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Histochemical and Electron Microscopic Study on the Zinc-containing Neurons in Rat Spinal Cord

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흰쥐의 척수에서 아연이 함유된 신경원에 대한 조직화학 및 전자현미경적 연구

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요 약

Sodium selenite를 피하주사하고 은 증폭시켜 흰쥐 척수에 있는 아연이 함유된 신경원의 세포체와 bouton을 표지하였다. 표지된 신경원 세포체는 회백질의 V, VI, VII 및 X층에 분포하였다. 8시간 생존시킨 경우 아연 셀레늄 반응물이 역행수송되어 세포체에 침전되었다. 이것은 척수에 있는 아연이 함유된 신경원의 전부 혹은 일부가 개재신경원인 것으로 생각된다. 1시간 생존시킨 경우 아연 침전물로 표지된 축삭 bouton들이 회백질과 백질의 척수전삭 및 복측삭에 있는 돌기에 분포하였다. 특히 AMG로 염색된 큰 형태의 bouton 이 IX층에 나타났다.

미세구조적으로 아연 침전물은 생존시간에 따라서 아연이 함유된 신경원의 세포질 리소좀이나 bouton 내의 vesicle에 위치하였다.

Key words : Zinc-containing neuron, Autometallography, Spinal cord

INTRODUCTION

Chelated zinc can be identified in the central nervous system at light and electron microscopic levels with by histochemical methods- the autometallographic (AMG) zinc sulphide method (Haug, 1973; Danscher, 1981) and the AMG selenium method (Danscher, 1982). The zinc is localized to the boutons and, more precisely, within the vesicles (Christensen *et al.*, 1992). Neurons giving rise to these zinc-containing boutons are called zinc-containing neurons or zinc enriched (ZEN) neurons.

In rats that were treated with sodium selenite and allowed to survive for a period of time,

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Danscher (1984) demonstrated a retrograde transport of zinc selenium reaction products from the boutons of ZEN neurons to their somata. If rats are treated with sodium selenite and allowed to survive for 24 hours, most or all ZEN neurons show retrogradely transport of zinc selenium crystal lattices (Slomianka *et al.*, 1990; Christensen *et al.*, 1992; Mandava *et al.*, 1993). At survival time of 1 hour, the zinc selenium products are located in the synaptic vesicles of boutons (Danscher, 1984).

The purpose of the present study is to identify the presence of somata and boutons of the ZEN neurons in the spinal cord by selenium method (Danscher, 1982; Danscher and Montagnese, 1994). Additionally, patterns of the ZEN neurons were observed depend on the survival times.

MATERIALS AND METHODS

Twelve adult Kyoto rats weighing from 300 to 370 g, were used in this study. To label the somata of ZEN neuron, sodium selenite (Na_2SeO_3) 10 mg were dissolved in deionized water 1 ml, and the solution was used for intraperitoneal injection. The rats were intraperitoneally injected with sodium selenite 8 mg/kg body weight and allowed to survive for 8, 24, 48 and 65 hours. For the detecting the axonal boutons of ZEN neurons, sodium selenite 20 mg/kg body weight were intraperitoneally infused and the survival time was 1 hour.

After the survival times, the rats were deeply anesthetized with sodium pentobarbital and sacrificed by transcardial perfusion with 3 % glutaraldehyde in 0.1 M Sφrensen's phosphate buffer (pH 7.4) at 140 mmHg for 15 min or decapitation. The vertebral canal was carefully opened by total laminectomy and the spinal cord was removed. The second and fourth cervicals (C2 and C4), seventh thoracic (T7) and third lumbar (L3) segments were selected as representative spinal cord.

Light Microscopy

The spinal cords of the perfused rats were placed in 30% sucrose and, when it had sunk to the bottom of the container, frozen by immersing the container in CO_2 gas. In case of the decapitated rats, the spinal cords were directly frozen with CO_2 gas. Transverse or sagittal cryostat sections of 30 μm thickness were cut, thawed onto slides and air-dried. The sections were processed with silver amplification procedure and counterstained with toluidine blue that described by Danscher (1982) and Danscher and Montagnese (1994).

