SECOND ATTACKS OF POLIOMYELITIS

An Experimental Study*

By HOWARD A. HOWE, M.D., and DAVID BODIAN, M.D.

(From the Department of Anatomy, Johns Hopkins University, Baltimore)

Plates 7 and 8

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It is generally conceded that a relatively substantial and lasting immunity to poliomyelitis is conferred through invasion of the nervous system by active virus; nevertheless, well authenticated second attacks of the disease do occur both in man and in experimental animals (1–9). This puzzling contradiction, which has led some to a denial of the existence of active immunity (1), arises in part at least from a lack of precise knowledge regarding the mechanisms of immunity production in the disease. For a solution of the problem one must have recourse to the experimental animal. It must be recognized at the outset that many strains of poliomyelitis virus do not produce consistent immunity against others (2, 4–9): that an animal is resistant to reinoculation by the same strain of virus is also accepted by most workers (7–11, *contra* 3). At the same time this substantial type of immunity which weathers large testing doses is thought to be a local one, residing in the central nervous system. There is a possibility, however, that such an immunity of the nervous system may be further restricted to include only portions of it. The following experiments and discussion will show that this is actually the case, which means that limited reaches only of the nervous system may be refractory to one strain of virus at a given time.

In the course of a series of investigations dealing with the influence of fiber tracts upon the propagation of poliomyelitis virus within the central nervous system of the monkey, it became apparent that although certain neurone systems were unquestionably highly susceptible to virus, nevertheless they did not always suffer visible signs of invasion (12). These differences in the total distribution of virus could be correlated with the portal of entry, and it was apparent that there were definite limits to the spread of virus even during a severe paralytic attack of poliomyelitis. Additional observations on this point are the subject of the present paper since the question immediately arose whether one invasion of the nervous system immunized it as a whole, or whether some of those portions which were susceptible but did not show lesions might

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still remain susceptible. This concept rapidly reduces to the proposition of whether a single attack of poliomyelitis automatically closes all portals of entry.

In order to establish the thesis that one attack of poliomyelitis does not immunize the entire nervous system it is necessary to produce paralytic poliomyelitis by one portal of entry and then subsequently attempt reinfection by a new portal which has not been involved in the first spread of the disease. Experiments designed to elucidate such a point are subject to certain limitations imposed by the fact that the host animal, the *rhesus* monkey, has only one "natural" portal. In this animal (contrast the chimpanzee (13)) intact skin and mucous membrane furnish an effective barrier to the entrance of virus at every point except the olfactory area, which may be regarded as the portal of choice for experiments dealing with immunity. Nevertheless when the barrier of the non-olfactory epithelium is broken and virus is brought into contact with any considerable part of the nervous system, infection regularly ensues. Once virus has gained access to the neuraxis there is no reason to doubt that it is disseminated through it with the same readiness as though it had been introduced through the olfactory portal. This being the case, it is possible to consider various portions of the nervous system as legitimate portals of entry which can be employed to induce an initial attack of paralytic poliomyelitis.

In order to control the second or testing inoculation it is necessary to show beyond all reasonable doubt that virus has not been present in the regions of the central nervous system which are to be exposed at this time. This necessitates the careful examination of the nervous tissues in many cases. Since the olfactory portal was to be used in most instances for the testing inoculation, the olfactory bulbs were examined in forty cases in which routes other than the olfactory were employed for the production of paralytic poliomyelitis. The ciliary ganglia were also studied in seven cases paralysed after intranasal inoculation since the intraocular route was also to be used for testing immunity in intranasal convalescent animals. All the tissues were examined in serial sections 10 to 20 μ in thickness stained with gallocyanin after the method of Einarson (14). Normality was determined by the presence or absence of lesions—a method of assay which in respect to a susceptible tissue like the olfactory bulb we have come to regard as more sensitive than subinoculation. The first group in Table I is composed of thirteen cases of severe paralysis induced by inoculations into various regions of the cerebral cortex, both motor (areas 4, 6, and 8), and sensory (area 5). On examination of the olfactory bulbs it was not uncommon to find a slight meningitis at the pial surface which was consistent with the presence of a general meningitis, but no true lesions were present in the bulbs except in one questionable case where a large inoculum was placed into one frontal pole. It seems very likely that this single positive case represented a direct contamination of the olfactory bulbs. Fur-

thermore in each of two cases following intracerebral inoculation into area 4, the ipsilateral olfactory bulb was sectioned serially while the other was subinoculated intracerebrally into a monkey. No lesions were present in the sectioned bulbs and there was no indication of active virus in the subinoculated ones. These findings are in complete agreement with those of Sabin and Olitsky (15) who after intracerebral, intrasciatic, and subcutaneous inoculation found no lesions in the olfactory bulbs.

The second group in Table I concerns the status of nine pairs of olfactory bulbs after intraocular inoculation. Infections of this type run a characteristic clinical course which has been described elsewhere (12), and it has been shown that the virus reaches the brain along the fibers of the autonomic nervous system. The histological picture in the brain indicates an invasion, usually *via* the ciliary ganglion, which is maximal in the midbrain and less intense in the basal portions of the forebrain. Although a slight meningitis was again present around some of the olfactory bulbs of this series, there were no lesions within their substance, indicating that if virus does reach this portal, it produces no reaction in the tissue. Also included in this group are two cases representing the inoculation of other cranial nerves, the vagus and hypoglossus respectively. The technique for neural inoculation has been previously described (16) and consists of moistening the cut end of the nerve with virus emulsion. It has been employed for all inoculations of this type. The animals showed initial bulbar symptoms and later limb paralyses. Their olfactory bulbs were histologically normal.

The third group (seventeen cases) is a composite one containing sciatic, intracutaneous, intraperitoneal, and intraspinal inoculations. They are included under one heading since they all represent infections ascending to the brain from the spinal cord. The Rockefeller MV strain was employed for all inoculations except the intracutaneous and intraperitoneal which were done respectively with the Wallingford virus, 17th generation (17), and an etherized human stool (18). The intraspinal inoculations were performed by direct piqûre into the cord. In every instance but two (a non-paralytic case showing cord lesions and a case of leg paralysis only), extensive involvement of both arms and legs resulted. A number of cases of the ascending type have already been described (12). They are characterized by a diminution in the severity of the lesions in the rostral portions of the forebrain. Each pair of olfactory bulbs belonging to this group was histologically normal.

