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ULTRASTRUCTURAL OBSERVATIONS ON THE EFFECT OF THE INSECTICIDE "ALDRIN" ON SOME NEUROSECRETORY CELLS OF THE CEREBRAL GANGLION OF THE EARTHWORM *APORRECTODEA CALIGINOSA*

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التركيب الفوق مجهري للخلايا العصبية الإفرازية في العقدة المخية لدودة الأرض *أبريكتودا كاليجينوسا* تحت تأثير المبيد الحشري «الألدرين»

شعـــاع اليوســـف

تمت تربية ديدان الإرض *أبريكتودا كاليجينوسا* في الحقل والمعمل بإضافة المبيد الحشري (الألدرين) بتركيزين مختلفين (١٪ ، ٣٪) حيث أظهر التركيز الأخير تغيرات كبيرة في التراكيب الخلوية المختلفة وبخاصة التحلل التام للحبيبات الإفرازية . أما التركيز الأقل فقد سبب تغيرات طفيفة في التراكيب الخلوية منها التحلل البسيط للحبيبات الإفرازية . يشير هذا البحث إلى أهمية الفحصوات المتعلقة بتغيرات الأنسجة والخلايا كمقياس دقيق للتلوث البيئي .

Key Words: Earth worms, Insecticide, Ultrastructure.

ABSTRACT

The effect of the insecticide "Aldrin" on the neuroglia and neurosecretory cells of the earthworm *Aporrectodea caliginosa* was studied. The fine changes on the organelles of certain neurosecretory cells was observed by light and transmission electron microscopes. Earthworms kept in contaminated soil with 3% aldrin show a prominent degeneration of neurosecretory granules. Less degeneration was observed when 1.5% aldrin was used. This study indicates the importance of histopathological bioassay as an indicator of environmental pollution . Further studies in histopathological bioassay is needed to valuator the effects of insecticides in earthworms.

INTRODUCTION

The wide use of insecticides in agriculture has harmful effects on man and animal populations [1-3]. Many workers have emphasized the beneficial role of earthworms in affecting the physicochemical properties of the soil [4-8]. This beneficial role depends on the burrowing and other activiteies of earthworms. These worms swallow large quantities of soil during their lifetime and therefore they are exposed to the side-effects of insecticides.Damage to the nervous system could affect normal behaviour and survival and therefore reduce their beneficial role.

A few light microscopical studies deal with the effects of pesticides on the earthworm nervous system. The effects of dimethoate and curacron on the histological structure of central nerve cord and intestinal cells of *Allolobophora caliginosa* have been studied by Banhawy *et al.*, [9,10]. These pesticides induced body contraction and severe coiling. These signs of poisoning appear with small amounts of these chemicals on longer term exposure. In recent years, a number of studies for assessing the toxicity of chemicals to earthworm mortality have been published [11, 12]. Neurotoxic effects of pesticides on earthworms have also been described by Drewes *et al.* [13].

The present study deals with the effect of the insecticide "Aldrin" on the ultrastructure of three types of neurosecretory (Nsy) cells and neuroglia within the cerebral ganglion of the earthworm *Aporrectodea caliginosa.* Many Nsy types were studied in detail by Al-Yousuf [14]; however, the neuroglia cells were

studied by Aros [15]. This is the first ultrastructural study which deals with the effect of an insecticide on various organelles of Nsy and neuroglia cells within the cerebral ganglion of the earthworms.

The insecticide "Aldrin" (1-4: 5,8-dimethino-naphthalene) is usually used to control a range of soil-dwelling pests and termites to protect a wide variety of crops especially palm trees [16].

MATERIAL AND METHODS

For toxicity (related to mortality) studies it is necessary to study specimens of similar age. One hundred and fifty specimens of the oligochaete, Aporrectodea caliginosa were cultured in the laboratory and field under normal conditions. Thus the various stages in the life cycle were known prior to experimentation. It was ascertained that the soil in which the worms were kept had not been subjected to insecticidal application. Mature earthworms (measuring about 8-10 cm) were divided into three cylindrical vessels in 10% moistened soil of pH = 6 with tree leaves (organic matter =15%).

The first cylinder was left as a control of non-treated soil. Watery solutions of 3% and 1.5% of the insecticide "Aldrin" were prepared. The 3% (30 g/L) solution were well mixed with dry soil in a concentration of 3 litres of the insecticidal solution in one cubic metre of the soil [1400 kg]. (As used by the Ministry of Agriculture). Thus, each kg of the soil contains 85 mg of the insecticide. To get 1.5% of Aldrin, half of previous concentration was used. The earthworms were kept in all soils for 3 months to assess the effect of the insecticide.Soil was watered regulary (1/2 L evry 48h) and temperature was controlled at 25c to ensure normal burrowingand feedin behaviour . The experiments were repeated twice.

