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Manganese Neurotoxicity in *Oreochromis niloticus*

Annabelle A. Herrera* and Elena S. Catap
Institute of Biology

ABSTRACT

Manganese is not an acutely hazardous environmental contaminant at low levels, but increased dose produces serious degenerative disorders in Oreochromis niloticus. *Sublethal exposure of fry to 2000 mg/L manganese chloride for eight days displays evidences of poisoning, and hard hit is the brain. Light microscopy shows appearance of gaps between brain layers and cell destruction. Electron microscopy shows damage to subcellular structures.*

INTRODUCTION

Manganese (Mn), although important in different industries, is among the metals which have contributed to the increasing possibilities of health hazards due to exposure. Thus, while some metals are classified as essential nutrients, like Mn, they can also serve as environmental hazards if the mechanisms which maintain them within their functional limit are unbalanced.

Manganese is one of the more abundant elements in the earth's crust and is widely distributed in soils, sediments, rocks, water and biological materials. The major sources of man-made environmental pollution by Mn arise in the manufacture of alloys, steel and iron products (1). Other sources include mining operations (2), the production and use of fertilizers and fungicides (3), and the production of synthetic manganese oxide and dry cell batteries (4). Organomanganese, fuel additives and anti-knock ingredients (5), though only a minor source at present, could significantly increase exposure. This means that for the first time, the general population

* Author to whom correspondence should be addressed.

can be exposed daily to low levels of this essential but potentially neurotoxic metal.

Occupational exposure to Mn has led to the discovery that it can affect the respiratory tract (Mn pneumonitis due to acute exposure) and the central nervous system (chronic Mn poisoning), also known as manganism (6). Non-occupational cases of manganism have also been reported (7).

Most studies on Mn toxicity are concerned with its effects on the central nervous system (CNS). Investigations have shown that the symptoms and signs of Mn encephalopathy share several features in common with Parkinson's disease and have directly linked the metabolism of catecholamines and the concentration of Mn in the brain (8,9,10,11,12). It has been observed that rats chronically treated with high oral load of $MnCl_2$ showed decreased concentrations of dopamine and homovanillic acid (HVA) in the brain. A return to normal value was observed after L-DOPA injections (13).

Studies on neuropathology of Mn are quite limited (7,14,15). Understandably, research works on Mn neurotoxicity were done using mammalian species. Investigations on the toxicity of Mn on aquatic organisms have been done but they are relatively few. This metal can be released into the water by industries like steel and mining and through fungicides from run-off water (16). Hematological abnormalities in *Colisa fasciatus* exposed at sublethal concentrations were observed (17). Decreased spermatogenic activity and hemorrhage in the testes were also reported (18).

The objective of this research is to investigate the extent of damage inflicted by Mn exposure on the brain of *Oreochromis niloticus* by observing the histopathological and ultrastructural alterations. These findings would be significant in understanding fully the mechanisms of manganese neurotoxicity.

MATERIALS AND METHODS

Oreochromis niloticus fingerlings from the Central Luzon State University, Muñoz, Nueva Ecija, were acclimated for one week prior to Mn exposure. The 96-hr LC50 was determined to be 3000 mg/L, after which 30 fingerlings were exposed to a sublethal concentration of Mn (as $MnCl_2$) at 2000 mg/L while another group of 30 fingerlings served as control. Water spiked with $MnCl_2$ was changed everyday. Brain from both control and treated groups were then dissected after 8 days of exposure. Brain tissue was then processed

using standard EM technique. Sections were observed using a JEOL 100 EM. Photomicrographs and electron micrographs were then prepared.

RESULTS AND DISCUSSION

Under the light microscope, limited histological alterations are revealed in the metencephalon, mesencephalon and other brain regions. Fig. 1 shows the normal histoarchitecture of the metencephalon with its intact gray and white matter. The gray matter is made up of the outer molecular layer, a central thin layer of Purkinje cells and the granular layer. The neurons of the granular layer are about the smallest in the organism and have a typical structure of perikaryon and nerve-fibers (Fig. 2). The granule cell (cerebellar granule) has several dendrites and one axon.

The Purkinje cells are large. The most superficial layer of the gray matter (molecular layer) has few perikaryons and nerve fibers are mostly unmyelinated (Fig. 2).

