VIREMIA IN HUMAN POLIOMYELITIS*

By DOROTHY M. HORSTMANN, M.D., ROBERT W. McCOLLUM,‡ M.D., AND ANNE D. MASCOLA

(From the Section of Preventive Medicine, Yale University School of Medicine, New Haven)

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The present study is concerned with the isolation of poliomyelitis virus from human blood during the incubation period, or early in the minor illness phase of infection.

The search for virus in the blood of patients with poliomyelitis has been taken up periodically by investigators since 1909 (1). Early efforts all yielded negative results, and it was not until 1946 that the first successful isolation was reported from this laboratory (1). In 1949 a second isolation of virus from human blood was reported by Koprowski *et al.* (2). The single positive test obtained in our laboratory was with blood from a 9 year old girl, drawn within 6 hours from the onset of a minor illness or abortive attack of the disease. Since it represented only 1 positive of 111 tested, we inferred that viremia occurred rarely in the human disease and was probably of no significance in its pathogenesis. Subsequently, however, it became apparent that by the time a clinical diagnosis of poliomyelitis is made, the patient may already have antibodies to his own strain of poliomyelitis virus (3, 4), and perhaps even to a heterologous type (5). Furthermore, in primates infected by the oral route, antibodies were demonstrated to be already present by the time paralysis occurs (6). It seemed likely, therefore, that in general, we, as well as other investigators, had looked for viremia too late in the course of the human infection; *i.e.*, after the appearance of the 2nd phase, or major illness, and at a time when instead of viremia, one could expect to find antibodies. It was decided, therefore, to reinvestigate the problem of viremia in poliomyelitis.

At first, efforts were directed toward demonstrating viremia early in the course of experimental infection after a natural route of transmission; *i.e.*, orally, using chimpanzees and *cynomolgus* monkeys. It was found, in this laboratory (7) and by Bodian (8), that virus can be isolated from the blood of these animals with some regularity several days after its ingestion, and it may persist there for as long as 5 days. In *cynomolgus* monkeys, which became paralyzed after feeding, viremia occurred relatively early in the incubation period and as long as 6 days *before* the onset of paralysis. In all animals showing viremia antibodies were prompt to appear, being present by the time signs of the experimental disease developed.

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‡Now 1st Lieutenant Medical Corps, United States Army.

With these experimental results as a background, the attempt to demonstrate viremia in the human infection was taken up once more in 1952. Efforts were concentrated not on frank clinical cases occurring in a poliomyelitis epidemic but on contacts of a known patient who might be in the incubation period, and on children showing the minor illness syndrome. Bodian and Paffenbarger (13) undertook a similar investigation during the same summer, and succeeded in isolating virus from the blood of 3 children. A preliminary report covering some of our results has also appeared (12).

Materials and Methods

A sharp outbreak of poliomyelitis which occurred in rural Ohio in 1952 was selected for study. Most of the cases occurred in small towns of several hundred to a thousand population, and in some of these extremely high attack rates were noted (9).

Collection and Preservation of Specimens.—Between June 20 and July 11, 1952, specimens were collected by one of us.[1] The procedure followed was that as soon as a patient was admitted to a hospital, or reported to the health officer, a visit was made to the family and neighborhood contacts of the case. Histories were taken and any child with symptoms was examined, and his temperature recorded. An effort was made to obtain a throat swab, a rectal swab, and 20 ml. of blood from all contacts, particularly those under 15 years of age, as well as from any non-contacts in the area who gave a history suggestive of the minor illness syndrome. In all, 119 blood specimens, 111 throat swabs, and 83 rectal swabs were obtained. All but 6 individuals from whom specimens were collected were contacts of a known case.

Throat and rectal swabs were placed in 1 ml. of 50 per cent sterile glycerine and frozen on dry ice shortly after collection. Blood was collected in vacutainer tubes containing potassium oxalate as anticoagulant. The tubes of whole blood were centrifuged at 3000 R.P.M. for 10 minutes, thus layering the plasma, white cells, and red cells before freezing. All specimens were transported to New Haven in the frozen state, and kept frozen until tested for the presence of poliomyelitis virus.

Testing of Specimens for Poliomyelitis Virus. Bloods.—All were tested in tissue culture tubes, and a certain number were tested in *rhesus* monkeys (*Macaca mulatta*) by the intracerebral route. Approximately 0.6 ml. amounts of plasma, or plasma and whole blood mixed, were inoculated into monkeys, 0.3 ml. being directed into the right and left sides of the thalamus. If the monkey failed to develop signs of poliomyelitis, it was reinoculated with the same specimen 3 to 4 weeks after the first injection. Any animals showing signs suggestive of the disease were sacrificed for histological study. The tissue culture techniques employed varied during the course of the work, always in efforts to make the tests more senstive.

