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The Toxicity and the Ultrastructural Modifications Induced by Indium in the Brain and in the Placenta Tissues of Pregnant Rat

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Research Article

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ABSTRACT

Indium is a chemical element (with symbol In), being the 61st element in abundance in the earth's crust with a proportion of 0.24 ppm per unit of weight. It has several physicochemical properties that allow it to be used in different fields such as: medicine and industry, which places the human being in direct and indirect contact with this metal. In can be source of some toxicity, noting that its toxicity depends on the absorption route and the compounds studied, embryotoxic or embryologic and teratogenic effects with different compounds (Pilliére, 2003). The aim of this work was to determine the impact of indium in the brain and placenta tissues of a pregnant rats using transmission electron microscopy (TEM). The ultrastructural study of brain tissue (neuron, glial cells) and placenta (maternal and fetal face) of treated rats with indium sulfate showed the presence of dense electron deposits in the lysosomes, altered mitochondria and dilated endoplasmic reticulum, on the other hand, no deposit in the control tissue. It is concluded that indium is a toxic element that can cause some intracellular damages.

INTRODUCTION

The brain is one of the most complex natural systems. Its function, intimately linked to its structure, is to coordinate the whole organism and to integrate the organism in the environment. The placenta is a complex organ essential to fetal development and growth [1]. It allows the survival of the blastocyst and physically and biologically connects the developing embryo to the uterine wall. The brain and the placenta have been chosen because they are two important organs in life, in addition the brain controls all the functions of all organs such as placenta. We have noted that these two organs are necessary, it is also noted that there are problems of toxicity introduced by the using of certain chemical elements such as indium in medicine and in industry.

The objective of this study was to determine the behavior of indium in the brain and placenta tissues of a pregnant rat treated with indium and to determine the cell organelle that is responsible in the sequestration phenomenon as well as some ultrastructural changes induced by the introduction of this metal. To solve these aims we have used Transmission Electron Microscopy.

MATERIALS AND METHODS

We used in this study 24 pregnant female Wistar rats, weighing around 250 g. These rats were divided into two groups (M1, M2), they were kept for 15 days on a 12 h dark/12 h light cycle in the department of Experimental Medicine of Faculty of Medicine of Tunis in order to be acclimated. The rats were fed ad libitum and have no restriction to water. Each cage contained two females, and one male to make the coupling. A vaginal smear test was done to check the pregnancy gestation. Beginning with the 16th day of gestation, the female rats underwent daily injections as follows:

- The first group (M1) of 12 rats received 4 intraperitoneal injections of 1 mL containing 1 mg of soluble solution of indium sulfate [$In_2 (SO_4)_3$] (Merk, France), in order to accumulate a total dose of 16 mg/kg of body weight.

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- The second group (M2) of 12 control rats received physiological serum in the same experimental conditions.

Twenty-four hours after the last injection, all rats were anesthetized, the two organs (brain and placenta) were removed, and all rats were killed by rapid decapitation.

The rates used in this study were maintained and treated according to internal institutional rules, based on the principles of ethics in animal experimentation.

Method of Sampling

The cortical region of the brain and the placenta were cut into fragments of 1 mm^3. These fragments were immediately immersed in a 3% glutaraldehyde solution in sodium cacodylate buffer for 24 hours at 4°C. After rinsing in the same buffer, the fragments were post-fixed in 1% osmium tetroxide for 17 hours at an ambient temperature of 25°C.

All fragments were dehydrated in ethanol baths of increasing concentration, and in two baths of propylene oxide, then embedded in Resin Epoxy (Epon) and incubated for 48 h at 45°C and for 24 h at 60°C. Semithin sections of 100 to 150 nm thicknesses were obtained. Tissues areas selected on semithin sections were then cut to obtain ultrathin sections that were collected on 300 mesh copper grids. These cuts were contrasted with uranyl acetate and lead citrate, and examined with the transmission electron microscopy.