The white matter makes up the central area of the metencephalon (Fig. 3). In this region are found nuclei of gray matter. Supportive cells, the neuroglia, and fibers of myelinated and unmyelinated kind are revealed in this area.

Figure 4 shows a part of the treated metencephalon. With the low magnification possible under light microscopy, no apparent change in the histoarchitecture of the granular layer is revealed. Figure 5, however, shows that a gap appears between the gray and the white matter.

In the white matter, scattered axon cross-sections are observed (Figs. 6 & 7). It is possible that improper myelination of the developing axons had taken place or demyelination might also have occurred as an effect of the metal Manganese on the enzymes for fat metabolism. This finding has also been reported in studies on pesticide toxicity in fish brain (19).

Cardozo and Bonilla reported that Manganese poisoning was able to produce cortical and striatal alterations in rats. The results of this study also suggest that relatively low concentrations of Mn orally ingested can induce morphological alterations observed through light microscopy (14).

Analysis of the results using electron microscopy revealed ultrastructural changes due to manganese intoxication. Fig. 8 shows the soma or perikaryon of a granular layer neuron of the metencephalon. The large nucleus is euchromatic. The chromatin is finely dispersed which is a reflection of the intense activity of the neuron. In the cytoplasm is the rough endoplasmic reticulum which is often organized into aggregates of cisternae. Between

cisternae are polysomes forming rosettes. These cells synthesize structural proteins and proteins for export. Mitochondria are scattered in the perikaryon.

Figure 9 shows that the axon hillock of the normal neuron typically lacks ribosome and ER. Parallel arrangement of microtubules is initiated in the axon hillock and becomes more pronounced in the initial segment of the axon. The neuron surface is completely covered by synaptic endings of other neurons or by processes of glial cells. There is virtually no intracellular material in nervous tissue (Fig. 10).

Figs. 11 and 12 show that Mn-treated optic tectum perikarya are heavily damaged as seen with electron microscopy. The typically spherical nucleus forms an evagination to present a dumbbell shape. The cell membrane appears ruptured and cell organelles can not be identified. In some neurons, as shown in Fig. 13, structures around the nucleus are reduced to scattered debris.

It has been suggested that the swelling and rupture of the cell and other cell organelles is caused by the effects of heavy metals on the membrane ATPase system, and that a disruption would lead to a reduction in the synthesis of ATP, resulting to an increased permeability of these membranes (20). It has been reported that brain mitochondria can accumulate Mn^{++} and Ca^{++} (21), thus, this influx of cations and water as well will cause the swelling and rupture of the membrane-bound structures. These cellular changes do occur in many cells following injury.

It was also reported, in a related study, that administration of $MnCl_2.4H_2O$ to rats for a period of 90 days produced 10% inhibition in the activity of Mg^{++}-ATPase, a response which could initiate cytotoxic effects (22). Another enzyme, hexokinase, which is one of the key and rate limiting enzymes of brain glucose metabolism, has been demonstrated to be inhibited by several potentially neurotoxic metals, including manganese (23).

A study on the uptake and accumulation of Mn in the brain regions of rats show that it is taken up by striatal, midbrain and thalamic regions at a greater rate than other brain areas. These data support the contention that the neuronal damage inflicted by Mn occurs as a direct result of the metal being selectively concentrated by, and thus more quickly reaching toxic levels in certain nuclei of the extrapyramidal system. Also, the metal has been shown to be able to initiate redistribution of magnesium (Mg) within the CNS, causing it to become increased in myelinated areas and reduced in certain areas. Mg concentrations were observed to increase significantly in

the corpus callosum, a region rich in myelinated fiber and had one of the lowest concentrations of Mg (11).

This study shows that histological alterations in the brain cells can be induced by Mn intoxication as revealed by light and electron microscope observations, along with biochemical and physiological responses reported from previous studies.

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Fig. 1. Photomicrograph of the control metencephalon with intact gray (g) and white matter (w) (x 100). Fig. 2. Higher magnification of Fig. 1. The gray matter is made up of the outer molecular layer (m) middle Purkinje cell (p) layer and inner granular layer (g) (x 400).

