LOCALIZATIONS OF THE VIRUS OF POLIOMYELITIS IN THE CENTRAL NERVOUS SYSTEM DURING THE PREPARALYTIC PERIOD, AFTER INTRANASAL INSTILLATION*

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The view that the virus of poliomyelitis enters the body through the nasal mucous membrane, first suggested by Flexner (1) in 1910 and consistently advocated by him and his associates since that time, appears now to be widely accepted. It is based on an impressive body of experimental evidence (2) showing that the nasal mucosa is unique among the body surfaces in permitting the ingress of virus without preliminary trauma. The relationship of this phenomenon to certain anatomical arrangements in the nose has been almost overlooked. Flexner in 1912 (3) commented on the fact that "the small olfactory filaments are advantageously placed to act as the means of transportation" (of the virus). It was not, however, until the discovery that poliomyelitis virus is propagated through the nerve cells and their axons, rather than through the blood, lymph or cerebrospinal fluid, that the nervous anatomy of the region took on unique significance in relation to poliomyelitis. Faber (2) has called attention to the importance of the unbroken connection of the olfactory nerves between the very surface of the nasal mucosa and the central nervous system proper. Hopkins (4) has shown that the terminal processes (olfactory hairs) of the olfactory cells are exposed to the air in the upper part of the nasal cavity, and the afferent axons of these cells pass through the olfactory nerves directly into the olfactory bulbs. No similar arrangement exists elsewhere on the body and no more

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ideal condition can be imagined for the entrance of a neurotropic virus deposited on the surface.

Assuming that the nasal mucosa, or, more correctly, the olfactory cells and nerves, constitute the normal gateway through which poliomyelitis virus gains access to the central nervous system in man, the subsequent pathways by which it reaches the site of its maximum effects, the anterior horns of the spinal cord, still remain unknown. Only a few recorded attempts have been made to determine experimentally the various localizations of virus during the incubation period after intranasal inoculation, most of which have given negative results and apparently discouraged further study.[1] In 1912, Flexner and Clark (7) showed that 48 hours after swabbing the nasal mucosa of a monkey with virus, the olfactory bulb contained a demonstrable amount of virus, while none could be detected in the medulla or in the spinal cord. The earlier demonstration by Landsteiner and Levaditi (8) of virus in the olfactory bulbs 11 days after nasal inoculation (submucous injection) did not include the study of other possible points of infection in the central nervous system. In 1920, Flexner and Amoss (9) reported an experiment in which they left a cotton pledget soaked with virus in the nose of a monkey for 24 hours. The animal was sacrificed 88 hours later and the olfactory lobes, postrolandic convolutions, medulla, cervical cord and lumbar cord were tested for the presence of virus, all giving negative results. In 1930, Fairbrother and Hurst (10) reported the results of four experiments with intranasal inoculation, in two of which virus was not detected anywhere in the central nervous system. In the other two, positive results were obtained. In one animal examined 12 days after inoculation, virus was found in the homolateral olfactory lobe and in the cervical cord; not in the left anterior frontal cortex, right olfactory lobe, lumbar cord

[1] The essential difficulty has been that experimental infection by the nose was not regularly obtained by the methods generally used until within the last 2 or 3 years. Howitt (5) estimates that only 53 per cent of intranasal inoculations are successful. The percentage is certainly less than this at times. Flexner (6) has recently been able to obtain much more uniform takes by instillations repeated over a period of several days. In our own work, no progress could be made for several months until the technique was modified (or the conditions changed spontaneously for unknown reasons), after which we had almost no failures and were able to go ahead with the present study.

or cerebrospinal fluid. In the other, examined 11 days after inoculation, virus was found in the cervical and lumbar cord; not in the left anterior frontal cortex. In both these experiments, however, the disease had progressed to the point of paralysis and the results give no clear indication of the localizations of infection during the incubation period proper. The histological study of the animals showed lesions particularly marked in the nasal cortex of the homolateral hemisphere, in the amygdaloid nucleus (one of the olfactory relays) and the cornu Ammonis (hippocampus); less in the homolateral thalamus and globus pallidus; none in the putamen and caudate nucleus. We find no other comparable experiments in the literature.

The experiments of Flexner and Clark, therefore, appear to be the only ones affording any positive information on the localization of virus during the preparalytic period of infection in animals intranasally infected (the presumptively normal route), and these experiments show only its very earliest localization, in the olfactory bulbs.

The demonstration of the axonal mode of propagation by Fairbrother and Hurst (10), since confirmed by Jungeblut and Spring (11), and by later experiments of Hurst (12) justifies the expectation that the natural route followed by virus from this initial point of attack should be along the tracts leading out from the olfactory bulbs and along others with which they connect. The present study is based on such an assumption and the experiments have been planned in accordance with it. We have attempted to map the distribution of virus in the central nervous system on successive days of the incubation period; to determine which areas are spared as well as those which are infected, and thus to discover whether or not a definite and continuous route of propagation can be discerned, leading from the primary site of implantation in the central nervous system down to the spinal cord.

