POLIOMYELITIS IN THE CYNOMOLGUS MONKEY

III. Infection by Inhalation of Droplet Nuclei and the Nasopharyngeal Portal of Entry, with a Note on This Mode of Infection in Rhesus*

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(Received for publication, April 3, 1944)

In a recent paper (1) we presented data on experimental poliomyelitic infection by the alimentary route. The present study deals with experimental infection by the respiratory route.[1] It seems likely that as a rule the natural infection in man enters the body by one or perhaps by either of these routes; that is, through the mouth or nose. As in the alimentary study, non-traumatic methods of infection were employed in the present study, following the thesis that human infection is ordinarily introduced without gross trauma. The two known exceptions to this assumption — poliomyelitis after adenotonsillectomy (3) and after injections for the purpose of active immunization (4) — may be considered as proving the rule.

Both *cynomolgus* and *rhesus* monkeys were used. The former, which in important aspects of infectibility are more closely related to man than are the latter, could no longer be obtained after Pearl Harbor, and for this reason certain supplementary experiments which we had planned could not be carried through when our current supply was exhausted.

Methods

Based on the apparatus of W. F. Wells (5) and with the help of some suggestions which he kindly made, an inhalation chamber was constructed (Figs. 1 and 2), of monel metal, measuring 18 × 18 × 24 inches with a capacity of approximately 4.5 cubic feet (128 liters), designed to accommodate 4 monkeys at a time. Compressed air, mixed with 5 to 10 per cent CO_2 (to increase the depth of respiration) from cylinders with flow meter attached, was run through a special atomizer (Fig. 3) for the virus suspension and into the chamber. By direct tests the atomizer was shown to deliver an even suspension of *dry* droplets ("nuclei"), the wet, unevaporated droplets falling back into the flask. Test runs with suspensions of *Chromobacterium prodigiosum* showed an even distribution of colonies in Petri dishes placed on the head rests inside the chamber, demonstrating a uniform admixture of droplet nuclei in the air entering the chamber. A window at the top of the apparatus filled with several layers of cotton sheet wadding served to equalize the pressure within the chamber and at the same time prevented outward escape of virus. The main body of air passed out from the chamber

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

[1] A preliminary report was published in 1941 (2).

through an outlet led through a series of 3 cylinders filled with 0.2 per cent $HgCl_2$ solution, connected at the end with a vacuum pump. The rate of flow through the exhaust apparatus

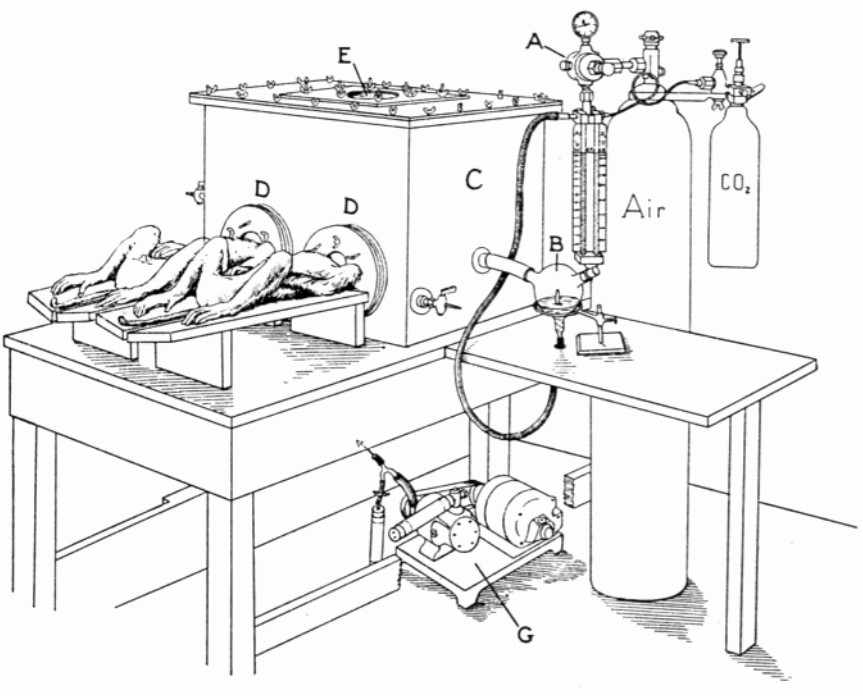

FIG. 1. Inhalation apparatus viewed from inlet side. Air and CO_2 from compression cylinders are run through flow meters connected with pressure gauge (*A*) into the atomizer flask (*B*) containing virus suspension. The air-CO_2 mixture containing atomized virus suspension is delivered through the connecting hose into the infecting chamber (*C*) in two opposite sides of which are port-holes (*D*). Monkeys are placed supine on frames with their heads inside the chamber. In the port-holes rubber diaphragms fit around the necks of the monkeys. The lid of the chamber is separate and fits over the flanged top of the side walls on which is fitted a rubber gasket; tight closure is obtained with wing-bolts screwed down on threaded posts attached to the flanges. In the center of the lid is a window (*E*) filled with several layers of cotton wadding which permit air to enter and equalize the internal pressure; this is necessary because air is exhausted at a slightly greater rate than that at which it enters at the atomizer inlet. The exhaust pump (*G*) is shown below and connects directly with the air-purifying cylinders (*F*) shown in Fig. 2.

was slightly greater than through the inlet, thus minimizing the chances of virus escaping into the room.

The monkeys, under nembutal anesthesia and usually in groups of 4, were placed in the supine position with the head projecting inside the chamber supported on a metal head rest. Abbott's veterinary nembutal, a solution containing 1 grain per cc., was injected intraperi-

toneally in dosages of 1 cc. per 5 pounds of body weight. It was found safer to dilute the solution, which contains 10 per cent alcohol, with 4 volumes of 0.85 per cent NaCl solution. A

