STUDIES ON ENTRY AND EGRESS OF POLIOMYELITIS INFECTION

III. Excretion of the Virus during the Presymptomatic Period in Parenterally Inoculated Monkeys*

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While the excretion of poliomyelitis virus in the nasopharyngeal secretions and stools of apparently healthy contacts (1–11), of "abortive" cases (12), and during the presymptomatic, acute, and convalescent periods of the overt disease is a well substantiated phenomenon, no general agreement has yet been reached on its basic mechanisms, and the sources and routes of egress of the excreted virus can still be regarded as controversial.

The relation of excretion to the time of onset of symptoms is a matter of special interest. Before onset, in the small number of tests that have been reported on human nasopharyngeal secretions, the following were positive: 5 days (Taylor and Amoss) (13); 4 to 6 days (Gordon *et al.*) (14); 1 day (Zintek, (15), and Rubenstein *et al.* (16)). After the onset, we have found 47 recorded positive tests in which the interval was stated: 1 to 3 days, 30 (1, 8, 9, 13, 17, 18, 20, 21); 4 to 6 days, 10 (9, 18, 21, 22); 8 to 9 days, 2 (22, 23); 11 to 14 days, 3 (10, 19); 17 days, 1 (24); and 4 months, 1 (25).[1]

In human stools, virus has been detected in 3 cases at 19, 12, and 9 days before onset, respectively, by Schabel *et al.* (26) and in 1 case, 12 days before the onset of symptoms and 19 days before paralysis, by Brown, Francis, and Pearson (27). After the onset, fecal excretion of virus is an almost regular occurrence, being most frequent at 1 to 2 weeks, later becoming progressively less so, and rare after 8 weeks (28). However, one positive test was reported at 12 weeks by Hortsmann, Ward, and Melnick (28) and another, the latest on record, at 123 days by Lépine, Sédallian, and Sautter (29).

Considering the much greater ease of detecting virus in the stools, as compared

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[1] This is the unique case reported by Lucas and Osgood (25) and the positive test we believe bears critical scrutiny; the subinoculated monkey developed typical paralysis, and passage was successful as shown by typical paralysis and pathologic lesions. The patient was convalescent from a second attack of poliomyelitis and the test material was nasal secretion which was said to be excessive. The patient was evidently a chronic carrier.

with nasopharyngeal secretions, the periods during which it has been found in the two sources are not too strikingly different. It is, however, general opinion today that virus tends to disappear sooner from the pharynx than from the intestine, a phenomenon which has been related by Howe (30) to the time of appearance of specific antibodies in the nasopharyngeal secretions.

Experimental study of excretion has been of limited scope but has demonstrated its occurrence in laboratory primates both in the nasopharynx and intestine in a manner closely paralleling the human case. For present purposes, only positive observations after parenteral inoculation will be reviewed. In 1910, Flexner and Lewis (31) detected virus in the nasopharyngeal mucous membrane of monkeys following intracerebral inoculation. In 1911, Osgood and Lucas (32) also recovered it from the same tissue in a monkey dying during the acute stage; and from 2 others dying 6 weeks and 5 months, respectively, after the acute stage, at a time when the cord and brain were negative. In the latter intriguing observation of prolonged persistence subinoculations were successful in two successive passages, one from the nasopharyngeal tissues of the subinoculated monkey. This was corroborated by Flexner and Clark (33) who in the same year reported finding virus in the same tissues 4 weeks after paralysis; in this animal, too, the cord was negative.

In 1911, Landsteiner, Levaditi, and Danulesco (34) found virus in the nasal secretions of 2 *cynomolgus* monkeys paralyzed after intracerebral and intraperitoneal inoculation; and also in the nasopharyngeal membrane and tonsils of *cynomolgus* monkeys inoculated intracerebrally. In 1912, Thomsen (35) obtained a take with nasal secretions from a monkey dying after intraperitoneal inoculation, which were simply rubbed on the nasopharynx of a second monkey.

Excretion into the intestine after parenteral inoculation has been less often studied. In 1940, Burnet and Backhouse (36) found virus in the washed walls of the intestine of a monkey paralyzed after intracerebral inoculation. Melnick's (37) study (1946) of excretion after parenteral inoculations is of great interest and significance. Using monkeys of 7 different species inoculated by various routes, virus was detected in the feces 15 times in 49 tests (31 per cent), sometimes before the onset of symptoms, as follows: intracutaneous and subcutaneous, $^{10}/_{27}$ positive tests from 32 animals; intracerebral, $^{1}/_{14}$ positive tests from 28 animals; intracerebral and intraperitoneal $^{1}/_{4}$ positive tests from 5 animals; all 4 routes, $^{1}/_{4}$ positive tests from 4 animals. In 4 chimpanzees inoculated intra- and subcutaneously (only 1 of which showed symptoms of infection) there were $^{3}/_{4}$ positive tests. In 1947 (38) we reported positive tests on both nasopharyngeal washings and stools of monkeys inoculated intraneurally into the infraorbital nerve in a preliminary report of the present study.

