FATE OF NASALLY INSTILLED POLIOMYELITIS VIRUS IN NORMAL AND CONVALESCENT MONKEYS WITH SPECIAL REFERENCE TO THE PROBLEM OF HOST TO HOST TRANSMISSION

By ALBERT B. SABIN, M.D., and PETER K. OLITSKY, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research)

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From an epidemiological point of view it is essential to know what happens to poliomyelitis virus which finds its way into the nose of a normal, susceptible animal and of one which is resistant by virtue of a previous attack of the disease. This question has not been investigated experimentally hitherto because until recently nasal instillation of poliomyelitis virus in monkeys caused the disease infrequently and irregularly. It now appears quite probable that of the various neural connections of the nasal mucosa (olfactory, trigeminal, sympathetic, and parasympathetic) poliomyelitis virus instilled into the nose of *Macacus rhesus* monkeys can invade the CNS (central nervous system) only along the olfactory pathway since mechanical interruption of this pathway prevents the development of the disease, as was shown in experiments reported simultaneously by Brodie and Elvidge (1) and Schultz and Gebhardt (2).[1] Additional support is found (*a*) in the early observation of Flexner and Clark (5) that in a monkey sacrificed 48 hours after having its nose swabbed with virus, the olfactory lobes contained the virus at a time when the medulla and spinal cord were not yet infectious, (*b*) in the experiments of Faber and Gebhardt (6) on the progression of nasally instilled virus, and (*c*) in the pathological

[1] See also Lennette and Hudson (3) and Howe and Ecke (4) for confirmation of these findings. The nature of the operation performed by Howe and Ecke, *i.e.*, section of the olfactory tracts by a lateral approach through the medial orbital walls, was such that it probably avoided the minute and little known nervus terminalis or 13th cranial nerve; the failure of their monkeys to develop paralysis may suggest, therefore, that this nerve also does not ordinarily supply a pathway for this virus.

studies of Sabin and Olitsky (7). But while it is thus known by what neural route virus in the nose invades the CNS of monkeys, nothing whatever is known about its fate locally in the nasal mucosa, *i.e.*, whether or not, or to what extent it multiplies in it before invading the CNS; how soon such multiplication begins and for how long it continues; how long the virus persists once the infection is established; and other related questions which are of obvious importance when one considers the nasal and associated passages as the sites from which infection may be disseminated in nature. For the same reason it is essential to know to what extent animals which have once had the disease can carry the same strain of virus as a result either of the first attack or of subsequent nasal instillations. While the results obtained in monkeys need not represent the course of events in human beings, they may, nevertheless, help in correlating the behavior of the virus in the only available experimental animal with the manifestations of the disease in man.

The literature records but a single attempt to determine the fate of virus applied to the nasal mucosa, that of Flexner and Amoss (8) at a time (1920) when the number of monkeys which developed paralysis with this mode of infection was quite small (8). These investigators applied the virus by means of a cotton plug which was left in the naris for periods varying from 2 to 24 hours, and sacrificed a number of monkeys at varying intervals after removal of the plug to test for the presence of virus in the excised nasal mucosa. One monkey was tested after 40 hours, two after 60 hours, and one each at 88 hours, 8 days, and 16 days. Virus was found in the nasal mucosa of only one of these animals, and that was in one of the two sacrificed at 60 hours. None of the monkeys exhibited any signs of disease at the time they were killed and in two of them (the 88 hour and 16 day animals) in which the olfactory lobes and other parts of the CNS, in addition to the nasal mucosa, were tested for virus, none was found. These results were interpreted as indicating that the nasal mucosa of certain normal monkeys possesses the power to destroy or otherwise render ineffective the virus applied to it; it was thought that that might, perhaps, be the reason for the failure of many animals to become infected by this route.

In recent years, as a result of further work by Flexner and others, it has become increasingly easy to produce paralytic poliomyelitis in monkeys by nasal instillation of the virus. The practice has been to give repeated nasal instillations of at least 1 cc. of suspension in each nostril and the incidence of poliomyelitis among monkeys receiving such instillations has gradually risen, for reasons as yet insufficiently clear, from 50 per cent to 75 per cent and in the past 2 years in our hands to 100 per cent (over 100 monkeys), especially when the virus suspensions were

prepared and given in a definite way. When repeated tests showed that one could predict that practically every monkey which received the virus intranasally in a certain way would develop the disease, it was possible to undertake a study of its fate in the nasal mucosa at different stages of the infection. Similarly one could also determine what happened to an infective amount of virus which was given intranasally to monkeys which had recovered from a distinct paralytic attack of the disease.

