ANTIBODY RESPONSE OF PATIENTS WITH POLIOMYELITIS TO VIRUS RECOVERED FROM THEIR OWN ALIMENTARY TRACT*

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(Received for publication, June 13, 1949)

The present investigation was undertaken to obtain conclusive data on the development and status of antibodies in human beings during the course of infection with the virus of poliomyelitis. Previous studies on this problem have been extensively reviewed by Paul and Trask (1, 2), Harmon and Harkins (3), Brodie, Fischer, and Stillerman (4), Burnet and Jackson (5), Kessel, Stimpert, and Fisk (6), Aycock (7), Turner and Young (8), and Sabin (9). The main issues and controversial findings will be summarized, however, to indicate how they determined the design of the present study.

Tests with strains of poliomyelitis virus which had undergone many passages in monkeys ("MV," "Aycock," or others) (1–7) or in mice (Lansing) (8, 10–15) invariably gave a similar pattern, which varied only quantitatively in different investigations. The sera obtained during the acute stage of the disease either had no antibody and, with certain exceptions, developed none during convalescence, or else neutralized the virus and, when tested quantitatively, only rarely increased in titer during convalescence.

Tests with strains of poliomyelitis virus of recent human origin (1, 2, 4–7, 16, 17) especially when the virus and the patients were derived from the same outbreak, yielded different results in different investigations. Thus, Paul and Trask (1), Howitt (16), Kessel, Stimpert, and Fisk (6), and Aycock (7) reported that a considerable number of patients' convalescent sera which failed to neutralize standard, laboratory, multiple-passage virus possessed antibodies for a strain of virus recovered during the same outbreak from which the patients were derived. Using these freshly isolated strains, it was occasionally possible to demonstrate absence of antibody in the serum obtained during the acute phase of the illness and presence during convalescence, when similar tests with the multiple-passage, laboratory strains either revealed no difference or yielded negative results. On the other hand, Brodie, Fischer, and Stillerman (4) and Burnet and Jackson (5) found no difference in the results of the neutralization tests with "standard" laboratory strains as compared with virus recovered during the same outbreak from which their patients were derived. The difficulties in appraising these data, which were appreciated by many of the investigators, stemmed from one or more of the following circumstances:

(*a*) the tests carried out in monkeys were usually done with single animals or small numbers of animals, and one could not be certain of occasional differences between acute and convalescent sera; (*b*) the viruses of recent human origin might have been of either low or high potency and virulence, and since the tests were carried out with unknown quantities of virus (in terms of paralytic or infective doses), the comparative studies were consequently neither

* Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

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definitive nor conclusive; (*c*) the accumulating evidence that different strains of poliomyelitis virus of recent human origin differed in antigenic constitution, and the lack of certainty that any one epidemic was caused by a single strain of virus, were regarded by many as the most probable explanation for the discrepant and often incomprehensible results.

Since the extent of antigenic variation among different strains of poliomyelitis virus was still unknown, it was apparent to a number of investigators that the antibody response in human poliomyelitis had to be elucidated first of all, by using virus recovered from the individual patient under investigation. At the time the present study was planned this had been done in only two instances. Trask and Paul (2) recovered virus from the throat of a child with a minor illness (abortive poliomyelitis) and showed that while the serum obtained during the 1 day of illness failed to protect a monkey, the serum obtained 25 days later tested in another monkey prolonged the incubation period sufficiently to suggest partial protection. Sabin and Ward (18) recovered a virus from the stools of a laboratory worker, who acquired paralytic poliomyelitis, and showed that it was immunologically identical with a strain of virus of recent human origin ("Per.") to which she was exposed and different from the "M.V." virus; this patient's acute phase serum failed to neutralize both the "M.V." and the "Per." viruses, while the 1 month, 2 month, and 10 month convalescent sera neutralized the "Per." virus (tested in 5 monkeys) but not the "M.V." virus (tested in 3 monkeys). It should be stated, however, that the potency of the "Per." virus used in this test was not established and it might have been in the minimal infective range.

The review of previous work made it abundantly clear, that any definitive study of the antibody response in human poliomyelitis would require not only the use of strains of virus recovered from the individual patient, but also, with less regard for the cost of large numbers of monkeys than was necessary in earlier years, the same quantitative techniques which are commonly utilized in the study of other virus infections.

Plan of Study and Methods

The goal was to collect approximately 20 patients with paralytic or non-paralytic poliomyelitis from whose alimentary tract virus would be recovered during the first days of the illness and from whom serum specimens would be available within 4 days after appearance of the first symptom, however slight, and at 2, 4, and 12 weeks after onset. The monkeys to be used for recovery of the virus from any one patient would be kept isolated to prevent them from picking up virus from the stools of monkeys inoculated with material from other patients. Material from the first monkeys to succumb with poliomyelitis was to be passaged to another group of monkeys (at least 2) and if these developed poliomyelitis, their spinal cords and medullae were to be used for the preparation of a centrifuged 20 per cent suspension in saline. This second generation virus material was to be stored frozen in a box containing solid CO_2 and used for preliminary titration of the potency of the virus. Any strain with a 50 per cent poliomyelitis dose (PD_{50}) of approximately 10^{-3} or more, would be considered suitable for the projected quantitative neutralization tests with the patient's sera. Since one could not predict the behavior of these strains on storage or the reproducibility of the titrations, the virus was to be

retitered each time a neutralization test was carried out and sera to be compared were to be tested simultaneously. All sera were to be stored in a dry ice refrigerator. In order to evaluate the specificity of the antibody response to the patient's own virus, the same sera were also to be tested in mice against the Lansing strain of poliomyelitis virus. This plan was strictly carried out and extended when the unexpected results of the study required further elucidation.

Selection of Patients.—In order to achieve the goal just described it was expected that approximately twice the number of patients ultimately desired would have to be studied. Accordingly, material was finally collected from 40 patients during the course of moderate outbreaks of poliomyelitis in Cincinnati, Ohio, Akron, Ohio, and Boise, Idaho and vicinity in 1947. Each of these patients was examined by either one of us before selection and subsequently until the clinical diagnosis of poliomyelitis and ultimate paralytic or non-paralytic nature of the disease were established.[1] Pleocytosis, with a minimum of 20 leukocytes per c. mm. of cerebrospinal fluid, along with nuchal-spinal rigidity and the other clinical manifestations usually seen in the non-paralytic and paralytic forms of the disease were present in all the selected patients. The time of onset was calculated from the appearance of the first relevant symptom, and the study was limited to patients admitted to the hospital within a few days after onset.