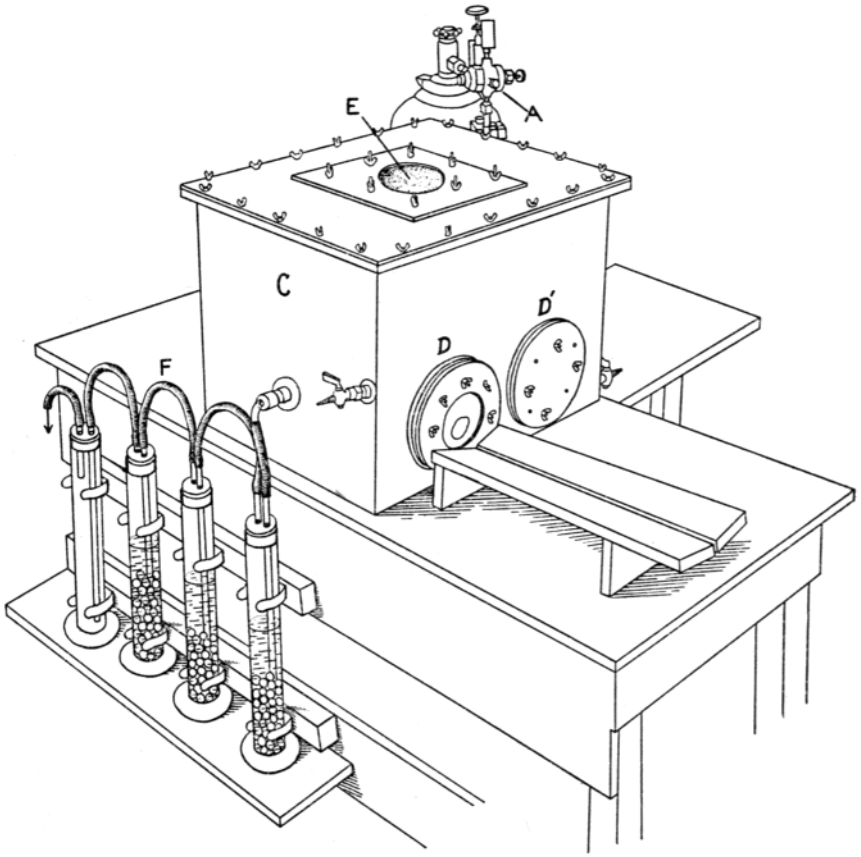

FIG. 2. Inhalation apparatus viewed from exhaust side. The exhaust air is drawn through three tall purification cylinders (*F*) containing a layer of marbles and filled with 0.2 per cent $HgCl_2$ solution. The fourth cylinder which is empty catches any bichloride solution that might be carried over, protecting the pump. The nipples with valve shown on the two sides of the infecting chamber are for air-sampling. After each experiment, live steam is run through the apparatus for an hour, assuring thorough sterilization.

rubber diaphragm fastened in the porthole (Fig. 1) fitted snugly about the neck. Satisfactory closure was obtained by means of wet absorbent cotton wrapped about the neck outside the diaphragm.

The atomizing apparatus was then started and continued for the periods stated in the protocols (1 to 2 hours): during this time the animals breathed regularly and quietly, the amplitude being somewhat increased over normal by the CO_2 added to the air. Approximately

390 liters of air-CO_2 mixture containing 6 to 21 cc. of virus suspension supernate was delivered each hour into the chamber. The amount of virus actually inhaled by each monkey cannot be accurately estimated. On the basis of the even distribution of droplets which we believe obtained we have divided the amount of virus suspension known to have been delivered into

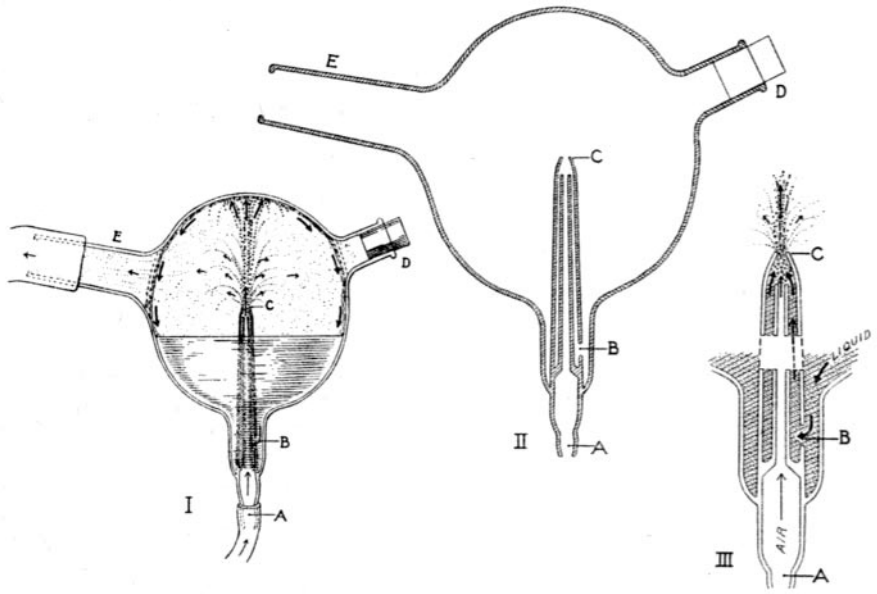

FIG. 3. Atomizing flask (adapted from a design by W. F. Wells). Detail of the nozzle is shown in III. Air under positive pressure enters at point *A*, passes upward through the inner tube creating negative pressure in the outer sleeve, where a small aperture (*B*) permits the entrance of virus suspension; this is drawn upward and mixed with air; the mixture is expelled at the upper opening (*C*) into the flask as a spray. The coarser droplets tend to ascend vertically while the finer droplets tend to diverge laterally. II shows the design of the flask as a whole. Arm *D* is for inserting virus into the flask and is fitted with a rubber cork. *E* is the outlet and is connected with a rubber hose leading to the infecting chamber. I shows the flask in operation. The coarse droplets strike the top and sides of the flask and run down them back into the fluid reservoir. The fine droplets are expelled into the outlet (*E*) connected with the infecting chamber. Direct tests have shown that the droplets are dry at the time they enter the chamber.

the chamber by the number of animals exposed at a given time to obtain the figure mentioned in the protocols and tables as "available per monkey." This is a maximum. Since without question a large amount of virus escaped in the exhaust uninhaled, the actual amount must have been much less than the "available" amount.

Blockade of the olfactory mucosa with 1 per cent zinc sulfate solution was applied to part of the animals by the technique of Schultz and Gebhardt (6). In this, the animal is held with the head downward, 15 to 30 cc. of the solution is forced into the nares with a rubber tipped

syringe, and the position is maintained for about 2 to 3 minutes. Obviously, the solution is not confined to the olfactory area but must to some extent also reach and affect the respiratory portion of the nasal cavities as well as the retropharynx, thus affording some possibility of damage to the endings of the fifth and the sympathetic nerves. The respiratory portion is, however, probably better protected by mucus than is the olfactory area, and the blockading effect is doubtless greatest in the latter. A more satisfactory and critical method would have been to section the olfactory tracts, a method which we had planned to use but were prevented from doing because of the failure of supply of *cynomolgus* monkeys. We suspect that our failure to infect some of our *cynomolgus* monkeys by inhalation may have been due to damage to the fifth and sympathetic nerve endings by zinc sulfate.

