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Chronic Recrudescent Rabies in a Cat (39847)

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Introduction. The concept of the inevitability of fatal outcome associated with rabies infection has achieved wide acceptance, although early investigators found much evidence to the contrary (1, 2) and that evidence has been confirmed repeatedly (3).

Bell (4) investigated nonfatal infection using the term "abortive rabies" and introduced experimental models for induction of rabies virus infection with prolonged survival. These studies were performed primarily on mice using intraperitoneal inoculation of street virus. Surviving mice were characterized by the development of prominent residual limb paralyses associated with high titers of rabies virus neutralizing antibodies (RVNA) in the cerebrospinal fluid (CSF) and brain tissue (5). Based on experience in mice, presence of central nervous system (CNS) RVNA in high titer, without demonstrable rabies virus or antigen, was considered reliable evidence of prior rabies encephalitis. Surviving animals were resistant to intracerebral challenge with 3 to 4 dex LD_{50} of fixed virus.

In extension of these studies to other animal species, five striped skunks (*Mustelidae mephitis*) and five cats were inoculated intraperitoneally with low-passage rabies virus. This resulted in an apparent chronic, slowly progressive encephalitis in one of the cats. Observations on this animal are the subject of this report.

Materials and methods. Animals. Five wild-caught, mature young striped skunks (*M. mephitis*) and five mature young cats were inoculated intraperitoneally. The animals were separated and observed daily. Blood, saliva, and CSF were obtained at regular intervals.

Inoculum. Each animal was inoculated intraperitoneally with 1.0 ml of a 10% hamster brain suspension in saline. The isolate was originally obtained from a bat and passed three times in suckling mice prior to the hamster inoculation. The infectious titer of the virus was 6 dex LD_{50}/0.03 ml in mice inoculated intracerebrally.

Assay of virus neutralizing antibody in serum, CSF, and brain tissue. Blood, saliva, and CSF were obtained under general anesthesia. Rabies virus neutralizing titers were determined by mixing dilutions of serum or CSF with fixed rabies virus to yield a final virus concentration of about 100 LD_{50} per unit of inoculum (0.03 ml), incubating the mixtures at room temperature for 1 h, and inoculating into mice by the intracerebral route (5). Antibody titers were calculated from the differences between test fluids and normal control serums (6).

Necropsy of cat C-3. The single affected cat was sacrificed with an anesthetic overdose. Tissues were fixed in formalin for paraffin embedding and histologic studies. Selected regions of the CNS were fixed in phosphate-buffered glutaraldehyde, post-fixed in OsO_4 and embedded in Epon for electron-microscopic study.

Attempts at viral isolation. Fresh tissue was obtained from the hippocampus, brain stem, cerebellum, cervical and lumbar spinal cord, and salivary glands. These tissues were homogenized in phosphate-buffered saline to yield a 10% suspension. After centrifugation, the supernatant fluid was injected intracerebrally into suckling mice. The mice were observed daily for 1 month. Several surviving mice were subsequently inoculated intracerebrally with fixed virus. Explants of CNS tissue were established in tissue culture and cocultivated with BHK-21 cells. Used media from these cultures were inoculated intracerebrally into mice.

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Fluorescent antibody studies. Direct fluorescent antibody testing (7) was applied to CNS tissues obtained at necropsy as well as tissue culture samples.

Results. Animals. Three of the skunks became paralyzed and died of acute typical fulminant rabies following an appropriate incubation period (14 days). Fluorescent antibody testing of brain tissues from the dead skunks revealed the typical, strongly positive reaction to rabies virus. The two remaining skunks and four of the cats continued entirely well. On the 11th day after the inoculation, cat C-3 developed paresis of the hind limbs, coarse tremors, irritability, and apparent anorexia. Over the next several days, the animal's condition stabilized and complete hind limb paralysis was never reached; however, considerable difficulty in walking was noted. In the next few weeks the coarse tremors ceased, irritability diminished, and the limb weakness improved but remained clinically obvious. A serum sample, taken 3 weeks after inoculation, had an RVNA of 960 (Fig. 1). Another serum sample and a CSF sample taken at 10 weeks had titers of 1920 and 256, respectively. Samples also were taken from the surviving skunks and other cats on that date; the highest serum titer was 44 and CSF titers were 2. Samples of serum and CSF from cat C-3 showed declining titers of RVNA to the 20th week (Fig. 1), as encountered in dogs that survived patent infection (see example, Fig. 2) (8). However, in the 16th week the titers were 1754 and 140, respectively, and in the 28th week were 2240 and 435. At 91 weeks, a peak serum RVNA concentration was found, followed by marked decline to 2560 at 118 weeks. CSF titers did not closely parallel those of the serum except that there was a gradual increase from the low of 140 at 28 weeks. No significant decline of CSF titer occurred when serum titer declined sharply.