Any valid explanation of viral excretion in poliomyelitis must rest on the demonstration of the source of virus in relation to the pharyngeal and intestinal surfaces. That it is present in the walls both of pharynx and intestine during human poliomyelitis infection has been abundantly demonstrated, notably by Sabin and Ward (39). The simplest postulation—and one that is equally difficult to prove and to disprove—is that the virus multiplies in the extraneural tissues of the walls of the upper and lower alimentary tracts and is simply

extruded from them into their lumina. Evans and Green (40) have argued, admittedly without proof, in favor of this hypothesis, pointing out that the period of excretion probably extends beyond the time when virus is still demonstrable in the CNS, an assumption that is not, however, supported by the observations of Kessel and his associates (41). Whether excretion occurs before infection has reached the *central* nervous system has not been determined, although this seems at least possible and perhaps probable in the case of asymptomatic contacts and also of patients excreting virus long before the onset of symptoms. Judgment as to whether the results obtained by Enders and his associates (42) with virus-tissue suspensions have any bearing on *in vivo* multiplication should, we believe, be suspended until the factors responsible for the apparent *in vitro* multiplication have been carefully analyzed. In the intact organism, it is to be noted, no crucial evidence has as yet been provided of a host-cell affinity for poliomyelitis virus nor of a capacity for supporting its growth in any extraneural tissue, while, on the other hand, these conditions have been abundantly proved for nerve cells (43), and equally convincing evidence is at hand for the axonal conduction of the virus (43, 44). Moreover, the insusceptibility of intestinal mucosa to infection by direct and fairly prolonged contact with virus is indicated by our experiments (45) with *cynomolgus* monkeys in which heavy exposures confined to the lower alimentary tract were not followed by continuing viral excretion beyond the immediate period after feeding. We believe that this constitutes evidence against extraneural surface implantation, while the fact that the one animal in this series that excreted virus later on did so only when coming down with paralysis points to excretion derived from an inner source in the nervous system. Further, the experimental studies already cited in which excretion followed parenteral inoculation clearly demonstrate that primary surface infection is not essential to the elimination of virus in the pharynx and intestine. Finally, the usual absence of local signs and symptoms, and of histopathological lesions of mucosal inflammation in the majority of cases of human poliomyelitis is somewhat inharmonious with the hypothesis of viral infection in the extraneural tissues of the alimentary tract.

For these reasons, we feel that an exploration of another hypothesis is called for: that the primary and continuing source of the excreted virus is the infected nerve cell and that its outgoing pathway to the surface is the peripheral process of this cell. The present study is devoted to an investigation of this mechanism, giving special attention to the peripheral nervous system.

In the present study, five methods of inoculation, all parenteral, have been employed: (*a*) infraorbital nerve dip (46); (*b*) direct inoculation into the semiunar (Gasserian) ganglion; (*c*) direct inoculation into the celiac ganglion; (*d*) intrathalamic inoculation; (*e*) intravenous injection. All these procedures exclude primary infection of the surfaces and walls of the alimentary tract.

Methods

Primary Inoculations

The *strains of virus* used are noted in the protocols. Both Cam and Wis '45 have been found by us to infect by feeding. *Cynomolgus* monkeys (*Macaca irus*) were used for primary inoculations in all but one experiment in which *rhesus* (*Macaca mulatta*) were employed. For subinoculations, *cynomolgus* and *rhesus* were used.

Intrathalamic Inoculation.—Through a trephine opening in the calvarium, the needle was inserted into the thalamus and virus suspension in stated amounts was injected.

Infraorbital Nerve Dip.[2]—This procedure has been previously described (46). The central end of the divided nerve (right in all instances) was exposed by direct contact with the virus suspension for a period of approximately 10 minutes, care being taken to avoid as far as possible contamination of the wound, which was carefully sutured at the end of the operation. In two experiments, the seventh nerve had previously been divided intracranially to confine infection to the fifth at the time of dip.

Inoculation into the Gasserian Ganglion.—The ganglion was exposed by the subtemporal approach, after incision of the dura. About 0.05 ml. of virus suspension was injected directly into the ganglion in two or three places; a small proportion escaped into the surrounding area.

Inoculation into the Celiac Ganglion.—By retroperitoneal approach on the left, the splanchnic nerve was identified and followed down to the ganglion, which was then injected with 0.03 to 0.05 ml. of virus suspension. As determined by subsequent dissections, the injections were made not into the main mass of the ganglion, but into one of its smaller components. As in the case of the Gasserian inoculations, a certain amount of inoculum escaped into the surrounding tissues.

Intravenous Inoculation.—Virus suspension, clarified by brief high speed centrifugation, was injected into the right femoral vein in amounts stated in the protocol.

Collection and Preparation of Materials

Nasopharyngeal washings (hereafter abbreviated as NPW) were collected by three methods, as follows:—

1. The animals were lightly anesthetized with ether. The plastic mouthpiece used with a blood-counting pipette was attached to a Luer syringe and introduced into one nostril. Saline was gently washed through, to return through the other nostril, and sometimes also the mouth. The same saline was used repeatedly for each washing, which lasted about 2 to 3 minutes. The procedure was repeated 3 to 4 times daily.

2. Under nembutal anesthesia, a fine plastic catheter was inserted through one side of the nose into the pharynx. Saline was dripped at a very slow rate over the pharyngeal wall and returned through both nostrils. After an hour, the head was turned on the opposite side, the catheter introduced through the other nostril, and washing continued for a 2nd hour.

3. Under nembutal anesthesia, about 5 ml. of saline, from an original volume of 10 to 15 ml., was forced rapidly through the nose from a Luer syringe attached to a close fitting

[2] The following working hypothesis forms the basis of the infraorbital dip procedure. The peripheral fibers of the infraorbital branches of the maxillary division of the trigeminal nerve supply the skin and other tissues of the face but not the mucous membranes of the nose, palate, and mouth, which are supplied by other branches of the same division of the trigeminal and hence by cells of the same region of the Gasserian ganglion. Exposure of the infraorbital nerve to poliomyelitis virus would therefore permit centripetal spread of infection to the ganglion from which the virus, after primary multiplication and after secondary cross-infection of neighboring nerve cells, could spread centrifugally to the nasal, palatal, and oral surfaces and be excreted.

smoothed glass tube. The same saline was used repeatedly for each washing. The procedure was repeated at about 5 minute intervals for a total of 2 hours, 1 hour with the head on one side and 1 hour, on the other. By this method, the entire oronasopharyngeal surface could be thoroughly washed, since the saline returned through both the nose and the mouth. We regarded this as the most satisfactory technic.

