THE NATURAL HISTORY OF EXPERIMENTAL POLIOMYELITIS INFECTION

I. Studies on the Centrifugal Spread and Elimination of Virus in Intrasciatically Inoculated Rhesus Monkeys*

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(Received for publication, September 2, 1941)

The studies to be presented in this communication form part of an investigation into the behavior of poliomyelitis virus and the natural history of infection produced by various strains of the virus in the various susceptible hosts. A correct understanding of the events which come to pass from the moment the virus is introduced in a certain part of the body to the time when the disease is fully developed is possible only when the pathways of viral dissemination in the infected host are known. The information which can be derived from a map of virus distribution during the terminal phases of the infection is, of course, dependent on the degree of centrifugal spread from the central nervous system or generalized dissemination in the body. With some viruses this centrifugal spread and dissemination is so extensive that the distribution of the virus in the body postmortem can throw little or no light on the pathways it pursued during the early stages of infection. Other viruses, however, are more limited in their migrations and different patterns of virus distribution can be obtained when the pathways of the virus are varied as a result of inoculation into different regions of the body. Although recent studies (1–4) tend to indicate that poliomyelitis virus belongs to the latter category, certain concepts, which have been almost axiomatic for years and are based chiefly on observations made on monkeys inoculated by the intracerebral or combined intracerebral and intraperitoneal routes, need reexamination before one can proceed with the evaluation of accumulating data. Among these concepts is the one in which the nasal mucosa is regarded as an avenue for the exit of virus from the nervous system of poliomyelitis-infected animals. If it were true that in a given host, poliomyelitis virus, after invading the central nervous system, could spread centrifugally along nervous or other pathways to localize in various peripheral tissues, it would either preclude or significantly affect

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

any attempt to deduce the natural history of the infection from a given pattern of virus distribution.

In 1910, Flexner and Lewis (5) reported that Berkefeld filtrates of the mucous membrane of the nasopharynx obtained from recently paralyzed monkeys produced paralysis in other monkeys. In 1911, Landsteiner, Levaditi, and Danulesco (6) reported that a number of attempts to find virus in the nasal secretions of paralyzed monkeys failed until they inserted cotton plugs for 24 hours into the nostrils of 2 paralyzed *cynomolgus* monkeys, and inoculated the pooled filtrate intracerebrally and intraperitoneally into another monkey which developed typical poliomyelitis in 10 days. In three tests on the tonsils and peritonsillar mucosa obtained from three paralyzed *cynomolgi*, which were infected by the intracerebral route, one yielded the virus. In 1912, Levaditi and Danulesco (7) continued these studies and recorded the following observations: (1) tests with filtrates of the nasal mucosa obtained from 4 intracerebrally inoculated, paralyzed *cynomolgus* monkeys were all negative and they stated that similar tests on a larger number of monkeys were also negative; (2) filtrates of the nasal secretions obtained on tampons from 5 intracerebrally inoculated, paralyzed monkeys (2 *rhesus* and 3 *cynomolgus*) yielded negative results when the secretions from each paralyzed monkey were subinoculated separately into a new monkey; (3) when the nasal secretions from two other *cynomolgus* monkeys were pooled and subinoculated into a single monkey, virus was demonstrated; (4) six experiments in which tampons from infected monkeys were placed in the noses of normal monkeys were negative; (5) tests on the tonsils from 4 intracerebrally inoculated, paralyzed monkeys (1 *rhesus* and 3 *cynomolgus*) yielded one positive result with the material from one of the *cynomolgus* monkeys.

