INFLUENCE OF HOST FACTORS ON NEUROINVASIVENESS 
OF VESICULAR STOMATITIS VIRUS 

III. EFFECT OF AGE AND PATHWAY OF INFECTION ON THE 
CHARACTER AND LOCALIZATION OF LESIONS IN THE 
CENTRAL NERVOUS SYSTEM 

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PLATES 6 TO 9 

(Received for publication, October 16, 1937) 

The experimental data of the preceding investigations (1, 2) suggested that the failure of older animals, which readily succumb after intracerebral injection of the virus, to exhibit any clinical manifestations of central nervous system (CNS) involvement after peripheral inoculations was possibly due to the presence of certain barriers to the invasiveness of the virus, encountered at the site of inoculation when the intramuscular or intraocular routes were employed and within the CNS when the virus was given by way of the nose. The hypothesis of localized barriers developing with increasing age of the host depends to a great extent on evidence that the distribution or movement of this virus in the nervous system of mice occurs by different mechanisms after peripheral and intracerebral inoculations. The purpose of the present investigation was to determine whether a detailed study of the localization of lesions in the nervous systems of susceptible and resistant mice after different modes of inoculation could throw additional light on the movement of the virus and thus on the nature of the resistance of older animals.

If the movement of vesicular stomatitis virus in the nervous system of mice occurs along the pathways suggested by the preceding experimental studies, one would expect that the distribution of the lesions would vary with intracerebral and peripheral inoculation, and that after peripheral injection they would also vary with the central connections of the nerves along which the virus gained
entrance. The successful demonstration of such specific variations in the localization of CNS lesions could add not only to the evidence for the axonal and trans-synaptic movement of this virus but also to the hypothesis of localized barriers along such a path. If, on the other hand, the distribution of the lesions should prove to bear no relationship to the route of inoculation or not to depend upon the central connections of the neurons supplying the sites of peripheral injection, then the above concepts would become untenable.

Histopathological studies have been successfully employed by a number of investigators (Goodpasture and Teague (3); Marinesco and Draganesco (4); Pette (5); Hurst (6); Sabin and Hurst (7)) to show that with certain viruses the primary lesions in the CNS are determined by the peripheral nerve supply of the inoculated area. The relation of secondary and subsequent localizations of lesions in the CNS to the known central connections of the site of primary attack has been studied but little. Seifried and Spatz (8) recently stated that the spread of the viruses of poliomyelitis, "epidemic encephalitis," rabies, and Borna's disease within the CNS was by way of the spinal fluid. Fairbrother and Hurst (9) and Hurst (10) believed from their studies with the virus of poliomyelitis that both after intracerebral and intrasciatic inoculation the localization of secondary lesions within the CNS was determined by the tract connections of the site of primary attack. With equine encephalomyelitis, however, Hurst (11) stated that in the guinea pig "lesions were substantially the same whether the virus was introduced intracerebrally, intramuscularly, subcutaneously or intradermally," and in a subsequent communication (12), that the localization within the CNS was frequently essentially the same after intranasal as after intramuscular inoculation.

Methods

The mouse is particularly suited for this type of study because (a) its size readily allows sectioning of almost the entire CNS, and (b) in an investigation such as this it is important to determine not only that lesions are present in certain definite regions but also that they are absent in others.

In the beginning most of the work was done with brains taken out of the cranial cavity and fixed in Zenker's fluid containing 5 per cent glacial acetic acid. Later on, it was found that satisfactory fixation occurred through the bone, so that the entire head minus the skin and lower jaw was fixed in the Zenker-acetic mixture for 24 hours. The acid caused sufficient decalcification to permit the sectioning of all the structures, including the nasal mucosa, eyes, and cranial ganglia, in situ. In a few instances sections were cut parasagittally, extending from the nose to the medulla. As a rule, however, the entire skull was embedded (paraffin) with the anterior end down, the sections being cut serially in the frontal transverse plane, at a thickness of 5 to 6μ. Ribbons of four to six sections out of each twenty to thirty were taken for staining. The entire spinal column was fixed in the same manner so that sections at various levels showed not only the cord, roots, and
spinal ganglia but frequently also the sympathetic ganglia. The sections were stained with phloxine and methylene blue, eosin and methylene blue, or hematoxylin and eosin.

**Remarks on Spontaneous Encephalitic Lesions in Mice**

Pathological investigations on the CNS of rabbits were often confusing until the occurrence of spontaneous, asymptomatic, encephalitic lesions generally referred to as being caused by *Encephalitozoon cuniculi* was recognized. That similar difficulties complicate studies on the CNS of mice has not as yet been sufficiently appreciated. Cowdry and Nicholson (13) found meningoencephalitic lesions in the brains of twenty-five of 132 mice studied, and in five of the twenty-five positive cases they noted the coexistence of protozoan-like parasites. Smadel and Moore (14), in their report on the pathology produced by the virus of St. Louis encephalitis in mice, also noted the presence in some of their animals of these spontaneous encephalitic lesions. In the present investigation it was observed that the occurrence of spontaneous meningoencephalitic changes in the Rockefeller Institute mice was distinctly related to the age of the animal; while none was detected in about fifty young mice (less than 1 month old), practically all the old animals (about 9 to 12 months of age) showed these lesions when many sections of the same brain were examined. The pathology consisted of dense perivascular cuffs of small, round cells, meningeal infiltration with similar cells, and small glial and occasionally granulomatous foci in the brain substance. These lesions could be found scattered irregularly throughout the CNS from the tips of the olfactory bulbs all the way down to the sacral cord. Sometimes, however, many sections of the same brain had to be examined before one or the other of these changes was found. Although no protozoan-like parasites were observed in any of the present sections, one cannot exclude the possibility of their having been in the brains at some previous time. In view of the fact, however, that the spontaneous virus encephalomyelitis described by Theiler (15) is endemic in the Rockefeller Institute stock (1), one must consider the possibility that some of these chronic meningoencephalitic reactions (particularly those which do not show granulomatous foci) may, perhaps, represent the residual lesions of a subclinical attack with this virus.

**General Aspects of Vesicular Stomatitis Virus Lesions**

In the CNS, primary necrosis of nerve cells is the outstanding lesion. The process is acute and the reaction to it consists chiefly of an invasion of leucocytes into the necrotic zones. Perivascular cuffing is either absent or extremely rare and inconspicuous; only occasionally does one find a vessel surrounded by a single layer of polymorphonuclear and mononuclear cells. There is no definite evidence

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1 In the past 2 years the incidence of the apparent form of this type of encephalomyelitis was found to be 1 or 2 per 1000.
of a direct attack of the virus on the meninges after intracerebral or peripheral inoculation. There is, however, some infiltration of the meninges with mononuclear and polymorphonuclear cells, representing a response to injury of the nervous tissue, although the distribution of the exudate bears no particular relationship to the presence or absence of cerebral changes in a particular region. There is no difficulty in distinguishing the rather mild, acute, inflammatory reaction in the virus lesions from the diffusely scattered, chronic, and intense perivascular and meningeal infiltration encountered in the spontaneous cases. Small, round, acidophilic, intranuclear "inclusions" have been described as occurring in nerve cells affected by vesicular stomatitis virus (16). In the present study these were found so rarely as to be of no practical aid in mapping out sites affected by the virus. A more frequent change preceding the complete disintegration of the cell consists of a marked increase in the nuclear acidophilic material which shrinks from the membrane, giving rise to a clear halo. The nucleolus, however, remains in situ, and there is no margination of the basophilic material. This picture resembles nerve cell changes in mice injected with yellow fever virus (17), and with other viruses in nerve cells of other hosts, but does not appear to us to have the characteristics of a specific inclusion body.