Changes in serum and CSF antibody concentrations were not accompanied by any evident change in clinical condition or attitude until the 120th week, when food intake decreased and limb weakness became more pronounced. In the following weeks there was further gradual deterioration of the animal's condition with ultimate virtual complete hind limb paralysis and atrophy and fasciculations of the musculature of the hindquarters (Fig. 3). The cat was sacrificed and necropsied 126 weeks after inoculation. Serum and CSF samples taken at the time of

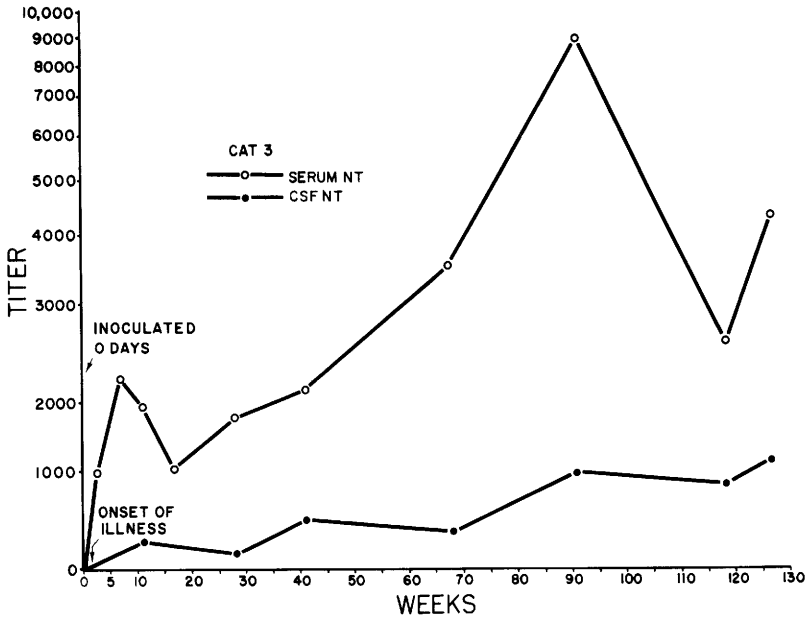


FIG. 1. Titers of rabies virus neutralizing antibodies in serum and cerebrospinal fluid of cat C-3 at various intervals after inoculation.

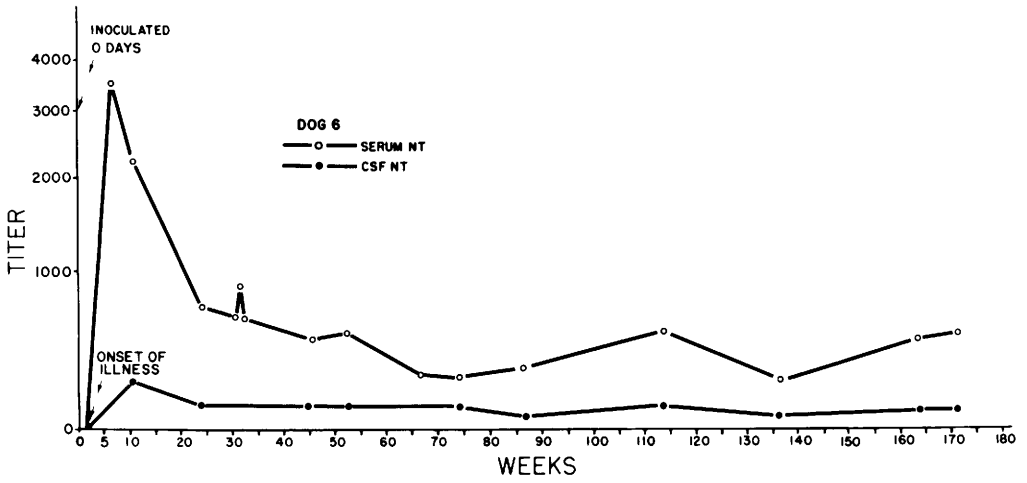


FIG. 2. Titers of rabies virus-neutralizing antibodies in serum and cerebrospinal fluid of a dog that recovered from experimental acute paretic rabies.

euthanasia had titers of 4326 and 1120, respectively.