Collections by the three methods thus represented NP secretions for 6 to 12 minutes, 2 hours and 2 hours, respectively. In the protocols and charts the day of collection has been designated according to the interval between the original inoculation and the time of starting the collection: 1 day, ± 24 hours; 2 days, ± 48 hours, etc. All washings were held on dry ice until prepared for subinoculation. They were then pooled by days and centrifuged either at 18,000 R.P.M. for 4 hours or at 30,000 R.P.M. for 1 to 1½ hours, after which the pellets were resuspended in saline and inoculated intrathalamically.

Stools were collected for approximate 24 hour, 9 a.m. to 9 a.m., periods and designated by day of collection in reference to day of original inoculation: thus, day 1 is the next after inoculation; day 2, the 2nd day, etc. Contents of the large intestine removed at autopsy were added to the stools for that day. All stools were stored on dry ice until used. Aqueous suspensions, pooled by days, were prepared for subinoculation by high speed centrifugation in the same manner as the NPW.

Gasserian ganglia were aseptically removed, pooled by side, and prepared for inoculation by grinding in a mortar with alundum and about 2.5 ml. of saline.

Results in animals used for subinoculation were based on the following criteria:—

1. Apparent Infection.—Typical symptoms developed, *i.e.* tremors and weakness or paralysis, with typical pathological lesions in the spinal cord.

2. Inapparent Infection.—The animals remained well, but typical lesions were found in the CNS. All symptom-free animals were sacrificed at 21 to 28 days. Sections were examined from two levels each of cervical and thoracic cords and one level of lumbar cord. The brain stem was examined at 1.25 mm. intervals. The lesions noted, although less extensive, were similar in type and distribution to those found in known poliomyelitic infection. Occasionally, animals were found with a few scattered, minute infiltrative foci, both perivascular and parenchymal, highly suggestive of poliomyelitic infection, but insufficient in number or size to make a definite diagnosis. Such animals are designated as questionable inapparent infections.

3. No Infection.—The animals remained well and histological examination of the brain stem and cord was negative.

PROTOCOLS OF INDIVIDUAL EXPERIMENTS

Infraorbital Dip

EXPERIMENT 1.—4 *rhesus* monkeys, sacrificed on the 5th day.
Strain.—Cam, 20 per cent suspension.
NPW collected by Method 1, and pooled from the 2nd to the 5th day.
Results of Primary Inoculation.—No symptoms; CNS lesions in 3/4.
Results of Subinoculations.—Gasserian ganglia: right, negative (only one subinoculation); left, positive. *NPW:* pooled 2nd through 5th day, positive.

EXPERIMENT 2.—4 *cynomolgus* monkeys, sacrificed on the 4th day.
Strain.—Cam, 20 per cent suspension.
NPW collected by Method 1.
Results of Primary Inoculation.—No symptoms; CNS lesions in 2/4 (minimal in 1).
Results of Subinoculations.—Gasserian ganglia: right, positive; left, negative. *NPW:* 3 and 4 day, positive; 2 day, negative. *Stools:* 4 day, positive; 2 and 3 day, negative.

EXPERIMENT 3.—4 *cynomolgus* monkeys, sacrificed on the 4th day.
Strain.—Cam, 20 per cent suspension.
NPW collected by Method 1.
Results of Primary Inoculation.—No symptoms; minimal CNS lesions in 1/4.
Results of Subinoculations.—Gasserian ganglia: right, positive; left, negative. *NPW:* 2, 3, and 4 day, negative. *Stools:* 2, 3, and 4 day, positive.

EXPERIMENT 4.—4 *cynomolgus* monkeys, sacrificed on the 4th day.
Strain.—Cam, 20 per cent suspension.
NPW collected by Method 2.
Results of Primary Inoculation.—No symptoms; minimal CNS lesions in 1/4.
Results of Subinoculations.—Gasserian ganglia: right positive; left, negative. *NPW*: 4 day, positive; 2 and 3 day, negative. *Stools*: 2, 3, and 4 day, negative.

EXPERIMENT 5.—4 *cynomolgus* monkeys, sacrificed on the 4th day.
Strain.—Wis '45, 50 per cent suspension.
NPW collected by Method 3.
Results of Primary Inoculation.—No symptoms; *no* CNS lesions.
Results of Subinoculations.—Gasserian ganglia: right, positive; left, positive. *NPW*: 3 and 4 day, positive; 2 day, negative. *Stools:* 2 and 4 day, positive; 3 day, negative.

EXPERIMENT 6.—4 *cynomolgus* monkeys, sacrificed on the 3rd day.
Strain.—Wis '45, 20 per cent suspension.
NPW collected by Method 3.
Results of Primary Inoculation.—No symptoms; *no* CNS lesions.
Results of Subinoculations.—Gasserian ganglia: right, positive; left, positive. *NPW:* 2 day, positive; 3 day, negative. *Stools:* 2 and 3 day, negative.

EXPERIMENT 7.—4 *cynomolgus* monkeys, in which N. VII had previously been divided intracranially, sacrificed on the 4th day.
Strain.—Cam, 20 per cent suspension.
NPW collected by Method 2.
Results of Primary Inoculation.—No symptoms; minimal CNS lesions, considered specific for poliomyelitic infection, in 1/4; lesions resulting from surgical trauma produced at the time of intracranial nerve section in 3/4.
Results of Subinoculationa.—Gasserian ganglia: right, positive; left, negative. *NPW:* 2, 3, and 4 day, negative. *Stools:* 2, 3, and 4 day, negative.

