POLIOMYELITIS IN THE CYNOMOLGUS MONKEY

II. Resistance to Spread of Infection in the Central Nervous System Following Exposures of the Mucous Membranes to Virus, with Comments on Non-Paralytic Poliomyelitis*

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Sabin and Ward (1) in a recent review and original study of non-paralytic and transitory poliomyelitis in *rhesus* monkeys pointed out that "the non-paralytic infection was almost invariably associated with the destruction of an appreciable number of nerve cells in the spinal cord." Howe and Bodian's (2) experiments similarly showed that destructive nervous lesions, sometimes extensive, are present in the central nervous system in non-paralytic poliomyelitis but not necessarily in the spinal cord. These observations clearly demonstrate that failure of the virus to produce overt manifestations of disease does not necessarily imply its complete inability to implant itself and multiply in the central nervous system of the host but rather its tendency to die out or to stop multiplying and invading in certain cases. This tendency—which is not too uncommon in experimental work with poliomyelitis virus—is presumably due in some instances to factors inherent in a particular strain of virus (virulence, invasiveness, adaptivity); in others to factors in the host (resistance, refractoriness, immunity), and in still others to a balance between the two. Since the same tendency is often seen in human beings reacting to the attack to poliomyelitis virus, the majority of whom suffer from non-paralytic forms of the disease, it is a matter of importance for us to discover whether and how the tendency can be artificially induced or enhanced. The present report deals with a few observations on host resistance under experimental conditions. The *cynomolgus* monkey (*Macaca irus*) was selected for the experiments because, unlike the *rhesus* (*M. mulatta*), it has been shown to be readily infectible by simple contact with the mucous membranes of the alimentary (3) and respiratory (4) tracts, presumably like man.

EXPERIMENTAL

Five *cynomolgus* monkeys of a series of 26 (reported in the preceding paper) were exposed to poliomyelitis virus (Sabin's *Per* strain, 4th to 9th passages) successively by capsule feeding, lingual swabbing, enema, oronasal spray

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(twice), and inhalation, over periods ranging from over 9 months to over 14 months. Some resistance to infection was evident at the time of inhalation. None of the 5 having shown any manifestations of poliomyelitis up to 19 days after the last exposure, all were then inoculated intracerebrally with 0.5 cc. of a 10 per cent suspension supernate of the same strain as a test of immunity. One animal (C20) succumbed 8 days later to typical paralytic poliomyelitis; the other 4 showed no evidence of infection. These were sacrificed 31 days after inoculation and a comprehensive histological survey of the central and peripheral nervous system was made in each. Included in this examination were the olfactory bulbs, secondary olfactory centers, brainstem, and spinal cord; Gasserian, geniculate, petrosal, and nodose ganglia of the cranial nerves; the superior, intermediate, and inferior cervical, the upper and lower thoracic, the lumbar and the celiac sympathetic ganglia; and the cervical, thoracic, and lumbar spinal ganglia. The olfactory bulbs and all the ganglia were studied by serial section; the midbrain, pons, and medulla, at 0.6 mm. intervals. 3 to 5 consecutive sections each of the upper, middle and lower levels of the cervical, thoracic, and lumbar cord (a total of 9 levels) were examined. 1 to 3 sections each of the thalamus and hypothalamus and of the secondary olfactory centers were studied, including the anterior perforated substance, amygdaloid nucleus, parolfactory area, and olfactory trigone. Einarson's gallocyanin counterstained with eosin was used throughout. Myelin stains were not done. The results of the histological examination are given in detail in the following protocols and in Table II of the preceding paper.

Cynomolgus 17.—In the anterior perforated substance, a large space filled with red cells apparently marked the site of inoculation. Near it was a large parenchymal infiltration and also a large perivascular infiltration. A collection of microglial cells probably marked an area of neuronophagia, though the nerve cells in it could not be definitely identified. In the substantia nigra and reticular substance of the midbrain several perivascular and parenchymal infiltrations with neuronophagia were seen. In the pons, the central gray matter, and the nucleus centralis superior each showed a small area of parenchymal infiltration. Caudally from the pons no further lesions were found, either in the medulla or spinal cord. A few minute lesions were found in one lower cervical spinal ganglion, in one inferior cervical sympathetic, and in one Gasserian, and in three of the abdominal sympathetics. Larger foci, probably with neuronophagia, were present in the other Gasserian ganglion. The celiac ganglion was entirely negative.