Postmortem studies. Gross and histologic examination of the visceral organs revealed no significant abnormalities. Fresh tissue was obtained from the hippocampus, brain stem, cerebellum, cervical and lumbar spinal cord, and salivary glands for attempted viral isolation and FA studies. Inoculation of homogenates of the material intracerebrally into suckling mice and onto BHK-21 tissue culture cells failed to reveal the presence of an infectious agent. Explant cultures of the tissues were established and cocultivated with BHK-21 cells at 37° only, without emergence of infectious virus as monitored by IC suckling mouse inoculation. Direct rabies FA studies of the tissues were negative except for a faint positive reaction of one imprint of lumbar spinal cord. Saliva samples taken at 37 weeks and on three other occasions did not contain virus detectable by mouse inoculation. Some of the mice that survived inoculation of saliva were given inoculations of known rabies virus, and all succumbed. Blind passages were made from the brains of other survivors without result.

Brain tissue RVNA titer was 522; C5 and L4 spinal cord titers were 92 and 150, respectively.

Neuropathologic studies. Histologic examination of the CNS of the affected cat revealed prominent lesions which differed markedly from those typically associated with classic acute rabies encephalitis (9, 10). Classic rabies is characterized by relatively scant perivascular infiltrates of lymphocytes and plasma cells, focal microglial nodules, and prominent neuronal cytoplasmic virion production (demonstrable as Negri bodies by light and electron microscopy and FA studies) (9, 11), despite little accompanying evidence of neuronal destruction.

The CNS of cat C-3 contained extensive dense perivascular inflammatory cell infiltrates consisting primarily of lymphocytes and plasma cells with rare macrophages and polymorphonuclear cells. Infiltrates were so thick and densely packed that the vascular lumen was frequently obliterated (Fig. 4). Lesions were widespread and most prominent in the temporal cortex, thalamus, entire brain stem, and lumbar spinal cord. In adjacent parenchyma, neurons were noted undergoing shrinkage and apparent destruction. Focal gliosis and neuronophagia were extensive, particularly in regions containing perivascular infiltrates (Fig. 5a and b). Several shrunken neurons were surrounded along their cell membranes by plasma cells.

A careful search was made for Negri bodies, but the characteristic intracytoplasmic inclusions of acute fatal rabies were not seen. The hippocampus and Purkinje cell layer of the cerebellum, sites of prominent

