# POLIOMYELITIS IN THE CYNOMOLGUS MONKEY

## I. Comparison of the Upper Portion of the Alimentary Tract with Its Lower, Gastrointestinal Portion As a Portal of Entry, with Special Reference to the Peripheral Ganglia*

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The present study was planned to test the relative susceptibility of different portions of the alimentary tract to penetration by poliomyelitis virus. For this purpose the *cynomolgus* monkey (*Macaca irus*) was selected because of its proved susceptibility to infection by the alimentary route.

Levaditi, Kling, and Lépine (1); Levaditi, Kling, and Hornus (2); Vignec, Paul, and Trask (3), and, most recently, Sabin and Ward (4) have all produced poliomyelitis in *cynomolgus* monkeys by simple feeding, the last of these having obtained infection in 6 of 15 animals fed virus in banana. In addition, Burnet, Jackson, and Robertson (5) induced two takes in 6 *cynomolgus* monkeys by dropping 1 cc. of thick virus suspension into the mouth, and Saddington (6), and also Flexner (7) each succeeded in infecting a *cynomolgus* by dropping large amounts in the mouth over a period of several days. All these experiments were non-traumatic, involving no injury to the mucous membranes and are reasonably comparable with "natural" infection. The use of the stomach tube, or the abrasive application of virus, as in the earlier experiments of Burnet and Jackson (8) introduces complicating and possibly misleading factors which we desired to avoid.

The methods of inoculation which we employed (excepting the final intracerebral injection) were designed with three points in mind: they were to be as nearly non-traumatic as possible; they were to exclude the olfactory pathway when necessary, and they were either to limit exposure as far as possible to a single area of the alimentary tract or to exclude certain portions of it from exposure at a given time. At first we depended for diagnosis on overt clinical manifestations of the disease, such as fever, weakness, tremors, or frank paralysis corroborated by histological examination; later, we sacrificed some animals without overt or characteristic clinical manifestations and depended upon histopathology. Following McClure's (9) report on the importance of lesions in the peripheral ganglia, we included a systematic study of these structures in most of our cases. Sabin's *Per* strain, proved to be highly virulent for *cynomolgus* (92 per cent of paralytic takes after intracerebral inoculation in 25 normal monkeys)

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was used throughout, in the 4th to 9th passages. This is the same strain as was used by Sabin and Ward (4) in their feeding experiments referred to above.

The experiments were carried out in successive stages as described below and summarized in the accompanying table.

TABLE I

*Summary of Exposures to Virus, Fate of Animals, and Evidences of Poliomyelitis Infection*

| Monkey No. | Capsules | First tongue swab | Second tongue swab | Enema | First spray | Inhalation | Second spray | Intracerebral inoculation | Died/killed | Clinical poliomyelitis | Lesions | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | CNS | Ganglia |
| | 1941 | | | 1942 | | | | | | | | |
| C6 | 4/5 | | | | | | | | D 5/15/41 | 0 | 0 | −− |
| C7 | 4/5 | | | | | | | | D 5/18/41 | 0 | 0 | −− |
| C10 | 4/5 | | | | | | | | D 5/18/41 | 0 | 0 | −− |
| C11 | 4/5 | | | | | | | | D 5/12/41 | 0 | 0 | −− |
| C15 | 4/5 | | | | | | | | D 9/15/41 | 0 | 0 | −− |
| C62 | 9/15 | | | | | | | | K10/29/41 | 0 | 0 | −− |
| C56 | 11/1 | | | | | | | | K 1/ 9/43 | 0 | 0 | ± |
| C57 | 11/1 | | | | | | | | K 6/14/43 | 0 | −− | −− |
| C8 | 4/5 | 5/20 | | | | | | | D 7/1 /41 | 0 | −− | −− |
| C13 | 8/15 | 9/15 | | | | | | | D12/ ?/41 | 0 | −− | −− |
| C18 | 4/5 | 5/20 | | | | | | | K 6/ 2/41 | + | + | + |
| C23 | 4/5 | 5/20 | | | | | | | D 7/ 1/41 | 0 | −− | −− |
| C48 | 8/15 | 9/15 | | | | | | | D11/ 3/41 | 0 | 0 | −− |
| C49 | 8/15 | 9/15 | | | | | | | D12/ 3/41 | 0 | 0 | −− |
| C51 | 8/15 | 9/15 | | | | | | | K10/10/41 | 0 | 0 | ± |
| C19 | 4/5 | 5/20 | 9/15 | 1/22 | | | | | K 2/13/42 | 0 | 0 | + |
| C21 | 4/5 | 5/20 | 9/15 | 1/22 | | | | | D 3/19/42 | 0 | 0 | + |
| C52 | 8/15 | 9/15 | | 1/22 | | | | | K 2/13/42 | 0 | 0 | + |
| C54 | 8/15 | 9/15 | | 1/22 | | | | | D 3/14/42 | 0 | 0 | + |
| C9 | 4/5 | 5/20 | 9/15 | 1/22 | 3/25–28 | | | | K 3/30/42 | + | + | + |
| C53 | 8/15 | 9/15 | | 1/22 | 3/25–28 | 4/24 | | | K 5/ 8/42 | + | + | + |
| C17 | 4/5 | 5/20 | 9/15 | 1/22 | 3/25–28 | 4/24 | 5/25–28 | 6/16 | K 7/17/42 | 0 | + | + |
| C20 | 4/5 | 5/20 | 9/15 | 1/22 | 3/25–28 | 4/24 | 5/25–28 | 6/16 | K 6/25/42 | + | + | −− |
| C24 | 8/15 | 9/15 | | 1/22 | 3/25–28 | 4/24 | 5/25–28 | 6/16 | K 7/17/42 | 0 | + | + |
| C50 | 8/15 | 9/15 | | 1/22 | 3/25–28 | 4/24 | 5/25–28 | 6/16 | K 7/17/42 | 0 | + | + |
| C55 | 8/15 | 9/15 | | 1/22 | 3/25–28 | 4/24 | 5/25–28 | 6/16 | K 7/17/42 | 0 | + | + |

### *Esophageal Administration of Capsules Containing Dried Virus*

The purpose of this procedure was to introduce virus into the alimentary tract without contamination of the mouth and pharynx, thus excluding the oropharyngeal portal of entry and confining exposure to the gastrointestinal tract. In past experiments the stomach tube has been used for this purpose. To this there are two objections: first, possible trauma in the mouth, pharynx,

and esophagus permitting entry at the site of trauma, and, second, contamination of the mouth and pharynx with virus when the tube is withdrawn. By placing the virus contained in capsules covered with a digestible fat, directly into the esophagus contamination of the oropharynx was, we believe, entirely avoided (none of the animals vomited) and esophageal peristalsis might be expected to carry the material beyond the point of any possible trauma in the esophagus (in one control monkey, however, the capsules remained *in situ* until dissolved). Exposures to virus then would be limited to the stomach and intestines. Finally, the use of virus in small bulk and solid form would permit the material to stay in the stomach and to be subjected to the normal digestive processes in a more natural manner than when a large amount of liquid is injected into the stomach as is the case with gavage.