Summary.—Recent active lesions were found descending from the point of inoculation heaviest in the midbrain and not extending beyond the pons. Lesions in the peripheral ganglia appear to be most reasonably explained on the basis of slight infection from previous procedures.[1]

[1] The character and significance of lesions in the peripheral ganglia have been discussed in the preceding paper of this series.

Cynomolgus 24.—A small perivascular infiltration was seen near the substantia nigra in the midbrain; a small parenchymal infiltration in the superior olivary nucleus in the pons, and three small perivascular infiltrations in the dorsal region of the lower medulla. No lesions were found in the spinal cord. A few minimal lesions were present in the lumbar spinal ganglia, the Gasserian ganglia, the nodosal ganglia, the upper and middle cervical sympathetics, and a large one in one lower cervical sympathetic.

Summary.—Slight but definite lesions were discovered in the brainstem but not in the spinal cord. The lesions in the peripheral ganglia probably resulted from previous inoculations.

Cynomolgus 50.—Well marked perivascular and parenchymal infiltrations were found in the massa intermedia of the thalamus. In the midbrain there were parenchymal and perivascular lesions in the substantia nigra, tegmentum, and reticular formation. In the latter place, many neurons were absent. In the pons, parenchymal infiltrations were seen in the reticular substance, in or near the motor nucleus of the 5th nerve and in the nuclei pontis. Definite neuronophagia was seen in the motor nucleus of the 5th nerve. In the medulla an intense focus with neuronophagia was seen lateral to the solitary nucleus, probably in the spinal vestibular nucleus, and there was marked parenchymal and perivascular infiltration in the lateral reticular nucleus. Perivascular and intense parenchymal infiltration with neuronophagia was found in the posterior horn in one level of the upper cervical cord. The rest of the spinal cord was negative. Minimal lesions were found in the Gasserian, nodosal, cervical, and thoracic spinal, lumbar sympathetic, upper and middle cervical sympathetic ganglia; and somewhat heavier ones in the lower cervical sympathetic and celiac ganglia.

Summary.—Well defined lesions, with neuronophagia, were present in the brainstem and in one place in the cervical cord. Lesions in the peripheral ganglia probably resulted from previous exposures.

Cynomolgus 55.—In the olfactory bulbs two small areas of meningeal infiltration and one doubtful perivascular infiltration near the edge suggested olfactory route infection but, in the absence of neuronophagia and heavy infiltrations were of doubtful significance. In the anterior perforated substance there was a small perivascular infiltrate; in the olfactory trigone, another; in the amygdaloid nucleus, another with a small area of parenchymal infiltration; and in the parolfactory area, a small spot of perivascular infiltration. In the thalamus and hypothalamus parenchymal and perivascular lesions were more abundant, but without neuronophagia. In the midbrain, similar lesions were still more intense and abundant, in the substantia nigra, tegmentum, superior colliculus, and central gray and other regions, together with considerable neuronophagia. Destruction of neurons was especially marked in the substantia nigra. In the pons, the reticular formation and central gray matter showed similar heavy lesions. In the medulla, the lesions were lighter and were present in and near the dorsal motor nucleus of the vagus, reticular substance, and olive. The cord was negative down to the lower thoracic level, where there was very heavy parenchymal infiltration, neuronophagia, and perivascular infiltration, much

more marked in one lateral half than in the other, but without cell damage in the intermediolateral columns. A few small foci were also present in the upper lumbar cord. Minimal lesions were present in the spinal ganglia at all levels, and in the upper cervical and lower thoracic sympathetics; somewhat heavier ones were seen in one Gasserian ganglion, in the lower cervical and lumbar sympathetics and in the celiac. In the celiac, one very large infiltration was seen.

Summary.—Minimal lesions were present in the olfactory bulbs, somewhat heavier ones in the secondary centers. Since these appeared to be of recent origin and since (see protocol of C17) the point of intracerebral inoculation may reach the anterior perforated substance, it is improbable that they had their origin in earlier exposure from inhalation. Zinc sulfate blockade of the olfactory membrane had preceded the latter. There was extensive and severe involvement of the brainstem, heaviest in the midbrain, extending through much of the medulla, and of a rather narrow segment of the lower thoracic and upper lumbar cord. Lesions in the peripheral ganglia corresponded with earlier surface exposures.

