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An Electron Microscopic Study of the Liver in the *Rhacophorus leucomystax maculatus (Gray)* Tadpole after Treatment with Aflatoxin B 1

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Abstract: Tadpoles, being visible to the naked eye, were chosen to observe embryonic material. *Rhacophorus leucomystax maculatus* tadpoles were treated with LD 50 of aflatoxin B1. Tadpoles were moribund on the 2nd, 3rd and 4th days after treatment. Those that survived the treatment were transfered into fresh distilled water. The livers of control tadpoles and those that were moribund on the 2nd, 3rd and 4th days were dissected by microdissection, processed and observed with an electron microscope. The livers of those tadpoles that survived the treatment were also processed after two weeks. The hepatocytes of the treated tadpoles showed nucleolar capping and in the cytoplasm there was fatty infiltration, structural changes in the organelles, presence of crystals and complete absence of glycogen; glycogen was present in the hepatocytes of control tadpoles and also in those tadpoles that survived treatment. The structural changes in the hepatocytes of treated tadpoles were probably due to the toxicity of aflatoxin on the cell.

1. Introduction

Several workers have observed the ultrastructural changes in the livers of various animals due to toxicity of aflatoxin B1. These observations have been made in the rat, [3,4,5,9] in the rat and in the monkey[8] and in the duckling. [10,11]

Arsecularatne *et al*[2] used tadpoles of the *Rhacophorus leucomystax maculatus* for the bioassay of aflatoxin B1. This was the first time that observations were made on the toxic effects of aflatoxin B1 on visually observable embryonic material when compared to other animals whose embryos were either in uteri or in eggs. Jayatilaka and Maxwell[6] observed electron microscopic changes in livers of *Xenopus laevis* (Daudin) tadpoles after treatment with aflatoxin B1. This paper presents the ultrastructural changes, caused by LD 50 of aflatoxin, in the liver of the *Rhacophorus leucomystax maculatus* tadpoles, in the same larval stages, as those used by Arsecularatne *et al*[2] in their study of the liver by light microscopy.

2. Materials and Methods

Tadpoles from a single spawn nest were reared and measured as described by Joseph and Jayatilaka.[7] 20 mm tadpoles (total length) were treated with 1·6 μg/ml (LD 50) aflatoxin B1 for 4 days as described by Arsecularatne *et al*.[2] Death

of tadpoles commenced about the 2nd day and was most frequent on the 3rd and 4th days. The livers were dissected by microdissection in control tadpoles and in moribund tadpoles on the 2nd, 3rd and 4th days. Tadpoles that survived were transferred to fresh distilled water on the 5th day and fed with spinach leaves thereafter. After a period of two weeks, the livers of these tadpoles were also dissected by microdissection. The dissected livers were immersed in a mixture containing 4% paraformaldehyde, 0·5% gluteraldehyde, 0·01% calcium chloride in 0·1 M sodium cacodylate at a pH of 7·3 for 24 hours at a temperature of 4°C. They were then postosmicated in a mixture containing 1·0% osmium tetroxide, 0·1% calcium chloride in 0·75 M sodium cacodylate at a pH of 7·37 for 2 hours at a temperature of 4° C. The specimens were then dehydrated in graded alcohols and finally embedded in epon. Ultra thin sections were cut on a Porter-Blum Ultramicrotome, floated on to copper grids, doubly stained with uranyl acetate and lead citrate mixture and examined under a Hitachi 12 U electron microscope operated at 75 KV.

3. Results

In the control tadpole, the hepatocytes showed a rounded nucleus with a nucleolus (Figure 1). Organelles such as mitochondria, rough endoplasmic reticulum, ribosomes, vacuoles and glycogen were observed in the cytoplasm (Figure 1). A large number of microvesicles were also observed in the cytoplasm (Figure 2). Tight junctional complexes were seen between adjoining hepatocytes which formed the bile canaliculi. Microvilli were seen to project into the lumen of a bile canaliculus (Figure 1).