Freshly dissected cerebral ganglia were fixed at 4°C in 1% OsO4 in vernal acetate buffer pH 7.2 for one hour. The material was then washed in the buffer for 15 minutes, dehydrated in ascending series of ethanol, and processed for epoxy embedding. All semi-thin sections of 1 µm thickness were stained with 1% toluidine blue (TB) and examined with the light microscope. Ultrathin sections (50-70 nm) were cut with Nova ultramicrotome, double stained with uranyl acetate and lead citrate, and examined with an Hitachi electron microscope, H300 at 100 KV. (Department of anatomy, Kyoto University, Japan) .

RESULTS

The External Symptoms

The earthworms treated with 3% of "Aldrin" exhibited external signs of poisoning such as body contraction, immobility, severe coiling and decreasing in body weight. A number (12 out of 50) of the worms treated with the insecticide died during the trial. Such external signs of poisoning nearly disappeared when 1.5% of "Aldrin" was used although significance (statistically different from control) decreases in body weight were noticed . Moreover 9 out of 50 worms died in this case, whereas only 3 worms died in the control cylinder (+ or - worm from replicate cylinders) .

Observations on semi-thin sections of control specimens

Over fifty semi-thin sections stained with TB clearly revealed the presence of many Nsy cell types within the control cerebral ganglion. However, only three types of Nsy cells (A1, A2 and B1) and the neuroglia were distinguishable in both control and treated ganglia. In control ganglia, many of type A1 cells appeared . These are relatively small (13 x 21 µm) with variable shape and intensely stained by TB . Type A2 cells are large (15 x 26 µm) and tend to be pear-shaped. They have less affinity to TB compared with type A1 cells. Type B1 cells are large rounded cells measuring about 38 x 41 µm and stained lightly with TB (Fig. 1a). The neuroglia cells show wide distribution outside the control cerebral ganglion. , some being found close to the brain capsule as well as in the surrounding tissues within the perineurium . Other neuroglia cells are found within the perineurium close to the blood vessels or muscles . Neuroglia cells vary in size (8-15 x 12-20 µm) and are characterized by their spherical nuclei . The nuclear chromatin of these cells stands out prominently as equally distributed particles of moderate sizes (Fig. 1a).

Observations on semi-thin sections of treated specimens

In 3% aldrin-treated cerebral ganglia, the affinity of types A1, A2, B1 and neuroglia cells to TB was markedly decreased. The nuclear chromatin was decreased or no longer shown in most cells (Fig. 1b). The neuroglia cells exhibited marked shrinkage (compare Fig. 1a with Fig. 1b). In addition, large vacuoles were occasionally shown among Nsy cells. Similar structural changes were noted in 1.5% aldrin-treated cerebral ganglia , but these were pronounced .

The ultrastructure study of control specimens

The cytoplasm of type A1 cells is packed with moderate to highly electron dense granules (130 - 250 nm) with a thin clear halo beneath their irregular limiting membrane. Variations in the density of granules did not appear to be correlated with their size. The granules are mainly condensed in the axonal side of the perikaryon (Fig. 2a).

The cytoplasm of type A2 cells is packed with oval shaped and moderately electron -opaque granules (140 - 260 nm in diameter). Clearly, these granules are less electron dense than those of type A1 cells. Moreover, the bounding membranes of type A2 granules could not be resolved (Fig. 3a).

Type B1, cells have a distinctive ultrastructure, being highly vaculated. Large cytoplasmic areas occupied by rough endoplasmic reticulum (RER) which form huge vacuoles enclosing a fine material of low electron density. The Nsy granules were rounded, measuring about 90 - 210 nm in diameter and contained moderately dense homogeneous cores surrounded by irregular narrow halos. Type B1 granules are characterized by forming prominent clusters throughout the cytoplasm (Fig. 4a).

Neuroglia cells contain few very dense granules which vary in size (300 - 500 nm) and shape. Some of their short fibres are associated with various Nsy fibre types within the perineurium or within the cerebral neuropile.