For *cynomolgus* monkeys, the *Per* strain of virus was used; for *rhesus*, the *OH* strain. Both these were of proved high infectivity for the respective species. The *Per* strain was used in its 4 to 8th passage; the *OH* in its 2 to 5th passage.

Histological examination in series I and II was limited to the central nervous system and was less complete than in series III: in the former two, it consisted of serial sections of the olfactory bulbs and some selected areas in the brainstem and cord and was designed mainly to show the presence or absence of olfactory entry. Some of the tissues were also used for subinoculation. In series III we used the same plan of fairly complete study of the central nervous system and peripheral ganglia which we employed and described in our previous paper on alimentary infection (1). The plan was designed to elucidate the portals of entry by examining for lesions the cell stations in the peripheral ganglia and their central connections. The secondary olfactory centers examined, unless otherwise specified, were the anterior perforated substance, the amygdaloid nucleus, the parolfactory area, and the olfactory trigone.

Series I. No Olfactory Blockade

Seven *cynomolgus* monkeys (C39, C40, C41, C43, C44, C45, C46) were exposed by inhalation under nembutal anesthesia for 1 hour periods on July 31 and August 1, 1941. The virus preparation was the sharply centrifuged supernate from a 20 per cent suspension of the *Per* strain, 6th passage. A total amount of 84 cc. of the supernate was atomized into the infecting chamber, or an average of 12 cc. per monkey. Only one animal (C43) failed to succumb to clinical poliomyelitis. The results are shown in Table I.

HISTOLOGICAL EXAMINATION.[2]—The central but not the peripheral nervous tissues were examined histologically in this series, using Einarson's gallocyanin stain, as described by Bodian (7). The distribution of lesions follows. None was found in C43, the only animal which failed to come down with poliomyelitis.

Olfactory Bulbs.—These were involved in all cases with infection but to varying degrees. The usual lesions consisted of PvI and PrI with Nph. In C41, only one bulb showed lesions and in C45, they were minimal in one bulb.

Uncinate gyrus.—Lesions, consisting of PrI and Nph were found only in C41. The other secondary olfactory centers were not inspected in this series.

Thalamus.—No lesions were found in C43 and C45. In C41 and C46, there were some PvI and in C44, also some PrI.

Hypothalmus.—PvI were found in C41, C44, C45, and C46.

[2] The following abbreviations of poliomyelitic lesions are used throughout the histological descriptions. PvI, perivascular infiltration (cuffing). PrI, parenchymal infiltration. Nph, neuronophagia. Pmn, polymorphonuclear leucocytes. CNS, central nervous system.

Midbrain.—PrI and PvI were found in C39, C40, and C41. No involvement of the mesencephalic nucleus of the V nerve was noted.

Pons.—PrI and PvI were noted in the reticular formation in C41, C44, C45 and C46. PrI was noted in the nucleus of the spinal tract of the V nerve in C39 and PvI only was seen in this nucleus in C41. PrI in the nuclei of the VI and VII nerves was seen in C41. No involvement of the motor nucleus of the V nerve was found in any case in which this nucleus was examined.

Medulla.—The reticular formation showed lesions—PrI, PvI—in the medulla in the same 4 animals as in the pons. In C44 the lesions were very heavy and included Nph. PrI and Nph were noted in C39 and C44 in the nucleus ambiguus. No lesions were found in the dorsal nucleus of the vagus, or the nucleus solitarius. In one case (C44) PvI was seen in the spinal vestibular nucleus. Minor lesions were found in the olive in 3 cases. The hypoglossal nucleus contained PvI, PrI, and simple degeneration in C41 and PrI in C44. In C41 also, Nph and simple degeneration were observed in the cuneate nucleus and PrI was observed in this nucleus in C44.

TABLE I

Results of Inhalation in Cynomolgus Monkeys with Intact Olfactory Mucosa

No.	C39	C40	C41	C43	C44	C45	C46
Onset of fever*	5	4	7	0	4	4	6
Onset of symptoms	8 R	4 Tr Ex	10 Tr	0	8 R	7 W P	7 Tr
Onset of weakness or paralysis	10 W	7 W	11 W	0	13 W	7 W P	10 W
Site of initial weakness or paralysis	Legs	General	Legs	0	Legs	Legs	Legs
Day killed	11	7	11	49	13	8	10
Poliomyelitic lesions in CNS	+	+	+	0	+	+	+
Subinoculations	—	0‡	0‡	—	—	+§	+§

R, ruffled fur. Tr, tremors. Ex, excitability. W, weakness. P, flaccid paralysis.

* Day after first exposure.

‡ Pooled samples: (*a*) from peripheral ganglia; (*b*) from lungs, of C40 and C41. No CNS tissue was tested.

§ Spinal cord.

Spinal Cord.—In C39, C44, C45, and C46 typical lesions were noted in the anterior horns at practically all levels. In C40 and C41, on the other hand, invasion was not yet apparent throughout the full length of the cord. In C40 severe lesions were found in the cervical and middle thoracic segments and mild lesions in the upper lumbar, while in C41 they were not found lower than the midcervical level. The freshness of the lesions in C41 was shown by the presence of many polymorphonuclear leucocytes. The posterior horns showed moderate lesions in three cases (C44, C45, and C46) and minor lesions in one, C40. The sympathetic intermediolateral columns showed small PrI only in 2 cases (C40 and C46); no lesions in 4.

Summary.—6 of 7 *cynomolgus* monkeys, without olfactory blockade, developed clinical poliomyelitis after exposure by inhalation of virus. In all instances infection occurred through the olfactory portal. The onset of fever occurred 4 to 7 days after the first exposure, and of characteristic symptoms, 4 to 10 days after exposure. Typical lesions were discovered in all. In the brainstem, the most frequent and severe lesions were found in the reticular formation of the

pons and medulla (4 cases). No bulbar paralysis was noted in any case, and in 5 animals the first motor impairment was in the legs. The data substantiate previous observations (8) that poliomyelitis infection commonly descends through the medulla without producing bulbar paralysis, to cause primary motor involvement of the legs.