The hepatocytes of larvae who were moribund on the 2nd day showed nucleolar changes and fatty infiltration of the cytoplasm (Figure 2). The nucleolus showed nucleolar capping with nucleolar material arranged in a peripheral very dense and less dense zones. The very dense zone was thicker than the less dense zone and the entire nucleolus gave the appearance of a signet ring (Figure 2). The centre of the nucleolus showed a clear area with fine granular material and extremely electron dense material in the middle of the clear area (Figure 2). A similar area was seen in the very dense part of the peripheral nucleolar material with fine granular material within it (Figure 2). The fat infiltration was seen as large ovoid cavities which were lined with electron dense material (Figure 2). The centre of the fat vacuole was clear. Macrovesicles were also observed in the cytoplasm. The hepatocytes of the tadpoles who were moribund on the 3rd day showed irregularly shaped nuclei and in the cytoplasm there were more fatty vacuoles either completely filled or

partly filled with electron dense material (Figure 3). Other hepatocytes showed fatty infiltration where the vacuoles were filled with dark electron dense material (Figure 3). The mitochondria in these cells were swollen and contained dark electron dense material (Figure 3). The rough endoplasmic reticulum and ribosomes were not observed but multivesicular and autophagic vacuoles were beginning to appear. The appearence of crystals bounded by membrane was another feature (Figure 3). The hepatocytes in tadpoles who were moribund on the 4th day were the seat of marked cellular degeneration. They also showed markedly distorted nuclei and the cytoplasm showed the presence of dilated vacuoles, some empty and others with membrane material, while autophagic vacuoles were distinctly more than in the hepatocytes of those tadpoles that were moribund on the 3rd day (Figure 4). In some hepatocytes of tadpoles, who were moribund on the 4th day, the cytoplasm showed dilated rough endoplasmic reticulum and were circular in cross section (Figure 5). These were identified as R. E. R. due to the presence of ribosomes attached to their membranes (Figure 5). Some hepatocytes showed densely osmophilic chromatin in the nucleus and the cytoplasm showed the presence of fatty infiltration, swollen mitocondria, dilated smooth endoplasmic reticulum and multivesicular bodies. Other hepatocytes showed only the presence of multivesicular and autophagic vacuoles, fat globules, myelin figures and membrane bound vesicles (Figure 6). In other hepatocytes, the nucleus was prominent with a well marked condensed nucleolus. In these the cytoplasmic organelles were not readily recognisable except for fat globules, distended vacuoles and myelin figures (Figure 7). Another marked feature was the presence of a large number of microvilli projecting into the lumen of each bile canaliculus when compared to a bile canaliculus in a control tadpole (Figures 1 and 8). Some bile canaliculi were grossly distended with few microvilli projecting into the lumen. In these hepatocytes the nucleolus was swollen but did not show nucleolar capping. Glycogen was not present in the hepatocytes of those tadpoles moribund on the 2nd, 3rd and 4th days after treatment.

In those tadpoles that survived the treatment and who were sacrificed after two weeks, the hepatocyte showed completely normal cellular architecture and was similar to the hepatocytes of the control tadpoles. The nucleolus appeared normal and the cytoplasm showed the presence of all other organelles and the presence of glycogen.

4. Discussion

The electron microscopic findings in this study support the observations of Arsecularatne *et al*[2] by light microscopy. Nuclear changes and cytoplasmic vacuolation was very well marked in the present study. The hepatocytes showed various stages of cellular degeneration. The vesicular nuclei observed by Arsecularatne *et al*[2] was probably due to the dispersal of chromatin which showed as vacuolation in light microscopy.

The findings in this study closely resembled the observation of Bassir and Babamunmi,[3] Butler [4, 5] and Svoboda and Higginson[9] in the rat, in the rat and in the monkey by Svoboda, Grady and Higginson[8] and in the duckling by Theron[10] and Theron, Liebenberg and Joubert.[11] Similar changes were observed by Jayatilaka and Maxwell[6] in the hepatocytes of *Xenopus laevis* (Daudin) tadpole. Nucleolar capping, dilatation of rough endoplasmic and smooth endoplasmic reticula, mitochondrial enlargement, multivesicular and autophagic vacuoles and bile canaliculi hyperplasia have also been observed in this study.

