SURVIVAL OF THE VIRUS OF POLIOMYELITIS IN THE ORAL AND NASAL SECRETION OF CONVALESCENTS*

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Certain epidemiologic concepts of poliomyelitis still rest on very sparse data. The detection of the virus in the oral and nasal secretions and tissues of living human beings is necessary to substantiate some of these concepts. In that category belong the detection of a carrier in the normal healthy population to account for the widespread immunity to poliomyelitis (1) and the detection of the virus in the nasal secretions of the so called mild characteristic illness of Paul and Trask (2), occurring in close proximity to frank cases of the disease. The data presented below attempt to answer a similar, though perhaps a somewhat more practical epidemiologic question, namely, how long does the virus of poliomyelitis persist in the nasal and oral secretions after the onset of the disease?

The number of times the virus has been isolated from the oral and nasal cavities of human beings, is very small. In a recent publication Paul, Trask and Webster (3) review the literature and estimate that the virus has been isolated 13 times from the nasal and oral secretions and tonsils of living human beings. Of these, 8 positive results were obtained from patients during or prior to the acute stage of the disease; only 2 from convalescents, one 17 days (4) and a second 4 months (5) after the onset of illness; 2 (6, 7) from healthy contacts, and one (1) from the tonsils and adenoids of a child without any history of contact with a case.

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In this paper we wish to record 2 additional instances of successful isolation of the virus of poliomyelitis from 2 recovered patients 16 and 13 days respectively after the onset of the illness, and to describe a simple method for the bacterial sterilization and the concentration of nasal washings.

A number of methods have been employed for obtaining and treating oral and nasal secretion preparatory to inoculation into animals. A major problem in the preparation of the inoculum has been the sterilization of the heavily infected material. This has been accomplished by the different authors in a variety of ways; by filtration through a Berkefeld candle; by treatment with ether; by phenolization or by glycerolation.

Since the amount of virus in the secretion is probably small, attempts have been made to concentrate the material either by vacuum distillation at 35–38°C. or by ultrafiltration. In most instances the concentration has reduced the volume from a quarter to one-tenth of the original quantity. The volume and type of fluid employed has varied. Kling and Pettersson (7) employed large quantities of sterile water (1 to 2 liters), reducing this large volume by vacuum distillation to from 100 to 200 cc. Taylor and Amoss (8), Paul and Trask (9) and Flexner, Clarke and Fraser (10) used quantities varying from 30 to 150 cc. Amoss and Taylor (11) carried their concentration to a final volume of 2 cc. Positive takes have been reported by the use of all these methods, though the takes have been few in number. When filtration was not employed, brain abscesses have resulted from time to time.

A somewhat modified, and we believe simplified, technique described below yielded sterile material concentrated to sufficiently small volume to permit intracerebral inoculation. Efforts were made to obtain a maximum amount of virus by the use of moderately large quantities of sterile water and the procedures followed for sterilization and concentration were those considered least likely to be injurious to the virus.

EXPERIMENTAL

Nasal washings were performed on patients[1] during the acute stages of the illness and at varying intervals after the acute symptoms had subsided. 50 to 75

[1] The patients were obtained from the poliomyelitis wards of the Kingston Avenue Hospital in Brooklyn, New York.

cc. of sterile distilled water was introduced by means of a soft rubber catheter passed into one nostril, and the nostril gently compressed against the catheter to prevent back flow and leakage. The water was slowly forced through the catheter with a 50 cc. syringe. With the patient lying prone, and the head held somewhat to one side, little difficulty was encountered in obtaining the return of most of the water introduced. The flow from the opposite nostril and the mouth of the patient was caught in a sterile pus basin.

The amount of the washing was measured, 10 per cent of anesthetic ether added, well shaken for 5 to 10 minutes and left overnight in the ice chest. The following day the washing was placed in a specially prepared sterile glass container, as shown in Text-fig. 1, and attached by glass seal to a vacuum system. The containers were then dipped in an acetone-CO_2 mixture until frozen solid, and desiccation accomplished by means of a hi-vac pump in series with a mercury pump.