At first, monkey testicular roller tubes were employed (10) usually 2 tubes being inoculated with plasma, and 2 with the sedimented red and white cell mixture containing some plasma; the red cells had partially hemolyzed as a result of being frozen on dry ice and thawed. The inoculum was 0.1 ml. in 0.9 ml. of media. The cultures were examined microscopically at 4

[1]We are grateful for the cooperation and help given us by Dr. E. E. Kleinschmidt, then Health Commissioner of the Wayne and Medina General Health District, and to Dr. Frederick Wentworth, Chief of the Communicable Disease Division, Ohio State Department of Health and their staffs, to Dr. James Kramer, Chief of Staff, Children's Hospital, Akron, and to Mr. Edward Tulley, Chairman of the Summit County Chapter of The National Foundation for Infantile Paralysis. We are also indebted to Dr. Claude Reed, Miss Ilah Kauffman, and Mrs. Berttina Orsborne, for assistance in the collection of specimens.

and 8 days at which time the fluid media was changed. Fluids from all positive or questionably positive tubes, *i.e.* those showing a cytopathogenic effect, were passed. The final reading was made on the 12th day; if no signs of degeneration had appeared, the 8 and 12 day harvests were pooled and passed blindly to 2 to 4 testicular roller tubes. Subsequently a shift was made to the use of monkey kidney tubes, both roller and stationary (11). All blood specimens from individuals who were known to be infected, as evidenced by a positive throat and/or rectal swab, were retested in monkey kidney tubes, using 8 to 10 tubes per specimen, and passing blindly the 8 and 12 day harvests of negative specimens. Finally, the remainders of a certain number of specimens (usually less than 5 ml. in amount) were ultracentrifuged at 30,000 R.P.M. for 1 hour, and the sediment tested in 10 monkey kidney tubes. All positive specimens were retested, when possible, returning to the original specimen.

Throat and Rectal Swabs.—These were prepared for inoculation by removing the cotton and squeezing it out thoroughly in 1 ml. of sterile saline. After light centrifugation, monkey testicular tubes were inoculated with 0.1 ml. of the suspension. Two tubes were used for each specimen; they were examined microscopically at 4, 8, and 12 day intervals, and passed in the same manner as the tubes inoculated with blood. Subsequently, when the greater sensitivity of kidney tissue was appreciated, all negative specimens were retested in 4 to 6 monkey kidney tubes.

Neutralization Tests.—All strains isolated were typed by means of tissue culture neutralization tests, using either monkey testicle or monkey kidney tubes (10). When kidney tubes were employed a high titer was obtained so rapidly that in order to avoid overdosing the system with virus, it was usually necessary to set up tenfold dilutions of the virus to be typed with hyperimmune monkey serum against Types 1, 2, and 3 poliomyelitis virus. Equal amounts of the unknown virus and hyperimmune serum diluted 1:5 were arranged in a series of tubes. A control tube contained virus and balanced salt solution. The mixtures were incubated at room temperature for 1 hour before being inoculated into 2 tubes each. The tests were read at 4 and 8 days when testicular tubes were employed, and 2 and 4 days when kidney tubes were used. Complete inhibition of degeneration by serum of one type only was taken to indicate specific neutralization of the virus.

Neutralization tests on the blood specimens collected were carried out in similar fashion, using equal amounts of a 1:5 dilution of plasma and 100 tissue culture doses of the standard Brunhilde Type 1 strain.

RESULTS

Isolation of Virus from the Blood.—Type 1 poliomyelitis virus was isolated from the blood of 6 of 33 individuals infected with this type as evidenced by positive throat and/or rectal swabs. Blood specimens, throat, and/or rectal swabs from the other 86 individuals tested were all negative for poliomyelitis virus. In the positive blood specimens, the virus was present in both plasma, and in the partially hemolyzed red and white cell mixtures which also contained some plasma.[2] Of the 6 with viremia, throat and rectal swabs were available from 5, and throat swab alone from one; all these specimens also yielded Type

[2]An effort to determine with which fraction of the blood the virus is associated was subsequently carried out using blood from a *cynomolgus* monkey with viremia after ingestion of a Type 1 strain of virus (16). No significant difference in titer was detected among serum, plasma, and sedimented red and white cell mixtures containing some plasma; however, no virus was found in association with the thoroughly washed red cells. Washed white cells alone were not tested.

1 poliomyelitis virus. The clinical and epidemiological circumstances under which viremia was detected in the 6 individuals were various.

Four of the positive blood specimens were obtained from 4 children in one family, the W. family, residing in Burbank, a town of approximately 400 inhabitants which had a high attack rate. The family had had no known contact with a case of poliomyelitis, but at the time specimens were collected, on June 23, 3 of the children had symptoms suggestive of the

