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Neuronal Pathways as Determining Factors in Dissemination of Poliomyelitis in the Central Nervous System.*

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There has been hitherto no clear-cut evidence regarding the mode of progression of the virus of poliomyelitis from the portal of entry into and through the central nervous system (CNS) of man. In the experimental animal more information is available and there is now a general belief, supported by considerable evidence, that the principal rôle in the dissemination of the virus is played by neuronal pathways rather than by humoral ones, and that within the CNS there occurs a progression of the virus from the point of entry to certain susceptible regions, especially the motor centers in the hindbrain and spinal cord, where the most serious effects of the virus-host reaction become apparent. Little is known as yet concerning the determining factors in the transmission and localization of the virus throughout the CNS, although studies of some of the neuronal pathways involved have been made by Fairbrother and Hurst,[1] and by others. In this report, additional evidence bearing on these problems will be presented.

The material examined up to the present time consists of some 50 brains of Rhesus monkeys in preparalytic and paralytic stages of poliomyelitis, induced by introduction of the MV virus intranasally, intracerebrally, intraocularly and intraneurally. In a few cases the Wallingford strain (Trask and Paul[2]), inoculated intracerebrally and by skin rub, was used. The brains were prepared under optimal conditions for histological study chiefly by the gallocyanin method of Einarson,[3] which satisfactorily demonstrates nerve cells, neuroglia, and inflammatory cells. In some cases various experimental procedures, such as section of the olfactory tracts, the corpus callosum, the bulbar pyramids, or the spinal cord, were carried out in order to modify if possible the mode of dissemination of the virus. In many cases microscopic sections of the olfactory bulbs, sympathetic ganglia, and other peripheral nervous struc-

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1 Fairbrother, R. W., and Hurst, E. W., *J. Path. and Bact.*, 1930, **33**, 17.

2 Trask, J. D., and Paul, J. R., *J. Bact.*, 1936, **31**, 527.

3 Einarson, L., *Am. J. Path.*, 1932, **8**, 295.

tures were available for study, in addition to the routine serial sections of brain and spinal cord. The analysis of the material has been made by study of the distribution of lesions and from the neuro-anatomical point of view, with the purpose of further elucidating the factors which govern the growth and dissemination of the virus in the CNS.

That the relation between the severity of the histopathological changes and the concentration of virus is not a strictly proportional one has been noted by Fairbrother and Hurst,[1] Schultz and Gebhardt,[4] and others, and indeed is clearly apparent in our material. Nevertheless, there is usually a sufficiently close correspondence between the distribution of lesions in the CNS and the distribution of virus to permit a reasonably accurate analysis of the paths of dissemination of the latter, by means of histological studies. Fairbrother and Hurst in fact have regarded histological examination as superior in some instances to inoculation tests for detecting the presence of virus, especially in regions such as the cerebral cortex, which are highly resistant to the virus. Especially in the pre-paralytic stage, following the onset of fever, the absence of lesions in any particular center, although not strictly exclusive of the presence of virus, may be safely assumed to be correlated either with very low concentration or with the absence of virus.[4]

It is possible to mention only briefly some observations which serve to emphasize the value of careful study of typical lesions as evidence of virus passage along neuronal pathways in well-controlled material. (1) In monkeys in which poliomyelitis is induced by intranasal instillation, after section of one olfactory tract, there is a marked preponderance of lesions on the side of the intact tract in olfactory centers and in the hypothalamus as far back as the midbrain tegmentum. This constant finding further supports previous evidence that the entry of virus into the CNS after intranasal instillation is by way of the olfactory tracts.[5]

(2) In pre-paralytic stages, after inoculation of virus into the leg region of area 4 (motor cortex), lesions are found in that portion of the dorsal thalamus, and that portion only, which has been shown

[4] Schultz, E. W., and Gebhardt, L. P., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 577.

[5] Schultz, E. W., and Gebhardt, L. P., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 728; Brodie, M., and Elvidge, A. R., *Science*, 1934, **79**, 235; Lennette, E. H., and Hudson, N. P., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1444; Howe, H. A., and Ecke, R. S., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 125; Gordon, F. B., and Lennette, E. H., *J. Bact.*, 1938, **35**, 43.

by experimental anatomical studies to be directly connected with the site of inoculation (lateral part of nucleus ventralis posterolateralis of Walker[6]). The constancy and unexpectedly sharp localization of the lesions in these cases, along a well-established neuronal system, offer convincing evidence of the propagation of virus along neuronal pathways and of the validity, in certain instances at least, of the use of typical lesions as stigmata of even early virus invasion.