Monitoring Methods: Transmission Electron Microscopy (TEM)

TEM based on the emission in a column or a satisfactory vacuum of electron beams which pass through only an object of very small thickness. The resulting image will be recorded on a sensitive plate [2].

RESULTS

Ultrastructural Study of the Effect of Indium in Brain Tissues

In this paper we studied the brain tissues: two varieties of cells were studied: the nerve cell (the neuron) and the glial cell (oligodendrocytes).

• The neurons

Ultrastructural study of brain tissues from rats which received intraperitoneally a soluble solution of indium sulfate revealed numerous lysosomes present within the cytoplasm; they displayed varied shapes and sizes, and most important, they were charged with an electron-dense material. Some mitochondria in the studied cells were swollen, with no visible cristae, and containing a very electron lucent matrix. In some cells, the endoplasmic reticulum profiles were also altered, while in other only a few profiles were identified **(Figure 1)**. The general architecture of these cells remained otherwise unchanged, still keeping their shape, and the normal distribution of organelles. However, the neurons from control rats displayed a normal ultrastructure of nuclei and cell organelles such as mitochondria and endoplasmic reticulum. No overloaded lysosomes were observed in different territories of brain cells and no altered mitochondria **(Figure 2).**

Ultrastructural study of the axons from rats treated with indium sulfate showed the presence of damaged mitochondria **(Figure 3).** No deposits were observed in the control axons and normal structure of mitochondria **(Figure 4).**

• Oligodendrocytes

The detailed observation of brain cells showed oligodendrocytes with irregular membrane and nucleus with abnormal distribution of chromatins, some mitochondria altered and dilated rough reticulum endoplasmic **(Figure 5)**. In the control tissues no deposits were observed and normal architectures of cells was found **(Figure 6).**

Ultrastructural Study of the Effect of Indium in Placenta Tissues

Two face of placenta were studied in this work: maternal and fetal face.

• Maternal face

The investigation of maternal face from rats receiving intraperitoneally a soluble solution of indium sulfate showed a more accentuated reaction of these epithelial cells to the experimental treatment. Most of the nuclei were still round and euchromatic, but with irregular outline. In the cytoplasm, round, oval lysosomes were present, all being charged with an electron-dense material in different amounts. Some mitochondria were altered, presenting rarefied matrix and abnormal cristae **(Figure 7)**. No deposits in the lysosomes of maternal face of control rats were found, and we observed a normal structure of reticulum and mitochondria **(Figure 8).**

• Fetal face

Ultrastructural study of fetal face of placenta from rats treated with indium sulfate starting with the 16th days of gestation cycle showed important degenerative changes. Lysosomes were characterized by various shapes and sizes, in all cases being charged with electron-dense material and altered mitochondria were observed in the cytotrophoblast and in the syncyotrophoblast **(Figure 9).** No ultrastructural modifications were observed in different studied territories of fetal cells control sections **(Figure 10).**

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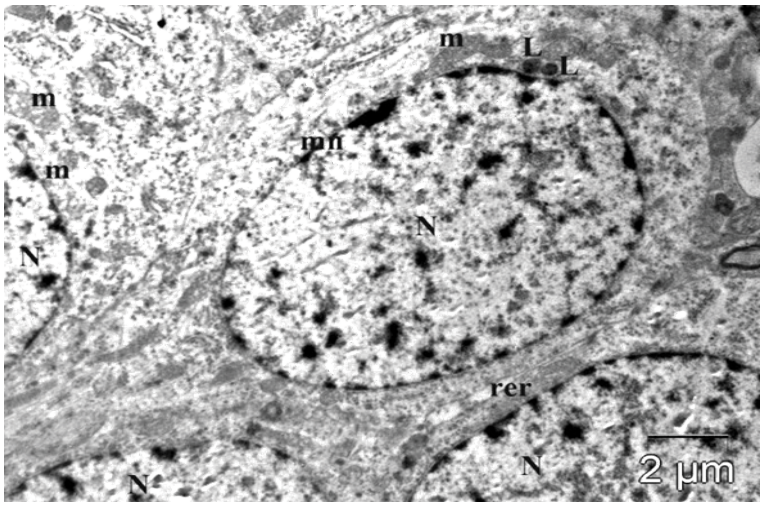

Figure 1. Ultrastructural micrograph of neuron of indium-treated rates. The image shows electron-dense deposits in lysosomes (L) of nerve cells with their round, euchromatic nucleus (N) and their membrane (mn), dilated rough endoplasmic reticulum (RER) and altered mitochondria (m) (magnification X 15 000).

