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THE EFFECT OF MANGANESE ON THE ULTRASTRUCTURE OF PITUICYTES *IN VITRO*

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Manganese (Mn) is widely distributed in nature but occurs only in trace amounts in biological material, particularly in animal tissues. A deficiency of this essential trace element results in a wide variety of structural and physiological defects which reflect altered biochemical functions (Underwood 1977). It is known that Mn plays an important role in the maintenance of normal function of central nervous system (CNS). It seems likely that the metal fulfils this role in conjunctions with enzymes acting as their dissociable cofactor (Utter 1976). In excess Mn is toxic. Manganese toxicity induces disorders in the function of the CNS in experimental animals and humans with symptoms similar to those of Parkinson's disease (Cotzias 1958; Chandra 1970; Śmiałek, Mossakowski 1981). It was recently reported by Rehnberg et al. (1980) that chronic Mn exposure of the young rats results in its high brain and pituitary concentrations. As it was revealed, the relative levels of Mn in cerebrum, hypothalamus and pituitary were 16—30 times higher than those in control tissues for the same age. It is also supposed that an unusual accumulation of manganese may be controlled by genetically conditioned processes (Ulmer 1973). Manganese produces a dose-dependent depression of proliferation, inhibition of the colony forming ability and DNA synthesis in mammalian cell lines *in vitro* (Fisher, Škreb 1980). It also stimulates the misincorporation of both ribo- and desoxyribonucleotides in various *in vitro* systems (Murray, Flessel 1976), and reduces phagocytosis in alveolar macrophages (Graham et al. 1975). Manganese is not considered a carcinogenic metal (Sunderman 1978).

The site and the mode of action of manganese at the cellular level are yet little known. The aim of present study was to determine on a ultrastructural basis the susceptibility of neurohypophyseal glial cells

—pituicytes—to manganese toxicity. Using the technique of organotypic culture of 14 day hypophyseal neural lobes derived from newborn rats, the direct effects of manganese on the morphological structure of neurohypophyseal glial cells, were investigated.

MATERIAL AND METHODS

Organotypic cultures were prepared from newborn Wistar rat neural lobes. The neural lobes were separated from the pars intermedia and pars distalis of the hypophysis. After removal of the capsular connective tissue, neural lobes were explanted on collagen-coated coverslips and maintained in Carrel flasks. The nutrient medium, renewed twice weekly, consisted of 50% human serum, 40% Earle's solution (pH 7.2), and 10% 9-day-old chicken embryo saline extract. It was supplemented with glucose to a final concentration of 600 mg/100 ml and penicillin of 100 units/ml. The pH of the medium ranged from 7.0 to 7.3.

Selected 14 day cultures were subjected to manganese chloride for three days. Manganese chloride was added to the incubating medium in two doses: 6 and 12 mg%.

For electron microscopy the cultures were briefly rinsed in Locke's solution. The fixation was carried out in 1.5% glutaraldehyde in 0.2 M cacodylate buffer, pH 7.2, for 30 min at 4°C. The tissues were then rinsed in the same buffer for 1 h, and postfixed for 1 h in 1.5% osmium tetroxide in 0.2 M cacodylate buffer, pH 7.2. The cultures were embedded in Epon 812, and ultrathin sections counterstained with uranyl acetate and lead citrate and examined with a JEM 7 A electron microscope.

RESULTS

Group I

Fourteen day cultured pituicytes of newborn rats, exposed for three days to manganese chloride in a dose of 6 mg% in culture medium, revealed ultrastructural abnormalities in comparison to control cultures of the same age. The general morphological features of cultured pituicytes have been described previously (Zaręba-Kowalska et al. 1983; Gajkowska, Zaręba-Kowalska 1983), so they will not be repeated in the present report.

Two categories of pituicytes could be distinguished in experimental cultures. The majority of pituicytes belonged to the first category. In these cells a significant increase in smooth endoplasmic reticulum (SER) was observed in contrast to relative scarcity of RER profiles. The SER formed a three-dimensional network of channels, frequently dilated and filled with an amorphous substance of moderate electron-density. Also

observed was a profusion of smooth-surfaced vesicles in close association with the SER system (Fig. 1). Striking changes affected the mitochondria. Most of them were swollen and exhibited an electron lucid matrix and a reduced number of cristae often placed in peripheral position. In some pituicytes with swollen mitochondria numerous glycogen particles as well as lipid droplets were observed (Figs 2, 3). The cellular nuclei of some pituicytes belonging to this cell population showed changed nucleoli. Observed changes resembled segregation of nucleolar material on granular and fibrillar parts.