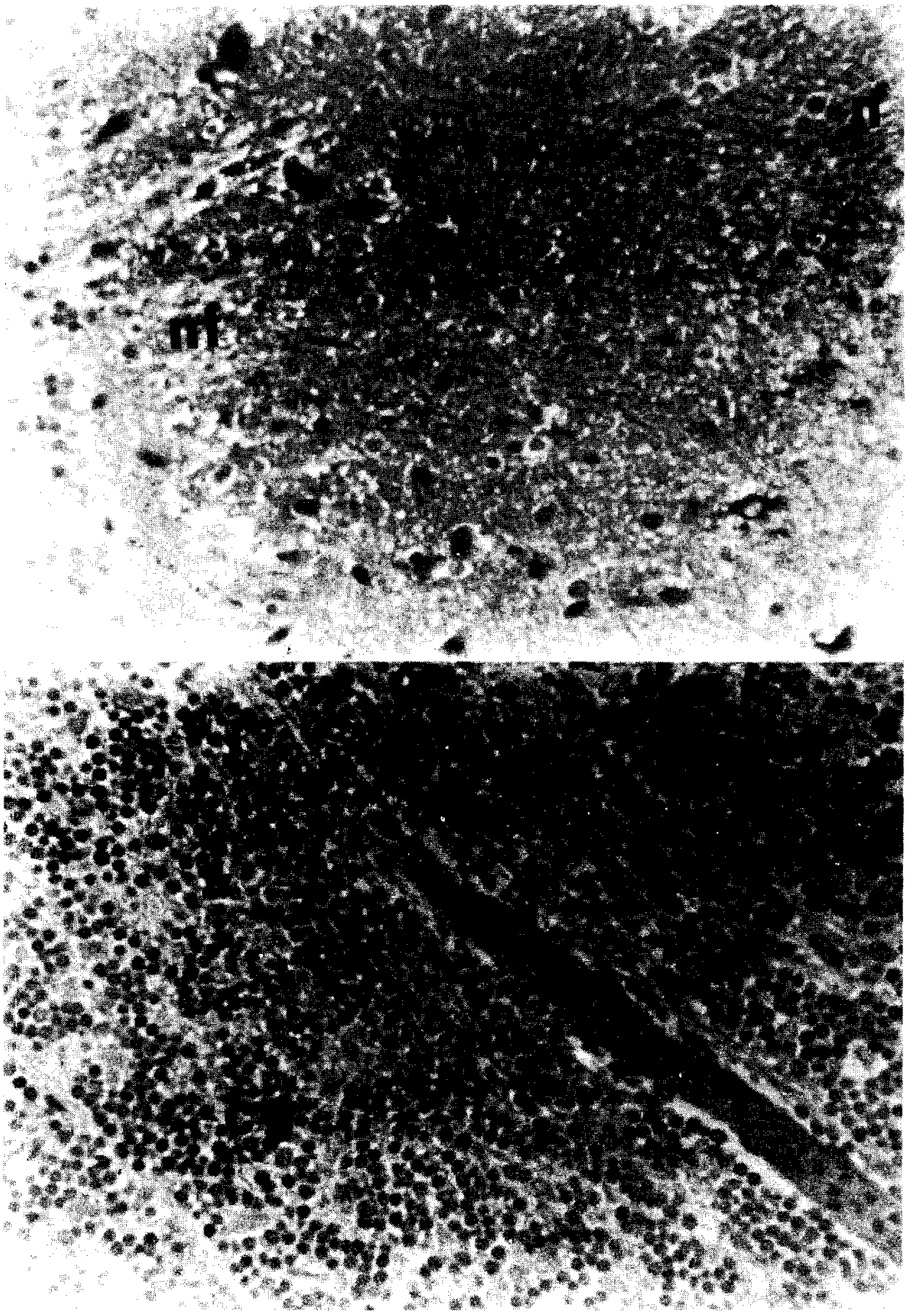
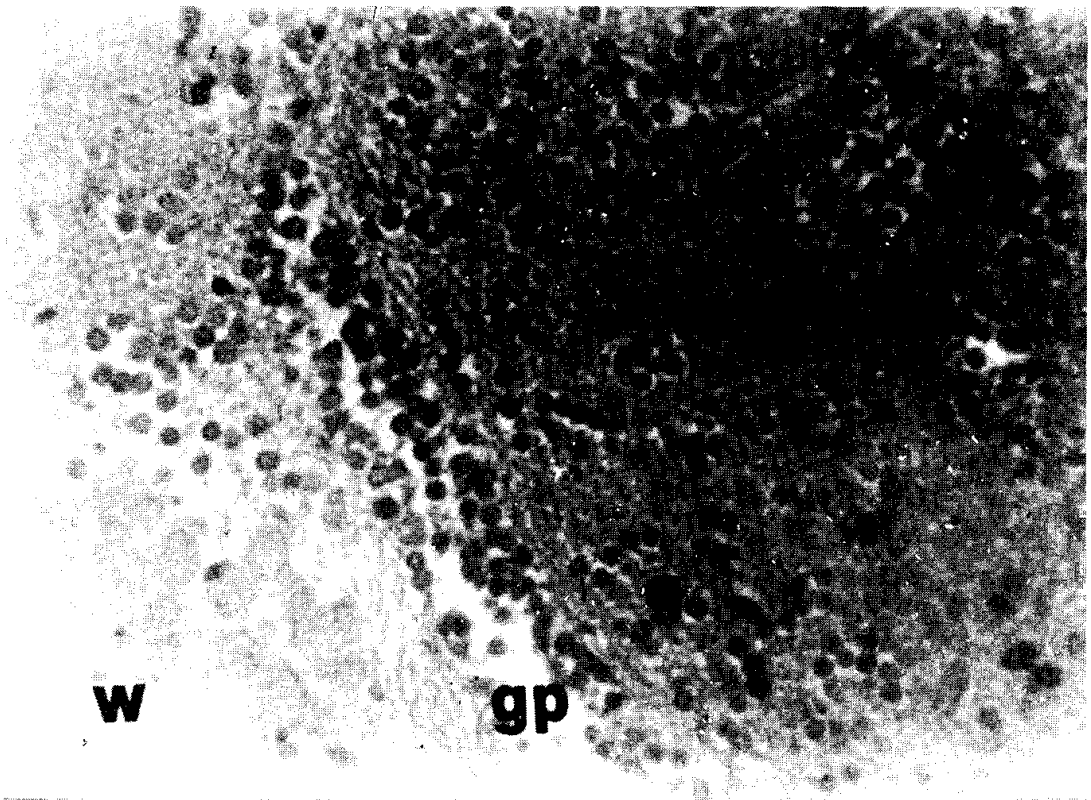


