STUDIES ON ENTRY AND EGRESS OF POLIOMYELITIC INFECTION

VII. EARLY LESIONS IN PERIPHERAL GANGLIA AFTER SIMPLE FEEDING: WITH COMMENTS ON THE POSSIBLE VALUE OF IMMUNIZATION IN PREVENTING NEURAL ENTRY*

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PLATES 41 TO 44

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The view that poliomyelitis is primarily a neural disease has been questioned by those who believe that it is primarily extraneural and only invades the nervous system during a later stage, mainly as a result of viremia (1, 2). The significance of our discovery (3) that as early as 2 days after gentle swabbing of the oropharynx with virus typical acute neuronal lesions are already present in the peripheral ganglia supplying that area, and that virus can be recovered from them at 3 days and later, has been discounted1 on the grounds that the method of exposure was traumatic and not comparable with "natural" exposures. While the method consisted of rolling, rather than rubbing, virus on the mucous membranes with cotton swabs and still seemed to us comparable with the normal friction involved in mastication, we (4) later presented evidence, in order to meet criticism, that after simple feeding of virus mixed with ordinary food, virus could be recovered from the regional ganglia at 3 days and later, thus duplicating the results of swabbing. However, we did not, in that study, examine the ganglia histologically in a parallel series, and were unable to state that after simple ingestion of virus, lesions also appeared at a comparably early period. In the present study, this feature

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1 Bodian (1) has criticized our method of swabbing as unnatural and not valid for comparison with human exposures. Further details of the method are therefore furnished. Cotton swabs dipped in virus suspension were gently rolled (not actually rubbed) over the mucosa of the posterior cheek and tonsillar region for approximately 1 minute at intervals of 5 minutes over a total period of 2 hours. Since the monkeys were in the side-lying position much of the 21,000 to 31,000 PD_{50} estimated total amount of virus applied ran out of the mouth, and very little was swallowed. Our contention that the amount of friction involved was no greater than that occurring during normal mastication still seems to us to be correct. Moreover, the total time of exposure was little greater than would occur under natural conditions from virus-contaminated food.
of the disease process has been investigated. The importance of characteristic lesions as an essential part of proof of viral invasion and multiplication in any tissue is obvious. It may be pointed out that such histopathological proof has not been produced for the "alimentary mucosa" which is regarded by Bodian (1) as the primary site of poliomyelitic infection.

The problem of the primary focus of infection is important not only in reference to the theory of pathogenesis but also to the potential value of prophylactic immunization, a subject which we shall presently discuss.

**Methods**

**Virus.**—The same Wiss '45 strain (Type 1) employed in previous studies of this series was used throughout. As indicated in the protocols, part of the suspensions was stock, with PD₅₀, 4.9; and part was an untitrated but active preparation, similarly prepared.

**Monkeys.**—*Macaca irus* (cynomolgus) monkeys supplied by Okatie Farms, Pritchardville, South Carolina, were used. They were of average weight (5 to 8 pounds; 2.3 to 3.6 kg.).

**Technic of Subinoculation.**—Blood was prepared and inoculated intraperitoneally and intracerebrally, and ganglia were prepared and inoculated intracerebrally, all according to methods previously described (4). Results were determined only by the occurrence or non-occurrence of paralysis, without histological examination for inapparent takes.

**Preparation of Tissue for Histological Examination.**—Under ether anesthesia the animals were exsanguinated and then perfused with 10 per cent formalin containing 1 per cent acetic acid. Ganglia were imbedded in paraffin; complete serial sections 10 μ thick were made and stained with gallocyanin.

**PROTOCOLS OF INDIVIDUAL EXPERIMENTS**

**Series I.**—**Histopathology and Subinoculation of Blood at 56 Hours after Feeding.**—Eight cynomolgus monkeys, received 4 days previously, were fed poliomyelitis virus mixed with their ordinary food on Dec. 20, 1952. Each animal received 3 ml. of a 33 ⅔ per cent stock, titrated suspension, or approximately 80,000 PD₅₀. All animals were sacrificed on Dec. 22, approximately 56 hours after feeding was started.

