Morphologic and immunoperoxidase study of neurologic lesions in naturally acquired rabies of raccoons

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Abstract. Histopathologic (hematoxylin and eosin [HE]) and immunoperoxidase (streptavidin-biotin complex) methods were used for examination of formalin-fixed tissues of rabid raccoons from an enzootic area of Pennsylvania. Extensive morphologic lesions of rabies encephalitis were present in the cerebrum and the brain stem regions. Negri bodies were detected by both methods and were present in the brain (cerebral cortex, hippocampus, brain stem, cerebellum, and cervical spinal cord) and in the ganglia of the trigeminal nerves. The viral inclusions were also seen in ganglion cells in the tongue, parotid salivary glands, pancreas, intestines, and adrenal glands. These sites were not associated with any inflammatory cellular infiltrate. The immunoperoxidase method was superior to HE for the detection of Negri bodies. Because lesions of rabies encephalitis were consistently observed in the cerebrum, brain stem, and cervical spinal cord regions, these areas of the brain should be included when raccoons are examined by the fluorescent antibody test for rabies.

Materials and methods

Two raccoons (nos. 1 and 2) that were showing neurologic signs and were from a rabies enzootic area of York County, Pennsylvania, were utilized for this investigation. Raccoon 1 was seen exhibiting neurologic signs during daylight hours and was shot by the local wildlife authorities. The second raccoon was also seen during daylight and was trapped alive. It appeared lethargic, had mucoid diarrhea, and died on the second day of captivity. Both carcasses were submitted to the University of Pennsylvania New Bolton Center for necropsy. A complete postmortem examination was done, and representative tissues of major organs, including the whole brain, were fixed in 10% buffered formalin. Fresh specimens of anterior cervical spinal cord were submitted to The Wistar Institute, Philadelphia, for rabies fluorescent antibody testing. The fixed brain was cut at approximately 2-mm transverse sections. All brain sections and tissue sections of the other organs were embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin (HE). Selected paraffin sections of cerebral cortex, hippocampus, cerebellum, brain stem, trigeminal (Gasserian) ganglion, tongue, parotid salivary gland, pancreas, adrenal glands, and intestines (duodenum) were also stained by the streptavidin-biotin complex immunoperoxidase method. This technique involved a mixture of 3 different monoclonal antibodies (502-2, 377-7, and 801-3) against rabies nucleocapsid protein to detect Negri bodies in the brain and in ganglion cells of the nonneural tissues. The extent of the morphologic distribution of brain lesions (HE sections) was recorded on preprepared diagrammatic representations of cross sections of brain. The presence of Negri bodies was scored from + (for occasional, l-2 bodies/section) to ++++ (for numerous, several bodies per high-power field) inclusions within a section of either HE- or immunoperoxidase-stained slides.

Results

Gross lesions were not seen in either of the raccoons. Both animals were positive for rabies antigen by the
fluorescent antibody test. Microscopic examination of HE-stained sections revealed extensive nonsuppurative encephalitis, which was more severe and more extensively distributed in raccoon 2. The detailed distribution of the brain lesions is shown in Figs. 1 and 2. In both brains, there was extensive involvement of cerebrum (bilateral but not necessarily symmetrical), brain stem, and cervical spinal cord areas. Cerebellar lesions were present in 1 of the raccoons (Fig. 2). However, the major portion of the cerebellum was not involved, and the lesions were confined to the corpus medullare region. The neuropathologic lesions consisted of perivascular cuffing with variable numbers of mononuclear cells, multifocal areas of gliosis, and, within the severely affected regions, some neuronal degeneration (Fig. 3A, 3B). Usually, in association with these lesions, the neurons had eosinophilic cytoplasmic (Negri) bodies of various sizes (Figs. 4-6, Table 1). When present singly, these bodies were usually larger than when present in multiple aggregations. The inclusions were also present in neuronal and axonal cytoplasm in areas such as the cerebellum (Purkinje cells), where encephalitis was not seen. Negri bodies were also seen in ganglion cells in the tongue, salivary glands, pancreas, para-adrenal gland ganglion (1 raccoon), submucosal plexus of duodenum (1 raccoon), and in the Gasserian ganglion (Figs. 7, 8). Except for the Gasserian ganglion, no inflammatory cellular response indicative of an infectious process was seen in the ganglia of the parenchymatous organs. Besides the changes seen in ganglion cells of the salivary glands, no inflammatory or degenerative changes suggestive of viral infection in these organs were seen in either animal.

The immunoperoxidase-stained preparations showed many more Negri bodies than were observed in the corresponding HE-stained sections (Table 1), except for intestinal and adrenal gland sections of 1 animal (raccoon no. 2), where neither of the HE-stained sections had inclusions but Negri bodies were observed on the immunoperoxidase preparations (Table 1).