Fresh cords from *cynomolgus* monkeys that had been inoculated intracerebrally and had shown typical symptoms, were cut into small pieces and frozen in a salt-ice bath within 48 hours of removal from the animal. They were then dried from the frozen state over calcium chloride in a vacuum desiccator, kept in the refrigerator at all times. Cords were kept *in vacuo* 2 to 7 days before preparation of capsules.

Three dried cords in the case of the first experiment, and four for the others, were cut into very small pieces and thoroughly mixed. No. 4 (2 grain) gelatin capsules were tightly packed with this material. On the basis of the weight of an average cord, and the number of capsules filled, approximately 0.6 gm. of wet cord were contained in each. While it was impossible to avoid contamination of the outside of the capsules with virus, visible pieces of tissue were removed and each capsule was then coated heavily with a digestible fat ("Vream"[1]). Administration of the capsules was accomplished by inserting a metal cannula into the esophagus of the unanesthetized monkey and pushing them into place through it by means of a closely fitting obturator. Three capsules were given each monkey, the total amount of dried material being the equivalent approximately of 1.8 gm. or ¼ to ⅓ of a fresh cord.

Test capsules filled with barium and coated with fat and inserted esophageally in monkeys in the same manner were observed under the fluoroscope. In three instances the capsules entered the stomach almost immediately, dissolving in 10 to 20 minutes. In one, barium entered the small intestine in 1 hour and 15 minutes after feeding and in another about an hour later. It required more than 7 hours to reach the large intestine, throughout which it was present at 24 hours. In one instance the capsules remained in the esophagus for over an hour and dissolved; at 2 hours the barium was in the stomach.

The contents of 2 capsules were suspended in 10 cc. of saline solution and 0.5 cc. of the suspension was inoculated intracerebrally in a *cynomolgus* monkey. Paralysis began on the 12th day and was complete on the 14th. A similar test on a *rhesus* monkey was negative but the *Per* strain has proved in our hands to be much less virulent for *rhesus* than for *cynomolgus*. In a previous study, we found that dried virus kept *in vacuo* retains its activity for over a year.

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[1] Vream, described on the label as "a shortening made from hydrogenated vegetable oil," is made by Swift and Company, Chicago, Ill.

Twenty-six *cynomolgus* monkeys were given virus by capsule in the manner above described. One of them, C19, 5 days after ingestion of capsules, had a temperature of 103.5° for one day, a rise from the previous levels of 101.5–102.1°; and again during the period of 9 to 14 days after capsules, had a temperature ranging from 102.5–103.0°. No other accompanying symptoms of any sort were observed. This animal was killed and studied later. Our experience agrees with that of Sabin and Ward (10) in finding that rise in temperature alone is dependent on activity and climatic conditions rather than on infection. We therefore did not sacrifice this animal at once. None of the others showed any signs of infection and all survived 37 days or more. Five of those fed in the first experiment of April 5, 1941, died between May 12 and May 18 of unknown cause, and autopsy revealed no lesions in the CNS. Another, C62, fed on September 15, 1941, was well until October 13; by October 28 it had become very thin and generally weak, and was sacrificed. Autopsy showed no lesions in the CNS. C56, fed on November 11, 1941, displayed coarse tremors and spasticity on November 29 and the next 2 days and again on January 9, 1943, when it was killed. The serum calcium level of 5.6 mg. per cent showed that the animal was suffering from tetany. No lesions were found in the CNS. Minimal (+) lesions were found in one of the cervical and in one of the thoracic spinal ganglia; in one of the lower cervical and in one of the lumbar sympathetic ganglia. None was found in the Gasserian, nodose, geniculate, upper cervical sympathetic, celiac, thoracic sympathetic, or lumbar spinal ganglia. C57, fed on November 1, 1941, never showed any signs of infection and survived to June, 1943.

Attempts were made to recover virus from the stools of some animals after administration of capsules.

*Cynomolgus* monkeys C13, C15, C24, C48, C49, C50, C51, C52, C53, C54, and C55 were fed capsules on August 15, 1941. Stools collected from these monkeys on August 17 were pooled, made into a 20 per cent suspension, and the supernate from this was treated with ether, according to the method of Trask, Vignec, and Paul (11). 6 cc. of the material, after removal of ether, was injected intraperitoneally into *cynomolgus* monkey C70. The animal died 4 days later. No lesions were found in the cord or medulla. 6 cc. of the same material were injected intraperitoneally into *rhesus* monkey R246; this animal remained well.

*Cynomolgus* C62 was fed capsules in the morning of September 15, 1941. All stools passed between that afternoon and 9 a.m., September 17, were pooled, made into a heavy suspension, and similarly treated. 10 cc. of the material were injected intraperitoneally into *cynomolgus* monkeys C56 and C57 on September 18. In addition, 1 cc. of the same material was inoculated intranasally in the same animals on 3 successive days, September 18 to 20. Both animals remained well. *Cynomolgus* monkeys C56 and C57 were fed capsules on October 31, 1941. Stools passed by them 5 to 48 hours later were pooled, made into 100 cc. of suspension, and not treated with ether. On November 8, 9, 10, 11, 12, and 14, 0.5 cc. of the supernate from the suspension was

inoculated intranasally in *cynomolgus* monkeys, C85 and C86, the mucous membranes being rubbed with a pipe cleaner by the method of Howe and Bodian (12). C85 had a slight elevation of temperature on November 14 and 15 and on the afternoon of the 15th was found lying on the floor of the cage. It died on November 17. Sections of the cord and medulla were negative. C86 remained well and was sacrificed on November 22. Sections of the olfactory bulbs, cord, and medulla were negative. In the Gasserian ganglia, two perivascular infiltrations, a few minute small-cell infiltrations, some degenerated neurons, and one neuronophagic focus were seen, representing possible invasion of the fifth nerve from the nasal mucosa.