Fig. 3. Higher magnification of Fig. 1. The control white matter is made up of normal myelinated and unmyelinated nerve fibers (mf) and neuroglia (n) (x 400).
Fig. 4. The treated granular layer contains cerebellar granule cells (cg) (x 400).



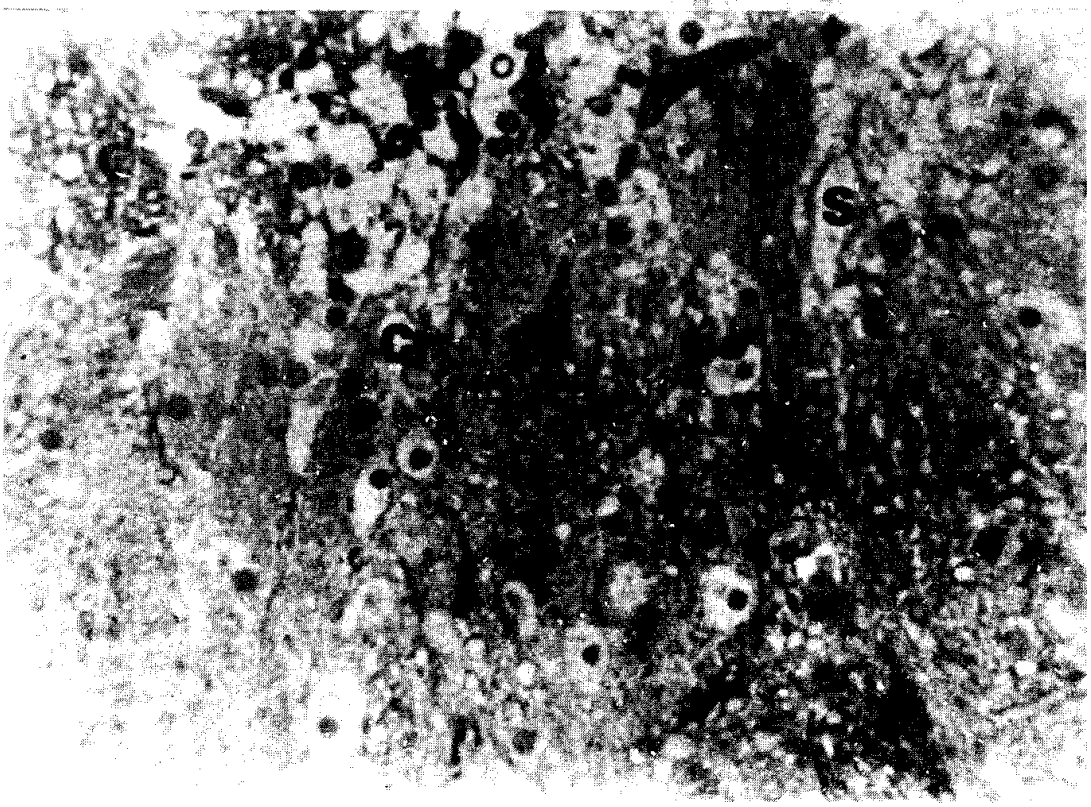


Fig. 5. A gap (gp) appears between the granular layer (g) and the white matter (w) in the treated brain (x 400). Fig. 6. Spaces appear between nerve fibers in treated brain. Neuron cytoplasm (c) degenerates (x 400).