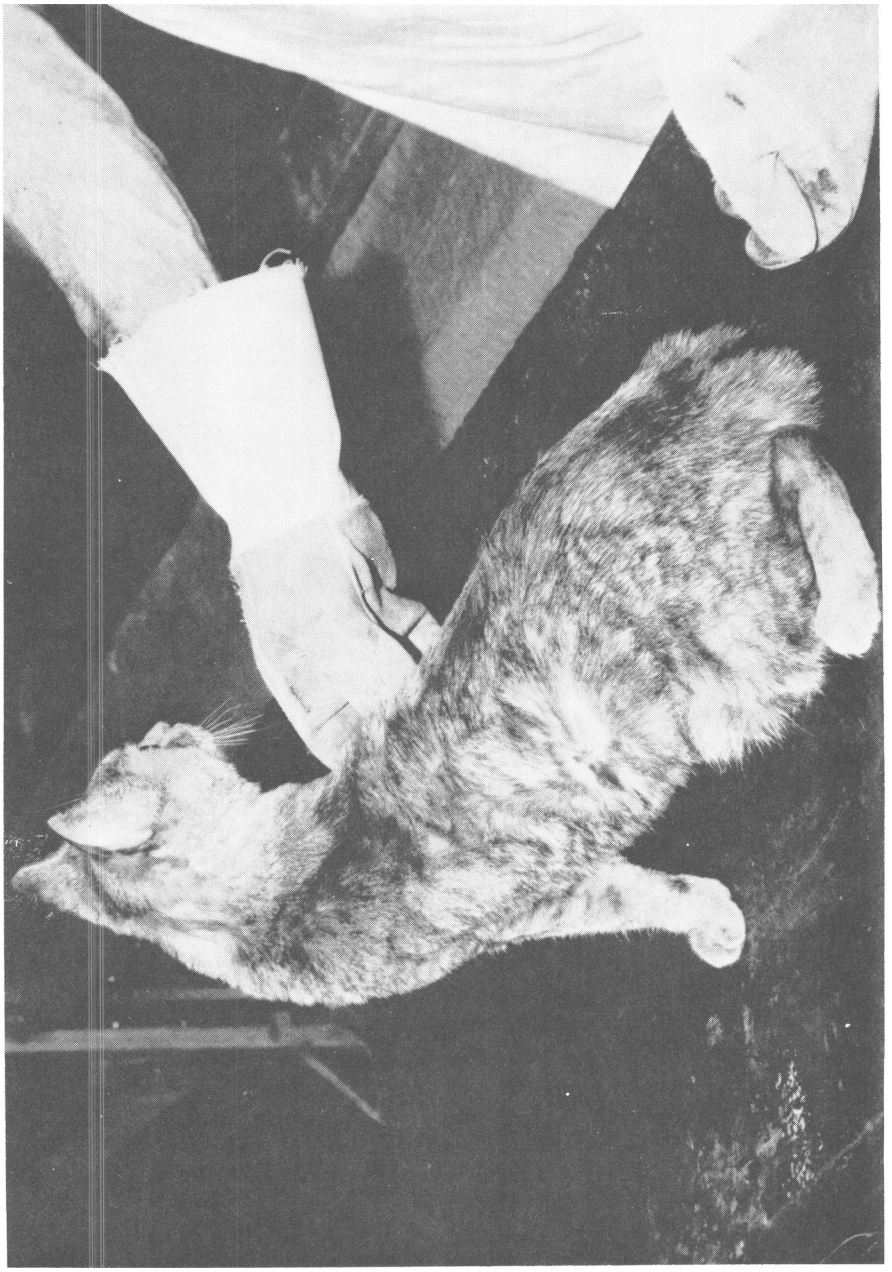


FIG. 3. Splayed paretic hindquarters of cat C-3 6 months after patent onset of rabies.

FIG. 4. Photomicrograph of the prominent, dense perivascular inflammatory infiltrates seen in cat C-3. Pons, hematoxylin & eosin, 140×.

involvement in acute rabies, were virtually free of inflammatory cell involvement or neuronal degeneration. Extensive ultrastructural studies were performed on tissues derived from the affected cat. These studies confirmed many of the light-microscopic findings including disruption of perikaryal cytoplasm with dispersion of Nissl bodies, mitochondrial swelling, gliosis, and focal myelin breakdown. No recognizable rabies virus virions could be identified.

Discussion. We believe that this animal suffered from an unusual form of chronic rabies infection.

Figure 2 illustrates the typical course of abortive rabies infection in a similarly inoculated dog (8). The graph, showing serum and CSF rabies neutralizing titers over a 3-year period, is similar to that seen in several comparable animals. During the initial phase, the typical animal exhibits limb weakness and irritability followed by an elevation of serum and CSF neutralizing titers, but soon recovers completely with lowering of the serum and CSF titers. Two such dogs have been examined at necropsy 2 years after initial inoculation and neither has shown any of the CNS lesions encountered in the affected cat described in this report.

We believe that cat C-3 suffered from an unusual form of rabies, namely, reactivation of abortive CNS infection. Changes in antibody titers in the CSF suggest that the animal initially achieved control over the adverse effects of the inoculated virus, however, about 5 months later the infection became reactivated and was intermittently progressive over the next 2 years. Failure to isolate virus may have been the result of the presence of high concentrations of neutralizing antibody in the CNS. Failure to demonstrate virus by electron microscopy could be attributable to the relatively small portion of the CNS examined by that technique. Even in acute rabies virus may, at times, be limited in distribution in the CNS (12). Whether the lesions represented allergic reaction or inflammatory response to rabies virus proliferation is uncertain, but the exalted antibody titers and neuronal destruction suggest the latter. In any case, the clinical deterioration and extensive active CNS lesions suggest that the course would have proceeded to death.