Recovery of Virus from the Alimentary Tract.—Material from the oropharynx on 2 cotton-tipped applicators, and stools or saline enema returns were obtained daily for a period of 3 days. Each pair of cotton swabs was placed in a screw-capped lusteroid tube containing 1 ml. of sterile, distilled water and stored frozen in a box with solid CO_2. The stools or enema returns were similarly stored in the frozen state in waxboard cartons. The liquid expressed from the thawed cotton swabs was centrifuged at 4,000 R.P.M. on an angle centrifuge for 30 minutes, treated with anesthetic ether, recentrigued at the same speed and time, and the supernatant liquid, after being shown to be free of bacteria by culture on blood agar, was used for intracerebral inoculation into each of 2 monkeys. The same 2 monkeys received the same patient's untreated stool suspension intranasally once a day for 4 to 10 days, and the ether-treated, centrifuged extract of the stools intraabdominally in amounts of 20 ml. daily for 1 to 4 days (19). Monkeys which developed paralysis were sacrificed within less than 24 hours to obtain material for passage at the optimum time and for histologic confirmation of the diagnosis. Monkeys which failed to develop paralysis were sacrificed 30 to 35 days after inoculation for histologic study to detect the presence of lesions associated with experimental non-paralytic poliomyelitis (20). When both monkeys, receiving material from the same patient, developed paralysis, their tissues were pooled for passage into 2 additional monkeys. The second generation virus was then used for intracerebral titration as shown in the protocols of the appendix. Each strain of virus was stored in the frozen state as a 20 per cent centrifuged suspension in saline, which was regarded as the 1:5 dilution. Further dilutions for titration and neutralization tests were prepared in saline.

Neutralization Tests in Monkeys.—Virus dilutions of 1:5, 1:50, 1:500, etc. were mixed with equal amounts of saline for control or with the undiluted sera to be tested, to yield final dilutions of virus of 10^{-1}, 10^{-2}, 10^{-3}, etc. The mixtures were left at room temperature (20 to 26° C.) for 1 hour, in a refrigerator at approximately 4 to 6° C. overnight for about 15 hours, and were

[1] We are indebted to the staffs of The Children's Hospital, Akron, Ohio, and the St. Luke's Hospital, Boise, Idaho, for permission to study their patients. Special thanks are due Dr. Margaret Baker in Akron and Mr. A. W. Klotz in Boise, for the collection and forwarding of convalescent serum specimens.

kept in an ice bath during the course of the inoculations. Each of 3 *rhesus* monkeys received 0.5 ml. of each mixture intracerebrally, the inoculation being carried out with a 1 inch long needle deep into the frontal lobes of one side. Monkeys which failed to develop typical paralysis were sacrificed 28 to 35 days after inoculation, and if histological changes compatible with non-paralytic poliomyelitis were found, they were so recorded and counted in the calculation of the PD_{50} of the virus. As might have been expected the PD_{50} titer of aliquots of the same virus preparation varied in different titrations within approximately one tenfold dilution without reference to the time of storage in the frozen state. For this reason the neutralization indexes (ratio of titer of virus in saline and titer of virus in mixture with test serum) were calculated both from the control titer of the virus in the simultaneous test and from the cumulative titer obtained in 3 separate titrations. The neutralization index calculated from the cumulative control titer is used in the summaries, because it is believed to be closer to the actual value.

When it proved necessary to carry out tests in which the virus dose was constant and the dilution of serum was varied, an attempt was made to use 50 PD_{50}, based on two preceding titrations of the virus preparation, although the final data for this calculation were occasionally not available because the histologic studies had not been completed. However, the dose so selected, turned out to vary from 13 to 1,000 PD_{50}, as calculated from a third, simultaneous titration of the virus, and from 32 to 200 PD_{50} as calculated from the cumulative control titer derived from the 3 titrations. The selected dose of virus (in twice the concentration to allow for dilution upon addition of an equal amount of saline or indicated dilutions of serum) was mixed with saline or the fourfold dilutions of serum, incubated in the same manner, and 0.5 ml. of each mixture was injected intracerebrally into each of 4 monkeys. The 50 per cent protective serum dilution end-point was calculated by the method of Reed and Muench (21).

Neutralization Tests with Lansing Virus in Mice.—This strain of virus was obtained in its 198th mouse passage from Dr. H. A. Howe. The virus used in these tests consisted of a large pool of centrifuged 10 per cent suspension in saline (regarded as the 1:10 dilution) prepared from the spinal cord and brain stems of several hundred mice. It was stored in 1 ml. amounts in sealed glass ampoules, frozen with solid CO_2. Titrations using 8 mice per dilution (prepared in saline) were carried out each time a test was done. The cumulative LD_{50} of one lot of virus based on 23 individual titrations over a period of 6 weeks was $10^{-3.5}$ with a range of $10^{-2.8}$ to $10^{-4.2}$ and 74 per cent of the titers in the range of $10^{-3.3}$ to $10^{-3.7}$. In the neutralization tests the 1:10 and 1:50 dilutions of virus were mixed with equal parts of the undiluted sera, yielding final dilutions of virus of 1:20 and 1:100, and after 1 hour at room temperature, 8 mice were inoculated intracerebrally with 0.03 ml. of each mixture. The LD_{50} titers of the virus in the presence of a given serum were estimated from the number of mice which died between 2 and 28 days after inoculation. The neutralization indexes were estimated by comparing these LD_{50} titers with the cumulative LD_{50} titer of the lot of virus used in the test. Under the conditions of the test a neutralization index under 30 was regarded as negative (0), between 30 and 50 as questionable (±), between 50 and 100 as positive (+), and over 100 as positive (++). When it was necessary to test various dilutions of some of the positive sera against a constant amount of virus, fourfold dilutions of the sera were mixed with equal amounts of virus diluted to contain 100 LD_{50} per 0.03 ml., so that the final dose of virus used was 50 LD_{50}. The 50 per cent protective serum dilution end-point was then estimated by the method of Reed and Muench (21). The acute phase sera from each of the 40 patients were tested first. Those which yielded negative or questionable results were then retested simultaneously with the 3 month convalescent serum from the same patient.