(3) Brodie[7] has shown that after inoculation of virus into the motor cortex the concentration of virus at the height of paralysis is greater in the opposite motor cortex than in that of the side of inoculation. This agrees with our frequent finding in late paralytic stages that lesions are more numerous in the motor cortex opposite the side of inoculation. However, after experimental section of the corpus callosum, the great preponderance of lesions in the late paralytic stage is on the side of the inoculation. This indicates that the corpus callosum is the principal, but not exclusive, route for spread of the virus from one cerebral hemisphere to the other.

Since the predominant histopathological changes occur in the gray centers of the CNS rather than in the fiber pathways which interconnect them, it is necessary always to keep in mind the patterns of interconnections of the nerve cells, in order to be able to interpret the paths of progression of the virus from center to center, if it be assumed that the virus is transmitted along neuronal pathways. This has been rendered highly probable by evidence that nerve cells rather than any other components of nervous tissues are necessary as a substrate for virus growth.[8] In our material this fact is most clearly manifested by an interesting and previously unreported finding,—namely, that there is a complete absence of any signs of virus invasion in those centers of the optic thalamus in which all of the nerve cells have undergone previous retrograde degeneration and have disappeared following destruction of their axonic terminals in the cerebral cortex. Many instances of this phenomenon have been observed, even in those thalamic centers which always contain lesions in paralytic stages following intracerebral inoculation.

The lesions produced by the virus of poliomyelitis in the CNS of the Rhesus monkey are found principally along 2 preferential systems of neuronal pathways. This is best demonstrated as a rule

[6] Walker, A. E., *The Primate Thalamus*, 1938, University of Chicago Press, Chicago.

[7] Brodie, M., *J. Immunol.*, 1933, **25**, 71.

[8] Hurst, E. W., *J. Path. and Bact.*, 1929, **32**, 457.

only in preparalytic and in early paralytic stages, in which the sharpness of localization of lesions along these pathways is especially evident. Later, the elementary pattern of progression of the virus may be obscured by its spread into secondary or tertiary pathways leading from the principal ones. The pre-paralytic intranasally inoculated animals, following the onset of fever, show the most important preferential pathway of virus passage in the CNS. This is the pathway from olfactory bulb to the spinal cord by way of the anterior perforate substance (tuberculum olfactorium), the preoptic area, the hypothalamus and the descending pathways from these centers. These appear to be the olfacto-tegmental and hypothalamo-tegmental tracts, and the periventricular bundle of Schütz, which connect with the midbrain tegmentum and the reticular formation of the hindbrain. From the latter regions virus passes to the motor centers of the medulla and spinal cord, apparently by way of many short connections.

After intranasal instillation of virus, in the late preparalytic stage, lesions not on the primary pathways are found chiefly in the parolfactory regions and in the amygdaloid nuclei, due to spread of virus from the olfactory bulb and the tuberculum olfactorium, and also in the midline gray of the dorsal thalamus. The virus appears to reach the latter by passage along the periventricular fiber system, which connects the hypothalamus with the midline centers of the thalamus. There is rarely seen any spread of lesions from the midline thalamic centers to the more laterally placed sensory portions of the thalamus, and then only in late paralytic stages. The anterior thalamic nuclei are occasionally the site of inflammatory infiltrations, apparently due to spread of virus from the midline thalamic centers, since the mammillary bodies which also connect with the anterior thalamic nuclei are, with rare exceptions, conspicuously free of lesions. There is no evidence in our material that the principal path of virus spread to the spinal cord in these intranasal cases is the spinothalamic tract, as suggested by Faber,[9] since lesions are not found in the somatic sensory (ventrolateral) portions of the optic thalamus until paralysis is extreme, and then only when lesions are also present in the cortex around the central sulcus. Occasionally in cases of extreme paralysis, lesions in the thalamus may be present only in the midline centers, which have no direct connections with lower levels of the CNS. It is thus apparent that subinoculation tests made with material from unspecified portions of the thalamus

[9] Faber, H. K., *Medicine*, 1933, **12**, 83.

can not be critical in the analysis of virus propagation to or from the thalamus.