40 ml. of blood was removed by cardiac puncture from each animal for subinoculation. Gasserian, nodose, and superior cervical sympathetic ganglia were removed from each animal. From four animals (C 1-065, 1-066, 1-067, and 1-068) pooled blood and pooled like ganglia were subinoculated; and from the remaining four (C 1-069, 1-070, 1-071, and 1-072), like ganglia were examined histologically by complete serial sections. From the second series, pooled blood was also tested by subinoculation.

**Results of Subinoculation.**—Each pool of blood produced typical paralysis in each of two animals tested; the incubation periods were 9, 5, 5, and 8 days respectively. Each pool of like ganglia was tested on two monkeys and all results were negative.

**Results of Histological Examination.**—C 1-069. In the Gasserian ganglia rare, widely scat-
tered PrI were found, all but one of minimal size; no PVI or NpH was discovered. In the nodose ganglia, no lesions were detected. In the superior cervical sympathetic ganglia, only two PrI, both of minimal size, were seen.

C 1-070. In one of the Gasserian ganglia, an extensive area of infiltration involving both the neuronal part of the ganglion and the adjacent nerve bundles could be followed through eight successive sections ending in a PVI; in this area the neurons had disappeared (Fig. 9). Apart from this lesion, only a few minimal PrI were found in the Gasserians. In the nodose ganglion, only a single PrI of moderate size was seen. In the superior cervical sympathetic several PrI of minimal and one of moderate size were encountered, but no NpH.

C 1-071. In one of the Gasserian ganglia, an extensive area of infiltration involving both the neuronal part of the ganglion and the adjacent nerve bundles could be followed through eight successive sections ending in a PVI; in this area the neurons had disappeared (Fig. 9). Apart from this lesion, only a few minimal PrI were found in the Gasserians. In the nodose ganglion, only a single PrI of moderate size was seen. In the superior cervical sympathetic several PrI of minimal and one of moderate size were encountered, but no NpH.

C 1-072. In the Gasserian ganglia, three PrI of moderate size were found, but no NpH. In the nodose, several PrI of moderate size were observed, also one PVI; NpH was detected in one lesion. In the superior cervical sympathetic ganglia, a single PrI, containing a chromatolytic neuron, was seen.

C 1-073. In the Gasserian ganglia, there were only rare PrI of minimal size. Three minimal PrI were found in the nodose ganglia. In the superior cervical ganglia, however, there were several moderately large PrI, one containing necrotic neurons and microglial cells; a single PVI was observed; all the lesions were in one ganglion.

Summary of Results.—The minimal lesions were about the same in extent as those previously (5) noted in normal ganglia of newly received monkeys. The large, sharply localized lesions in the Gasserian ganglion of C 1-070 and the similar, though smaller one, in the superior cervical sympathetic of C 1-072, were strongly suggestive of axonally conducted rather than of blood-borne infection. Some of the other lesions of moderate size might have had either origin.

Series 2.—Histopathology at 60 Hours after Feeding.—Three monkeys received in the laboratory Apr. 22, 1953, were fed 1 gm. of untitrated virus, one (C 1-086) on May 24 and two (C 1-091 and 1-092) on May 31. All were sacrificed approximately 60 hours after feeding.

Sections from like ganglia were mounted together. Of 26 slides from the Gasserian ganglia, 19 were free from lesions. In the remainder, there were occasional PrI of small to moderate size. In one of these there was a focus containing a chromatolytic neuron, (Fig. 2), with several pyknotic neurons nearby. In another slide, there was a large PrI containing necrotic neurons; this focus could be followed through several sections. No lesions were found in the nodose ganglia. In the superior cervical sympathetic ganglia there was a single large focus containing necrotic neurons.