Methods

Infection of Monkeys by Nasal Route.—It is not yet possible for us to single out the factors to which can be attributed the constancy with which nasal instillation of poliomyelitis virus in monkeys has produced the paralytic disease in our laboratory for the past 2 years. It may, therefore, be worth while to describe in detail the method used and to indicate the effects of certain deviations from it. The virus suspensions are prepared exclusively from the spinal cords and medullae of monkeys which had developed paralysis following nasal instillation of the M.V. strain which has been used only for nasal infection in the past 3 years. Whether or not repeated passage by the nasal route is an essential factor has not been investigated. It appears to be important that the tissue should not have been in glycerol longer than 1 month; in one experiment in which 3 month old cords were used the incidence of successful infection was reduced to 80 to 90 per cent of thirty-six monkeys. Tissue from at least six different monkeys is used to prepare the virus suspension. 10 per cent and 5 per cent suspensions are equally effective but it should be stated that in the preparation of the virus the tissue is first minced finely and ground to a paste with alundum before the necessary amount of diluent is gradually added; after centrifugation at very low speed for only 2 to 3 minutes to remove the alundum and gross pieces, the supernatant liquid and loose sediment are poured off and stirred to yield a milky suspension. The dose is 1 cc. for each nostril instilled with the aid of a pipette fitted with a rubber urethral tip, forcefully expelling the total amount in the direction of the olfactory mucosa (Fig. 1), immediately drawing it back into the pipette, and repeating the process 2 to 3 times. The monkey is then made to take several deep breaths through the nose to aid further in carrying some of the suspension to the olfactory part of the mucosa. It has been the practice for some time to repeat the dose 48 hours later and many of the monkeys used in the present study were infected by this method. We found, however, that the second dose could be given on the same day, either several hours or even a few minutes after the first, and still induce infection in all the monkeys. When, however, the second dose was eliminated in one experiment, so that only 1 cc. per nostril was administered, two of four monkeys failed to develop the disease. Whether the effectiveness of two doses depends upon the larger volume that is administered, increasing the chances of covering more of the olfactory mucosa, or upon the additional virus, has not been determined. The weight and age of the monkeys apparently make little difference, as was pointed out in a previous communication (9).

As is the case with other viruses, so in the intracerebral titration of poliomyelitis virus, there is a minimal dose which is infective for practically all monkeys. When less than this dose is injected, the incidence of infection drops until, with one-tenth of it, there is practically no infection at all. For the M.V. virus, the constantly minimal infective dose by the intracerebral route is in the zone of 0.01 cc. to 0.005 cc. of a 5 per cent suspension.

FIG. 1

One may estimate, therefore, that 4 cc. of a 5 per cent suspension contains about 400 to 1,000 constantly infective minimal cerebral doses. This compares quite favorably with the number of minimal cerebral doses of other viruses which are required to produce infection regularly by the nasal route in the most susceptible animals (9). Thus, with vesicular stomatitis virus in young mice, about 100 to 1,000 minimal cerebral doses are required and with equine encephalomyelitis

virus (also in mice) 1,000 to 10,000 cerebral doses are necessary to produce infection regularly by way of the nose.

Course of Fever in Experimental Poliomyelitis Induced by Nasal Instillation of the Virus.—The clinical course of the disease induced by nasal instillation of the virus differs from that which follows infection by the intracerebral, subcutaneous, and intrasciatic routes in that the period of fever preceding the onset of nervous signs is distinctly longer in the former. When both doses of virus are given on the same day, the first rise in temperature (2°F. or more) occurs in the majority of monkeys 3 or 4 days after nasal instillation. There is, then, a period of at least 3 or 4 days before the appearance of nervous signs, during which the temperature either remains elevated or drops (frequently almost to normal) for a day or two and then rises again, *i.e.*, the dromedary type of curve which is exhibited by about half the number of monkeys.

Preparation of Nasal Mucosa for Inoculation.—The technique employed was influenced by the fact that it was considered important to avoid (*a*) contamination with the olfactory bulbs and (*b*) loss of virus by filtration. The monkeys were exsanguinated and the brain and olfactory bulbs removed. The two fossae harboring the olfactory bulbs were then swabbed out with absorbent gauze and all membranes cut as close to the cribriform plate as possible. The soft tissues were dissected away from the face and the eyes were enucleated to permit cutting through the orbits and maxillary bones for the removal of the nose and palate from the rest of the skull. Further cuts with bone scissors through the nasal bones and palate separated the septum from the other nasal structures and exposed the entire nasal mucosa (olfactory and respiratory). The olfactory portion was cut distal to its connections with the intracranial membranes. The entire mucosa, stripped from the septum, conchae, and lateral walls of the nose, usually weighed about 1 gm. It was finely minced, ground to a paste with alundum, followed by the gradual addition of 10 cc. of physiological salt solution. After horizontal centrifugation at about 2,000 R.P.M. for 10 minutes, the supernatant liquid was spun on the angle centrifuge at 4,000 R.P.M. for 40 minutes. 2 cc. of this supernatant liquid were drawn off for intracerebral injection of a monkey and the rest was thoroughly mixed both with the angle and horizontal centrifuge sediments and used for nasal instillation in the same monkey. Usually there were 7 to 8 cc. of the latter thick suspension which was divided into three portions, one being instilled daily. The nasal mucosa of twenty-two monkeys was prepared in this manner and in only four instances was the intracerebral injection followed by a fatal, bacterial (pneumococcus) meningitis, three cases occurring in a single experiment.