Pathology after Intracerebral Injection

Young Mice.—The CNS of three mice, 15, 21, and 30 days of age, were examined. Mice of this age die within less than 48 hours after intracerebral injection of 100 or more minimal infective doses. When many sections are made the site of inoculation can be recognized as a tract of necrotic cells extending through the neopallial cortex and into the underlying diencephalon or mesencephalon. The lesion is sharply demarcated and appears to correspond roughly to the size of the injecting needle. From the site of inoculation, lesions are found to extend anteriorly and posteriorly in close relation to the ventricular system and its extension anteriorly, the rhinocerele, and posteriorly along the ependyma of the central canal.

The brain of the 15 day old mouse showed no changes anterior to the lateral ventricles. In the other brains, the olfactory bulbs exhibited extensive necrosis, most marked in and about the rhinocereles and involving the cells of the internal granular and mitral layers. The outer layers of the bulbs (external granular, glomerular, and layer of nerve fibers) appeared generally well preserved, suggesting that the spread of the lesions was from the rhinocerele outwards. Longitudinal and partial transverse serial sections indicated that the lesion was a continuous one, extending from the lateral ventricles to the tips of the olfactory bulbs. At the level of the lateral ventricles there was evidence of involvement of the ependymal cells as well as necrosis of a few layers of periventricular nerve cells, more marked ventrally but occurring also at the sides and dorsally in the corpus callosum. Anterior to the optic chiasm there were no other discernible lesions and the meninges showed no evidence of being attacked by the virus or of having permitted it to pass through it to the underlying brain tissue. Beyond the level
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of the optic chiasm the periventricular distribution of the lesions was again apparent. There was necrosis of the cornu Ammonis where it forms the floor and inner sides of the lateral ventricles, as well as of the parts of the neopallial cortex which form the roof and outer sides. These changes did not extend for any appreciable distance beyond the ventricular walls. With the exception of a few necrotic cells and some polymorphonuclear leucocytes in the habenular nuclei, there was little involvement of the tissues surrounding the third ventricle. In one case (15 day old mouse) there was a considerable invasion of tissues around the third ventricle with polymorphonuclear leucocytes but without any evidence of cellular necrosis. Again with the exception of a small area of necrosis in the tectum of one brain, apparently forming a part of the needle tract, there were no significant lesions in the midbrain.

The meningeal reaction was slight in two instances, consisting chiefly of the infiltration of polymorphonuclear and a few mononuclear leucocytes, and rather marked in the third which exhibited an exudate containing a great deal of fibrin and polymorphonuclear leucocytes, particularly around the midbrain and in the region of the dorsal portion of the third ventricle. After intensive study this severe reaction appeared to be due neither to an attack of the virus on the meninges themselves nor on the underlying brain tissue, but rather to the trauma of inoculation.

Continuing posteriorly, one found an occasional necrotic focus in relation to the fourth ventricle, and definite involvement of the ependyma of the central canal and the contiguous nerve cells. Strangely enough the lesion was very slight in the cervical and thoracic spinal cord, but quite extensive in its lumbar portion.

No lesions were found in any of the following structures connected with the CNS: the sensory ganglia of the cranial nerves, the spinal ganglia, the submaxillary, ciliary, otic, and superior cervical sympathetic ganglia, the hypophysis, pineal body, retina, and nasal mucosa.

In summary it can be stated that after intracerebral injection of vesicular stomatitis virus in young mice the recognizable CNS lesions present shortly before death of the animal are situated along the ventricular system and its extensions in the brain, and along the central canal of the spinal cord. The indications are that the primary spread of the virus occurred along this open pathway. There is no evidence that the meninges are attacked by the virus (not even at the site of inoculation), nor that virus spreads along the subarachnoid space.

Old Mice.—It has already been indicated that old mice (about 8 months to 1 year of age) and young mice are equally susceptible to intracerebral inoculation of vesicular stomatitis virus in the sense that the minimal infective dose is the same
for both, but that the old mice develop signs and succumb a day or two later than
the young ones. The brains of two intracerebrally injected, 1 year old mice,
sacrificed when prostrate and near death, were studied. The CNS of the mouse
sacrificed 4 days after inoculation showed no lesions which could be attributed to
the effect of the virus. The changes present corresponded entirely to those of
“spontaneous encephalitis” encountered in uninoculated old mice. The CNS
of the mouse sacrificed 3 days after inoculation also showed the lesions of spon-
taneous encephalitis with several granulomatous nodules in one olfactory bulb
and widespread lymphocytic perivascular and meningeal infiltration, but there
were, in addition, two slight and very sharply limited lesions of the type encoun-
tered in the young mice. One of these was in one of the olfactory bulbs and con-
sisted of an area of acutely necrotic nerve cells infiltrated with polymorphonuclear
cells, extending from the rhinencephalon to and including a few cells of the mitral
layer. Sections of the same olfactory bulb just anterior or posterior to this area
appeared entirely normal. The other zone was ventral and lateral to the fourth
ventricle and consisted of a few necrotic nerve cells and a few polymorphonuclear
cells.

It appears from these data that the nerve cells of the old mice are
generally more resistant to necrosis than are those of the young
animals, in spite of the fact that sufficient multiplication of virus
and change occur in these cells to give rise to signs of encephalitis
and death. This relative absence of significant lesions is even more
remarkable since the infective process lasts twice as long in the
old as in the young mice.

**Pathology after Nasal Instillation of Virus**

*Young Mice.*—Nine mice (seven 15 days and two 21 days old) were studied.
Mice of this age succumb 4 or 5 days after nasal instillation of virus. Two 15
day old animals were sacrificed 2 days after instillation when they still appeared
entirely well. The sections through the nasal mucosa did not permit any definite
conclusion about the state of the cells in the olfactory or respiratory mucosa.
There was, however, neither necrosis nor inflammation. The olfactory nerve roots
in the mucosa and at their junction with the olfactory bulbs showed no inflam-
matory or other visible change. The meninges over the olfactory bulbs and the
rest of the brain appeared entirely normal, as did the nerve tissue itself, with
the possible exception of some of the mitral cells in the olfactory bulbs. These cells
showed what may perhaps represent the early changes of virus action.