Electron microscopy

The spinal cords of the perfused rats were transversely cut on a vibratome sections (150 μm thickness) and the sections were silver amplificated for 1 hour according to Danscher method (1982). The sections were osmicated, dehydrated, embedded Epon, cut into 3 μm semi-thin sections and stained with 1% toluidine blue. The selected sections were re-embedded, cut in an ultratome and stained with uranyl acetate and lead citrate.

RESULTS

The cytoarchitectonic laminar scheme of the spinal cord was identified by references of Molander *et al.* (1989), Paxinos and Watson (1986) and Rexed (1952, 1954). The transeverse sections of spinal cord C2, C4, T7 and L3 segments were figured by camera lucida (Fig. 1). In the 24 hours of survival time the labeled

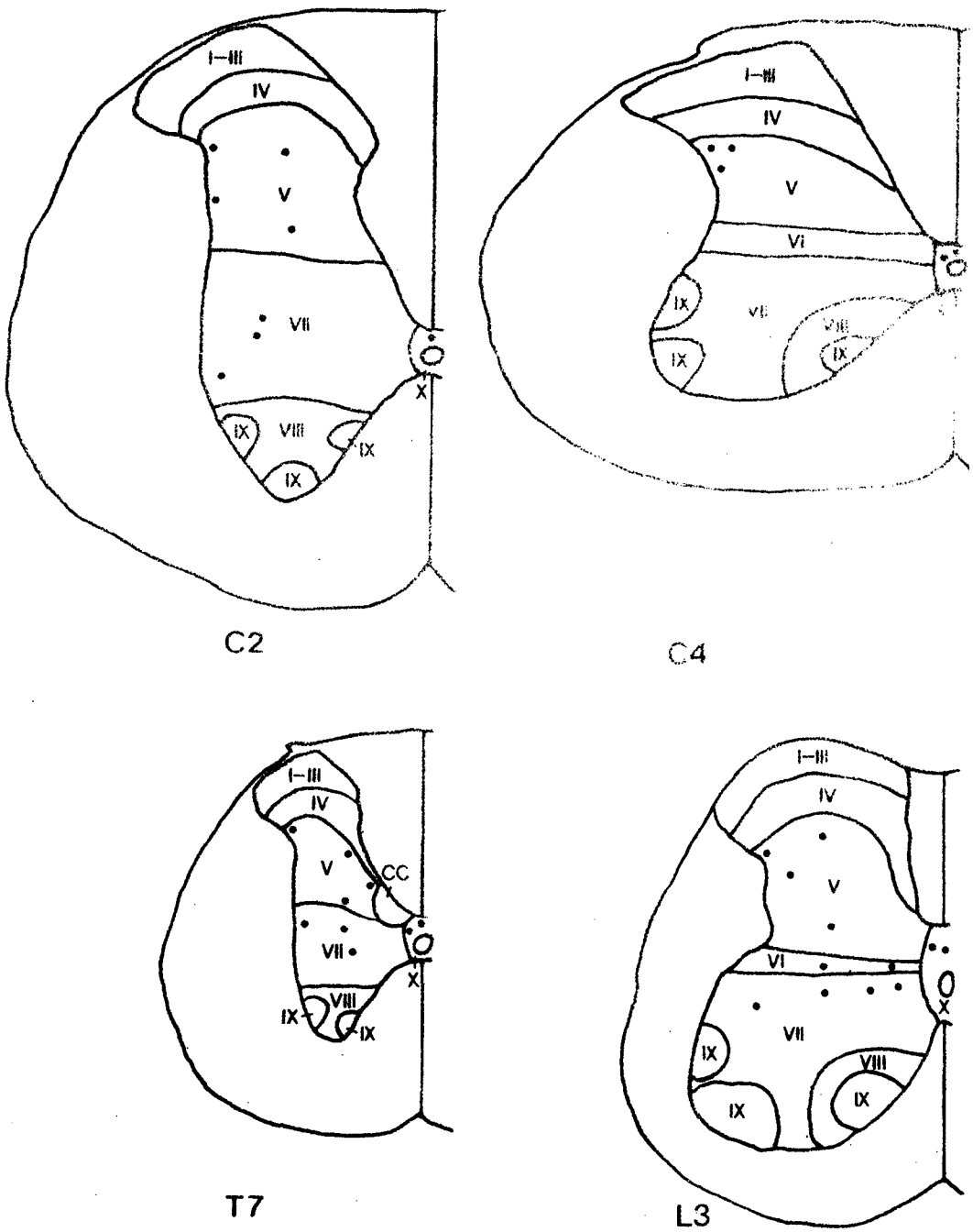

Fig. 1. Drawings of transverse sections of the spinal cord with survival 24 hours. C2, C4, T7 and L3 indicate the second and fourth cervicals, seventh thoracic, and third lumbar segments respectively. Dot denotes the localization site of retrogradely labeled ZEN neuron in the gray matter. Roman numerals refer to lamination. CC means column of Clarke.

somata of ZEN neurons were located in laminae V, VI, VII and X of gray matter whereas not in white matter. The somata were loaded with AMG silver amplified zinc selenium crystals in the lysosomes examined by electron microscope (Fig. 6a). Laminae I, II, III, IV and IX were void of ZEN neuronal somata (Figs. 1, 2). Labeled somata were only distributed in the gray matter (Figs. 1, 3). The huge ZEN neurons were appeared in laminae V and X. In particular, somata of the neurons showed big and elongated form at above site of central canal in lamina X (Fig. 2). In spite of the survival 24 hours or more than the time, some of zinc selenium crystals in boutons were not retrogradely transported to the somata of their neurons. The crystals were precipitated in the cytoplasm of somata at survival 8 hours (Fig. 4).

ZEN neuronal boutons with the zinc precipitates were distributed in the gray matter and in the lateral and ventral funiculi of the white matter at exposure time 1 hour (Fig. 5a). In the white matter, the boutons were located along the axons in the lateral and ventral funiculi but not in cuneate fasciculus, dorsal corticospinal cord and gracile fasciculus. Size of the boutons within the lamina IX was big compare to those in another laminae (Fig. 5b).