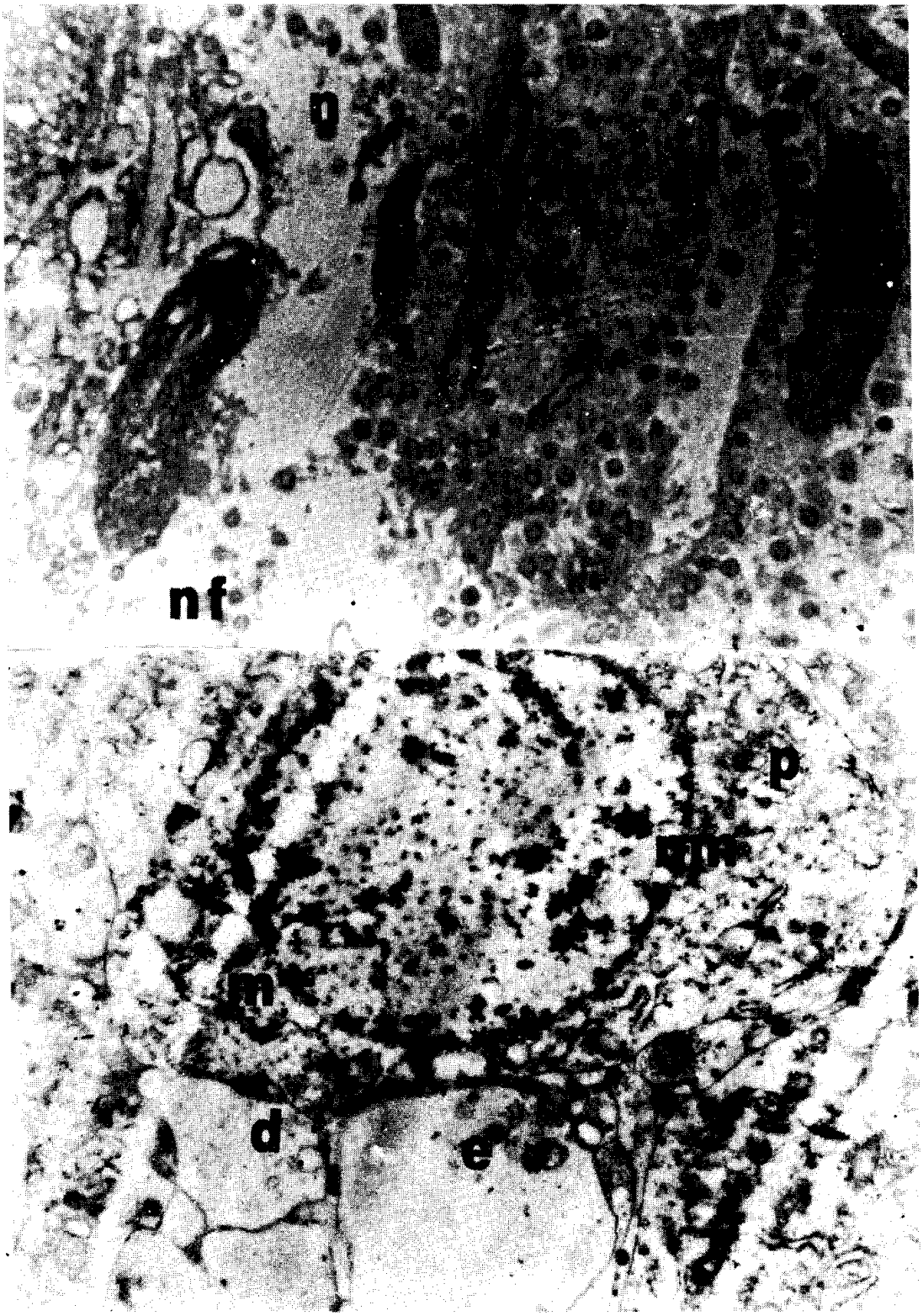


Fig. 7. Other areas show scattered nuclei (n) and nerve fiber (nf) cross-sections (x 400). Fig. 8. Electron micrograph of one of the granular cells of Fig. 2. The untreated metencephalon soma or perikaryon of the granular layer shows well-defined nuclear membrane (nm). Polysomes (p) form rosettes in the cytoplasm. A neuron synaptic ending (e) and dendrite (d) are found at the bottom mitochdrion (m) (x 6,000).