All mentioned cell types contain a large number of small smooth or coated vesicles; RER which attached to numerous ri-

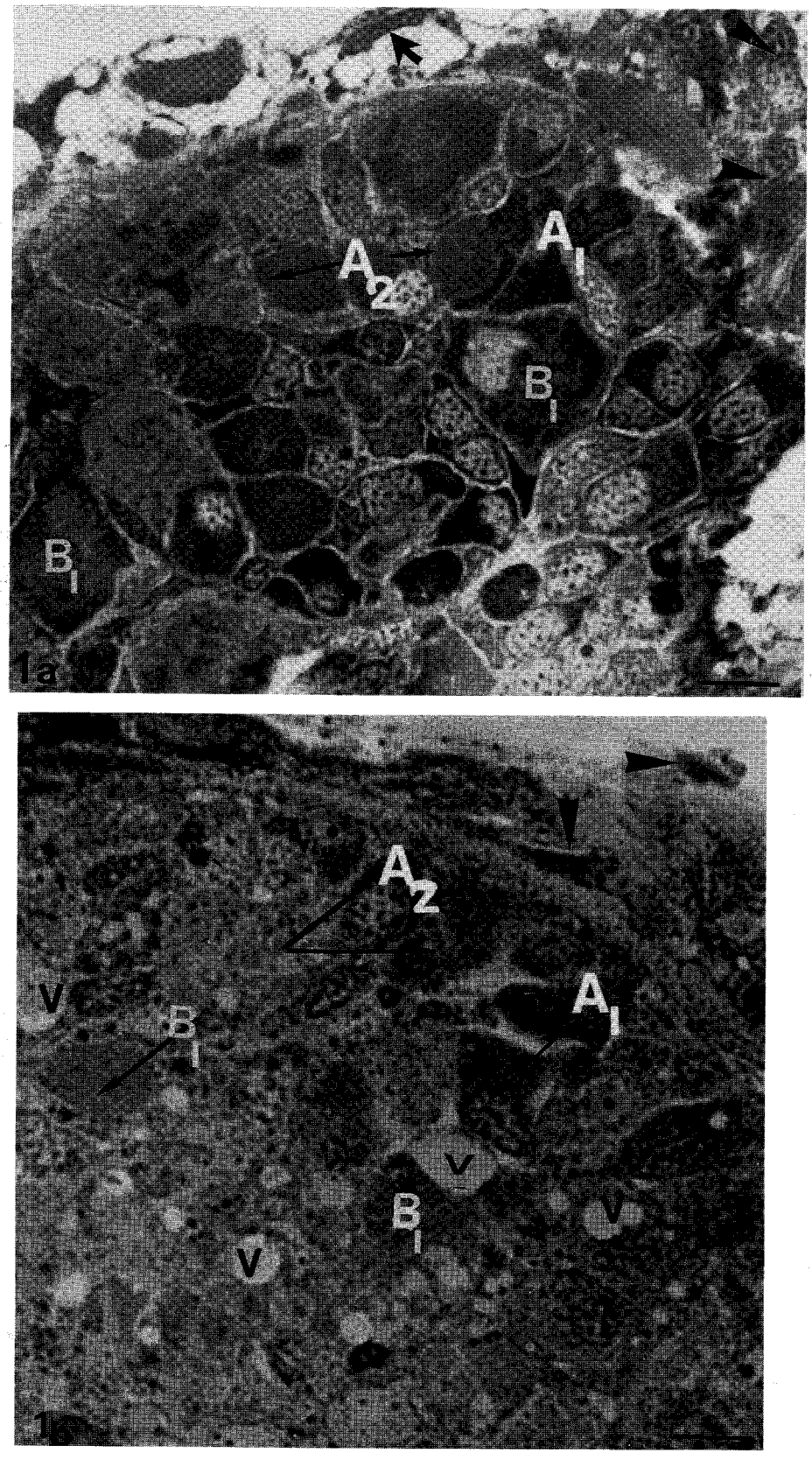


Fig. 1a: Transverse semi-thin section of control cerebral ganglion stained with TB. Note locations of Nsy cell types (A1, A2 and B1) and neuroglia cells (Large arrow). Scale bar = 10 µm.

Fig. 1b: Sagittal semi-thin section stained with TB showing effect of 3% Aldrin at Nsy cell types (A1, A2 and B1) and neuroglia cells (small arrows). Note number of empty vacuoles (V) among cell types. Scale bar = 10 µm.