RESULTS

Recovery of Virus from the Alimentary Tract and Status of Antibodies for Lansing Virus in Individual Patients.—Twenty strains of virus were recovered

TABLE I

Recovery of Virus and Status of Antibodies for Lansing Virus in Individual Patients

Type of illness	City	Patient No.	Patient Name	Patient Age	Time after onset 1st specimen obtained	Virus recovery + or −	Virus recovery Titer in monkeys‡	"Lansing" antibody in serum Acute phase	"Lansing" antibody in serum Convalescent 3 mos.	Estimated N.I.* Acute phase	Estimated N.I.* Convalescent 3 mos.
				yrs.	*days*		*10⁻*				
Paralytic	Cincinnati 1947	1	Fro	0.6	3	+	3.3	++		200+	
		2	Ric	0.6	5	+	3.6	−; −	++; ++	10−; 5−§	140+; 160+
		3	Ris	1.5	3	+	3.8	±; −	−	32−; 13−	4−
		4	Hopk	2	2	+	4.0	++		320+	
		5	Fin	8	0.5	+	2.9	−; −	−	16−; 13−	6−
		6	Hopp	1.5	4	+	1.0+?	−; −	−	20−; 5−	5−
		7	Wol	2	3	−		++		320+	
		8	Bre	12	5	−		−; −	−	20−; 15−	10−
		9	Ten J	7	2	+	4.1	±; −; −	+; +	50−; 25−; 25	63+; 80+
	Akron 1947	10	Obe	13	1	+	3.6	−; −	−	10−; 5−	5−
		11	Kau	11	3	+	<1.0	++		320+	
		12	Smi	13	3	+	<1.0	±; −	−	50−; 10−	16−
		13	Gaf	12	4	−		++		320+	
Non-paralytic	Cincinnati 1947	1	Hof	4	3	+	4.3	−; −	−	20−; 6−	5−
		2	Vau	8	5	+	2.3	−; −	+; ++	16−; 6−	50+; 100+
		3	Cah	3	2	−		−; −	−	20−; 16−	6−
		4	Red	7	4	−		−; −	−	10−; 5−	13−
		5	Sea	7	1	−		−; −	−	20−; 5−	4−
		6	Gil	11	2	−		++		200+	
		7	Hal	12	3	−		++		250+	
		8	Bru	15	1	−		++		250+	
		9	Wal	3	2	+	4.2	−; −	−	20−; 5−	4−
		10	Pet	13	2	+	2.5	+; ++	+	80+; 140+	80+
		11	Fal	36	3	+	2.3	−; −	−	13−; 6−	10−
		12	Kni	2	1	+	<2.0	±; −	−	50−; 10−	20−
		13	Yea	2	0.3	+	<2.0	±; −; −	±; ±	50−; 8−; 6	40+; 32
		14	Col	12	1	+	1.0+?	++		250+	
		15	Sic	15	1	+	<1.0	+; +	++	100; 80	140+
	Akron 1947	16	Cur	3	4	−		−; −	−; −	10−; 4−	20−; 16
		17	Let	5	1	−		−; −	−	20−; 6−	4−
		18	Sny	5	1	−		−; −	−	20−; 10−	5−
		19	Wor	7	1	−		+; −	−	63−; 13−	20−
		20	Hud	11	3	−		++		200+	
		21	Ten S	12	4	−		−; −	++	20−; 13−	125+
		22	Fru	12	4	−		++		250+	
		23	Gra	16	4	−		−; −	−	13−; 6−	5−
		24	Den	5	0.3	+	1.0	++		125+	
	Boise, Idaho 1947	25	Mac	5	3	−		−; −	±; +	20−; 6−	40+; 63+
		26	Ack	18	1	−		++		250+	
		27	Kra	66	3	−		++		320+	

* N. I. = neutralization index.

‡ This refers to the cumulative PD_{50} based on 3 intracerebral titrations in monkeys of a single lot of virus consisting of second generation monkey material, except in paralytic patient 5, where third generation virus was titrated.

§ Two or more values separated by a semicolon indicate the results of repeated tests; the minus sign after a neutralization index indicates that the estimated value might be less than the actual value, while the plus sign indicates that it might be greater.

from the 40 patients under investigation—10 strains from the 13 patients who ultimately developed the paralytic form of the disease, and 10 from the 27 patients with a non-paralytic illness. Using 20 per cent suspensions of the spinal

TABLE II

Antibodies in Undiluted Acute and Convalescent Sera against Virus Recovered from Patient's Alimentary Tract during Acute Phase of Illness Contrasted with Those against Lansing Strain of Poliomyelitis Virus

Ultimate clinical course	Patient	Time after onset	Neutralization index of undiluted serum *versus*	
			Patient's virus (Tests in monkeys)	Lansing virus (Tests in mice)
		days		
Non-paralytic	Wal	2	16	5−
		92	1600	4−
	Hof	3	10−	6−
		91	63	5−
Paralytic	Obe	1	400+	5−
		94	800+	5−
	Ris	3	200	13−
		89	63+	4−
	Fin	0.5	80+	13−
		93	250+	6−
	Ric	5	800+	5−
		91	1250+	140+
	Ten.J.	2	2500+	25
		91	4000+	80+
	Hopk	2	2000+	320+
		92	3200+	
	Fro	3	630+	200+
		90	400+	

cords and medullae of the monkeys which succumbed after inoculation with the human material, each of these strains produced paralytic poliomyelitis in the intracerebrally inoculated monkeys used for passage. However, when the second generation virus material was titrated in monkeys, only 9 of the 20 strains proved to have titers of approximately 10^{-3} or more (Table I). It may be note-

worthy that of the 10 strains derived from paralytic patients, 7 were of the more potent variety, while only 2 of the 10 strains from non-paralytic patients were in this category.

The tests for Lansing antibody in the serum of these 40 patients (Table I) indicated that 24 or 60 per cent had no antibody for this strain of poliomyelitis virus during the acute phase of the illness. Among the 20 patients from whose alimentary tract poliomyelitis virus was recovered in monkeys, 13 or 65 per cent had no antibodies for the Lansing virus during the acute phase. When the acute phase sera were retested simultaneously with the 3 month convalescent sera of the 24 patients who had no Lansing antibody early after onset of the illness, it was found that 5 (21 per cent) had become definitely positive and one questionably so. Among the 13 patients from whom virus was recovered and whose serum was without Lansing antibody during the acute phase, 3 and possibly 4 developed this antibody during convalescence while 9 did not. Accordingly, it appeared possible that the infections we studied might have been caused by viruses of different antigenic constitution and that some of them were probably related immunologically to the Lansing strain. However, the tests to be reported did not support this assumption.

Tests with Undiluted Acute and Convalescent Sera versus Homologous Strains of Virus.—The tests performed with the 9 strains of virus which had an intracerebral PD_{60} of approximately 10^{-3} or more are summarized in Table II, and the individual protocols are presented in the appendix (Tables VIII to XVI). The 2 patients with the non-paralytic illness had no significant amounts of antibody for the virus recovered from their own alimentary tract early after onset, while the 3 month convalescent sera neutralized the homologous virus but not the Lansing virus. In these 2 patients it might be possible to conclude on serologic grounds that the strains of virus recovered from them were responsible for the infection under investigation. The results obtained with the sera of the 7 paralytic patients were unexpected, however, in that all the acute phase sera, even those obtained 12 to 24 hours after appearance of the first symptom, neutralized almost completely the maximum amount of the homologous virus. The tests on the undiluted, 3 month convalescent sera yielded essentially the same results, and it became apparent that the neutralization test, employing undiluted sera against varying amounts of the homologous virus, could not indicate the character of the antibody response in these paralytic patients. It was of interest to note that 5 of these 7 acute phase sera, which had antibodies for the patient's own virus, failed to neutralize the Lansing virus, and that Lansing antibodies became demonstrable in the 3 month convalescent sera of 2 patients in this group. In former years when such tests were not carried out with the patient's own virus, antibody was not infrequently found in sera obtained during the earliest stages of infection, but it was generally believed that such antibody was not against the infecting strain of virus and was probably

present prior to the infection under investigation. Since the present data showed that such findings were even more frequent in tests with strains of virus recovered from the patient's own alimentary tract, and since there was no way of determining whether this antibody was present before the present infection it became necessary to determine whether the homologous antibody found so early after onset was static or changed during convalescence. Since