Preparation of Nervous Tissue for Inoculation.—The olfactory bulbs (from either normal or convalescent monkeys) were ground in a mortar without any abrasive and taken up in 1 cc. of saline solution, all of which was injected intracerebrally in a monkey. When other regions of the CNS of convalescent monkeys were tested, several grams of the tissue were ground with alundum and made up to

10 per cent suspension with phosphate buffer of pH 7.4. After very light centrifugation, 12 cc. of the milky suspension was drawn off for intracerebral (2 cc.) and intraperitoneal (10 cc.) inoculation of a monkey. To the remainder of the suspension sufficient phosphate buffer (usually not more than an equal volume) was added to bring the volume up to 100 cc., which was distributed among three U tubes and submitted to cataphoresis.

Cataphoresis.—The technique was essentially that employed by Olitsky, Rhoads, and Long (10), except that 3 per cent agar was used in the bridges and a milliamperage of 2.5 to 5 for a period of 4 to 5 hours.

Controls.—Whenever any of the normal or immune convalescent monkeys (which were to be sacrificed in an experiment) received nasal instillations of virus, there were at least three or more other monkeys which were given the same amount of the same suspension of virus and allowed to develop the paralytic disease, as an index of the infectivity of the virus used in each experiment. Daily rectal temperatures were taken on all animals.

EXPERIMENTAL

Sensitivity of Method.—When several tests with nasal mucosa from paralyzed monkeys failed to reveal virus, it became essential to determine approximately how many minimal cerebral infective doses (M.C.I.D.) had to be present in the nasal mucosa in order to be demonstrable by the method employed.

To the ground up nasal mucosa of a normal monkey was added 0.05 cc. of a Berkefeld N filtrate of a 5 per cent virus suspension (about 5 to 10 M.C.I.D.), and then 10 cc. of saline, the final mixture being submitted to the same horizontal and angle centrifugation as was described under Methods. Another mixture containing 0.2 cc. of the same virus filtrate (about 20 to 40 M.C.I.D.) and the nasal mucosa of another monkey was similarly prepared. Mixtures of 0.05 cc. and 0.2 cc. of virus filtrate each with 10 cc. of salt solution, similarly centrifuged, served as controls. Each preparation was inoculated into a monkey intracerebrally (2 cc.) and intranasally (7 to 8 cc.).

The results shown in Table I indicate that the monkeys inoculated with the mixtures containing nasal mucosa succumbed much more rapidly than those which received equivalent amounts of saline-virus mixtures. It is evident, therefore, that little or no virus is lost during the various steps of the procedure and that when as little as 5 to 10 M.C.I.D. are present in the nasal mucosa, it should be possible to detect the virus.

Virus in Nasal Mucosa and Olfactory Bulbs at Various Intervals after Nasal Instillation in Normal Monkeys.—The object of these tests was

TABLE I

Sensitivity of Method for Detecting Poliomyelitis Virus in Normal Nasal Mucosa

Medium	Amount of virus added	Dose and route of inoculation	Monkey No.	Result*
Entire nasal mucosa of normal monkey in 10 cc. saline	0.05 cc. (about 5 M.C.I.D.)	2 cc. intracerebrally 8 cc. intranasally	1	Paralysis 5, dead 6
	0.2 cc. (about 20 M.C.I.D.)	" "	2	" 5, prostrate 6
10 cc. saline	0.05 cc. (about 5 M.C.I.D.)	" "	3	Paralysis 15, " 17
	0.2 cc. (about 20 M.C.I.D.)	" "	4	" 11, " 12

* Numbers represent the day following inoculation or first instillation of virus on which paralysis or death occurred.

to determine (*a*) how much of the nasally instilled virus remained in the nasal mucosa within the first few hours, *i.e.*, the amount of virus which may be present without local increase; (*b*) whether or not the virus multiplied or increased in the nasal mucosa and in case it did, whether or not it occurred before invasion of the CNS; (*c*) how soon virus could be detected in the olfactory bulbs, particularly in relation to the time when fever first appeared and paralysis developed; (*d*) for how long a period virus could be detected in the nasal mucosa after the CNS had become invaded.

Thirteen monkeys were sacrificed at different stages of the experimental disease, beginning with 4 hours after nasal instillation and including animals which were completely paralyzed on the 8th or 9th day. The nasal mucosa and olfactory bulbs were tested for virus as described under Methods, and the results are shown in Table II.