The last control group consists of seven animals which received their inoculations intranasally. A wide variety of virus strains was used, ranging from the MV and Wfd to four different human stools (19). All the animals were severely paralysed and their olfactory bulbs showed characteristic lesions. Sections of the ciliary ganglia, however, revealed no histological abnormalities. This series thus forms a parallel with those which showed no abnormalities in

TABLE I

Distribution of Lesions after a Single Series of Inoculations

Experiment	Virus	Portal	Outcome	Other portals
6-27	MV	Area 4	Paralysis. CNS +	Olfactory bulbs normal
6-39	"	" "	" " +	" " "
6-91	"	" "	" " +	" " "
7-75	"	" "	" " +	" " "
9-39	"	" "	" " +	" " "
9-40	"	" "	" " +	" " "
9-41	"	" "	" " +	" " "
9-42	"	" "	" " +	" " "
9-67	"	" "	" " +	" " "
9-68	"	" "	" " +	" " "
7-87	"	" 5	" " +	" " "
5-92	"	" 6	" " +	" " "
1-09	"	Frontal pole	" " +	" " + ?*
7-26	"	Intraocular	Paralysis. Ciliary ganglion +	Olfactory bulbs normal
7-93	"	"	" " " +	" " "
8-51	"	"	" " " +	" " "
8-53	"	"	" " " +	" " "
9-20	"	"	" " " +	" " "
9-21	"	"	" " " +	" " "
A8-1	"	"	" " " +	" " "
A7-5	"	"	Paralysis	" " "
A7-7	"	"	"	" " "
A7-4	"	Vagus nerve	Bulbar paralysis	" " "
A1-36	"	Hypoglossal nerve	" "	" " "
A-8	MV	Sciatic nerve	Paralysis. CNS +	Olfactory bulbs normal
A2-7	"	" "	" " +	" " "
A6-5	"	" "	" " +	" " "
A6-6	"	" "	" " +	" " "
A8-2	"	" "	" " +	" " "
A8-7	"	" "	" " +	" " "
7-90	Wfd	Intracutaneous	" " +	" " "
8-66	"	"	" " +	" " "
8-67	"	"	" " +	" " "
8-68	"	"	" " +	" " "
8-69	"	"	" " +	" " "
8-46	Stool	Intraperitoneal	" " +	" " "
8-47	"	"	" " +	" " "
A9-4	MV	Intraspinal	" " +	" " "
A9-7	"	"	" " +	" " "
A9-8	"	"	" " +	" " "
A1-08	"	"	" " +	" " "

* Bulbs probably infected by direct penetration of inoculum.

TABLE I—*Concluded*

Experiment	Virus	Portal	Outcome	Other portals
9-43	MV	Intranasal	Paralysis. Olfactory bulbs +	Ciliary ganglia normal
A1-4	Wfd	"	" " " +	" " "
A1-7†	MV	"	" " " +	" " "
A3-2	Stool	"	" " " +	" " "
A4-6	"	"	" " " +	" "
A4-9	"	"	" " " +	" " "
A8-3‡	"	"	" " " +	" " "

† Guenon.
‡ Chimpanzee.

the olfactory bulbs after intraocular inoculation, although the portals are reversed.

All of the foregoing cases may be regarded as controls for the observations which follow. They indicate that even in animals suffering paralytic poliomyelitis there are apparent limits to the centrifugal spread of virus in the central nervous system. It is thus possible with reasonable care to produce clinically definite poliomyelitis by a wide variety of routes without the invasion of the first two neurones in the olfactory system. It is also clear that after olfactory inoculation such far flung portions of the nervous system as the ciliary ganglia are not invaded. Whether neurone systems more contiguous to the pathways commonly used by virus in the CNS (12) are involved, depends upon the severity of the initial infection, and other unknown factors.

It now remains to demonstrate to what extent virus invasion may take place in a convalescent monkey through a previously untouched portal. A series of nineteen monkeys has been tested for immunity in this fashion. In the majority of cases the testing inoculation was given by the intranasal route for the obvious reason that it should be the one portal where humoral and neural sources of immunity could be expected to cooperate most effectively. Two possibilities present themselves for the assay of this untouched portal—homologous and heterologous strains of virus. The results of the tests are summarized in Table II.

Heterologous Virus Inoculations—Two Portals

Group A contains three animals, which were reinfected with a heterologous strain of virus. In two of them (9-26, 9-27) the olfactory portal was inoculated with MV virus after previous intracutaneous (abdominal skin) and intracerebral (area 4) inoculations with Wfd virus.

No. 9-26. Mar. 2, 1939. Abdomen cleaned and an area the size of a half-dollar scraped raw, but not bleeding in the gross. 1.5 cc. Wfd 8-18—8-19 (17th generation) virus rubbed into this area. Mar. 6. Repeated.

TABLE II

Inoculation of Convalescent Animals

First inoculation				Second inoculation		
Experiment	Virus	Portal	Outcome	Virus	Portal	Outcome
Group A. Heterologous virus strains. Different portals						
9-26	Wfd	Intracutaneous	Paralysis	MV	Intranasal	Paralysis*
9-27	"	Intracerebral	"	"	"	" *
A4-5	Pest	Intranasal	"	"	"	Fever; ciliary ganglion +
				Wfd	Intraocular	
Group B. Homologous virus strains. Different portals						
A9-3	MV	Below spinal transection	Knee jerk lost. L cord +	MV	Intranasal	Paralysis.* Olfactory bulbs +
A9-6	"	" "	Knee jerk lost. L cord +	"	"	Paralysis.* Olfactory bulbs +
A9-9	"	" "	Knee jerk lost. L cord +	"	"	Olfactory bulbs −. Old lesions in medulla
A1-17	Wfd	Intraocular	Paralysis	Wfd	Intranasal	Olfactory bulbs +
A1-34	"	"	"	"	"	" " −
A1-35	"	"	"	"	"	" " +
A1-74	Pool	Intracerebral	"	Pool	"	" " +
A1-07	Pool	Intranasal	Paralysis	Pool	Intraocular	Ciliary ganglion −
A1-12	"	"	"	"	"	" " +

Group C. Identical virus. Parallel portals

First inoculation				Second inoculation				
Experiment	Virus	Portal	Outcome	Virus	Portal	Fever	Olfactory bulbs	
							Right	Left
A1-26	A8-3	Right nostril	Paralysis				+	−
A1-28	"	" "	"				+	−
A1-31	"	" "	"				+	−
A1-30	"	" "	"				+	+
A1-24	"	" "	"	A8-3	Left nostril	+	+	+
A1-25	"	" "	"	"	" "	0	+	+
A1-27	"	" "	"	"	" "	+	+	+
A1-32	"	" "	"	"	" "	+	+	+

* Paralysis of muscle groups not involved in first attack.

Mar. 16. Right leg completely paralysed.

Mar. 17. Left leg 5 per cent functional (has a trace of knee jerk—can feebly abduct digits). Right leg as before, arms normal.

Apr. 18, 19. Extremities as before—climbs actively with arms only. Inoculated intranasally Wfd 9-28 (18th generation). Animal and control remained well.

May 3, 4. Inoculated intranasally Wfd 9-28. Animal and two controls remained well.

June 3, 5. Inoculated intranasally, fresh Wfd 9-66 (18th generation). Animal and two controls remained well.

June 18, 19. Inoculated intranasally Wfd A1-4 (19th generation). Animal and two controls remained well.

July 12, 13. Inoculated intranasally, fresh MV virus A2-2,[1] 0.5 cc. per nostril.

July 16–21. Dromedary temperature spike.