In more recent years a number of attempts to demonstrate the virus in the nasal or nasopharyngeal mucosa and secretions of paralyzed *rhesus* monkeys have yielded negative results. In 1938, Sabin and Olitsky (8) observed that even after nasal instillation of highly potent "M.V." virus in *rhesus* monkeys none was found in the nasal mucosa and secretions at the time of paralysis, although virus was demonstrable at 72 hours after instillation by the method used. In the same year Sabin (2) reported that no virus was found in the nasal mucosa of 4 *rhesus* monkeys which succumbed with typical poliomyelitis after injection of "M.V." virus by the tonsillopharyngeal route. In 1939, Kramer, Hoskwith, and Grossman (9) reported negative results when they subinoculated the nasopharyngeal and oral washings together with nasal and pharyngeal tissue obtained from 7 paralyzed *rhesus* monkeys some of which had been inoculated intracerebrally with "M.V." virus and others intranasally with the "Armstrong" virus.

The early experiments probably differed from those carried out later on in that strains of virus of more recent human origin or *cynomolgus* monkeys were used, and in so far as larger amounts or virus might have been injected intracerebrally and intraperitoneally. Theoretically, it is possible that after injection of large amounts of virus intracerebrally some may escape along the known existing connections between the subarachnoid space and the interstitial tissues of the nasal mucosa, although Yoffey and Drinker (10) reported that "M.V." virus inoculated intracerebrally or intranasally in *rhesus* monkeys could not be demonstrated in the lymph from the nasopharynx.

In so far as one is searching for corollaries to help explain events in the human disease, it is more relevant to know what happens when the virus has reached the central nervous system from a peripheral site than after intracerebral injection. Fully realizing that ultimately one will have to consider separately the behavior of different strains of virus in different hosts, it was decided to begin the experimental inquiry into the centrifugal spread of poliomyelitis virus with the monkey-adapted "M.V." strain in *rhesus* monkeys. The intrasciatic route of inoculation was selected chiefly because we had reason to expect that poliomyelitis could be produced regularly in this manner, and despite the fact that, according to a report by Hurst (11), virus can sometimes be found in the spinal fluid of such monkeys when the disease is fully developed although not in the earlier stages. Since poliomyelitis virus has never been demonstrated in the cerebrospinal fluid of human cases and rarely, if ever, in monkeys inoculated by other peripheral routes, the intrasciatic route of infection might thus perhaps be expected to favor the possibility of escape of virus from the subarachnoid space into the nose.

General Plan

The plan of investigation was as follows: (1) inoculate "M.V." virus into the sciatic nerve of a group of *rhesus* monkeys; (2) collect the nasal secretions on absorbent cotton plugs (the method by which successful isolation of virus from intracerebrally inoculated *cynomolgus* monkeys was reported by Landsteiner Levaditi, and Danulesco (6) and by Levaditi and Danulesco (7)) every 24 hours during life; (3) pool the secretions collected during each 24 or 48 hours from several monkeys and test for virus; (4) when the monkeys were either prostrate or dead as a result of poliomyelitis infection a number of tissues selected for their capacity to indicate the extent of centrifugal spread of virus, are to be obtained with special precautions to avoid contamination and tested for virus by subinoculation into new *rhesus* monkeys. The spinal cord was included in the group of tissues from each monkey to serve as a positive virus control. The tests on the olfactory bulbs were expected to indicate not only whether the virus had spread that far, but also, in case virus were found in the nasal secretions or mucosa, whether the virus had reached the nose by a neuronal pathway or otherwise. The presence or absence of virus in the tonsils and pharyngeal tissue might be an index either of lymphatic absorption from the nose or centrifugal spread from the medulla. Tests on the superior cervical sympathetic ganglia, the abdominal sympathetic ganglia of the celiac plexus, the adrenals, small intestine, and salivary glands were expected to show how far virus localized in the central nervous system wandered into peripheral sympathetic ganglia or tissues containing collections of nerve cells of the parasympathetic system. It is well known that by the time a monkey is prostrate as a result of poliomyelitis infection specific neuronal lesions may be found in almost all the spinal ganglia and also in the sensory cranial ganglia regardless of the route of inoculation (Pette, Demme, and Környey (12), Bodian and Howe (3), and personal observations). It is apparent, therefore, that the virus spreads to the sensory neurons close to the spinal cord and medulla and

might thus perhaps be expected to involve also some of the nerve cells in the ganglia of the sympathetic trunk along the vertebral column in the thorax and abdomen although Pette, Demme, and Környey (12) stated that in general no neuronal lesions were found in these ganglia. Whether or not it can spread as far as the collateral ganglia, such as the celiac, or the terminal ganglia located within the viscera or glands that they innervate, is a question of considerable importance in any attempt to interpret a given pattern of virus distribution in cases where the original portal of entry of the virus is unknown.