At the ultrastructural level, the zinc selenium products were distributed in vesicles of the bouton (Fig. 6b).

DISCUSSION

This study demonstrated the presence of ZEN neurons in the spinal cord of rat. At the survival 24 hours or more than the times, the loaded somata of the neurons were located in laminae V, VI, VII and X of spinal cord gray matter. The neurons were labeled by retrograde transport of zinc selenium crystal lattices created in the synaptic vesicles of boutons after an intraperitoneal injection with sodium selenite (Danscher, 1982; Howell and Frederickson, 1989; Frederickson and Danscher, 1990). Such neurons have been described to take place also in the brain by Slomianka *et al.*(1990). At 24~48 hours, the AMG staining seems to be at its maximum and then it starts to disappear. However after 65 hours, zinc precipitates can still be AMG silver amplified in the larger ZEN neurons.

As shown in Fig. 4, nearly after the survival 8 hours, a little amount of zinc selenium crystals were retrogradely transported from the boutons to somata of the ZEN neuron and the crystals were precipitated in the cytoplasm of the perikarya. The 8 hours appearence of zinc granules in the ZEN somata suggest that the neurons have very short axons supporting the notion that all or part of the spinal cord ZEN neurons are interneurons.

Even 48 hours after the sodium selenite exposure, boutons staining could still be recognized around neuronal somata and along axonal ramifications. This observation intimate that not all of the ZEN vesicles are retrogradely transported or release their contents into the lysosomes. In the original selenium method paper (Danscher, 1982) it was demonstrated that in central nervous system the contents of ZEN boutons are released into the synaptic clefts as are the case after local intracerebral injection of sodium sulphide (Danscher, 1984; Pérez-Clausell and Danscher, 1985). It can be concluded that ZEN vesicles might be present in two different forms 1) a form that result in release of zinc ions, albeit zinc selenium crystal lattices, into the synaptic clefts 2) vesicles that return to the somata of the ZEN neurons through axonal transport, or

release their content into the smooth endoplasmic retriculum wherein crystals are transported to end up in the lysosomes.