In eukaryocyte organisms, a definite number of chromosomes of the cell take part in organising the nucleolus--called nucleolar organisers. It is seen as perinucleolar chromatin. For this purpose, the chromosomes were located close to the nucleolus. Allison and Paton[1] have shown that enzymes liberated from lysosomes may enter the nucleus and produce chromosomal aberrations. These two workers have shown that lysosomal enzymes produce chromatid breaks and play a part in carcinogenesis. Lysosomal enzymes could gain access to genetic material and cause changes without impairing mitosis. Lysosomes can be affected by carcinogenic agents like aflatoxin B1. The granular element have been shown to be RNA and the fibrillar elements the DNA strands. Thus nucleolar capping may be the earliest changes seen in the nucleolus of those cells that may become cancerous due to a changed genetic structure.

The other observations were mainly due to the acute toxicity of the hepatocytes. The second day tadpoles showed fatty infiltration in the cytoplasm which was a characteristic feature in the hepatocytes of those tadpoles who were moribund on the 3rd and 4th days. The hepatocytes of the tadpoles who were moribund on the 2nd, 3rd and 4th day after treatment showed alteration of the structure of the organelles in various degrees, while in some hepatocytes the picture was that of cell death.

The hepatocytes of those tadpoles who survived the treatment showed normal structure This perhaps was due to the fact that the hepatocytes either had undergone regeneration in the liver or that these hepatocytes were not affected at all. Arsecularatne *et al*[2] reported that those tadpoles which survived became giant tadpoles and that the period of their metamorphoses was delayed. Thus it may be postulated that the metabolism of the tadpole was in some way deranged by the toxicity of the hepatocytes by aflatoxin B1 and this in turn may have caused disruption of the hypothalamo - hypophysio - thyroid axis thereby producing giant tadpoles and also delay in metamorphosis.

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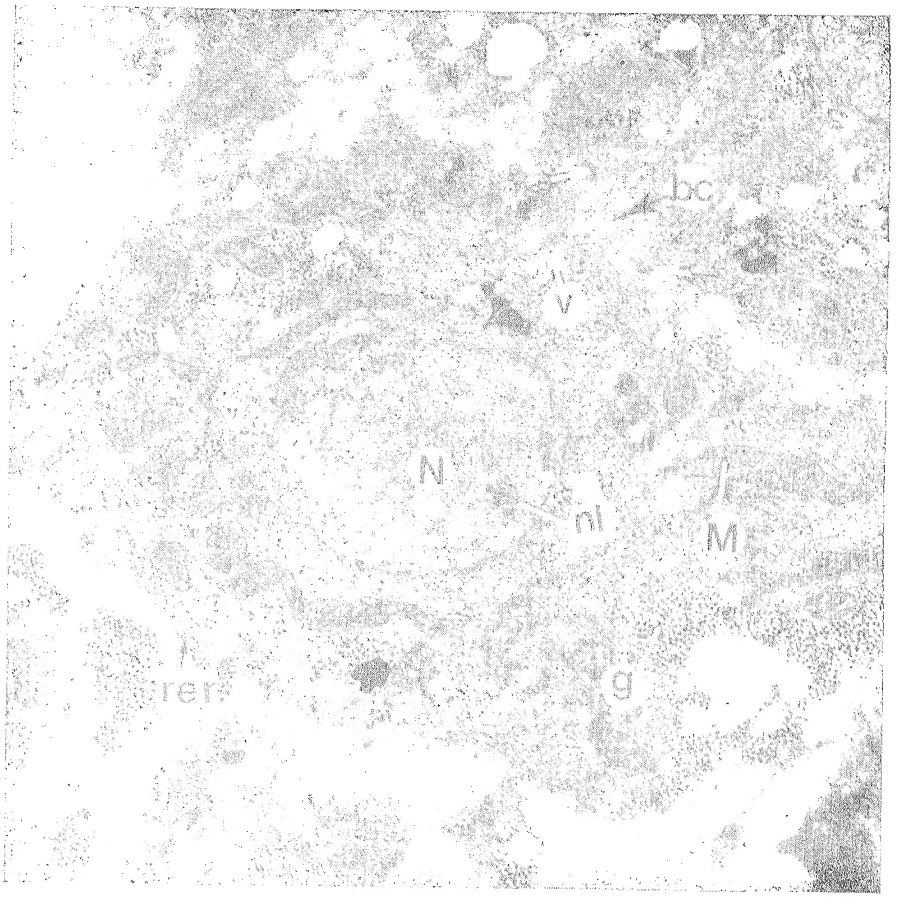