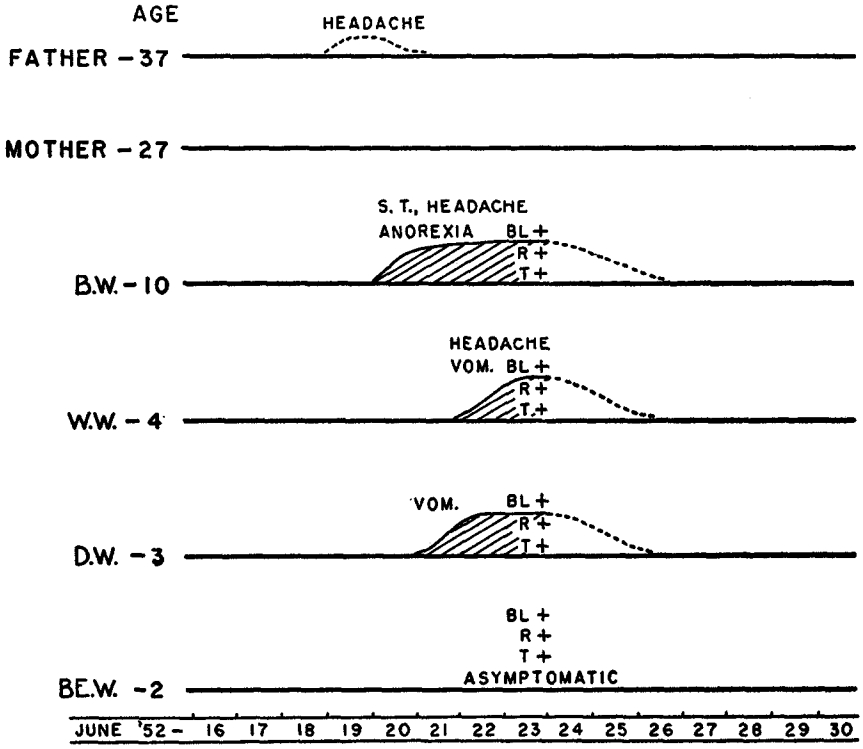

FIG. 1. An outbreak of Type 1 poliomyelitis infection in the W. family. Shaded areas indicate periods of fever; S. t., sore throat; Head., headache; Vom., vomiting; + the isolation of Type 1 virus from blood (Bl), throat swab (T), and rectal swab (R).

minor illness and one was asymptomatic (Fig. 1). The father had complained of headache several days previously, but there were no associated symptoms; the mother remained well throughout. *B.W., age 10 years*, had an onset with sore throat, fever, and anorexia, beginning on June 20. She complained also of soreness of the calves of the legs. On June 23, the day on which specimens were collected, her rectal temperature was 101.2°F. *W.W., age 4 years*, had her first symptoms on June 22, beginning with fever and vomiting. On June 23 her throat was sore, tonsils appeared enlarged and red; the rectal temperature was 102°F. *D.W. age 3 years*, began with fever, nausea, and vomiting on June 21. His temperature on June 23 was 99.5°F., and there were no physical signs. *Be.W., age 2 years*, remained asymptomatic. Her rectal temperature on June 23 was 99.5°F.

The children recovered promptly, and when a follow-up visit was made on June 25, all 4 were well and outdoors playing. Fig. 1 illustrates diagrammatically the course of events in the family, and the time at which the positive specimens were obtained. Tests for Type 1 antibody in the blood of the 4 children made at the time of viremia were all negative.

Viremia was also detected in an infant who subsequently developed mild non-paralytic poliomyelitis: *N.A., aged 10 months,* was a household contact of a 5 year old girl who had been

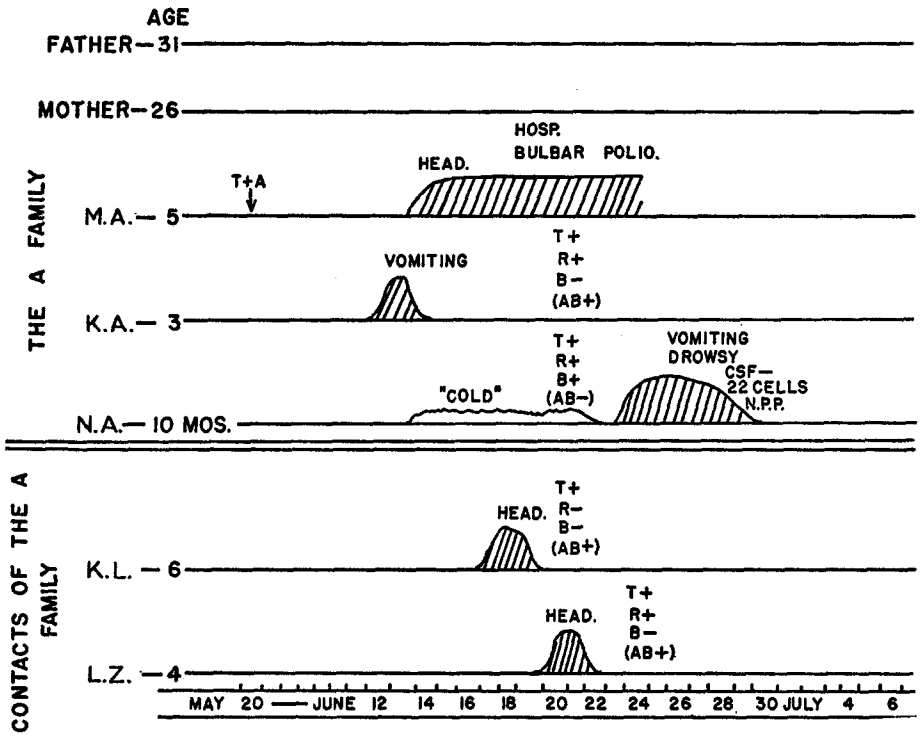

FIG. 2. Type 1 poliomyelitis virus infection in the A. family, and two neighborhood contacts. Legends as in Fig. 1. T + A indicates tonsillectomy + adenoidectomy; Hosp., hospitalized; N.P.P., non-paralytic poliomyelitis. AB + or —, Type 1 neutralizing antibodies positive or negative.

admitted to the hospital on June 18 with a diagnosis of bulbar poliomyelitis (Fig. 2). He was well until about June 14, when he developed a "cold;" *i.e.*, cough and runny nose, which persisted for about 8 days and was present on June 21 when throat and rectal swabs, and a blood specimen were collected. By June 22, the child seemed well, and remained so until June 24, when he developed fever, which persisted over the next 4 days, the maximum temperature being 102°F. During this time he was drowsy, slept a great deal, and vomited occasionally. On June 27 he was admitted to the hospital at which time his temperature was found to be 101.2°F., and physical examination revealed an inflamed pharynx and tonsils, and moderate anterior cervical adenopathy. The neck was supple. Spinal puncture, however, yielded fluid containing 22 WBC per c. ml., with 60 per cent lymphocytes, and a positive test for globulin. His recovery was uneventful; muscle examination on July 3 revealed slight tightness of neck

and back muscles. He was discharged as well on July 10. As indicated in Fig. 2, poliomyelitis virus was isolated from all three specimens taken on June 21 from N.A., —blood, throat swab, and rectal swab. This was 3 days before the onset of the major illness, which in his case turned out to be mild non-paralytic poliomyelitis. At the time the specimens were collected just prior to the "second phase" of his illness he was afebrile, although he still had a "cold" which had been present for 8 days.