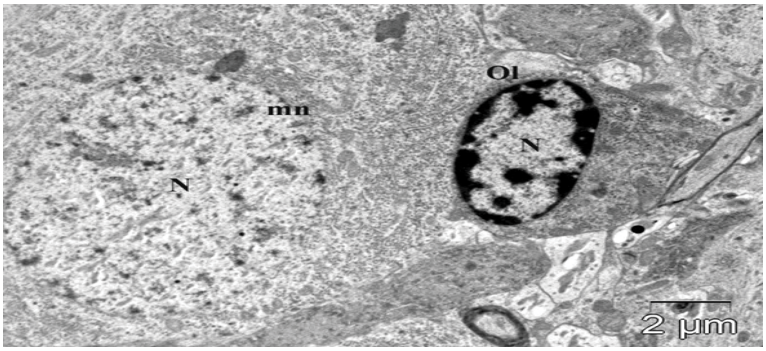

Figure 2. Ultrastructural micrograph of neuron of control rates showing the presence of normal structure of nerve cells with their nuclei (N) and their membrane (mn), oligodendrocyte (Ol) with their nuclei (N) (magnification X (15 000).

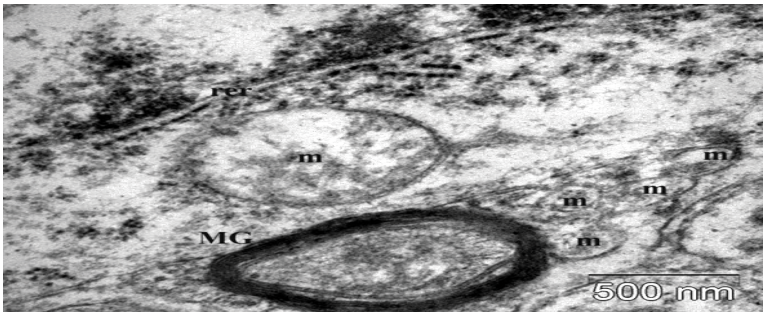

Figure 3. Ultrastructural micrograph of nerve cells of indium-treated rates. The image shows axon with myeline gaine (MG), altered mitochondria (m) and dilated rough endoplasmic reticulum (RER) (magnification X 50 000).

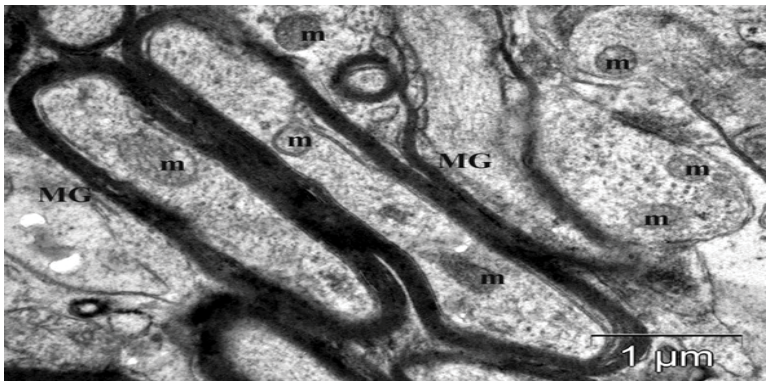

Figure 4. Ultrastructural micrograph of nerve cells of control rates. The image shows many axons with myeline gaine (MG) and normal mitochondria (m). (magnification X 30 000).