In 1961 Soave *et al.* (13) reported "reactivation" of rabies infection in 1 of 6 guinea pigs 5 months after inoculation with street virus and subsequently (14) reported "reactivation" of infection in 1 of 10 guinea pigs 8½ months after inoculation, attributing the "reactivation" to the stress of overcrowding, although no evidence of initial infection was reported. Those observations probably were attributable to very long incubation periods frequently seen in rabies (3). Sulkin *et al.* (15) identified brown fat as a potential long-term storage site for rabies virus in bats with reactivation of latent infection related to pregnancy or hibernation.

Reactivation of latent viral infections of the central nervous system is a concept that has been accepted for many years, and has recently received increasing interest because of relationships to certain chronic degenerative disorders of the nervous system (16, 17). It has been noted that viruses which give rise to these persistent infections in general tend to cause minimal cytopathology (18). Rabies virus, despite its supreme lethality to the organism itself, may be characterized in this way on a cellular level (9).

The observations reported here indicate that rabies virus may be capable of establishing a form of persistent latent infection with the capability of reactivation. Study of the pathophysiologic mechanisms underlying the disease associated with reactivation of this infection may yield important information about the nature of virus vs host interactions in the nervous system, as well as possibly provide clues to the survival of rabies virus in nature.

Summary. In an attempt to induce nonfatal rabies infection, five cats and five skunks were inoculated intraperitoneally with low-passage rabies virus. Three of the skunks died of acute fulminant rabies. Only one of the cats showed any evidence of illness. The affected cat developed hind limb paresis, coarse tremors, and irritability 11 days after inoculation of the virus and survived the apparent infection with nondisabling sequelae. High rabies virus neutralizing antibody (RVNA) titers were found in the serum and cerebrospinal fluid several weeks after the onset of illness and declined subsequently, as is common in abortive rabies encephalitis. However, after the 16th week, RVNA