Fig. 2, which is concerned with the A. family and two contacts of the children, shows the time relationships of symptoms in the group, as well as the results of tests for virus in blood, throat, and rectal swabs, and Type 1 antibody determinations on the blood specimens. All 4 children who were contacts of

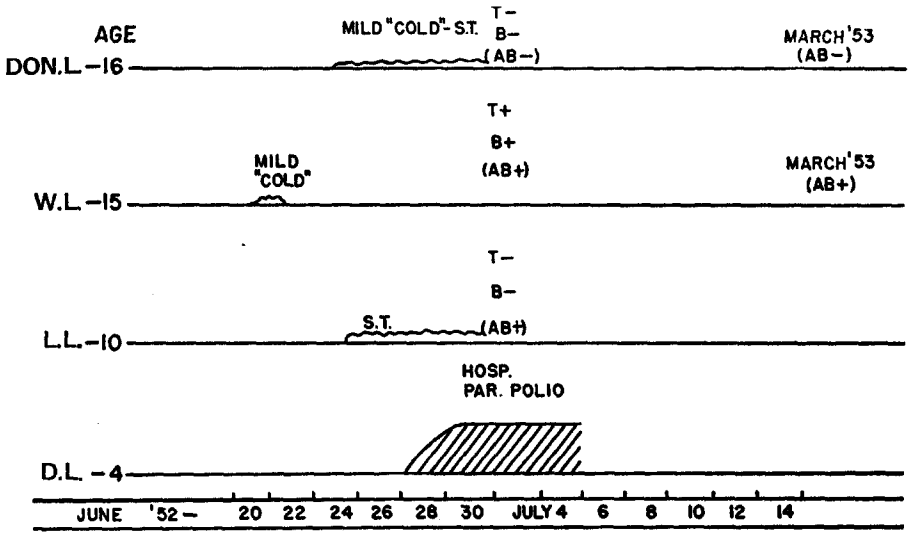

FIG. 3. Type 1 poliomyelitis infection in the L. family. Legends as in Fig. 1. Par. Polio., paralytic poliomyelitis.

the index case, 2 familial and 2 extrafamilial, were infected, and all but 1 (N.A.) from whose blood poliomyelitis virus was isolated, had antibodies to Type 1 virus. The time from onset of the minor illness to the collection of specimens was 10, 5, and 5 days respectively in those positive for antibodies but negative for viremia, or 8, 2, and 2 days after the minor illness symptoms had disappeared.

The 6th virus isolation from blood, was from W.L., an asymptomatic 15 year old boy, a household contact of a 4 year old paralytic case whose onset date was June 28 (Fig. 3). W.L. gave a history of "a mild cold" on June 22, which apparently lasted 1 day and cleared completely. He was well on July 1 when a throat swab and blood specimen were collected, both of which yielded Type 1 poliomyelitis virus. No virus was isolated from the 2 other siblings, one of whom, age 10 years, had Type 1 neutralizing antibodies. Don. L., age 16, gave a history

suggestive of a minor illness, but she was negative for both virus and antibodies on July 1. That her illness was not poliomyelitis is suggested by the fact that her blood was negative for Type 1 poliomyelitis antibodies in March 1953, 9 months later.

The strain of virus isolated from the blood of W.L. behaved peculiarly in its early passages. Subsequently it was found that antibodies were present in a titer of 1:100 in the same specimen from which virus was isolated. The circumstances under which the isolation was made were as follows:

The plasma and hemolyzed red cell suspension were inoculated separately in 0.1 ml. amounts into monkey testicular tissue culture tubes containing 0.9 ml. of media. After 4 days, the fluids were removed and discarded, and 1 ml. of fresh media was added to each tube. 8 days after inoculation the tubes showed no degeneration on microscopic examinations; the fluids were harvested and replaced with fresh media. At 12 days, still no degeneration was seen in the fibroblastic outgrowth; the fluids were therefore harvested, pooled with the 8 day harvests, and passed blindly. It is not possible to calculate accurately the dilution of the original specimen in the final harvest, but it was at least greater than 1:1000. On blind passage to 2 testicular tissue culture tubes, degeneration began to appear in both tubes by the 4th day, but was still not complete by the 8th day when the fluids were harvested. A 2nd blind passage was made from the original passage and this resulted in a 4+ degeneration in 1 of 4 tubes. 3rd passage of both of these lines resulted in degeneration in all tubes, less coarse in type than usually seen with poliomyelitis virus. The titer of the two 3rd passages was low, less than 10^{-1}. Two *rhesus* monkeys inoculated with undiluted 2nd and 3rd passage fluids failed to develop any signs of poliomyelitis. Attempts to neutralize the 3rd passage with antisera to the 3 types gave unsatisfactory results because of the low titer of the virus. After a 4th passage in testicular tubes a shift was made to monkey kidney tissue culture tubes; and after 4 rapid passages, each showing degeneration characteristic for poliomyelitis, a titer of 10^{-5} was achieved. In a neutralization test set up against the 3 types of hyperimmune serum using 100 tissue culture doses of virus, Type 1 antiserum completely inhibited virus growth, while Types 2 and 3 serum had no effect. Undiluted 9th passage virus produced paralytic poliomyelitis in one monkey, and non-paralytic disease in another, both results being verified by histological examination. Attempts to repeat the isolation of virus from the blood of W.L. were made, using monkey kidney tubes, and undiluted and diluted blood and plasma. All these efforts produced negative results. It may be said therefore that the strain of virus which was isolated was obtained with difficulty.