TABLE III

Demonstration of Increase in Titer of Homologous Antibodies during Convalescence by Tests on Varying Dilutions of Serum against Constant Amount of Virus

Patient	Time after onset	50 per cent serum dilution end-point	PD_{50} of virus in test based on		
			2 Preceding titrations	Simultaneous titration	Cumulative titer
	days				
Obe	1	Undiluted?	63	200	80
	94	1:32			
Ris	3	1:3	63	630	125
	89	1:13			
Fin	0.5	1:6	32	160	40
	93	1:97+?			
Ric	5	1:16	50	32	40
	91	1:107+?			
Ten J	2	1:1	63	1000	125
	91	1:26			
Hopk	2	1:16	50	13	32
	92	1:107+?			
Fro	3	0?	100	320	200
	90	1:3			

the tests on the undiluted sera failed to show a significant, quantitative difference between the acute and convalescent sera in the 7 paralytic patients, the study was repeated by using various dilutions of sera against a constant amount of virus.

Neutralization Tests with Varying Dilutions of Serum against a Constant Amount of Virus.—The results of the tests (summarized in Table III and shown in detail in the appendix, Tables X to XVI) on the acute and convalescent sera of the 7 paralytic patients indicate that the antibody for the homologous virus was in each instance present in lowest titer soon after onset of the

illness and in increased concentration 3 months after onset. It can be seen that the antibody titers vary considerably in individual patients and that they are highest when the dose of virus, calculated from the cumulative titer of the lot used, was 32 to 40 PD_{50}. Smaller amounts of virus were not used in these tests, because it was believed that the number of monkeys employed for titration did not lend sufficient certainty to the fate of animals in the minimal infective range, and it was desirable that the incidence of poliomyelitis in the absence of antibody be as close to 100 per cent as possible. However, it is noteworthy that even when 80 to 125 PD_{50} of virus were used, increases in antibody titer up to 32-fold could be demonstrated in the convalescent sera. The 50 per cent

TABLE IV

ntibody Titers vs. Lansing and Patient's Own Virus during Convalescence in Individuals Possessing Both at Onset of Illness

Patient	Time after onset	Antibodies for Lansing virus		Antibodies for patient's virus		
		Neutralization index of undiluted serum	50 per cent protective serum dilution end-point *vs.* 50 PD_{50} of virus	Neutralization index of undiluted serum	50 per cent protective serum dilution end-point *vs.* indicated PD_{50} of virus.	
	days					
Hopk	2	320+	1:52	2000+	1:16	32 PD_{50}
	92		1:27	3200+	1:107+?	
Fro	3	200+	1:20	630+	0?	200 PD_{50}
	90		1:8	400+	1:3	

protective serum dilution end-point is undoubtedly affected by the quantity of virus used. Accordingly only sera tested simultaneously against the same amount of virus can be compared. Although the results in patient Fro. (appendix, Table XVI) were especially irregular, the difference in antibody titer between the acute and 3 month convalescent sera in the other patients was from 4- to 32-fold. It appeared, therefore, that antibody for the homologous virus was not static during the course of infection.

On the other hand, antibody for the Lansing strain of virus failed to appear in 5 of these 9 patients, appeared sometime during convalescence in 2, and in 2 others (Hopk. and Fro.) it was obviously necessary to perform neutralization tests with varying dilutions of serum against a constant amount of virus in order to determine the status of the antibody. The results of this test (Table IV) show that in these last 2 patients who, soon after onset of illness, possessed antibodies for both the Lansing strain and their own virus, there was no increase in titer of the Lansing antibody when that for their own virus increased during

convalescence; the somewhat lower titers of the Lansing antibody in the convalescent sera are probably within the range of experimental error. This specific change in antibody titer for the homologous virus in at least 7 of the 9 patients studied suggested that the antibody response in human beings may perhaps begin very early during the course of infection with poliomyelitis virus.

Time of Appearance or Increase in Titer of Antibody against Patient's Own Virus and against Lansing Virus.—The data on 4 patients, 2 with and 2 without

TABLE V

Time of Appearance and Increase in Titer of Antibodies for Patient's Strain of Virus

Group	Patient	Time after onset of 1st symptom	Neutralization index of undiluted serum	50 per cent protective dilution of serum *vs.* indicated No. of PD_{50} of virus	
				Serum dilution	PD_{50} of virus
		days			
Antibody tests negative in acute phase	Wal	2	16		
		14	1000		
		28	500		
		92	1600		
	Hof	3	10−		
		34	6		
		91	63		
		250	40		
Antibody present in acute phase	Obe	1	400+	Undiluted?	
		14		1:6	80
		94	800+	1:32	
	Ten J	2	2500+	1:1	
		14		1:8	125
		91	4000+	1:26	

antibody for their own virus early after onset, are summarized in Table V (details in Appendix, Tables VIII, IX, X, and XI). In one (Wal) of the 2 non-paralytic patients with little or no antibody soon after onset there was a marked increase at 14 days, while the other (Hof) who showed no antibody at 31 days had a rather poor response at 91 and 250 days. In the case of patient "Hof," one cannot be certain whether one is dealing with an individual who produces antibody poorly or whether the strain of virus which was recovered from the alimentary tract was the one actually responsible for the infection under investigation. In the other 2 patients it appeared that the antibody they had soon after onset was increasing progressively during the course of the infection but had not yet reached its peak at 14 days, although clinical improvement had begun at least 1 week before.

TABLE VI

Time of Appearance of "Lansing" Antibody in Patients Who Developed It during Convalescence

Type of illness	Patient	Time after onset 1st specimen obtained	Neutralization indexes of undiluted serum obtained at indicated intervals after onset			
			1st specimen	2 wks.	1 mo.	3 mos.
		days				
Paralytic	Ric	5	10−; 5−*	20−	16−	140+; 160+
	Ten J	2	50−; 25−; 25	20	10	63+; 80+
Non-paralytic	Ten S	4	20−; 13−	20−	8−	125+
	Vau	5	16−; 6−	13−	6−	50+; 100+
	Mac	3	20−; 6−	4−	125+	40+; 63+
	Yea	0.3	50−; 8−; 6	4	20	40+; 32

* See Table I for legends.