Series II. Olfactory Blockade

Eight *cynomolgus* monkeys (C67, C68, C69, C71, C72, C73, C76, C77) were treated with intranasally instilled 1 per cent zinc sulfate solution for olfactory blockade on August 25, 1941. On September 5, they were exposed in groups of 4, under nembutal anesthesia, by inhalation for a single period of 2 hours. The virus preparation was the supernate from a 15 per cent suspension of *Per* strain, 8th passage. Approximately 5 cc. were available per monkey, considerably less than half the amount in series I. 2 monkeys, C71 and C76, died during and shortly after exposure respectively, and are not included in the tabulations. C68 came down, and the others remained well. C67, C69, C73, and C77 were treated again with zinc sulfate on October 30, 1941 and re-exposed by inhalation on November 13; 5 cc. of supernate from a 20 per cent suspension for *Per* virus in the 4th passage were delivered into the infecting chamber per monkey. None of these developed clinical poliomyelitis.

Protocol, C68.—At 9 a.m., Sept. 16, 1941, 11 days after exposure, right facial paralysis, incoordination of the ocular movements, excitability, deep, labored breathing, and hoarseness were noted. The temperature was 102.3°. The animal was sacrificed at 3 p.m. on the same day, no extension of paralysis having occurred during the interval.

Parts of the nervous tissues were set aside for subinoculation; for this reason the histological survey was incomplete.

Olfactory Bulbs.—(Examined serially) showed no lesions.

Diencephalon.—No lesions were found in the thalamus. The hypothalamus contained a few mild lesions, including foci of PrI containing Pmn.

Midbrain.—Foci of PrI with Pmn, simple neuronal degeneration, acidophilic degeneration, and Nph were seen in the reticular formation.

Pons.—The reticular substance contained several PrI, throughout. The nucleus of the spinal tract of the V nerve showed many PvI and some PrI. The motor nuclei of the V and VI nerves and the main sensory nucleus of the V nerve were not in the level examined. The nucleus of the VII nerve showed extensive cell destruction on one side and heavy PrI containing Pmn together with PvI on both sides.

Medulla.—One section only was examined. Lesions of moderate intensity were found in the reticular formation, nucleus ambiguus, and dorsal motor nucleus of the vagus. The nucleus of the spinal tract of the V nerve showed PvI, PrI, and Nph. No lesions were observed in the solitary nucleus.

Cord.—Typical and moderately severe lesions were found in the anterior horns of the cervical and upper thoracic levels only. No lesions were found in the intermediolateral columns.

The peripheral nervous system was not examined histologically.

Subinoculations.—Material from the medulla was inoculated intracerebrally into a *rhesus* monkey, which developed fever, tremors and weakness of the legs 15 days later. No paralysis ensued. The animal was killed on the 32d day and showed typical poliomyelitic lesions.

Material from the cervical, thoracic, and lumbar portions of the ganglionated cord and from the celiac ganglion was inoculated intracerebrally into *rhesus* monkeys without resulting infection.

Summary.—The bulbar character of the symptoms, the positive subinoculation from the medulla, the negative results of subinoculation from the sympathetic ganglia, the absence of lesions in the olfactory bulbs, the presence of lesions in the pons and medulla and in the upper but not the lower portion of the spinal cord suggest entry through the connections of the nasopharynx with the lower brainstem. The presence of lesions in the spinal tract of the V nerve and the absence of lesions in the solitary nucleus and in the intermediolateral columns of the cord suggest that entry was through the sensory portion of the fifth nerve rather than through the pharyngeal afferent components of the VII, IX, and X nerves or the sympathetics. Unfortunately the peripheral ganglia were not examined in this case.

Series III. Olfactory Blockade

Four *cynomolgus* monkeys (C63, C64, C66, and C75) were treated with intranasally instilled 1 per cent zinc sulfate solution for olfactory blockade on January 5, 1942. On January 15 they were exposed, under nembutal anesthesia, by inhalation for a single 2 hour period, with the supernate from a 20 per cent suspension of *Per* virus in its 4th passage. Approximately 3 cc. per monkey was delivered into the infecting chamber. One monkey, C75, remained well. The other 3 developed clinical poliomyelitis.

Protocol, C63.—The animal remained well and afebrile until Jan. 28, 13 days after exposure. At that time the fur became ruffled, the posture was bent, dyspnea was present, and some intercostal weakness was suspected. The temperature was not elevated (101.4°). On the next day, the animal was prostrate. There was aphonia, incomplete right facial paralysis, complete paralysis of the intercostal and back muscles, and weakness of the arms and legs. The temperature was 94°. The animal was promptly sacrificed, about 24 hours after the beginning of symptoms.

HISTOLOGICAL EXAMINATION.—

A. CNS.—

The infiltrative lesions contained many Pmn characteristic of early poliomyelitic infection.

Rhinencephalon.—The olfactory bulbs (serial sections) and secondary olfactory centers (anterior perforated substance, amygdaloid nucleus, parolfactory area, and olfactory trigone) contained no lesions.

Diencephalon.—The thalamus was negative. In the hypothalamus near the margins of the III ventricle some fairly large foci of PrI were found.

Midbrain.—In the oculomotor nucleus there was intense PrI. Smaller foci of PrI were seen in the reticular formation. Elsewhere lesions were few and slight.

Pons.—Many scattered areas of PrI were present especially in the reticular formation. The main sensory nucleus and the nucleus of the spinal tract of the V nerve were free of lesions, as was the nucleus of the VI nerve. Slight PvI and Nph but no PrI were seen in the

motor nucleus of the V nerve. Marked PvI, PrI, and Nph were found in the nucleus of the VII nerve. Severe lesions were present in the vestibular nuclei, especially Deiters'.

Medulla.—Intense lesions were found in the reticular substance at all levels, and in the vestibular nuclei. PvI, PrI, and some Nph were present in the hypoglossal nucleus, and the nucleus ambiguus showed PvI and PrI. The dorsal motor nuclei of the vagus were almost entirely spared except for one focus of PrI. In or near the solitary nucleus there were a few small PrI and a single PrI of moderate size was found in the region of the nucleus of the spinal tract of the V nerve. There were no significant lesions in the olive, gracile, and cuneate nuclei.

Spinal Cord.—Typical severe lesions, characterized by large numbers of Pmn were found at all levels. In the upper and middle thoracic levels the anterior horn motor cells were almost entirely phagocytosed. In contrast with the usual absence of lesions in the intermediolateral sympathetic columns, PrI was found in them more intense on one side, at three levels of the thoracic cord. In the uppermost level, corresponding with the entrance of fibers from the cervical sympathetics, active Nph was observed. Throughout the intermediolateral columns the number of neurons appeared to be markedly diminished. PrI was seen in the posterior horns at all levels.