Summary of Results.—The isolated large focus in the Gasserian and the similar one in the sympathetic ganglion were suggestive of axonally conducted infection. The remaining lesions, mostly small, were like those seen in un inoculated monkeys kept in the laboratory more than 2 weeks. Their origin could not be identified.

Series 3.—Histopathology at 72 Hours after Feeding.—Two monkeys, received Aug. 4, 1953, were fed 1 gm. of untitrated virus on Sept. 15, and were sacrificed approximately 72 hours later.

Results of Histological Examination.—C 1-116. In the Gasserian ganglia there were scattered PrI of minimal to moderate size, like those seen in the previous series, but mainly confined to one of the ganglia. In addition, in this ganglion, there was an extensive and heavy infiltrate, with NpH and PVI, which could be followed through more than 20 sections, originating within
the neural area and finally debouching into the nerve bundles of the peripheral side of the ganglion along a blood vessel. In the nodose ganglion, rare PrI, only one of more than minimal size, were found but no Nph; in one place there was lymphocytic infiltration in a nerve bundle.

In the superior cervical sympathetic ganglion a few PrI were seen, two of moderate size; no Nph was observed.

C 1-117. Both Gasserian ganglia were extensively and heavily involved, one more than the other. Most of the lesions were, however, discrete. Many necrotic neurons, some with Nph and some without, were observed (Fig. 7). In addition, there was a heavy infiltration of the nerve bundles adjacent to the neuronal area of the ganglia (Fig. 10), which appeared to be confined to the side of the peripheral, ingoing fibers and which was not present in the outgoing, central fiber bundles of the sensory root. One end of each ganglion was uninvolved. In sharp contrast to the Gasserian ganglia, the nodose and superior cervical ganglia showed only rare, small PrI, without signs of neuronal damage.

Summary of Results.—The heavy lesions found in both monkeys, and particularly those in C 1-117, largely confined to the Gasserian ganglia, appeared almost certainly to have been due to axonally conducted infection from the oropharyngeal area. The involvement of the ingoing nerve bundles of the ganglia in contrast to the lack of involvement on the central side (sensory root) lends support to this view. Further support is given by the contrastingly slight involvement of the nodose and superior cervical sympathetic ganglia.

Discussion

These experiments have shown that simple feeding of poliomyelitis virus produces, and with equal promptness, essentially the same type of histopathological reaction in the peripheral ganglia supplying the mouth and pharynx as does simple oropharyngeal swabbing; including chromatolysis, necrobiosis, and neuronophagia, as well as interstitial, perineuronal, and paravascular infiltrations. Examples of lesions following both types of exposure are shown in Figs. 1 to 10. The severity and extent of the pathological changes were on the whole greater after feeding than after swabbing, probably because of the larger dosage of virus. With both types of exposure significant lesions had appeared at a very early period (2 to 2½ days) and had increased in numbers and intensity at 3 days. In one fed animal (C 1-117) the lesions in the Gasserian were exceptionally severe and numerous, and were accompanied by marked infiltrations in the adjacent nerve trunks.

Not only did lesions appear at too early a period to be secondary to viremia, which is itself a secondary phenomenon, but their pattern of distribution, after both feeding and swabbing exposures, lacked the random character to be expected from blood-borne origin. On the contrary, they were largely confined to those ganglia which supply the main areas of exposure in the mouth and pharynx, and particularly to those of the trigeminal system which supplies both areas. The vagus and cervical sympathetic ganglia which pro-
vide a more limited and less abundant nerve supply to these areas, were less heavily involved, particularly after feeding.

The explanation of our failure in both the swabbing and feeding series to recover virus by subinoculation from the earliest specimens of ganglia, despite the presence of lesions in the parallel histological series, is obscure. Obviously, infection of the ganglia could not have accounted for the viremia observed 56 hours after feeding. Under the experimental conditions, such early viremia is, we believe, due to passive intestinal absorption following ingestion of massive quantities of virus. The subject will be dealt with more fully in another communication (8).