TABLE VII

Immunologic Relationship of Viruses Recovered from Patients Who Developed "Lansing" Antibody during Convalescence

Strain of virus	Serum used	Incidence of poliomyelitis at indicated final dilutions of virus					
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Ten J (Tests in monkeys)	None—saline	—	9/9	8/9	3/8	4/9	0/3
	"M.V." antiserum (*rhesus*)	3/3	3/3	—	—	—	—
	"Ten J" patient's convalescent serum	0/3	0/6	0/3	0/3	—	—
Ric (Tests in monkeys)	None—saline	—	9/9	7/9	3/8	0/6	—
	"M.V." antiserum	3/3	3/3	—	—	—	—
	"Ric" patient's convalescent serum	0/3	0/5	0/3	0/3	—	—
Lansing (Tests in mice)	None—saline	—	36/39	24/40	4/40	—	—
	"M.V." antiserum	2/8	0/8	—	—	—	—
	"Ric" patient's convalescent serum	0/8	1/8	—	—	—	—
	"Ten J" patient's convalescent serum	3/8	0/8	1/8	—	—	—
	"Ten J" *rhesus* convalescent sera	14/16	14/16	—	—	—	—
	"Vau" *rhesus* convalescent serum	7/8	5/8	—	—	—	—

On the other hand, among the 6 patients, whose 3 month convalescent sera neutralized the Lansing virus, while those obtained soon after onset did not, none of the 14 day and only one of the 28 day convalescent sera had significant

amounts of antibody (Table VI). In 2 of these patients ("Ten J" and "Ric") the antibody response was so different for the homologous virus, that it seemed doubtful that the virus recovered from their own alimentary tract could be immunologically identical with the Lansing strain. Tests shown in Table VII indicate that an "M.V." monkey antiserum which fully neutralized the Lansing virus failed to neutralize the viruses derived from patients "Ten J" and "Ric." Furthermore, the convalescent sera of 2 monkeys paralyzed with the "Ten J" virus and of one monkey paralyzed with "Vau" virus obtained 3 months after inoculation also failed to neutralize the Lansing virus. It would appear, therefore, that in most if not all the patients who developed the Lansing antibody during convalescence, both the time of appearance of the antibody and the nature of the recovered viruses indicate that the illness under investigation was not caused by a virus of the Lansing type. It is, of course, possible that these patients may have had a subsequent inapparent or clinically unrecognized infection with a Lansing type virus. On the other hand, it may also be that some strains of poliomyelitis virus may be only partially related to the Lansing type so that at the peak of the homologous antibody response which seems to occur some weeks after onset of the illness, the serum may also acquire the capacity to neutralize the Lansing virus.

DISCUSSION

The data presented in this communication permit a better understanding of the immune response of human beings to infection with poliomyelitis virus. It can be concluded first of all, that when tests are performed with virus derived from the patient's own alimentary tract, it is possible in each instance to demonstrate that antibody, specific for this strain, either develops or increases in titer *early* during the course of infection. The totally unexpected finding that, as early as 12 to 24 hours after appearance of the first symptom of illness, the undiluted serum of 7 of the 9 patients was capable of neutralizing the maximum amounts of the patient's own virus presented special problems. Thus it became clear that it was not possible to evaluate the immune response of human beings to poliomyelitis virus merely on the basis of presence, absence, or slight variations in amounts of antibody demonstrable in undiluted serum, but that it would be necessary to investigate it quantitatively by determining the maximal dilution of serum capable of neutralizing a specified number of infective doses of virus. At the conclusion of the present investigation Hammon and Roberts (22) reported similar results in tests on the acute phase sera of 7 paralytic patients, all of which neutralized an undetermined number of infective doses of the patient's own virus. These investigators also demonstrated an increase of antibody titer in sera of 3 patients obtained 45 to 80 days after onset. The question requiring elucidation was whether the antibody present soon after onset was specific and the result of a rapid response of the patient

to the strain of virus responsible for the illness under investigation, or whether it was present as a result of a previous infection with a partially related or even the same strain of poliomyelitis virus. In the present investigation, tests for Lansing antibody on the same sera showed no correlation with the results obtained in the tests against the patient's own virus. Furthermore, the antibody for the homologous strains of virus was present in the lowest titer during the acute phase and increased progressively during convalescence, suggesting that what was found soon after onset might represent the beginning of the antibody response. However, since no sera were available on these patients prior to the onset of the present illness, the evidence cannot be conclusive with regard to the prior origin of the antibody found early after onset.

The pattern of beginning antibody development during the acute phase of the illness or within 14 days after onset, which this study suggests for human poliomyelitis, is different from the results obtained in monkeys infected by the intracerebral or intranasal routes but remarkably similar to those in monkeys infected by the oral route. Sabin and Olitsky (23) found that the sera of convalescent paralyzed monkeys failed to neutralize as little as 20 infective doses of virus at 4 to 5 weeks after the attack and that the antibodies did not appear until 2 or 3 months after intracerebral or intranasal inoculation of "M.V." virus. However, von Magnus and Melnick (24) showed that after oral or pharyngeal administration of virus 13 of 17 monkeys, regardless of species, *developed* neutralizing antibodies in varying titer by the first day of paralysis. It is perhaps also noteworthy that the clinical course of the disease in our patients could not be correlated with the rate of development of homologous antibody, since improvement and arrest of paralysis occurred early after onset when the titer of antibody in the serum is low and some weeks before it apparently reaches its peak. It may appear paradoxical that the only 2 patients without significant homologous antibody shortly after onset turned out to have a mild non-paralytic illness, while the 7 patients in the present study as well as the 7 reported by Hammon and Roberts (22), possessing such antibody, were all paralytic. However, while it is not certain that future studies will yield similar results, these observations may perhaps suggest that the rate and quantity of antibody production as well as the paralytic or non-paralytic outcome of the infection may depend on the extent of viral multiplication.

One other observation, having a bearing on the significance of the appearance during convalescence of antibodies for the Lansing virus, is worthy of special comment. Although most investigations for Lansing antibody in acute and convalescent sera from patients with poliomyelitis have yielded inconclusive data, there have been some (12, 13) which might be interpreted as possibly indicating infection with a Lansing type virus. In the present study, among 24 patients without Lansing antibody during the acute phase, 6 or 25 per cent were found to have such antibody 3 months later. It was of interest therefore,

that unlike the early appearance of the antibody for the patient's own virus, that for the Lansing virus, with one exception, was absent at 1 month after onset in those who had it at 3 months. Furthermore, in at least 2 of these, it was possible to show that the virus recovered from the patient during the acute phase of the clinically recognizable illness was not antigenically of the Lansing type. Since the peak homologous antibody response apparently occurs more than 2 weeks after onset, it appeared possible that the late appearance of Lansing antibody could be the response to a common antigen. However, the possibility of a subsequent inapparent or clinically unrecognized infection with a Lansing type virus must also be considered.

