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role in the etiology of lymphoproliferative disease in the goldfinches studied here. It is more likely that immunosuppression resulting from the neoplasia permitted the development of coccidiosis.

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Intracytoplasmic Neuronal Inclusions in the Hippocampus of Non-rabid Moose, *Alces alces* (L.)

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Intracytoplasmic acidophilic neuronal inclusions in the brain are characteristic of rabies virus infection, but they are also described in non-rabid animals of several species including domestic cat (Szlachta and Habel, 1953, *Cornell Vet.* 43: 207–212), dog (Cameron and Conroy, 1974, *Vet. Pathol.* 11: 29–37), cattle and sheep (Stovall and Pessin, 1942, *Am. J. Public Health* 32: 171–175), skunk (Jubb and Kennedy, 1970, *Pathology of Domestic Animals*, Vol. 2, Academic Press, New York, pp. 414–416), fox, and laboratory mouse (Smith et al., 1972, *Veterinary Pathology* (4th Ed.), Lea and Febiger, Philadelphia, pp. 351–356). Such descriptions have provided useful baseline data for histological interpretation of diseased brains. This paper provides the first description of intracytoplasmic neuronal inclusions in the hippocampus of non-rabid adult moose.

In August 1979, a cow moose (Case 1) showing abnormal behavior was reported by tourists to officials of Prince Albert National Park, Saskatchewan, Canada. The animal was killed and the head was removed, frozen, and submitted for post mortem examination 1 mo later. In

October 1980 a 2½ yr old cow moose (Case 2) raised at the Wyoming Game and Fish Department's Sybille Wildlife Research Unit became acutely ill. Clinical signs included weakness, anorexia, and bilateral limbal corneal opacity. The moose would drink water and eat willow branches when these were held for her. After 2 days the moose was killed due to her deteriorating condition and a post mortem examination was conducted. Intracytoplasmic neuronal inclusions were observed in the brains of these two moose, and additional moose brains were obtained for comparison. These included a cow moose (Case 3) which died of chronic enteritis at Sybille, and two free-ranging cow moose from Wyoming, one with no history of clinical disease (Case 4) and one with severe keratoconjunctivitis (Case 5).

At necropsy, brain tissue was fixed in 10% neutral buffered formalin. Half of each brain was frozen unfixed and submitted either to the Western Animal Disease Research Institute (Agriculture, Canada) or to the Wyoming State Veterinary Diagnostic Laboratory for possible detection of rabies virus antigen by fluorescent antibody technique (FAT). Mouse inoculation studies were conducted in Cases 2–5. Samples of fixed brain, which included frontal and occipital cortex, basal ganglia, hippocampus, thalamus, mesencephalon, cerebellum, and medulla oblongata in most cases, were embedded in

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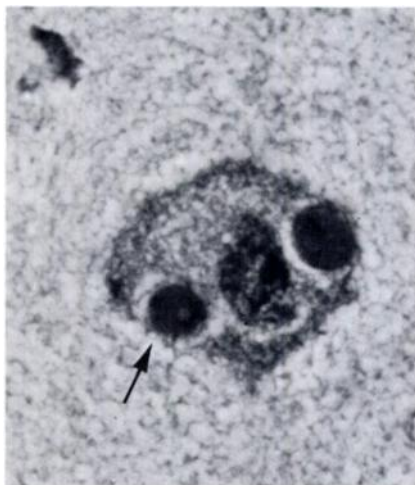


FIGURE 1. Two large eosinophilic intracytoplasmic inclusions in a hippocampal neuron from an adult moose. Each contains internal granules. H&E, $\times 2,000$.