*Summary.*—Administration of dried virus to 26 *cynomolgus* monkeys in a manner excluding contact with the mucous membranes of the oropharynx failed to produce any evidence of poliomyelitic infection. Tests of the stools of animals so treated failed to demonstrate the presence of virus except in one doubtful instance.

### *Lingual Application of Virus*

The ends of the gustatory fibers of the VII, IX, and X cranial nerves in the taste buds are directly exposed to fluid materials in the mouth and pharynx and might therefore constitute a point of special vulnerability to virus contained in ingested food or drink. To test this possibility the 18 surviving monkeys were subjected to the following procedure:

The olfactory mucosa was blockaded with zinc sulfate. 8 to 11 days later tight cotton swabs were dipped in the supernate of a 20 per cent suspension of *Per* virus (4th passage) and gently rolled over the dorsal surface of the tongue. An estimated amount of less than 0.8 cc. was applied to the tongue of each monkey. In spite of efforts to prevent it, some trauma to lips or gums was caused by the tongue depressor in 4 of the monkeys but the bleeding points were at once touched with 5 per cent tincture of iodine. This occurred in C18, described in the protocol below, which developed poliomyelitis.

The animals whose tongues were swabbed, and the dates, are shown in Table I. It will be noted that 5 monkeys received a second swabbing 4 months after the first. One animal, C18, developed poliomyelitis after a single treatment. One animal, C51, 25 days after the second swabbing, showed coarse tremors and spastic phenomena, mainly in the arms, but without fever, lasting about 2 hours. The symptoms were essentially the same as those seen in another animal with proved low-calcium tetany, but it was sacrificed for histological study. Excepting a single minimal lesion in one cervical sympathetic ganglion, the peripheral and central nervous systems were negative. The other 16 animals remained free of symptoms.

*Protocol, C18.*—1 per cent zinc sulfate solution was applied to the upper nasal passages on May 12, 1941, (olfactory blockade). On May 20, the tongue was swabbed with virus suspension. On May 26, the temperature which previously had not been

higher than 101.2° rose to 102.2° and on the following days remained above this level (maximum 102.8°). In the afternoon of June 1, the legs became almost completely paralyzed and the arms were weak. Marked coarse tremors of the entire body were observed. The next morning paralysis was complete excepting in the hands and the animal was sacrificed by exsanguination. Subinoculations were made of the following tissues, the result and the day of paralysis in the subinoculated animal being noted in parentheses: cervical cord (+, 13); thoracic cord (+, 14); lumbar cord (+, 8); lumbar spinal ganglia (+, 11); thoracic sympathetic ganglia (reinoculation, +, 16); cervical spinal ganglia (0); thoracic spinal ganglia (0); celiac ganglion (0); lumbar sympathetic ganglia (0).

Histological examination was made of tissues not used for subinoculation. In the CNS, the olfactory bulbs (examined by serial section) and cerebellum were negative. Marked, typical lesions, including perivascular and parenchymal infiltrations and neuronophagia were found throughout the brainstem, including thalamus, hypothalamus, midbrain, pons, medulla (where they were heaviest), and throughout the spinal cord. Nearly all portions of the medulla were involved, including the hypoglossal nucleus, reticular substance, olive, nucleus ambiguus, spinal nucleus of the fifth nerve, dorsal motor nucleus of the vagus (slight), and the nucleus of the tractus solitarius. In the peripheral system, the spinal ganglia showed lesions (probably centrifugal) in the thoracic and lumbar levels but none was found in the cervical. The celiac ganglion showed none. Unfortunately, the geniculate, petrosal, and nodosal ganglia (which receive fibers from the taste buds) were not examined.

Six animals of the series died or were killed 2 months or more after lingual swabbing without undergoing further exposures, at the times shown in Table I. None had shown any symptoms of poliomyelitis. In 3 no autopsy was performed, in the other 3 no lesions were found in the CNS, and in one of them no lesions were found in the peripheral system (this was not examined in the other two).

*Summary.*—One of 18 *cynomolgus* monkeys exposed to virus by application to the tongue developed poliomyelitis. So rapid and extensive was the spread of the disease after symptoms appeared that, although the animal was sacrificed about 18 hours after the onset of symptoms and less than that after paralysis had begun, the portal of entry was suggested better by the sites where lesions were absent than by those where they were present. Thus, the absence of lesions in the celiac ganglion and olfactory bulbs indicates that entry had not been through the intestine and olfactory mucosa. Under these conditions, the lingual site of exposure and the very heavy lesions in the medulla probably permit the inference that entry had occurred through the upper part of the alimentary tract. It is interesting that the legs were most heavily involved while no paralysis of the cranial nerves was noted.

### *Administration of Virus by Enema*

As a test of the permeability of the lower intestinal tract, the 11 monkeys surviving from preceding experiments were given enemas of 5 cc. of the super-

nate from a 20 per cent suspension of *Per* virus in its 4th passage, on January 22, 1942.

A No. 13 French catheter was inserted as far as it would go. In a test under the fluoroscope, the catheter was seen to reach the cecum. In some cases it perhaps was not inserted so far but probably reached the hepatic flexure in all. None of the animals had symptoms but 2, C52 and C19, showed a rise of temperature to 103.5° and 103.7° on February 10 (the room temperature was high on that day). The temperature returned to its previous levels on the next day but they were both sacrificed on February 13 for investigation, 22 days after the enema.

The pooled contents of the large intestines of these 2 monkeys were suspended in water and the supernate instilled intranasally daily for 8 successive days into two *rhesus* monkeys with negative results. Histological examination of the CNS was negative in both C19 and C52. In C19 minimal lesions were found in the thoracic and lumbar spinal ganglia, in the cervical thoracic and lumbar sympathetic ganglia, in the celiac ganglion (one small lesion only), while foci of moderate size with neuronophagia were found in the Gasserian and nodose ganglia. No lesions were found in the cervical spinal, geniculate, or petrosal ganglia. In C52, minimal lesions were found in the cervical, thoracic, and lumbar spinal ganglia and in the thoracic sympathetic ganglia. Moderate lesions with neuronophagia were present in the cervical sympathetic, Gasserian and nodose ganglia, and marked lesions with neuronophagia were found in the celiac. No lesions were found in the lumbar sympathetic and geniculate ganglia. Two other monkeys of the series, C21 and C54, showed no symptoms and died about 2 months later, shortly after zinc sulfate had been given them in preparation for the next procedure. In C21 the CNS was entirely negative. Minimal lesions were found in the cervical, thoracic, and lumbar spinal ganglia, in the lumbar sympathetic and in the nodose ganglia, with neuronophagia in the first two and last of these. The Gasserians showed moderate lesions with neuronophagia and neuron degeneration, and the celiac showed moderate lesions with neuron degeneration only. No lesions were found in the cervical and thoracic sympathetic, geniculate, and petrosal ganglia. In C54, minimal lesions were present in the thoracic spinal, cervical sympathetic (with neuronophagia), and Gasserian ganglia; moderate lesions in one upper thoracic sympathetic (with neuronophagia), and in two lumbar sympathetic ganglia (without neuronophagia). No lesions were found in the cervical and lumbar spinal, in the celiac, in the geniculate, in the petrosal, or in the nodose ganglia.