We have now, we believe, accumulated sufficient experimental evidence from this and our previous studies, to prove that the oral and pharyngeal mucosae constitute an important, if not necessarily the exclusive, primary portal of entry under non-traumatic conditions reasonably comparable with natural exposures; and that such entry leads through axonal pathways into regional ganglia, notably the trigeminal, where initial foci of viral multiplication and inflammatory reaction are set up. The experimental evidence is consistent with pathological findings in human patients (7).

In relation to the prospects for successful passive and active immunization against poliomyelitis, the concept of primary neural entry and propagation of infection poses certain theoretical difficulties not inherent in the hypothesis that viremia is the sole means by which the CNS becomes infected. Since viremia can be promptly combatted by antibody, even in relatively low concentrations (9), this second hypothesis appears to offer a much more hopeful outlook for successful results from either active or passive immunization. In view of the evidence we have obtained favoring primary neural infection, however, the possible ways in which this mode of entry might be prevented or modified must be taken into account.

The observations of Hammon and his associates (10) on the effects of gamma globulin suggest that relatively small amounts of antibody given shortly in advance of exposure have some protective effect against the acquisition of infection, and also that when infection is not entirely prevented its paralytic effects may be comparatively mild. These observations, which remain, at the time of writing, to be expanded, suggest that antibody may (1) act at the portal of entry, and (2) modify already established infection.

There is some evidence to support the view that specific antibody might block primary neural entry in the mouth and throat. After virus has been first deposited on the mucosal surfaces of the upper alimentary tract, there must be a brief interval before it can make contact with the superficial epithelium and its nerve fibers. During this period it must penetrate the superficial mucus and be exposed to such immune substances as may be present therein. It is probable that in poliomyelitis, as in other infectious diseases,
the number of infective particles entering the body to initiate infection is extremely small. In 1917, Amoss and Taylor (11) demonstrated the presence in human nasopharyngeal secretions of poliomyelitic neutralizing antibody. More recently Bell (12) has measured the concentrations of Type 2 antibody in these secretions and simultaneously in the blood of the same individuals. The mean ratio between the two was about 1:25, and the median (our calculation), 1:13. Antibody was almost constantly present in the secretions when the serum level was 1/4 (antilog 1.8) or higher; below this level it was detected in 12 of 33 cases at dilutions of 1/2 to 1/8 (antilogs 0.3 to 0.9). In another study Bell (13) demonstrated a straight line logarithmic relationship between the quantity of virus and the quality of immune serum required to neutralize it. Thus, for very small amounts of virus correspondingly small amounts of antibody sufficed. It is therefore possible that even minimal concentrations of specific immune substances in the overlying mucus could oppose a barrier to initial invasion of the surfaces of the mouth and pharynx by a few particles of poliomyelitis virus under natural conditions of exposure. It should be noted, however, that excretion of antibody on the intestinal surfaces has not been demonstrated and may not occur. If so, there is no comparable barrier to primary infection of nerve elements in this area, but it is encouraging that our previous studies (14) indicated that paralytic poliomyelitis rarely follows exposures confined to the gastro-intestinal mucosa.

It is generally supposed that once poliomyelitis virus has entered nerve fibers and cells it is inaccessible to circulating antibody. However, the important possibility remains that a damaged, in contrast to an intact cell, might admit antibody to the intracellular space. Bodian has shown (6) that recovery of infected, visibly damaged, neurons occurs spontaneously in many instances (thus accounting in part at least for the not infrequent recovery of patients from paralysis). Experimental exploration of this possible mode of action of antibody in poliomyelitis is desirable.

SUMMARY

At 56, 60, and 72 hours after simple feeding of poliomyelitis virus, typical, discrete lesions were found in the ganglia supplying the mouth and pharynx, which were most numerous and severe in the Gasserian ganglia. Lesions were also found in the nerve bundles adjacent to the infected ganglia.