The lysosomal presence of the retrogradely transported zinc selenium crystals has been observed also in central nervous system (Slomianka *et al.*, 1990; Howell *et al.*, 1991; Danscher and Montagnese, 1994).

At one hour after intraperitoneal injection of high dose of sodium selenite (20 mg per kg body weight) the gray matter demonstrates a dense innervation with boutons. Already a light microscopical level it is immediately noticeable that the AMG stained boutons not only are of different size and sometime reveal a kind of grouping ZEN boutons, but also that they are arranged in relation to the surface of the neurons. In the white matter rows of ZEN boutons are seen radiating into the lateral and ventral funiculi. This arrangement is in good agreement with the known protrusions of axons from neurons in the gray matter. The dorsal funiculus do not process such invading axons and do in accordance not contain ZEN boutons.

ABSTRACT

The somata and boutons of the zinc-containing neurons in the spinal cord of the rats were labeled by intraperitoneal injection of sodium selenite and silver amplification. The labeled somata of the neurons were located in laminae V, VI, VII and X of the gray matter. The zinc selenium reaction products were retrogradely transported and precipitated into somata of the neuron with survival 8 hours. This observation suggest that all or part of the spinal cord zinc-containing neurons are interneurons. At survival 1 hour, the loaded axonal boutons with zinc precipitates of zinc-containing neurons were distributed in the gray matter and in the processes of lateral and ventral funiculi of the white matter.

In particular, AMG stained boutons with huge form were appeared in the lamina IX.

Ultrastructurally, the zinc precipitates were located in the cytoplasmic lysosomes or the vesicle within boutons of the zinc-containing neuron in accordance with survival times.

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FIGURE LEGENDS

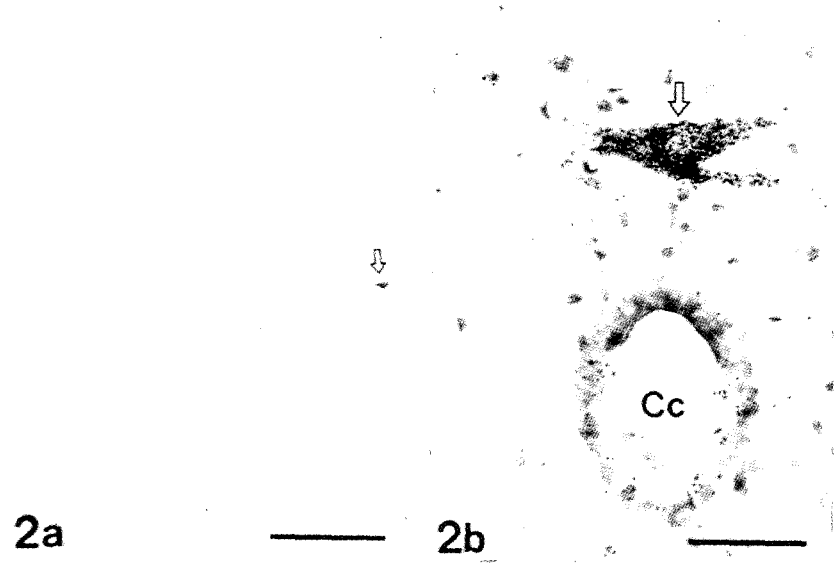
Fig. 2. a, Labeled somata (arrow) of the ZEN neuron was located at above site of central canal in lamina X obtained from C4 segment of perfused rat with survival 48 hours. Scale bar indicates 500 μm.
b, Higher magnification of the labeled neuron (arrow) in the lamina X of a.
Abbreviation: Cc, central canal. Scale bar indicates 50 μm.

Fig. 3. Sagittal section of segment from the decapitated rat with survival 24 hours.
Labeled somata (arrows) were located in the gray matter. Abbreviations: Gm, gray matter; Wm, white matter. Scale bar indicates 200 μm.