Figure 1. An electron micrograph of a hepatocyte in a control tadpole showing nucleus (N), nucleolus (nl), mitochondria (M), rough endoplasmic reticulum (rer), vacuole (V), and glycogen (g). Arrows show tight junctions near bile canaliculus (bc). x 10,250

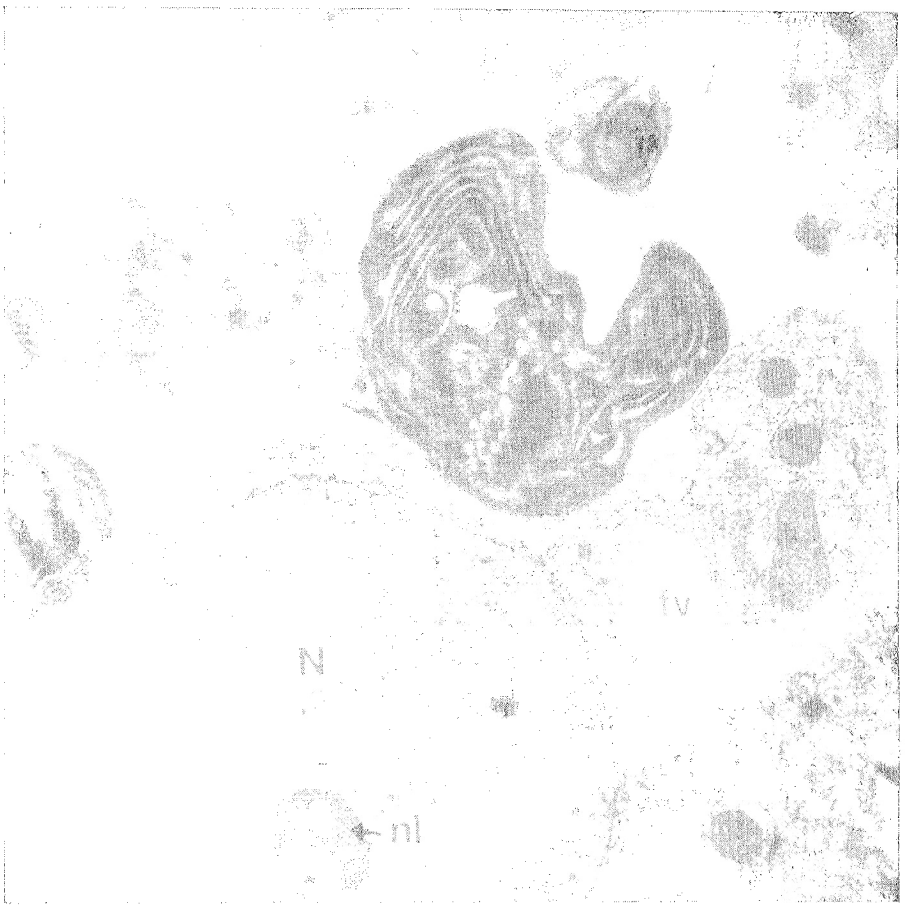

Figure 2. An electron micrograph of a hepatocyte in a tadpole moribund after two days of treatment showing nucleolar capping (nl) and a fat vacuole (fv). x 16,000