B. Peripheral Ganglia.—

The ganglia of the cranial nerves showed very few lesions: none at all was found in the Gasserian, geniculate, and petrosal and one nodose; the other nodose ganglion contained one small focus with a single cell in early phagocytosis. The cervical sympathetic ganglia, on the other hand, showed marked lesions. Lesions of moderate severity with Nph were found in two while in a third a large, intense focus in which the capsular structure was destroyed was seen. In this lesion a few degenerated nerve cells were seen and many empty spaces, with a considerable amount of granular eosinophilic material. The upper thoracic and the lumbar sympathetic ganglia were negative, and only one minimal lesion was found in a single lower thoracic ganglion and another in the celiac ganglion.

The cervical spinal ganglia were free of lesions; but all the thoracic and lumbar ganglia were heavily infiltrated.

Summary—The clinical as well as the pathological evidence in this case points rather convincingly, we believe, to entry of infection through the sympathetic nerves of the nasopharynx to the upper thoracic cord, with subsequent spread both upwards to the cervical cord and brainstem and downwards to the lower thoracic and lumbar cord. It will be noted that the first observed weakness was in the intercostal muscles, which was followed by bulbar palsies and symptoms and by weakness of the legs. Involvement of the cervical sympathetic ganglia was marked, and the discovery of neuronophagia in the upper thoracic intermediolateral column completes the chain of lesions from periphery to CNS.

This case also throws some light on the frequently difficult problem of distinguishing between centrifugal and centripetal spread of lesions found in the peripheral ganglia. Here it is noteworthy that the spinal ganglia of the thoracic and lumbar levels were nearly all involved. On anatomical grounds we believe that in poliomyelitis, lesions in these ganglia are always centrifugal. It is therefore of special interest to find that in the present instance, with marked centrifugal spread in the spinal ganglia, there were practically no lesions of

significance in the ganglia of the cranial nerves (despite the presence of lesions in the brainstem), nor in any of the sympathetic chain other than the cervical group. The lesions in the latter therefore appear to be centripetal.

Protocol, C64.—The animal remained well and afebrile until Jan. 26, 11 days after exposure, when the temperature rose to 103.3° without any other evidences of infection. The next day, at 9 a.m. there was marked hyperpnea but no other abnormal signs. At 11 a.m., head tremors were noted, and the animal seemed generally a little weak. At 1:30 p.m. the condition was unchanged and the animal was sacrificed; *i.e.*, 4½ hours after the first symptoms other than fever were noted. No paralysis had occurred.

HISTOLOGICAL EXAMINATION.—

A. CNS.—

Rhinencephalon.—No lesions were found in the olfactory bulbs or secondary olfactory centers.

Diencephalon and Midbrain.—The thalamus was negative and only a few PvI were found in the hypothalamus. Intense foci of PvI, PrI, and Nph were found in the oculomotor nuclei, central gray, substantia nigra, and reticular formation of the midbrain.

Pons and Medulla.—The main sensory and the motor nuclei of the V nerve were not present in the sections examined. Heavy foci of infiltration were found in the nucleus of the spinal tract of the V nerve in the pons, in the motor nuclei of the VI and VII nerves, in Deiters' nucleus, and in the reticular formation. The inflammatory reaction in the medulla was most marked in the reticular formation. The hypoglossal nucleus was heavily involved. The dorsal motor nucleus of the vagus showed no lesions other than some PvI. PrI and occasional Nph were seen in the nucleus ambiguus. One small focus of PrI was found in the solitary nucleus and several in the nucleus of the spinal tract of the V nerve in the medulla. The spinal vestibular nucleus was negative. Occasional PvI were seen in the olive.

Spinal Cord.—Marked lesions were found in the anterior horns at all levels, but neuronal destruction was most severe in the cervical. The infiltrations in the posterior horns were also most marked in the cervical region. A minute PvI was seen in the intermediolateral column in the upper thoracic level and a somewhat larger one in the lower thoracic, but the sparing of these columns was in sharp contrast with the adjacent lesions in the anterior horns.

B. Peripheral Ganglia.—

Of the ganglia of the cranial nerves, only the Gasserian showed marked lesions. In both of these there were numerous foci of infiltrations, with many Pmn, especially at the margins. There was little intercapsular infiltration but some Nph of the marginal cells was noted. Minimal lesions were found in both nodose ganglia and in one geniculate; the former contained Nph, the latter none. Many, but not all, of the spinal ganglia at all levels showed moderately severe infiltrative lesions.

In the sympathetic ganglia, there were no lesions in the thoracic and lumbar; lesions of moderate severity were present in one cervical sympathetic ganglion containing an example of acidophilic degeneration and another Nph; and in the celiac there were several fairly large and intense lesions with neuron degeneration and Nph.

Summary.— This is a striking example of a phenomenon to which we have previously (9) called attention, the great extent of the lesions sometimes seen in experimental poliomyelitis before paralysis appears. The cord was involved throughout its length with extensive cell damage and infiltration in the anterior

horns. As in most very early cases, the infiltrating cells were mainly Pmn. Centrifugal spread was already marked in the spinal ganglia. On the other hand the marked involvement of the Gasserian alone of the cranial nerve ganglia suggests that it was probably centripetal; and the absence of lesions in the intermediolateral columns of the thoracic cord suggests a similar origin for the lesions found in one of the cervical sympathetic ganglia, and in the celiac. The fairly marked involvement of the celiac ganglion in this case is almost unique in our *cynomolgi* exposed by inhalation, but the absence of anything more than a single cuff — a lesion of no localizing value — in the corresponding portion of the intermediolateral columns, indicates that it was not a source of CNS infection. On the whole, the evidence points most strongly in this case to central involvement originating in the afferent fibers of the fifth nerve with passage into the Gasserian ganglion and thence into the pons and medulla.

Protocol, C66.—In the morning of Jan. 28, 13 days after exposure, the temperature was 103° but no suggestive symptoms were noted. In the late afternoon, hyperpnea was observed. The next morning breathing was still rapid and deep and the legs were completely paralyzed. No other weakness or paralysis was noted and there was no tremor. The temperature had declined to 100.9°. The animal was then sacrificed.

HISTOLOGICAL EXAMINATION.—

A. CNS.—

Rhinencephalon.—The olfactory bulbs and the secondary olfactory centers were entirely normal.

Diencephalon.—The thalamus showed no lesions. In the hypothalamus one small PrI was seen. The midbrain was not examined.