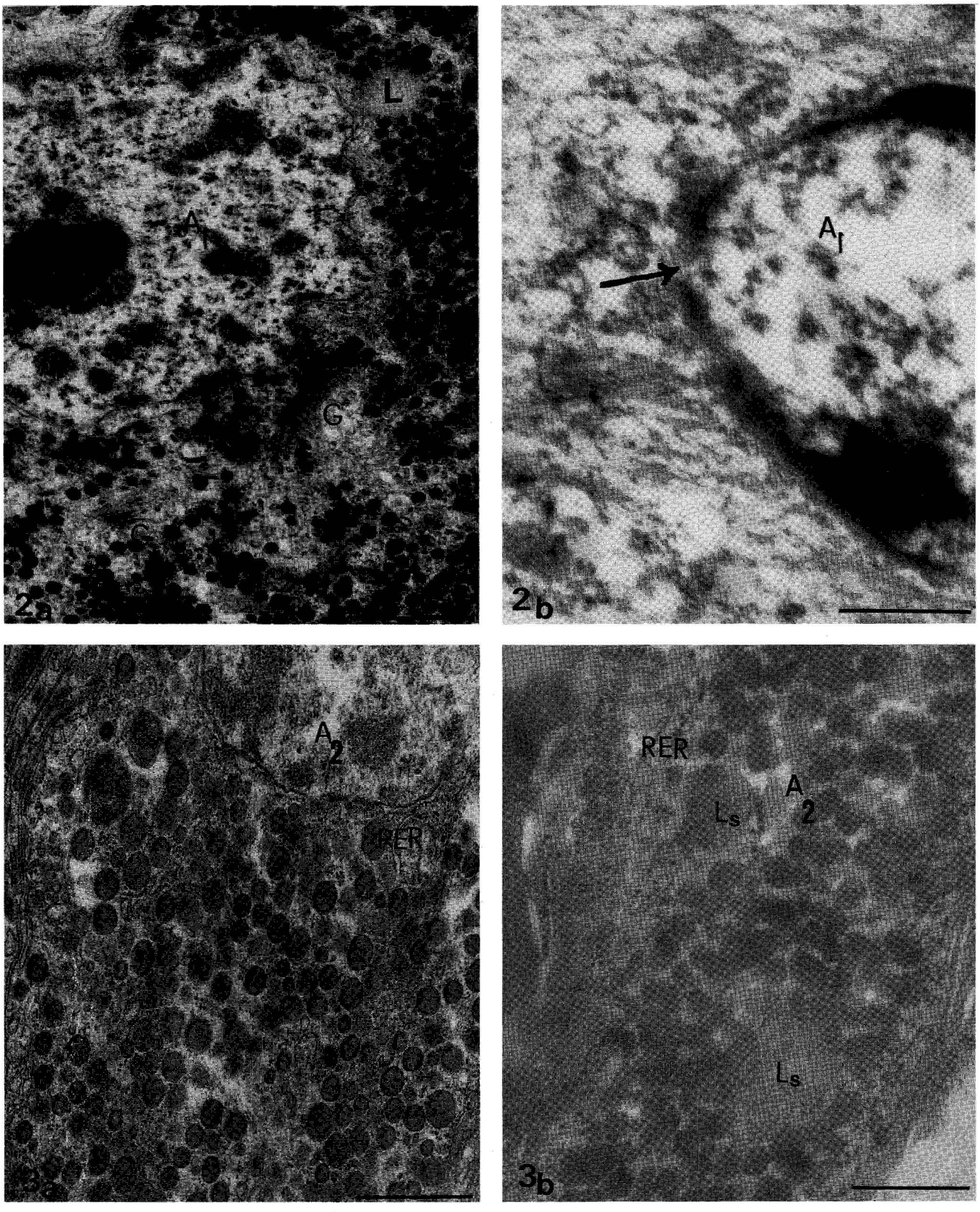


Fig. 2a: Type A1 cell of control cerebral ganglion. Note fine structure of their secretory granules; Golgi bodies (G), lipid droplet (L); and mitochondria (M). Scale bar = 0.25 µm.

Fig. 2b: Type A1 cell of 3% Aldrin treated cerebral ganglia: Note high level of granules degeneration. Arrow indicates break in the nuclear envelope. Scale bar = 0.25 µm.

Fig. 3a: Type A2 cell, note fine structure of their secretory granules. Note possible lysosome (Ls). Scale bar = 0.25 µm.

Fig. 3b: Type A2 cell within cerebral ganglion treated with 3% aldrin. Note degeneration of secretory granules and increase of lysosome's size (Ls); Scale bar = 0.25 µm.