SUMMARY

Of 20 strains of virus recovered from 40 patients with poliomyelitis only 9 possessed a titer of 10^{-3} or more, permitting significant quantitative neutralization tests in monkeys. Seven of the 9 high titer strains were derived from patients whose illness was ultimately paralytic, and tests with their undiluted sera indicated that the acute phase as well as the 3 month convalescent specimens neutralized maximum amounts of the patient's own virus. However when varying dilutions of the sera were tested against a single dose of virus, it was found that the antibody was present in lowest concentration early after onset and progressively increased in titer over a period of weeks during convalescence. The 2 remaining high titer strains were recovered from patients with a nonparalytic illness, and in both of these the acute phase sera were without significant amounts of antibody for their own virus. Antibody was demonstrable at 14, 28, and 92 days after onset in one of these patients, while the other had none at 1 month and only a minimal amount at 3 and 8 months.

Tests with the Lansing virus on the same sera, clearly established the specificity of the antibody response to the strain of virus recovered from each patient under investigation. Five of the 9 patients, whose sera were studied with both viruses, had no antibody for the Lansing virus during the acute phase and none 3 months later. Two had antibody during the acute phase but serum dilution tests showed no increase in titer in the 3 month convalescent specimen. In 2 others, who were without antibody for the Lansing virus during the acute phase but had it at 3 months after onset, it was possible to show that this antibody appeared later than 1 month after the illness and that the virus recovered from these patients during their illness was not antigenically of the Lansing type.

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APPENDIX

Protocols of Neutralization Tests in Monkeys with Each of the 9 High Titer Strains of Virus

TABLE VIII

Patient "Wal:" Non-paralytic: 8–25–47—onset with "fever," fatigue, nuchal "soreness;" 8–26—103.6°F., nuchal-spinal rigidity, cerebrospinal fluid (CSF)—27 lymphocytes, no paralysis. Fever and clinical signs abated in succeeding few days.

Virus used in all tests: Generation II, pool of spinal cord and medulla of 2 *rhesus* monkeys —cumulative $PD_{50} = 10^{-4.2}$.

Type of test and date	Serum or saline	Time after onset	Poliomyelitis in monkeys at indicated final dilutions of virus					PD_{50} 10^{-}	Neutralization index based on	
			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}		Titer of virus in same test	Cumulative virus titer
		days								
Initial virus titration 4–29–48	Saline	—	—	8,10,12	8,8,④	11,13,0	0,0,0	4.2	—	—
Undiluted sera	Saline	—	—	—	8,8,0	5,10,0	0,0,0	4.0	—	—
vs. varying	Serum	2	7,8,10	7,12,12	8,0,0	7,0,0	—	3.0	10	16
doses of virus 6–3–48	Serum	92	7,0,0	7,0,0	0,0,0	0,0,0	—	1.0	1,000	1,600
Undiluted sera	Saline	—	—	—	6,8,10	8,8,0	9,0,0	4.5+?	—	—
vs. varying	Serum	14	8,9,0	0,0,0	0,0,③	0,0,⑰	—	1.2	2,000+?	1,000
doses of virus 8–29–48	Serum	28	8,8,24	0,0,0	0,0,0	0,0,⑰	—	1.5	1,000+?	500

Legends for Tables VIII to XVI:

Figures refer to day after inoculation paralysis first noted in individual monkeys.

0 = neither clinical nor histological evidence of poliomyelitis.

Encircled figure, ④, indicates day of death of monkey exhibiting neither clinical nor histological evidence of poliomyelitis.

NP = non-paralytic poliomyelitis based on histological changes in sacrificed monkey.

TABLE IX

Patient "Hof:" Non-paralytic: 9–2–47—onset with headache, red throat, 105°F., questionable nuchal rigidity, CSF—no cells; 9–4—irritable, muscular pains, nuchal rigidity, CSF—474 leukocytes (90 per cent lymphocytes); 9–5—first specimens collected, fever, nuchal rigidty, pain subsiding. Complete recovery without paralysis in succeeding few days.

Virus used in all tests: Generation II, pool of spinal cord and medulla of 2 *rhesus* monkeys—cumulative $PD_{50} = 10^{-4.3}$.

Type of test and date	Serum or saline	Time after onset	Poliomyelitis in monkeys at indicated final dilutions of virus						PD_{50} 10^{-}	Neutralization index based on	
			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}		Titer of virus in same test	Cumulative virus titer
		days									
Initial virus titration 2–27–48	Saline	—	4,5	6,9,11	6,7,9	7,7,9	6,0,0	—	4.7+?	—	—
Undiluted sera *vs.* varying doses of virus 3–15–48	Saline	—	—	—	—	8,0,0	0,0,0	0,0,0	3.7	—	—
	Serum	3	8,8,0	6,8,10	7,8,0	20,0,0	—	—	3.3+?	3—	10—?
	Serum	91	8,8,9	12,13,0	9,0,0	0,0,0	—	—	2.5	16	63
Undiluted sera *vs.* varying doses of virus 5–20–48	Saline	—	—	—	6,6,7	9,11,0	—	—	4.2+?	—	—
	Serum	34	6,7,7	6,7,13	6,12,12	0,0,0	—	—	3.5	5+?	6
	Serum	250	7,8,8	6,10,NP	20,0,0	0,0,0	—	—	2.7	32+?	40

TABLE X

Patient "Obe." Paralytic: 8-27-47—onset with nausea, headache, "fever;" 8-28—first specimen collected, nuchal-spinal rigidity, CSF—27 leukocytes (63 per cent polymorphs); 8-30—102°F., complete inability to swallow. Ultimately recovered completely.

Virus used in all tests: Generation II, spinal cord and medulla of one *rhesus* monkey—cumulative $PD_{50} = 10^{-3.6}$.