The material from one of the remaining seven mice was obtained immediately
after death, while the others were sacrificed either when they showed pronounced
nervous signs or when prostrate and near death. While there was a certain
amount of individual variation in the extent and location of lesions, their dis-
TABLE I

Distribution of Necrotic Lesions in Various Regions of the Central Nervous Systems of Mice Succumbing after Nasal Instillation of Vesicular Stomatitis Virus

<table>
<thead>
<tr>
<th>Region</th>
<th>Age of mice and remarks</th>
<th>15 days old</th>
<th>21 days old</th>
<th>1 yr. old</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dead 4½ days (no circling)</td>
<td>Sacrificed 4th day (circled left)</td>
<td>Sacrificed 5th day (no circling)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory mucosa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Olfactory mucosa</td>
<td></td>
<td>++</td>
<td>++++</td>
<td>n.s.</td>
</tr>
<tr>
<td>Lateral olfactory gyrus</td>
<td></td>
<td>+++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Septum (ventromedial aspect)</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Tuberculum olfactorium</td>
<td></td>
<td>++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Priform lobe (including amygdaloid complex)</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Corn Ammonia</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hypothalamus (tuber cinereum)</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Thalamus</td>
<td></td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Mamillary body</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>habenular nuclei</td>
<td></td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Lateral and medial geniculate bodies</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Superior and inferior colliculi</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tegmental nuclei, anterior</td>
<td></td>
<td>± (red nucleus)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gudenh's and other posterior tegmental nuclei</td>
<td></td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Nuclei of pons</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Neopallial cortex</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Corpus striatum</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Sensory nucleus of fifth nerve</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Motor nucleus of fifth nerve and reticular formation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vestibular nucleus of eighth nerve</td>
<td></td>
<td>-</td>
<td>++ (left side)</td>
<td>-</td>
</tr>
<tr>
<td>Delters' and Bechterew's nuclei</td>
<td></td>
<td>-</td>
<td>++ (left side)</td>
<td>-</td>
</tr>
<tr>
<td>Cerebellum—deep nuclei</td>
<td></td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>—rest of medulla and cortex</td>
<td></td>
<td>-</td>
<td>n.s.</td>
<td>-</td>
</tr>
<tr>
<td>Spinal cord</td>
<td></td>
<td>-</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Gasserian ganglia</td>
<td></td>
<td>-</td>
<td>-</td>
<td>n.s.</td>
</tr>
<tr>
<td>Superior cervical sympathetic ganglia</td>
<td></td>
<td>-</td>
<td>-</td>
<td>n.s.</td>
</tr>
<tr>
<td>Submaxillary ganglia</td>
<td></td>
<td>-</td>
<td>-</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

n.s. = no sections.
± = necrosis of occasional cell, found with difficulty.
+ = small necrotic focus present in only a few of a series of sections from the same region.
++ = limited zone of necrosis present in most sections of series from same region.
+++ = extensive necrosis seen in all sections of region.
++++ = almost complete necrosis of region.
- = no evident neuronal lesion.
distribution corresponded to so definite a plan that they can all be described together. Some idea of the variations can be obtained from Table I, in which the presence or absence of lesions in certain regions of the CNS of five young mice is indicated.

The olfactory mucosa constitutes the greater portion of the nasal membrane of mice and it appeared to be primarily attacked by the virus. The lesion manifested itself as a necrosis of scattered patches of olfactory mucosa with little or no inflammatory response. At this stage many areas were denuded of cells, the necrotic debris lying free in the nasal cavities. No definite evidence could be found of involvement of the respiratory mucosa, although the occasional presence of suspicious intranuclear acidophilic dots was somewhat confusing. No lesion of any kind was found in the roots of the olfactory nerve in the mucosa, nor as a matter of fact in the outer layer of the olfactory bulbs consisting of the lamina fibrorum nervi olfactorii. The chief lesion in the olfactory bulbs was in the mitral cell layer, where a varying number or practically all the cells may be necrotic. Frequently there were only empty spaces left where the mitral cells had been present originally. Depending upon the severity of the process, there was necrosis of the cells in the internal granular and gelatinous layers, and only rarely in the cells of the external granular layer surrounding the glomeruli which, as a rule, were entirely spared. The distribution of lesions in these olfactory bulb sections was quite different from that seen after intracerebral injection. After nasal instillation of virus the mitral cells appeared to be the center of the lesions and, even when practically the entire bulb was necrotic, the rhinocoele remained undamaged, while after intracerebral injection it was clear that it was from this structure that the lesion extended.

Beyond the olfactory bulbs where the neopallial cortex joins the rhinencephalon, the former appeared entirely normal and in the latter there was usually a zone of varying extent which showed no lesions until the region of the tuberculum olfactorium and anterior perforated space was reached. Here there was a varying amount of necrosis in every case, while the lateral olfactory gyrus showed definite necrosis in only one and involvement of a very small patch of cells in two others; the septal region was involved in only one case. There were no lesions whatever around the rhinocoele or the lateral ventricles, nor was there necrosis of tissue adjacent to the meninges. The lesions just described were practically always separated from either the ventricles or the meninges by normal appearing tissue.

Posterior to the optic chiasm the further distribution of necrotic foci appeared to depend upon the position of the lesions anterior to it. Thus, the cornu Ammonis was unaffected in all but one instance, and in that instance there was also necrosis in the ventromedial aspect of the "septum." Again, in only one case was there necrosis in the piriform lobes, and then it was unilateral and on the same side as extensive involvement of the lateral olfactory gyri. The tuber cinereum of the hypothalamus was affected in all mice and some slight necrosis could always be found in the mammillary body. The habenular nuclei showed foci of necrosis in two mice. No lesions were found in the thalamus or other diencephalic structures. The tectum of the midbrain was negative in all cases, as was the anterior
portion of the tegmentum, with the possible exception of a few necrotic cells in one red nucleus of one brain. The interpeduncular nucleus showed necrosis in two cases and it is to be noted that it occurred only in the brains with involvement of the habenular nuclei. There were always, however, lesions in the posterior part of the tegmentum in the region of Gudden's nucleus and to a varying extent ventrally in the nuclei of the raphé and occasionally laterally, involving the mesencephalic nucleus of the fifth nerve. The nuclei of the pons exhibited no lesions. In the medulla there was necrosis in one or both motor nuclei of the fifth nerve and in varying regions of the formatio reticularis in all but one case. There was never any evidence of involvement of the sensory nucleus of the fifth nerve. In two instances which corresponded with circling as the outstanding clinical sign, there was unilateral necrosis in the area of the vestibular nucleus of the eighth nerve, and the region of Deiters' and Bechterew's nuclei. In one of these brains, the nuclei of the cerebellum showed necrosis, while in all other instances the cerebellum appeared normal. The neopallial cortex was never involved. The spinal cord was examined in three cases and, with the exception of a few polymorphonuclear and necrotic nerve cells, there were no significant changes.

Special attention was paid to the nuclei of the other nerves supplying the nasal mucosa but no lesions were found in the Gasserian (sensory fifth), submaxillary ("parasympathetic"), or superior cervical (sympathetic) ganglia. It may also be stated that lesions were seen neither in the other sensory or autonomic cranial ganglia nor in the spinal ganglia. The lungs, liver, spleen, kidneys, and suprarenals of mice sacrificed on the 2nd and 4th days of the disease showed no changes attributable to the action of the virus.

Relation between Central Connections of Nerve Supply of Nasal Mucosa and Distribution of Lesions.—From the foregoing description, it is evident that of the various nerves connected with the nasal mucosa (sensory fifth, sympathetic and parasympathetic fibers, and olfactory nerves) only the olfactory pathway showed signs of having been traversed by the virus. The choice of this special pathway by vesicular stomatitis virus cannot be regarded merely as a natural consequence of special anatomical relations (the large number of exposed olfactory neurons and possibly other direct connections with the CNS), since, as will be shown in another communication, not all viruses given intranasally to mice of the same breed and age invade the CNS along the olfactory nerves, but select instead the other nerves of the nasal mucosa. The use of the olfactory pathway should, perhaps, be regarded, therefore, as the result of some special affinity of vesicular stomatitis

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2 With regard to the involvement of the various regions in the midbrain, pons, and medulla reference may be made to Wallenberg's studies on the ramifications of the basal olfactory tract or bundle in the rabbit. (Wallenberg, A., Anat. Anz., 1901-02, 20, 175.)