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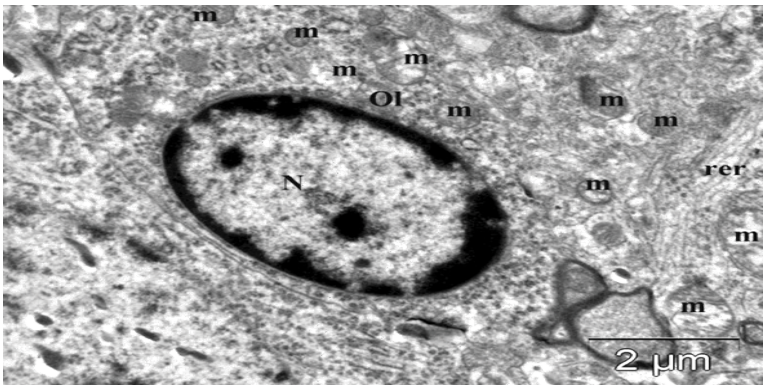

Figure 5. Ultrastructural micrograph of oligodendrocyte of indium-treated rates. The image shows oligodendrocyte (Ol) with their nucleus (N), altered mitochondria (m) and dilated rough endoplasmic reticulum (RER) (magnification X 10 000).

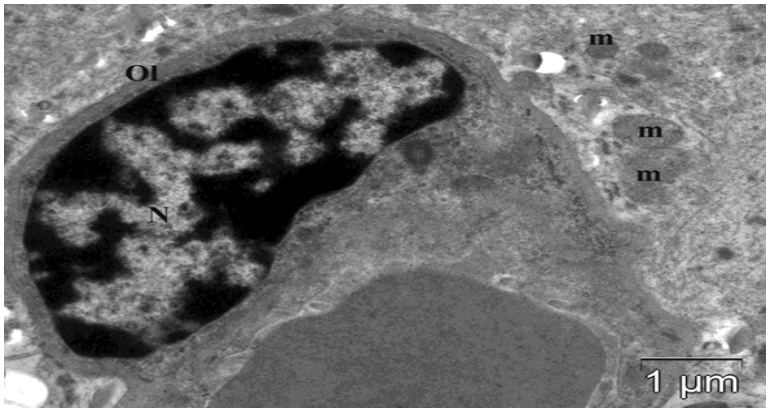

Figure 6. Ultrastructural micrograph of oligodendrocyte of control rates. The image shows one oligodendrocyte (Ol) with their nucleus (N) and normal mitochondria (m) (magnification X 30 000).

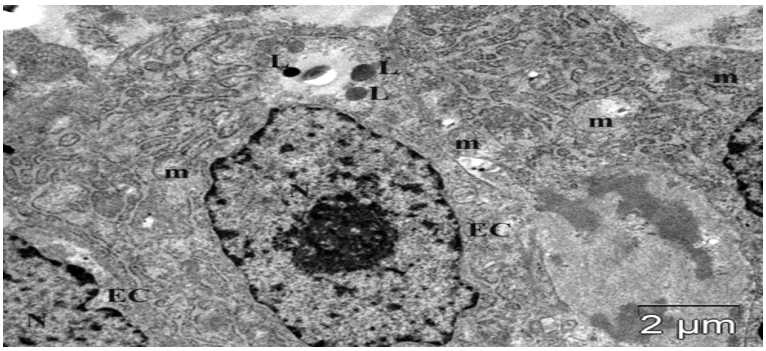

Figure 7. Ultrastructural micrograph of maternal face of indium-treated rates. The image shows electron-dense deposits in lysosomes (L) of endothelial cells (EC) with their nucleus (N) and altered mitochondria (m) (magnification X 10 000).

