficient material remained in 3 instances to inoculate fresh monkeys; in the other 6, the tests remained incomplete.

#### RESULTS

Poliomyelitis virus was detected in the blood of 1 patient. The sample was obtained from a child with non-paralytic, or abortive, poliomyelitis not more than 6 hours after the onset of symptoms. 74 other tests were entirely negative.

*Clinical history of positive case:* E. Bun., a 9-year old girl living in New Haven, Conn., was perfectly well until the morning of August 12, 1943. (The week of Aug. 12 to 18 marked the peak of the epidemic in New Haven.) At 11 a.m. while playing outdoors she was struck on the back of her neck by her brother's fist. Immediately after this she went home and complained of her neck hurting badly and of pain along the inner aspect of her right thigh. She also complained of feeling chilly and was noted to be feverish. She was nauseated but did not vomit. On admission to the hospital in the afternoon her temperature was 38.4° C. She exhibited stiffness of the back and neck and hamstring muscles but no alteration of the reflexes and no weakness or paralysis. The remainder of the physical examination was essentially negative.

*Laboratory data:* Lumbar punctures on August 12 and 16 revealed clear samples of cerebrospinal fluid devoid of cells on both occasions, and normal with respect to quantities of protein and sugar and to bacteriologic study. Blood culture was negative. The red blood cells numbered 4.69 million, white blood cells, 7000 per cubic millimeter, with a differential count of 77 per cent polymorphonuclear leukocytes, 19 per cent lymphocytes, 3 per cent large monocytes, and 1 per cent eosinophiles. Routine urinalysis was negative.

*Course in the hospital:* On August 13, her temperature was 38.2° C. On August 14 it dropped to normal remaining so during the rest of her hospital stay. No paralysis or weakness developed and she was discharged on August 16.

*Identification of poliomyelitis virus in the blood:* On August 12, not more than 6 hours after the appearance of first symptoms, 40 ml. of blood were withdrawn from an arm vein and frozen almost immediately. Forty-eight days later the blood sample was prepared for inoculation as described. Two ml. of the concentrated suspension was injected intracerebrally into a rhesus monkey which was reinoculated with 1 ml. on the 7th day. On the 16th day the monkey exhibited fever 106.3° F., complete left facial paralysis and weakness of the extremities. The latter appeared to be weaker on the following day when the animal was sacrificed. Histological examination of several levels of the spinal cord and medulla at the level of the 7th nerve nucleus revealed the characteristic lesions of acute poliomyelitis, viz., neuronal necrosis, neuronophagia, round cell infiltration, and perivascular cuffing. Passage of spinal cord and medulla to 2 rhesus monkeys resulted in paralysis with typical microscopic lesions in both. Ten mice, 2 guinea pigs, and 2 rabbits inoculated intracerebrally with the same material remained negative for 6 weeks.

*Detection of virus in the stool:* Poliomyelitis virus was also found in this patient's stool passed on August 14, the third day of the disease, two days after the positive blood sample had been obtained. A sample of stool passed October 13, 9 weeks after onset, failed to yield virus. Of 30 patients whose blood was negative for virus and whose stools have been tested in connection with another study (14), 18 were found positive for virus.

## DISCUSSION

Although the detection of poliomyelitis virus in human blood has not been reported heretofore, nevertheless the rarity of the event (1 positive of 111 patients tested) is in itself an indication as to its probable lack of importance. In other words, it is possible to say that the presence of poliomyelitis virus in the blood stream appears to be neither a common event nor a necessary factor in the pathogenesis of the human disease. This is in keeping with what is known concerning the pattern of distribution of lesions (11) and of virus (9) in the human central nervous system. This pattern does not correspond to that expected from indiscriminate spread of virus across blood vessels as occurs, for example, in equine encephalomyelitis (12). The explanation for the occasional detection of poliomyelitis virus in the human blood stream or in certain viscera is not altogether clear. A likely possibility is that as happens in other virus diseases, during active multiplication in certain tissues, virus may be liberated into the blood stream (13).

It is felt that this thorough search for poliomyelitis virus in human blood is sufficiently important to record, and that the single positive test may be considered an exception.

## SUMMARY AND CONCLUSIONS

Tests for poliomyelitis virus were carried out on blood samples (individual and pooled) of 111 patients ill with poliomyelitis. The paralytic and non-paralytic or abortive forms of the disease were about equally represented, and over half the patients were bled on or before the third day of the disease.

Virus was detected in the blood of 1 patient—a girl aged 9 years with abortive poliomyelitis, whose blood sample was obtained within 6 hours of onset of illness.

The results of this study suggest that the presence of poliomyelitis virus in the human blood stream is not a common event.

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