In contrast, the strain of virus isolated from the throat of this same patient, behaved in a more regular fashion. It was negative in 2 testicular tubes, but produced characteristic degeneration in 7 of 10 kidney tubes on 1st passage, 4 of 4 on 2nd passage, and was completely neutralized by Type 1 antiserum on 3rd passage, its titer being $10^{-5.5}$. A second trial in kidney tubes resulted in a similar series of positive passages. Two *rhesus* monkeys, given undiluted 2nd passage material, developed paralytic poliomyelitis.

When both the original specimen, and a follow-up blood sample from W.L., collected in March 1953, 9 months after the original specimen was obtained were titered for Type 1 neutralizing antibodies in the same test, the original had a titer of 1:100, and the later one a titer of greater than 1:500, suggesting that the first specimen was taken at a time when the titer was rising.

Amounts of Virus Present in the Positive Blood Specimens.—As Table I indicates, the virus isolations from blood were made with some difficulty. The

TABLE I

The Isolation of Type 1 Poliomyelitis Virus from the Blood, Throat, and Rectal Swabs of 6 Children

Name	Age	Date of onset minor illness	Date specimen collected		Results of tests for poliomyelitis virus					
					Direct monkey inoculation	Tissue culture passage				Monkey inoculation, tissue culture passage
						1st	2nd	3rd	Later	
B.W.	10 yrs.	6/20/52	6/23/52	Blood	0/1*	2/3‡	4/4	2/2§		1/1(3rd)
					0/3‖					
				Throat swab		2/2	2/2	2/2§		
				Rectal swab		1/2	2/2	2/2§		
W.W.	4 yrs.	6/22/52	6/23/52	Blood	0/2	0/4	0/2			
						3/5	3/3	2/2§		1/1(3rd)
						1/3	1/2			
				Throat swab		2/2	2/2	2/2§		
				Rectal swab		2/2	2/2	2/2§		
D.W.	3 yrs.	6/21/52	6/23/52	Blood	0/2	0/4	0/2			
						0/4	0/3			
						0/5	1/5	2/2§		1/1(3rd)
						3/10K¶	2/2§K			
				Throat swab		4/4	2/2§			
				Rectal swab		2/2	2/2	2/2§		
Be.W.	2 yrs.	—	6/23/52	Blood	0/2	0/4	0/2	0/2		
						0/4	0/4			
						1/6	5/6			
						9/10§K**	2/2K			1/1(3rd)
				Throat swab		2/2	2/2	2/2§		
				Rectal swab		2/2	2/2	2/2§		
N.A.	10 mos.	?6/14/52	6/21/52	Blood	0/2	0/6	0/2			
						0/5	0/4			
						1/8K**	5/5K	2/2§K		1/1(3rd)
				Throat swab		4/4	2/2	2/2§		
				Rectal swab		2/2	2/2	2/2§		
W.L.	15 yrs.	?6/22/52	7/ 1/52	Blood	0/2	0/4	2/2	4/4	2/2§K(9th)	0/2(2nd, 3rd)
							2/4	2/2		2/2(9th)
						0/4				
						0/10K	0/7K			
						0/10K	?1/7K	0/4K		
						0/10K**	0/8K			
				Throat swab		0/2	0/2			
						7/10K	4/4K	2/2§K		2/2(2nd)
						4/5K	4/4K	2/2§K		

* Numerator indicates number positive; denominator, number tested.

‡ Numerator indicates number of tubes showing degeneration; denominator, number of tubes inoculated.

§ Neutralization by Type 1 antiserum.

‖ These monkeys received 0.3 intracerebrally once only.

¶ K indicates monkey kidney tissue culture tubes used, if no K is marked, monkey testicular tubes were used.

** Specimens ultracentrifuged before testing in monkey kidney tubes.

quantity of virus present must have been minimal, for it was insufficient to produce poliomyelitis in *rhesus* monkeys on direct intracerebral inoculation of the undiluted plasma, although after passage in tissue culture, the speci-

mens tested in monkeys produced typical poliomyelitis. Only 2 of the 6 specimens were positive on the first trial in monkey testicular tissue culture tubes, one (W.L.) only on blind passage; with the others several separate tests were carried out before positive results were obtained. 1st passages were often difficult to interpret because of the tendency of certain plasma specimens to produce non-specific toxic effects particularly in testicular tissue tubes; on 2nd passage, however, degeneration typical for poliomyelitis occurred, and neutralization tests demonstrated the agents to be Type 1 poliomyelitis virus. Kidney tissue tubes proved more sensitive in detecting virus than testicular tissue tubes, and some specimens which were negative or only suggestively positive on 1st passage with the latter, were clearly positive on 1st passage in kidney.