EXPERIMENT 8.—4 *cynomolgus* monkeys, in which N.VII had previously been divided intracranially, sacrificed on the 4th day.
Strain.—Wis '45, 50 per cent suspension.
NPW collected by Method 3.
Results of Primary Inoculation.—No symptoms; *no* CNS lesions specific for poliomyelitis; lesions resulting from surgical trauma produced at time of intracranial nerve section in 4/4.
Results of Subinoculations.—Gasserian ganglia: right, positive; left, negative. *NPW:* 4 day, positive; 2 and 3 day, negative. *Stools:* 4 day, positive; 3 day, negative.

Summary of Results.—(Chart 1). After infraorbital nerve dip, excretion of virus was demonstrated in 7 of 8 experiments, 2 to 4 days after exposure. In 6

EXPERIMENT	PRIMARY INOCULATIONS			SUBINOCULATIONS					
	Virus (conc.)	Symptome	CNS lesions	Material	Day 2	Day 3	Day 4	Gasserian ganglia Right	Gasserian ganglia Left
N.VII INTACT									
1. (4Rh)*	Cam (20%)	0	●●●○ †5	Npw / St		■ □ (+5) ND		□	■■ †5
2. (4Cyn)	Cam (20%)	0	●●○○ †4	Npw / St	□□ / □□	◪ / □□	■■ / ■	■	□ †4
3. (4Cyn)	Cam (20%)	0	●○○○ †4	Npw / St	□□ / ◪□□	□ □ / ■ □	□□ / ■	■	□□ †4
4. (4Cyn)	Cam (20%)	0	●○○○ †4	Npw / St	□□ / □□	□□□ / □□	■□ / □□□	■■	□□ †4
5. (4Cyn)	Wis'45 (50%)	0	○○○○ †4	Npw / St	□□ / ◪□	■■ / □□	■□ / ■	■	■□ †4
6. (4Cyn)	Wis'45 (20%)	0	○○○○ †3	Npw / St	◪□ / □□	□□ / □□		■	■□ †3
N.VII DIVIDED									
7. (4Cyn)	Cam (20%)	0	●○○○ †4	Npw / St	□□ / □	□□ / □	□□□ / □□	■■	□□ †4
8. (4Cyn)	Wis'45 (50%)	0	○○○○ †4	Npw / St	□□ / ND	□□ / □□	■■ / ■□	■	□□ †4

*Number of animals and species

LEGEND
- □ No symptoms or lesions
- ◪ Lesions; no symptoms
- ■ Symptoms and lesions
- ○ No lesions (CNS)
- ● Lesions (CNS)

Npw: nasopharyngeal washings
St: Stools
ND: not done
Rh: rhesus
Cyn: Cynomolgus
†Sacrificed on stated day
Each square or circle: 1 monkey

CHART 1. Inoculation of right infraorbital nerve.

experiments, the NPW were positive and in 4, the stools. In 3, both were positive; in 2, the NPW only, and in 1, the stools only. In 3 experiments, no involvement of the CNS was found, indicating that excretion originated in the ganglia. In 7 cases, the ipsilateral ganglia were positive, and in 3, the contralateral also. Of the latter, 2 were unaccompanied by CNS lesions, suggesting viral reinvasion from the surface following excretion. No symptoms had occurred in any of the animals.

Inoculation into the Gasserian Ganglion

EXPERIMENT 1.—4 *cynomolgus* monkeys, sacrificed on the 4th day.
Strain.—Cam, 20 per cent suspension.
NPW collected by Method 2.
Results of Primary Inoculation.—No symptoms; minimal lesions in 1/4.
Results of Subinoculations.—Gasserian ganglia: right, positive; left, positive. *NPW:* 2, 3, and 4 day, negative. *Stools:* 4 day, negative (others not tested).

EXPERIMENT 2.—4 *cynomolgus* monkeys, sacrificed on the 4th day.
Strain.—Wis '45, 20 per cent suspension.
NPW collected by Method 3.
Results of Primary Inoculation.—No symptoms; minimal CNS lesions in 1/3; CNS from fourth animal lost.
Results of Subinoculations.—Gasserian ganglia: right, positive; left, positive. *NPW:* 4 day, ? positive; 2 and 3 day, negative. *Stools:* 2, 3, and 4 day, negative.

EXPERIMENT 3.—4 *cynomolgus* monkeys, sacrificed on the 4th day.
Strain.—Wis '45, 20 per cent suspension.
NPW collected by Method 3.
Results of Primary Inoculation.—No symptoms; *no* CNS lesions.
Results of Subinoculations.—Gasserian ganglia: right, positive; left, positive. *NPW:* 4 day, positive; 2 and 3 day, negative. *Stools:* 4 day, positive; 2 and 3 day, negative.

EXPERIMENT 4.—4 *cynomolgus* monkeys, tested for early excretion of virus and for infectibility by the method.
Strain.—Wis '45, 20 per cent suspension.
NPW collected by Method 3, from approximately 17 to 24 hours after inoculation, as a test of early excretion.
Results of Primary Inoculation.—No symptoms at 1 day; 2/4 died at 18 hours and 4 days, respectively; 2/4 developed typical paralytic poliomyelitis after 8 and 9 days incubation periods respectively.
Results of Subinoculations.—Gasserian ganglia: not tested. *NPW:* 1 day, negative. *Stools:* not tested.

EXPERIMENT 5.—(Control). 2 *cynomolgus* monkeys, in which virus was dropped into the subarachnoid space directly over the ganglion, but without trauma to it, as a control of infectibility of both the ganglion and the CNS from the cerebrospinal fluid when no susceptible centers were traumatized.
Strain.—Wis '45, 20 per cent suspension.
NPW not collected.

Results of Primary Inoculation. C3-93. Symptoms: none; sacrificed at 1 month. Histological examination: Gasserian ganglia, no lesions greater than in normal controls (47); CNS, negative for polio (a few minute infiltrative foci were found in areas not usually associated with poliomyelitic infection). C3-94. Symptoms: none, sacrificed at 1 month. Histological examinations: Gasserian ganglia, no lesions greater than in normal controls; CNS, negative.