3 This does not include the small nervus terminalis about which relatively little is known and which was not readily distinguished in our sections.
vesicular stomatitis virus. III

Virus for the olfactory neurons in the nasal mucosa. These neurons are in synaptic relation with the mitral cells in the olfactory bulbs, the synaptic junction occurring not directly on the bodies of these neurons but rather at some distance on the dendrites entering the glomeruli. It is significant, therefore, and perhaps indicative of the mode of virus progression that one can find necrosis of the olfactory mucosa with no visible change in the olfactory nerve fibers, glomeruli, and the susceptible cells of the external granular layer which surround them, and yet see almost complete destruction of the mitral cells beyond. In an advanced lesion there is, of course, necrosis of granular cells (they are connected with the mitral cells), particularly of the internal layer, but even then most of the glomeruli and the surrounding cells remain intact.

The axons of the mitral cells grouped in the lateral, intermediate, and medial olfactory striae are said to terminate, either directly or after preliminary synapse in the anterior olfactory nucleus, chiefly among the neurons of the lateral olfactory gyrus, tuberculum olfactorium (region of anterior perforated substance), and questionably in the septum, and it is in these areas also that the further localization of lesions occurs. It is noteworthy that the olfactory bulb lesions are not continuous with those further posteriorly, i.e., that there is between them, almost invariably, an apparently undamaged zone. An analysis of the subsequent localization of lesions (Table II) indicates that the regions involved depend upon the major central connections of olfactory areas which have been previously affected. Thus, in accord with the constant involvement of the intermediate olfactory nucleus (anterior perforated substance, tuberculum olfactorium), one finds always affected the hypothalamus (tuber cinereum and mammillary body), and tegmental nuclei (constituting one of the descending olfactory correlation pathways). Occasionally the pathway through the habenular nuclei and the interpeduncular nucleus is used. In view of the fact that the neurons in the interpeduncular nucleus participating in the olfactory relay are in relation proximally only with cells in the habenular nuclei, it should be noted that when lesions are observed in the interpeduncular nucleus they are invariably also found in the habenular, even though they may be so limited as to require many sections to disclose them. The cornu Ammonis was involved but once and that occurred in a brain which showed necrosis in the ventromedial, septal region anterior to the optic chiasm. The piriform lobes also showed necrosis in only one case and that in association with a lesion of the lateral olfactory gyrus on the same side. Why the tuberculum olfactorium or anterior perforated substance should be so constantly involved, while the lateral olfactory gyrus, piriform, and septal regions

4 Any simple or short description of the central connections of the olfactory neurons of the second order is necessarily incomplete. The termination of such fibers in the septum of higher mammals is especially open to question. The occasional presence of necrosis in the ventromedial aspect of the septum (parolfactory area, paraterminal body) (see Fig. 6) in some of these mice should, therefore, be interpreted with that in mind.
The purpose of this scheme is to show how the localization of lesions deep within the CNS occurred in accord with the involvement of certain definite, intermediate zones. Thus there were no lesions in the interpeduncular nucleus without involvement of the habenular nuclei and tuberculum olfactorium; none in Ammon's horn without damage to the septum, etc.
are only occasionally affected, is not clear. According to the view of axonal and trans-synaptic progression of the virus, it could perhaps be accounted for if the axons of the greater number of mitral cells were included in it, or if the greater number or the more suitable synaptic connections were in this relay. The diencephalic and tegmental mesencephalic structures which are most frequently affected are the ones which are chiefly concerned in the correlation of olfactory with other impulses and in the function of relay stations on a reflex pathway to the motor and perhaps other nuclei of the hind-brain.

Findings in Old Mice Showing No Clinical Signs of Disease.—Nine mice (eight were approximately 1 year old and one was 31 days old) were studied at various intervals after nasal instillation of virus. Two were sacrificed on the 2nd day, two on the 4th, and one on the 5th, 6th, 7th, 10th, and 14th days, none showing any signs of disease. In none of these animals did the CNS show any changes which could be attributed to the effect of the virus. No definite effect ascribable to the action of the virus on the nasal mucosa was demonstrated. In some of the mice the presence of large numbers of pus cells in the sinuses, tissues, and nasal cavities was perplexing until the same picture was observed in two “normal” old mice which received no virus. No definite conclusion could be reached about the significance of occasional intranuclear, acidophilic bodies.

The absence of obvious lesions in these mice, in spite of the fact that virus can be demonstrated in the rhinencephalon anterior to the optic chiasm between the 2nd and 5th days, is not surprising in view of the fact that they may be practically absent in old mice succumbing with encephalitis after intracerebral injection of the virus.

Findings in Old Mice Showing Signs of Encephalitis or Myelitis.—A varying number of old mice are not resistant to nasal instillation of the virus and develop, after a relatively prolonged incubation period of 8 to 14 days, either signs of encephalitis or merely flaccid paralysis of the posterior extremities. The CNS of two such mice were studied, and the extensive lesions which they exhibited were in marked contrast to the findings in the intracerebrally injected animals. One was sacrificed on the 8th day, when it showed signs of encephalitis for the first time. Lesions quite as extensive as those found in the young mice were present here and in the same regions (see Table I). It should be noted that these extensive lesions clearly due to virus action were present side by side with those of spontaneous encephalitis. The other mouse studied histologically was the only one of a group of eleven 1 year old, similarly instilled mice to develop nervous signs. These appeared in the form of flaccid paralysis of the posterior extremities on the 8th day after nasal instillation, and the mouse was sacrificed 9 days after the onset of paralysis. The striking fact here was that while there was rather marked destruction and neuronophagia in both lateral regions of the anterior horns of the lumbar cord, there was no evident involvement of either the ependyma
and periependymal tissue, the posterior horns of this level, or of any part of the
cervical, thoracic, and sacral levels. While insufficient sections of the brain
were available to determine the exact tracts by which the cord might have been
reached, clearly defined foci of necrosis were found in the hypothalamus, thalamus,
and habenular nuclei. It is noteworthy that the spinal ganglia, even of the
lumbar level, showed no lesions, and also that there was neither meningeal nor
perivascular reaction in the cord.

Pathology after Intraocular Injection

Young Mice.—15 day old mice uniformly succumb after intraocular (vitreous)
injection of the virus and are almost as susceptible to inoculation by this route as
by the intracerebral. The incubation period is considerably prolonged in 21 day
old mice and many of them are completely resistant, even to the highest concen-
tration of virus, while 1 year old animals, with only few exceptions, exhibit no
clinical signs of disease whatever. It should also be recalled that while in sus-
ceptible mice, 21 days of age or older, it was possible to show by subinoculation
methods that the primary spread of the virus was probably along the optic nerve
with an early localization in the contralateral diencephalon and mesencephalon,
no conclusion could be drawn from the experiments with the 15 day old animals
because the virus, when first demonstrated in the CNS, was already widely scat-
tered (2). One of the questions, therefore, was whether the marked and uniform
susceptibility of the 15 day old mice is due to a mode of virus progression different
from that which obtains among the older animals, or whether the same pathways
are pursued in all, the rate of progression being so much greater in the 15 day old
mice that the entire nervous system is rapidly invaded by varying amounts of
virus.