July 22. Right arm zero function, left arm 10 per cent functional.

July 23. Arms zero—left leg as on Mar. 17.

July 25. No change—left leg as before. Killed.

Olfactory bulbs—each shows extensive recent invasion. There were also fresh lesions along with chronic ones throughout the brain.

Summary.—An animal with complete paralysis of the right leg and only a few muscle fibers functional in the left leg following intracutaneous inoculation of Wfd virus, suffered complete arm paralysis after subsequent intranasal inoculation of MV virus. There was no further invasion of the lumbar cord. The olfactory bulbs showed extensive fresh lesions.

No. 9-27. Mar. 2, 1939. Inoculated into motor area of cortex cerebri with Wfd virus 8-18—19 (17th generation).

Mar. 8. Temperature 106°.

Mar. 12. Extremities in terms of normal strength: right arm zero, left arm 60 per cent; right leg zero, left leg, 60 per cent.

Mar. 24. Convalescent. Gets around without difficulty. Arms and left leg are nearly normal. Right leg 5 per cent. Inoculated intranasally Wfd 9-28 (18th generation). Remained well.

Apr. 18, 19. Inoculated intranasally Wfd 9-28. Animal and two controls remained well.

May 3, 4. Inoculated intranasally Wfd 9-28. Animal and two controls remained well.

June 3, 5. Inoculated intranasally fresh Wfd 9-66 (18th generation). Animal and two controls remained well.

June 18, 19. Inoculated intranasally Wfd A1-4 (19th generation). Animal and two controls remained well.

July 12, 13. Inoculated 0.5 cc. per nostril fresh MV A2-2.

July 17, 18. Temperature 106.2°.

July 20. Weak and uncertain—barely able to climb.

[1] MV virus has been consistently used as a 20 per cent cord emulsion.

July 22. Still barely able to climb. Right arm 50 per cent, left arm 30 per cent left leg 50 per cent. Killed.

Olfactory bulbs—recent maximal invasion left, patchy invasion right.

Summary.—An animal showing extensive paralysis following an intracerebral inoculation of Wfd virus, made a good convalescence except for an almost completely paralysed right leg. Following subsequent intranasal inoculation with MV virus it developed fever, and paralysis in the arms and left leg. The olfactory bulbs showed extensive recent lesions and there were fresh lesions as well as chronic ones throughout the brain.

The remaining animal in Group A was reinfected by the intraocular route following an original olfactory infection.

No. A4-5. Oct. 13, 14, 16, 17, 1939. Received 1 cc. per nostril of human stool.

Oct. 20–23. Temperature 106.8°.

Oct. 24. Extremities in terms of normal strength. Right leg 20 per cent, left leg 10 per cent, arms 100 per cent, still climbing.

Nov. 30. Gets about actively—biopsy of motor cortex shows characteristic lesions.

Feb. 8, 9, 10. Arms stronger than previously—legs useless though some movement is possible. Inoculated intranasally 1 cc. per nostril MV A7-7.

Feb. 13, 14. Temperature up 2° from level base line—no other reaction. Three of four controls paralysed.

Feb. 20, 21, 23. Inoculated intranasally 0.5 cc. per nostril MV A8-2—no reaction. Two controls paralysed.

Mar. 18. 0.1 cc. Wfd A6-2 injected into vitreous of left eye.

Mar. 25. Temperature up 1.5°—no other reaction. Control paralysed.

Apr. 5. Symptomless. Killed.

Pathological findings: Superior cervical ganglia normal. Right ciliary ganglion normal. *Left ciliary ganglion showed many foci of lymphocytic infiltration and a possibly cuffed vessel* (Fig. 1). The olfactory bulbs showed relatively fresh lesions.

Summary.—An animal convalescent from an intranasal inoculation of human virus, reacted to subsequent intranasal inoculation of MV virus with fever. Following intraocular inoculation of Wfd virus there was slight fever but no further paralysis. The appropriate ciliary ganglion, however, showed lesions.

The foregoing cases show clearly that a highly virulent virus such as the Rockefeller MV strain is capable of almost completely overriding any immunity induced by a relatively weaker strain such as the Wfd if it is introduced by a previously uninvaded portal. The single case, A4-5, would indicate that the MV virus probably "took" in the already extensively invaded olfactory bulbs and that the first neurone of the intraocular portal was still susceptible to a third less virulent strain of virus, the Wfd. Kessel and Stimpert (7) found that a mild strain inoculated intracerebrally was also capable of producing further paralysis in an animal convalescent from a virulent strain previously given by the intracerebral route.

Homologous Virus Inoculations—Two Portals

Group B is concerned with nine tests of immunity by the inoculation of the same virus strain through previously uninvaded portals. This group falls into two categories, that in which two surgically isolated portions of the CNS were inoculated (three animals) and that in which the intact animal was used (six animals).

The first category contains three spinal animals which were inoculated below their transections, subsequently showed signs of poliomyelitis in both legs, and after a 26 to 34 day interval were reinoculated intranasally. Two reacted as though they had never been in contact with virus and became completely paralysed above the transection. The protocols are given below.

No. A9-3. Jan. 10, 1939. Spinal cord transected at lower level of T_9—muscle and fascia inserted between cut ends.

Jan. 22. Inoculation of 0.4 cc. MV A2-3 in two piqûres into lumbar enlargement.

Jan. 23. Knee jerks no longer present on either side.

Feb. 17. Animal in excellent general condition despite several decubitus ulcers over malleoli and pelvic bones. Very active and aggressive despite transection. Inoculated intranasally 0.5 cc. per nostril with MV virus on this day, and also Feb. 18, 19, 21, 23 with MV A7-7 and A8-2.

Feb. 23. Control paralysed.

Feb. 27. Control paralysed.

Mar. 1. Very tremulous—arms still strong.

Mar. 2. Arms 20 per cent functional—unable to support animal in sitting position. Killed.

Pathological findings: Spinal cord at L_{6-7} shows loss of practically all cells in grey matter. The process is an old one. The cervical cord shows extensive fresh lesions. Olfactory bulbs show heavy recent invasion on the left, light invasion right.

Summary.—An animal in which the spinal cord was transected was inoculated with MV virus intraspinally 12 days later below the transection. Knee jerks were lost but no signs of poliomyelitis developed in the arms. 26 days later the animal was inoculated intranasally with MV virus. It became paralysed above the transection. The lumbar cord showed old lesions, the cervical cord and olfactory bulbs fresh ones.

No. A9-6. Jan. 11, 1940. Spinal cord transected at T_9. Muscle and fascia inserted between cut ends.

Jan. 26. Lumbar enlargement inoculated 1.5 cc. MV A2-3; knee jerks and ankle jerks active.

Jan. 29. Knee jerks +; ankle jerks absent.

Feb. 29. Animal in excellent condition. Active and aggressive—arms very strong—has shown no signs of poliomyelitis above the transection. Inoculated this day and Mar. 1, 2 intranasally with 0.75 cc. per nostril MV virus (A8-2, A7-7, 8-38, 9-05 pooled).