Methods

Virus and Mode of Inoculation.—The "M.V." strain of poliomyelitis virus was used. Before each experiment glycerinated cord was passaged and fresh virus was used for intrasciatic injection. With the monkey under ether anesthesia an incision through skin and muscle was made midway over the posterior aspect of the thigh and the sciatic nerve was then exposed by blunt dissection. 1 cc. of a 10 per cent lightly centrifuged suspension of the virus was then injected into the nerve using a 20 or 22 gauge needle which was moved back and forth within the sheath of the nerve for the purpose of cutting a certain number of nerve fibers and exposing their axis cylinders. While ballooning of the nerve occurred during the injection, most of the inoculum escaped into the surrounding tissues. It is noteworthy that of 8 monkeys so inoculated all developed poliomyelitis. In view of the fact that some investigators (Harrison and Woolpert (13), Toomey (14)) reported that in their experience most monkeys failed to develop poliomyelitis after intrasciatic injection and in view of the fact that it has been suggested that vitamin D deficiency may facilitate progression of poliomyelitis virus along peripheral nerves, it should be pointed out that 6 of the 8 monkeys in the present experiments either had no vitamin D deficiency to begin with or else had been given vitamin D (drisdol) for a period of 2 weeks prior to inoculation; in the other two monkeys both chemical and roentgen evidence of D deficiency were present. The relationship between vitamin D nutrition (as determined by the concentration of phosphorus in the blood and roentgen examination of the bones) and the invasiveness of poliomyelitis virus along peripheral nerves was studied on these monkeys but the data will be presented in another communication (15).

Collection and Preparation of Nasal Secretions for Subinoculation.—Directly the intrasciatic inoculations were completed, absorbent cotton was plugged into each nasal cavity. At the end of 24 hours these plugs were removed and new ones were inserted. The moist plugs were kept in a refrigerator or frozen by means of solid CO_2. This process was repeated every 24 hours until the monkey was dead or sacrificed. In the first series of tests the cotton plugs for each 24 hours from each of 4 monkeys were pooled for subinoculation into a new monkey. In the second series of tests the cotton plugs for each 48 hours from each of 2 monkeys were pooled. As much as possible of the original nasal juice was expressed from the cotton plugs and further extracts were obtained by maceration with physiological salt solution following the same procedure of extraction, centrifugation, and etherization that was used in similar tests on human beings and described in a previous communication (16). The untreated (unetherized) centrifuged sediments were instilled intranasally into the same monkeys which received the etherized supernatant liquids intracerebrally and

intraperitoneally. Some of the etherized material was saved for a second intracerebral inoculation 4 to 7 days later. Several monkeys died of a bacterial pneumonia until the ether anesthesia used for the intracerebral inoculation was replaced by local anesthesia.

Collection and Preparation of Tissues for Subinoculation.—The various tissues were obtained when the paralyzed monkeys were either dead or prostrate. The adrenals, abdominal sympathetic (celiac) ganglia and plexus, the superior cervical sympathetic ganglia, salivary glands (parotid and submaxillary), spinal cord, and olfactory bulbs were all obtained with aseptic precautions and suspensions prepared of them were