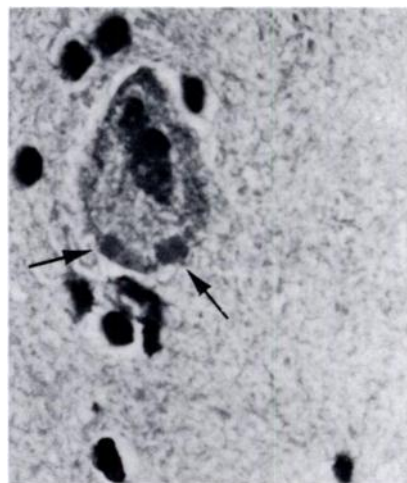


FIGURE 2. Two small eosinophilic intracytoplasmic inclusions in a hippocampal neuron from an adult moose. H&E, $\times 800$.

paraffin, sectioned at 6 μm and stained with hematoxylin and eosin (H&E). Sections of hippocampus were subsequently stained with Massigiani and Malferrari's stain (1961, *Stain Technol.* 36: 5-8) and Schleifstein's stain (Luna, 1968, *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*, McGraw-Hill Book Co., New York, pp. 194-195) for rabies virus inclusion bodies, with Perl's Prussian Blue stain for iron and with Ziehl-Neelsen and Periodic Acid Schiff (PAS) stains (Culling, 1963, *Handbook of Histopathological Techniques*, Butterworths, London, 553 pp.). For ultrastructural study of neuronal inclusion bodies seen by light microscopy, fixed hippocampal tissue was selected, dehydrated, and embedded in Epon/Araldite. Ultrathin sections were cut, stained with uranyl acetate and lead citrate and examined by transmission electron microscopy. For comparative purposes, paraffin-embedded brain from a FAT-positive case of rabies in a striped skunk (*Mephitis mephitis*) and in a domestic bovid were sectioned and stained as above for light microscopy.

The brain from Case 1 was autolysed and had suffered further histologic distortion because of freezing. There were no gross lesions in the brain or skull and no malacia or encephalitis suggestive of parasite migration or other infection. Many neurons in or adjacent to the pyramidal layer of the hippocampus contained

prominent round to oval eosinophilic intracytoplasmic inclusion bodies (H&E) which were not present elsewhere in the brain. These ranged in size from 2 to 20 μm in diameter and one to several inclusions were present in affected cells (Figs. 1, 2). Some of the larger inclusion bodies had distinct golden-brown internal granules and most were present in cells that also contained brown granular pigment. Microscopic changes in the brain of Case 2 were characterized by severe vasculitis with fibrinoid degeneration of the tunica media, and marked lymphoid cell perivascular infiltrates. Neuronal necrosis and gliosis were minimal and a diagnosis of malignant catarrhal fever (MCF) was made. Many neurons in the pyramidal layer of the hippocampus, and occasional neurons in the thalamus and medulla oblongata contained inclusions similar to those in Case 1. Inclusions similar in location, light microscopic morphology, and stain affinity were found in the hippocampi of Cases 3, 4, and 5. No other histologic changes were present in these brains.

The stain affinities of these inclusions and of intracytoplasmic neuronal inclusions from cases of rabies (Negri bodies) are given in Table 1. No virions or fibrillar matrix were present in the moose inclusions studied ultrastructurally. Rather, they were homogeneous masses of finely granular, electron-dense material (Fig. 3). Small electron-lucent bodies and a few dense

TABLE 1. Stain affinities of intracytoplasmic neuronal inclusion bodies from the hippocampi of non-rabid moose compared with those from two rabid animals. Tissue was fixed in formalin, embedded in paraffin and sectioned at 6 μ m prior to staining.

Stain ^a	Usual substrate (positive reaction)	Stain affinity ^b			
		Non-rabid moose		Rabid skunk	Rabid bovid
		n	Result		
Hematoxylin and eosin	Acidophilic material (red), basophilic material (blue)	5	+ (red)	+ (red)	+ (red)
Massignani and Malferrari	Acidophilic material, Negri body (red)	3 1	+ -	+	+
Periodic acid Schiff	Glycosaminoglycans (red)	5	+	-	-
Perl's Prussian blue	Ferric iron (blue)	4	-	-	-
Schlieffstein	Negri body (deep magenta)	2	+	+	Not determined
Ziehl-Neelsen	Acid-fast material (red)	4	-	-	-

^a See text for methods.

^b + = positive reaction, - = negative reaction.

masses resembling aggregates of granular material were present within one inclusion. No rabies virus antigen was demonstrated in the brains of Cases 1–5 by FAT and mouse inoculation studies in Cases 2–5 were negative.