It appears from these data that within 4 hours after nasal instillation of at least several hundred M.C.I.D. of virus, less than 5 to 10 M.C.I.D. remain in the entire nasal mucosa since no virus was detected by subinoculation. This finding is in accord with our observations on nasally instilled vesicular stomatitis virus in mice (11) and guinea pigs (12), in which no virus could be detected in the nasal mucosa even 1 to 3 hours after nasal instillation of as much as 100,000 M.C.I.D. Since one can be reasonably certain that animals in which the virus disappears in this manner would have succumbed, the question arises whether the subsequent infection develops from an undetectably small amount which becomes fixed to the cells of the nasal mucosa (although a relatively large amount is necessary to initiate a successful take), or whether there is, perhaps, an early non-infective stage in the attack of virus upon cells—a question which at the present state of our knowledge cannot be answered one way or another.

At 24 and 48 hours virus was still undetectable in the nasal mucosa and olfactory bulbs, the latter apparently still containing less than a single M.C.I.D., since both bulbs were transferred practically without loss into the brain of another monkey. In both monkeys which were sacrificed 3 days after nasal instillation virus was found both in the nasal mucosa and the olfactory bulbs. The animals inoculated with the nasal mucosa did not develop paralysis until the 15th or 13th day,

TABLE II

Tests for Virus in Nasal Mucosa and Olfactory Bulbs at Various Intervals after Nasal Instillation in Normal Monkeys

Time after nasal instillation of poliomyelitis virus	Monkey No.	Fever or paralysis	Effect of inoculating monkeys with	
			Nasal mucosa	Olfactory bulbs
4 hrs.	1	None	0	n.t.*
24 "	2	"	Bacterial contamination	0
	3	"	0	0
2 days	4	"	Bacterial contamination	0
	5	"	0	0
3 "	6	1st day of fever (105.6°F.)	Paralysis 15th day (poliomyelitis histology)	Paralysis 5th day
3 days (after 1st dose) (1 day " 2nd ")	7	None	Paralysis 13th day (poliomyelitis histology)	" 6th "
4 days (after 1st dose) (2 " " 2nd ")	8	Fever 3rd and 4th days	0	Paralysis 5th day
7 days (after 1st dose) (5 " " 2nd ")	9	Fever since 4th day	Bacterial contamination	" " "
8 days	10	Paralyzed 7th day	0	" " "
	11	" " "	0	n.t.
	12	" " "	0	"
9 days	13	" " "	0	"

* n.t. = not tested.

as compared to 5 or 6 days in the monkeys inoculated with the olfactory bulbs from the same animals. The clinical course of the disease in the monkeys inoculated with the nasal mucosa as well as the histological examination of their olfactory bulbs suggested that they succumbed as a result of the intracerebral inoculum (*i.e.*, 1/5 of the total nasal mucosa) and not as a result of the remainder of the material instilled intranasally. Sections of small pieces of the olfactory bulbs of one of the monkeys sacrificed on the 3rd day revealed distinct pathological changes consisting of acidophilic necrosis of a number of mitral cells and an infiltration of the outer layers of the bulbs with mononuclear and polymorphonuclear cells (7). It is also noteworthy that the first detection of virus in the nasal mucosa and olfactory bulbs corresponded to the first appearance of fever in one monkey but was unassociated with any rise in temperature in the other. In monkeys sacrificed later than the 3rd day after nasal instillation, virus was demonstrable in the olfactory bulbs but not in the nasal mucosa. It may be interesting to recall here that in the experiments of Flexner and Amoss (8), already described in a preceding section, the one positive result in recovering virus from the nasal mucous membranes was 60 hours after the administration of the virus by means of a cotton plug.

It appears, therefore, that within a few hours of and for 2 days after the nasal instillation of an amount of poliomyelitis virus which is capable of inducing paralysis in practically all monkeys, none can be detected in the excised nasal mucosa. It then becomes demonstrable simultaneously in the olfactory bulbs and nasal mucosa on the 3rd day, and while it subsequently remains in the bulbs and progresses through the rest of the CNS to produce the complete paralytic disease, it again either disappears from the nasal mucosa or diminishes in amount to such an extent that it cannot be again recovered. One cannot be certain whether the transitory presence of detectable, though small, amounts of virus in the nasal mucosa on the 3rd day is the result of local multiplication in the olfactory neurons of the first order or of an overflow of virus multiplying in the olfactory bulbs, although the failure to find it in the mucosa on the 4th day or later at a time when the bulbs were highly infective may, perhaps, be considered as evidence against the latter assumption. The present data are also sig-

nificant in showing that virus and lesions may be demonstrable in the CNS (at least the olfactory bulbs) before the onset of fever, and that the interval between involvement of the bulbs and development of paralytic signs is about 4 to 5 days. It is furthermore evident that the nasal mucosa of susceptible monkeys is not a site where the virus of poliomyelitis can lodge passively in any appreciable amount and that even when it becomes demonstrable for a single day during the entire experimental disease, the amount present is so small that if the entire nasal mucosa were instilled intranasally into another monkey it would be insufficient to infect it.

Although the numerous unsuccessful attempts to transmit poliomyelitis to monkeys by contact infection were made with animals inoculated by routes other than the intranasal, one can readily understand from the results obtained why contact infection should not occur even with monkeys infected by the nasal route. Such an experiment was, nevertheless, carried out.