TABLE II

*Results of Tests for Viremia in Blood Specimens of 119 Individuals in an Epidemic Area**

Age	Group 1—33 persons with positive throat and/or rectal swab		Group 2—78 persons with negative throat and/or rectal swab		Group 3—8 persons, throat and rectal swabs not tested	
	Antibody −	Antibody +	Antibody −	Antibody +	Antibody −	Antibody +
yrs.						
0– 4	**4/8**‡	0/8	0/8	0/10		
5– 9	0/2	0/5	0/4	0/15		0/1
10–14	**1/3**	0/5	0/5	0/19		0/1
15–19	0/1	**1/1**	0/6	0/5	0/1	
20+			0/3	0/3	0/1	0/4
Totals.....	5/14 (36 per cent)	1/19 (5 per cent)	0/26	0/52	0/2	0/6

* All strains isolated from blood, throat, and rectal swabs belonged to Type 1. Antibody status refers to Type 1 neutralizing antibodies.

‡ Numerator, number positive for viremia; denominator, number tested.

tubes. Ultracentrifugation also probably aided in increasing the sensitivity of the tests; one specimen (N.A.) which had been negative on direct inoculation of testicular tissue tubes, after ultracentrifugation was positive in kidney tubes.

The Incidence of Viremia in the Group Studied.—In all, 6 blood specimens from 33 individuals known to be infected, yielded virus. The question arose whether the negative results might be due to the blood having been drawn too late in the course of infection, at a time when antibodies were already present, since, ordinarily, in spite of the instance of viremia in the presence of antibodies reported above, one would not expect to find circulating virus in the presence of specific antibodies.

The blood specimens of the 33 infected individuals, as well as those of the 86 from whom no virus was isolated, were, therefore, all tested for the presence of Type 1 neutralizing antibodies. The results are shown in Tables II and III.

Table II relates viremia to the antibody status and age of the individuals tested. In group 1, consisting of the 33 individuals known to be infected with Type 1 virus, viremia was detected in 5 of 14 who were bled early enough in the course of infection to be without antibody levels sufficient to neutralize 100 tissue culture doses of virus. Of the 19 infected individuals who had detectable Type 1 antibodies, 1 was found to have virus in the blood as noted above. No virus was isolated from the blood of the 78 non-infected individuals, 26 of whom were antibody-negative, and 52 antibody-positive, nor from the 8 from whom only blood specimens were collected.

DISCUSSION

In these studies poliomyelitis virus has been demonstrated in human blood in association with three types of infection: the minor illness, the asymptomatic infection, and preceding the non-paralytic form of the disease. As to how commonly viremia occurs in the human infection, one cannot say, but the fact that it was found in 5 of 14 individuals who were bled early enough in the course of infection to be without antibodies, suggests that it may occur with some frequency (Table II).

The finding of virus in the blood of children with the minor illness confirms the earlier observation from this laboratory (1), and is in line with Bodian and Paffenbarger's recent results (13); it would seem to establish beyond a doubt that the minor illness—or first phase of the diphasic cause —is a specific infection with poliomyelitis virus, and not merely a non-specific and unrelated disease which precipitates poliomyelitis, a view which is sometimes still put forth (14). The speed with which antibodies appeared after the minor illness in some of the children in this study also supports the specificity of the syndrome. In recent years antibodies have been demonstrated to be present at the time of onset of the 2nd phase or major illness (3, 4), and also as early as 9 days after the minor illness (15); the present findings indicate that they may appear even earlier—as soon as 3 and 4 days after the onset of the minor illness (Fig. 2, Table III).

Two striking features of viremia in orally infected monkeys and chimpanzees are first, its early appearance—as soon as 3 or 4 days (occasionally only 24 hours) after virus ingestion, and as long as a week before paralysis (16); and second, its constant association with the early appearance of antibodies. The time at which viremia has been demonstrated in human poliomyelitic infection agrees well with the pattern found in orally infected animals. Two instances of viremia have, however, been reported, by Koprowski *et al.* (2) and Jungeblut (17) in which virus was detected *after* the onset of the major illness. One can speculate that in these individuals antibody-producing mechanisms were slow in responding, and antibodies failed to appear as they usually do, by the time of onset of symptoms. Jungeblut has suggested that in his patient, the fact

that she was pregnant at the time of infection, may have involved adrenal mechanisms, so that the situation was analogous to poliomyelitis with viremia induced in hamsters by the administration of ACTH or cortisone as reported by Schwartzman (18).

TABLE III

Type 1 Poliomyelitis Virus Isolations and Neutralizing Antibody Status of 33 Infected Individuals