Summary of Results.—(Chart 2). In 1 of 4 experiments, direct inoculation into the Gasserian ganglion was followed, at 4 days, by excretion demonstrated in both the NPW and stools, and in another, a questionable positive was obtained, also on the 4th day, from the NPW. No symptoms of poliomyelitis had occurred in any of the animals. No excretion was demonstrated in the first 24 hours. In one case, no CNS lesions were present, while NPW, stools, and

EXPERIMENT	PRIMARY INOCULATIONS			SUBINOCULATIONS						
	Virus Am't Conc.	Symptoms	CNS lesions	Material	Day 1	Day 2	Day 3	Day 4	Gasserian ganglia Right	Gasserian ganglia Left
1. (4 Cyn)*	Cam <0.05 20%	0	Day 4 ●○○○	Npw / St		□□ / ND	□□ / ND	□□ / □□	Day 4 ■■	■■
2. (4 Cyn)	Wis '45 <0.05 20%	0	Day 4 ●○○	Npw / St		□□ / □□	□□ / □□	? ■□ / □□	Day 4 ■	■□
3. (4 Cyn)	Wis '45 <0.05 20%	0	Day 4 ○○○○	Npw / St		□□ / □□	□□ / □□	■■ / ■■	Day 4 ■	■■
4. (4 Cyn)	Wis '45 <0.05 20%	0		Npw	□□					

* Cynomolgus

CHART 2. Inoculation of right Gasserian ganglion.

both ganglia were positive, suggesting, as in the comparable infraorbital dip experiments, that excretion was followed by reinvasion from the surface. The control experiments indicate that virus escaping into the subarachnoid space at the time of operation probably played no part in the infective process.

Inoculation into the Celiac Ganglion

EXPERIMENT 1.—4 *cynomolgus* monkeys.

Strain.—Cam, 20 per cent suspension.

Results of Primary Inoculation.—C2-90. Symptoms: 5 days, coarse intention tremor of limbs when disturbed; 9 days, same, plus occasional head tremor; 11 days, marked tremor, slight weakness legs. Histological examination: left (ipsilateral) celiac ganglion, ++ (not greater than normal controls (47)); right celiac ganglion, +++; sympathetic columns, lower thoracic cord, negative, but not examined at all levels. C2-91. No symptoms; not sacrificed. C2-92. No symptoms, not sacrificed. C2-93. Symptoms: 13 days, occasional tremor of legs; 15 days, right hand paralyzed, right arm weak, no tremor. Histological

examination: celiac ganglia, negative; sympathetic columns thoracic cord, T_{10} to T_{12}, negative.

Results of Subinoculations.—Stools: pooled 6 and 7 days, pooled 8 and 9 days, pooled 10 and 11 days, and 12 days, negative. Stools from C2-90 were not included in the 11 and 12 day pools.

EXPERIMENT 2.—4 *cynomolgus* monkeys.

Strain.—Wis '45, 20 per cent suspension.

Results of Primary Inoculation.—C3-00. No symptoms, not sacrificed. C3-22. Symptoms: none, but found dead at 9 days with heavy filarial infestation. Histological examination: celiac ganglia, negative; anterior horns cord, positive; sympathetic columns thoracic cord, T_{10} to T_{12}, negative. C3-24. Symptoms: 9 days, slight tremor, aphonia, right facial weakness, legs weak. Histological examination: left (ipsilateral) celiac ganglion, ++; right celiac ganglion, + (neither greater than normal controls); sympathetic columns thoracic cord, T_{10} to T_{12}, positive. C3-25. Symptoms: 11 days, tremor, legs markedly weak, arms slightly weak. Histological examination: celiac ganglia, negative; sympathetic columns thoracic cord, T_{10} to T_{12}, positive.

Results of Subinoculations.—Stools: 7, 8, and 9 days, positive; pooled 1 and 2 days, pooled 3, 4, and 5 days, and 6 days, negative.

EXPERIMENT 3.—4 *cynomolgus* monkeys.

Strain.—Wis '45, 20 per cent suspension.

Results of Primary Inoculation.—C3-97. Symptoms: 10 days, aphonia, arms weak, legs paralyzed. Histological examination: celiac ganglia, negative; sympathetic columns thoracic cord, T_6 to T_{12}, positive. C3-98. Symptoms: 6 days, left arm paralyzed, right arm weak; 7 days, found dead. Histological examination: left (ipsilateral) celiac ganglion, +; right celiac ganglion, ++ (neither greater than normal controls); sympathetic columns thoracic cord, T_6 to T_{12}, positive. C4-10. Symptoms: 7 days, legs paralyzed, arms weak. Histological examination: left (ipsilateral) celiac ganglion, +; right celiac ganglion, +; (neither greater than normal controls); sympathetic columns thoracic cord, T_6 to T_{12}, positive. C4-11. No symptoms, not sacrificed.

Results of Subinoculations.—Stools: 4, 5, and 6 days, positive; 1, 2, and 3 days, negative.

Summary of Results.—(Chart 3). In 2 of 3 experiments, virus was detected in the stools beginning at 4 and 7 days respectively after inoculation into one of the components of the celiac ganglion and 2 days before the onset of symptoms. In both, excretion was demonstrated for a period of 3 consecutive days. While the NPW were not tested, it seems probable that excretion followed sympathetic pathways to the intestinal surface. It is probable (see under Methods) that the portion of the celiac ganglion histologically examined was not the same as that originally inoculated.

Intrathalamic Inoculation

EXPERIMENT 1.—4 *cynomolgus* monkeys.

Strain and Amount.—Cam, 0.4 ml. 20 per cent suspension, 0.2 ml. each side.

NPW collected by Method 2.