Pons.—Lesions were widespread and intense, containing many Pmn and numerous microglial cells. Nph was marked. The heaviest lesions were found in the main sensory nucleus and the nucleus of the spinal tract of the V nerve. The vestibular nuclei were heavily involved. The reticular formation showed lesions of less severity. The motor nuclei of the V and VII nerves contained some lesions, but that of the VI nerve did not.

Medulla.—The most severe lesions were found in the reticular formation. Heavy lesions were found in the nucleus of the spinal tract of the V nerve, decreasing caudally in intensity; in the hypoglossal and spinal vestibular nuclei. Scattered foci of PvI and PrI were seen in the dorsal motor nucleus of the vagus and in the nucleus ambiguus. Some PrI was seen in or near the solitary nucleus. No lesions of significance were found in the olive or in the gracile and cuneate nuclei.

Spinal Cord.—The anterior horns were involved at all levels examined (the upper and lower thoracic levels were not examined), most heavily in the lumbar. Many Pmn were found in the infiltrations, as well as microglial cells. The posterior horns were free of lesions in the cervical levels, one focus of PrI was seen in the midthoracic level; they were heavily involved in the lumbar cord. The intermediolateral columns in the midthoracic level were normal.

B. Peripheral Ganglia.—

One Gasserian ganglion was heavily involved, showing many large and moderate foci of PrI, many containing Pmn, both within the cell groups and in the emerging fibers. Widespread Nph was shown by the presence of infiltrating cells within empty capsules. The other

Gasserian ganglion showed only minimal lesions. The geniculate, petrosal, and nodose ganglia were entirely normal.

Many of the spinal ganglia showed intense PrI with Nph; the involvement was most severe and extensive in the cervical and lumbar levels. Of the sympathetic ganglia, the celiac showed only minimal lesions and there were none at all in the cervical, upper and lower thoracic, and lumbar members of the ganglionated cord.

Summary.—This is a particularly clear cut example of entry of infection from the mucosa through the afferents of the fifth cranial nerve into one Gasserian ganglion with involvement of its central connections and spread to the lower brainstem and cord. In the face of the histological evidence, the clinical aspects are of special interest. Apart from hyperpnea, probably correlated with lesions in the reticular formation, no bulbar phenomena were noted, and the primary site of paralysis was in the legs. It is noteworthy that while primary paralysis in experimental poliomyelitis may be segmental to the portal of entry (*cf.* C68 and C63), this is by no means always so and was not true in this case, in which clear proof is presented that primary leg paralysis was not due to primary entry through the intestine. The same thing is usually true of olfactory entry, as has already been noted.

Protocol, C75.—This monkey remained well for 22 months when it developed low calcium tetany and was killed. A systematic examination of the CNS and peripheral ganglia showed no evidence of poliomyelitic infection.

Experiments with Inhalation in Rhesus Monkeys

Our first 32 tests (series I and II) employed a technique which proved inadequate and gave no positive results; these have been previously described (2) and are not included in the present report. With the improved atomizing technique already described on p. 39 and using the OH strain of virus, we exposed 39 *rhesus* monkeys to "available" amounts of 2.5 to 10 cc. each. (series III to VIII). Several of the animals were repeatedly exposed, the total number of exposures being 63, of which 56 were with and 7 without olfactory blockade. The results, shown in Table II, may be summarized as follows:—

Of 35 monkeys with olfactory blockade given a total of 56 exposures, 4 developed clinical poliomyelitis and were found to have typical poliomyelitic lesions in the CNS; of these 2 were shown by examination of the olfactory bulbs to have acquired the disease through the olfactory route (despite blockade) and 2 (R1-99 and R2-41) through another route, possibly the trigeminal (see the protocols below). Of the 7 monkeys without olfactory blockade, 5 developed symptoms and typical lesions in the CNS, all shown to have been acquired through the olfactory route.

The 2 monkeys which acquired poliomyelitis of the CNS by non-olfactory channels are of particular interest. Unfortunately, the histopathological

examinations are not complete, but in one the localization of lesions suggested entry through the sensory portions of the V nerve.

Protocol R1-99.—1 per cent zinc sulfate was applied to the olfactory mucosa on May 12' 1941. The animal (in series III) was exposed to virus for 1 hour on May 20, 21, and 22. The total "available" amount was 8.5 cc. of a 20 per cent supernate. On June 6, 17 days after the first exposure, the temperature was 105.2°, the voice was hoarse, tremors were present, and the legs were weak. Both legs were paralyzed the next day when the animal was sacrificed for histological examination and subinoculation.

TABLE II

Results of Inhalation in Rhesus Monkeys: Series III to VIII

	Olfactory blockade			No blockade		
		Histopathology			Histopathology	
		No. examined	No. with lesions in CNS		No. examined	No. with lesions in CNS
No. of monkeys	35			7		
No. of exposures	56			7		
No symptoms	18	2	0	2	0	
Fever, 103.2–104°	8	7	0	0	0	
Fever, 104°+	3	3	1*	2	2	2*
Tremors or weakness	1	1	1‡	1	1	1*
Flaccid paralysis	2	2	2§	2	2	2*
Died of other causes	3	3	0	0	0	
Totals		18	4		5	5

* Olfactory take: lesions in olfactory bulbs.

‡ Non-olfactory take.

§ One olfactory, one non-olfactory take.

Temperatures of 103.2° or over not considered as fever unless sharply over previous base-line.

No lesions were found in serial sections of the olfactory bulbs. The secondary olfactory centers were not examined. The thalamus and hypothalamus were normal. One section each was examined from the midbrain, pons, and medulla. Lesions were found in the lateral vestibular nucleus and reticular formation, but not elsewhere. The main sensory nucleus of the V nerve was not examined, but no lesions were seen in the nucleus of the spinal tract of the V nerve, nor in the solitary nucleus. The anterior horns of the cord were involved in the three levels examined (midcervical, thoracic, and lumbar). The intermediolateral columns of the thoracic cord were spared. Of the peripheral nervous system, only a few of the spinal ganglia were studied histologically, and all showed lesions.

Positive subinoculations were obtained with the spinal cord, and thoracic and lumbar spinal ganglia. Subinoculations of the cervical spinal ganglia, cervical, thoracic, and lumbar sympathetic ganglia, celiac ganglion, tongue, bronchial washings, and stool were all negative.

Conclusions with regard to the portal of entry in this animal are impossible, except to state that it was undoubtedly not by the olfactory pathway. While

no lesions were found in the sensory nuclei of the cranial nerves or the intermediolateral columns of the thoracic cord, the examination was not complete. The peripheral ganglia of the cranial nerves were not examined.

