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# THE EFFECT OF THE INSECTICIDE (PRIMICID) ON NEUROSECRETORY CELLS OF THE EARTHWORM *APORRECTODEA CALIGINOSA*

By

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## تأثيرات المبيد الحشري (بريميسيد) على الخلايا العصبية الإفرازية في العقدة المخية لدودة الأرض أبوركتوديا كاليجينوزا

شـــعاع اليوســـف

تمت تربية ديدان الأرض أبوركتوديا كاليجينوزا في الحقل والمعمل مع إضافة المبيد الحشري (بريميسيد) بتركيزين مختلفين (٥ر١٪ و ٣٪). وقد أظهر استخدام التركيز الأخير تغيرات كبيرة ذات دلالة واضحة على العضيات الخلوية المختلفة للخلايا العصبية الإفرازية وبخاصة التحلل شبه التام للحبيبات الإفرازية، أما التركيز الأقل فقد سبب تغيرات طفيفة في التراكيب الخلوية منها التحلل البسيط للحبيبات الإفرازية. وعند المقارنة فإن هذه التغيرات أقل بكثير من تلك الحادثة عند استخدام مبيد الألدرين في الدراسة السابقة (اليوسف ١٩٩٤).

يشير هذا البحث إلى أهمية الدراسات المتعلقة بتغيرات الأنسجة والخلايا كمقياس دقيق للتلوث البيئي ومن ثم مقارنة أي المبيدات الحشرية أخف ضررا على البيئة .

*Key Words* : Earthworm, Insecticide (Primicid), Neurosecretory cells

## ABSTRACT

The effect of the insecticide "Primicid" on the neurosecretory cells of the cerebral ganglion of the earthworm *Aporrectodea caliginosa* was studied. The fine changes in the organelles of certain neurosecretory cells was studied by light and transmission electron microscopy. Earthworms kept in soil contaminated with 3% Primicid showed prominent degeneration of neurosecretory granules. Less degeneration was observed when 1.5% Primicid was used.

This study aims at a comparison with the previous study and to identify the less effective insecticides on the environment. The study confirms that earthworms accumulate a high concentration of insecticides in their bodies and so they may be used as indicators of pollution hazards.

## INTRODUCTION

Insecticides have harmfull effects on man and animal populations. Since earthworms swallow large quantities of soil during their life time, they are constantly exposed to the effect of insecticides and pesticides [1,2]. Neurosecretory (Nsy) cells were found through the whole nervous system of many earthworm species [3]. The cerebral ganglion contains special Nsy cells which are easily recognised.

Few studies dealt with the effect of insecticides and pesticides on the earthworm nervous system at the ultrastructure level. However, the effects of dimethoate and curacron on *A.caliginosa* have been studied by the light microscope only [4–5]. These pesticides induced body contraction and severe coiling. Severe signs of poisoning appeared with small amounts of the above mentioned chemicals after longer periods of time [6,7,8].

A few authors published the results of ultrastructural studies of various insecticides effects on the organells of Nsy cells.

In a previous ultrastructure study, the insecticide, Aldrin was found to be very harmfull, even when small amounts were used for a short period of time[9].

The present study deals with the effect of the insecticide, Primicid on the Nsy cells within the cerebral ganglion of the earthworm *A.caliginosa.*

## MATERIAL AND METHODS

For toxicity studies it is necessary to study specimens of similar age. One hundred & fifty specimens of the oligochaetes, ***Aporrectodea caliginosa*** were cultured in the laboratory . It was ascertained that the soil in which the worms were kept had not been subjected to insecticide application. Equal numbers of mature earthworms (measuring about 9–10 cm) were kept in three cylindrical vessels in 10% moistened soil of pH = 6 with three leaves (organic matter = 15%).

The first cylinder was left as a control with non-treated soil. Watery solutions of 3% and 1.5% of the insecticide "Primicid" were prepared. The 3% (30 g/L) solution was 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 Minstry of Agriculture). Thus, each kg of the soil contains 85 mg of the insecticide. To get 1.5% of Primicid, half of previous concentration was used. The earthworms were kept in all soils for 3 months to assess the effect of the insecticide. The soil was watered regulary (1/2 L every 48h) and temperature was controlled at 25°c to ensure normal burrowing and feeding behaviour. The experiments were repeated twice every three months.

Freshly dissected cerebral ganglia were fixed at 4°C in 1% $OsO_4$ in veronal acetate buffer pH 7.2 for one hour. The material was then washed in the buffer solution 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, provided by the Faculty of Medicine, Department of Anatomy, Kyoto University, Japan.

## RESULTS

### The External Symptoms

Earthworms treated with 3% Primicid exhibited extrenal signs of poisoning (immobility, severe coiling). Moreover a number (15 out 50) of the worms treated with the insecticide died during the trial.

Such external signs of poisoning nearly disappeared when 1.5% Primicid was used although signs of inactivity and decrease in body weight were noticed. Moreover (nine out of fifty) worms died in this case whereas only three worms died in the control cylinder (1 + or 1 – worm from replicated cylinder).