Results of Primary Inoculation.—C2-76: 8 days, tremor; 9 days, legs paralyzed, arms weak. C2-77: 9 days, slight tremor; 11 days, tremor more marked, no weakness noted. C2-78: 9 days, slight tremor, left arm slightly weak; 12 days, marked tremor, slight incoordination legs. C2-79: 9 days, tremor; 10 days, legs weak.

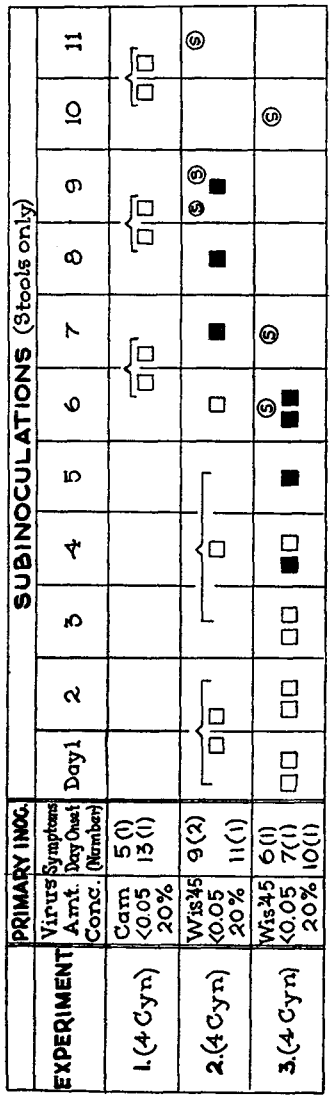

CHART 3. Inoculation of celiac ganglion.

Results of Subinoculations.—NPW: pooled 4 and 5 days, positive; 4 and 5 days tested separately, and 6 days, negative. *Stools:* pooled 4 and 5 days, and 6 days, negative.

EXPERIMENT 2.—4 *cynomolgus* monkeys.
Strain and Amount.—Wis '45, 0.1 ml. 20 per cent suspension into right thalamus only.
NPW collected by Method 3.
Results of Primary Inoculation.—C3-57: 6 days, arms, upper back paralyzed. C3-58: 4 days, right arm paralyzed, died under nembutal. C3-59: 5 days, tremor; 6 days, tremor, incoordination; 7 days, complete paralysis. C3-60: 6 days, tremor, right leg paralyzed; 7 days, complete paralysis.
Results of Subinoculations.—NPW: 5 days, positive; 2, 3, and 4 days, negative. Washings from C3-58 were included in the negative 4 day pool; on the other hand, washings from C3-59 were *not* included in the positive 5 day pool, which was from C3-57 and C3-60 only. *Stools:* 2, 3, and 4 days, negative; no stool was available on the 5th day.

EXPERIMENT 3.—4 *cynomolgus* monkeys.
Strain and Amount.—Wis '45, 0.1 ml. of 1 per cent suspension into right thalamus only.
NPW collected by Method 3.
Results of Primary Inoculation.—C4-62: 5 days, tremor, ? palatal paralysis; 6 days, tremor, left hand extensors paralyzed, ? palatal or masticator paralysis; 7 days, complete paralysis. C4-63: 5 days, tremor; 6 days, tremor, legs weak; 9 days, complete paralysis. C4-64: 5 days, ? mild bilateral ptosis; 7 days, tremor, still ? ptosis, left internal strabismus, right leg weak; 8 days, complete paralysis. C4-65: 5 days, ? tremor; 6 days, marked tremor; 7 days, extreme tremor; 9 days, same plus both legs very weak, left arm slightly weak.
Results of Subinoculations.—NPW: 2, 3, and 4 day, negative; none collected on the 5th day because of the condition of the animals. *Stools:* 2, 3, and 4 day, negative. (The 4th day collection was extended to 12 noon of the 5th day.)

Summary of Results.—(Chart 4). In 2 of 3 experiments intrathalamic inoculation was followed by nasopharyngeal excretion of virus, once at 4 to 5 days and once at 5 days. In both of these experiments, excretion preceded the onset of symptoms.

Intravenous Inoculation

EXPERIMENT 1.—4 *cynomolgus* monkeys.
Strain and Amount.—Wis '45, 10 per cent suspension, clarified by brief high speed centrifugation, 6 to 10 ml. (24,000 to 40,000 P. D_{50}).
NPW collected by Method 3.
Results of Primary Inoculation.—C4-48: 15 days, tremor; no progression. C4-49: 11 days, left arm paralyzed, shoulder girdle and back weak. C4-50: 16 days, tremor; no progression. C4-51: 8 days, almost complete paralysis.
Results of Subinoculations.—NPW: 2 to 4 hours and 24 to 27 hours after inoculation, negative. *Stools:* 0 to 24 hours and 24 to 48 hours after inoculation, negative.

Comment.—This experiment was designed as a rigorous control test of the possibility that excreted virus might reach the surfaces by way of the blood stream, an event which under the experimental conditions would be expected to occur, if at all, within 48 hours after injection. The negative results, in view

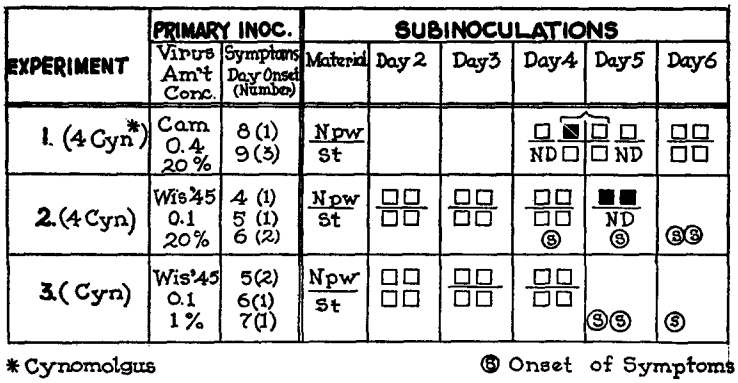

CHART 4. Intrathalamic inoculation.