Less numerous pituicytes belonging to the second category had normally developed RER and SER but in some of them an increased number of lipid droplets appeared. The majority of mitochondria in these cells were normal and only some of them contained osmophilic inclusions. The nuclei and nucleoli of these cells did not reveal any abnormalities (Fig. 4).

It is worth noting that some pituicytes in this experimental group contained numerous filaments scattered between cytoplasmic organelles (Fig. 5).

Group II

Fourteen day cultured pituicytes exposed for three days to manganese chloride in a dose of 12 mg% revealed more marked changes in ultrastructure. Nearly all pituicytes possessed altered nucleoli, showing segregation of its material while in their damaged, electron-lucent cytoplasm inclusion bodies containing osmophilic material were observed. Also observed was the decrease in the number of ribosomes and polyribosomes (Fig. 6).

In some pituicytes with less damaged cytoplasm, apart from normal mitochondria there were also abnormal mitochondria, mostly swollen with shortened and aggregated cristae or devoid of cristae (Fig. 7).

In some cells large lipid droplets were visible in the cytoplasm (Fig. 8).

DISCUSSION

In the present experimental conditions dose-related cellular effects of manganese intoxication could be demonstrated in neurohypophyseal glial cells, i.e., pituicytes. Our studies revealed ultrastructural alterations both in the cytoplasm of pituicytes as well as in their nuclei. The most striking changes concerned mitochondria. It was possible to follow the subsequent stages of these changes: swelling, accumulation of osmophilic bodies and final transformation into inclusion bodies. Our results confirm previous observations that manganese enters mitochondria where it causes morphological alterations (Cotzias 1958: Autissier 1974;

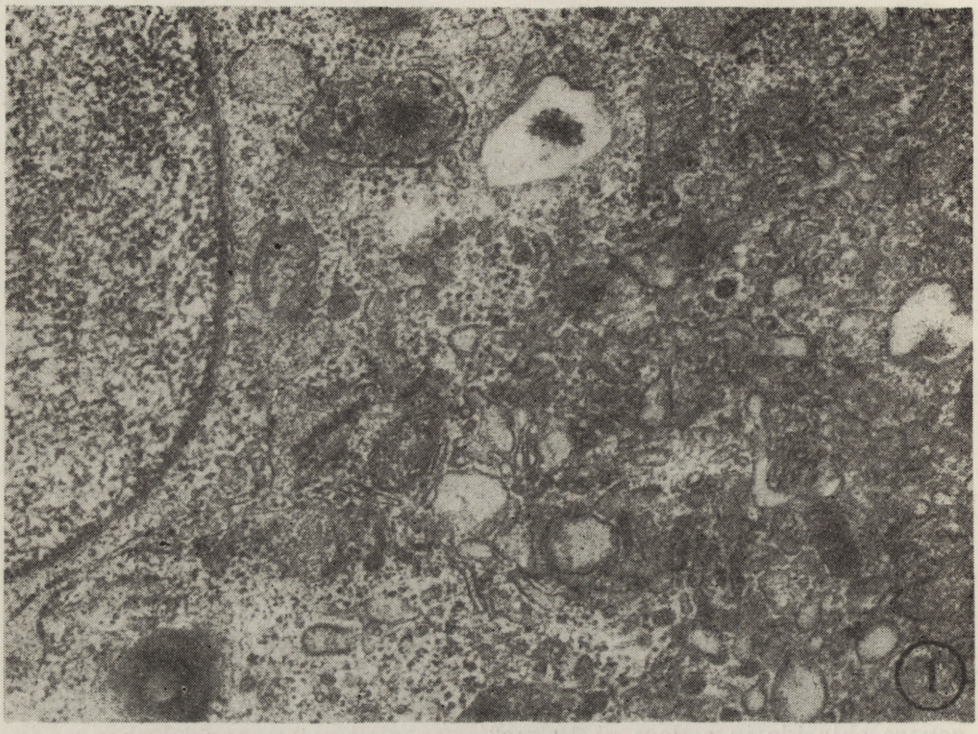

Fig. 1. Group I. Pituicyte treated with manganese chloride showing extensively developed SER. × 13 350