Three monkeys were given the usual nasal instillations of poliomyelitis virus and immediately put into a small cage (84 x 71 x 76 cm.) in intimate contact with six normal monkeys. The inoculated monkeys developed the disease in the usual time, while the six contacts exhibited neither fever nor other signs of illness during a 5 week observation period. It may be added that between the 10th and 20th days after the beginning of this experiment, fifteen additional monkeys, intracerebrally injected and in the preparalytic or paralytic stages of the disease, were crowded into the same cage with the normal contacts, without influencing the outcome.

Fate of Nasally Instilled Poliomyelitis Virus in Immune, Convalescent Monkeys.—The main purpose of the following tests was to determine whether animals which are resistant to reinfection by virtue of a previous attack of the disease can act as carriers of the same strain of virus. Secondarily it was of interest to determine whether in such animals nasally instilled virus can invade the CNS as it does in normal monkeys and in the greater number of vaccinated animals whose immunity appears to be limited only to the presence of neutralizing antibodies in their blood (13, 14).

Six convalescent *M. rhesus* monkeys were used in these tests. Four of these had their primary infection following nasal instillation and two following intracerebral injection of the virus (M.V. strain). All had had distinct paralysis as a result of the primary infection, all had received additional virus instillations

TABLE III

Protocols of Convalescent, Immune Monkeys

Monkey No. and History	Date	Treatment	Remarks
	1936		
43	Feb. 1	2 cc. 10% virus intranasally	4 control monkeys developed poliomyelitis
	" 3	" " " " "	
	↓	Remained well	
Dec. 3, 1935, and Dec. 5, 1935, virus intranasally. Paralysis limited to face. Complete recovery	Mar. 5	2 cc. 10% virus intranasally	12 " " " "
	" 7	" " " " "	
	↓	Remained well	
	Apr. 13	2 cc. 10% virus intranasally	3 " " " "
	" 15	" " " " "	
	↓	Remained well	
	May 11	2 cc. 10% virus intranasally	3 control monkeys developed poliomyelitis. Sacrificed (4 hrs. later). Nasal mucosa—no virus found
7-31	Mar. 5	2 cc. 10% virus intranasally	
	" 7	" " " " "	
	↓	Remained well	
	Apr. 13	2 cc. 10% virus intranasally	
Jan. 31, 1936, virus intracerebrally. Paralysis of all extremities. Recovery with residual paralysis	" 15		Sacrificed (48 hrs. after virus)
			Nasal mucosa, Olfactory bulbs, Pons, medulla, and cord, before and after cataphoresis — no virus detected
			0.9 cc. 10% CNS suspension failed to neutralize 10 and 20 M.C.I.D. of virus

7-20	Mar. 5	2 cc. 10% virus intranasally	
	" 7	" " " " "	
	↓	Remained well	
	Apr. 13	2 cc. 10% virus intranasally	
Jan. 2, 1936, and Jan. 4, 1936, virus intranasally. Paralysis of left upper extremity only. Recovered with residual paralysis of left arm	" 15	" " " " "	
	↓	Remained well	
	May 11	2 cc. 10% virus intranasally	
	" 13	" " " " "	
	" 14	Temperature normal; monkey well	Sacrificed
			Nasal mucosa, Olfactory bulbs, Pons, medulla, and cord, before and after cataphoresis — no virus detected
			0.8 cc. of spinal fluid failed to neutralize 20 M.C.I.D. of virus

TABLE III—*Concluded*

Monkey No. and History	Date	Treatment	Remarks
	1936		
7-19	Mar. 5	2 cc. 10% virus intranasally	
	" 7	" " " " "	
	↓	Remained well	
	Apr. 13	2 cc. 10% virus intranasally	
	" 15	" " " " "	
	" 17	Temperature normal; monkey well	Sacrificed
Jan. 2, 1936, and Jan. 4, 1936, virus intranasally. Complete paralysis of lower extremities and partial paralysis of upper extremities. Recovered with residual paralysis			Nasal mucosa Olfactory bulbs Hypothalamus Pons, medulla, and cord, before and after cataphoresis — no virus detected 0.9 cc. 10 per cent CNS suspension failed to neutralize 10 and 20 M.C.I.D. of virus
42	Feb. 1	2 cc. 10% virus intranasally	
	" 3	" " " " "	
	↓	Remained well	
	Mar. 5	2 cc. 10% virus intranasally	
	" 7	" " " " "	
Dec. 3, 1935, and Dec. 5, 1935, virus intranasally. Paralysis of right arm and weakness of other extremities. Recovered with residual paralysis of right arm	" 10	Temperature normal; monkey well	Sacrificed Nasal mucosa Olfactory bulbs Hypothalamus Pons, medulla, and cord, before and after cataphoresis — no virus detected