Methods

Macacus rhesus monkeys were used in all the experiments. The source of virus was a 10 per cent pooled suspension of cord, or cord and medulla, from 3 to 5 monkeys infected with the MV strain, each lot having been tested in the usual manner for virulence. For the present series and the controls a uniform technique of inoculation was followed. The left naris was always used, and an effort was made to confine the preliminary treatment as well as the inoculum to this side. First the naris was flooded with a slightly acid buffer solution at approxi-

mately pH 5.0,[2] with the animal's head in the dependent position; immediately thereafter 0.5 cc. of the 10 per cent virus suspension was dropped into the nose. 3 hours later and, again, 6 hours later, virus suspension in the same amount was dropped into the left naris. The acid wash was not repeated. No traumatization was involved in any part of the procedure.

23 of 26 animals[3] thus treated and permitted to survive developed typical poliomyelitis. The average period of incubation was 8.2 days; the shortest individual period was 7 days and the longest, 10 days. The availability of a method giving such almost uniformly positive results and permitting the dating of infection from a single day was essential to the study of the problem.

Animals thus treated were killed by ether on the 3rd, 4th, 5th, 6th and 7th days of incubation, respectively. They were immediately dissected under strictly aseptic precautions and the brain, and the spinal cord with its ganglia, removed. The brain was divided into two lateral halves, with particular care to divide the brain stem and cerebellum in the midline. Pieces were then excised from the various areas to be described. All instruments were sterilized before being used for another excision. When specimens were to be taken from closely adjoining areas such as the hypothalamus, thalamus and midbrain, the precaution was taken of leaving a bridge of tissue between the excised areas. This precaution could not, however, be taken in separating the anterior and posterior halves of the cord.

The selection of areas for testing was primarily based on a study of the olfactory tracts and their connections, discussed elsewhere (2). The reader is reminded that from the olfactory bulb the main tract connections are (*a*) with the hypothalamus, thalamus and midbrain, and (*b*) with the olfactory cortex (hippocampus, uncus, gyrus fornicatus, etc.), and thence with the hypothalamus, thalamus and midbrain. From the hypothalamus down to the medulla is a tract or series of tracts, in the formatio reticularis which appears to have special importance in poliomyelitis, since it has been found to be frequently and heavily involved in the human pathology (14). Connections with the spinal cord and its ganglia are traceable through the great sensory tracts, especially the spinothalamic from the thalamus, to the posterior horns and ganglia; and through various motor pathways having relays in the midbrain and medulla, directly to the anterior horns of the cord. For certain reasons, mainly clinical, discussed in

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[2] With reference to the value of this method, which was introduced by Schultz and Gebhardt at the Department of Bacteriology of Stanford University, Schultz in a recent personal communication to me states that the results thus far obtained indicate that the incidence of takes is somewhat increased by preliminary nasal washes with acid phosphate solutions. A note on the subject is being submitted to the *Proceedings of the Society for Experimental Biology and Medicine* for publication.

[3] These were used in other experiments by Schultz and Gebhardt.

another paper (2), it has been surmised that the chief pathway of infection in man is along the great spinothalamic sensory pathway to the posterior horns and thence to both the dorsal root ganglia and anterior horns.

On the basis of the hypothesis just outlined, specimens were removed from the following areas: the olfactory bulbs; the hypothalamic area; the thalami; the midbrain; the medulla with the lower half of the pons (designated medulla in the protocols); the spinal cord at different levels, in some instances separating the anterior and posterior portions approximately at a line just behind the posterior edge of the grey commissure (in this case it was impossible because of the smallness of the cord to leave a space between the excised portions; the two sections were therefore immediately contiguous); the dorsal root ganglia, right and left separately. While the neopallial cortex (the portion exclusive of the olfactory cortex) and the cerebellum are not in the direct line of connection with the olfactory tracts, portions of these were also removed: the frontal pole of the frontal lobe; the precentral (prerolandic) gyrus of the frontal lobe (cortical motor area); the postcentral gyrus of the parietal lobe (cortical sensory area, connected directly with the thalamus); the lateral lobes and vermis of the cerebellum.

The portions removed were weighed (or with very small specimens the weight was estimated); physiological salt solution was added sufficient to make a 10 per cent suspension; the mixtures ground in mortars for ½ hour, according to the method of Schultz and Banham (13), and stored until used. In testing for virus, measured amounts of the suspensions (1.5 and 2.0 cc.) were injected intracerebrally according to standard technique. The first observed typical symptoms, usually preparalytic (tremor, nervousness, apprehensiveness, occasionally local weakness) have been used to date the time of onset of the disease. In the protocols the designation poliomyelitis has been used for the appearance of these first typical symptoms and should be so interpreted. The time when paralysis became complete or nearly so has also been noted in the protocols. The amount of virus in a given suspension is assumed to be in rough inverse proportion to the time of onset and of complete paralysis. For this reason, a tabulation of these data is given for each of the series of protocols.

Many specimens were examined histologically throughout the study and the typical changes of poliomyelitis found in all instances. It is hoped that at a later time the distribution of lesions in the central nervous system on various days of the incubation period after intranasal inoculation can be studied. The present study deals only with the distribution of virus in amounts capable of detection by subinoculation. It should, of course, be recognized that virus may have been present in smaller amounts than this in other areas than those giving positive subinoculation tests.

In several instances stated in the protocols animals used for subinoculation and found negative were later found to be susceptible to poliomyelitis.