Ryc. 1. Grupa I. Pituicyt poddany działaniu chlorku manganawego. Silnie rozwinięta SER. Pow. 13 350 ×

Gajkowska et al. 1983). These mitochondrial abnormalities are a result of manganese accumulation leading to their functional disorder and reflect an especially important relationship between manganese and mitochondria. It is also worth noting that within untreated, normal cells mitochondria are organelles, which are richest in Mn content and have served as starting material for the isolation of several manganese-containing metalloproteins (Leach, Lilburn 1978). Our attention was also drawn to the appearance of excessive amounts of glycogen and/or lipids associated with mitochondrial abnormalities in some manganese treated pituicytes. Similar observations were previously made in astrocytes in manganese treated cultures of rat striatum by Gajkowska et al.

Fig. 2. Group I. Pituicytes exhibiting swollen mitochondria devoid of cristae (m) and numerous glycogen particles in cytoplasm. × 13 350

Ryc. 2. Grupa I. W cytoplazmie pituicytów obrzęknięte mitochondria pozbawione grzebieni (m) i liczne ziarna glikogenu. Pow. 13 350 ×

Fig. 3. Group I. Pituicyte with altered nucleolus and well developed SER is visible. × 11 500

Ryc. 3. Grupa I. Pituicyt ze zmienionym jąderkiem i dobrze rozwiniętą SER. Pow. 11 500 ×

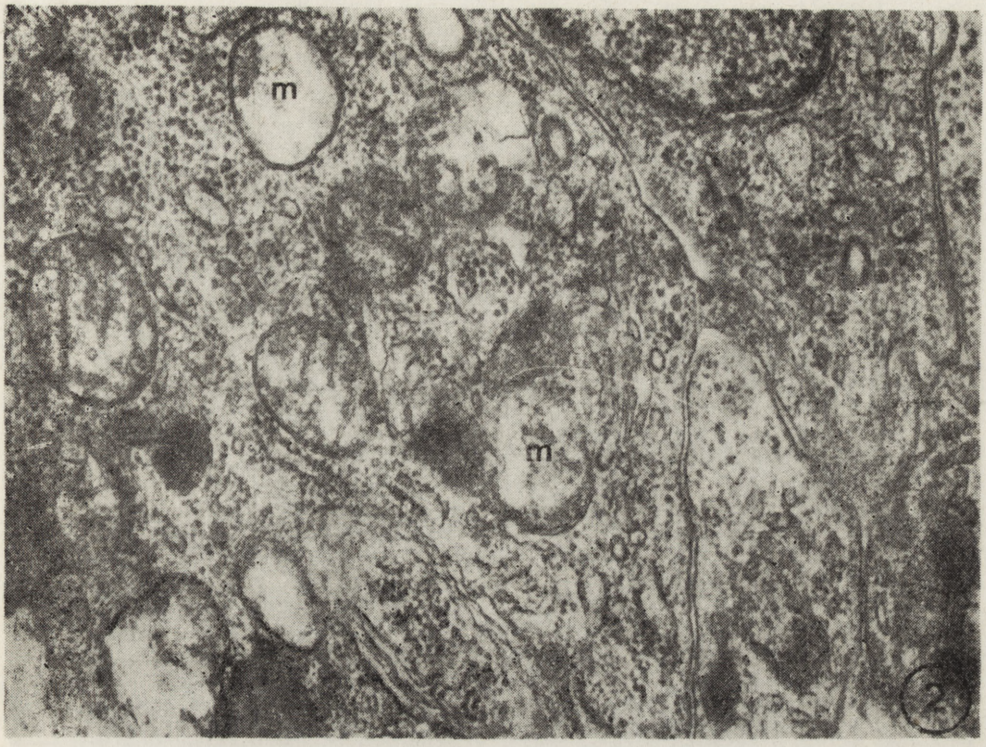

Fig. 4. Group I. Pituicyte. Note well developed RER, numerous lipid droplets and mitochondrium containing osmophilic bodies (arrow) in cytoplasm. × 13 350