34-15	Jan. 3 ↓	Virus intracerebrally Remained well	Intracerebral control—poliomyelitis
	Mar. 5	2 cc. 10% virus intranasally	
	" 7	" " " " "	
Dec. 31, 1935, virus intracerebrally and intraperitoneally. Widespread partial paralysis. Recovered with slight residual paralysis	↓	Remained well	
	Apr. 13	2 cc. 10% virus intranasally	
	" 15	" " " " "	
	" 20	Temperature normal; monkey well	Sacrificed
			Nasal mucosa Olfactory bulbs Hypothalamus Pons, medulla, and cord, before and after cataphoresis — no virus detected
			0.9 cc. 10 per cent CNS suspension failed to neutralize either 10 or 20 M.C.I.D. of virus

The control normal monkeys which were given the same amount of virus simultaneously with each test on the convalescents all developed poliomyelitis; the numbers of animals used in the various tests are recorded only in the protocol of M 43.

which they resisted, and all had neutralizing antibodies in their serum at the time of the last test when they were sacrificed. Normal monkeys which were given virus each time any of the convalescent animals received it, invariably succumbed with typical poliomyelitis. All but two of the convalescents had two doses of virus, 48 hours apart, and individual animals were sacrificed at the following intervals for tests on the nasal mucosa and the nervous system: 4 hours after a single dose (M 43), 48 hours after a single dose (M 7-31), 72 hours after the first and 24 hours after the second dose (M 7-20), 96 hours after the first and 48 hours after the second dose (M 7-19), 120 hours after the first and 72 hours after the second dose (M 42 A), and 7 days after the first and 5 days after the second dose (M 34-15). The results are summarized in the protocols presented in Table III.

It is clearly apparent that in none of the six immune monkeys, sacrificed at intervals of 4 hours to 7 days after nasal instillation of amounts of virus which regularly produced the disease in normal animals, was the infective agent demonstrable either in the nasal mucosa or in different parts of the CNS. It should be further noted that neither by direct test nor with the aid of cataphoresis was it possible to demonstrate the presence of virus in the CNS,[2] which points not only to its rapid inactivation relatively early after the appearance of paralysis but also to the inability of newly instilled virus (of the same strain) to invade and multiply in the CNS of such animals. It may be of interest to point out in this respect that (*a*) tests with extracts of the nasal mucosa of two of these monkeys failed to neutralize completely 10 M.C.I.D. of virus (Table IV), (*b*) tests with suspensions of CNS of three of the convalescent monkeys failed to reveal that the nervous tissue of these resistant animals had any capacity to inactivate or inhibit the effects of small amounts of virus, and (*c*) that vaccinated monkeys which possess as much neutralizing antibody in their blood as these convalescent animals (16) are not, as a rule, resistant to similar amounts of nasally instilled virus.

Apart from these considerations, it is clear that monkeys which are resistant by virtue of a previous attack of the disease quickly rid

[2] The demonstration by means of cataphoresis of virus in a single monkey 23 days after infection has been brought forth by some investigators as evidence that persistence of immunity in poliomyelitis may be correlated with persistence of virus. The present tests, taken together with those performed by Levaditi and Lepine (15), indicate that as regards experimental poliomyelitis there is no evidence for such an assumption.

themselves of virus which may again be introduced on their nasal mucosa, and thus cannot act as carriers (or be a source of infection) of the same strain of virus which caused the primary infection. The fact that this applies only for the same strain of virus is stressed because there seems to be little doubt now that monkeys which have recovered from a distinct paralytic attack with one strain of poliomyelitis virus, and are resistant to further inoculations with the same strain, can be reinfected by the nasal instillation of another strain of

TABLE IV

Effect of Extracts of Nasal Mucosa from Normal and Immune, Convalescent Monkeys on Small Amounts of Poliomyelitis Virus

Nasal mucosa of	Amount of extract	Amount of virus (about 10 M.C.L.D.)	Monkey injected intracerebrally with incubated mixture	Result*
	cc.	*cc.*		
Normal monkey A	0.9	0.1	1	Paralysis 5, dead 6
" " B	"	"	2	Bacterial meningitis
Convalescent, immune monkey C	"	"	3	Paralysis 16, prostrate 16
" " " D	"	"	4	" 16, " 17
Control				
Serum of normal monkey A	0.9	0.1	5	Paralysis 7, prostrate 8

* Numbers as in Table I.

virus. Monkeys convalescent from and resistant to the M.V. strain have been shown to develop typical poliomyelitis a second time following the nasal instillation of the Philadelphia (1932) strain of the virus (17).[3]

While probably it would be generally agreed that an occasional convalescent monkey may not be resistant to reinoculation with the homologous strain of

[3] Personal communication by Dr. Flexner; this also occurred in five of six such animals which we transferred to Dr. Flexner for further study.