Mar. 5. Temperature has risen from 103° to 106.2°.

Mar. 7. Control paralysed.

Mar. 10. Weak and tremulous.

Mar. 11. Both arms 30 per cent functional, head tremor. Killed.

Pathological findings: Spinal cord at L_6 shows extensive old lesions wiping out practically all grey matter. Cervical cord, moderately heavy fresh lesions. Right olfactory bulb, heavy recent invasion. Left olfactory bulb, perivascular cuffing only.

Summary.—An animal in which the spinal cord had been transected was inoculated directly with MV virus into the lumbar cord 15 days later. Poliomyelitis resulted as evidenced by loss of leg reflexes and lesions in lumbar cord, but the animal remained symptomless above the transection. 34 days later intranasal inoculation of MV virus resulted in arm paralysis. Fresh lesions were present in the olfactory bulbs and cervical cord.

No. A9-9. Jan. 12, 1940. Spinal cord transected at T_9. Muscle and fascia interposed between cut ends.

Jan. 25. Removal of right sympathetic ganglia at $T_{8,\ 9,\ 10}$.

Jan. 29. Removal of left sympathetic ganglia at $T_{8,\ 9,\ 10}$. Inoculation of cord in lumbar enlargement 0.3 cc. MV A2-3.

Jan. 31. Knee jerks and ankle jerks present.

Feb. 4. Temperature has risen from 102.4° to 105.8°. Has low grade infection of incision but is active and alert. While being carried for treatment had attack of syncope.

Feb. 7. Control paralysed.

Feb. 11. Has been lying down. Both arms are weak and the left hand has practically no grasp. Virus has entered the cervical cord and probably the medulla[2] (*cf.* fever and fainting spell of Feb. 4).

Feb. 23. No change. Inoculated intranasally on this day and Mar. 1, 2 with MV A8-2, A7-7, 8-38, 9-05—0.075 cc. per nostril.

Mar. 13. No reaction of any sort. (Controlled by A9-3 and A9-6.) Attempt to remove olfactory bulbs by biopsy abandoned and animal killed.

Pathological findings: Lumbar cord showed extensive old lesions. The medulla and midbrain contained lesions which were lighter in the latter, indicating an ascending infection. The olfactory bulbs were badly traumatized so that accurate examination was impossible. They contained no heavy lesions but there were some questionable infiltrations.

Summary.—An animal in which the spinal cord was transected and the sympathetic chains bilaterally interrupted at the same level was inoculated into the cord with MV virus below its transection 15 days later. It subsequently developed arm paralysis and showed symptoms referable to a disturbance of the medulla. Following intranasal inoculation of MV virus 31 days subsequently it remained symptomless. There were very questionable lesions in the olfactory bulbs, but old lesions in the cervical cord and medulla.

In the foregoing cases there is no reason to question the first attack of poliomyelitis following cord piqûre. The lesions present in the lumbar cord

[2] Progression of virus around a transection of the spinal cord has occurred in eight of eleven animals (44).

could not have been caused by indirect traumatic or vascular injury incidental to the cord transection. The fact that tendon reflexes were present until a few days after inoculation rules out this possibility. Furthermore the obvious chronicity of the lesions with respect to those in the olfactory bulbs and cervical cord indicates that two separate invasions must have taken place. The presence of lesions in the olfactory bulbs removes the possibility that the animals suffered merely an exacerbation of their original disease. Whether the longer incubation periods as compared with the controls indicate a certain degree of immunity cannot be determined from the limited material at hand.

The olfactory portal was deliberately chosen for the testing inoculation because it seemed more physiological than the intracerebral route. The amounts of virus given intranasally were no doubt excessive, but in the case of the second animal (A9-6) no more than that usually employed for a routine inoculation where success must be definitely assured. The relativity of immunity with respect to the dosage which can be tolerated has been brought out by Toomey (4) who reported numerous second attacks in previously paralysed animals following large intracerebral inoculations of homologous virus. Nevertheless when extensive virus dissemination has taken place, the animal may show no paralysis after large doses of the infective agent administered intranasally (2, 20). This point is illustrated also by A9-9 which did not succumb to a second infection and will become clearer in the light of the cases which follow.

Returning to Table II it will be seen that the second category of homologous inoculations employing two different portals of entry, deals with two groups of intact animals.

Résumé of Histories of Animals Convalescent Following Intraocular or Intracerebral Inoculations and Reinoculated Intranasally.—

No. A1-17. Mar. 18, 1940. Received 0.1 cc. Wfd A6-2 (15th generation) into vitreous of left eye.

Mar. 25–27. Fever, followed by partial paralysis of left arm and leg.

May 22–24. Extremities in terms of function: Arms 100 per cent, right leg 80 per cent, left leg zero. Inoculated 0.5 cc. per nostril Wfd A6-2.

June 6. No reaction to inoculation. Killed. Two controls paralysed.

Pathological findings: Ciliary ganglia show no characteristic lesions (nearly 3 months after intraocular inoculation). The olfactory bulbs show extensive recent lesions (Fig. 2).

Summary.—See Table II, Group B.

No. A1-35. Mar. 29, 1940. Received 0.1 cc. Wfd virus A6-2 (15th generation) into vitreous of left eye.

Apr. 7. For several days has had bilateral ptosis, disappearing on excitement, and gradually mounting temperature which today reached 105.8°.

Apr. 13. Prostrate. Extremities in terms of function: right arm 30 per cent, left arm 10 per cent, legs zero.

May 22, 23, 24. Can pull himself into sitting position. Right arm 40 per cent, left arm 20 per cent. Legs zero except for feeble toe movements. Inoculated intranasally 0.5 cc. per nostril Wfd A6-2.

June 12. Temperature has been more irregular than before the last inoculation, but the arms have gained steadily in strength so that the animal is now able to climb all over the cage. No change in legs. Killed. (Control paralysed.)

Pathological findings: Right ciliary ganglion normal, left ciliary shows one cuffed vessel and two areas of lymphocytic infiltration. Olfactory bulbs show extensive recent lesions of the same character as those of the control (Fig. 3).

Summary.—See Table II, Group B.

No. A1-34. Mar. 29, 1940. Received 0.1 cc. Wfd virus A6-2 into vitreous of left eye.

Apr. 6. Temperature 105.4°. Tremulous—bilateral ptosis.

Apr. 8. Slight bilateral ptosis. All extremities 60 per cent functional.

May 22, 23, 24. In excellent condition—runs and climbs without difficulty. Extremities in terms of function: left arm 100 per cent, right arm 80 per cent, left leg 100 per cent, right leg 70 per cent. Inoculated intranasally 0.5 cc. per nostril, Wfd A6-2.

June 11. Has shown no reaction of any sort. Killed. (Two controls paralysed.)

Pathological findings: Right ciliary ganglion normal, left ciliary ganglion shows cuffed vessel. Olfactory bulbs normal (Fig. 4). Control bulbs show extensive lesions.

Summary.—See Table II, Group B.