DISCUSSION

In a series of 25 previously unexposed *cynomolgus* monkeys inoculated intracerebrally with the same strain of virus (*Per*) in approximately the same amounts, 23 (92 per cent) came down with paralytic poliomyelitis. Of the 5 monkeys here reported which had been previously subjected to a series of non-traumatic exposures, only one (20 per cent) came down with paralysis, while the remaining 4 showed no clinical manifestations of the disease. All 4, however, showed typical poliomyelitic lesions of various degrees of severity descending varying distances from the level of inoculation and therefore suffered from non-paralytic poliomyelitis. It seems reasonably certain that these 4 had acquired a considerable degree of refractoriness consisting, not of complete immunity to infection but rather of a capacity to limit its spread and severity short of paralysis, or of any other clinical manifestation of poliomyelitis. The protocols show strikingly the various degrees in which this limitation was displayed. In one animal (C24) the lesions in the central nervous system were mild. In another, (C17) they were marked in the upper brainstem but had not extended downward beyond the pons. In a third (C50) they were also marked in the brainstem, and had descended to the medulla and upper cervical cord (posterior horn only) but no lower. In the fourth (C55) they were marked in the brainstem including the medulla and had involved a small segment of the lower thoracic and upper lumbar cord. It is noteworthy that lesions of various portions of the peripheral nervous ganglia were found in all the animals, at levels which corresponded with the probable portals of entry from previous exposures of the surfaces of the alimentary tract. It should be emphasized that careful scrutiny of the central nervous tissue

revealed no signs of an earlier central infection. For this reason, we are unable to explain the refractory state of the central nervous system on the basis of its previous infection in accordance with the observations of Kessel and Stimpert (7) and of Howe and Bodian (2). Kessel and Stimpert noted that a certain proportion of monkeys failing to develop poliomyelitis from an intracerebral inoculation may be resistant to another, even of a different strain. Howe and Bodian found that a local refractory state (tissue resistance) was set up by inoculation and infection of a given area of the central nervous system, while previously uninfected areas remained susceptible to later injections or exposures.[2] In our experiments the mechanism appears to be of a different nature, since no infection of the central nervous system had, as far as we could determine, previously occurred. The curious limitation of spread of infection after intracerebral inoculation recalls an hypothesis which one of us suggested in 1933 (6) in explanation of the common tendency seen in human poliomyelitis for the disease to come to a halt at various stages of its evolution. This hypothesis was that in the synaptic spaces virus escaping from infected neurons might be exposed to neutralizing antibodies in the tissue fluids and so prevented from infecting connecting neurons; thus the spread of infection might be prevented. The experiments of Schultz and Gebhardt (5) failed to give support to this theory of humoral resistance. They injected relatively large amounts of hyperimmune serum into *rhesus* monkeys before intranasal and intracerebral inoculation with virus and found that, while about one-fourth of them were completely protected, the rest came down with unmodified paralytic poliomyelitis.

The presence of lesions, often quite extensive, in the peripheral ganglia of the 4 non-paralytic cases in the present series and the probability that these peripheral lesions resulted from earlier exposure suggest that preceding infection and perhaps the continuing presence of virus in the peripheral ganglia may have been responsible for the modification of infection in the central nervous system resulting from intracerebral inoculation. No exactly comparable studies with poliomyelitis or other viruses are known to us. Rivers, Sprunt, and Berry (8) found that dermal infection of monkeys with vaccinia virus resulted in immunity of the central nervous system. Hurst (9) found that in guinea pigs systemic infection with equine encephalomyelitis virus pro-

[2] It is possible that an extension of Howe and Bodian's theory to include the peripheral ganglia might explain some of the failures of exposure to cause poliomyelitis in the central nervous system. Thus, supposing that a ganglion such as the Gasserian had been previously infected, it is conceivable that later virus contacts of the mucous membranes supplied by the 5th nerve would fail to infect, not because the surfaces themselves were immune but because the nerve paths leading from them were now refractory. This phase of the refractory state does not, however, bear directly on the main thesis of the present paper.

duced immunity of the central nervous system, but this was not true in the case of monkeys. Comparable results with poliomyelitis are not on record to our knowledge and the opinion, recently expressed by Howe and Bodian (2), is widely held that a general tissue immunity of the central nervous system to poliomyelitis is difficult if not impossible to achieve.