The second important preferential pathway is that from the motor cortex to the medulla and spinal cord. Along this pathway lesions are found not only after intracerebral inoculation into any part of the cortex, but also, in late paralytic stages, after inoculation by any other portal, including the intranasal. In fact, unlike other parts of the cerebral cortex, the motor cortex (areas 4 and 6) appears to be a favorable site for virus growth. In prodromal stages after intracerebral inoculation, the distribution of lesions suggests that the pathway from the motor cortex to lower centers is by way of the globus pallidus, the zona incerta, the field of Forel, the substantia nigra, and the midbrain tegmentum, rather than by way of the more direct cortical-spinal route. Although the latter is not excluded as a possible route of dissemination of the virus, it is by no means necessary for virus spread from motor cortex to the opposite side of the spinal cord, since the pattern of crossed initial paralysis does not appear to be materially affected by section of the appropriate bulbar pyramid.[10] The chief pathways of virus spread from the subthalamic and midbrain regions, as evidenced by the distribution of lesions, are apparently the same as those involved after intranasal instillation.

In summary, the sequence of events appears to be as follows: In the preparalytic stage, after the onset of fever, the pattern of propagation resulting from inoculation by different portals may be recognizably different, with sharp localization of lesions along certain neuronal pathways. By the time paralysis has set in, regardless of the portal of entry, the virus has reached the preferential pathways from the olfactory centers to the spinal cord, and from the motor cortex to the spinal cord, and has passed both rostrally and caudally along these pathways to the centers involved in a typical intranasal or intracortical inoculation. In late paralytic stages the primary pathways of transmission of the virus are obscured by the fact that evidences of virus invasion are found in centers other than those along the preferential systems of nerve cells and fibers. This indicates a spread, after the initial invasion, from the more susceptible primary pathways, to less susceptible centers. Finally, unless recovery occurs, an overwhelming spread of the virus occurs from the centers primarily and secondarily involved, so that even some cen-

[10] Howe, H. A., and Ecke, R. S., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 123; also Howe, H. A., and Ecke, R. S., unpublished experiments.

ters which are rarely or never affected in the early stages (that is, centers with high immunity) are reached by the virus and succumb to it. Thus, the final pathological picture in all cases is similar, as apparently the susceptibility to virus of various parts of the CNS is independent of the portal of entry (see also Pette, Demme, and Környey[11]). However, even terminally it is possible to observe in certain cases differences in the distribution of lesions, depending on the portal of entry. For example, if the virus is inoculated by some other portal than the intranasal one, signs of virus invasion in the late paralytic stages may occasionally be observed as far forward in the olfactory system as the tuberculum olfactorium, but, in contrast with intranasal cases, apparently never in the olfactory bulbs.[12]

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The Pia-Arachnoid as a Barrier in Experimental Poliomyelitis.*

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The occasional demonstration of poliomyelitis virus in the cerebrospinal fluid of experimental animals has given rise to a series of experiments and speculations regarding the rôle of this body liquid in the dissemination of the disease through the central nervous system. Such investigations as those of Clark and Amoss[1] and Hurst[2] who produced experimental poliomyelitis regularly by intracisternal and intrathecal inoculation have led to the assumption recently made articulate by Schaeffer and Muckenfuss[3] that intracerebral inocula may be effective at sites far distant from the point of introduction. In previous observations the permeability of the ependyma and the possibility of injury to the pia-arachnoid are factors which have not been properly controlled. The following experiments indicate that under ordinary conditions the leptomeninges

11 Pette, H., Demme, H., and Környey, St., *Deutsche Z. f. Nervenh.*, 1932, **128**, 125-252,

12 Sabin, A. B., and Olitsky, P. K., *J. Am. Med. Assn.*, 1937, **108**, 21.

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1 Clark, P. F., and Amoss, H. L., *J. Exp. Med.*, 1914, **19**, 217.

2 Hurst, E. W., *J. Path. and Bact.*, 1932, **35**, 41.

3 Schaeffer, M., and Muckenfuss, R. S., *Am. J. Path.*, 1938, **14**, 227.