TABLE I

Virus in Nasopharyngeal Washings and Stools

Route of inoculation (No. of experiments)	Days										Total
	0	1	2	3	4	5	6	7	8	9	
1. Infraorbital nerve (8)											
NPW		—	1/7	2/7	5/7*						8/21 (38 per cent)
Stools		—	2/6	1/7	4/6						7/19 (37 per cent)
2. Gasserian ganglion (4)											
NPW		0/1	0/3	0/3	1/3						1/10 (10 per cent)
Stools		—	0/2	0/2	1/3						1/7 (14 per cent)
3. Thalamus (3)											
NPW		—	0/2	0/2	0/3	2/2‡	0/1				2/10 (20 per cent)
Stools		—	0/2	0/2	0/3	0/1	0/1				0/6 (0 per cent)
4. Celiac ganglion (3)		0/2	0/2	0/2	1/2	1/2	1/3	1/2	1/2	1/2	6/19 (31 per cent)
5. Intravenous (1)											
NPW	0/1	0/1									0/2 (0 per cent)
Stools	0/1	0/1									0/2 (0 per cent)

Numerator: number of pools positive. Denominator: number of pools tested.

* 2 to 5 day pool (positive) from *rhesus*, entered as 4th day.

‡ 4 and 5 day pooled in one positive experiment, entered as 5th day.

of the very large amounts of virus administered, appear to us to carry considerable evidential weight against excretion from the blood.

The results of all the experiments are summarized in Table I.

DISCUSSION

Since primary exposure of the respiratory and alimentary surfaces and walls was excluded in our experiments, all inoculations being parenteral, the excretion of virus which occurred both in pharynx and intestine as early as the 2nd and with some regularity by the 4th day after inoculation and always before the onset of symptoms, cannot be ascribed to extraneural infection and multiplication in these structures. The focus of infection immediately responsible for the excretion of virus in all four types of experiment, including the intrathalamic, can be placed with some assurance in the peripheral ganglia supplying nerve fibers to the walls of the alimentary tract. In another study, as yet unpublished, of intrathalamic inoculation we found virus in the Gasserian ganglia 5 and 7 days after exposure; hence, while these ganglia were not tested in the present experiments with this route, we have reason to believe that here, too, the source of virus excreted into the pharynx was ganglia supplying this region. It seems reasonable to suppose that other ganglia with surface connections, such as the nodose of the vagus, the superior cervical sympathetic and the submucous plexus of Meissner[3] might likewise become infected and serve as sources of excreted virus.

In this process, as shown by four of our experiments (infraorbital dip series, 5, 6, and 8, direct Gasserian inoculation series, 3), the CNS need not be implicated at all, the sole source of eliminated virus being the peripheral ganglia. This observation, applied to the human disease, may well clarify some of the puzzling and controversial features such as the site of infection and the source of excreted virus in asymptomatic cases, in the early presymptomatic phases, in the initial phase of diphasic ("dromedary," "Bactrian") cases, in mild cases without central nervous symptoms and with negative spinal fluids, and in the cases of "summer grippe or sore throat" without CNS symptoms reported by Sabin and Steigman (48). It seems probable to us that in at least some such instances, no CNS involvement is present and that in all of them the immediate source of excreted virus is the peripheral ganglia. Moreover, when CNS invasion has occurred and centrifugal spread to the ganglia has ensued, these structures may again serve, as suggested by our observations with intrathalamic inoculation, as sources for excretion. However, the relatively small percentage of experiments with demonstrated excretion after intracerebral inoculation both in our study and in that of Melnick (37) suggests that the CNS may be somewhat less important as a source of excretion than the peripheral ganglia.

The broad concept of poliomyelitic disease which regards the peripheral

[3] The Gasserian ganglion supplies the nasal and oral cavities, including the soft palate and uvula; the nodose, the posterior pharyngeal wall, the tracheobronchial tree, and the alimentary tract from the esophagus to the mid portion of the large bowel; the superior cervical sympathetic ganglion, the surface of the nose, mouth, and pharynx; and the submucous plexus, the alimentary surfaces from the upper esophagus to the rectum.

ganglion both as the site of the initial infection (as previously stressed by us) (49, 50) and as the immediate source of excretion of virus affords a unitarian explanation of its various peculiar and characteristic clinical and pathological features, including secondary invasion of the CNS, that is consistently based on the established neurotropic properties and axonal conduction of the virus. The older explanation of CNS invasion as formulated by Draper (51) and apparently still widely accepted, in which the initial infection is regarded as "systemic" or "general" (that is, extraneural with some vague, undefined locus) rests, it should be noted, on the now untenable (52) assumption of penetration of the "blood-brain barrier" by blood-borne virus.

The question whether virus appears in the stools as a result of swallowing pharyngeal secretions or is directly excreted into the gut is perhaps largely academic, but poses a problem of some interest. In our first three groups of experiments, the former was probably the case, taking into account the primary foci and early stages of infection, even when virus was detected only in the stools. Negative subinoculation tests generally carry less weight than positive, especially with pharyngeal washings. After celiac inoculation excretion was probably directly into the gut, although it occurred relatively late in two experiments at a time when infection may have reached the brain stem and the cranial nerve ganglia. In human poliomyelitis we are inclined to believe that as a rule infection enters through the pharynx and that the first excretion of virus occurs in the same area, reaching the intestine by ingestion from that source. That excretion occurs at later stages of infection directly or exclusively into the intestine seems possible, but not necessarily true. Our present studies do not decide the point.[4]