Throughout the period of desiccation, the washings were kept frozen. While no accurate check was made of the exact temperature of the frozen material, the vacuum reading on a McCleod gauge varied from 0.03 to 0.001 mm. of pressure after all the ether had been volatilized. From 4 to 6 hours were required to re-

TEXT-FIG. 1

duce the volume of the washings to 1 cc. or less. The container was then sealed off under vacuum. The concentrated material was removed by breaking one of the sealed ends and poured off aseptically into a test tube. The mucoid material adhering to the sides of the container was emulsified with a little sterile distilled water by rubbing a sterile pipette gently along the sides of the container. The total amount, usually 2 to 4 cc., was then inoculated intracerebrally into a normal animal, a small portion being retained for culture. The cultures (aerobic) were sterile on blood plates and in broth and there was no instance of brain abscess.

A series of controls were included. These consisted of from 0.05 to 0.2 cc. of 5 per cent suspension of stock virus, diluted to 50 cc. with sterile water and treated with ether and desiccated in the same manner as the washings. Our technique was considered adequate when these controls yielded positive takes in test animals in at least 3 consecutive desiccations (Table I).

20 nasal washings including 2 from physicians in charge of the patients and 3 second or repeat washings of children were studied

TABLE I

Results of Intracerebral Inoculation of Concentrates in Rhesus Monkeys

Case	Patient	Age	Date of onset	Date of nasal washing	Time from onset to washing	Date of inoculation	Amount of inoculum	Monkey No.	Outcome			
									Fever*	Symptoms	Lesions	Neutralization test
		yrs.	*1935*	*1935*	*days*	*1935*	*cc.*					
1	G. B.	5	Sept. 27	Sept. 30	3	Oct. 2	2	J2	—	—	—	
2	S. D.	6	Oct. 5	Oct. 14	9	Oct. 16	3.6	J2-3	—	—	—	
3	A. S.	10	Oct. 3	Oct. 14	11	Oct. 16	3.4	J2-5	+22	Generalized weakness. Refused to climb	Not sacrificed	Failed to neutralize
4	F. G.	9½	Oct. 2	Oct. 15	13	Oct. 17	3.9	J2-7	—	—	—	
5	H. LaG.	8	Sept. 30	Oct. 15	16	Oct. 17	3.5	J2-8	+8	Monkey's right arm paralyzed. Animal sacrificed	Typical experimental poliomyelitis	
6	R. McK.	13	Sept. 14	Oct. 22	36	Oct. 24	2	J6-6	+28	Left upper limb weak. Animal sacrificed	Suggestive but not typical of experimental poliomyelitis	
7	J. S.	13	Oct. 16	Oct. 22	6	Oct. 24	2	J6-7	—	—	—	
8	M. B.	8	Oct. 8	Oct. 24	16	Oct. 26	1.6	J6-9	—	—	—	
9	R. B.	7	Oct. 4	Oct. 24	20	Oct. 26	1.6	J7-0	—	—	—	
10	J. H.	13	Sept. 22	Oct. 29	37	Oct. 31	1.8	J7-3	—	—	—	
11	D. A.	5	Oct. 26	Oct. 29	3	Oct. 31	1.5	J7-5	—	—	—	
12	R. C.	14	Oct. 16	Oct. 29	13	Oct. 31	3	J7-6	+21	Weakness of right arm. Animal sacrificed	Hemorrhage, infiltration, satellitosis, neuronophagia. Disappearance of ganglia	
13	R. F.	4	Oct. 11	Oct. 30	19	Nov. 1	1.5	J9-8	—	—	—	