The pathology after intraocular injection was therefore studied chiefly in 15
day old mice. It was early observed that the CNS lesions in these animals could
apparently be accounted for on the basis of primary virus progression only along
the decussating optic nerve pathway. In each of three mice, for example, whose
right eye was inoculated marked necrotic lesions were present in the left superior
colliculus, while the right one appeared entirely uninvolved. To eliminate the
possibility that the left superior colliculus may be particularly susceptible to the
action of this virus and to establish more convincingly that the decussating path-
way is used, the brains of two 15 day old mice injected into the left eye were
studied. In these two animals the right superior colliculi showed marked necrosis,
while the left ones remained uninvolved, thus indicating rather conclusively that
in 15 day old mice the virus follows the decussating pathway suggested by the
experimental observations on older animals.

A detailed tabulation of the localization of lesions in various regions of the CNS
of each of the five mice is presented in Table III. Four were sacrificed 3 days
and one 4 days after inoculation at a time when they showed advanced nervous
signs or were prostrate. Sections of the inoculated eyes revealed in each instance
TABLE III

Distribution of Necrotic Lesions in the Central Nervous Systems of 15 Day Old Mice Succumbing after Intraocular (Vitreous) Injection of Virus

<table>
<thead>
<tr>
<th>Region</th>
<th>Eye inoculated and day on which mice were sacrificed</th>
<th>Right eye</th>
<th>Left eye</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3rd</td>
<td>3rd</td>
</tr>
<tr>
<td>Inoculated eye</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retina</td>
<td>Cornes, iris, etc.</td>
<td>+±</td>
<td>++</td>
</tr>
<tr>
<td>Uninoculated eye</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>n.s.</td>
</tr>
<tr>
<td>Optic nerve near chiasm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>Left</td>
<td>+</td>
<td>n.s.</td>
</tr>
<tr>
<td>Left</td>
<td>n.s.</td>
<td>n.s.</td>
<td>+</td>
</tr>
<tr>
<td>Optic chiasm and tract</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right beyond decussation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lateral geniculate body</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>Left</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Medial geniculate bodies</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Superior colliculus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>Left</td>
<td>++++</td>
<td>+++</td>
</tr>
<tr>
<td>Inferior colliculi</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Region of oculomotor and trochlear nuclei</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tegmentum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior (including red nuclei)</td>
<td>?</td>
<td>-</td>
<td>+ (raphé)</td>
</tr>
<tr>
<td>Posterior (to red nuclei)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hypothalamus (contiguous to optic tract)</td>
<td>-</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>Thalamus</td>
<td>+ (left lateral nucleus)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mammillary body</td>
<td>-</td>
<td>-</td>
<td>±</td>
</tr>
<tr>
<td>Habenular nuclei</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Interpeduncular nucleus</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ganglion basale opticum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>Left</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Cornu Ammonis</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Piriiform lobes</td>
<td>-</td>
<td>-</td>
<td>± (left side adjacent to optic tract)</td>
</tr>
<tr>
<td>Septum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>Left</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

See legends of Table I.
a lesion of varying extent in the retina with no evidence of any specific action on any of the other structures. In one mouse (sacrificed on the 4th day) more than one-half of the entire retina was destroyed, leaving not a trace of its original structure or cell outlines. In the other animals the retinae exhibited scattered foci of necrosis involving all the layers to a varying degree. Small numbers of polymorphonuclear and occasional mononuclear leucocytes infiltrated these foci and particularly the outer layers of nerve fibers and ganglion cells. Only a few of the invading cells were found free in the posterior or anterior chambers. The uninjected eyes appeared normal. Sections of eyes removed 4 days after inoculation of broth, or normal mouse brain suspension for control showed only a few polymorphonuclear and mononuclear cells in the posterior and anterior chambers but no changes whatever in the retina. Some inflammatory exudate in the outer coats and muscles of the eye apparently near the site of inoculation was found both after virus and control inoculations.

Examination of the optic nerve of the inoculated eye revealed no change except in the portion near the optic chiasm. This change, consisting of a varying degree of disorganization of the normal structure with necrosis of some of the large interstitial glial cells and infiltration with polymorphonuclear leucocytes, was also found in the middle of the optic chiasm and after the decussation on the side opposite to that of the injected eye. Pathological changes in the optic chiasm beyond the decussation were noted on the left side in mice, which received the virus in the right eye, and on the right in those inoculated in the left eye.
some of the mice the same sort of change continued in the contralateral optic tract beyond the chiasm.

The one other lesion constantly exhibited by all the mice consisted, as already indicated, of extensive necrosis of the contralateral superior colliculus. In one mouse, sacrificed when prostrate on the 3rd day, this was practically the only finding apart from those already mentioned. Of the three mice which received virus in the right eye, two showed no lesions in the lateral geniculate bodies of either side, while in the third one only the left (i.e., contralateral) lateral geniculate body was affected, showing extensive necrosis of both the dorsal and ventral nuclei with practically no infiltrating inflammatory cells. Here were found many neurons which exhibited the early cytoplasmic and nuclear changes which have already been described. In this mouse the same changes were also present in the left lateral nucleus of the thalamus. Of the two mice, in which the inoculation was made in the left eye, one showed distinct, though not as extensive, necrosis of the right lateral geniculate body with no perceptible involvement of the left one, and in the other there was no lesion in either. It was thus clear that the localization of the most obvious lesions depends on the distribution of the greater number of the axons of the retinal ganglion cells.

Two of the five mice exhibited significant unilateral lesions in the olfactory pathway. The chief interest of this involvement lies in the fact that in both cases it was the contralateral olfactory pathway which was affected. In one of these mice, whose right eye was inoculated, the right olfactory pathway appeared entirely normal, while moderately extensive foci of necrosis (not contiguous with one another) were present in the tuberculum olfactorium, lateral olfactory gyrus, and olfactory bulb of the left side. In the other mouse the virus was injected in the left eye, and the left olfactory pathway appeared normal, while the tuberculum olfactorium and olfactory bulb of the right side showed distinct foci of necrosis. It is apparent from this localization that these lesions cannot be explained on the basis of an escape of some of the virus from the conjunctival sac into the nose, for then they should have been on the same side as the inoculated eye. The explanation for the involvement of the contralateral olfactory pathway may, perhaps, be found in the changes spreading from the optic tract, beyond the decussation of the optic chiasm, to the structures with which it is intimately connected by contiguity, i.e., the tuber cinereum, the ganglion basale opticum (an olfactory ganglion), and the piriform lobe. Varying numbers of necrotic cells were found in all these structures in the zones which were in contact with the necrotic portion of the optic chiasm and tract. These changes were least noticeable in the mice sacrificed on the 3rd day, but quite marked in the one killed on the 4th day. It is apparent, however, that the further distribution of lesions from these olfactory centers anteriorly or posteriorly is not by contiguity, but apparently axonal and trans-synaptic from one level or station to the next. The two mice just described also showed necrosis of a small number of cells in the mammillary body and extensive destruction of the ventral portion of the posterior tegmentum (i.e., posterior to the level of the red nuclei).
In none of the four mice sacrificed on the 3rd day were there any perceptible lesions just ventral to the aqueduct of Sylvius (region of nuclei of third and fourth cranial nerves and Edinger-Westphal nucleus), nor in any other part of the anterior tegmentum, (i.e., portion containing red nuclei). The mouse sacrificed on the 4th day (left eye injected) showed marked necrosis in the region of the nuclei of the 3rd and 4th cranial nerves on the right side and almost complete destruction of the anterior and posterior regions of the tegmentum. The pons showed a varying number of necrotic cells in three mice, and the medulla some foci in the reticular substance of two.