A phenomenon of considerable interest, showing the rapidity of excretion and of reinvasion, without CNS involvement, noted in three of our experiments after infraorbital nerve dip, deserves comment. In these, virus was found on the 3rd or 4th day not only in the ipsilateral (right) ganglion but also in the contralateral. Virus had been detected in the nasopharyngeal washings, stools, or both in all three cases, and careful examination of the CNS had shown no lesions. Since no cross-connections outside the CNS between the paired ganglia are known, it can be deduced that virus had descended from the right, primarily infected, ganglion to the pharynx whence it had reinvaded peripheral nerves leading to the left ganglion, all within a 3 to 4 day period. This observa-

[4] It is theoretically possible that virus continues to be excreted in the pharynx for a fairly long time but cannot as a rule be detected by ordinary subinoculation beyond the few days after onset of symptoms because it has been neutralized by specific antibodies in the nasopharyngeal secretions that begin to appear toward the end of the first week (53). The neutralization process does not necessarily destroy virus, which can be released from the antibody-virus complex by centrifugation or dilution; the possibility may therefore be considered that virus may be freed during the course of digestion in the stomach or upper intestine and then become detectable in the stools by subinoculation. This possibility remains to be explored.

tion suggests the existence of an aspect of the pathogenesis of poliomyelitis that has not, we believe, been previously envisioned, that *the invasive process consists not merely of the initial entry of virus but also of subsequent reinvasions*, following the initial excretion of virus, into peripheral ganglia other than those first involved, each of which in turn could act as a fresh source of excretion and as another potential pathway to the CNS—a serial process that could hardly be checked until the body's defenses, humoral or cellular, had formed and become effectively mobilized.

That excretion from ganglia to the mucosal surfaces occurs *via* peripheral nerves seems probable for several reasons. Comprehensive studies by Ward Horstmann, and Melnick (54) and by others (55, 56) have led to the conclusion that blood invasion by poliomyelitis virus is extremely rare and not an important part of the disease process in man. Our own experiments show that even enormous quantities of virus in the blood stream failed to result in excretion in the nasopharyngeal secretions and stools. Excretion *via* lymphatic channels also seems improbable, since these normally drain into the regional lymph nodes and thence into the lymph ducts and blood stream (57). Yoffey and Drinker (58), however, were unable to detect poliomyelitis virus in the lymph ducts of monkeys infected intracerebrally. Moreover, the practical importance of excretion of virus from the cerebrospinal fluid through the perineural lymph spaces to the nasopharyngeal surfaces, even if, as recently suggested by Yoffey (59), it were theoretically possible, must be minimal since human cerebrospinal fluid virtually never contains poliomyelitis virus (41). On the other hand, positive evidence of centrifugal nerve-borne spread of poliomyelitis virus has been obtained by Burnet and Jackson (60) who detected it in peripheral nerves (sciatic, vagus, sympathetic) of monkeys at the time of paralysis after intraocular and intracerebral inoculation; its presence in the sciatic can only be reasonably explained by centrifugal spread from the infected cord since this nerve has no mucous membrane connections to serve as sources of centripetal propagation. We have repeated and confirmed these observations with both brachial and sciatic nerves of monkeys sacrificed at the time of paralysis, in experiments that will be reported in another study.

The precise manner in which virus in the terminal nerve filaments is extruded into the lumen of the alimentary tract, while not demonstrable by direct observation, would appear to find an adequate explanation in the constant and lively desquamation of the superficial mucosal epithelium, in which the finest nerve teledendria between these cells must participate.

Further research is required to explain the prolonged, though not indefinite, period over which poliomyelitis virus continues to be excreted. Perhaps virus persists longer in the peripheral than in the central nervous system, and perhaps longer in the peripheral nerves than in the ganglia. In nerves, as shown by Weiss and Hiscoe (61), a very slow centrifugal movement of the axoplasm occurs. These are problems which we propose to investigate in the near future.

It remains to be said that while our experiments do not disprove the extraneural theory of virus excretion—and we know of no practicable *in vivo* method by which it can be disproved—they do make its postulation unnecessary to account for excretion, which can be adequately explained on the basis of the proved neurocytotropic properties of poliomyelitis virus and its proved mode of spread through axons.

The present study again emphasizes the importance of the peripheral nervous system in the pathogenesis of poliomyelitis.

SUMMARY AND CONCLUSIONS

Excretion of poliomyelitis virus has been demonstrated in monkeys after four different parenteral routes of inoculation. Virus has been found in both the pharyngeal secretions and the stools after infraorbital nerve dip and after inoculation of the Gasserian ganglion; in the pharyngeal secretions after intrathalamic inoculation; and in the stools after inoculation of the celiac ganglion.

Excretion began as early as the 2nd and as late as the 7th day after inoculation, in all instances before the onset of symptoms.

The immediate source of the excreted virus appeared to be infected peripheral ganglia with neural connections to the mucous membranes of the upper and lower portions of the alimentary tract, notably the pharynx.

Primary infection of the body surfaces was excluded in the experiments and therefore could not account for the excretion of virus.

The mode of elimination was probably by centrifugal spread through axons of peripheral nerve fibers and not by way of the blood stream or lymphatics.

Evidence was obtained that when excretion of virus has once occurred, reinvasion from the implicated surface to other, previously uninfected peripheral ganglia ensues, thus providing new sources for excretion and other potential pathways for invasion of the CNS. It is suggested that such reinvasion may occur serially until the immunological defenses come into play.

Our experiments lend support to the view that during the initial stage of poliomyelitis, and perhaps throughout its course in some cases, *e.g.* the asymptomatic and the mild cases without central nervous symptoms, infection is confined to the *peripheral* nervous system. Involvement of the CNS when it occurs is a secondary phase of the infective process and is not a necessary prelude to elimination of the virus.

Excretion is explainable on the basis of the established neurocytotropism and axonal conduction of the virus without resort to the hypothesis of extraneural infection.

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