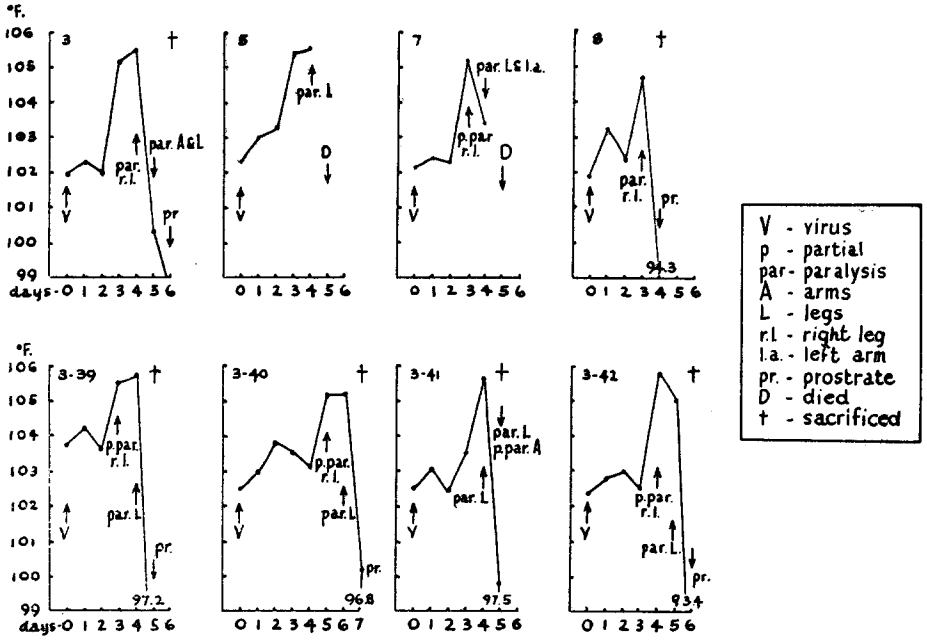

CHART 1. Clinical course of *rhesus* monkeys inoculated into right sciatic nerve.

injected intracerebrally or intracerebrally and intraperitoneally when sufficient material was available. The nasal mucosa (obtained by a method previously described (8)), the tonsils with the attached pharyngeal tissue, and the small intestine were ground with sand and physiological salt solution and the centrifuged supernatant liquids were treated with 15 per cent of anesthetic ether before intracerebral and intraperitoneal inoculation.

RESULTS

The clinical course of the experimental disease produced in the 8 monkeys by intrasciatic injection of poliomyelitis virus is shown in Chart 1. It is noteworthy (1) that the incubation period was relatively short, paralysis being observed first on the 3rd day in 3 monkeys, on the 4th day in 4 monkeys, and on the 5th day in 1 monkey, and (2) that the first rise in temperature occurred

TABLE I

Search for Poliomyelitis Virus in Nasal Secretions of Rhesus Monkeys Inoculated with the "M.V." Strain

Donor monkeys	Period after inoculation	Test on nasal secretions		
		Dose and route	Monkey No.	Result
	days	*cc.*		
	0 to 1	2 i.c. 1.8 i.p. 3 i.n.	18	D_2 (pneumonia)
	1 to 2	2 i.c. 3 i.p. 3 i.n.	19	D_1 (pneumonia)
Intrasciatic Rh. 3—par. 4 Rh. 5— " 4 Rh. 7— " 3 Rh. 8— " 3	2 to 3	2 i.c. 4 i.p. 9 i.n.	20	Remained well—no poliomyelitis
	3 to 4	2 i.c. 3 i.p. 12 i.n.	21	" " " "
	4 to 5	2 i.c. 4 i.p. 9 i.n.	49	D_3 (pneumonia)
	0 to 2	2 i.c. (× 2) 8 i.n.	3-61	D_1—enteritis
	2 to 4	2 i.c. 8 i.n.	3-62	D_7—enteritis—no poliomyelitis
Intrasciatic Rh. 3-40—par. 5 Rh. 3-42— " 4		2 i.c.	2-82	Remained well " "
	4 to 6 (Rh. 3-42) 4 to 7 (Rh. 3-40)	2 i.c. (× 2) 10 i.n.	3-63	" " " "
	0 to 2	2 i.c. (× 2) 6 i.n.	2-68	" " " "
Intrasciatic Rh. 3-39—par. 3 Rh. 3-41— " 4	2 to 4	2 i.c. (× 2) 6 i.n.	3-87	D_6—pyogenic meningitis
	4 to 5	2 i.c. (× 2) 6 i.n.	3-88	Remained well—no poliomyelitis
Intracerebral Rh. 4—par. 5	5 to 7	2 i.c. 0.5 i.p. 6 i.n.	2	" " " "