*Summary.*—In these experiments no clinical or histological evidence was obtained of infection involving the CNS following the introduction of virus into the large intestine. Four animals were sacrificed or died and were examined histologically. In all, lesions of varying degree were found in some of the peripheral ganglia. In all, the Gasserian ganglion showed lesions, which were of moderate degree (++) with neuronal damage in three and minimal (+) in one. In three, the celiac ganglion showed lesions, which were minimal in one, moderate (++) with neuron degeneration in one, and heavy (+++) with neuronophagia in one; no lesions were found in it in the fourth. No

lesions were found in the geniculate or petrosal ganglia (gustatory route). It would appear that invasion may have occurred in some of the monkeys through the intestine and also through the fibers of the fifth nerve through the mouth, but not through the nerves of taste. It cannot be stated with assurance whether the lesions in the celiac ganglia were derived from the initial administration of virus in capsules or from the enemas, but the lesions in the Gasserian ganglia presumably resulted from the earlier oral application.

### *Application of Virus to the Nose and Mouth by Spray*

The first oral (lingual) application of virus was in very small amounts. We desired to try larger amounts over a larger area.

For this purpose, we first treated the olfactory surfaces of the 7 surviving monkeys with zinc sulfate solution on March 14, 1942, and on 4 successive days, March 25 to 28, we sprayed the nasal and oral passages with an atomizer. The animals were under light ether anesthesia. The supernate from a 10 per cent suspension of pooled virus from the 4th to the 9th passage was used and the total amounts applied to each animal were 4 to 6 cc. One of the monkeys, C9, on the afternoon of March 30, 5 days after the first spray and 2 days after the last, showed typical preparalytic symptoms consisting of a rise of temperature, tremors, weakness of the arms, hands, legs, neck, and back. It was sacrificed immediately. This case has already been made the subject of a preliminary report (13), but the histological data are recapitulated below.

*Protocol, C9.*—The olfactory bulbs (serial sections), secondary olfactory centers, and brainstem down to the pons were negative. In the superior olivary nucleus there was a small perivascular infiltrate. In the dorsal motor nucleus of the vagus there was a small parenchymal infiltration, and in and near the nucleus of the tractus solitarius there were five lesions: three perivascular infiltrations, one of large size, and two parenchymal infiltrations. A perivascular infiltration was also seen in the spinal nucleus of the fifth nerve. Extensive lesions (+ + +) were found in the Gasserian and nodose ganglia and moderately large ones (+ +) in the petrosal ganglion and one upper thoracic sympathetic. Small lesions (+) were found in one geniculate ganglion, the cervical and lumbar sympathetics, and thoracic spinal ganglia, and moderate sized ones in one upper thoracic sympathetic. No lesions were found in the cervical spinal, lumbar spinal, or celiac ganglia. The lesions in the Gasserian, nodose, geniculate, and petrosal ganglia all showed neuronophagia. In the geniculate many of the neurons were pyknotic. In the petrosal, there was extensive nerve cell destruction, as well as neuronophagia and interstitial invasion with small cells and microglia. The nodose ganglion showed very extensive and severe involvement including degenerative changes of various degrees in the nerve cells, neuronophagia, and heavy infiltrations.

*Summary.*—The heavy involvement of the ganglia connected with the mouth and pharynx alone (Gasserian, geniculate, and petrosal) was in sharp contrast with the absence of lesions in the celiac, which is connected only with the intestines. These facts together with the prompt onset of infection after oropharyngeal spraying and the sharp localization of lesions in the region of the

tractus solitarius of the medulla make us believe that entry was almost certainly from the upper alimentary tract and that the lesions in the nodose ganglion were derived from involvement of its pharyngeal (and possibly its esophageal) fibers. The experiment is of special and probably crucial importance because histological examination and analysis were referable almost exactly to the moment of beginning invasion of the central nervous system when the pathways of infection from the mucous surfaces to the brainstem could be clearly defined.

### *Inhalation of Virus in Droplet Nuclei*

In a previous study (14) we had found that *cynomolgus* monkeys were very susceptible to inhaled virus administered in the form of droplet nuclei according to the method of Wells (15), even when the olfactory route had been blockaded with zinc sulfate. The 6 monkeys surviving the first mouth spraying were tested by this method on April 24, 1942, 27 days after the last exposure. Since zinc sulfate blockade had been performed on them 6 weeks previously (March 14), we did not repeat the procedure.

For the 6 animals a total of 30 cc. of 20 per cent supernate was atomized into the infecting chamber, an average of 5 cc. per monkey. The actual amount inhaled is unknown. Five of the animals remained well. On May 8, 14 days after exposure, one (C53) showed slight tremors of the head; the left lower leg was completely paralyzed and the right leg was weak. The arms were normal and there was no rise in temperature. The animal was sacrificed for examination.

*Protocol, C53.*—Heavy, typical lesions were present in one olfactory bulb, but no lesions were found in the other. The secondary olfactory centers were heavily involved, as were the thalamus, hypothalamus, and midbrain. Lesions of lesser intensity were found in the pons and medulla, most marked in the reticular substance. Typical lesions were found in all levels of the spinal cord, heaviest in the upper cervical and upper and middle segments of the lumbar cord. In the peripheral system, the heaviest lesions with nerve cell destruction were found in the spinal ganglia at all levels; these were probably in large part centrifugal in origin. Moderate lesions (++), with neuronophagia and cell degeneration were present in the Gasserian and cervical sympathetic ganglia. Minute lesions, with one or two examples of cell destruction, were found in the celiac and lower thoracic sympathetic ganglia, and, without cell damage, in one lumbar sympathetic. No lesions were found in the upper thoracic, geniculate, petrosal, or nodose ganglia.