No instance of secondary infection—brain abscess, meningitis, etc.—referable to contamination of the inocula, occurred.

EXPERIMENTAL RESULTS

Three entirely negative experiments should be mentioned, in which the experimental conditions were alike save in respect to the day on which the animals were sacrificed. Three instillations were made at 3 hour intervals in the left naris.

Monkey F78-1 was killed 3 days after virus instillation: no virus was detected in the left olfactory bulb, the left thalamus, the medulla, the cervical and lumbar cord (pooled) nor in the pooled cervical and lumbar ganglia. Those were the only places tested. A control animal treated in exactly the same way, on the same day and with the same suspension of virus, developed typical poliomyelitis on the 10th day. In these experiments only 0.25 cc. of suspension was instilled each time. 0.5 cc. was used in the other experiments. It is, of course, possible that Monkey F78-1 would have succumbed to the disease and that the virus had not yet reached the olfactory bulb in sufficient concentration on the 3rd day of incubation to permit detection.

Monkey F85-1 was killed 5 days after intranasal inoculation. No virus was found in the left olfactory bulb, the left hypothalamus, the left thalamus nor in the medulla (both sides pooled). These were the only places tested.

Monkey F80-4 was killed 6 days after intranasal inoculation. No virus was detected in the left olfactory bulb, the left hypothalamus nor in the medulla (both sides pooled). These were the only places tested.

In the last two experiments it seems probable that no infection had occurred.

PROTOCOLS

Series A. 4th Day of Incubation

Monkey F80-3.—Oct. 21, 1932: 3 instillations in left naris, of 0.5 cc. of a 10 per cent suspension of MV virus (3 cords pooled), at 3 hour intervals. Killed Oct. 25. Specimens ground and diluted to approximate 10 per cent suspension in normal saline solution.

Subinoculations

Left Olfactory Bulb.—Monkey F80-5, inoculated Oct. 26; 2.0 cc. Oct. 30, poliomyelitis. Oct. 31, complete flaccid paralysis; killed.

Hippocampus, Right and Left, Pooled.—Monkey F81-1, inoculated Nov. 8; 2.0 cc. Remained well.

Left Hypothalamus.—Monkey F80-6, inoculated Oct. 26; 2.0 cc. Remained well.

Medulla, Right and Left Sides, Pooled.—Monkey F80-7, inoculated Oct. 26; 2.0 cc. Remained well. Susceptible in later experiment (F84-0).

Lumbar and Cervical Cord and Dorsal Root Ganglia, Pooled.—Monkey F81-2, inoculated Nov. 8; 2.0 cc. Remained well.

Summary.—Virus detected in left olfactory bulb only, and in extremely high concentration. The first signs of poliomyelitis in the test monkey appeared on the 4th day, and paralysis was complete on the 5th.

Series B. 5th Day of Incubation

Monkey F88-4. Dec. 15, 1932: 3 instillations in left naris, each of 0.5 cc. of a 10 per cent suspension of MV virus (pooled), adjusted to pH 7.0, at 3 hour intervals. Dec. 19, temperature 105.6°; no other symptoms. Killed Dec. 20, 1932. Specimens ground and diluted to approximate 10 per cent suspension in normal saline solution.

TABLE I

4th Day of Incubation. Monkey F80-3. Positive and Negative Results

Side tested	Left	Right	Both
Olfactory bulb	+(4)	−	
Cortex, hippocampus			0
Interbrain			
Hypothalamus	0	−	
Thalamus	−	−	
Midbrain	−	−	
Pons-medulla			0
Cord			0
Dorsal ganglia			0

+: tested by intracerebral inoculation and found positive by development of typical poliomyelitis in test animal; the first definite signs appearing on day after inoculation indicated by figure in parentheses. 0: tested and found negative, test animal remaining well. −: not tested. "Both" indicates that the specimens from the two sides were pooled and tested together.

Subinoculations

Left Olfactory Bulb.—Monkey F88-6, inoculated Dec. 21; 1.5 cc. Dec. 27, poliomyelitis. Dec. 29, complete paralysis. Dec. 30, very low; killed.

Right Olfactory Bulb.—Monkey F93-1, inoculated Jan. 28, 1933; 1.5 cc. Feb. 4, tremors. Feb. 6, complete paralysis; killed.

Hippocampus, Right and Left, Pooled.—Monkey F89-1, inoculated Jan. 5, 1933; 2.0 cc. Remained well.

Left Hypothalamus.—Monkey F88-7, inoculated Dec. 21, 1932; 1.5 cc. Dec. 29, poliomyelitis. Dec. 31, complete paralysis. Jan. 3, very low; killed.

Thalamus, Right and Left, Pooled.—Monkey F88-9, inoculated Jan. 5, 1933; 2.0 cc. Remained well.

Midbrain, Right and Left Sides, Pooled.—Monkey F92-8, inoculated Jan. 23, 1933; 1.5 cc. Remained well.