### The ultrastructural study

Ultra-thin sections stained with TB clearly revealed the presence of many Nsy cell types within the control cerebral ganglion (Fig. 1). The ultrastructure of the Nsy cell types show prominent changes in worms kept in contaminated soil with 3% Primicid. The most clear change was within Nsy granules which showed high levels of degeneration and became less electron dense as compared with the control cell types- Golgi bodies appeared as broken sticks which lost their characteristic shape, and became indistinguishable in many cases. The ground cytoplasm together with many cytoplasmic organelles were severely degenerated, consequently a number of lipid droplets were pre-formed (Fig.2).

Possible lysosome and autophagic vacuoles appeared throughout the cytoplasm. Most membranes of the rough endoplasmic reticulum (RER) appeared in high degenerative state (Fig.2).

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. Moreover severe degeneration of synaptic membranes was observed.

Exposure to 1.5% Primicid induced less ultrastructural changes than the higher concentration. However, shrinkage of many organelles was clearly observed (Fig.3). RER was not greatly affected but in some cases appeared as small scattered vacuoles. Moreover, the ribosomes showed decreased affinity to the osmic acid. Furthermore, lipid droplets and ribosomal particles were accumulated in several regions of the cytoplasm (Fig. 3). Many lysosomes were observed, in addition to autophagic vacuoles with degenerated organelles (e.g. parts of RER, mitochondria and Nsy granules) (Fig. 4). However, degenerative synaptic membranes were rarely observed in this treatment.

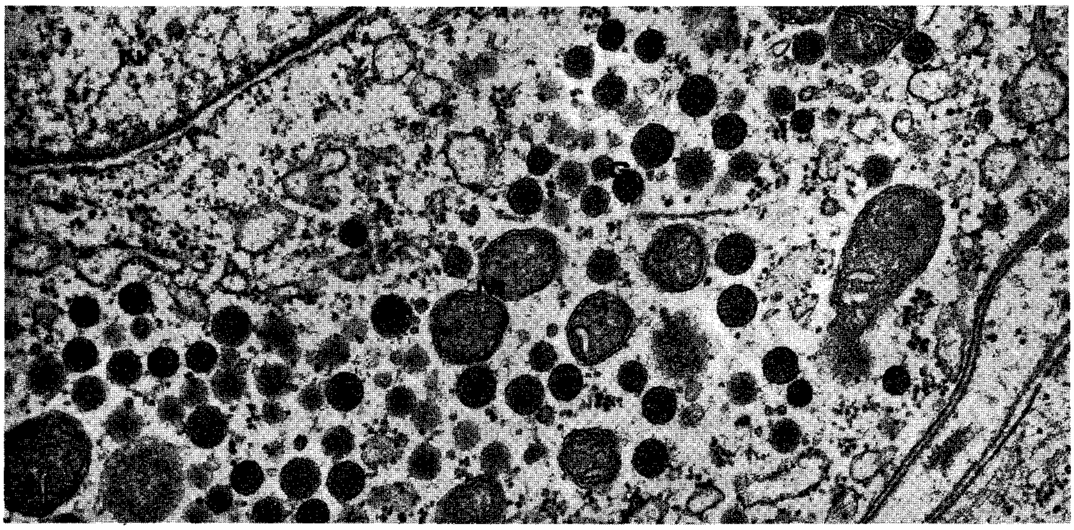

Fig. 1. Section of control cerebral ganglion showing Nsy cell in normal case. Note Nucleu (N), mitochondria (M), secretory granules (G). X = 55000

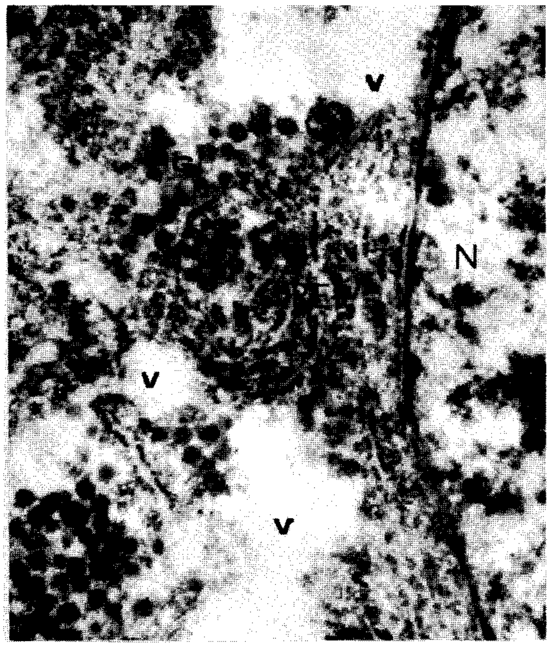


Fig. 2. Nsy cell within cerebral ganglion treated with 3% Primicid. Note large vacuoles (V) degeneration of secretory granules (G) ruptured (RER). Nucleus (N). X = 55000