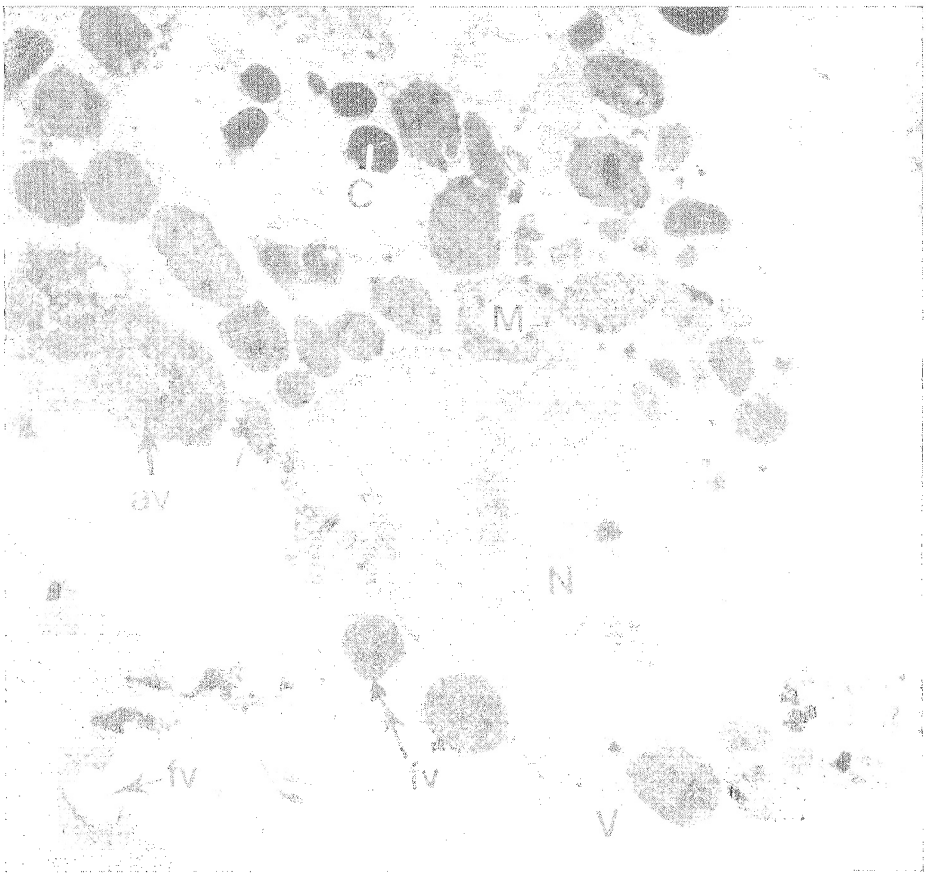

Figure 3. An electron micrograph of a hepatocyte in a tadpole moribund after three days of treatment showing irregularly shaped nucleus (N), swollen mitochondria (M), electron fat vacuoles (fv), vacuoles (V) autophagic vacuoles (av) and membrane bound crystals (C). x 16,000

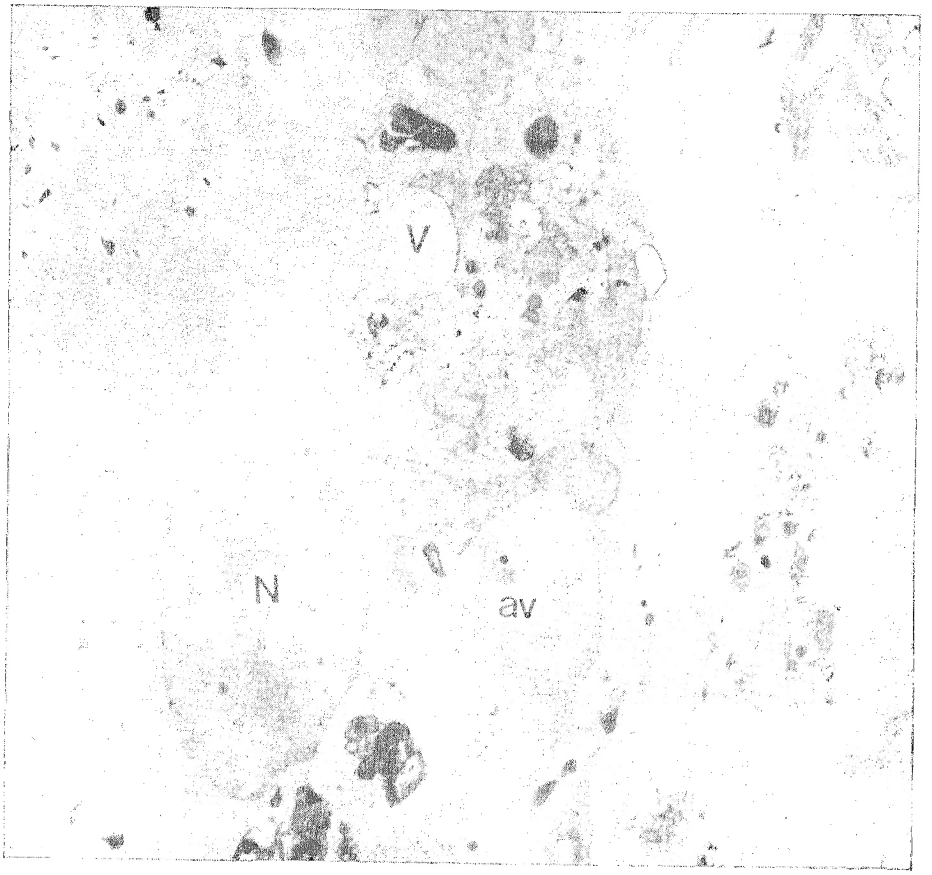

Figure 4. An electron micrograph of a hepatocyte in a tadpole moribund after four days of treatment showing distorted nuclei (N), dilated vacuoles (V) and autophagic vacuoles (av). x 12,750