Ryc. 4. Grupa I. Pituicyt. W cytoplazmie widoczna dobrze rozwinięta RER, liczne krople lipidowe i mitochondrium zawierające ciała osmofilne (strzałka). Pow. 13 350 ×

(1983). Following the hypothesis of Tassin and Brucher (1982) concerning the pathogenesis and etiological classification of mitochondrial disorders, these morphological pictures may be interpreted as a result of an enzyme deficiency within the Krebs cycle or in the respiratory chain. When there is an enzyme deficiency, carbohydrates, lipid metabolites and their precursors accumulate above the normal level. The findings of Fisher and Škreb (1980) that lactic acid production is stimulated in manganese

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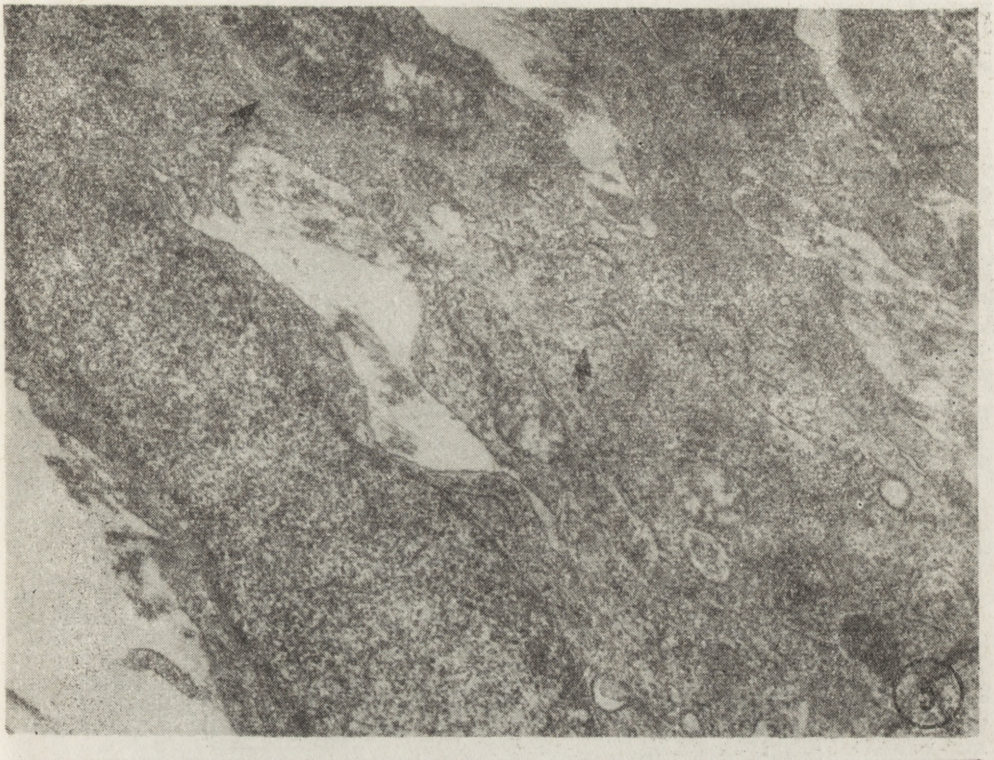
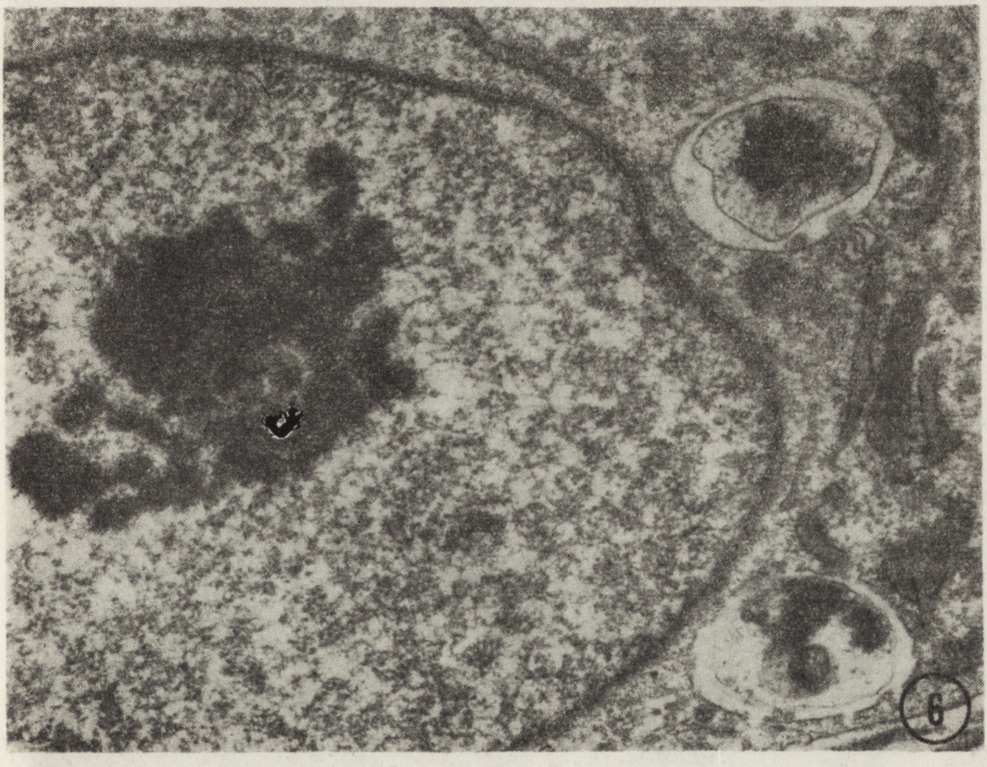
Fig. 5. Group I. Pituicytes. Swollen mitochondria and numerous filaments (arrows) are visible in cytoplasm. × 9 000