14	G. B.	5	Oct. 8	Oct. 30	22	Nov. 1	2	J9-9	+10	All limbs weak. Monkey sacrificed	Suggestive but not typical of experimental disease	
15	I. F.	6	Sept. 22	Nov. 4	43	Nov. 6	1.2	J1-03	+5	Monkey appeared sick. Hind limbs weak. Tremors, but no definite paralysis	—	Neutralized stock virus on 2 occasions
16	M. B.	8	Repeat	Nov. 19	22	Nov. 21	1.5 cc. intracer., 2.5 cc. intraper.	J1-22	—	—	—	
17	G. B.	5	Repeat	Nov. 19	42	Nov. 21	1.5 cc. intracer., 2.5 cc. intraper.	J1-23	—	—	—	
18	H. LaG.	8	Repeat	Oct. 30	31	Nov. 1	1.4	J9-7	+10	General weakness. Disinclination to climb	Not sacrificed	Failed to neutralize
19	K.**			Nov. 4		Nov. 6	2	J1-02	+9	Refused to walk or climb. General weakness	—	Failed to neutralize
20	R.**			Nov. 4		Nov. 6	2.3	J1-01	+5	Generalized weakness	—	Failed to neutralize

Controls

Monkey No.	Date of inoculation	Amount of inoculum	Outcome
	1935		
J3	Oct. 2	2 cc. concentrate containing 0.05 cc. 5% suspension of virus	Poliomyelitis, Oct. 9, 1935
J2-4	Oct. 16	3.8 cc. concentrate containing 0.2 cc. 5% suspension of virus	Poliomyelitis, Oct. 24, 1935
J2-6	Oct. 17	1.8 cc. concentrate containing 0.2 cc. 5% suspension of virus	Poliomyelitis, Oct. 25, 1935
J6-5	Oct. 24	2 cc. concentrate containing 0.2 cc. 5% suspension of virus	Poliomyelitis, Nov. 15, 1935

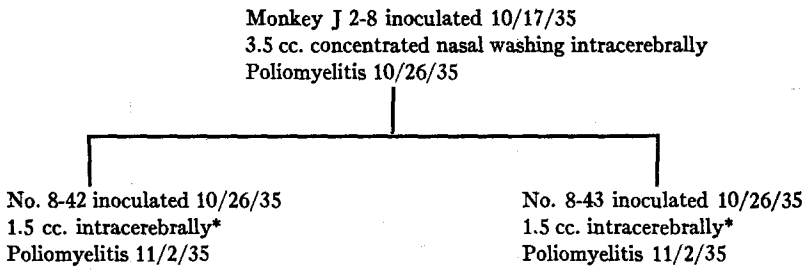
*+ Indicates fever. Numeral indicates day of fever. ** Adult contacts.

(Table I). 2 positive takes were obtained, and the virus subjected to passage (Text-figs. 2 and 3).

The 3½ cc. of concentrated nasal washing from which the first strain was isolated, was obtained from a child, H. La G., 16 days after the onset of illness.

The concentrate was inoculated intracerebrally into monkey J2-8 on Oct. 17, 1935. On Oct. 26 the right upper extremity was paralyzed. The animal was sacrificed on that day and 1½ cc. of a 10 per cent suspension of the cord was inoculated intracerebrally into 2 normal monkeys (8-42, 8-43). On Nov. 2 both these animals developed rapid progressive paralysis, were prostrated the same day and were sacrificed. The histologic sections of the cords of all 3 animals were typical of experimental poliomyelitis.

The hospital record of the child, H. La G., reveals that she had become ill on Sept. 30, 1935, with typical symptoms and physical findings and a spinal fluid cell count of 160. She made a good recovery without residual paralysis. A second