i.c.—intracerebral; i.c. (× 2)—reinoculated intracerebrally in 4 to 7 days; i.p.—intraperitoneal; i.n.—intranasal; par. 4—initial paralysis 4th day after inoculation; D_2—dead 2nd day.

on the same day as the paralysis in 6 monkeys and on the day preceding paralysis in 2 monkeys (to be contrasted with the disease following nasal instillation of the virus when the first rise in temperature occurs 3 to 5 days before the onset of paralysis). The appearance of paralysis first in the inoculated leg of 6 monkeys and in both legs of two others is in accord with the usual course of events. The disease was purposely allowed to run its full course

TABLE II

Tests for Centrifugal Spread of Virus in Rhesus Monkeys Succumbing with Poliomyelitis Following Intrasciatic Injection of M.V. Virus

Tissues tested	Result of test for virus on tissues of donor monkey No.							
	3	5	7	8	3-39	3-40	3-41	3-42
Spinal cord	+	+	+	+	+	+	+	+
Olfactory bulbs	0	0	0	0 [o]	0	0	0	0
Nasal mucosa	0	0	0	0	0	0	0	t.u.
Tonsils + pharyngeal tissue	0	0	0	t.u.	0	0	0	0
Salivary glands	0	0 [o]	t.u.	0	0	0	0	t.u.
Small intestine	t.u.	0	t.u.	0	0	0	t.u.	0
Abdominal sympathetic-celiac ganglia and plexus	t.u.	0	0	0	0	0	0	0
Adrenals	0	0	0 [o]	0	0	0	0	0
Superior cervical sympathetic ganglia	0	0	0 [o]	0	0	0	0	t.u.

$\overset{0}{[o]}$ refers to monkeys whose nervous tissues were passaged to other monkeys with negative results.

t.u.—test unsatisfactory because monkey died of extraneous causes before poliomyelitis could have developed.

in these monkeys in order to permit the greatest possible spread of the virus.

The results of the search for virus in the nasal secretions of these monkeys are shown in Table I. While a number of the subinoculated monkeys died of non-poliomyelitic causes, a sufficient number survived in the several series to indicate that no virus is demonstrable in the nasal secretions of the intrasciatically infected *rhesus* monkeys during any stage of the preparalytic or paralytic phases of the disease. The tests on the tissues listed in Table II indicate that by the time the terminal phase of the disease is reached or at death, the virus had not spread sufficiently either in the central nervous system

to involve the olfactory bulbs and the adjacent nasal mucosa, or peripherally to affect the collateral sympathetic ganglia, such as the superior cervical sympathetic, celiac, or the nerve cells in the adrenals, the collections of nerve cells of the parasympathetic system such as those of Meissner's and Auerbach's plexuses in the small intestine or those in the salivary glands and pharyngeal wall around the tonsils. The negative tests with the tonsils offer confirmatory evidence for the absence of virus in the tissue spaces of the nasal mucosa and other regions whose lymphatics drain into these nodes. The positive results with the spinal cord of each monkey lend weight to the negative tests with the other tissues.