Nevertheless and regardless of its correct explanation, a limited degree of tissue resistance in the central nervous system appears to have been acquired by our experimental animals and it is of some clinical interest to note its resemblance to the "halting phenomenon" in human poliomyelitis to which we have just referred. In man, a wide range of variation is seen in the clinical manifestations of the disease which, on analysis (6), appears to be based on the same tendency of the central nervous system to limit the spread of infection. In the mildest cases, no characteristic symptoms appear ("subclinical" and "abortive" cases). In others, there are nervous symptoms but no paralysis (non-paralytic cases). In others, (the commonest form) paralysis occurs but is restricted to one or a few segments of the cord. In others, involvement of one or more bulbar centers appears but the infection does not descend to the cord, at least in sufficient intensity to cause paralysis. In a certain proportion of cases, of course, no tendency to halting is seen, and widespread paralysis with death supervenes.

In further analogy to human disease, we call attention to the possible similarity between our method of exposure precedent to the final intracerebral inoculation and the kinds of exposure presumably common in man and presumably causing his resistance to poliomyelitis. Our program consisted of repeated exposures of various portions of the upper respiratory and of practically all of the alimentary tract extending over a period of 9 months in three instances and of nearly 14 months in the other two. Since the normal span of life in monkeys is 20 to 25 years (10), these periods of exposure corresponded with about $2\frac{1}{2}$ to $3\frac{1}{2}$ years for man. The exposures were non-traumatic and consisted of simple application of virus to the mucous surfaces. The evidence in favor of similar conditions in the human case are not only the occasional discovery of virus in the nasopharyngeal secretions and stools (11–13) of normal contacts but the general increase in the population of resistance to poliomyelitis with advancing age as shown by the curve of declining incidence of the disease in later childhood and adult life, and by the roughly corresponding increasing prevalence of neutralizing antibodies, (14, 15) which is itself testimony to past exposures. Finally, the common occurrence of inflammatory lesions in peripheral ganglia of human beings (16, 17), resembling those seen in our monkeys, may have a bearing on the subject.

While these human lesions are regarded by pathologists as non-specific for particular diseases, it is at least possible that in some instances they are due to poliomyelitis. The subject deserves further study.

SUMMARY AND CONCLUSIONS

1. Repeated non-traumatic exposures to poliomyelitis virus of the mucous membranes of the upper respiratory tract and of various portions of the alimentary tract in 5 *cynomolgus* monkeys, extending over a period of 9 to 14 months, were followed by a definite, though limited resistance to intracerebral inoculation to the homologous strain of virus in 4 of them. Only one monkey developed paralysis (20 per cent incidence) and the other 4 remained free of signs and symptoms of the disease. 92 per cent of 25 previously untreated monkeys developed paralysis with the same strain after intracerebral inoculation.

2. Microscopic examination of the central and peripheral nervous systems of the 4 non-paralytic cases revealed in all instances typical lesions in the central nervous system descending from the level of inoculation into the brainstem but in only 2 into the spinal cord and then only in limited, small areas. Lesions were found in the peripheral ganglia in all animals which corresponded in distribution with the surface membranes previously exposed to virus. No lesions were found in the central nervous system indicative of invasion preceding the intracerebral inoculation.

3. Our observations point to the acquisition by the immune animal, as a result of surface exposure, of the power to limit the spread of infection in the central nervous system rather than of the capacity entirely to prevent implantation and multiplication of virus.

4. This limitation of spread, it is suggested, resembles the tendency so commonly seen in human poliomyelitis of the disease to "halt" at some stage of its evolution, resulting in the various clinical gradations in extent and severity of involvement (subclinical, abortive, non-paralytic, mild paralytic forms, etc.)

5. The experimental methods of exposure, previous to intracerebral inoculation, employed in our study are compared with the natural exposures of human beings during the course of life, which, as age advances, lead to increasing resistance to poliomyelitis and to an increasing incidence of specific neutralizing antibodies in the blood.

6. The mechanism of resistance is obscure. It may be related to the continuing presence of infection in the peripheral ganglia but this is as yet unproved.

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