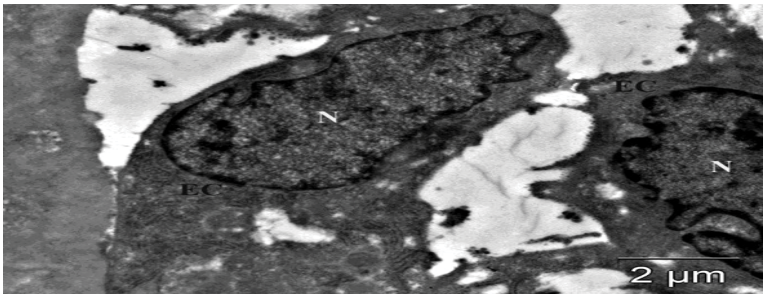

Figure 8. Ultrastructural micrograph of maternal face of control rates. The image shows endothelial cells (EC) with their nucleus (N) (magnification X 10 000).

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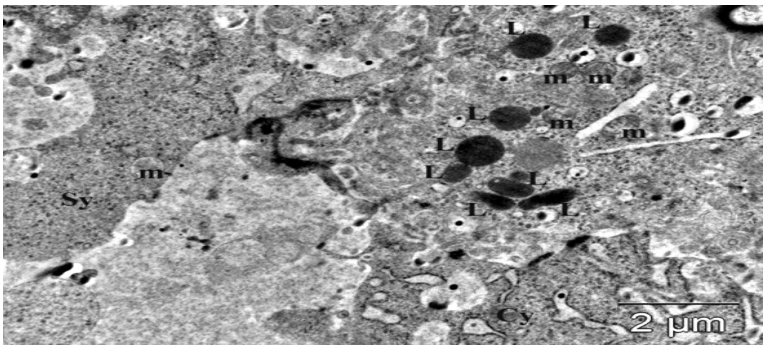

Figure 9. (Ultrastructural micrograph of fetal face of indium-treated rates. The image shows electron-dense deposits in lysosomes (L) of Syncyotrophoblst (Sy) and Cytotrophoblast (Cy), altered mitochondria (m) (magnification X 10 000).

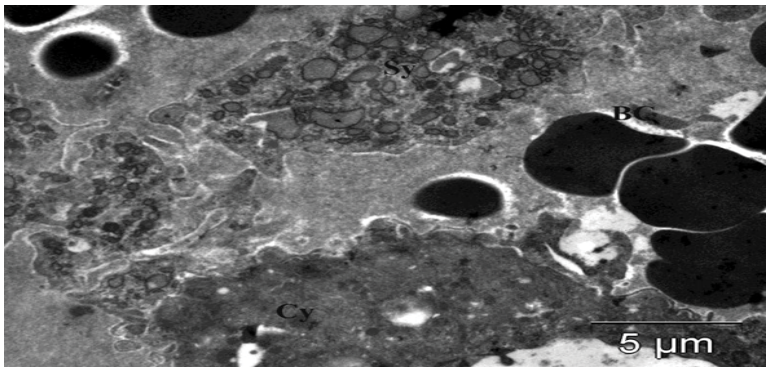

Figure 10. Ultrastructural micrograph of fetal face of control rates. The image shows Syncyotrophoblast (Sy), Cytotrophoblast (Cy) in normal structure and blood capillary (BC) (magnification X 6 000).

DISCUSSION

Previous Studies

There are several studies which have dealt with the behavior of indium within certain organs, as examples: hepatic and bone marrow cells [3], testicle [4-5], ovary and uterus [6], showing that this chemical precipitates in insoluble form in the lysosomes of these different organs, always associated with phosphorus and causes some modifications in the architecture of tissues such as vacuolization, dilated endoplasmic reticulum and altered mitochondria.

Other elements belonging to the same chemical group as indium have a similar behavior. Thus, gallium precipitates in the mammary gland epithelial cells and in the tumor cells of the bone marrow [3,7-9]; aluminum precipitates in the macrophages of the marrow [10], liver [11], and kidney [12]. Hafnium and zirconium have been found in the lymph nodes [3,13]. Further work was subsequently carried out on gold showing that this element accumulates in a wide variety of cells, in particular the steroidogenic cells of the adrenal cortex and the Leydig interstitial cells of the testis [3,14]. Niobium, palladium and platinum precipitate in the lysosomes of proximal renal cells [3]. Nickel was localized within lysosomes of cultured tumor cells [3] and indium within lysosomes of proximal renal cells [3,12,15,16].