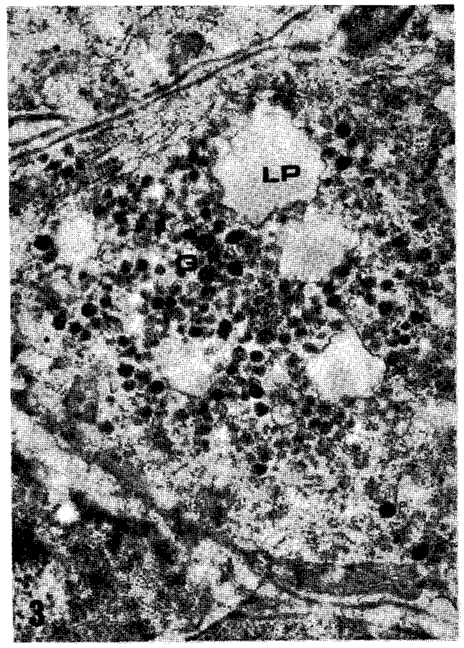


Fig. 3. Nsy cell within cerebral ganglion treated with 1.5% Primicid. Note, shrinkage of secretory granules (G) and accumulation of lipi droplets (LP) .. X = 33000

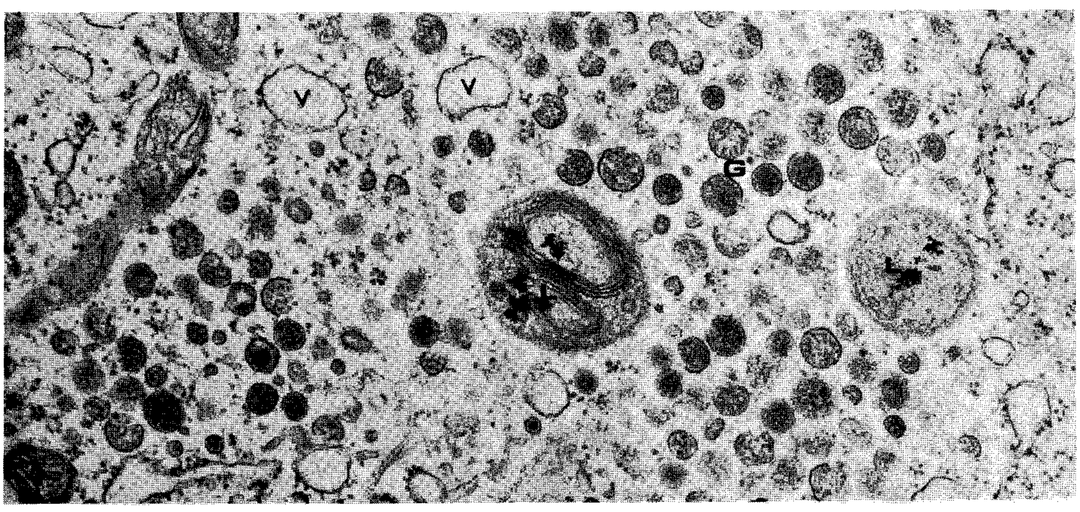


Fig. 4. Nsy cell within cerebral ganglion treated with 1.5% Primicid.

Note, scattered groups of secretory granules (G), vacuolated appearance of RER (V) and lysosome (L). X = 55000

## DISCUSSION

It is generally accepted that the symptoms of insecticide intoxication indicate nervous impairment. In the present work, Primicid was found to induce external toxicity symptoms and may cause death if used at high concentrations. Lesser signs of toxicity are caused by lower concentrations. Other insecticides (e.g. Aldrin) induced sever signs of poisoning to earthworm - nervous system, even when used at low concentrations [8].

However, the deleterious effects of primicid will greatly depend on the frequency of its use and on the persistence and mobility of earthworms in the soil. As shown in a previuos study [9] the use of low concentration of aldrin resulted in deleterious effects while a higher concentration resulted in lethal effects. Previous studies also showed similar effects of insecticides on the nervous system of insects [10,11]. Cytopathological changes were also observed in the vertebrate nervous tissue using organochlorine and organophosphorous insecticides [12]. Curacran and Dironethoate induced harmful effects on the distribution of cholinesterases in the brain and Gasserian ganglion of the albino rat [13]. On the other hand, DDT caused degeneration of Golgi and mitochondria in neurones of white rats [6]. Severe changes were reported on Golgi bodies and mitochondria in the liver cells of rats treated with Dieldrin and Sevin [8].

The cytopathological changes produced by various insecticides proved that the effects of an insecticide are most likely not specific [9–16].

Earthworms have the ability to concentrate high levels of metals in their bodies, and therefore they are used as indicators of soil contamination or as undicators of pollution hazards [17–20]. However, It is difficult to draw a specific relationship between the chemical structure of an insecticide and the cytopathological changes.

It is interesting to note that earthworm species differ in their tolerance to toxic chemicals [14–18]. The differences of such sensitivities is a reflection of different ways of life among various species [19–20]. So, in a laboratory test the ecology of the species must be the considered when designing a chemical test.

This study is a comparison with a preveious one of Al-Yousuf [9]. Both studies aim to identily the less harmful effective insecticides to the environment.

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