*Summary.*—One animal succumbed to poliomyelitis after inhalation. In this case infection had penetrated by the olfactory route, the effect of zinc sulfate having worn off. As has previously been noted with olfactory infections, paralysis in this case began in the legs although infection had to pass through the brainstem, including the medulla, and upper cord to reach the lumbar cord. Since in our experience with exposure by inhalation infection ensues in about 50 per cent of *cynomolgus* monkeys with olfactory blockade

and nearly 100 per cent without blockade, the low incidence in this series of exposures (1 in 6) suggests that at this period of our experiments some resistance to infection had been acquired by the test animals.

### *Second Oronasal Spraying*

The surviving 5 *cynomolgus* monkeys were subjected daily, May 25 to 28, 1942, to a second series of sprayings of the mouth and nose by the technique described on page 506. None of them displayed any signs of infection and none was sacrificed until after the following inoculation:—

### *Intracerebral Inoculation*

On June 16, 1942, 18 days after the last procedure, we inoculated all 5 surviving monkeys intracerebrally with 0.5 cc. of the supernate from a 10 per cent suspension of *Per* virus. One of them, C20, showed tremors 4 days later, which persisted and increased during the next days. On June 23 the temperature rose to 102.1°; on June 24, the right arm became paralyzed, and the back was weak and the next morning paralysis was practically complete. Sections showed typical lesions in the cord, but no detailed study of the distribution of the lesions was made. The remaining 4 monkeys showed no clinical evidences of infection up to July 17, 1942, when they were sacrificed for detailed study. The results of the examination and comments on the resistance to infection shown by these 4 animals are reported in the next paper of this series.

### *Note on Lesions in the Peripheral Ganglia*

Since our conclusions are based in part on the presence or absence of lesions in the peripheral ganglia, some comments on these structures, the pathological changes in them, and their relation to poliomyelitis are necessary.

If, as now seems to be thoroughly proved, poliomyelitis virus is strictly neurotropic, it is highly probable that its entry into the body occurs from the surfaces, particularly the mucous, into the endings of the peripheral nerves and thence along the axons to their nerve cells where it finds its first opportunity to multiply. With a few exceptions, these primary potential foci of infection are situated in the peripheral nervous ganglia and not in the CNS. The principal exceptions are: (1) the olfactory fibers, ending in the olfactory bulbs; (2) the efferent fibers of the vagus, derived from the dorsal motor nucleus of the vagus in the medulla; and (3) the motor fibers of superficially placed skeletal muscles, such as those of the pharynx. The last of these are of some special importance, probably constituting the route of invasion in poliomyelitis after adenotonsillectomy. The regional distribution of the fibers from several of the peripheral ganglia is such that the presence and absence of lesions in the latter afford clues to the portal of entry. Reference to the accompanying table (Table III) will reveal the value and limitations of this method of detection. Particularly useful are the Gasserian, geniculate, petrosal, and cervical sym-

pathetic ganglia, all of which are limited to the head area; and the celiac ganglion, which is limited to the stomach and intestine.

Further information on the portals of entry may be supplied by comparing the presence or absence of lesions (or virus) in particular ganglia with the presence or absence of lesions in the regions of the CNS with which they connect. Of special interest are the sensory nuclei of the fifth nerve; the solitary nucleus in the medulla with which afferent fibers from the geniculate ganglion of the seventh nerve (gustatory portion), the petrosal ganglion of the ninth nerve, and the nodosal ganglion of the vagus connect; and the intermediolateral column of the thoracic cord, with which the celiac ganglion connects through the fibers of the splanchnic nerves. Although the latter pass through the lower six thoracic sympathetic ganglia, they do not make synaptic connections with their cells; hence major lesions in these ganglia should not occur from centripetal spread from the celiac. The only sympathetic ganglia which appear to have connections with the mucous membranes or glands are the cervical, the fifth and sixth thoracic (esophagus), and the last thoracic and first lumbar (lower colon and rectum). So far as we can determine the spinal ganglia have no connections with the mucous membranes and their involvement points to centrifugal spread (see C53).

After infection is well established in the CNS and particularly after it has reached the stage of paralysis, secondary involvement of some ganglia, at least, may occur by centrifugal invasion. For this reason human postmortem material and material from paralyzed monkeys is often difficult to interpret, unless the corresponding central ganglionic centers are uninvolved. In such material, too, another, as yet unproved, possible secondary involvement of the celiac and perhaps some other ganglia, might occur from reabsorption of virus excreted into the gut. This might also account for the occasional presence of virus in mesenteric lymph nodes.

Precise grading of lesions for tabulation has been somewhat difficult. For tabulation (Table II), we have adopted the plan of indicating the number and size of lesions by signs (+, ++, +++, ++++) and the more important histological abnormalities by letters (N, neuronophagia; D, simple neuron degeneration; V, perivascular infiltration; P, polymorphonuclear infiltration). When there is no accompanying letter, it is understood that the lesions consisted only of infiltration of small cells, usually lymphocytes, in or adjacent to the neuron sheaths. Minimal, or +, lesions consist of minute aggregations of such cells, not more than three in any one ganglion and not exceeding the diameter of four neurons. When more than three such minute infiltrations or a single infiltration of five or more neuron diameters were present, involvement was graded as ++. When two or more of the larger infiltrations were present, it was graded +++; and when the infiltrations were very large and extensive, usually with neuronophagia, it was graded ++++.