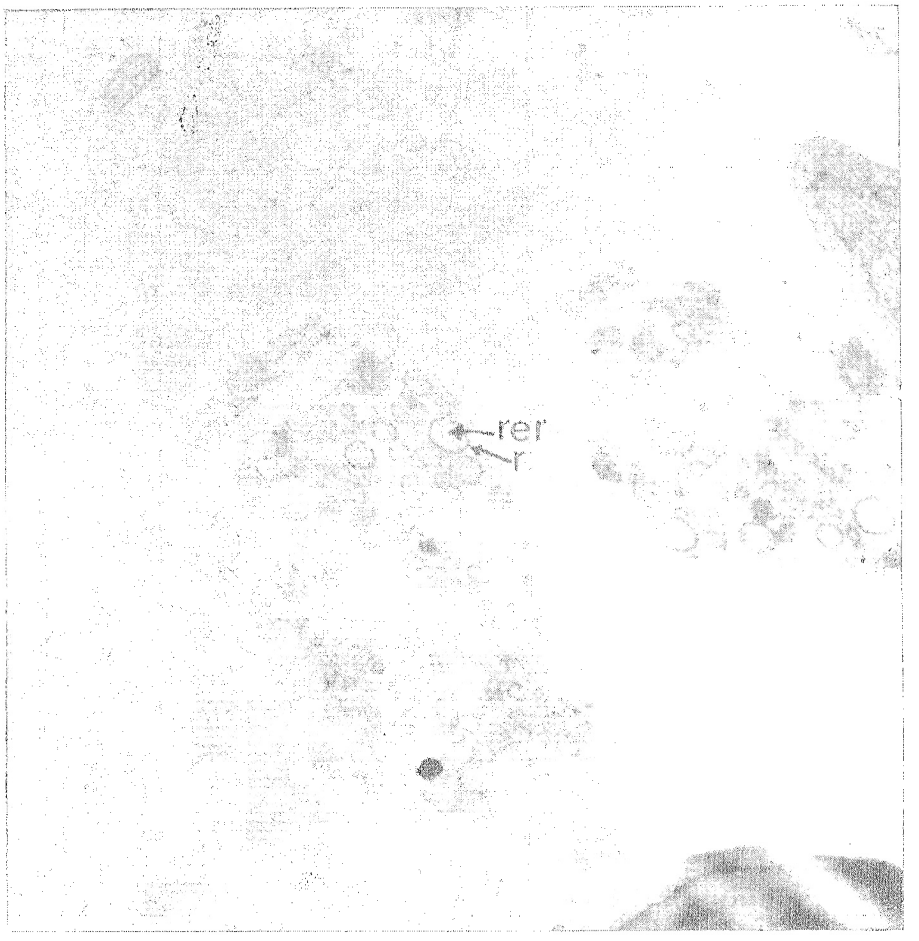

Figure 5. An electron micrograph of a hepatocyte in a tadpole moribund after four days of treatment showing distended rough endoplasmic reticulum (rer) and ribosomes (r). x 25,000