No. A1-74. Chimpanzee. Dec. 6, 1940. Inoculated into motor cortex with 0.5 cc. cord emulsion from chimpanzee A8-3.

Dec. 10. Temperature elevated from base line of 101° to 104.8°.

Dec. 13. Temperature dropping—weakness of left arm—clumsy and listless.

Dec. 16. Left arm useless, right arm about 20 per cent functional—barely able to pick up food. Legs 40 to 50 per cent functional.

Feb. 7, 1941. Because of pulmonary tuberculosis animal was anesthetized with nembutal and reinoculated intranasally with the same specimen of virus previously employed, 0.5 cc. per nostril, on Feb. 7 and 8. Preinoculation physical examination showed considerable recovery. Left arm: shoulder 15 per cent, biceps 20 per cent, triceps 10 per cent, grasp 10 per cent, tendon reflexes all easily obtained but very feeble. Marked muscular atrophy of upper arm. Right arm: shoulder 25 per cent, biceps 40 per cent, triceps 20 per cent, grasp 25 per cent in fingers only, thumb zero. Tendon reflexes all brisk but not forceful. Uniform muscular atrophy. Legs essentially normal.

Feb. 14. Despite tuberculosis twice daily temperature records showed a fairly steady base line at 101.5°. Temperature began to climb on Feb. 10 reaching 102°, and on Feb. 14, 103°. The next day it dropped to 101°.

Feb. 17. Right arm reflexes hyperactive—grasp 5 per cent in finger, thumb 0. (Weaker than on Feb. 7.) Intranasal *rhesus* control showed light paralysis.

Feb. 20. Left arm: biceps 20 per cent, triceps 2 per cent (triceps reflex now very feeble and difficult to get), grasp 10 per cent, flexion at wrist 20 per cent. Right arm: biceps and triceps 10 to 15 per cent, flexion at wrist 20 per cent, grasp 2 per cent. Reflexes all obtained. Legs as before. Killed.

Pathological findings: There were fresh lesions in both olfactory bulbs (Fig. 5). The cervical cord (C_7 and C_8) showed extensive lesions but it was impossible to be sure that any were of recent origin.

Summary.—A chimpanzee which was extensively paralysed following an intracerebral inoculation into the motor cortex was reinoculated intranasally 7 weeks later. There was a slight but definite febrile response to the second inoculation and increased weakness in certain portions of the arms. Microscopic sections showed fresh lesions in the olfactory bulbs, though a new process could not be established in the cervical cord (Fig. 5).

Résumé of Histories of Animals Convalescent Following Intranasal Inoculation and Reinoculated Intraocularly.—

No. A1-07. Feb. 13, 14, 15, 1940. Inoculated intranasally as a control for chimpanzee A1-05. Received 1 cc. per nostril per day of a pool of six potent human stools (17).

Feb. 18. Temperature 106.4°.

Feb. 25. Completely prostrate. Right leg and tail still 10 per cent functional. Neck strong and cranial nerves intact.

Mar. 25. The animal is incontinent of urine and completely paralysed except for the tail, head, neck, and diaphragm. There are great muscular atrophy and decubitus ulcers over the trochanters which have been arrested by keeping him face down over an inflated automobile inner tube. Received 0.1 cc. of A1-05 virus (chimpanzee for which he was control) into vitreous of left eye.

Apr. 17. Has shown no reaction attributable to second inoculation. Killed. (Control paralysed, lesions in left ciliary ganglion.)

Pathological findings: Right ciliary ganglion normal, left ciliary ganglion shows two rather large cuffed vessels at its margin and focal accumulations of round cells within it (Fig. 6). Olfactory bulbs show definite old lesions but much of the debris has been cleared up.

Summary.—See Table II, Group B.

No. A1-12. Feb. 26–29, Mar. 1, 1940. Inoculated intranasally (1 cc. per nostril) stool of chimpanzee A1-05.

Mar. 4. Temperature 105.6°.

Mar. 9. Prostrate—no function in arms and legs.

Mar. 25. Completely paralysed except for head, neck, diaphragm, and tail—marked muscular atrophy and contracture. Received 0.1 cc. chimpanzee virus A1-05 into vitreous of left eye.

Mar. 27. Temperature up from base line of 102° to 104°. Eye red, lid swollen.

Mar. 28. Eye more red—lids swollen, exudate. Temperature normal.

Mar. 29. Eye much better, still opaque but lids nearly normal.

Apr. 8. Found dead. Had shown no clinical signs of poliomyelitis, although control came down 1 week previously.

Pathological findings: Ciliary ganglia normal. Bulbs show old lesions.
Summary.—See Table II, Group B.

The foregoing series of cases shows a second invasion of homologous virus into a new portal in four out of the six. In only one instance was this "second attack" accompanied by any clinical manifestation. There is a suggestion that some of the cases of second paralytic attacks following intracerebral inoculation reported by Toomey (3, 4) fall into a category intermediate between our spinal animals and these which had no further paralysis. Some of his animals which were convalescent from subserosal and intrasciatic inoculations showed little resistance to paralysis from relatively small intracerebral inoculations. This state may have been favored by a light dissemination of virus from the spinal cord rostrally into the brain. In the present instance it seems fairly clear that the virus was not effective at any point beyond the convergence of the pathways from the new portal with those in the brain which had already been invaded. The difficulty of establishing the existence of fresh lesions in the brain discouraged attempts in this direction, but it is not impossible that the virus made further advances without producing symptoms. It would be desirable to repeat the experiments with a series of lightly paralysed convalescent animals in an effort to quantitate the amount of invasion necessary to prevent further paralysis from a given testing dose.

A suggestive case of this sort is found in a chimpanzee A4-8, which received by stomach tube 25 cc. of supernatant fluid from a pool of six potent human stools. On the 6th day following inoculation his temperature rose from a base line of 101–102° to 104.4°. No other abnormalities were noted but a lumbar puncture was not done. The animal subsequently was given 70 cc. of the same pool by stomach tube without further reaction. 8 months later he contracted a partial left facial paralysis after receiving 2 cc. of the same stool pool by mouth on 3 successive days. The lesions were sharply localized to the medulla, centering about the nuclei of the LT fifth and seventh nerves (42).

Homologous Virus Inoculations—Parallel Portals

Table II, Group C, indicates the findings in a small series of animals which are regarded as suggestive that inoculation of one olfactory bulb leaves the other susceptible to subsequent invasion. We shall give a résumé of the histories and advance an interpretation for what it is worth—possibly not a great deal in view of the nature of the problem and the few cases available.