## TABLE II

*Summary of Histopathological Data in Monkeys Exposed by Alimentary Route*

| Monkey No..... | C34 | C87 | C92 | C94 | C18 | C51 | C19 | C21 | C52 | C54 | C9 | C53 | C17 | C24 | C50 | C55 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Last exposure | 0 Control | 0 Control | 0 Control | 0 Control | Tongue swab | Tongue swab | Enema | Enema | Enema | Enema | Spray | Inhalation | Intracerebral | Intra-cerebral | Intra-cerebral | Intracere-bral |
| Paralysis | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0* | + | 0 | 0 | 0 | 0 |
| Olfactory bulbs | — | — | — | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | 0/2 | +++N,V 1/2 | 0/2 | 0/2 | 0/2 | 0/2 |
| Gasserian ganglia | — | + 1/2 | ++N 2/2 | 0/2 | — | — | ++D,N 2/2 | ++N,D 2/2 | ++N,D 2/2 | + 1/2 | +++N,D 2/2 | ++N,D 2/2 | +++N,D 2/2 | ++ 2/2 | +N 2/2 | ++N,V 2/2 |
| Geniculate ganglia | — | 0/2 | + 2/2 | 0/2 | — | — | 0/2 | 0/2 | 0/2 | 0/1 | +N 1/2 | 0/2 | 0/2 | 0/1 | 0/2 | 0/2 |
| Petrosal ganglia | — | — | +N 2/2 | 0/2 | — | — | 0/2 | 0/2 | — | 0/2 | ++N,D 2/2 | 0/1 | 0/2 | 0/2 | 0/2 | 0/2 |
| Nodosal ganglia | — | 0/2 | ++N 2/2 | 0/2 | — | — | ++V,N 2/2 | +N 2/2 | ++N 2/2 | 0/2 | ++++N,D 2/2 | 0/2 | — | + 2/2 | +N 1/2 | 0/2 |
| Cervical sympathetics | 0/5 | | | | — | + 1/4 | + 3/4 | 0/6 | ++N 2/6 | +N 2/6 | ++N 2/6 | ++N,D 2/4 | | | | |
| Superior | | 0/2 | +++N 2/2 | + 2/2 | | | | | | | | | ++ 1/2 | + 2/2 | +D 1/2 | +V 2/2 |
| Intermediate | | 0/2 | ++N 2/2 | 0/2 | | | | | | | | | 0/2 | + 1/2 | + 1/2 | 0/2 |
| Inferior | | 0/2 | +++N 2/2 | + 2/2 | | | | | | | | | + 1/2 | ++N 1/2 | ++N 2/2 | ++V 2/2 |
| Thoracic sympathetics | 0/7 | | | | +(S) | 0/8 | | | | | | | | | | |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Upper 6 pairs | | 0/11 | +N 1/5 | + 1/8 | | | 0/7 | 0/8 | 0/8 | ++N 1/6 | ++ 2/10 | 0/9 | 0/7 | 0/7 | 0/7 | 0/9 |
| Lower 6 pairs | | 0/9 | +N 3/7 | 0/7 | | | + 2/5 | 0/6 | + 1/6 | 0/8 | 0/11 | +D 2/6 | 0/10 | 0/8 | 0/6 | + 1/8 |
| Lumbar sympathetics | 0/8 | 0/7 | + 3/9 | + 1/11 | 0(S) | 0/6 | + 1/6 | + 1/7 | 0/7 | ++ 2/7 | + 1/6 | + 1/9 | +P 3/8 | 0/9 | + 2/9 | +N 3/10 |
| Celiac | 0 | 0 | ++ | + | 0 0(S) | 0 | + | ++D | +++N,D | 0 | 0 | +N,D | 0 | + | ++ | +++N,P |
| Cervical spinal | 0/3 | 0/13 | 0/14 | 0/13 | 0 0(S) | — | 0/11 | +N 2/11 | + 1/12 | 0/9 | 0/10 | +++N,D 9/13 | + 1/14 | 0/10 | +N 4/13 | + 1/14 |
| Thoracic spinal | 0/4 | 0/24 | 0/18 | + 1/17 | ++1/1 0(S) | — | + 1/11 | +N 1/12 | + 1/15 | + 1/17 | + 2/14 | +++N 4/21 | 0/20 | + 1/18 | +N,D 2/22 | +N,D 3/22 |
| Lumbar spinal | 0/4 | 0/11 | 0/12 | + 1/12 | +1/2 +(S) | — | +D 1/18 | + 1/14 | +N 2/15 | 0/10 | 0/14 | ++++N 6/12 | 0/12 | +N,D 2/13 | 0/14 | +N,P 4/12 |
| Brainstem | — | — | — | 0 | + | 0 | 0 | 0 | 0 | 0 | + | + | + | + | + | + |
| Spinal cord | — | — | — | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 | + | + |

S, subinoculation; N, neuronophagia; D, simple neuronal degeneration; V, perivascular infiltration; P, polymorphonuclear infiltration; O, no lesions found; —, not examined. Fraction notations: the numerator represents the number of ganglia containing lesions; the denominator, the number of ganglia examined. Explanation of grading of lesions as +, ++, +++, and ++++ is given in text (p. 509). + lesions are of doubtful significance. Lesions in the brainstem (diencephalon, midbrain, pons, and medulla) are not graded in the table. For the ganglia, the maximum grade of lesions found in any of the given group is shown.

* C9 showed preparalytic symptoms of tremors, weakness, and fever but was killed before paralysis had begun.

Neuronophagia in peripheral ganglia is sometimes difficult to determine with complete certainty. We have included as probable and minimal neuronophagia those instances in which the pericellular sheath has been invaded by small cells and the

TABLE III

*Peripheral Ganglia Examined in This Study, Their Regional Relations to the Mucous Membranes of the Alimentary Tract, and Their Central Connections*

| Ganglion | Surface distribution | Central connections |
|---|---|---|
| Gasserian (semilunar) V nerve | Nasal fossae, paranasal sinuses, rhinopharynx, palate, buccal surfaces, gums, tongue | Mesencephalic nucleus (midbrain); main sensory nucleus (pons); spinal nucleus (medulla and cord) |
| Geniculate VII nerve | Anterior ⅔ of tongue (gustatory fibers) | Nucleus of tractus solitarius in medulla |
| Petrosal IX nerve | Posterior ⅓ of tongue (gustatory fibers); Eustachian tube; pharynx; tonsils; palate | Nucleus tractus solitarius |
| Nodose X nerve (afferent portion) | Posterior tongue and epiglottis (gustatory fibers); pharynx; esophagus; stomach and intestines | Nucleus tractus solitarius |
| Cervical sympathetic | Salivary glands (and superficial mucous glands?) | Intermediolateral column, spinal cord, $T_1$, $T_2$ |
| Upper thoracic sympathetic | Upper esophagus | Intermediolateral column, $T_5$, $T_6$ |
| Lower thoracic sympathetic | (Probably few or no synaptic visceral connections)* | Intermediolateral column, $T_6$–$T_{12}$ |
| Lumbar sympathetic | Lower colon and rectum | Intermediolateral column, lowest thoracic and upper lumbar segments |
| Celiac (splanchnic nerves) | Stomach, small intestine, proximal colon | Intermediolateral column, $T_5$–$T_{12}$ |
| Spinal ganglia | No connections with mucous surfaces. Lower thoracics connected with Vater-Pacinian corpuscles of mesentery | Nucleus gracilis and cuneatus of medulla. Internuncial neurons of spinal cord |

* The preganglionic fibers of the splanchnic nerves, connecting the celiac ganglion with the spinal cord, merely pass through these ganglia, "at least in major part," according to Ranson (20); and, according to White and Smithwick (21), "for the most part without interruption."

neuron is indented in two or more places by them. Extreme degrees are shown in those lesions in which the entire sheath is occupied by invading cells and the remains of the neuron consist of amorphous background material, usually eosinophilic. Degeneration of neurons without neuronophagia occurred in varying grades, from shrinking of the cells with pyknosis to instances of acidophilic degeneration (the latter is rare in our experience). We have been cautious about including such phenomena as minor abnormalities of quantity and appearance and distribution of Nissl substance, and

eccentricity of the nucleus or nucleolus, since the nerve cells of the peripheral ganglia not uncommonly show such minor abnormalities in the absence of any other signs of infection.