No lesions were found in any of the mice in the cornu Ammonis, corpus striatum, neopallium, cerebellum, and spinal cord. It should also be noted that no lesions were seen in the ciliary, Gasserian, submaxillary, and superior cervical sympathetic ganglia.

**Old Mice.**—It has already been shown that in old mice which are resistant to intraocular injection, no virus can be demonstrated in the CNS (2). The chief interest, therefore, was in determining the effect of the virus in the eye, where its progression is apparently held up. The eyes of two old mice sacrificed on the 4th day showed no visible lesions in the retina nor any more cellular infiltration than was found in eyes injected with normal mouse brain suspension for control. The optic nerves and CNS similarly revealed no change.

**Pathology after Intramuscular Injection**

**Young and Old Mice.**—Young mice receiving virus in the muscles of one leg develop flaccid paralysis, first of the inoculated extremity, and succumb with signs of an ascending myelitis. Old mice, on the other hand, exhibit no signs of disease after intramuscular injection of the largest amounts of virus (10^7 M.C.L.D.). In the young mice the virus has been shown to multiply at the site of inoculation and to invade the spinal cord by way of the peripheral nerves, while in old mice neither local multiplication nor invasion of the peripheral nerves or CNS was demonstrable (2). One of the questions which arose during these experiments was whether the local multiplication of virus in the young mice occurred in nervous structures or in the muscle and other non-nervous elements. Another question was whether the intramuscular injection of large amounts of virus in old mice had any effect on the muscle or nervous tissue which might be demonstrable histologically though not by animal passage.

To answer these questions, histological studies were made of the muscles and peripheral nerves of the inoculated legs of young and old mice at various intervals after injection. For control, young and old mice were given similar amounts of normal mouse brain suspension and the above mentioned tissues removed at the same intervals after inoculation. To aid in the localization of the site of inoculation, a small amount of powdered charcoal was added both to the virus and normal brain suspensions. In mice sacrificed on the 2nd day the reaction to the normal mouse brain-charcoal powder mixture consisted of an infiltration of the interstitial connective tissue with polymorphonuclear and mononuclear cells. The
number of the former cells diminished on the 3rd day, and on the 5th day the inflammatory exudate consisted almost entirely of mononuclear cells. The reaction was practically the same in both the young and old mice. There was no evidence of any involvement of the muscle or nerve tissue at the injected site, and sections of the sciatic nerves appeared normal.

In the old mice injected with virus there was no perceptible difference from the reaction to normal tissue just described, while in the young mice, on the other hand, there appeared definite evidence of a direct attack of the virus on the muscle tissue. At 2 days after injection (the period when considerable multiplication of the virus was demonstrated and successful muscle to muscle passage was carried out (2)), there was as yet no evident necrosis of the muscle fibers and no appreciable difference in the inflammatory reaction from that observed in the control animals. On the 3rd day, fragmentation and hyaline-like necrosis of a small number of muscle fibers became apparent and an increase in the inflammatory exudate consisting mostly of polymorphonuclear leucocytes, many of which appeared to be phagocytizing the fragmented muscle fibers. On the 5th day practically the same picture was seen but in an exaggerated form: A large number of muscle fibers was completely necrotic and the phagocytosis of muscle fragments by numerous polymorphonuclear leucocytes (which may perhaps be defined as myophagia) closely resembled the picture of neuronophagia in the nervous system. Hypertrophy and proliferation of the sarcolemmal nuclei formed another prominent feature of the reaction at this time. No inclusions were seen in any of the cells on the 2nd, 3rd, or 5th days and no evidence could be found of a direct attack of the virus on the interstitial connective tissue, blood vessels, or nerve trunks lying in the vicinity of necrotic muscle tissue. Longitudinal sections through the sciatic nerves of the inoculated legs showed no inflammatory reaction at any time nor any other perceptible abnormality on the 2nd and 3rd days; on the 5th day the appearance was suspicious of a fragmentation of a certain number of nerve fibers, but no definite conclusion is possible with the method employed.

These histological findings thus proved to be in accord with the experimental results (2) and further elucidated the nature of local virus multiplication at the site of intramuscular injection in the young mice.

The CNS of two mice (15 days old) injected with virus into the right leg were examined; one on the 4th day, when it exhibited only paralysis of the posterior extremities, and the other on the 5th day immediately after death. In the animal sacrificed on the 4th day the outstanding lesion was found in the lower lumbar cord, where it seemed to be confined to the neurons of the anterior horns. These cells were in various stages of necrosis and early neuronophagia was present. A few of the blood vessels were surrounded by one or two layers of mononuclear and polymorphonuclear cells. The meninges showed no evidence of virus attack. The spinal and sympathetic ganglia at the lumbar levels of the cord having considerable anterior horn cell involvement exhibited neither neuronal necrosis nor inflammatory reaction. It would appear that either the virus entered the cord entirely by the efferent axons of the anterior horn cells distributed to the muscles
which it attacked, or if it also pursued the afferent sensory pathway, the neurons in the spinal ganglia must undergo necrosis much less readily than the motor cells. The thoracic and cervical levels of the spinal cord showed little change with the possible exception of some doubtful intranuclear inclusions in a few neurons. The only other neuronal lesions in the CNS were found in the ventral aspect of the medulla, slightly cephalad to the decussation of the pyramidal tracts, and particularly on the right side in the region of the lateral reticular and possibly of the olivary nuclei. Here there was evidence of early nerve cell necrosis, and some interstitial and perivascular infiltration with polymorphonuclear and mononuclear cells. A small number of these cells could be seen in the meninges of the ventral aspect of the brain as far cephalad as the olfactory bulbs.

In the second animal in which the infection was allowed to go on to a fatal termination, there was complete necrosis of almost all neurons, both in the anterior and posterior horns of the lumbar cord with very little inflammatory reaction. Thus neuronal destruction had already spread to most of the sacral and thoracic portions of the spinal cord, while in the cervical portion necrosis was still in its earlier stages. The only other nervous lesions noted were again in the reticular substance of the medulla, where foci of necrosis and neuronophagia could be found. The spinal ganglia appeared entirely uninvolved.

In summary it may be pointed out that the CNS lesions, after inoculation of the virus into the muscles of one leg, are distinctly different in distribution from those which follow intracerebral, intranasal, or intraocular injection of the virus. Their localization is in accord with a primary, insulated, axonal transmission of the virus, the most evident damage being observed not along the course of the axons but rather at the site of their cell bodies. The absence of lesions in the spinal ganglia, even at death, strongly suggests that the invasion of the virus into the spinal cord may have occurred chiefly along the efferent fibers supplying the affected muscles.