Type of test and date	Serum or saline	Time after onset	Poliomyelitis in monkeys at indicated final dilutions of virus						PD_{50} 10^{x}	Neutralization index based on	
			$10^{-1.0}$	$10^{-1.7}$	10^{-2}	10^{-3}	10^{-4}	10^{-5}		Titer of virus in same test	Cumulative virus titer
		days									
Initial virus titration 4-19-48	Saline	....	....	....	9,14,③	10,NP,⑳	0,0,0	0,0,⑭	3.2	....	....
Undiluted sera *vs.* varying doses of virus 5-18-48	Saline	....	....	....	8,8	9,11,12	10,0,0	....	3.7+?	....	....
	Serum	1	NP,0,0	....	13,0,⑧	....	....	....	1.0−?	500+	400+
	Serum	94	10,0,0	....	0,0,0	....	....	....	0.7−?	1,000+	800+
Various dilutions of sera *vs.* single dose of virus* 10-14-48	Saline	....	....	6,8,10,⑦	10,13,0	6,8,17	11,18,0	....	4.0+?	50 per cent protective serum dilution	
	Serum	1 Undiluted		9,10,17,0						Undiluted?	
		1 1:4		8,8,8,0							
		1 1:16		8,10,10,14							
		1 1:64		8,8,10,10							
	Serum	14 Undiluted		0,0,0,0						1:6	
		14 1:4		17,0,0,0							
		14 1:16		8,10,16,17							
		14 1:64		7,7,10,10							
	Serum	94 Undiluted		0,0,0,0						1:32	
		94 1:4		0,0,0,0							
		94 1:16		11,0,0,0							
		94 1:64		8,9,12,0							

* Dose of virus = 200 PD_{50} on basis of simultaneous titration and 80 PD_{50} on basis of cumulative titer.

TABLE XI

Patient "Ten J:" Paralytic: 8-22-47—onset headache; 8-23—lethargy, anorexia; 8-24—first specimens collected, headache, vomiting, nuchal spinal rigidity, questionable dysphagia, CSF—200 leukocytes (45 per cent polymorphs); 8-25—102.4°, complete inability to swallow, paralysis upper intercostal muscles. Gradual ultimate recovery.

Virus used in all tests: Generation II, pool of spinal cord and medulla of 2 *rhesus* monkeys—cumulative $PD_{50} = 10^{-4.1}$

Type of test and date	Serum or saline	Time after onset	Poliomyelitis in monkeys at indicated final dilutions of virus						PD_{50} 10^{-}	Neutralization index based on	
			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}		Titer of virus in same test	Cumulative virus titer
		days									
Initial virus titration 5-27-48	Saline	....	....	8,17,17	8,9,9	0,0,0	9,10,0	....	3.8	....	....
Undiluted sera *vs.* varying doses of virus 8-15-48	Saline	....	....	9,NP	8,9,9	7,0,0	0,0,0	0,0,0	3.7	....	....
	Serum	2	10,0,0	0,0,0	0,0,0	0,0,0	....	....	0.7−?	1,000+?	2,500+
	Serum	91	0,0,0	0,0,0	0,0,0	0,0,0	....	....	0.5−?	1,600+?	4,000+
	Saline	....	....	6,9,10,17	7,13,0	9,NP,②	9,12,0	....	5.0+?	50 per cent protective serum dilution	
Various dilutions of sera *vs.* single dose of virus* 11-2-48	Serum	2 Undiluted		9,9,0,0						1:1	
		2 1:4		10,12,17,0							
		2 1:16		9,9,9,⑦							
		2 1:64		9,9,10,12							
	Serum	14 Undiluted		6,7,0,⑲						1:8	
		14 1:4		9,9,0,0							
		14 1:16		0,0,0,⑥							
		14 1:64		7,7,10,⑰							
	Serum	91 Undiluted		0,0,0,⑮						1:26	
		91 1:4		17,43,0,0							
		91 1:16		11,0,0,0							
		91 1:64		13,13,0,0							

* Dose of virus = 1000 PD_{50} on basis of simultaneous titration and 125 PD_{50} on basis of cumulative titer.

TABLE XII

Patient "Ris:" Paralytic: 8-13-47—onset with irritability and drowsiness; 8-14—101.8°F., chills, anorexia, vomiting, questionable nuchal rigidity; 8-15—questionable weakness both legs; 8-16—first specimens collected; flaccid paralysis both lower extremities; CSF—107 leukocytes (97 per cent lymphocytes). No further progression of paralysis.

Virus used in all tests: Generation II, pool of spinal cord and medulla of 2 *rhesus* monkeys—cumulative $PD_{50} = 10^{-3.8}$.

Type of test and date	Serum or saline	Time after onset	Poliomyelitis in monkeys at indicated final dilutions of virus						PD_{50} 10^{-}	Neutralization index based on	
			$10^{-1.0}$	$10^{-1.7}$	10^{-2}	10^{-3}	10^{-4}	10^{-5}		Titer of virus in same test	Cumulative virus titer
		days									
Initial virus titration 4-29-48	Saline	....	....	....	6,7,7	8,11,0	0,0,0	0,0,0	3.2	....	....
Undiluted sera *vs.* varying doses of virus 6-17-48	Saline	....	....	....	8,0	7,7,8	10,10,0	....	4.0+?	....	....
	Serum	3	8,12,0	....	14,0,0	0,0,0	0,0,0	....	1.5	320+?	200
	Serum	89	6,⑤,③	....	15,⑰,0	12,0,0	0,0,0	....	2.0−?	100+?	63+?
	Saline	....	....	5,7,8	5,7,0	6,8,9	6,7,7	....	4.5+?	50 per cent protective serum dilution	
Various dilutions of sera *vs.* single dose of virus* 9-27-48	Serum	3 Undiluted		8,0,0,0						1:3	
		3 1:4		9,10,21,0							
		3 1:16		8,11,13,14							
		3 1:64		6,14,14,0							
	Serum	89 Undiluted		0,0,0,0						1:13	
		89 1:4		0,0,0,0							
		89 1:16		8,12,16,0							
		89 1:64		6,8,11,0							

* Dose of virus = 630 PD_{50} on basis of simultaneous titration and 125 PD_{50} on basis of cumulative titer.

TABLE XIII

Patient "Fin:" Paralytic: 8-27-47—onset with headache, 12 hours later nuchal-spinal rigidity, CSF—1070 leukocytes (93 per cent polymorphs); first specimens obtained; 8-28—paralysis left leg; no progression until 8-31—paralysis right leg, bowel, bladder, both arms, intercostal muscles; prolonged convalescence.

Virus used in all tests: Generation III, pool of spinal cord and medulla of 2 *rhesus* monkeys—cumulative $PD_{50} = 10^{-2.9}$.

Type of test and date	Serum or saline	Time after onset	Poliomyelitis in monkeys at indicated final dilutions of virus						PD_{50} 10^{-}	Neutralization index based on	
			$10^{-1.0}$	$10^{-1.3}$	$10^{-2.0}$	10^{-3}	10^{-4}	10^{-5}		Titer of virus in same test	Cumulative virus titer
		days									
Initial virus titration 3-16-48	Saline	....	....	....	13,13	0,0,0	9,0,0	0,0,0	2.7	....	....
Undiluted sera *vs.* varying doses of virus 4-8-48	Saline	....	....	....	9,9,NP	10,0,0	7,0,0	....	3.0	....	....
	Serum	0.5	8,0,⑥	....	0,0,0	0,0,0	0,0,0	....	1.0−?	100+	80+
	Serum	93	0,0,0	....	0,0,0	0,0,0	0,0,0	....	0.5−?	320+	250+
	Saline	....	....	9,12,18,19	10,14,16	10,0,0	7,24,0	....	3.5+?	50 per cent protective serum dilution	
Various dilutions of sera *vs.* single dose of virus* 6-13-48	Serum	0.5 Undiluted		0,0,0,0						1:6	
		0.5 1:4		7,7,11,0							
		0.5 1:16		12,15,18,0							
		0.5 1:64		14,14,0,0							
	Serum	93 Undiluted		0,0,0,⑪						1:97+?	
		93 1:4		0,0,0,0							
		93 1:16		0,0,0,0							
		93 1:64		11,0,0,0							

* Dose of virus = 160 PD_{50} on basis of simultaneous titration and 40 PD_{50} on basis of cumulative titer.