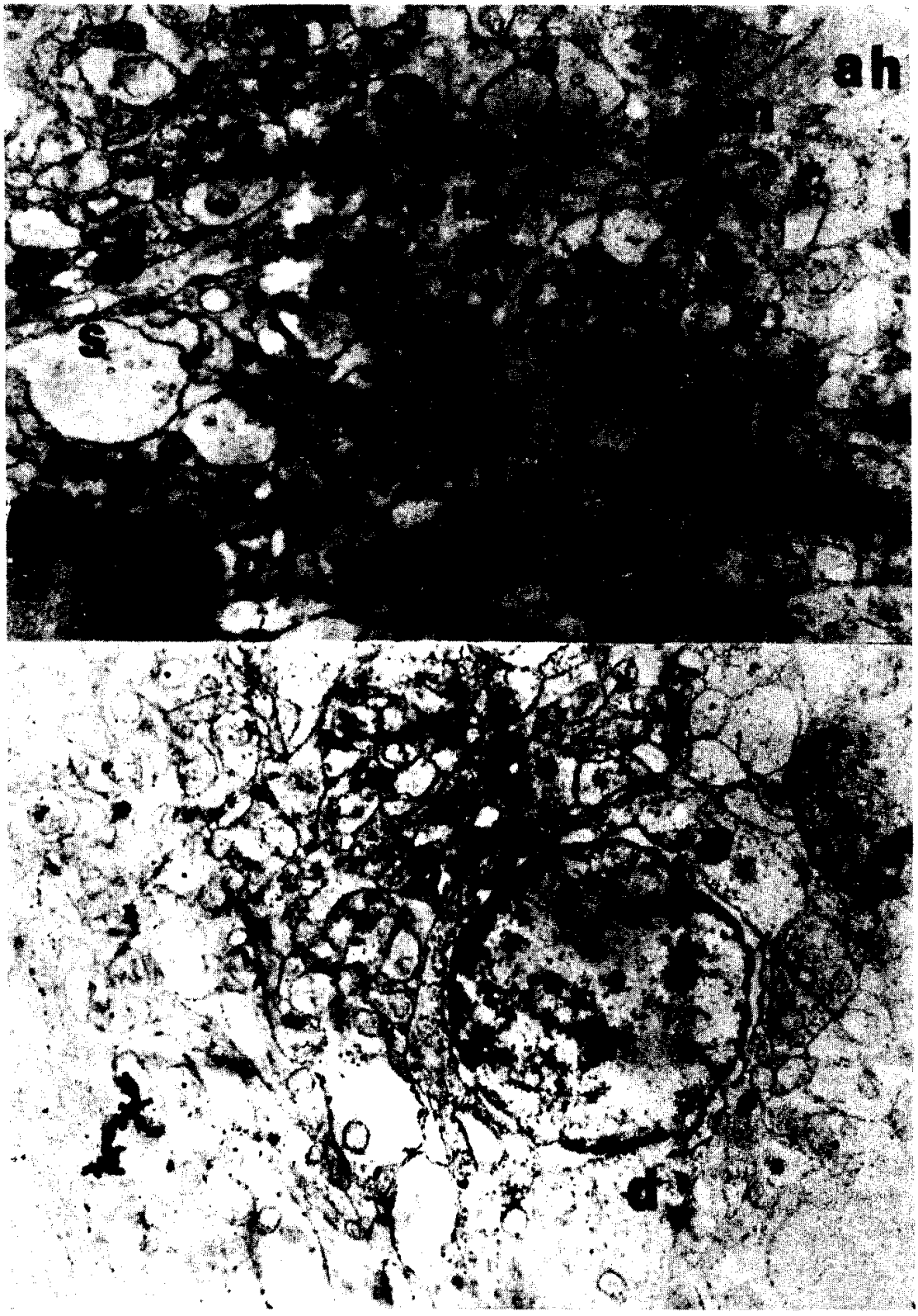


Fig. 9. Electron micrograph of the axon hillock (ah) shows lack of ribosomes and ER. Parallel microtubules (pm) are seen in the hillock and the first segment (IS). Other structures are synaptic endings of other neurons (s) and processes of glial cells (g) (x 6,000). Fig. 10. The perikaryon (p) is completely invested by synaptic endings of neurons and glial (g) process (x 6,000)