Protocol, R2-41.—Olfactory blockade was induced by zinc sulfate irrigation on Sept. 3, 1941. On Sept. 17 and 18, virus was administered by inhalation in the first exposure of the animals in series IV. Each exposure was for 1½ hours and the "available" amount was 10 cc. of a 20 per cent supernate per monkey. On Sept. 25, 8 days after the first exposure, the temperature was 104° in the morning, and slight head tremors were noted. The tremors were more marked in the afternoon, and the animal was excitable. The right leg was slightly weak the next morning. The symptoms had not progressed by the early afternoon, when the animal was sacrificed.

Only the CNS was studied histologically. No lesions were found in serial sections of the olfactory bulbs. The secondary olfactory centers were not examined. The thalamus was normal. PvI, without PrI, was seen in the hypothalamus. Minimal lesions were found in the midbrain. The vestibular nuclei of the pons were heavily involved, the reticular formation and motor nucleus of the VII nerve less so. The main sensory nucleus of the V nerve was not examined, but slight diffuse PrI, with an occasional Pmn, was seen in the nucleus of the spinal tract of the V nerve in the pons. Lesions of moderate severity were found in the reticular formation, nucleus of the hypoglossus, and nucleus ambiguus in the medulla. Foci of PrI were observed in or near the nucleus of the spinal tract of the V nerve. The other nuclei of the medulla, including the solitary, were normal. Lesions were found in all nine levels of the cord examined, although the intermediolateral columns of the thoracic cord were spared.

The portal of entry in this animal was definitely not by the olfactory route, although, here again, an exact analysis is impossible. The presence of lesions in or near the nucleus of the spinal tract of the V nerve, suggests that entry may have been by this route. The absence of lesions in the solitary nucleus and the intermediolateral columns of the thoracic cord is against entry by way of the VII, IX, or X cranial nerves, or the sympathetic system.

The peripheral ganglia of 15 exposed and of 7 unexposed animals were examined; in the exposed the olfactory bulbs and CNS were also examined. 5 of the controls were examined within 5 days after reception in the laboratory, and the other 2 had been housed for 12 days in a cage previously used by a chimpanzee which had been fed virus. The possibility of cage contamination with virus may be of importance in evaluating the lesions found. The results are given in Table III.

Eleven of the exposed animals showed symptoms typical or suggestive of poliomyelitis and 2, tabulated in a separate column, died of intercurrent causes 4 and 8 days after exposure. Only lesions that we regard as significant are tabulated.

Perhaps the most striking finding in the peripheral ganglia is the frequency of involvement of the Gasserians. Of 14 exposed animals in which they were examined, lesions were found in 11 (79 per cent); also in 1 of the 2 controls possibly exposed, but in none of the 4 definitely not exposed. The occurrence

of Gasserian lesions in both animals which died of other causes than poliomyelitis shortly after exposure is of added interest because no lesions were found elsewhere in the nervous system. Involvement of other ganglia (excepting the spinal, which probably were centrifugally involved) was much less frequent:

TABLE III

Inhalation in Rhesus Monkeys: Distribution of Lesions in Peripheral Ganglia

	Controls		Exposed animals		
			No olfactory lesions		Olfactory lesions present
	A*	B‡	C§	D‖	E**
No.	5	2	2	6	7
Symptoms	0	0	0	5(F)	7(3P,1W,3F)
CNS involved	—	—	0/2	0/6	7/7
Ganglia:					
Gasserian	0/4	1/2	2/2	3/5	6/7
Geniculate	0/4	0/2	0/2	0/3	1/6
Petrosal	0/4	0/2	0/2	0/3	0/7
Nodose	0/4	1/2	0/2	0/3	3/7
Cervical sympathetic	1/5	2/2	0/2	2/6	2/7
Thoracic sympathetic	0/5	0/2	0/2	0/6	1/7
Lumbar sympathetic	0/5	0/2	0/2	1/6	0/7
Celiac	0/5	1/2	0/2	2/5	1/6
Cervical spinal	0/5	0/2	0/2	0/4	4/6
Thoracic spinal	1/5	0/2	0/2	1/4	4/6
Lumbar spinal	0/5	0/2	0/2	1/4	3/6

F, fever. W, weakness. P, flaccid paralysis.

* Killed within 5 days after arrival in laboratory.

‡ Killed 17 and 23 days after arrival but housed in the cage in which a chimpanzee had been kept during feeding experiments.

§ Died of intercurrent causes 4 and 8 days respectively after inhalation.

‖ All of this series had had previous olfactory blockade.

** 2 of this series had had olfactory blockade; 5, had not.

Numerators: number of animals showing lesions in the designated structures.

Denominators: number of animals examined. —: not examined.

in order of diminishing frequency they were the cervical sympathetic, the nodose, and the celiac. The geniculate was involved only once and the petrosal in none, indicating that entry through the taste buds is exceptional.

The study of *rhesus* under inhalation is of some special interest because this species has been regarded as insusceptible to non-traumatic infection by any route excepting the olfactory. By inhalation, however, 2 of our animals developed poliomyelitis by non-olfactory routes and a significant proportion of the rest displayed lesions in various peripheral ganglia, especially those of the

trigeminal nerve. Of 7 with the olfactory mucosa unblockaded, 5 developed poliomyelitis of the CNS. This, like the statistically similar result with *cynomolgus*, illustrates the efficacy of inhalation as a mode of experimental infection.

DISCUSSION

Our experiments demonstrate the ease with which poliomyelitis is acquired by inhalation of virus. This occurs with considerable regularity by the olfactory route in both *rhesus* and *cynomolgus* monkeys but of special interest with reference to the human disease is the frequency with which it occurs through non-olfactory channels in *cynomolgus*, a species apparently more closely related to man than is *rhesus* in respect to its infectibility by non-traumatic methods. In two instances even *rhesus*—a species hitherto regarded as uninfectible without trauma except by the olfactory route—developed poliomyelitis after inhalation in the presence of effective olfactory blockade. The percentage (40 per cent) of non-olfactory takes in our *cynomolgi* is the same as that obtained by Sabin and Ward (10) after ingestion of the same strain of virus but is much higher than we ourselves found (1) after a series of exposures of various portions of the alimentary tract, most of which excluded the respiratory mucosae. It appears that the nasal mucosae, respiratory as well as olfactory, are quite highly vulnerable to poliomyelitis virus.