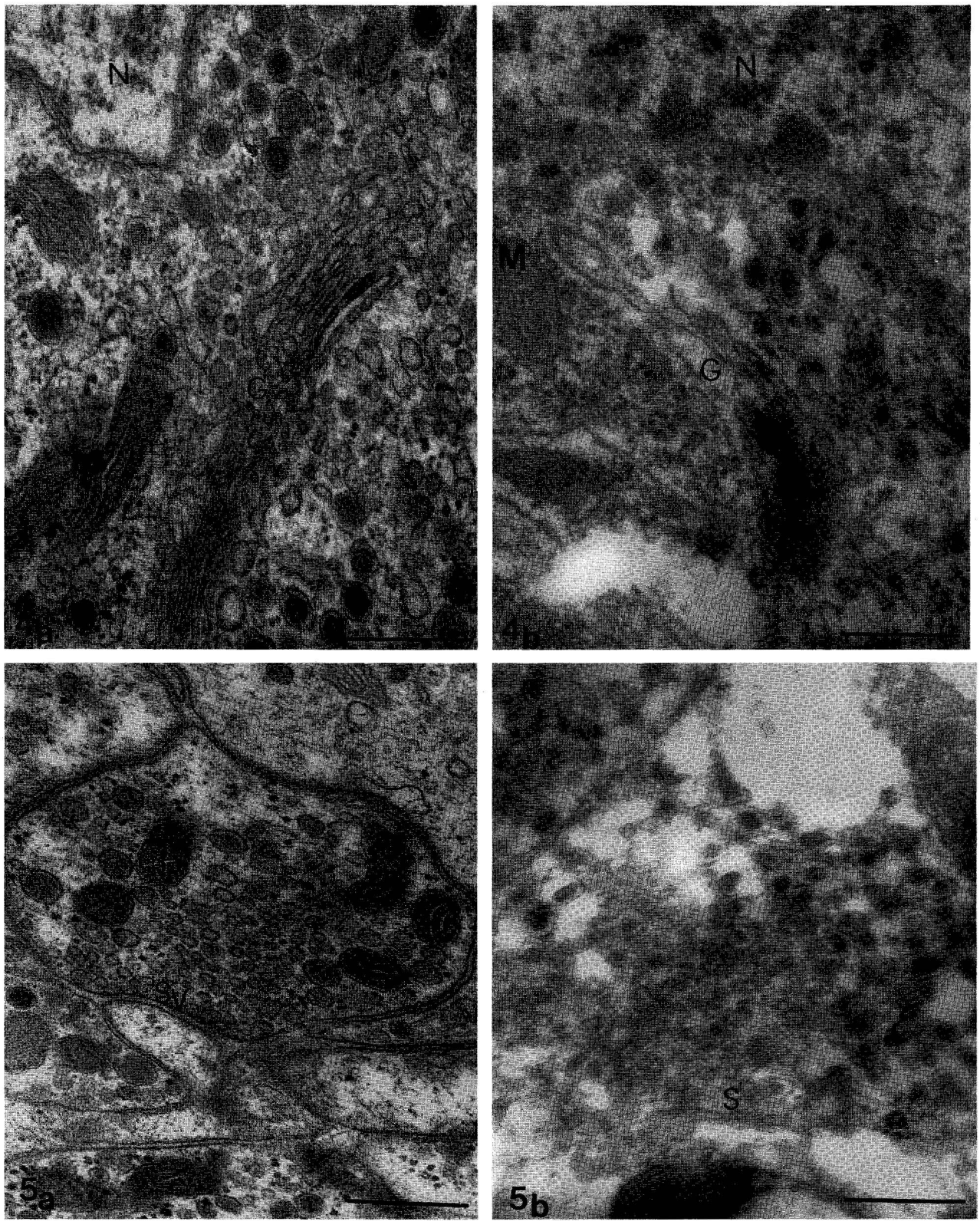
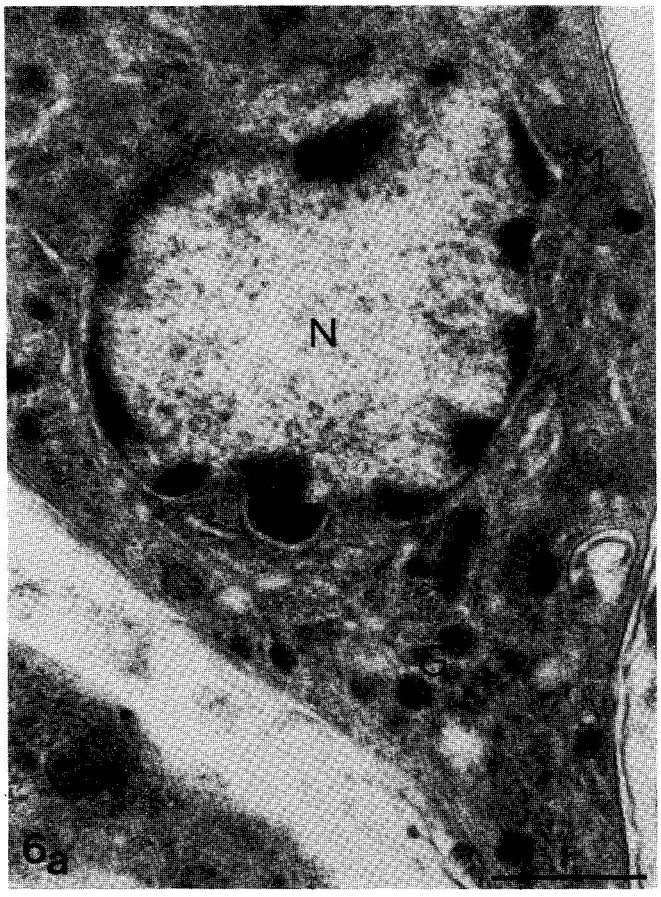