Discussion

Rabid animals usually exhibit dramatic neurologic signs without revealing any grossly visibly alteration

| Table 1. Presence or absence* of Negri bodies in neurons of various tissues of raccoons with naturally acquired rabies infection. |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| Tissues                                        | HE†             | Peroxidase†     | HE†             | Peroxidase†     |
| Cerebral cortex                               | I               | ++++           | ++             | ++++           |
| Hippocampus                                   | ++++           | ++++           | ++++           | ++++           |
| Brain stem                                    | +              | ++++           | ++++           | ++++           |
| Cerebellum                                    | ++++           | ++++           | ++             | ++++           |
| Gasserian ganglion                            | +              | ++++           | ++++           | ++++           |
| Tongue                                        | +              | ++++           | +              | ++++           |
| Salivary gland                                | NE§            | NE             | ++             | ++++           |
| Pancreas                                      | –              | –              | ++             | ++             |
| Adrenal gland                                 | ++++           | +              | –              | ++             |

* = negative for Negri bodies; ++ = 1-2 bodies/tissue section; +++ = 2-5/section; ++++ = 5-10/section; ++++ = >10/section; ++++ = several per high-power field (40×).
† Hematoxylin and eosin.
§ Streptavidin–biotin immunoperoxidase.
§ NE = not examined.
of the central nervous system (CNS) at postmortem examination. Both naturally infected rabid raccoons in this study followed this pattern. Both raccoons had shown CNS signs prior to their death, but no gross lesions were observed in either animal. However, severe and extensive histopathologic lesions of nonsuppurative encephalomyelitis with marked perivascular cuffing and the presence of neuronal inclusion (Negri) bodies were observed. These neurologic lesions were generally similar to those described in other commonly reported rabid animals.

In mammals, rabies virus has an affinity for salivary glands, in which it replicates in acinar cells, leading to focal lysis of the acinar cells, mononuclear cellular infiltration, and presence of Negri bodies in the ganglionic neurons. Salivary glands of rabid raccoons contain substantial amounts of infectious rabies virus. However, although many Negri bodies (both on HE and immunoperoxidase preparations) were observed in the ganglion cells of the salivary glands, no detectable degeneration was noted in acinar cells nor was there any inflammatory cellular infiltrate in the glands (Fig. 8).

The distribution of morphologic lesions of rabies...
encephalomyelitis in these raccoons (Figs. 1, 2) is different from that of previously reported cases of experimental raccoon rabies. In the present study, there was bilateral and extensive involvement of the cerebrum, brain stem, and cervical spinal cord regions in both raccoons. The more extensive lesions in the cerebrum suggest that the rabies virus in both raccoons had preferential affinity for the cerebrum. This pattern of distribution of neuropathologic lesions is quite different from that in raccoons inoculated with a dog isolate of rabies virus. Those experimental raccoons did not demonstrate any dramatic neurologic signs, and the lesions of encephalitis were confined to the brain stem region. Rabies diagnostic laboratories should therefore sample cerebrum, brain stem, and the cervical spinal cord regions of suspect rabid animals for the fluorescent antibody test for rabies and should attempt the immunoperoxidase technique with tissues that have been inadvertently fixed in formalin.

The detection of Negri bodies by HE and immunoperoxidase was generally comparable (Table 1). However, the immunoperoxidase method detected many more Negri bodies than were seen in the corresponding HE-stained sections. Also, in 2 tissues of raccoon no. 2 (adrenal gland and intestines), the immunoperoxidase method was able to demonstrate inclusions when the HE results were negative for Negri bodies. In this study, the Negri bodies observed were

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**Figure 4.** Photomicrograph of hippocampus of raccoon no. 2 showing Negri bodies (arrows) in neurons. Immunoperoxidase.

**Figure 5.** Photomicrograph of hippocampus of raccoon no. 1 showing Negri bodies in cytoplasm of some of the neurons. HE.

**Figure 6.** Photomicrograph of brain stem region of raccoon no. 1 showing many Negri bodies of various sizes. Immunoperoxidase.

**Figure 7.** Photomicrograph of para-adrenal ganglion cells of raccoon no. 1. Many Negri bodies of various sizes are visible in some of the ganglion cells. Immunoperoxidase.
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Figure 8. Photomicrograph of ganglion cells in parotid salivary gland of raccoon no. 2. Negri bodies (arrow) are present in 1 of the ganglion cells. Note there is no inflammatory infiltrate in the parenchyma. Immunoperoxidase.

of various sizes; some were quite large, especially those found singly in a neuron (Fig. 6). This finding is contrary to that of a previous study of raccoons in which only small-sized Negri bodies were found.9

The presence or absence of Negri bodies in tissues of rabid raccoons also appears to be related to the type of virus isolate to which the animals are exposed. In this investigation of a raccoon virus isolate, numerous Negri bodies were seen in various organs, whereas exposure to a nonraccoon strain of rabies virus, such as a dog or a bat isolate, may result in the formation of either no Negri bodies6 or the presence of only occasional cytoplasmic inclusions.11

The distribution pattern of morphologic lesions of rabies encephalitis and the presence or absence of Negri bodies in tissues of rabid raccoons may be related to the particular rabies isolate to which the animals are exposed, which is contradictory to the commonly held belief that the presence and distribution of Negri bodies is dependent on the host animal.12 Under experimental conditions, the choice of the rabies isolate used may influence the clinical signs that are produced in the raccoons. For example, the administration of a raccoon isolate to a raccoon may produce much more severe clinical neurologic signs (because of more cortical involvement of the brain, probably resulting from natural serial passage of the isolate in the same species) than when the raccoons are administered a nonraccoon, such as a dog, isolate where there is exclusive brain stem involvement. This hypothesis is currently being tested.

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References