As for lanthanides, the same phenomenon of precipitation was also found for some of them, such as cerium [3,17], lanthanum [18,19] and samarium [3], which have been localized in the spinal and medullar macrophages, as well as in the lysosomes of the liver cells [18,19] and kidney [3].

Contribution of this Work and Discussion

This paper is the first detached investigation of the brain and placenta tissues of pregnant rats treated with indium since the 16 day of gestation cycle. In this work, the transmission electron microscopy has been demonstrated to provide a detailed ultrastructural study of the brain and placenta tissues after intraperitoneal administration of indium sulfate to pregnant rats revealing that this element was found in the form of electron dense deposits within the lysosomes of different cellular varieties:

- The brain: Nerve cells The glial cells.
- The placenta: The maternal side The fetal face.

The brain

Our ultra-structural results provided additional information on the effects of indium administration on the cell structure of the tissues studied: nerve cells and glial cells. Indeed, indium is known for its high toxicity triggering intracellular damage

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manifested mainly by weight loss. At cellular level, mitochondrial alterations as well as dilation of endoplasmic reticulum and some vacuolization were also observed with doses used. The intralysosomal deposits appear to be composed of indium. But the direct demonstration of the presence of indium in lysosomes was not possible with the electron microscope. This technique only allowed an ultrastructural study. However, the dense granulations in lysosomes were present only in the brain cells in indium-treated rats, while they were absent in the lysosomes of control rats.

The intralysosomal appearance of the deposits observed in the brain of the treated rats is fairly comparable to that already described for this element in other organs such as mammary glands [20], kidney [4], testicle [4-5] strongly suggesting that the intralysosomal deposits observed most likely correspond to the indium previously administered.

These ultrastructural observations were in agreement with previous work showing that aluminum, a chemical element of the same group as indium, precipitated in the same way, as electron dense deposits in the lysosomes of a wide variety of cells: nervous cells [21-23], liver cells [10-11] and mammary gland cells [20], after its intraperitoneal administration, being always associated with phosphorus. Another work carried out by Maghraoui and collaborators showed that this element was sequestered in lysosomes of cell varieties such as duodenal and jejuna enterocytes and hepatocytes in insoluble form [4].

The placenta

In our study following intraperitoneal administration to pregnant rats for 4 days, electron dense deposits were observed in maternal and fetal face cells. Also, structural changes within placenta tissue included: mitochondrial alterations, rough endoplasmic reticulum dilatations. Absence of loaded lysosomes within placenta tissue was an important feature of the control samples.

These results were consistent with those observed in the Leydig and Sertoli testicular cells after intraperitoneal indium injection in rats. This element was precipitated with phosphorus in the lysosomes of the testicular cells, in insoluble form, under the action of the acid

phosphatase, as demonstrated using microanalytical techniques [4-5,21]. The same element was precipitated, in the same way after its oral administration, in the lysosomes of the duodenal cells [4]. Gallium, the element belonging to the same indium group was concentrated in lysosomes of tumor cells and bone marrow cells [3,10-11,24].

These results make it possible to demonstrate the crucial role played by the lysosome in the phenomenon of precipitation and sequestration of mineral elements. Recalling that its primary role is to degrade organic matter. Moreover, this phenomenon of precipitation prevents their diffusion in the organism and plays a role of "defense" to oppose the penetration of the elements into the internal environment.

CONCLUSION

This study made possible demonstration of the lysosomes role in the concentration phenomenon of indium at cellular level, revealing as well the structural modifications induced by the injection of the indium at the level of brain that at the level of placenta.

PERSPECTIVES

Determine the effect of prolonged accumulation of this element on hormonal secretion.

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