Fig. 4. Sagittal section of segment from the decapitated rat with survival 8 hours. The retrogradely transpotred zinc granules were started to precipitate in somata of the ZEN neurons. Arrows indicate the precipitated zinc in ZEN neuronal somata in the gray matter. Scale bar indicates 140 μm.

Fig. 5. a, The loaded bouton with zinc precipitates were distributed in the gray matter and along the axons of the lateral and ventral funiculi in the white matter obtained from C4 segment of perfused rat in survival 1 hour. Abbreviations: Cc, central canal; Cf, cuneate fasciculus; Dct, dorsal corticospinal tract; Lf, lateral funiculus; Vf, ventral funiculus. Scale bar indicates 400 μm.
b, Magnification of the framed area in a. In particular AMG stained boutons in lamina IX were big size compare to those in another laminae. Scale bar indicates 100 μm.

Fig. 6. a, Electron micrograph showed the zinc precipitates in the lysosomes ringing the nucleus of the ZEN neuron that located at above site of central canal in lamina X of L2 segment obtained from survival 24 hours. Abbreviation: N, nucleus. Scale bar indicates 2.5 μm.
b, The bouton with zinc precipitates in the gray matter. Zinc granules were confined to vesicles of the bouton from C2 segment of survival 1 hour. Scale bar indicates 0.02 μm.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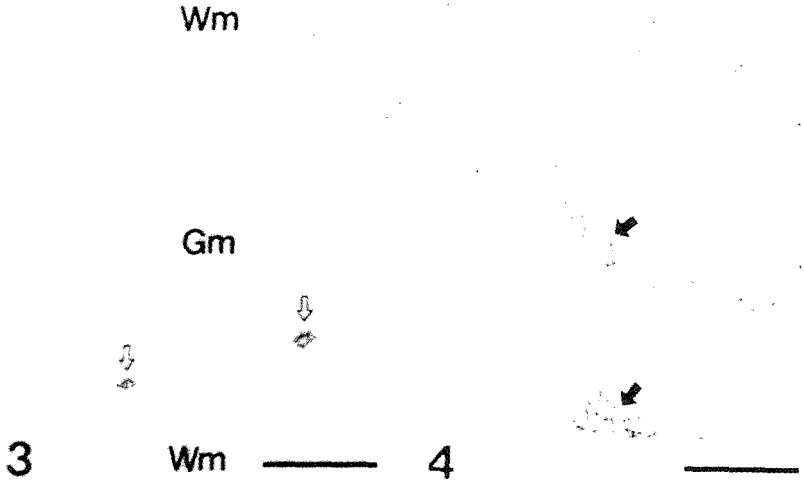
2b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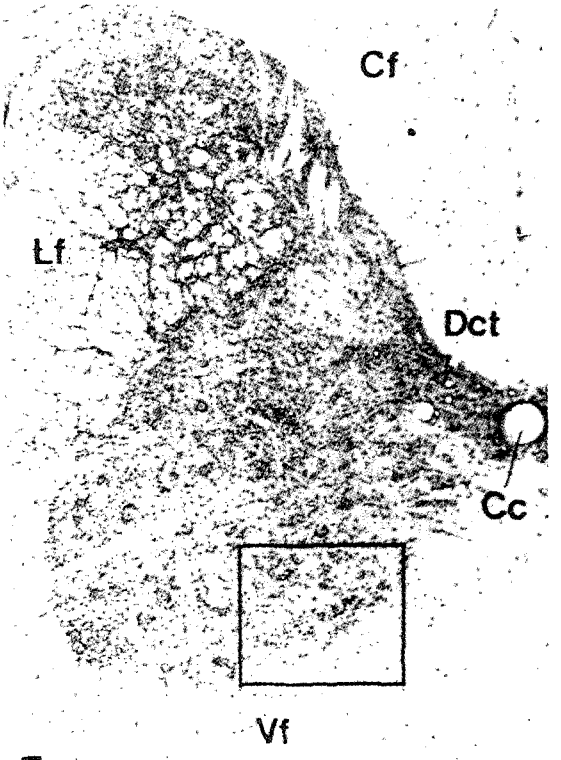