virus (18), a review of the literature indicates no concurrence on the rarity or frequency of such lack of resistance. In 1910, Flexner and Lewis (19) showed that none of ten previously paralyzed monkeys developed a second attack upon intracerebral reinoculation. In the protocols of a paper by Schultz, Gebhardt, and Bullock, 1931 (20), it appears that of twelve monkeys having had partial or complete paralysis of one or more extremities following intracerebral virus inoculation, all resisted further repeated intracerebral injections of homologous virus. Paul and Trask (21) stated that they failed to reinfect thirteen convalescent monkeys by intracerebral injection of the homologous strain of virus and concluded that "as others have often shown, such instances of reinfection must be uncommon." In 1936, Jungeblut (22) pointed out that of thirty-four animals with a previous history of distinct paralysis, all were refractory to intracerebral reinjection of 1 cc. of 10 per cent homologous virus suspensions (Aycock strain); of twenty others with a history of a febrile cycle following intracerebral injection of virus and certain inactivating agents, all failed to resist similar reinoculation. In the same year, Toomey (23) indicated that animals which received injections of virus into the intestine or brain and developed only paresis or limited palsies, contracted distinct paralytic poliomyelitis after reinjection with homologous virus (1 to 2 cc., 10 per cent M.V. suspensions), while animals that had severe quadriplegia seemed to be protected. In 1936 we described (16) nine monkeys convalescent from paralytic poliomyelitis (one or more extremities) which were given nasal instillation of homologous, M.V. virus, capable of inducing the disease in practically all normal animals. Six of the nine resisted repeated instillation (these are included in the present paper), while three became prostrate and died within 3 to 4 days; two of these monkeys were investigated and no virus could be demonstrated in their CNS and there were no acute pathological changes to indicate a second attack of the disease. Such demonstration of virus and pathological change should, whenever possible, be used to authenticate experimental second attacks.

In 1936 and 1937, Flexner (17), in elaborating his material from 1912 to 1933, described four monkeys with apparent second attacks following reinoculation with homologous virus. Two of the four had had paralysis following intracerebral injection (M.A. strain, 1912–1913) and during the course of repeated subcutaneous injections of virus months later, there was sudden development of paralytic symptoms and death. The third monkey had progressive symptoms of tremor, ataxia, and weak legs, accompanied by fever which followed intracerebral inoculation of M.V. virus; it later succumbed with characteristic poliomyelitis as a result of nasal instillation of the same virus. The fourth monkey had no clinical symptoms but spinal fluid pleocytosis of 335 cells following the first series of five nasal instillations of 1933 virus; it responded to another series of nasal instillations with the same virus given 40 days later, with paralytic poliomyelitis and death. Recently we observed reactions similar to those found in this fourth monkey: Two animals (one previously given immunizing injections of virus) reacted to later instillation of M.V. virus with fever (to 105.2 and to 105°F.), spinal fluid pleocytosis (from 35 to 95 and from 25 to 280 cells respectively), and with ques-

tionable signs of excitement and tremor, but without definite paralysis in either, and succumbed with prostrating paralytic poliomyelitis to a second dose of M.V. virus instilled 25 days later. Recently Kessel and Stimpert (24) stated that of four monkeys recovered from infection with M.V. virus, two developed a second attack after reinoculation with the homologous strain.

DISCUSSION

When poliomyelitis virus is instilled into the nares of normal monkeys, the greater portion of it can be seen to flow down into the throat and mouth and to be swallowed in short order. It is evident, however, from what has been said before, that enough must remain or be taken up by the nasal mucosa, and probably more specifically by the olfactory portion of it, to initiate the course of events which now leads almost constantly to the invasion of the CNS by the olfactory pathway. Yet, within 4 hours and for at least 48 hours after nasal instillation, no virus was demonstrable either in the entire excised nasal mucosa or in the olfactory bulbs. The sensitivity of the method was such that if about five minimal cerebral infective doses (M.C.I.D.) were present in the mucosa and one in the bulbs, it should have been possible to detect the virus. The first appearance of virus in demonstrable amounts in the nasal mucosa was about 72 hours after the first instillation and almost simultaneously with the first demonstration of virus and lesions in the olfactory bulbs. Subsequently, however, and as early as the 4th day after nasal instillation, while the virus remained in considerable quantities in the olfactory bulbs and was spreading elsewhere in the CNS, the nasal mucosa was no longer infective by the method employed. Repetition of this work on a larger scale, or with different quantities of virus, may, perhaps, modify the particular time relationship noted here, but the principle that the nasal mucosa is infective for a short and transitory period during the experimental disease induced by nasal instillation of virus appears quite clear. It is, furthermore, noteworthy that in the monkey the infectivity of the nasal mucosa during that transitory period is of such a low order that it is demonstrable by intracerebral inoculation only after a prolonged incubation period and probably could not infect another monkey by the nasal route even if the entire nasal mucosa were transferred to it. That virus is not demonstrable in the blood or spinal fluid of monkeys at various times after nasal instillation has

already been shown by Brodie and Elvidge (25) and as regards blood, by the extensive transfusion experiments of Gordon and Lennette (26). In view of all these observations, it was not surprising to find that experimental poliomyelitis induced by nasal instillation of the virus does not spread spontaneously to other monkeys.