**Correlation between Distribution of Lesions and Presence of Virus in the Central Nervous System**

One is impressed with the fact that many areas in the CNS which were shown to contain virus by animal passage (2) exhibited no evident histological changes. This is particularly apparent among the 15 day old animals which were injected intraocularly. Thus, while animal passage revealed virus among other zones in the homolateral diencephalon and mesencephalon, and in the occipital cortex at an early stage, no lesions were found in these regions in any of the mice. In general, the impression is gained that in the case of vesicular stomatitis, virus multiplication precedes recognizable cytological changes by a day or two and that lesions appear where the virus is delivered first and perhaps in largest amount,
TEXT-FIG. 1. Influence of route of inoculation on CNS pathology in young mice (vesicular stomatitis virus, N.J.). Diagrammatic representation of localization of lesions in CNS of young mice after different routes of inoculation. The outlines of the cross sections of the brain and cord are about 4 times actual size. The lesions found in several mice inoculated by the same route were combined on one series of cross sections.

R, right side; L, left side; a.s., aqueduct of Sylvius; c. am., cornu Ammonis; c.c., corpus callosum; cerv. c., cervical cord; g.b.o., ganglion basale opticum (preoptic nucleus); g.l.d., dorsal nucleus of lateral geniculate body; g.l.v., ventral nucleus of lateral geniculate body; h., habenula; i.c., inferior colliculus; inf., infundibulum; l.c., lumbosacral cord; l.g., lamina glomerulosa of olfactory bulb; l.m., layer of mitral cells; l.o.g., lateral olfactory gyrus; l.p., lobus piriformis; l.th., lateral nucleus of thalamus; m.g.b., medial geniculate body; n.c., neopallial cortex; opt. ch., optic chiasm; rh., rhinencephalon (anterior extension of lateral ventricle); s.c., superior colliculus; s.g., spinal ganglion; th.c., thoracic cord; v.l., lateral ventricle; III. third ventricle; IV, fourth ventricle; i. n., interpeduncular nucleus.
death terminating the disease before the other areas, infected later and perhaps less heavily, have developed demonstrable changes. It does not appear that in young mice certain areas are more resistant to necrosis than others, because by varying the route of inoculation almost all regions of the CNS can be shown to be susceptible. Considering the extensive connections between various regions of the CNS, it is easy to understand how virus can spread almost simultaneously to many remote parts of the brain, but the localization of lesions chiefly in the zones which receive the greatest number of axons from neurons primarily infected is in good agreement with the hypothesis of axonal and trans-synaptic progression of the virus. In intracerebrally injected old animals, on the other hand, animal passage clearly demonstrated that virus had multiplied in widely scattered areas of the CNS within 24 hours after injection (1) and yet in moribund mice sacrificed on the 3rd and 4th days recognizable lesions were either entirely absent or involved only a small number of isolated cells. This observation indicates that at least in some of the older animals the cells have become distinctly more resistant to necrosis resulting from virus multiplication. It is to be recalled, however, that while the cells retain their essential structure and show no other changes recognizable by the methods employed, there is, nevertheless, impairment of their function as evidenced by the clinical signs of encephalitis and death of the animal. Further study may reveal that the resistance of nerve cells of old mice to necrosis may not be limited to vesicular stomatitis virus. In describing the pathology of yellow fever encephalitis in two mice, one 6 days old and one adult, Goodpasture (17) noted that in the young mouse there was a great deal of neuronal necrosis with almost imperceptible inflammatory reaction, while in the adult mouse despite the abundant perivascular and diffuse cellular exudation, neuronic alterations were difficult to find. While the inflammatory reaction in the old animal may have occurred in response to imperceptible virus action on the cells, it resembles very closely the picture of spontaneous encephalitis.

SUMMARY AND DISCUSSION

It will be well to restate the main problem at this point and to examine how far the accumulated data can help to elucidate it. The problem is this: Why are old mice generally resistant to all forms of peripheral inoculation of vesicular stomatitis virus when intracerebral injection is equally fatal for mice of all ages? The results of experiments in which the presence of virus was demonstrated by animal passage suggested that the reason can perhaps be found in (a) the different mechanisms of virus progression after intracerebral and peripheral injection, and (b) the development with age of localized barriers capable of halting the spread of virus (1, 2). The present study sought histological evidence for the nature of virus progression
and for the changes observed in the older animals. The results clearly demonstrate that after intracerebral injection virus spreads along an open system, the lesions being distributed almost entirely in contiguity with the ventricles and their extensions, while after peripheral inoculations the evidence points to progression of the virus in a closed system of neurons and their processes, at least in the stage preceding neuronal necrosis, the distribution of lesions depending upon the central connections of the primary neurons connected with the inoculated site. Thus, in young mice, nasal instillation of the virus was followed by necrosis of a long chain of neurons, starting with those in the olfactory mucosa and progressing through specific zones of the olfactory pathway, pursuing the same order in which the various regions are known to have their major connections with one another. It is important to note that after nasal instillation the apparent lesions were present where the cell bodies of the neurons are situated, and not along the tracts connecting one group of neurons with another, which accounts for the lack of contiguity between the affected zones and the normal appearing, intervening areas. The assumption that the primary progression of the virus in this case occurs in a closed system is based on the absence of lesions in unrelated areas contiguous to those which are necrotic and to the tracts which connect one affected zone with another.

Additional evidence for the assumption that the initial dissemination of peripherally injected virus is in a closed system is found in the decussating optic nerve pathway primarily pursued by the intracocularly injected virus. The progression of the virus along this decussating pathway was indicated in the experimental data obtained on mice 21 days or older, while in younger animals the spread of virus was so rapid and diffuse that the pathways along which it might have occurred remained obscure (2). In the present study, in which 15 day old mice were used, the lesions in the retinal neurons and the constant involvement of only the contralateral superior colliculus left little doubt that the primary spread of the virus, even in these very young animals, must have occurred within the retinal neuron processes (axons) which decussate in the optic chiasm (in the mouse, as in the rat, very few of these go to the homolateral side) and synapse chiefly with the neurons of the contralateral superior colliculus and
also, apparently to a lesser extent, with those of the contralateral external geniculate body, where lesions were also demonstrated. Virus spreading in the optic nerve along the perineural subarachnoid space would be found at the base of the brain at the optic chiasm; virus extending along the interstitial spaces in the optic nerve should involve not only the nuclei of both sides of the optic pathway but also non-optic structures, such as the medial geniculate bodies, posterior colliculi, etc., by means of the commissures of von Gudden and of Meynert, whose fibers course through the chiasm. The highly specific localization observed in the present study is best accounted for by progression along the suggested closed pathway. Hurst (10) observed that poliomyelitis virus, after injection into the left sciatic nerve, may, after invading the lumbar cord, be found first in the contralateral motor cortex or thalamus and he suggested that this was evidence of progression along a decussating pathway and in favor of the axonal hypothesis of virus spread. It was not shown, however, that this particular localization was specifically related to the introduction of virus in the left sciatic nerve, or that it could be reversed by inoculating the sciatic nerve of the opposite side. The hypothesis proposed by Hurst, however, finds support in the present instance for (a) the superior colliculi never showed lesions after intracerebral, intranasal, or intramuscular inoculations, and (b) necrosis was produced in either the right or the left superior colliculus, depending on whether the virus was injected into the left or right eyes.