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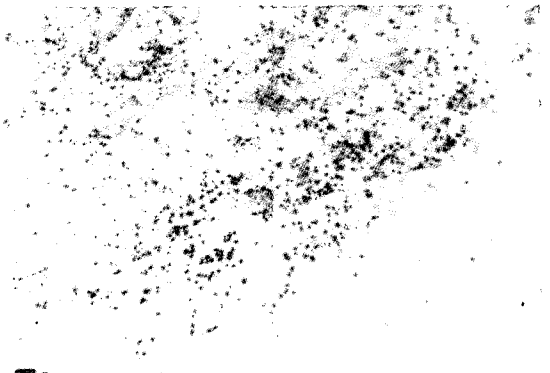

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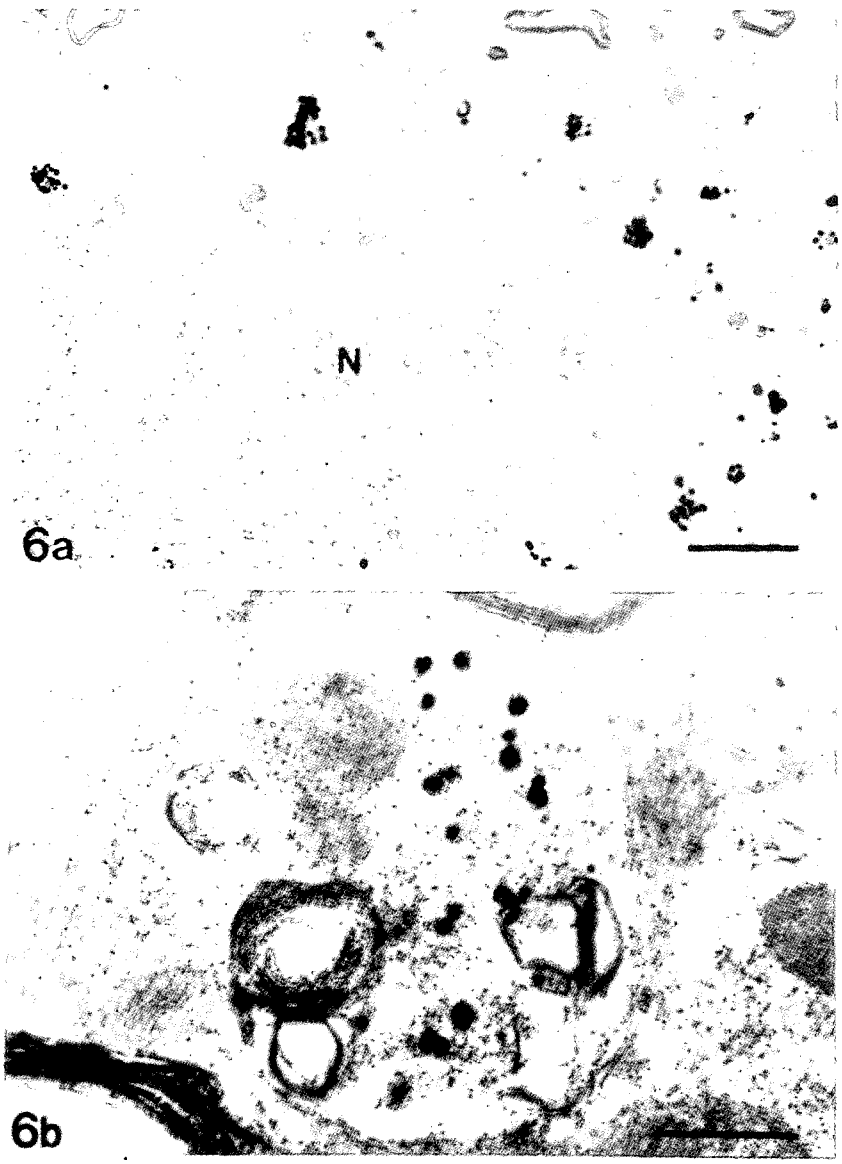
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