The character, localizations, and time of appearance of lesions point to nerve-conducted entry of infection from the mucosa of the mouth and pharynx.

The possibility is suggested that under natural conditions of exposure, in which only small amounts of virus are involved, artificially induced immunity, active and probably passive, may block primary neural entry at the oropharyngeal portal by virtue of antibodies in the overlying mucus.
BIBLIOGRAPHY

8. Faber, H. K., and Dong, L., unpublished experiments.
EXPLANATION OF PLATES

The sections, all from *cynomolgus* monkeys, were treated with Einarsen's gallocyanin stain. The Wis' 45 strain (Type 1) of virus was used throughout.

**PLATE 41**

**Fig. 1.** C 6-66. Primary lesions in the Gasserian ganglion 48 hours after atraumatic swabbing of the oropharynx with virus. A chromatolytic nerve cell with an eccentric nucleus is surrounded by a small infiltration, consisting mainly of lymphocytes with some microglial cells, which is invading the perineuronal space on one side. A capillary is seen at the right of the infiltration. There are several normal neurons in the outer portion of the section. $\times$ 368.

**Fig. 2.** C 1-086. Primary lesion in the Gasserian ganglion 60 hours after simple feeding of virus. A chromatolytic nerve cell is surrounded by a small infiltration, consisting mainly of lymphocytes with some microglial cells. Several normal neurons are seen in the outer portion of the section. Note the similarity of this lesion to that in Fig. 1. $\times$ 368.
(Faber and Dong: Entry and egress of poliomyelitic infection. VII)
C 6-66. Four successive, serial sections from the Gasserian ganglion showing a primary lesion 48 hours after atraumatic swabbing of the oropharynx with virus. × 220.

Fig. 3. In the center there is a chromatolytic nerve cell with an eccentric nucleus (a), and to the right and above is a second chromatolytic nerve cell (b). There is a small surrounding infiltration of lymphocytes, microglia, and probably proliferating capsular cells.

Fig. 4. Cell (a) is still well seen, but without its nucleus. The capsular space of cell (b) is seen but the cell itself has disappeared. The infiltration is somewhat denser.

Fig. 5. The top of cell (a) is still visible, now beginning to be overlaid with capsular cells, lymphocytes, and at least two microglial cells.

Fig. 6. The nerve cells in the focus have entirely disappeared, leaving only the infiltration of lymphocytes and microglia, together with proliferated capsular cells. The appearance of the focus is now that of an ordinary small, grade 1 lesion.
(Faber and Dong: Entry and egress of poliomyelitic infection. VII)
Both sections are from Gasserian ganglia of C 1-117, 72 hours after simple feeding of virus.

Fig. 7. A moderately large primary lesion with dense infiltration. Within it, six necrotic, pyknotic, deformed nerve cells are visible (indicated by arrows) in one of which early neuronophagia can be discerned. $\times$ 184.

Fig. 8. A dense lesion near the border of the nerve tracts shows a roughly circular central area containing nerve cell debris. Within the infiltrate below and to the right a deformed nerve cell is visible. $\times$ 184.
(Faber and Dong: Entry and egress of poliomyelitic infection. VII)
FIG. 9. C 1-070. Gasserian ganglion 56 hours after simple feeding of virus. The focus is large and dense. Within it no nerve cells are visible, in contrast to their abundance and normal appearance in the adjacent areas. Below and to the right (arrow) a paravascular infiltration is connected with the main focus. Above and at right (arrow) the infiltration leads into a nerve bundle. × 92.

FIG. 10. C 1-117. Gasserian ganglion 72 hours after simple feeding of virus. At the left is a rather small but very dense focus and above a somewhat larger but more diffuse one. Lymphocytic invasion of a nerve bundle is seen at the right (arrow). × 92.
(Faber and Dong: Entry and egress of poliomyelitic infection. VII)