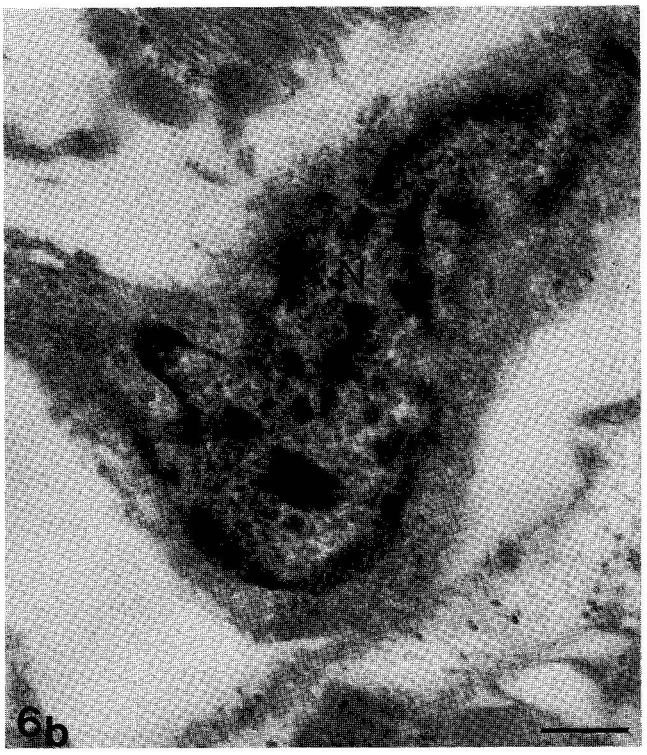
Fig. 4a: Type B1 cell within control cerebral ganglion, note distinctive ultrastructure of their secretory granules. Nucleus (N); mitochondria (m) and Golgi body (G). Scale bar = 0.25 µm.

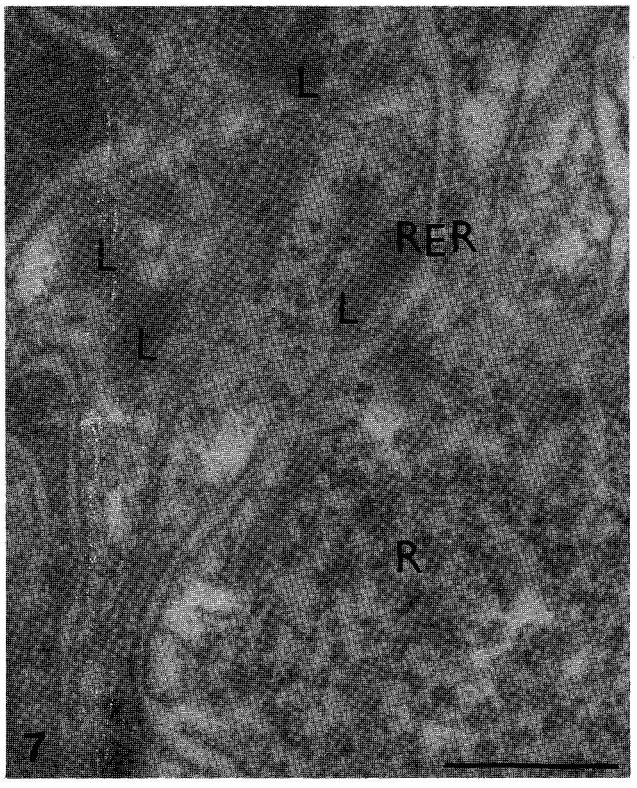
Fig. 4b: Type B1 cell of 3% aldrin treated cerebral ganglion. Note thick masses of heterochromatin within the nucleus (N) (Compare with Figure 4a). Golgi body (G) is difficult to be distinguished as well as the mitochonolria (M). Scale bar = 0.25 µm.

Fig. 5a: Typical synapse of type B1 cell within the neuropile of control cerebral ganglion. Note cluster of synaptic vesicles (SV) and thickness of synaptic membrane. Scale bar = 0.25 µm.