The pathways of infection in the four non-olfactory takes in *cynomolgus* are of considerable interest and confirm the thesis that infection is nerve-borne from the mucous surfaces to the appropriate peripheral ganglia and thence to the CNS. Clearly, the presence of "bare" nerve-endings, contrary to earlier belief (11), is not essential to penetration of virus. The afferents of the trigeminal nerve appear to be of special importance. Thus, in one case (C68) this was the most probable route; in another (C64) it was certainly the main route, and in a third (C66) it was clearly the only one. One case (C63) gave convincing evidence of entry through the *sympathetic fibers*, presumably those of the nasal mucosa and nasopharynx, into the superior cervical sympathetic ganglion, and thence into the intermediolateral column of the upper thoracic cord. So far as we know this is the first time that invasion by this particular route has been demonstrated.[3] Its actual importance in human poliomyelitis is uncertain. Sabin and Ward (13) were unable to find virus in the superior cervical ganglia in 6 human cases, a number not large enough to prove a negative

[3] As regards the mucous surfaces of the head, the internal carotid nerve, derived from the superior cervical ganglion, supplies sympathetic fibers to the soft palate, nasopharynx, and pharynx through the internal carotid plexus; and, through the cavernous plexus, the nasal mucosa. While the fibers pass through other ganglia such as the sphenopalatine, they do so without interruption (12). The trigeminal nerve supplies all the mucous surfaces of the head with exteroceptive fibers.

general rule. The route is of potential importance as constituting a channel directly to the spinal cord from the nose and mouth, while other nerve routes from these areas connect with the medulla.

In the present series of *cynomolgus* monkeys exposed by inhalation, we found evidence in only one case (C64) of involvement of the celiac ganglion. Here, as in the case of alimentary exposure previously reported (1), we found no significant lesions in the corresponding portion of the CNS (lower thoracic intermediolateral columns) and we have no reason to believe that the intestine was the site from which infection reached the CNS. The impression we gather from our own experiments of this and the previous study is that poliomyelitis infection occurs somewhat more readily by inhalation than by ingestion and that in both cases the virus implants itself with greater ease in and through the mucosae of the head area — nasal passages, pharynx, and mouth — than elsewhere.[4]

We should like to point out that infection by inhalation is not necessarily or exclusively dependent on droplet infection. While the demonstrated presence of poliomyelitis virus in the nasopharyngeal secretions in a significant number of cases (14) provides an obvious basis for air-borne infection from droplets, the further possibility of inhalation of dust contaminated with fecal matter cannot be disregarded. Direct evidence of this type of air contamination is to be found in the studies of Nolan and Reardon (15) and of Sawitz, D'Antoni, Rhude, and Lob (16) on the common pinworm, *Enterobius vermicularis*. Both of these groups of workers repeatedly found viable ova in dust from such places as the top moldings of doors, windows, and picture frames which were too high to have been contaminated by manual contact. It is conceivable that poliomyelitis virus, with its known resistance to drying, could be spread in the same way. Very little attention has been paid to this possibility,[5] although in one instance Neustaedter and Thro (17) recovered virus from sweepings from the sick room of a patient.

On the basis of our experiments and of the points just discussed we venture to suggest that the concept of poliomyelitis as an air-borne disease acquired by inhalation of contaminated air or dust deserves more consideration than it has hitherto received. Such a view, we wish to emphasize, does not, and is not intended to, exclude ingestive infection. Experimental work shows that infection may occur in both ways. Which mode of infection is the more important for man is a problem that still awaits solution.

[4] In our preliminary paper (2) we suggested that infection after inhalation might have entered through the lower respiratory tract. The later histological study and analysis of our material has failed to support such a view.

[5] An extensive investigation of house dust for poliomyelitis virus is now under way in this laboratory.

SUMMARY

1. Poliomyelitis virus suspensions were atomized so as to produce dry droplet nuclei which, suspended in air, were introduced into a special infecting chamber and inhaled by test animals, both *rhesus* and *cynomolgus* monkeys.

2. Without olfactory blockade, 5 of 7 *rhesus* and 6 of 7 *cynomolgus* monkeys developed poliomyelitis of the CNS with entry through the olfactory nerves.

3. With olfactory blockade, 2 of 35 *rhesus* and 4 of 10 *cynomolgus* monkeys developed this form of the disease by routes proved by serial sections of the olfactory bulbs not to have been olfactory.

4. The neural pathways of infection from the mucous surfaces to the CNS in the 4 *cynomolgus* monkeys with blockade were shown in 2 instances to have been the afferent fibers of the trigeminal nerve into the Gasserian ganglion and thence to its central connections in the pons-medulla; in another case this was the probable route. In one instance the pathway consisted of the sympathetic fibers of the nose or nasopharynx into the cervical sympathetic ganglia and thence into the uppermost levels of the thoracic cord. The routes in the 2 *rhesus* monkeys with non-olfactory takes were not accurately determined but in one there was suggestive evidence of entry through the trigeminal nerve.

5. Study of the peripheral ganglia in a number of exposed *cynomolgus* and *rhesus* monkeys, including several with no demonstrated involvement of the CNS, revealed lesions most constantly in the Gasserian ganglia; less so in the cervical sympathetics and still less so in the celiac. In 2 *rhesus* monkeys dying of other causes a few days after exposure, lesions were limited to the Gasserian ganglia. No evidence was found in any case of passage of infection from the celiac ganglia into the CNS.

6. The importance of the peripheral ganglia as intermediate stations in the centripetal passage of infection from the body surface is again emphasized.

7. Comparison of the present with a previous study suggests that infection by inhalation of virus occurs with greater ease than by ingestion.

CONCLUSIONS

Inhalation of virus produces poliomyelitis in animals with marked facility. This is especially true in the *cynomolgus* monkey. With the olfactory pathways blocked a considerable percentage of *cynomolgus* monkeys became infected and in two instances *rhesus*, also, succumbed to the disease. The method employed involved no mechanical trauma to the mucous surfaces.

In non-olfactory infection after inhalation, the most important route of entry appears to be the afferent fibers of the fifth cranial nerve. Entry by way of the sympathetic fibers of the nose and pharynx was demonstrated in one instance.

It is suggested that inhalation of virus deserves more serious attention as a

mode of human infection than it has hitherto received, since air and dust may be contaminated from fecal as well as from nasopharyngeal sources.

Grateful acknowledgment is made to Mr. W. F. Wells of the Laboratories for the Study of Air-borne Infection of the Department of Public Health and Preventive Medicine at the University of Pennsylvania for his helpful interest in our work and for valuable suggestions, particularly in the design of the atomizing flask.

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