Eight animals were inoculated into the right nostril with a single instillation of 0.5 cc. of a very virulent chimpanzee stool. All subsequently became paralysed (two were prostrate, one almost helpless, and the rest ambulatory). The two prostrate animals and one other were killed immediately as controls. 6 weeks later the nearly helpless animal had to be killed. The olfactory bulbs

of these four animals which served as controls were examined in serial sections. Three showed unilateral lesions. 3 months later the four remaining animals were inoculated as before, but into the left nostril with 0.5 cc. of stool from the same specimen previously employed. Within a week three of the animals showed fever between 104.8° and 106° but there was no extension of paralysis over a 2 week observation period, although one control became prostrate. Examination of the olfactory bulbs revealed bilateral lesions in every case,—a ratio differing markedly from that of the controls. It was thus strongly suggested that in the twice inoculated animals two separate invasions had taken place, the first through the right olfactory bulb, resulting in paralysis, and the second *via* the left, producing fever and lesions on this side. Perhaps the most confusing aspect of the whole problem was connected with the age of the lesions in the bulbs. There were available several pairs of olfactory bulbs from 2 to 3 month convalescent animals inoculated with various strains of virus and one bulb of a 6 week convalescent animal from the series in question. These indicated that inflammation is resolved relatively rapidly in the olfactory bulb, so that in 6 to 8 weeks little is left beyond remnants of cuffing in the marginal fiber layer and diffuse gliosis of the molecular and glomerular layers. Rod cells are no longer abundant at this stage and the mitral cell losses merely appear as vacancies in their respective layer. In the olfactory bulbs of the reinoculated convalescent animals it was not surprising to find massive cuffing with fresh neuronophagia and cellular infiltration on the side inoculated last, but to find these changes bilateral in two cases was totally unexpected. Since the series did not provide control material of the exact age of the bulbs in question, one can do little more than hint that fresh invasion had taken place on the side of first inoculation as well as on that inoculated more recently. It is hoped that further study will clarify this very important point.

Homologous Virus Inoculations—One Portal

It is generally true that in the monkey a paralytic attack of poliomyelitis following intracerebral or intranasal inoculation protects against paralysis from any but massive inoculations of the same virus by the same portal (3, 4, 7–11). Nevertheless, there are several nuances of this apparent fact which are worthy of consideration. For example one intracerebral inoculation is taken as the equivalent of another, but this is not necessarily true, since virus does not distribute itself uniformly through the entire cortex. This factor is probably of no practical importance as regards the immunizing and testing inoculations usually employed in the laboratory, since the overwhelming quantities of virus used ensure wide dissemination throughout the brain. However, it is conceivable that carefully controlled experiments closer to the monkey's threshold of susceptibility might reveal that two infections could

take place from cortical areas possessing different projection pathways, or even through a single olfactory bulb. This becomes a more realistic possibility when one considers human infections which may well occur from small quantities of virus entering the CNS on different occasions.

Heterologous Inoculations—One Portal

Several investigators attest the lack of immunity which intracerebral or intranasal inoculation of one strain may confer to another inoculation of a different strain (2, 4–9, 23).

A good illustration of this phenomenon is seen in monkeys A1-76 and A1-73. These animals both received intranasal inoculations of the same pool of human stools. They were both paralysed to about the same degree but remained ambulatory despite involvement of all extremities. 2 months after paralysis was first noted A1-76 received three daily intranasal inoculations of MV virus (0.5 cc. per nostril). The temperature went up to 107° on the 4th day and remained elevated in a dromedary spike for 1 week. Toward the end of this period the animal became so weak that it was barely able to climb. Examination of the olfactory bulbs showed massive invasion on both sides (Fig. 7). The olfactory bulbs of A1-79 which was allowed to live for the same period showed almost no lesions though many mitral cells had disappeared. Fig. 8 was taken from an area in one bulb which represented the maximal changes. It does not even approximate in severity the lesions found in the animal suffering the second attack.

Some virus strains appear to have a marked ability to override previous immunity, but according to Kessel and Stimpert (7), this is not a property of their virulence. It is again not unlikely that many animals which do not show additional paralysis may nevertheless suffer extension of lesions. A case in point is monkey A4-5 which has already been listed in Table II, Group A. The animal was given an intranasal inoculation of human stool which resulted in almost complete leg paralysis. 4 months later it received three daily intranasal inoculations of MV virus. On the 6th day following the first of the second series of inoculations its temperature rose from a steady base line of 102° to 105° and remained between 105° and 106° for 3 days. There was no visible extension of paralysis, and the animal was subsequently given an intraocular inoculation of Wfd virus to which it reacted with a slight temperature elevation. It was killed 2 months after the intranasal inoculation of MV virus, and 6 months after its original intranasal inoculation. The olfactory bulbs showed relatively fresh lesions which could scarcely have been present for 6 months. Flexner (2), and Lennette and Gordon (8) have recorded similar cases of animals which have suffered two paralytic attacks after two successive series of heterologous intranasal inoculations.

It now remains to consider the possible immunizing effects of virus which is

brought into direct contact with peripheral nerve axones but which does not produce a temperature reaction or any other sign of disease in the animal. This situation was encountered in six monkeys which remained symptomless following the moistening of the cut end of one sciatic nerve in a virus emulsion of demonstrated potency. Infection did not result because the neurones involved had been rendered temporarily refractory by repeated previous section of the nerve (41). The present point concerns the fact that probably every axone in the sciatic nerve was brought into protoplasmic contact with active virus, and that the transient state of resistance in the nerve thus permitted a much more extensive contact between virus and nerve tissue than would have been possible in the intact animal. The whole procedure might be considered as a sort of supervaccination of the nervous tissue together with a considerable amount of contact between the virus and the subcutaneous tissues. Nevertheless there was no evidence of virus proliferation.

Several weeks after this type of "vaccination" the animals were found readily susceptible to infection. Two became paralysed after the drastic procedure of exposing the opposite sciatic nerve to virus, but the remaining four were severely paralysed following routine intranasal inoculations. Furthermore there was no indication of increased resistance in the cells which had been in contact with virus. The series suggests that no general immunity results without overt virus activity.

DISCUSSION

The experiments just reported are not concerned primarily with humoral antiviral factors. Neither laboratory workers nor clinicians can agree on the rôle of serum antibodies in immunity to poliomyelitis (20–33). At best the degree of protection afforded by various methods of vaccination against intracerebral or intranasal inoculation is slight. Many of the observed inconsistencies may result from the fact that humoral protection and antibody titer vary not only from one individual to another but also in different tissues. The tests for immunity usually employed in the *rhesus* monkey have not brought out these subtle but important differences. It has been assumed throughout our own experiments that in each instance a more than adequate antigenic stimulus must have been provided by the first attack of poliomyelitis and that circulating antibodies were present. Nevertheless, despite the fact that the intranasal portal was used for the testing of immunity, it is possible that no opportunity was provided for the participation of humoral factors, since this type of inoculation brings virus into very intimate contact with nervous tissue in which it is rapidly fixed and thus removed from the action of antibody (43). Such a situation has been unavoidable because of the limitations imposed by the use of the *rhesus* monkey which can be infected readily only by direct neural or intranasal inoculation. In all probability these experiments have

been dealing primarily with a type of local immunity which appears to be largely a function of actual contact between virus and susceptible nerve cells (34). They have defined more clearly than heretofore the character of this immunity, showing it to be determined by the movements of virus along nerve fiber pathways. To what extent it may be modified by humoral factors is not at the moment apparent. Very delicately graded tests of the immunity of the central nervous system will be required to demonstrate such a relationship.