Fig. 5b: Synapse of type B1 cell within 3% aldrin treated ganglion. Note less number and low density of synaptic vesicles if compared with Figure (5a). Note also severe degeneration of synaptic membrane (S). Scale bar = 0.25 µm.







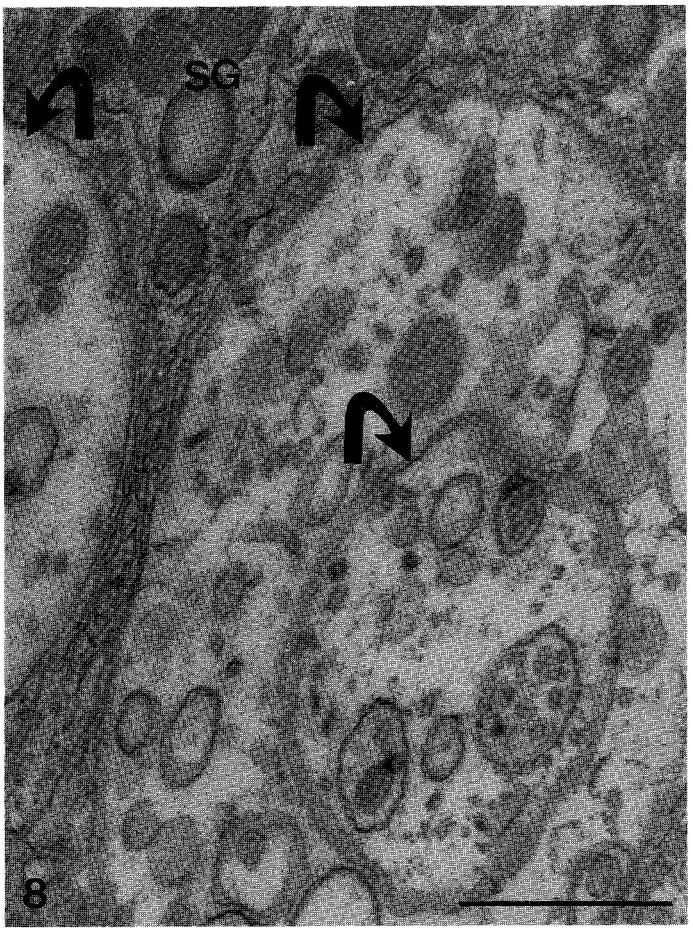


Fig. 6a: The ultrastructure of neuroglia cell within the perineurium of control cerebral ganglion. Note large prominent nucleus (N), mitochondria (m) and few dark granules (G). Scale bar = 0.25 µm.

Fig. 6b: Neuroglia cell treated with 3% aldrin. Note shrinkage of the whole cell and abnormality of the nucleus (N). Scale bar = 0.25 µm.

Fig. 7: Type B1 cell treated with 1.5% aldrin. Note cluster of pale ribosomes (R) which are mainly not attached to the rough endoplasmic reticulum (RER). Note also number of lipid droplets (L) throughout the cytoplasm. Scale bar = 0.25 µm.

Fig. 8: Type A1 cell treated with 1.5% aldrin. Note number of autophagic vacuoles which contain various inclusions of secretory granules (G). Scale bar = 0.20 µm.

bosomes; and many well developed Golgi stacks. The mitochondria are small in size , rounded or oval in shape with matrices of dark to moderate electron density. The nuclei of most cells are large and spherical, with one nucleolus. The hetero-chromatin is in the form of scattered clumps, besides evenly distributed euochromotin (Figs. 2a, 3a and 4a).

The ultrastructural study of treated specimens

The ultrastructure of the three Nsy cell types show prominent changes in worms kept in contaminated soil with 3% Aldrin. The most clear change is in Nsy granules which showed high levels of degeneration and became less electron dense if compared with the control cell types (2b, 3b and 4b). Golgi bodies appeared as broken sticks which lost their characteristic shape, and became indistinguishable in many cases (Fig. 4b). The ground cytoplasm together with many cytoplasmic organelles were severely degenerated, consequently a number of lipid droplets were pre-formed.

Many types of possible lysosomes appeared throughout the cytoplasm. Some of them are apparently autophagic vacuoles which contain various degraded organelles (Fig.3b). Most membranes of the RER appeared in high degenerative states and had lost most of their attached ribosomes, so many free ribosomes were found through the cytoplasm (Fig. 3b and 4b).

The double-membrane nature of nuclear envelope became almost indistinguishable or broken in some cases (Fig. 2b). Thick masses of hetero-chromatic clumps appeared adhering to the inner surface of the nuclear envelope, thus the core of the nucleus became devoid of such chomatin clumps (Figs. 2b and 4b).