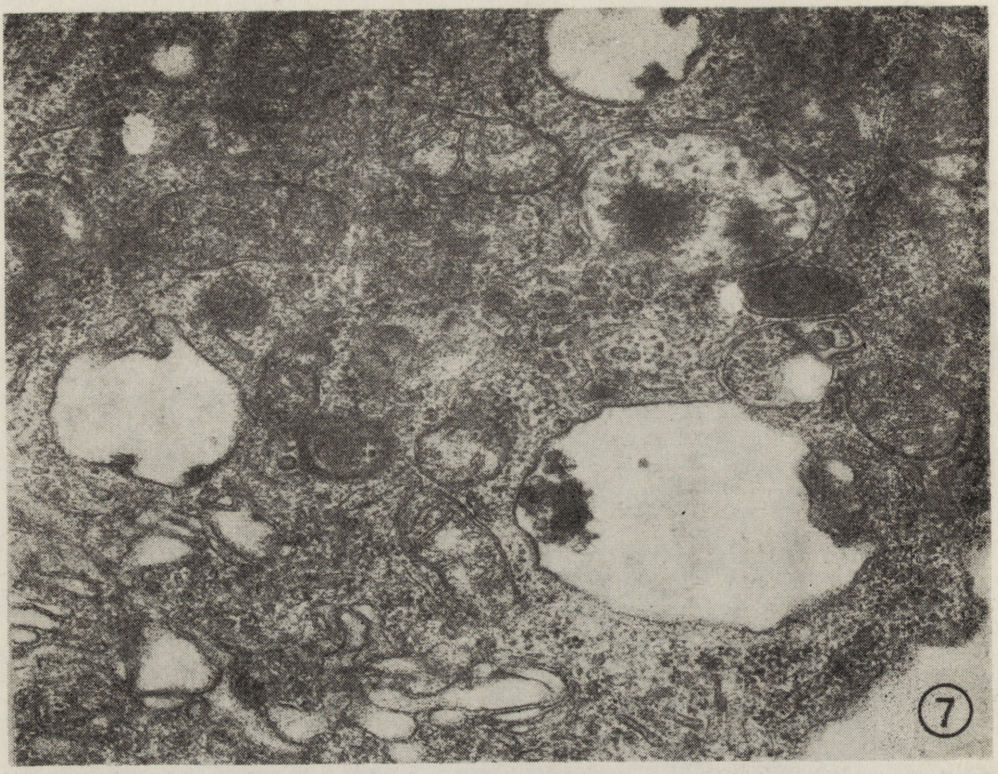
Ryc. 5. Grupa I. Pituicyty. W cytoplazmie obrzęknięte mitochondria i liczne filamenty (strzałki). Pow. 9 000 ×