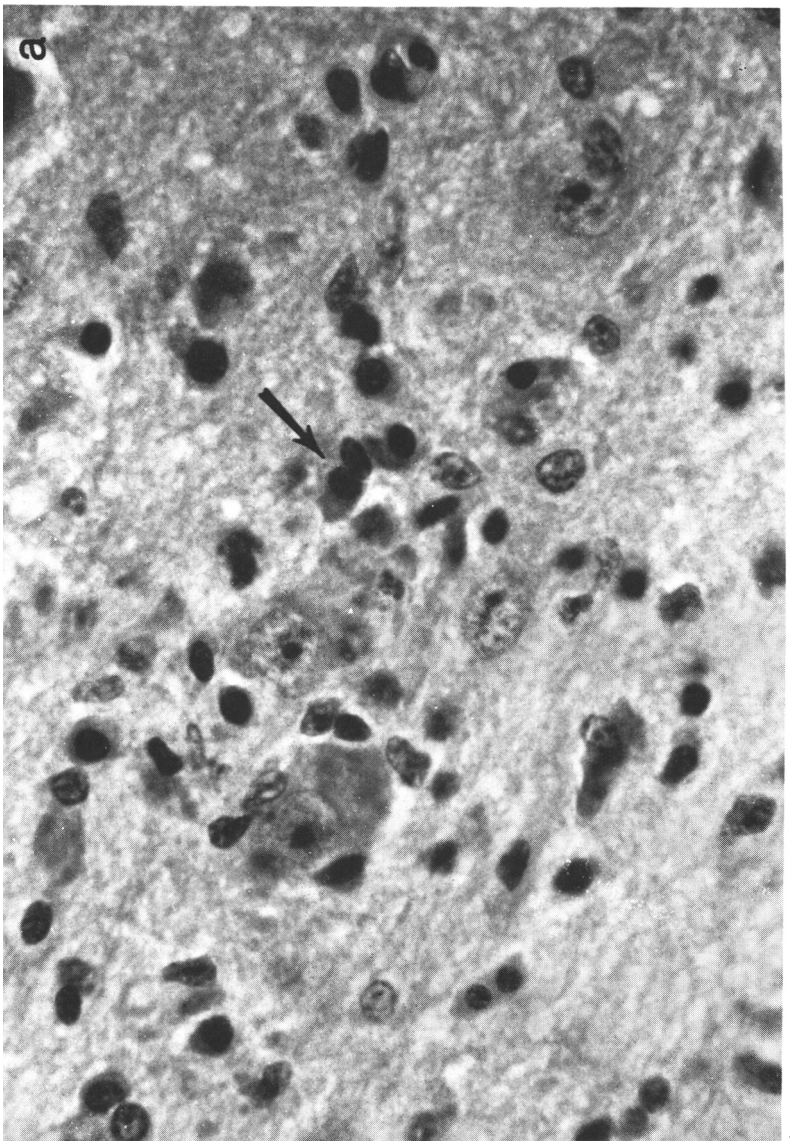
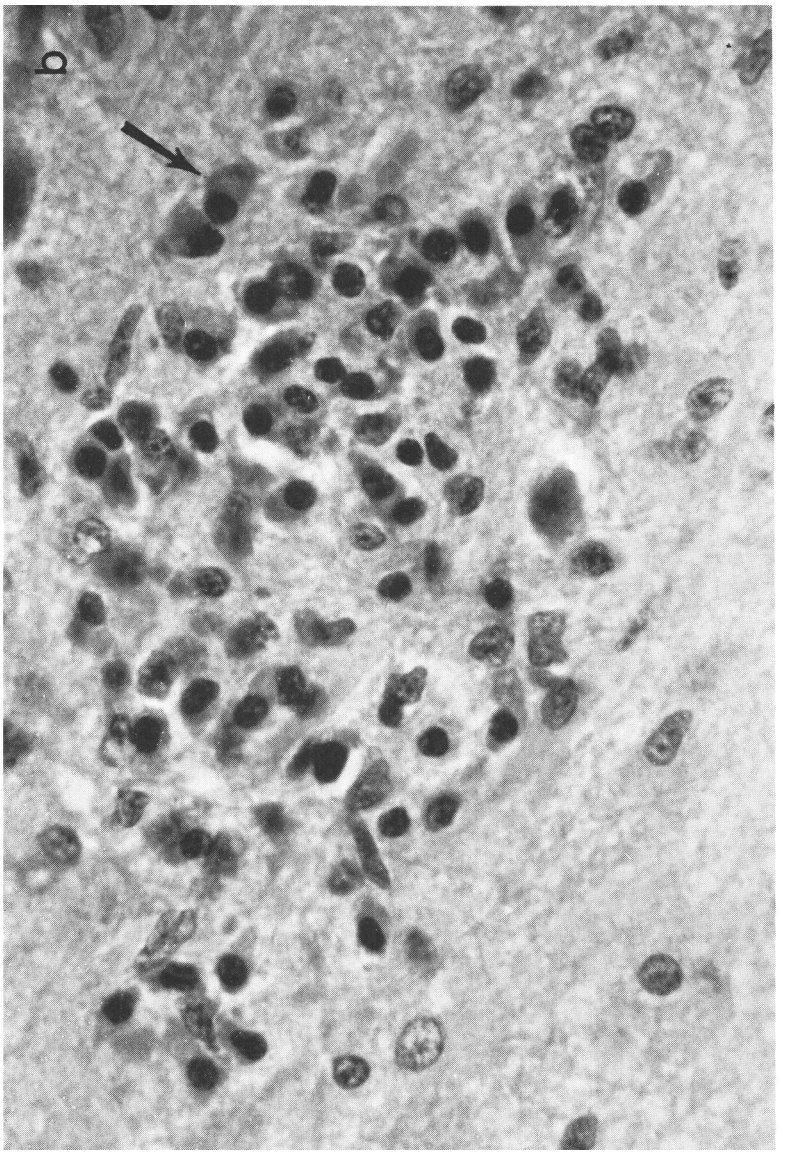


FIG. 5. (a) and (b). Examples of the extensive satellitosis and neuronophagia present in cat C-3. Note the numerous plasma cells within these leions (arrow). Midbrain, hematoxylin & eosin, 500×.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titers in both fluids again rose, and in the ensuing year and a half very high levels of antibodies were achieved. At about 120 weeks, the clinical condition worsened, and 6 weeks later the cat was sacrificed. At necropsy, lesions found in the central nervous system were not those of classical acute rabies, but consisted of an extensive chronic destructive encephalitis. Virus could not be isolated, probably because of the coexisting high antibody level. The observations are interpreted as consistent with a chronic recrudescent form of rabies encephalitis.

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