Perivascular infiltrations are much less common and conspicuous in the peripheral ganglia than they are in the CNS. In most instances they have been small and brief and seen better in longitudinal than in cross section. We have been unable to determine from histological texts whether or to what extent perivascular spaces, like those of Virchow-Robin of the CNS, normally exist in the ganglia.

Infiltrating cells usually have round or kidney-shaped nuclei, resembling those of lymphocytes. In some instances, and particularly associated with neuronophagia, they have the irregular, elongated contour with reentrant angles of microglia. Polymorphonuclear leucocytes have been seen mainly but not invariably in cases where centrifugal spread has probably occurred. Following Spielmeyer (16), we regard these cells as indicative of very recent involvement, and have several times found them associated with dilatation of the small blood vessels.

Caution has to be used against regarding as pathological the small collections of lymphocytes sometimes seen in the outer connective tissue sheath of a ganglion or at the emergence of nerve bundles from it.

The question whether the lesions found in the peripheral ganglia of our animals are necessarily due to poliomyelitis remains to be discussed. Observations in man (17, 18) have indicated that certain degenerative and inflammatory lesions in the peripheral ganglia commonly found are not specific for particular diseases. In *rhesus* monkeys, McClure (19) has noted mild lesions in animals not intentionally exposed to poliomyelitis, while more severe lesions occurred only in inoculated animals. Our own observations confirm his, with one exception. In 3 *cynomolgus* monkeys from our colony, all of which had been kept for many months in the same quarters with infected animals, only minimal lesions (+) were found. The same was true of 6 *rhesus* monkeys and 3 African mustache monkeys (*Cercopithecus cephus*) recently acquired. One *cynomolgus* monkey (C92) which had been in the colony for several months showed marked and extensive lesions with neuronophagia in the peripheral ganglia, particularly those of the head area. In this case, unfortunately, the CNS was not examined. No clinical signs of poliomyelitis had been observed. We are inclined to believe that this was a case of poliomyelitis, spontaneously acquired in the laboratory, although conclusive proof is lacking.

In interpreting the results of histological examination of the peripheral ganglia, as shown in Table II, we have considered it reasonable to regard lesions marked + as probably insignificant, and those of greater magnitude and severity as probably significant of poliomyelitis.

## DISCUSSION

In recent years, interest has been revived in the alimentary portal of entry for poliomyelitis as a result, among other things, of the virtual abandonment of

the olfactory portal for man (22, 23), the discovery that virus is found almost constantly in the stools of patients and the demonstrated ease of infecting the *cynomolgus* monkey (4–7, 24) and the chimpanzee (23) by oral and gastric (24) administration of virus. Earlier observers (25–27) had postulated that the probable site of entry is the intestine. Kling, Levaditi, and Lépine (24), Toomey (28), and Howe and Bodian (29) have stated that a natural route of penetration is through the sympathetic fibers of the gut to the celiac plexus and thence to the spinal cord. On the other hand, Flexner's (7) view was that the paralytic disease resulted from entry through the nasal and buccal membranes and not from the intestine. Sabin and Ward (30) and Howe and Bodian (23) believe that entry may occur anywhere in the alimentary tract; that is, either in the mouth and pharynx or in the intestine. It may be remarked here that the origin of virus in the stools and intestinal wall has not as yet been determined with certainty. That it may be due to *excretion* rather than to local invasion and multiplication in the intestinal wall remains a strong possibility, since two (31, 32) groups of workers have recovered virus from the stools of animals intracerebrally inoculated.

Close scrutiny of the available data reveals that while an excellent case can be made, at least experimentally, for entry of virus through the intact alimentary tract somewhere, the evidence has been inconclusive as to whether, under conditions reasonably comparable to the human disease, virus is more likely to enter through the upper levels (mouth, pharynx, esophagus) or—as is implied in the common use of the term "gastrointestinal"—through the lower levels (stomach and intestine). In our opinion, previous experimental approaches to the problem of the region of entry have been unsuitable to prove the point at issue. In order to exclude the upper alimentary tract from exposure and to confine it to the lower, gastrointestinal portion, two methods have been employed: first, tube feeding (23, 24) and, second, laparotomy and injection directly into the wall or lumen of the gut (33). The latter, as Flexner (7) has pointed out, is grossly traumatic and artificially introduces virus into direct contact with the ganglia and nerve endings in the intestinal wall. It is therefore not comparable with natural conditions of infection. The use of the stomach tube inevitably produces contamination of the oropharyngeal surfaces as the tube is withdrawn and may also involve some trauma of the exposed mucous surfaces. Washing the tube before withdrawal cannot remove virus from its outer surface. Hence, a positive result from gavage does not by itself exclude the possibility of oropharyngeal infection. Indeed, the protocols of the first 2 of Howe and Bodian's (23) tube-inoculated chimpanzees reveal lesions in the Gasserian ganglia and their central connections as well as in the celiac ganglia. In the first stage of the present experiments, we believe that contamination of the oropharynx was entirely avoided and of the esophagus, largely so. None of the 26 animals showed paralysis or other overt manifestations of

poliomyelitis. The same was true of the 11 monkeys to which virus was administered by enema. In both instances the amounts of virus to which the animals were exposed were large. The dried virus as fed in capsules was proved to be active. Our failure to recover virus from the stools of some animals fed virus-containing capsules may have been due to faults in method or technique, or possibly to some destructive effect of the gastric and enteric secretions.