Group 1—With symptoms								Group 2—Asymptomatic					
Name	Age	History	Days from onset* to collection of specimens	Results of virus tests‡ T	R	Bl	Type 1 antibody status	Name	Age	Results of virus tests T	R	Bl	Type 1 antibody status
B.W.	10 yrs.	S.t.§, fever, headache, anorexia, 3 days	3	+	+	+	−	B.W.	2 yrs.	+	+	+	−
								R.C.	7 "	+	−	−	−
W.W.	4 "	Fever, vomiting, S.t., 1 day	1	+	+	+	−	J.W.	17 "	+	ND‖	−	−
								C.S.	3 "	+	+	−	−
D.W.	3 "	Fever, nausea, vomiting, 2 days	2	+	+	+	−	M.W.	14 "	+	−	−	−
								D.M.	5½ mos.	−	+	−	+
N.A.	10 mos.	"Cold," 8 days	8	+	+	+	−	M.F.	10 yrs.	+	ND‖	−	+
W.L.	15 yrs.	"Cold," 1 day	9	+	ND‖	+	+	F.P.	12 "	+	−	−	+
B.S.	10 "	S.t., abdominal pain, 1 day	3	+	+	−	−	K.C.	14 mos.	−	+	−	+
								S.O.	10 yrs.	−	+	−	+
D.S.	7 "	Headache, fever, nausea, 2 days	2	+	+	−	−	G.O.	2 "	−	+	−	+
								J.B.	5 "	+	−	−	+
J.K.	3 "	Irritable 4 days, nausea, 1 day	4	+	+	−	−	T.S.	13 "	+	−	−	+
								C.W.	2 "	+	−	−	+
M.C.¶	3 "	Vomiting, 1 day	6	+	+	−	−	P.W.	11 "	+	−	−	+
M.C.¶	1½ yrs.	Vomiting, fever, 1 day	4	+	−	−	−						
D.J.	11 mos.	"Cold," 1 day	18	−	+	−	+						
L.S.	5 yrs.	S.t., fever, 3 days	13	+	−	−	+						
K.A.	3 "	Fever, 1 day	8	+	+	−	+						
K.L.	6 "	Headache, fever, 1 day	3	+	+	−	+						
L.Z.	6 "	Headache, fever, 1 day	4	+	+	−	+						
B.R.	6 "	S.t., fever, 1 day	13	−	+	−	+						
D.R.	4 "	Fever, "several days"	?	−	+	−	+						
G.H.	3 "	Cough, conjunctivitis, 7 days	7	−	+	−	+						

* Refers to onset of symptoms compatible with the minor illness.

‡ T, R, Bl, indicate throat swab, rectal swab, and blood.

§ S.t., sore throat.

‖ ND, not done.

¶ These two children developed mild non-paralytic poliomyelitis 2 days after the specimens were collected.

The direct evidence that viremia in humans is followed by a prompt antibody response consists of Bodian and Paffenbarger's demonstration that 3 children from whose blood virus was isolated during the minor illness all had significant antibody titers 2 weeks later (13). Further, 1 individual in the present study (W.L.) appears to have been bled at the moment that virus was disappearing and antibody was appearing. Both the early appearance of virus

in the blood, and the early appearance of antibodies, support the earlier hypotheses of primary extraneural growth of poliomyelitis virus (19–21), for it has been observed that when virus is growing only in nervous tissue, as presumably may happen after intracerebral inoculation of monkeys, antibody production may be long delayed (22–24). It seems likely that the very tissues responsible for antibody production may be the ones in which the virus may first multiply. Diffuse involvement of lymphoid tissue in the intestinal tract and in peripheral lymph nodes in the rapidly fatal human disease has been commented upon regularly since 1912 (25); and virus has been isolated from various lymph nodes with some frequency (26); the latter is especially striking if one considers that the tissues for testing were obtained at a time when antibodies were undoubtedly present. Faber and his colleagues have shown that virus multiplication may appear very early in the peripheral ganglia in *cynomolgus* monkeys infected orally (27). Multiplication in these sites does not, however, preclude its occurring in other and non-neural sites.

The relationship of viremia to CNS invasion is uncertain. It may be as we assumed in 1946 (1), and as Faber *et al.* suggested recently (28), that it represents simply a spill-over from areas of active multiplication, and has no significance in the pathogenesis of the disease. The latter authors hold firm to the concept that poliomyelitis virus is a strictly neurotropic agent. On the other hand, experimental poliomyelitis can be produced in the absence of intact nervous connections (29); and experiments of Bodian have shown the effectiveness of small amounts of passive antibody in protecting orally infected *cynomolgus* monkeys (8) and chimpanzees (30) against the development of CNS lesions, presumably by preventing viremia. Both these observations suggest that the appearance of virus in the blood stream may be vital in the pathogenesis of the disease. This is the interpretation which was proposed in 1917 by Draper (31) who pointed to the biphasic clinical course of poliomyelitis as evidence of a preliminary systemic or general infection, followed by the 2nd or CNS phase only when penetration of the blood brain barrier occurred.

The time at which poliomyelitis virus has been isolated from the blood, throat, and feces of patients, and the time at which antibodies have been found are indicated in Fig. 4, which is a schematic diagram of the so called "childhood type" of the disease (32). The findings suggest the following sequence of events as a likely possibility in the human biphasic infection: first, primary multiplication of virus, occurring during the few days after exposure. Such multiplication may be largely or entirely extraneural at this stage; second, the development of the "minor illness" in association with the presence of virus in blood, throat, and feces, with viremia persisting only for a few days, until antibodies make their appearance; finally, if the circulating virus has gained a foothold in the CNS, a neural phase of multiplication, followed by the characteristic signs of CNS involvement. A similar interpretation of the pathogenesis of