TABLE II

Monkey F80-4. 5th Day of Incubation. Positive Results. Periods after Intranasal Inoculation When Typical Symptoms Appeared, and When Paralysis Became Practically Complete

	Day first symptoms	Day complete paralysis
Left olfactory bulb	6	8
Right olfactory bulb	7	9
Left hypothalamus	8	10
Medulla	6	10

TABLE III

5th Day of Incubation. Monkey F88-4. Positive and Negative Results

Side tested	Left	Right	Both
Olfactory bulb	+(6)	+(7+)	
Cortex			
Hippocampus			0
Interbrain			
Hypothalamus	+(8)	−	
Thalamus			0
Midbrain			0
Pons-medulla			+(6)
Cerebellum			0
Cord, cervical			0

Medulla, Right and Left Sides, Pooled.—Monkey F88-8, inoculated Dec. 21, 1932; 1.5 cc. Dec. 27, poliomyelitis. Dec. 29, paralysis, right leg. Dec. 31, complete paralysis; killed.

Cerebellum, Right and Left, Pooled.—Monkey F89-2, inoculated Jan. 5, 1933; 2.0 cc. Remained well.

Spinal Cord, Cervical.—Monkey F88-9, inoculated Dec. 21, 1932; 1.5 cc. Remained well.

Summary.—Virus detected in left and right olfactory bulbs, in left hypothalamus and in medulla; highest concentration apparently in left olfactory bulb.

Series C. 6th Day of Incubation

Monkey F83-4.—Nov. 9, 1932: 3 instillations in left naris, each of 0.5 cc. of a 10 per cent suspension of MV virus (cord and medulla of 6 monkeys, pooled), at 3 hour intervals.

Subinoculations

Left Olfactory Bulb.—Monkey F83-6, inoculated Nov. 16, 1932; 1.5 cc. Nov. 22, poliomyelitis. Nov. 25, complete paralysis. Nov. 26, killed.

Right Olfactory Bulb.—Monkey F85-0, inoculated Nov. 26, 1932; 2.0 cc. Remained well.

Hippocampus, Right and Left, Pooled.—Monkey F83-9, inoculated Nov. 16, 1932; 1.5 cc. Remained well.

Precentral Gyri, Right and Left, Pooled.—Monkey F85-2, inoculated Nov. 29, 1932; 1.5 cc. Remained well.

Postcentral Gyri, Right and Left, Pooled.—Monkey F85-3, inoculated Nov. 29, 1932. Remained well.

Left Hypothalamus.—Monkey F83-8, inoculated Nov. 16, 1932; 1.5 cc. Nov. 21, poliomyelitis. Nov. 22, partial paralysis both arms. Nov. 23, complete paralysis; died.

Right Hypothalamus.—Monkey F88-2, inoculated Dec. 14, 1932; 1.5 cc. Remained well.

Left Thalamus.—Monkey F83-7, inoculated Nov. 16, 1932; 1.5 cc. Nov. 25, paralysis, both arms. Nov. 26, complete paralysis. Dec. 3, killed.

Right Thalamus.—Monkey F88-3, inoculated Dec. 14; 1.5 cc. Dec. 22, poliomyelitis. Dec. 25, complete paralysis. Dec. 28, died.

Left Caudate Nucleus.—Monkey F84-1, inoculated Nov. 16, 1932; 1.5 cc. Remained well.

Midbrain, Right and Left, Pooled.—Monkey F92-9, inoculated Jan. 23, 1933; 1.5 cc. Jan. 31, fine tremors. Feb. 1, complete paralysis, both arms; killed.

Medulla, Right and Left, Pooled.—Monkey F84-0, inoculated Nov. 16, 1932; 1.5 cc. Nov. 25, partial paralysis both legs. Nov. 28, complete paralysis; killed.

Cerebellum, Right and Left, Pooled.—Monkey F85-7, inoculated Nov. 29, 1932; 1.5 cc. Remained well.

Spinal Cord, Cervical.—Monkey F85-5, inoculated Nov. 29, 1932; 1.5 cc. Remained well.

Spinal Cord, Lumbar.—Monkey F85-6, inoculated Nov. 29, 1932; 1.5 cc. Remained well.

Dorsal Root Ganglia, Cervical and Lumbar, Right and Left, Pooled.—Monkey F85-4, inoculated Nov. 29, 1932; 1.5 cc. Remained well.

Summary.—Virus detected in left olfactory bulb, in left hypothalamus, in left and right thalami, in midbrain, in medulla; highest concentration apparently in left hypothalamus, and next highest in left olfactory bulb. Concentrations approximately equal in the thalamus (right and left), midbrain and medulla.

TABLE IV

6th Day of Incubation. Monkey F83-4. Positive Results

	Day first symptoms	Day complete paralysis
Left olfactory bulb	6	9
Left hypothalamus	5	7
Left thalamus	9	10
Right thalamus	8	11
Midbrain	8	9
Medulla	9	10

TABLE V

6th Day of Incubation. Monkey F83-4. Positive and Negative Results

Side tested	Left	Right	Both
Olfactory bulb	+(6)	0	
Cortex			
Hippocampus			0
Precentral gyrus			0
Postcentral gyrus			0
Interbrain			
Hypothalamus	+(5)	0	
Thalamus	+(9)	+(8)	
Caudate nucleus	0		
Midbrain			+(8)
Pons-medulla			+(9)
Cerebellum			0
Cord			
Cervical			0
Lumbar			0
Dorsal root ganglia			0

Series D. 7th Day of Incubation

Monkey F86-3.—Dec. 6, 1932: 3 instillations in left naris of 0.5 cc. of a 10 per cent suspension of MV virus (5 cords pooled) at 3 hour intervals. Dec. 13, temperature 105.4°; no other signs of poliomyelitis; killed.