The localization of lesions after injection of virus into the muscles of one leg indicated that in the young the invasion occurred along the local peripheral nerves, especially the motor fibers (neurons destroyed in the lumbar cord with those in the spinal ganglia intact), after a primary attack on the muscle itself. The only other lesions found at a late stage were in the reticular substance of the medulla, the olfactory portions of the brain appearing entirely normal. In this respect the mechanism of progression of intramuscularly injected vesicular stomatitis virus differs from that of eastern equine encephalomyelitis and pseudorabies viruses similarly injected into mice of the same age and breed: the former (E.E.E.) invades the central nervous system in the majority of instances, by being eliminated on the nasal mucosa and then along the olfactory pathways (18), while the latter
appears to employ chiefly the local sensory fibers, attacking primarily the neurons in the spinal ganglia (unpublished observations).

Because the CNS of old mice remain for the most part susceptible to vesicular stomatitis virus (although definite evidence of resistance to necrosis of the neurons was observed), and because after intracerebral injection the virus has been shown to spread in an open (ventricular) system, it is clear why young and old mice are equally susceptible to inoculation by this route. After peripheral inoculation, however, it has been amply demonstrated by experimental and histological methods that the spread of this virus begins and continues, at least until the cells disintegrate, in a closed system within the neurons and their processes and apparently also across the synapses. The halting of the virus somewhere in the anterior rhinencephalon after nasal instillation in resistant mice (1) would appear to be due to an arrest in an insusceptible neuron or an impenetrable synapse somewhere in the chain, and to the failure of the affected neurons to disintegrate (no lesions were found in the CNS of these mice) and thus to liberate the virus into the open system. After intramuscular injection, on the other hand, the virus encounters a different kind of muscle cell in the old mouse, and its inability to invade the nerves may perhaps be bound up with its demonstrated inability to attack and multiply in these changed muscle cells, although the rôle of a possible alteration in the terminal nerve endings themselves is not yet clear. After intraocular injection, the virus fails to affect visibly the retinal neurons of resistant old mice and the further invasion of the CNS is inhibited. The resistance of old mice to peripheral inoculations of vesicular stomatitis virus thus appears to be the result of (a) changes produced by age not in the whole animal but in certain specific, isolated structures, and (b) the special mode of progression of peripherally injected virus.

It may be of interest to point out two phenomena which may perhaps be related to the one investigated in the present study. Tobacco mosaic virus has been found to produce different types of disease in certain plants of different ages; thus a widespread, systemic necrosis leads to the death of young Nicotiana rustica plants, while in old plants it is possible to produce necrotic foci in many parts of the plant by direct inoculation, although generalization does not
occur from an isolated focus as it does in young specimens (19). In other words, age apparently does not change the whole plant, but it does transform something which allows the virus to spread easily from one site to another. MacNider (20) has observed that dogs which survive a severe type of hepatic injury from uranium, repair this injury with a special type of atypical, epithelial cell and become resistant not only to secondary intoxications by uranium but also by chloroform; he has also found that this change in epithelial cell type may be acquired as a product of senility, and that when it develops it imparts to the liver a degree of resistance to chloroform comparable to that induced by a process of repair following a severe hepatic injury from uranium nitrate.

CONCLUSIONS

The resistance of old mice to peripheral inoculations of vesicular stomatitis virus appears to be the result of (a) changes produced by age not in the whole animal but in certain specific, isolated structures, and (b) the special mode of dissemination of peripherally injected virus.

The equal susceptibility of young and old mice to intracerebral inoculation can be explained by the observation (a) that virus injected in this way spreads primarily in an “open system” (the lesions being distributed almost entirely in contiguity with the ventricles and their extensions), and (b) that in the old animals the central nervous system as a whole, although more resistant to neuronal necrosis, is still lethally affected by the multiplication of virus.

After peripheral inoculations the evidence points to progression of the virus in a closed system of neurons and their processes, at least in the stage preceding neuronal necrosis, the distribution of lesions in the central nervous system depending upon the central connections of the primary neurons connected with the inoculated site. In old mice the inability of the virus to involve the terminal processes or cell bodies of the neurons at the site of inoculation (as after intramuscular or intraocular injections) or to spread to other neurons along the chain in the central nervous system (as after nasal instillation) prevents the development of the clinically apparent and fatal disease.
BIBLIOGRAPHY

EXPLANATION OF PLATES

The sections in the plates are of material obtained from 15 day old mice and stained with phloxine and methylene blue.

PLATE 6

Fig. 1. 4 days after injection of virus into vitreous humor of left eye. A, uninoculated eye; note normal appearance of retina. B, inoculated eye; arrows point to necrosis of retina. × 28.

Fig. 2. Necrosis of right superior colliculus after inoculation of left eye. × 31.
Photographed by Joseph B. Haulenbeck and Louis Schmidt

(Sabin and Olitsky: Vesicular stomatitis virus. III)
PLATE 7

FIG. 3. Necrosis of left superior colliculus after inoculation of right eye. × 31.

FIG. 4. 4 days after nasal instillation of virus. Note necrosis and desquamation of part of olfactory mucosa. Left olfactory bulb shows extensive necrosis in all layers except the outermost one of nerve fibers; arrows point to almost complete disappearance of mitral cells. The right olfactory bulb shows a much earlier lesion, involving only a small number of the mitral cells and neurons in the lamina gelatinosa; note the normal appearance of the three outer layers: nerve fibers, glomeruli, and external granular layer. For normal architecture of olfactory bulbs, see also Fig. 1. × 35.
Photographed by Joseph B. Hauerbeck and Louis Schmidt

(Sabin and Olitsky: Vesicular stomatitis virus. III)
Plate 8

Fig. 5. Intracerebral inoculation. Arrow points to location of neuronal necrosis around lateral ventricle (see Fig. 7). Letters A and B point to areas to be compared with Fig. 6. × 16.5.

Fig. 6. 4 days after nasal instillation. Compare with Fig. 5 and note necrosis of neurons in the ventromedial aspect of the septum at A and of lateral olfactory gyrus at B. The tuberculum olfactorium (C) presents only a small number of necrotic cells at this level, which are not perceptible at this magnification. × 16.5.

Fig. 7. Periventricular lesion after intracerebral injection. Note necrosis and disorganization of ependyma and shrunken neurons with pycnotic nuclei directly around the ventricle. × 280.

Fig. 8. Higher magnification of necrotic zone in lateral olfactory gyrus shown at B in Fig. 6. × 115.
Photographed by Joseph B. Haulenbeck and Louis Schmidt

(Sabin and Olitsky: Vesicular stomatitis virus. III)
PLATE 9

Fig. 9. 4 days after nasal instillation. Arrows point to necrosis in piriform lobe at A, hypothalamus, B, habenular nucleus, C, and cornu Ammonis, D. × 15.

Fig. 10. Intracerebral inoculation. Arrows point to necrosis of portions of cornu Ammonis and neopallial cortex forming the walls of the lateral ventricles. × 15.