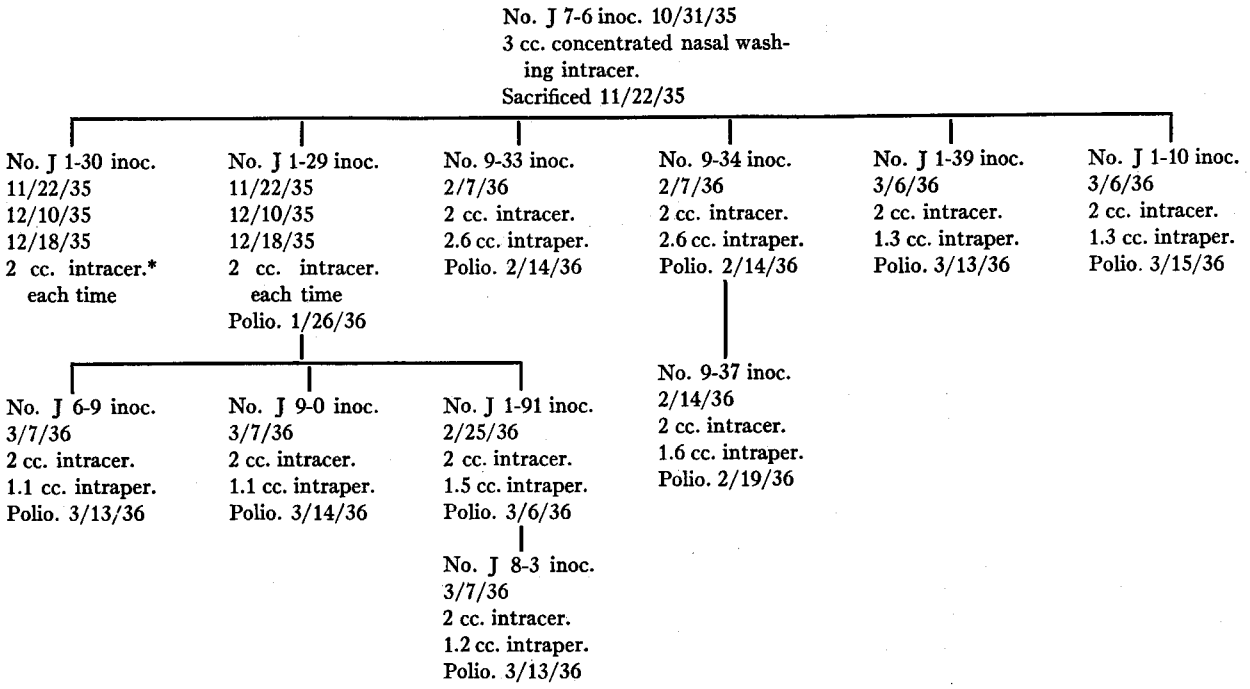
* A 10 per cent suspension of cord was used throughout.

TEXT-FIG. 2

nasal washing of this child taken 15 days later or 31 days from the onset of the illness yielded a negative result.

The second positive take was obtained from the nasal washings of a 14 year old boy, R. C.

This boy became ill Oct. 16, 1935, and was admitted to the hospital already paralyzed. Nasal washings were obtained on Oct. 29, or 13 days after the onset of illness, when the patient was convalescing. 3 cc. of the concentrated washing was inoculated intracerebrally into monkey J7-6 on Oct. 31, 1935. The animal had an elevation of temperature to 105°F. on Nov. 21, appeared ill and weakness of right arm was noted. The following day the temperature returned to 102.4°F. and the animal was sacrificed. Histologic study of the cord showed many hemor-



* A 10 per cent suspension of cord was used throughout.

TEXT-FIG. 3

rhages in the white and grey matter, moderate infiltration with lymphocytes, mononuclear and a few polymorphonuclear leucocytes. Many of the anterior horn ganglion cells showed degenerative changes, some satellitosis and neuronophagia.

Somewhat greater difficulties were encountered in subjecting this strain to further passage.