DISCUSSION

The results of the present investigation indicate quite clearly the limited spread of "M.V." poliomyelitis virus which has invaded the central nervous system of *rhesus* monkeys by way of a peripheral nerve such as the sciatic. While it has been known since Hurst's (11) observations on the histological changes found in intrasciatically inoculated monkeys that the virus invading the lumbar cord rapidly progresses as far as the thalamus and motor cortex, it is now clear that the absence of lesions in the olfactory bulbs of such monkeys, previously noted by Sabin and Olitsky (1) and Bodian and Howe (3), is also associated with an absence of virus. It is also noteworthy that no lesions were found in the anterior perforated substance which was examined in 4 of the monkeys used in the present study. It is interesting and helpful that this correlation between the presence of specific lesions and occurrence of virus obtains in poliomyelitis, since in most of the other neurotropic viruses studied no such correlation has been found chiefly because there usually is widespread dissemination of the viruses in the central nervous system during the terminal phases of the disease. This limited localization of poliomyelitis virus as well as its correlation with the localization of lesions in the central nervous system has recently been found to obtain also in human beings (4).

The negative results with the nasal secretions obtained during various stages of the disease, indicate that there is no justification for the generalization that elimination of virus by the nasal route is one of the consequences of poliomyelitis infection. That these negative results with the nasal secretions and nasal mucosa obtained in *rhesus* monkeys infected by a peripheral neural route with "M.V." virus are probably not unique is evident from the fact that similar results were recently obtained in studies on human poliomyelitis (4). The direct demonstration by Lennette and Hudson (17) and the indirect indications from the experiments of Armstrong (18) and of Sabin and Olitsky (1), that poliomyelitis virus may be eliminated on the nasal mucosa following intravenous injection of large amounts, apparently have no bearing on the events which take place when infection occurs by peripheral neural routes.

The absence of virus in the collateral sympathetic ganglia, the adrenals, the

salivary glands, tonsils and pharyngeal tissue, and the small intestine with its numerous nerve cells of Meissner's and Auerbach's plexuses, is ample evidence against the assumption that, at least in *rhesus* monkeys infected with the "M.V." strain, poliomyelitis virus after multiplication in the central nervous system spreads outward again to affect peripheral collections of nerve cells in various tissues. The recent tests on the same tissues of human beings with poliomyelitis indicated a similar absence of centrifugal spread, and for that reason the frequent finding of the virus in the human pharynx and intestine suggested these sites as the probable usual portals of entry in man (4).

The possibility that with certain strains of poliomyelitis virus in certain hosts, there may occur a more extensive centrifugal spread cannot be evaluated on the basis of existing data and certainly requires further investigation. For instance, in 1914, Flexner, Clark, and Amoss (19) reported two experiments in which virus was demonstrated in the celiac ganglia: (*a*) in the first experiment the celiac ganglia from 4 monkeys which died after inoculation with the "K" strain of virus were subinoculated into a *rhesus* monkey which developed clinically and histologically typical poliomyelitis; (*b*) in the second experiment subinoculation of the celiac plexus from a single monkey succumbing to the "K" virus also produced clinically and histologically typical poliomyelitis. The interpretation of these findings in relation to the question of centripetal versus centrifugal spread depends, however, on whether the monkeys from which these ganglia were derived were inoculated only intracerebrally or intraperitoneally as well. For if the donor monkeys had been inoculated only by the intracerebral route these findings would indicate a centrifugal spread to the collateral sympathetic (celiac) ganglia; if, on the other hand, they had also received virus intraperitoneally the virus may have been present in the celiac ganglia as a result of centripetal invasion. Bodian and Howe (3), for example, observed that lesions in the parasympathetic ciliary ganglia were present in monkeys succumbing after intraocular inoculation ("M.V." virus) but not after infection by other routes. Similarly one of us found no lesions in the superior cervical sympathetic and celiac ganglia of 5 *rhesus* monkeys infected intranasally with "M.V." virus, while after intraocular injection with the same strain of virus in the same species of monkey Bodian and Howe (3) found occasional lesions in the superior cervical sympathetic ganglia (which contain the sympathetic neurons supplying the eye) in unoperated animals and numerous lesions in monkeys whose ciliary ganglia were removed prior to infection. The recent report of Burnet and Jackson (20) that they found the virus in the sympathetic chain in 2 of 4 *cynomolgus* monkeys which had been inoculated intracerebrally or intraocularly with a strain of virus (Mar.) of recent human origin may perhaps be indicative of a certain degree of centrifugal spread to the paravertebral sympathetic ganglia. However, if the sympathetic chain which they tested included the cervical sympathetic ganglia and if the positive results were obtained in the animals which