TABLE XIV

Patient "Hopk:" Paralytic: 8-24-47—onset with vomiting, "fever;" 8-25—same plus drowsy, irritable; 8-26—first specimens collected, gait staggering, weakness abdominal, gluteal, extensor muscles right foot, nuchal-spinal rigidity, CSF—58 leukocytes (78 per cent lymphocytes) Gradual recovery without residual paralysis.

Virus used in all tests: Generation II, pool of spinal cord and medulla of 2 *rhesus* monkeys—cumulative $PD_{50} = 10^{-4.0}$.

Type of test and date	Serum or saline	Time after onset	Poliomyelitis in monkeys at indicated final dilutions of virus						PD_{50} 10^{-}	Neutralization index based on	
			10^{-1}	$10^{-2.0}$	$10^{-2.5}$	$10^{-3.0}$	10^{-4}	10^{-5}		Titer of virus in same test	Cumulative virus titer
		days									
Initial virus titration 3-16-48	Saline	....	....	7,8,12	....	7,8,0	8,9,0	0,0,0	4.0	....	....
Undiluted sera *vs.* varying doses of virus 4-6-48	Saline	....	....	6,6,11	....	9,11,0	6,8,12	0,0,0	4.3	....	....
	Serum	2	20,0,0	0,0,0	....	0,0,②	0,0,0	....	0.7−?	4,000+	2,000+
	Serum	93	0,0,0	0,0,0	....	0,0,0	0,0,0	....	0.5−?	6,300+	3,200+
	Saline	....	....	....	6,7,8,10	7,8,0	0,0,0	7,9,0	3.6+?	50 per cent protective serum dilution	
Various dilutions of sera *vs.* single dose of virus* 7-6-48	Serum	2 Undiluted			0,0,0,0					1:16	
		2 1:4			12,NP,0,⑲						
		2 1:16			12,0,0,0						
		2 1:64			6,7,9,12						
	Serum	93 Undiluted			0,0,0,⑤					1:107+?	
		93 1:4			0,0,0,0						
		93 1:16			12,0,0,0						
		93 1:64			0,0,0,⑰						

* Dose of virus = 13 PD_{50} on basis of simultaneous titration and 32 PD_{50} on basis of cumulative titer.

TABLE XV

Patient "Ric:" Paralytic: 8-9-47—onset with "fever" and "runny nose;" 8-12—generalized convulsion; 8-14—onset of paralysis-left facial, right leg, back muscles; CSF—39 leukocytes, first specimens collected; 8-15—extension of paralysis to left leg. Gradual recovery.

Virus used in all tests: Generation II, pool of spinal cord and medulla of 2 *rhesus* monkeys—cumulative $PD_{50} = 10^{-3.6}$.

Type of test and date	Serum or saline	Time after onset	Poliomyelitis in monkeys at indicated final dilutions of virus					PD_{50} 10^{-}	Neutralization index based on	
			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}		Titer of virus in same test	Cumulative virus titer
		days								
Initial virus titration 5-14-48	Saline	....	....	6,7,8	8,0,0	6,0,0	0,0,0	3.0	....	....
Undiluted sera *vs.* varying doses of virus 6-28-48	Saline	....	....	7,14	5,8,10	8,29,0	....	4.2+?	....	....
	Serum	5	25,0,0	0,0,0	0,0,0	0,0,0	....	0.7−?	3,200+	800+
	Serum	91	0,0,0	0,⑩,⑤	0,0,0	0,0,0	....	0.5−?	5,000+	1,250+
	Saline	....	....	6,9,9,10	9,10,11	0,0,⑥	0,0,0	3.5	50 per cent protective serum dilution	
Various dilutions of sera *vs.* single dose of virus* 9-20-48	Serum	5 Undiluted		0,0,0,0					1:16	
		5 1:4		10,NP,0,0						
		5 1:16		8,10,0,0						
		5 1:64		9,12,0,0						
	Serum	91 Undiluted		0,0,0,0					1:107+?	
		91 1:4		16,0,0,0						
		91 1:16		0,0,0,0						
		91 1:64		0,0,0,0						

* Dose of virus = 32 PD_{50} on basis of simultaneous titration and 40 PD_{50} on basis of cumulative titer.

TABLE XVI

Patient "Fro:" Paralytic: 8-9-47—onset with "fever," irritability, continued until 8-12—left facial paralysis, nuchal-spinal rigidity, 102° F., CSF—56 leukocytes (60 per cent lymphocytes); first specimens collected. No further paralysis; gradual recovery.

Virus used in all tests: Generation II, pool of spinal cord and medulla of 2 *rhesus* monkeys—cumulative $PD_{50} = 10^{-3.3}$.

Type of test and date	Serum or saline	Time after onset	Poliomyelitis in monkeys at indicated final dilutions of virus					PD_{50} 10^{-}	Neutralization index based on	
			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}		Titer of virus in same test	Cumulative virus titer
		days								
Initial virus titration 3-16-48	Saline	....	....	8,8,10	9,23,0	0,0,⑤	0,⑧,⑲	3.3	....	....
Undiluted sera *vs.* varying doses of virus 4-12-48	Saline	....	....	7,10,NP	0,0	6,0,0	....	2.7	....	....
	Serum	3	0,0,⑤	0,0,0	0,0,0	....	....	0.5−?	160+	630+
	Serum	90	20,0,0	0,0,0	0,0,0	....	....	0.7−?	100+	400+
	Saline	....	6,6,7,11	5,6,7	5,7,7	....	....	3.5+?	50 per cent protective serum dilution	
Various dilutions of sera *vs.* single dose of virus* 9-8-48	Serum	3 Undiluted	7,8,8,10						0?	
		3 1:4	6,7,7,10							
		3 1:16	7,7,7,11							
		3 1:64	6,7,7,8							
	Serum	90 Undiluted	10,11,0,0						1:3	
		90 1:4	7,11,11,0							
		90 1:16	7,7,13,0							
		90 1:64	7,20,21,0							

* Dose of virus = 320 PD_{50} on basis of simultaneous titration and 200 PD_{50} on basis of cumulative titer.