Subinoculations

Left Olfactory Bulb.—Monkey F86-4, inoculated Dec. 14, 1932; 1.5 cc. Dec. 20, poliomyelitis. Dec. 23, complete paralysis; killed.

Right Olfactory Bulb.—Monkey F89-5, inoculated Jan. 5, 1933; 2.0 cc. Jan. 12, poliomyelitis. Jan. 13, paralysis both arms. Jan. 14, complete paralysis.

Hippocampus, Right and Left, Pooled.—Monkey F89-6, inoculated Jan. 5, 1933; 2.0 cc. Jan. 17, poliomyelitis. Jan. 18, paralysis both arms. Jan. 19, complete paralysis; killed.

Cortex; Right and Left Sides Pooled; Precentral and Postcentral Gyri; Anterior Pole of Frontal Lobe.—Monkey F89-7, inoculated Jan. 5, 1933; 2.0 cc. Remained well.

Left Hypothalamus.—Monkey F86-5, inoculated Dec. 14, 1932; 1.5 cc. Remained well. Susceptibility shown by poliomyelitis on 9th day after intranasal inoculation, Jan. 10, 1933.

Right Hypothalamus.—Monkey F93-0, inoculated Jan. 28, 1933; 1.5 cc. Remained well.

Left Thalamus.—Monkey F86-6, inoculated Dec. 14, 1932; 1.5 cc. Remained well. Susceptibility shown by poliomyelitis on 8th day after intranasal inoculation, Jan. 10, 1933.

Right Thalamus.—Monkey F89-4, inoculated Jan. 5, 1933; 2.0 cc. Jan. 20, paralysis both arms. Jan. 21, complete paralysis.

Midbrain, Right and Left, Pooled.—Monkey F92-7, inoculated Jan. 23, 1933; 1.5 cc. Jan. 31, tremors. Feb. 2, complete paralysis; killed.

Medulla, Right and Left, Pooled.—Monkey F89-3, inoculated Jan. 5, 1933; 2.0 cc. Jan. 11, poliomyelitis. Jan. 12, paralysis both arms. Jan. 13, complete paralysis; killed.

Cerebellum, Right and Left, Pooled.—Monkey F89-8, inoculated Jan. 5, 1933; 2.0 cc. Remained well.

Spinal Cord, Cervical, Anterior Half.—Monkey F92-4, inoculated Jan. 23, 1933; 1.5 cc. Jan. 30, slight nervousness. Jan. 31, tremors, paralysis right arm. Feb. 2, complete paralysis. Feb. 4 , died.

Spinal Cord, Cervical, Posterior Half.—Monkey F92-4, inoculated Jan. 23, 1933; 1.5 cc. Jan. 30, nervousness. Jan. 31, tremors, paralysis left leg. Feb. 2, complete paralysis. Feb. 3, died.

Spinal Cord, Lumbar, Anterior Half.—Monkey F92-6, inoculated Jan. 23, 1933. Feb. 2, tremors; paralysis both legs. Feb. 3, complete paralysis; killed.

Spinal Cord, Cervical and Lumbar, Posterior Half.—Monkey F89-9, inoculated Jan. 5, 1933; 2.0 cc. Jan. 12, poliomyelitis. Jan. 13, almost complete paralysis. Jan. 13, killed.

Spinal Cord, Lumbosacral.—Monkey F86-7, inoculated Dec. 14, 1932; 1.5 cc. Remained well. Susceptibility shown by poliomyelitis 8 days after intranasal inoculation, Jan. 10, 1933.

Dorsal Root Ganglia, Left.—Monkey F88-0, inoculated Dec. 14, 1932; 1.5 cc. Dec. 26, poliomyelitis. Dec. 28, complete paralysis; killed.

Dorsal Root Ganglia, Right.—Monkey F88-1, inoculated Dec. 14, 1932; 1.5 cc. Dec. 23, poliomyelitis. Dec. 24, paralysis both arms. Dec. 25, died.

TABLE VI

Monkey F86-3. Positive results

	Day first symptoms	Day complete paralysis
Left olfactory bulb	6	9
Right olfactory bulb	7	9
Hippocampus	12	14
Right thalamus	15	16
Midbrain	8	10
Medulla	6	8
Cord		
Cervical, anterior half	7	10
Cervical, posterior half	7	10
Lumbar, anterior half	10	11
Cervical and lumbar, posterior half	7	8
Dorsal ganglia, left	12	14
Dorsal ganglia, right	9	10+

TABLE VII

7th Day of Incubation. Monkey F86-3. Positive and Negative Results

Side tested	Left	Right	Both
Olfactory bulb	+(6)	+(7)	
Cortex			
Hippocampus			+(12)
Pre-, postcentral gyri			0
Interbrain			
Hypothalamus	0	0	
Thalamus	0	+(15)	
Midbrain			+(8)
Pons-medulla			+(6)
Cerebellum			0
Cord			
Cervical anterior half			+(8)
Cervical posterior half			+(8)
Lumbar anterior half			+(10)
Lumbar posterior half			−
Cervical and lumbar, posterior half			+(7)
Lumbosacral			0
Dorsal root ganglia, cervical and lumbar	+(12)	+(9)	