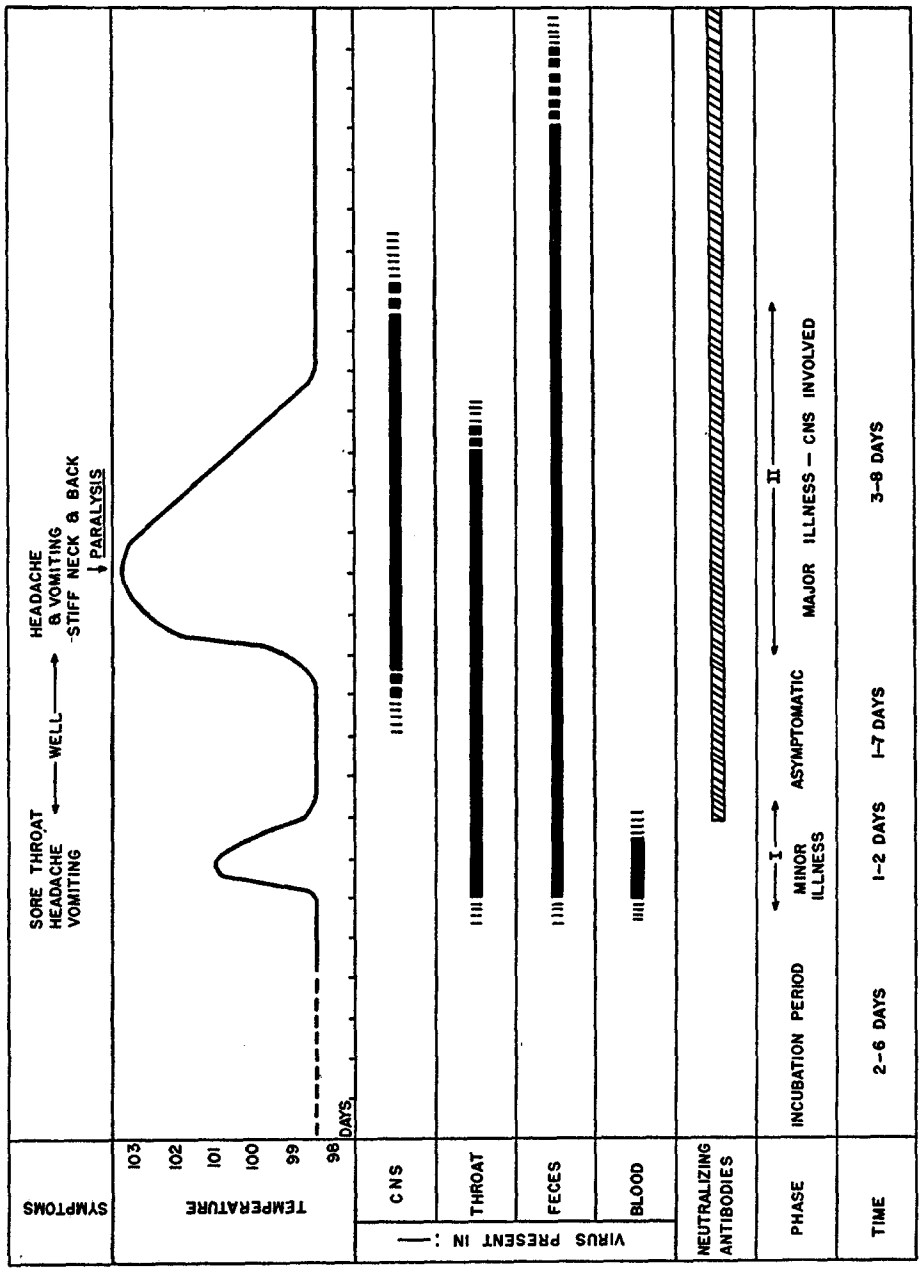

FIG. 4. Schematic diagram relating the presence of virus in various anatomic sites to the symptoms in a biphasic case of poliomyelitis.

poliomyelitis has been suggested by Bodian, who designates the three hypothetical phases as visceral, viremic, and neural (33), and by Pette (34); although there is considerable evidence to support this concept, it has not yet been proven.

The Isolation of Virus from the Blood in the Presence of Antibodies.—The finding of Type 1 virus in the blood of W.L., which also had Type 1 neutralizing antibodies was unexpected. However, although a laboratory error cannot be completely ruled out, on careful evaluation, there was no reason to suspect that any error had been made, especially inasmuch as none of the other 32 specimens in the same test was positive, either originally or on blind passage. Furthermore, the peculiar behavior of the virus isolated supports the theory that the agent was in a modified, and atypical state, which is a situation deserving further study. Failure to repeat the isolation may be due in part to the time which elapsed between the 1st and only successful test in tissue culture which was set up 6 days after the specimen was collected, and the 2nd and subsequent attempts at isolation begun after the specimen had been kept frozen at −20°C. for 6 months.

The isolation of infective particles from presumably neutralized mixtures of antiserum and virus is a well known phenomenon with other systems. Vaccinia virus has been thoroughly studied in this respect, and it was found by Andrewes that the virus can be recovered even in the presence of an excess of antibody by simple dilution (35). Coxsackie virus, which is in the same size range and has many biological and physical properties in common with poliomyelitis virus, can also be dissociated from its antibody by dilution, as shown by Melnick and Ledinko (36). Since the nature of the union of poliomyelitis virus and its antibody is unknown, one can only speculate as to the intimacy of the combination and the difficulty or ease with which it can be separated.

SUMMARY

Poliomyelitis virus was isolated from the blood of 6 of 33 individuals infected with a Type 1 strain. Of the 27 negative for viremia, 18 already had Type 1 antibodies.

Type 1 virus was isolated from the throat swabs of all 6 who had viremia, and from the 5 rectal swabs tested. These specimens were all collected at the same time as the positive blood specimens.

The clinical circumstances under which viremia was detected included the minor illness, the asymptomatic infection, and one instance in which the virus was isolated several days *before* the onset of a mild non-paralytic attack.

In one instance, virus was isolated from a blood specimen which also contained Type 1 antibodies.

The possible role of viremia in the pathogenesis of poliomyelitis is discussed.

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