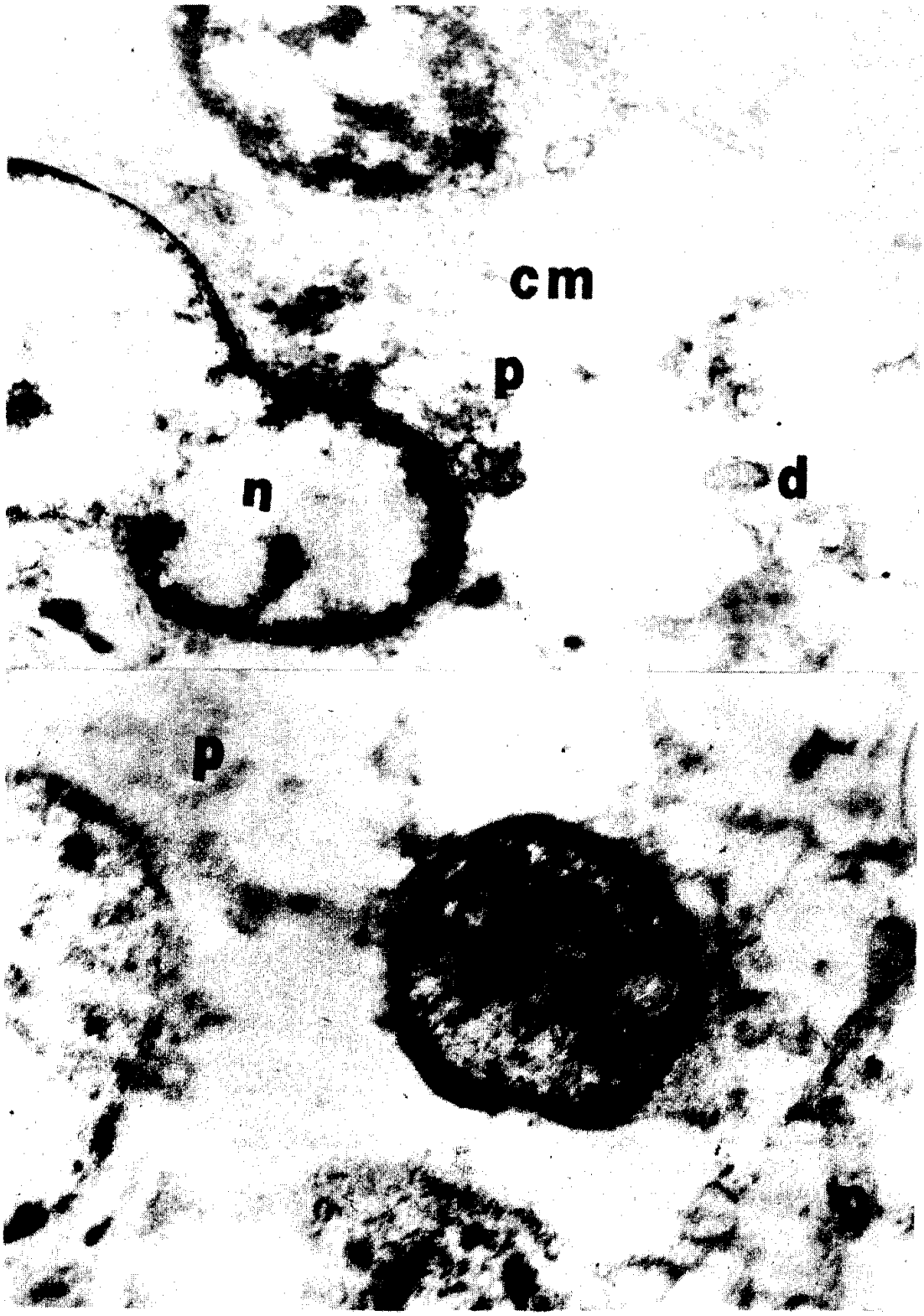


Fig. 11. The perikaryon (p) of a heavily damaged mesencephalon neuron. The spherical nucleus (n) becomes bilobed and the cell membrane (cm) ruptures, so that cell organelles are scattered as debris (d) (x 10,000). Fig. 12. The cell membrane and organelles are