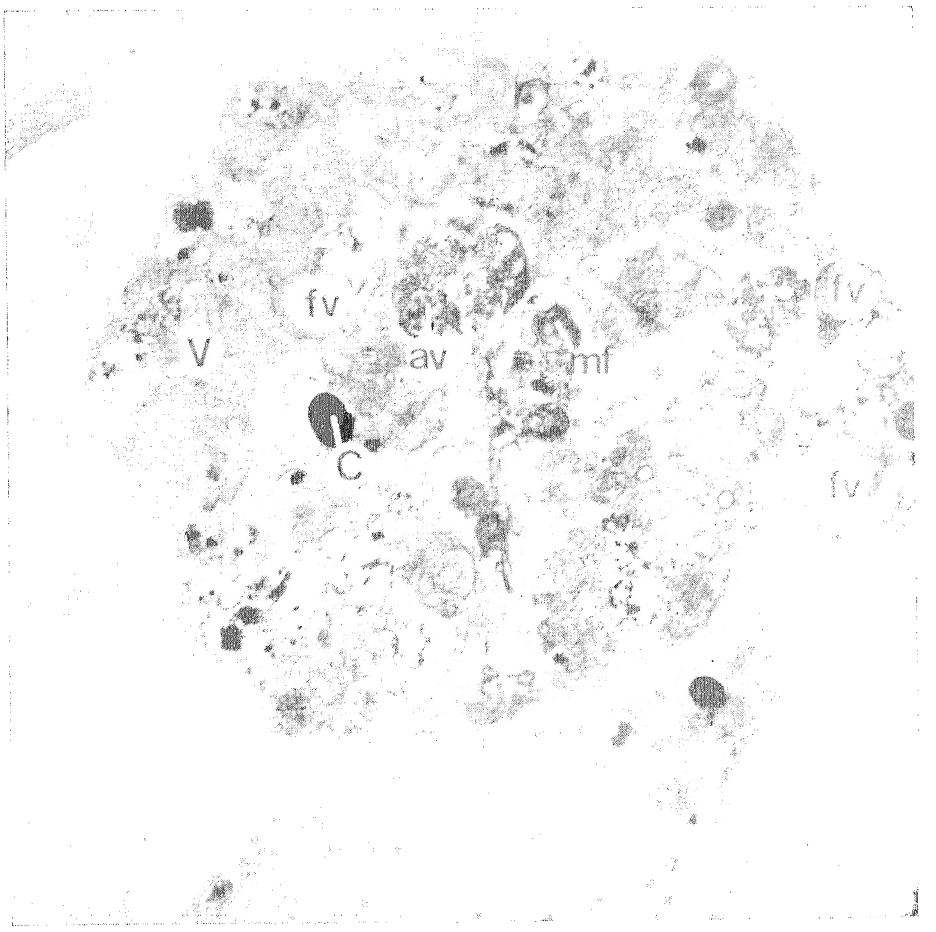

Figure 6. An electron micrograph of a hepatocyte in a tadpole moribund four days after treatment showing autophagic vacuoles (av), fatty vacuoles (fv), myelin figures (mf), vacuoles (V) and crystals (C). x 12,750