were inoculated intraocularly, the virus might have spread there centripetally along the sympathetic fibers and neurons connecting the eye with the central nervous system rather than centrifugally.

In view of the high incidence of positive virus isolations from the stools of human beings with poliomyelitis, and the demonstration that the virus is present in the intestinal wall of patients dying of the disease (4), it is of considerable importance to know whether or not poliomyelitis virus can reach the intestinal wall or stools as a result of centrifugal spread from the central nervous system. Clark, Roberts, and Preston (21), Howe and Bodian (22), and Toomey (23) reported negative results in tests on the stools of a total of 17 *rhesus* monkeys which were paralyzed as a result of intracerebral inoculation of "M.V." virus. These negative results are especially significant in view of the demonstration by several investigators that poliomyelitis virus can pass through the alimentary tract of *rhesus* monkeys without being inactivated (21), and the finding in the present investigation that demonstrable amounts of virus do not spread to the intestinal wall from the central nervous system. In an attempt to determine whether the same would obtain for a strain of virus of recent human origin and for *cynomolgus* monkeys, we have made some preliminary tests by searching for virus in the colon contents of 3 paralyzed *cynomolgus* monkeys, inoculated intracerebrally with our 1940 "Per." strain, and obtained negative results. In 1939, Kramer, Hoskwith, and Grossman (9) reported that they tested separately the contents of the small and large intestines (plus, in each instance, some of the mucosa obtained by curettage) of 7 *rhesus* monkeys some of which received the "M.V." virus intracerebrally and others the "Armstrong" virus intranasally. While the contents from the large intestine were negative in each case, they found virus in one instance in the contents of the small intestine. It would be easier to interpret the significance of this positive finding if we knew whether or not it occurred in a monkey which had received the "Armstrong" virus (presumably a strain of more recent human origin) intranasally (and therefore also swallowed). In work which we have recently completed on *cynomolgus* monkeys which developed paralysis after being fed a strain of virus of recent human origin ("Per.") we had no difficulty in demonstrating virus in the wall and contents of the small intestine (24). The possibility that strains of recent human origin may behave similarly in *rhesus* monkeys is now under investigation.

SUMMARY AND CONCLUSIONS

1. Eight *rhesus* monkeys with experimental poliomyelitis following intrasciatic inoculation of "M.V." virus were used to study the extent of virus spread in the central and peripheral nervous systems and the question of its elimination in the nasal secretions.

2. Tests on nasal secretions collected on absorbent cotton plugs daily and

continuously from the moment of inoculation to the end of the disease failed to reveal virus.

3. No virus was found in the olfactory bulbs, nasal mucosa, tonsils and adjacent pharyngeal tissue, salivary glands, adrenals, superior cervical sympathetic ganglia, abdominal celiac ganglia, and small intestine.

4. Elimination of virus by the nasal route was not one of the consequences of poliomyelitis infection resulting from invasion of the "M.V." virus by way of a peripheral nerve in *rhesus* monkeys.

5. No indiscriminate widespread dissemination of virus occurred in the central nervous system of the intraneurally inoculated *rhesus* monkeys nor did the virus spread outward sufficiently to involve the collateral sympathetic ganglia or the collections of nerve cells in various peripheral tissues. Under certain circumstances, therefore, the presence of virus in these ganglia and tissues may be used as an index to the portal of entry of the virus.

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