not distinct in some neurons of the treated brain. Surrounding nerve endings and glial cells are not evident and debris (d) is scattered all around (x 10,000).

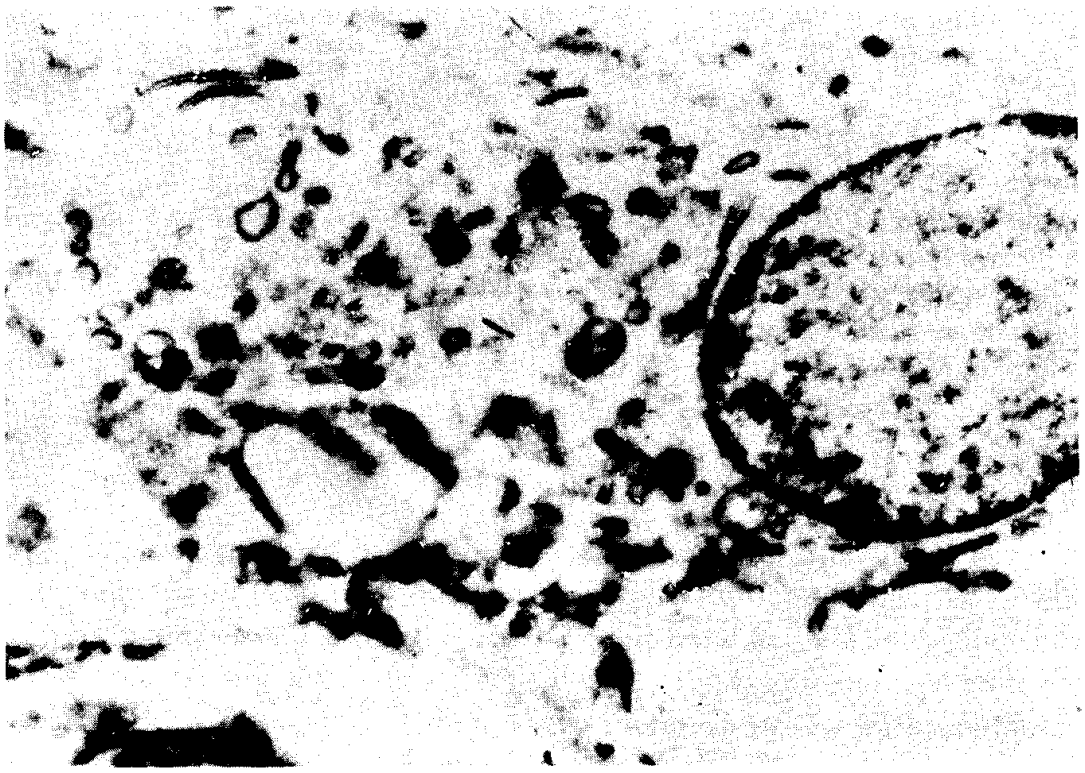


Fig. 13. The cell organelles around the nucleus are reduced to unidentifiable structures (x 6,000).