It is by no means clear how much of the experimental work on *rhesus* monkeys can be applied to the problem of immunity to poliomyelitis in man. There is little doubt that most individuals do acquire an effective immunity with increasing age and epidemiological evidence favors the idea that this is produced by contact with active virus, rather than by mere physiological maturation or other metabolic factors. The concept of "abortive" poliomyelitis as an immunizing agent, however, is based chiefly upon the postulate of a general immunity of the CNS produced by a limited invasion of virus from a single portal of entry. Actually nothing is known concerning the amount of invasion which may take place in human abortive and non-paralytic forms of poliomyelitis or their antigenic effects. Our own findings suggest that as regards the central nervous system an immunity of this type might not be very substantial since its influence probably would be restricted to the region of immediate exposure. Recent studies of human pathological material point increasingly to the utilization of several portals of entry in man (35–40), so that in order to produce effective immunity an antecedent attack must conceivably close a variety of portals leading to the neuraxis along various peripheral nerves. At the moment one can do no more than speculate on the means by which this might be accomplished.

There is no reason to believe that the central nervous systems of man and the *rhesus* monkey differ fundamentally in their reaction to poliomyelitis virus. The outstanding disparities between these two forms relate to the portals of entry. Thus the monkey, in which practically the entire nervous system is susceptible to virus, presents a very restricted portal of entry, the olfactory area. In this animal, however, it has been shown that paralytic poliomyelitis does not necessarily close this portal "from within" and that a second attack, whether paralytic or not, seems to depend upon the strains of virus used and the degree to which virus was disseminated through the neuraxis during the first exposure. The closure by an abortive attack of *multiple* portals of entry from within would therefore present even greater complications, involving extensive dissemination of virus in the neuraxis without the production of paralysis. It thus seems more logical to conclude that such immunity as can be demonstrated in the *rhesus* monkey by intracerebral in-

oculation has little bearing upon the problem of human immunity and that the immunity which is presumably produced by subclinical virus activity in man comes by the closure of the portals from without, either through the participation of humoral factors, or not inconceivably by alterations in the mucous membranes or other barriers against the entrance of virus into the nervous system.

SUMMARY

1. It was found in forty *rhesus* monkeys that intracerebral, intraocular, intracutaneous, intraperitoneal, intraspinal, and neural inoculations of poliomyelitis virus produced no lesions in the olfactory bulbs despite the fact that the animals contracted pronounced paralyses. This indicated that the virus could be restricted to certain neuronal systems.

2. Similarly intranasal inoculation of seven animals produced no lesions in the ciliary ganglia.

3. Two monkeys convalescent from an intracutaneous and an intracerebral inoculation respectively had further paralyses after intranasal inoculation of heterologous virus. A third animal convalescent from an intranasal inoculation showed extension of lesions after intranasal and intraocular inoculation with heterologous virus.

4. Two spinal animals in which an attack of poliomyelitis was limited to an isolated segment of spinal cord, contracted typical paralyses in the previously uninvaded portions of the CNS following intranasal inoculation of homologous virus.

5. Four of six convalescent monkeys showed extension of lesions but no clinical signs after homologous virus inoculation through a previously uninvaded portal.

6. Four animals convalescent from a unilateral intranasal inoculation showed evidence of new invasion in the opposite olfactory bulb but no extension of paralyses following a second inoculation of homologous virus into the appropriate nostril.

7. Two animals had second attacks after heterologous second inoculations. The intranasal portal was employed for both exposures.

8. It thus seems apparent that in the *rhesus* monkey a second attack of poliomyelitis, whether paralytic or not, seems to depend upon the strains of virus used and the degree to which virus is disseminated through the neuraxis during the first exposure.

9. The above experimental data emphasize the difficulty of utilizing the *rhesus* monkey for experiments seeking to elucidate the mechanisms of immunity in man and suggest that human immunity to poliomyelitis does not result from immunization of the nervous system but rather is the result of some

process which prevents infective quantities of active virus from reaching nervous tissue.