No satisfactory diagnosis was reached for Case 1. The animal was considered non-rabid because of a negative FAT and absence of encephalitis. Ultrastructural characteristics of its neuronal inclusions were not those of Negri bodies. Case 2 was diagnosed as MCF on the basis of the observed histopathology which was distinct from the encephalitis induced by rabies virus (Jubb and Kennedy, 1970, *op. cit.*). The initial concern about rabies in these cases was raised by the morphology of the inclusions seen in H&E sections. The largest of these inclusions would probably not be mistaken for Negri bodies, but the smaller inclusions could not be distinguished from Negri bodies by routine light microscopy. The laboratory diagnosis of rabies has relied heavily on the identification of neuronal inclusions since these were first described by Negri in 1903 (*Z. Hyg. Infektionskr.* 43: 507–528). A major characteristic of these Negri bodies was the presence of slightly basophilic internal granular structures. Inclusions identical to Negri bodies in all ways except the absence of internal granules were termed Lyssa bodies by Goodpasture in 1925 (*Am. J. Pathol.* 1: 547–582) and their relationship to Negri bodies and to rabies was controversial. Intracytoplasmic acidophilic neuronal inclusions were subsequently described in the brains of non-rabid animals of several species as noted above. With

the exception of the inclusions described in neurons of the lateral geniculate nucleus of cats, neuronal inclusions in non-rabid animals lacked basophilic internal structure, and possession of such internal structure became established as an important diagnostic feature of the inclusions caused by rabies virus (Jubb and Kennedy, 1970, *op. cit.*; Smith et al., 1972, *op. cit.*). In 1976, Sung et al. (*J. Neuropathol. Exp. Neurol.* 35: 541–559) showed that rabies virus could be found by electron microscopy in neuronal inclusions that lacked internal structure of any kind when viewed with the light microscope. Thus, internal structure is not a valid criterion for distinguishing inclusions that form in neurons infected with rabies virus from those that arise by other processes, and there are no criteria, at the light microscope level, that permit this distinction to be made with certainty. Immunological techniques and electron microscopy are means by which the distinction can be made.

The inclusions described in this paper occurred in the brains of five non-rabid moose, and pathologists should be alert to the possibility that similar inclusions may pose diagnostic dilemmas in the histopathologic assessment of moose brains. The PAS stain may be helpful in identifying these inclusions (Table 1) but our sample was too small to establish this stain as a reliable identifying criterion.

Moose were submitted for necropsy by the staff of Prince Albert National Park (Parks Canada) and by Tom Thorne, Wyoming Game and Fish Department. Technical assistance was ren-

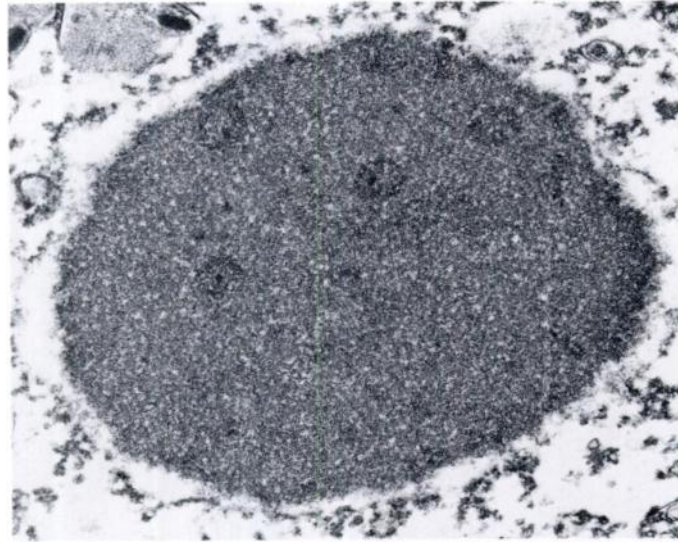


FIGURE 3. Electronmicrograph of intracytoplasmic neuronal inclusion body from the hippocampus of an adult moose. $\times 21,000$.

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