Electron microscopical examination of synapses within the neuropile of the cerebral ganglion showed synaptic vesicles to be reduced in number and density compared with those of the control specimens (compare Fig.5a and Fig.5b). Moreover, severe degeneration of synaptic membranes was observed (Fig. 5b).

Less ultrastructural degeneracy was seen within Nsy granules of type A2 cells compared with those of type A1 cells. (Compare Fig. 2b with Fig. 3b). However, less degeneration of synaptic membranes was observed in the axonal terminal of both types; on the other hand, severe changes were observed within type B1 fibres (Fig. 5b).

In worms treated with 3% Aldrin, the plasma membrane of the neuroglia showed marked shrinkage. Moreover, the cytoplasm exhibited severe shrinkage and many of the cellular organelles, including granules, were lost . The mitochondria became cup-shaped and their electron density decreased. The nuclei exhibited irregular and compressed shapes with subsequent condensation of the chromatin clumps (Compare Figs. 6a with 6b).

Exposure to 1.5% Aldrin induced less ultrastructural changes than the higher concentration . However, shrinkage of many organelles was clearly observed . Many of shrunken granules sticking together to form scattered groups.

RER was not greatly affected but in some cases appeared as small scattered vacuoles. Moreover, the ribosomes show decreased affinity to the osmic acid. Furthermore, lipid droplets and ribosomal particles were found accumulated in several regions of the cytoplasm (Fig. 7). Many lysosomes were observed, in addition to autophagic vacuoles which contain various degenerated organelles (e.g. parts of RER, mitochondria and Nsy granules) (Fig. 8). However, degenerative synaptic membranes were rarely observed in this treatment.

DISCUSSION

It is generally accepted that the symptoms of insecticide intoxication indicate nervous impairment [17]. In the present work, "aldrin" was found to induce overt symptoms of toxicity in earthworms (20% death at 3% concentrations) which is considered as a lethal dose. A sub-lethal dose 1.5% of this insecticide resulted in severe signs of poisoning to the nervous system; this has also been found in other studies using various insectivides [9, 10, 18]. However, to the best of my knowledge there is no literature concerning the ultrastructural changes on earthworms caused by pesticides, so this study is considered as the pioneer on this subject.

The deleterious effects of "Aldrin" will greatly depend on the frequency of use and on the persistence and mobility of "Aldrin" in the soil . As shown in this study the use of low concentration of "Aldrin" resulted in deleterious effect . On the other hand the use of high concentration resulted in a lethal effect [19]. Cytopathological changes were also observed in vertebrate nervous tissue using organochlorine and organophosphorus insecticides [20]. Curacron and dimethoate induced deleterious effects on the distribution of cholinesterase in the brain and Gasserian ganglion of albino rats [18], on the other hand DDT caused degeneration of Golgi and mitochondria in neurones of white rats [21]. Severe changes were reported in Golgi bodies in the neurones of the spinal ganglia of rats [22].

Khattab and Riad [18] studied the effect of dieldrin and sevin on Golgi and mitocondria of rat liver cells, they found severe deleterious effects . Some studies on the effects of insecticides were carried out on insects, Chang [23] for example found that DDT broke down the Golgi bodies. Furthermore, Moussa and Banhawy [24] found that BHC treatment caused deformed Golgi dictyosomes which finally disintegrated into granules.

The similarity of histopathological changes produced by various insecticides suggests that the effects of an insecticide are most likely not specific. It is difficult to draw a specific relationship between the chemical structure of an insecticide and the histopathological changes it produces [9, 10].

The most interesting results on this subject is the fact that earthworm species differ in their tolerance to toxic chemicals [11, 25]. The differences of such sensitivities is a reflection of different ways of life among various species [26, 27]. So, a laboratory test must consider the ecology of the species when designing a chemical test. By using contact filter paper test, Roberts and Dorough [13] found a good correlation between *Lumbricus rubellus* and *Eisenia foetida* with four pesticides while with anather six pesticides, the first species was more sensitive than the second one. However, the filter paper contact test might not be a suitable tool to determine earthworm toxicity, as bioavailability in soil is not taken into account in this test . Hence soil exposure as used in the present study is more realistic .

Earthworms have the ability to concentrate high level of heavy metals in their bodies, and have therefore been used as an indication of soil contamination [28]. The present study together with others on earthworms [11, 29] show that earthworms can be used as a sensitive means of detecting potential sublethal neurotoxic effects of pesticides and other environmental pollution.

Histopathlogical bioassay has advantage of showing various changing in cellular activity as effects of pesticides .Ultrastructure observations are the best in this field as cytochemistry applied to study enzymes activity .Gas chromatograms of animals extracted would also be helpfull in this respect.

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