Fig. 6. Group II. Pituicyte showing changed nucleolus. In cytoplasm mitochondria transformed in inclusion bodies, decreased number of free ribosomes and polyribosomes are visible. × 13 500

Ryc. 6. Grupa II. Pituicyt ze zmienionym jąderkiem. W cytoplazmie widoczne mitochondria przekształcone w ciała wtrętowe, zmniejszona liczba wolnych rybosomów i polirybosomów. Pow. 13 500 ×







treated cells *in vitro* may be explained as a compensatory mechanism following impairment of oxydative respiration. The same authors reported the inhibitory effects of manganese chloride on proliferation, colony formation and DNA synthesis in several mammalian cell lines. The depression of ^3H thymidine incorporation the authors explained by the direct effects of manganese on DNA or its influence on other cellular targets. Our morphological findings: alterations in cellular nuclei and decrease in the number of free ribosomes, polyribosomes and of RER profiles may suggest the inhibition of protein synthesis in treated pituicytes.

The appearance of filaments in large amounts in some pituicytes subjected to the influence of manganese chloride may be associated with unexpected change of the cytoskeleton organization. The possibility cannot be excluded, nevertheless, that this phenomenon may be related to adaptation of cells to specific culture conditions, which was previously reported (Weinstein, Kornblith 1971).

We do not know what quantities of manganese ions were taken up by the cells, during their exposure to metal action, but it can be supposed that considerable amounts of Mn must bind to the cells in a time and concentration-dependent manner. The ultrastructural abnormalities observed in the cells treated can be interpreted as a morphological expression of disturbances in cell metabolism caused by the toxic action of manganese. Ultrastructural alterations in cultured pituicytes were the sum of the effects resulting from numerous intracellular processes and membrane events. The rapidity and importance of mitochondrial reaction, however, must be stressed.

WPŁYW MANGANU NA ULTRASTRUKTURĘ PITUICYTÓW *IN VITRO*

Streszczenie

Badano wpływ jonów manganu na obraz morfologiczny komórek glejowych (pituicytów) płata nerwowego przysadki szczura *in vitro*. Mangan wywołuje zaburzenia metabolizmu komórkowego które objawiają się charakterystycznymi zmianami morfologicznymi: uszkodzeniem mitochondriów, pojawianiem się nadmiernej ilości glikogenu, ciał lipidowych oraz filamentów. Stopień nasilenia zmian jest wprost proporcjonalny do stężenia jonów manganu w medium hodowlanym.

Fig. 7. Group II. Fragment of pituicyte cytoplasm with vacuoles containing osmophilic material is visible. × 13 350

Ryc. 7. Grupa II. Fragment cytoplazmy pituicytu z wakuolą zawierającą materiał osmofilny. Pow. 13 350 ×

Fig. 8. Group II. Pituicyte containing large lipid bodies in cytoplasm (Li). × 13 350

Ryc. 8. Grupa II. Pituicyt. W cytoplazmie obecne duże ciała lipidowe (Li). Pow. 13 350 ×

ВЛИЯНИЕ МАРГАНЦА НА УЛЬТРАСТРУКТУРУ ПИТУИЦИТОВ *IN VITRO*

Резюме

Исследовано влияние ионов марганца на морфологическую картину глиальных клеток (цитуицитов) нервной доли гипофиза крысы *in vitro*. Марганец вызывает расстройства клеточного метаболизма, которые проявляются в характерных морфологических изменениях: повреждениях митохондрий, в появлении чрезмерного количества гликогена, липидных тел а также филаментов. Степень интенсивности изменений прямо пропорциональна к концентрации ионов марганца в культурной среде.

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