TABLE VIII

Summary of Experimental Results

Day after inoculation	4			5			6			7		
	Left	Both	Right	Left	Both	Right	Left	Both	Right	Left	Both	Right
Olfactory bulb	++++		−	+++		+++	+++		0	+++		+++
Cortex												
Hippocampus		0			0			0			+	
Pre-, postcentral gyri		0			0			0			0	
Interbrain												
Hypothalamus	0		−	++	−		++++		0	0		0
Thalamus		−			0		++		++	0		+
Midbrain		−			0			++			++	
Pons-medulla		0			+++			++			+++	
Cerebellum		−			0			0			0	
Cord												
Cervical, undivided		0(*a*)			0			0				
Cervical, anterior half											++	
Cervical, posterior half											++	
Lumbar, undivided		0(*a*)						0				
Lumbar, anterior half											+	
Lumbar, posterior half											−	
Cervical and lumbar, posterior half											++	
Lumbosacral, undivided											0	
Dorsal ganglia		0(*a*)			−			0		+		++

0(*a*): 3 specimens pooled; single test. ++++: incubation in test animal, 4 to 5 days. +++: incubation in test animal, 6 to 7 days. ++: incubation in test animal, 8 to 9 days. +: incubation in test animal, 10 days or more. −: untested.

Summary.—Virus detected in both olfactory bulbs, in hippocampal cortex (small amounts), in right thalamus (small amounts), in midbrain, in medulla, in both anterior and posterior halves of cervical cord, in anterior half of lumbar cord (in smaller amounts than in cervical), in dorsal root ganglia both right and left (more in right). The highest concentrations were found in the left olfactory bulb and in the medulla, and a little less in the specimen from the posterior half of the cervical and lumbar cord; other high concentrations were found in the right olfactory bulb, and the anterior and posterior half of the cervical cord.

DISCUSSION

After deposition on the nasal mucosa the virus of poliomyelitis apparently requires a period of 2 to 4 days for penetration into the olfactory cells, ascent through the olfactory nerves into the olfactory bulb and multiplication therein to amounts sufficient for detection. That this is the actual pathway of ascent is shown by the occurrence of virus in the olfactory bulb (homolateral side with the naris inoculated) before it could be detected in any other of the nervous areas examined; by its absence from the outgoing olfactory pathways (hypothalamus, hippocampus), and by the extraordinarily high concentration of virus in the olfactory bulb. So sharp a localization and heavy a concentration of virus cannot be interpreted otherwise than by axonal passage through the ascending olfactory neurons, since the other alternative—ascent by the perineural lymphatics into the subarachnoid space—must necessarily lead to much wider diffusion of the virus. *The olfactory bulb, therefore, is to be regarded as the initial focus of poliomyelitic infection within the central nervous system,* directly infected through the olfactory nerves from the nasal mucosa. By the 4th day of the incubation period it has become a heavily charged reservoir joined by an elaborate and extensive system of communications with other parts of the central nervous system through which infection can be rapidly discharged and distributed.

Throughout the incubation period the olfactory bulb continues to be heavily infected.

On the 5th day, virus is found, as yet in smaller amounts than in the primary focus, in one of the primary relays of the olfactory tracts,

the homolateral hypothalamus; in the contralateral bulb; and in a more distant area, the medulla oblongata. It is not found in the olfactory cortex (hippocampus), thalamus, midbrain, cerebellum or spinal cord. Its presence in the right olfactory bulb may have been due either to passage from the left bulb through the anterior commissure or to spread of infection from the nasal mucosa of the left naris to that of the right and so up the right olfactory nerves. Its presence in the homolateral hypothalamus was anticipated since this structure (the mamillary region, especially) is directly connected with the olfactory bulb through one of the olfactory tracts and also indirectly, by way of the hippocampus. It would appear that the virus had followed the outgoing olfactory fibers along their basal pathways, which are the shortest. The absence of virus from the olfactory cortex (hippocampus) in this and all but one of the other animals is extremely interesting, since the greater portion of the outgoing olfactory pathways in all higher vertebrates is long circuited through the cortex before they go in the interbrain and midbrain. To explain the phenomenon, it is necessary to refer to Fairbrother and Hurst's (10) conclusion that the cortex of the brain in general affords an unsuitable ground for implantation and survival of poliomyelitis virus.

That the medulla should show infection before the thalamus or midbrain had not been anticipated and called our attention to the possible existence of intimately connected tracts between the hypothalamus and medulla. Recent studies, elsewhere (2) reviewed, have shown important functional connections between the hypothalamus and medulla concerned largely in the higher control of various sympathetic and parasympathetic functions, and the probability that the corresponding anatomical tract is, in part at least, the formatio reticularis. In histological studies of human poliomyelitis, this structure of ganglion cells and white matter has been found to be quite constantly and heavily involved (Harbitz and Scheel (14)), and the prominence of various sympathetic and parasympathetic symptoms early in the disease may be referable to these lesions in and between the hypothalamus and medulla. The relatively large amount of virus in the medulla, indicated by the appearance of symptoms in the test animal on the 6th day (complete paralysis on the 10th day) indicates further that bulbar tissue (or at least the part in question) is favorable to implantation and multiplication of virus.