On the day the animal was sacrificed a 10 per cent suspension of cord was prepared and 2 cc. of the suspension was inoculated intracerebrally into 2 animals, (J1-29 and J1-30). Monkey J1-29 showed fluctuations in temperature between 102° and 105°, but not evidence of any palsy. Both animals were reinoculated with 2 cc. each of a 10 per cent suspension of the cord on Dec. 10, 1935, and again on Dec. 18. Monkey J1-30 remained unaffected by these inoculations, whereas monkey J1-29 continued to show fluctuations in temperature from time to time until Jan. 26, 1936, when the animal developed weakness of all four extremities and was sacrificed. A histologic study of the section of the cord showed extensive degenerative changes in the ganglion cells, and moderate inflammatory reaction. The findings were compatible with a diagnosis of poliomyelitis, but not typical of the experimental disease. Monkey J1-91 inoculated with a suspension of cord of this animal (J1-29) yielded typical poliomyelitis. A further passage from monkey J1-91 resulted in typical poliomyelitis in monkey J8-3.

2 additional animals (9-33 and 9-34) were again inoculated with a 10 per cent suspension of the original cord from monkey J7-6 on Feb. 7, 1936. 2 cc. was inoculated intracerebrally and 2.6 cc. was inoculated intraperitoneally into each of the animals. Both of these animals developed typical poliomyelitis. Injection of 2 cc. intracerebrally and 1.6 cc. intraperitoneally of a 10 per cent suspension of cord from monkey 9-34, yielded typical poliomyelitis in monkey 9-37. Histologic study of the 3 cords showed typical experimental poliomyelitis. Because of the unusual response of monkey J1-29, considerable effort was made in establishing this strain of virus in the monkey and Text-fig. 3 illustrates these efforts.

7 other animals, which received various inocula, presented suggestive temperature rises or generalized weakness (Table I).

These animals were healthy, vigorous animals at the time of the inoculation, had no cough or diarrhea. Within 1 to 3 weeks following inoculation, they developed elevations in temperature and appeared ill. They failed to evidence the normal activity of the healthy monkey, refused to run or jump and tired easily. Within 24 hours of the elevation in temperature 2 of these animals (J6-6, J9-9) were sacrificed and a 10 per cent suspension made from part of the cord and inoculated intracerebrally into 4 animals (2 for each suspected animal). The histologic pictures in both animals were suggestive of, and compatible with a

diagnosis of poliomyelitis, but were not typical of the experimental disease. We have thus far been unsuccessful in obtaining a positive take from either of these cords. The remaining 5 animals continued to show unusual fluctuations in temperature, but ultimately returned to normal temperature and vigor. These animals were bled from 1 to 2 months after the disappearance of the suggestive symptoms or temperature elevations, and neutralization tests were done. The serums of 4 of the 5 animals failed to neutralize small doses of the stock virus. The fifth animal, monkey J1-03, neutralized the virus on two separate occasions.

The nasal washings inoculated into this animal came from a 6 year old child, I.F., 43 days after the onset of illness. Since the virus was not actually isolated and subjected to passage, the presence of neutralizing substance in the serum of this monkey (J1-03) can be considered only as presumptive evidence of the presence of the poliomyelitis virus in the nasal secretion of this child. It is interesting to note that a second case appeared in this family. I.F., the child referred to above, became ill on Sept. 22, 1935, and within 3 days developed definite paralysis. A younger child (R.F., 4 years old) became ill with the disease on Oct. 11, 1935. Nasal washings taken from this second child yielded a negative result.

SUMMARY

The positive detection of the virus of poliomyelitis in the nasal secretions of 2 children, 16 and 13 days after the onset of the disease, is described. 7 animals which had been inoculated with other concentrates became ill with symptoms and temperature elevations suggestive of poliomyelitis, from 1 to 3 weeks following inoculation, but without definite paralysis. In 2 of these animals which were sacrificed, the histologic findings were compatible with the diagnosis of poliomyelitis but were not typical. Of the serums of the 5 remaining animals 4 failed to neutralize stock virus, whereas the serum of the fifth neutralized the virus on two different occasions. This serum was obtained from a monkey that had been inoculated with concentrated nasal secretions of a child 43 days after the onset of illness.

It is suggested that the present quarantine period of 3 weeks is compatible with the available data. It is further suggested that the methods of procedure described may be useful in similar investigations.

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