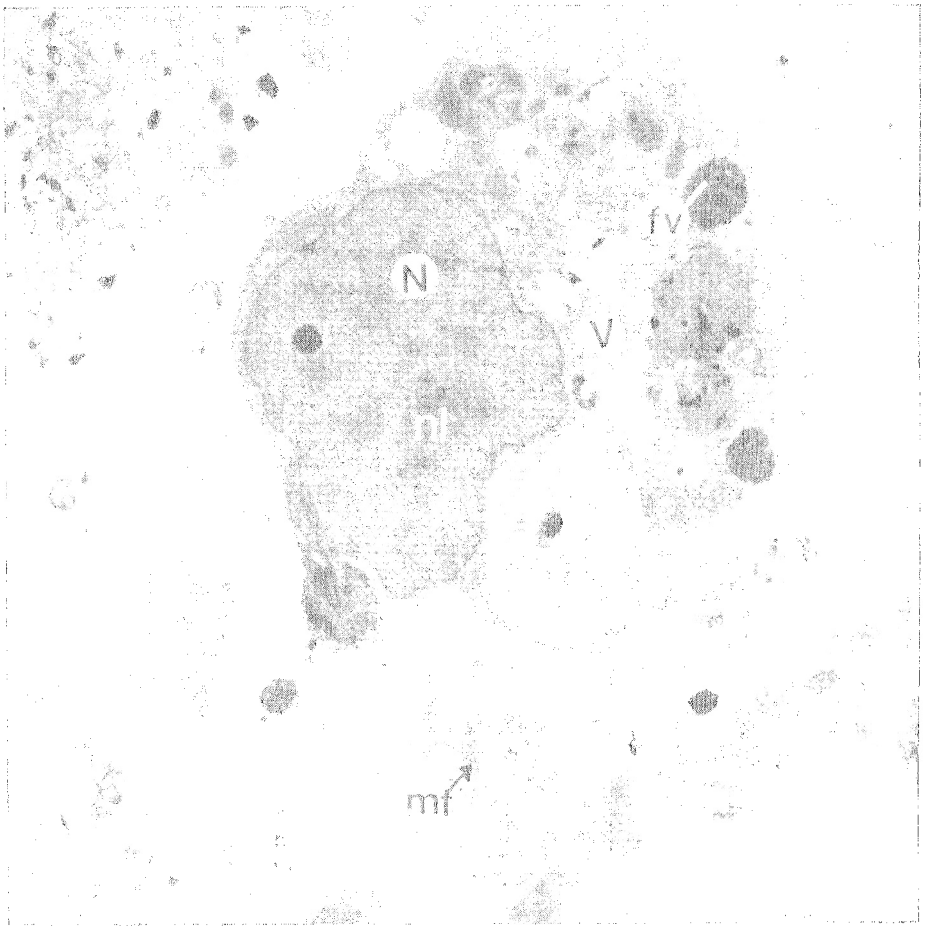

Figure 7. An electron micrograph of a hepatocyte in a tadpole moribund four days after treatment showing prominent nucleus (N), nucleolus (nl), fatty vacuoles (fv), vacuoles (V) and myelin figures (mf). x 12,800

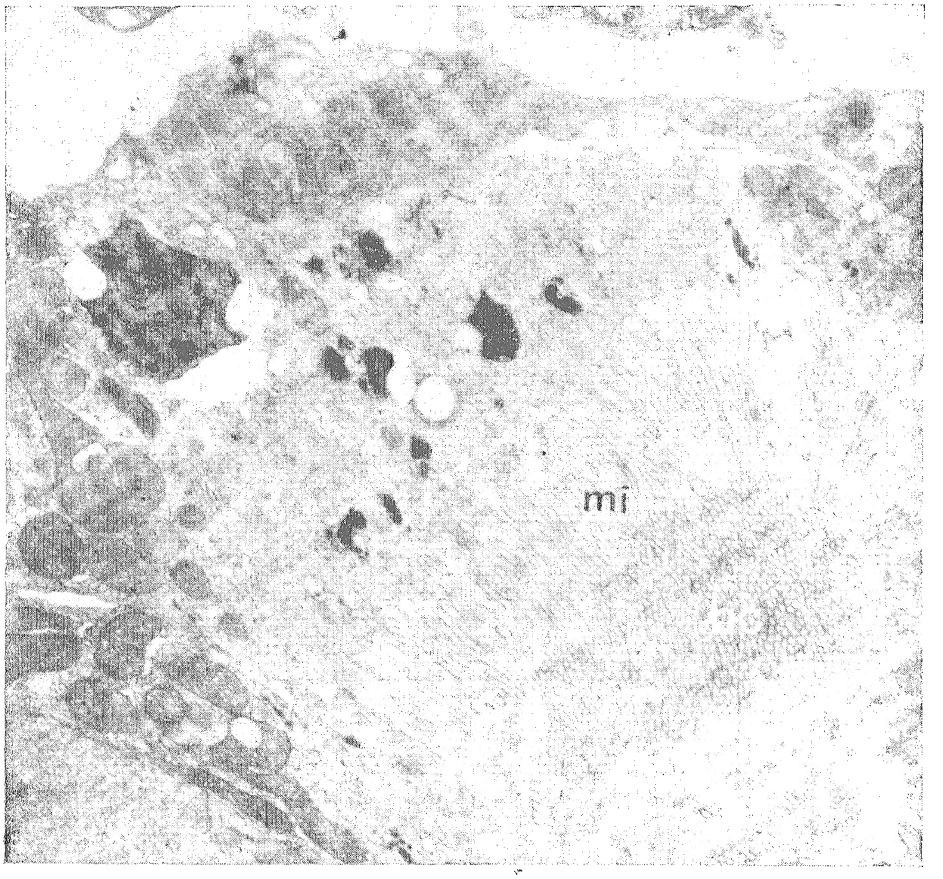

Figure 8. An electron micrograph of a hepatocyte in a tadpole moribund four days after treatment showing increased microvilli (vi), projecting into a bile canaliculus (bc). x 16,000

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