The experiments of the 6th day furnish additional information. At this time, it will be noted that virus was again found in the left olfactory bulb (same side as the inoculation), as it was on every day from the 4th to the 7th inclusive. Its absence from the right side (opposite side from the inoculation) in this experiment, and the smaller amounts on this side than on the left in the 5 and 7 day experiments have an obvious significance. The very high concentration of virus in the left (homolateral) hypothalamus (5 day incubation) is in good agreement with the postulated pathway of infection. The two thalami now show a moderate degree of infection, approximately equal to that also found in the midbrain and medulla. No virus was detected anywhere in the cerebral cortex, left caudate nucleus, contralateral hypothalamus, cerebellum, cervical or lumbar cord or intervertebral ganglia. The appearance of virus in previously uninfected areas—the thalamus and midbrain—would appear to indicate that infection was now following a separate channel from that noted on the 5th day (hypothalamus to medulla). On the basis of the known anatomy, this may have been through the mammillothalamic tract (bundle of Vicq d'Azyr) from the hypothalamus to the anterior nuclei of the thalamus (and the commissural connections between the thalami), and the mammillotegmental tract from the hypothalamus to the midbrain. The presence of virus in the midbrain and its absence in all experiments from the cerebellum are suggestive of a decidedly restricted localization within the relatively small mesencephalic area, since they apparently indicate that the red nucleus—a relay in the cerebellar pathways and a close neighbor of Gudden's nucleus and of the hypothalamus, also—is not involved.

The complete absence of virus in the spinal cord even in the cervical region, on both the 5th and 6th days when it is present in the medulla in fairly high concentration, is extremely interesting, suggesting that the infected areas in the medulla are perhaps not the main source of infection of the spinal cord. The inference was elsewhere made (2), based on considerations of anatomy, pathology and symptomatology, that the main route of invasion of the spinal cord was more probably from the thalamus than from the medulla, and this view is apparently supported by the present experimental observations.

On the 7th day virus is found abundantly and extensively in the

spinal cord. At the same time there is evidence that it has died out or disappeared from some of the previously infected areas, thus confirming the experience of Fairbrother and Hurst with intracortical inoculations. It is still present in high concentration in the left and right olfactory bulbs; indicating either that virus has continued to ascend from the olfactory nerves or that the tissues here are favorable to survival, as well as to initial implantation and multiplication, of the virus. The disappearance from the homolateral (left) hypothalamus is particularly interesting, since on the previous day, it had been present here in large amounts.

The contralateral side was negative. Virus had also disappeared from the homolateral (left) thalamus, but was still present, though in minimal amount (15 day incubation) in the contralateral thalamus. Its appearance in small amounts (12 day incubation) in the hippocampus—the only positive result in the entire series from the end brain—is of interest as evidence of the predominant infection of the olfactory tracts, but the lateness and slightness of this cortical involvement suggests that it is probably incidental and secondary.[4]

In the 7th day experiments, an attempt was made to determine what differences, if any, might be detected between the anterior (motor) and posterior (sensory) portion of the cord, as well as between different levels. If the anterior gray matter contained larger amounts of virus, it might be inferred that the virus had descended along the tracts connecting the higher centers in the brain stem directly with the anterior horn cells (rubrospinal, tectospinal, vestibulospinal, reticulospinal tracts, etc.). If, on the other hand, the posterior horns were more heavily infected, it might be inferred that it had descended along the main sensory tracts (spinothalamic tract, columns of Goll and Burdach). Clinical evidence, particularly the character of the preparalytic symptoms in man, points rather clearly to the spinothalamic (2).

The present experiments fail to answer this question unequivocally, perhaps due in part to the necessity of using immediately contiguous

[4] Harbitz and Scheel (14) have described inflammatory lesions in this area. However, no characteristic symptoms (olfactory hallucinations) derived from such involvement in man have been reported. The point has not been sufficiently studied by clinicians.

areas of cord for the tests. Unfortunately, too, one test could not be made that might have been critical—of the posterior half of the lumbar cord. The experiments actually made, however, show definitely that the posterior half is at least as heavily infected as the anterior at the earliest moment when virus can be detected anywhere in the cord. In the cervical region the two halves appear to be equally infected, since the test animals showed the first signs and developed paralysis at almost exactly the same time. In the anterior lumbar cord, the concentration was less than in the cervical cord, and no virus at all was detected in the lumbosacral segment. The highest concentration found anywhere in the spinal cord was in a pooled specimen of the posterior halves of cervical and lumbar segments. Unfortunately, no note was made of the exact levels from which the pieces were removed (the upper segment was well separated from the medulla) and we are therefore unable to state the point where maximum concentration occurred, except that it was outside the cervical area separately tested (Monkey F92-4).

The presence of virus in the dorsal root ganglia in somewhat smaller amounts than in the cord itself and the definitely larger amount in the contralateral (right) side from the original inoculation afford further evidence of involvement of the sensory tracts, and also suggest propagation along decussating pathways.