Despite the absence of clinical signs of infection following gastrointestinal exposures to virus, some histopathological evidence of penetration of the intestinal mucosa was found. Of the 12 cases histologically examined, 7 showed lesions in the celiac ganglia, 4 with simple neuronal degeneration or neuronophagia or both. All of the positive cases had received virus enemas as well as capsules. None of these, however, showed evidence of centripetal spread of infection into the CNS. It will be recalled that the pathway from the celiac ganglia to the CNS is along the fibers of the splanchnic nerves to their cells of origin in the intermediolateral columns of the thoracic cord. Of the 7 cases with lesions in the celiac ganglia, only 2 showed any lesions in the spinal cord. One of them (C53) had paralytic poliomyelitis following olfactory entry and the other (C55) had non-paralytic poliomyelitis following intracerebral inoculation. In neither of these were the cells of the intermediolateral columns involved. These observations strongly suggest that the sympathetics do not constitute a preferential route of invasion from the intestine to the CNS.

The other possible neural route of invasion, first suggested by Toomey (28), from the intestine *via* the vagus, is difficult to exclude entirely because of the wide distribution of the fibers of this nerve. The nodose ganglion which supplies afferent fibers to the entire alimentary tract, including the pharynx and esophagus, was involved in 6 of the 9 animals in which it was examined, most heavily in a case (C9) of upper alimentary infection in which the celiac ganglion was not involved. It contained no lesions in 2 cases in which the celiac ganglia were involved. The dorsal motor nucleus of the vagus in the medulla which supplies efferent fibers to the myenteric plexuses of the entire alimentary tract from the beginning of the esophagus downward showed lesions in only 2 cases of 12 in which it was examined and these consisted of but one or two perivascular cuffs without neuronal damage. The evidence of centripetal spread along the efferent fibers of the vagus is, therefore, negligible, and along the afferent fibers from the intestine, ambiguous.

Perhaps the most striking feature noted in the peripheral ganglia of our animals was the high relative frequency and severity of lesions in the Gasserian and cervical sympathetic ganglia, both of which supply the head area alone. The Gasserians were involved in all of the 10 cases in which they were examined and showed neurophagia in 8. The cervical sympathetics were involved in 10 of the 11 cases in which they were examined, with neuronophagia in 6. On the other hand, the geniculate and petrosal ganglia showed lesions in only

one case (C9) (they were examined in 10 and 9 instances, respectively), suggesting that entry through the taste buds and gustatory fibers is exceptional.

Four cases of symptomatic poliomyelitis occurred in the series.[2] Of these one (C53) resulted from olfactory entry and one (C20) from intracerebral inoculation: these have no direct bearing on the problem of the alimentary portal. Of the other 2, one (C18) showed no lesion in the olfactory bulbs nor in the celiac ganglion nor virus in the latter; in all probability it acquired the infection by way of the upper alimentary tract, and the other (C9) quite certainly did.

Our study, then, shows a notably greater tendency for poliomyelitis virus to enter through the upper portions of the alimentary tract (including the mouth, pharynx, and possibly the esophagus) than through its lower portions (stomach and intestines). It strongly suggests to us that infection resulting from ingestion of virus reaches the CNS and produces clinical manifestations of poliomyelitis mainly, and perhaps only, when entering through the oropharynx (and possibly the esophagus), and not when entering through the intestine. This seems to be true at least of the *cynomolgus* monkey which in its susceptibility to non-traumatic alimentary infection resembles the chimpanzee and perhaps man. Whether the same rule applies to man must await an answer from further study of human evidence, since experimental work can only indicate the potential modes and channels of infection in the human disease. In the light of present knowledge, however, we see no compelling argument against it while in favor are certain considerations, some of which we have previously discussed (34, 13), such as the constancy of symptoms referable to the brainstem during the preparalytic period pointing to involvement of this region before the spinal cord; the fact that with introduction of virus by ingestion the oropharynx is exposed first and most intensively; the greater probability of minor trauma to the mouth and gums, and some others. At a later time we expect to discuss the subject in greater detail.

## SUMMARY

1. *Cynomolgus* monkeys were subjected to a series of non-traumatic exposures of the mucous membranes of the alimentary tract, designed to test the relative permeability to poliomyelitis virus of its upper and lower portions.

[2] The proportion of clinical poliomyelitis in our series is smaller than in the experiments of Sabin and Ward (4) in which the same strain of virus administered in food caused paralytic infection in 6 out of 15 *cynomolgus* monkeys. There is reason to believe that our animals, during the progress of the experiments when they had been repeatedly exposed, developed a degree of resistance to infection. We shall discuss the subject in another paper of this series. This factor did not complicate the first stage of our experiments (capsule feeding). It is interesting to note that Sabin and Ward failed to recover virus from the celiac ganglion of any of their infected animals.

2. In the first stage, dried poliomyelitis virus of tested potency was administered in fat-covered capsules to 26 monkeys in such a way as to avoid contamination of the oropharynx but to permit thorough exposure of the gastrointestinal mucosae. No clinical evidence of poliomyelitic infection appeared.

3. Subsequent application of small amounts of virus to the tongues of 18 of the same monkeys caused paralytic poliomyelitis in one of them.

4. Virus given subsequently by enema to 11 of the monkeys caused no clinical manifestations of poliomyelitis.

5. Of 7 monkeys later treated with virus by oronasal spraying, one developed typical preparalytic signs of infection, and the distribution of lesions indicated that entry had occurred through the afferent nerves of the oropharynx and, possibly, the esophagus.

6. The 6 surviving monkeys were exposed to virus by inhalation. One of them developed paralytic poliomyelitis by olfactory entry. The others appeared to have acquired some resistance to infection.

7. The 5 surviving monkeys were inoculated intracerebrally, as a test of immunity. One of them developed paralytic poliomyelitis. The other 4 showed no clinical signs of infection, but all had typical lesions of varying extent and intensity in the central nervous system.

8. A histological examination of the peripheral nervous ganglia in 12 of the exposed monkeys sacrificed at various stages of the experiments revealed lesions compatible with poliomyelitis in all. Ganglia connected with the head alone (Gasserian, cervical sympathetic) were more constantly and, on the average, more severely involved than the celiac, which is connected only with the intestine.

9. While the celiac ganglion was involved in 7 cases, no evidence was found of the spread of infection from it to the spinal cord.

## CONCLUSION

The upper portion of the alimentary tract (mouth, pharynx, and perhaps the esophagus) appears to be more vulnerable and to constitute a more probable primary portal of entry for poliomyelitis than its lower, gastrointestinal portion.

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