The hypothesis that in the human disease the virus first attacks the olfactory mucosa and that it is from this site also that it is disseminated from man to man would require that a great deal more virus be produced locally than occurs in the monkey, or that each monkey infective unit be equivalent to more than 100 or 1,000 infective units of virus in man. The relatively low incidence of positive results obtained in attempts to demonstrate virus in the nasal washings of human cases, even during the acute stage of the disease, could be due, in addition to the presumable difficulties of experimental transmission, to the possibility that in the majority of human cases as in monkeys, the nasal mucosa was most infective before the disease became clinically apparent.

It is of interest to note in this connection that Taylor and Amoss (27) obtained virus from the nasal washings of a child 5 days before the onset of clinical signs of poliomyelitis and that in a large series of abortive cases studied by Paul and Trask (28), and Paul, Trask, and Webster (29), the three positive isolations of virus were all from cases on the 1st day of the clinical disease. One can find satisfactory reports of perhaps nine additional isolations of virus in the period of the 4th to the 17th days of the disease (see recent summary by Stillerman and Brodie, 30), but when one considers the total number of nasal washings that were examined to obtain these results, it is not improbable that so late a persistence of virus may perhaps be exceptional. The nasal mucosa itself has been studied by Flexner and Amoss (31) in three human beings dying during the 1st week of the disease, and virus was isolated from one of the three. In the same investigation they recovered the virus from the tonsils of five of ten patients dying within the 1st week of the disease, in one instance the tonsils yielded virus while the nasal mucosa from the same case did not, and in another virus was obtained from the nasal mucosa but not from the tonsils. Flexner and Clark (32), at an earlier date, reported that with unfiltered, phenolated suspensions they obtained virus from the tonsils of each of four human cases. It is important to recall here that the tonsils form part of a chain of lymph nodes into which the lymphatics from the nasal mucosa drain. It may be of interest in this respect that in guinea pigs the cervical lymph nodes draining the nasal mucosa were found to contain no vesicular stomatitis virus several hours after nasal instillation of 50,000 to 500,000 M.C.I.D. and little or none at the height of local (nasal) multiplication about 2 days later (12) but in the supervening few days, when the virus had practically disappeared

from the nasal mucosa, it was easily demonstrable in these lymph nodes (unpublished observations).

The studies on the convalescent, immune monkeys clearly showed that after nasal instillation of the same strain of virus, it was quickly "washed" away as in normal animals, but it failed to reappear later or to invade the CNS as is the case in normal monkeys. That the humoral antibodies probably do not determine this result is evident from the observations that vaccinated monkeys possessing the same amount of neutralizing antibody as convalescents (16) usually succumb when given the same amount of virus, as well as from the fact that monkeys, immune as a result of a previous paralytic attack of the disease, are resistant to subsequent nasal instillation of virus long before the antibodies become demonstrable in the serum (16). As regards the possible effect that neutralizing antibodies might have on the capacity to detect the virus in the nasal mucosa or CNS, it was shown that these tissues did not contain enough to mask the presence of as little as 10 M.C.I.D. It is important to stress here that this applies only to reinoculation with the same strain and to monkeys which have suffered a paralytic attack of the disease. The possible significance of these data from an epidemiological point of view is in the suggestion that individuals who are immune because of a previous attack of the disease (not to be confused with natural resistance which perhaps determines whether an attack of poliomyelitis will be apparent or inapparent) may no longer act as transmitters of the same strain or type of virus infection.

SUMMARY

With a method of intranasal instillation of poliomyelitis virus that brings about infection of all *M. rhesus* monkeys subjected to it, a study was undertaken of the fate of nasally instilled virus in normal and convalescent, immune animals. Control experiments revealed that nasal mucosa of normal monkeys contained no observable antiviral factors and that when five or ten minimal cerebral infective doses were added to the mucosa, virus could be detected by the employed procedure. In the olfactory bulbs even a single infective dose could be recovered, since suspensions of both bulbs could be transferred to the brain of a monkey without any loss of material.

After nasal instillation of virus in normal monkeys, it disappeared

quickly (4 hours or less) and could be recovered neither from the excised nasal mucosa nor from the olfactory bulbs during the first 48 hours. At 72 hours, just before or coincident with the first rise of temperature, virus was found in very small amounts in the nasal mucosa and for the first time also in the olfactory bulbs. At 96 hours, at least 3 days before the appearance of nervous signs, and later, while virus continued to be present in considerable amounts in the olfactory bulbs (and presumably elsewhere in the central nervous system), none was detected in the nasal mucosa. In convalescent, immune animals receiving the same strain of virus intranasally which caused the original infection, none could be recovered from the nasal mucosa or central nervous system at 4 hours, 1, 2, 3, 4, 5, and 7 days. The bearing of these observations on the problem of host to host transmission of poliomyelitis virus is discussed.

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