The experiments with tissues from the spinal cord therefore indicate invasion of both the anterior and posterior horns shortly (approximately 1 day) before paralysis was to have been expected. About 1 day earlier (6th) in the incubation period no virus was demonstrable anywhere in the cord or its ganglia. On the 7th day it was abundantly present in the cervical segments and in smaller amounts also in the lumbar. The maximum concentration in the cord was found in the posterior half (presumably in the posterior horns). We hope to investigate the localizations in the cord in greater detail in the near future.

SUMMARY

The results of our investigation indicate that in experimental poliomyelitis produced by intranasal inoculation it is possible to trace, always through the nervous system, the descending routes of infec-

tion in continuous series from the nose to the spinal cord, and, since the portal of entry is probably the same in man, it appears reasonable to suppose that the pathways of infection are similar in the human disease. Considerations of the human symptomatology and distribution of pathological lesions elsewhere (2) discussed point to the same conclusion.

All these considerations, experimental, pathological and clinical, appear to justify a conception of poliomyelitis as an infection of nervous tissues alone throughout its course, by a virus that is rather strictly neurotropic. Even within the nervous system itself a sharp tendency to preferential localization is clearly seen. The neopallial portions of the end brain and the cerebellum were never found to contain virus in our experiments, and the archipallial portion of the telencephalon (hippocampus) contained it but once and then late and in relatively small amounts. Moreover, the involvement of the diencephalon (hypothalamus, thalamus), while marked for a time, appeared to be transient. The suitability of the central nervous tissues to implantation of the virus appears to be limited to the olfactory bulbs, brain stem (exclusive of the cerebellum) and cord; and the conditions suitable for survival of the virus, to the olfactory bulbs, midbrain, medulla and spinal cord. The clinical course in the majority of human cases indicates (2) that the anatomical range of optimal conditions for survival of the virus may be much more limited than in the monkey, since permanent damage is usually left only in a portion of the anterior horns of the cord.

During the preparalytic period in the monkey (the same may be assumed to hold true of man), it may be convenient to distinguish four successive phases of poliomyelitic infection.

1. Implantation in the olfactory mucosa. Infection of the olfactory cells, and ascent through the axons of the olfactory nerves. First 3 days (approximately). No signs or symptoms.

2. Invasion and implantation in the olfactory bulb (initial central nervous focus). No signs or symptoms. 4th day (approximately).

3. Descent of virus through the olfactory tracts; establishment of secondary foci in the interbrain (hypothalamus, thalamus), midbrain and medulla. No disturbances except occasionally fever, and later, general nervousness and apprehensiveness. 5th and 6th days (approximately).

4. Invasion of the spinal cord and ganglia. Tremor, hyperesthesia. 7th day (approximately).

In direct succession to the fourth stage, is the stage of flaccid paralysis (8th day, approximately) in which the function of the anterior horn cells ceases as they are destroyed. The final stage of healing, common in man, is rarely seen in the monkey.

CONCLUSIONS

1. About 4 days after intranasal instillation, the virus of poliomyelitis establishes its initial focus, within the central nervous system, in the olfactory bulbs. It apparently reaches this structure through the axons of the olfactory nerves after primarily infecting the olfactory cells of the nasal mucosa.

2. From this initial focus, the virus spreads (on the 5th and 6th days) through the olfactory tracts and their connections in the brain stem. A secondary focus in the hypothalamus is first established. From this, two main channels can be discerned: first, to the medulla; second, to the thalamus and midbrain.

3. On the 7th day, virus can first be detected in the spinal cord. It is widespread but is found in larger amounts in the cervical than in the lumbar segments. It is present in both the anterior and posterior horns, either in equal amounts or in slightly larger amounts in the posterior. It is also present in the intervertebral ganglia. The surmise is presented that the main route of infection of the cord is not from the medulla (which had been infected as early as the 5th day) but along the sensory tracts, presumably from the thalamus (spinothalamic tracts).

4. Certain portions of the central nervous system were never found to contain demonstrable quantities of virus: these were the cortex of the frontal and parietal lobes (neopallium), and the cerebellum. The olfactory (archipallial) cortex (hippocampus) was only once found to contain virus; this occurred on the 7th day and in small amounts, and presumably had its source in the olfactory bulbs.

5. The experiments of the 7th day suggest that virus had died out in areas previously infected (in the hypothalamus and thalamus, particularly), while continuing, apparently undiminished, in the midbrain and medulla, and spreading to the cord. These observations are in

harmony with the general contentions of Fairbrother and Hurst that virus is better adapted to survival in the lower portions of the cerebrospinal axis than in the higher.

6. The conception here presented of the manner of entrance and routes of propagation of the virus of poliomyelitis in the experimental animal appears to be in essential agreement with the clinical and pathological characteristics of the disease in man. Both the experimental disease and the disease as it occurs in man appear to present the features of an infection spread through nervous tissue only. It is unnecessary to assume that at any stage of its progress, during the incubation period or later, systemic or general extranervous infection is present.

For a more detailed review of the human pathology and symptomatology, as well as of the anatomical and experimental data relevant to the subject, and for a more exhaustive discussion of the broad conception of poliomyelitis as primarily and essentially a disease of the nervous system, the reader is referred to another paper (2).

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