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EXPLANATION OF PLATES

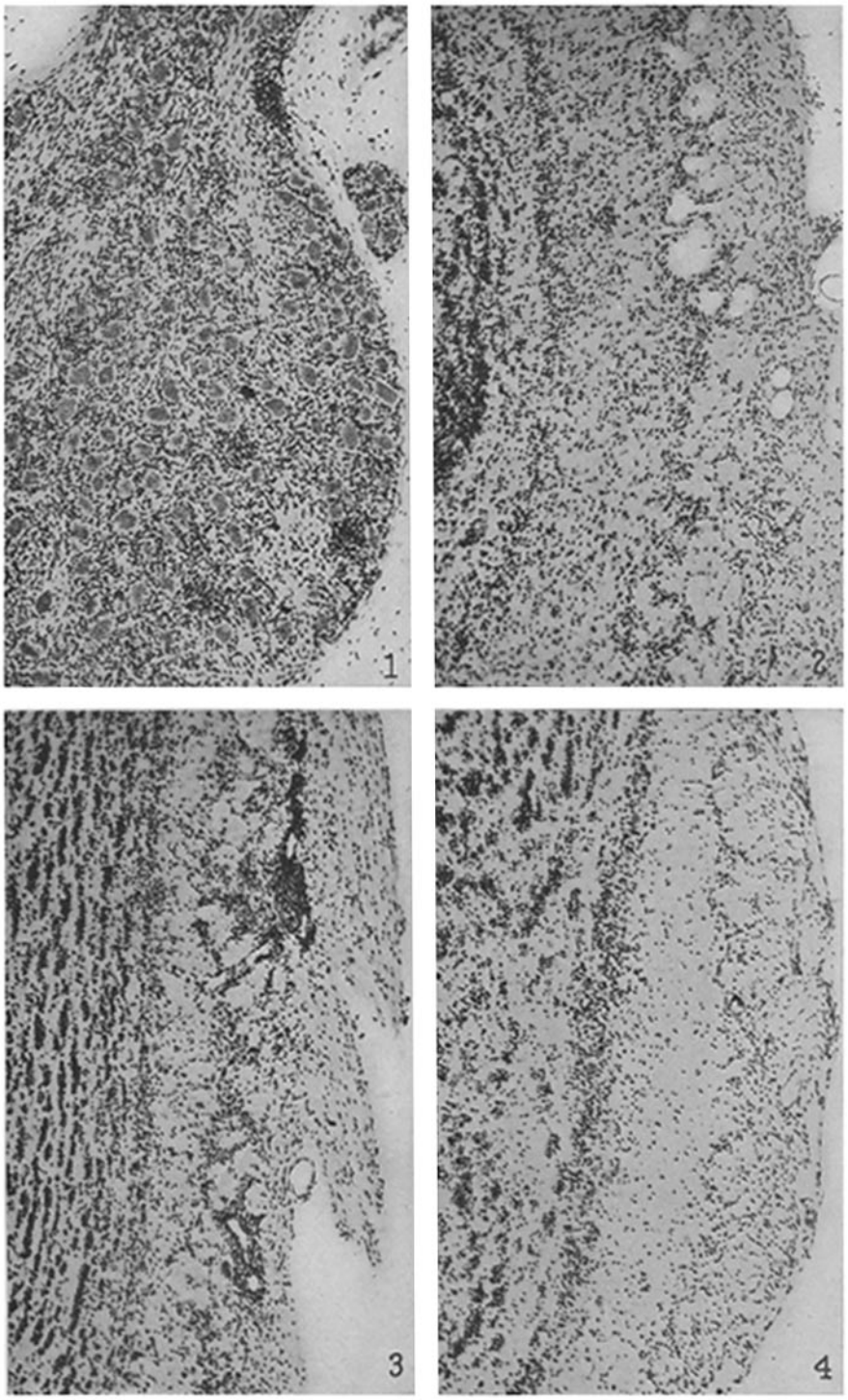
PLATE 7

FIG. 1. The left ciliary ganglion of A4-5, an intranasal convalescent monkey which was subsequently inoculated into the vitreous of the left eye with heterologous virus. Note lymphocytic foci which are the characteristic of virus invasion in this tissue.

FIG. 2. Olfactory bulb of A1-17, a convalescent from an intraocular inoculation subsequently given homologous virus intranasally. Compare with Fig. 4 which shows a normal olfactory bulb. Note dense cellular infiltration and complete loss of mitral cells. × 60.

FIG. 3. Olfactory bulb of A1-35, a convalescent from an intraocular inoculation subsequently given homologous virus intranasally. Note heavy perivascular cuffing and areas of neuronophagia in mitral cell layer. × 60.

FIG. 4. Olfactory bulb of A1-34. Treated as in the above cases A1-17 and A1-35 but showing no lesions. Note normal appearance of mitral cells in their respective layer and the acellular character of the molecular layer. × 60.



(Howe and Bodian: Second attacks of poliomyelitis)

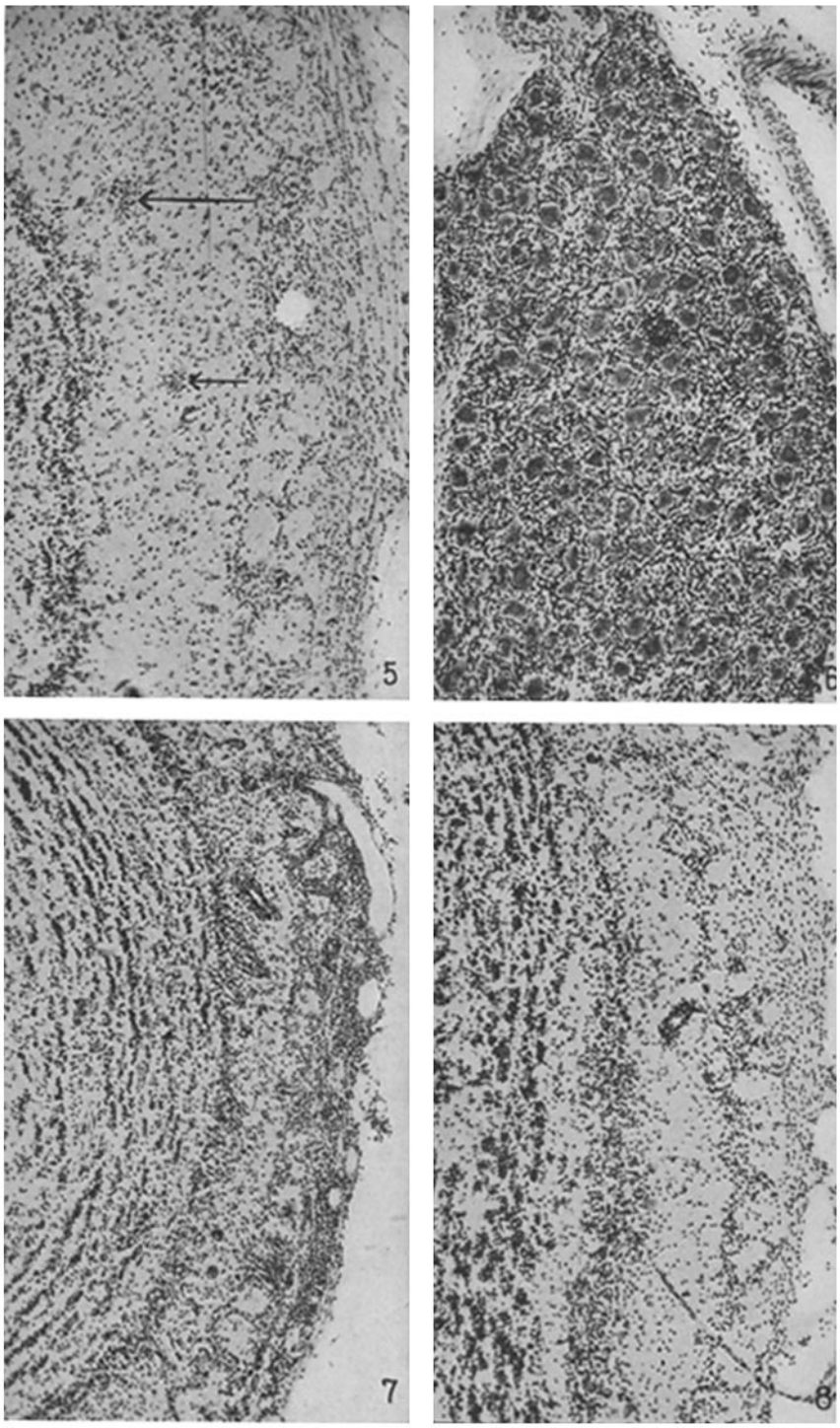
PLATE 8

FIG. 5. Olfactory bulb of chimpanzee A1-74. The animal was convalescent from an intracerebral inoculation and subsequently received homologous virus intranasally. Note clumps of phagocytes (designated by arrows) surrounding scattered mitral cells in the molecular layer. × 60.

FIG. 6. Left ciliary ganglion of A1-07, a convalescent from an intranasal inoculation subsequently receiving homologous virus in the vitreous of the left eye. Note focus characteristic of invasion in this tissue. × 60.

FIG. 7. Olfactory bulb of A1-76, a convalescent from an intranasal inoculation subsequently receiving heterologous virus by the same portal. Note the massive character of the invasion. Compare with control (Fig. 4). × 60.

FIG. 8. Olfactory bulb of A1-79, a convalescent from an intranasal inoculation. It received the same virus as A1-76, suffered approximately the same amount of paralysis, and lived the same number of days, but received no second inoculation. The cuffed vessel represents the maximal amount of change which could be found in the olfactory bulbs after this period of convalescence. × 60.



(Howe and Bodian: Second attacks of poliomyelitis)