A Micrographic Study of the Giant Nuclei of *Neoechinorhynchus* sp. (Acanthocephala)

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Abstract

Microscopic analyses of the giant nucleus of *Neoechinorhynchus* sp. indicate that these organelles possess a complex internal structure, and appear to be composed of 2 distinct structural regions: a peripheral lobed region and a central region. The peripheral region is composed of a series of lobes separated by invaginations of the nuclear membrane while the central region appears to contain tubular structures and a fibrous network. The giant nuclei are intimately associated with the transverse lacunar canal system via lacunar reservoirs situated above and below the nuclei. The giant nuclei of *Neoechinorhynchus* lack double unit membranes and nucleoli. The Feulgen reaction indicates the absence of DNA. The presence of a complex internal structure and absence of DNA indicate that giant nuclei have differentiated into new organelles.

Introduction

For almost 200 years investigators have observed the giant nuclei in the tegument of adult acanthocephalans. The unusually large size of the giant nuclei has presented problems in the identification of these organelles as nuclei to early investigators. Some giant nuclei have been measured up to 5 mm in length and 50 μm wide in *Macracanthorhynchus hirudinaceus*. Investigators have sought explanations for the unusual size of this organelle. Van Cleave (1951) postulated that this extreme size was due to polyploidy state. Marshall et al. (1973) and Robinson (1973) supported this postulate in their studies on the giant nuclei of *Moniliformis dubius*. Robinson clearly demonstrated the presence of a nucleolus, DNA, and a double membrane enclosing the giant nucleus.

In observing whole mounts of *Neoechinorhynchus*, stained with Harris' hematoxylin, unusual structural forms within giant nuclei were noted. These observations prompted this study of giant nuclei using a combination of light and electron microscopic techniques.

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Materials and Methods

Acanthocephala used for this study were obtained from turtles purchased from

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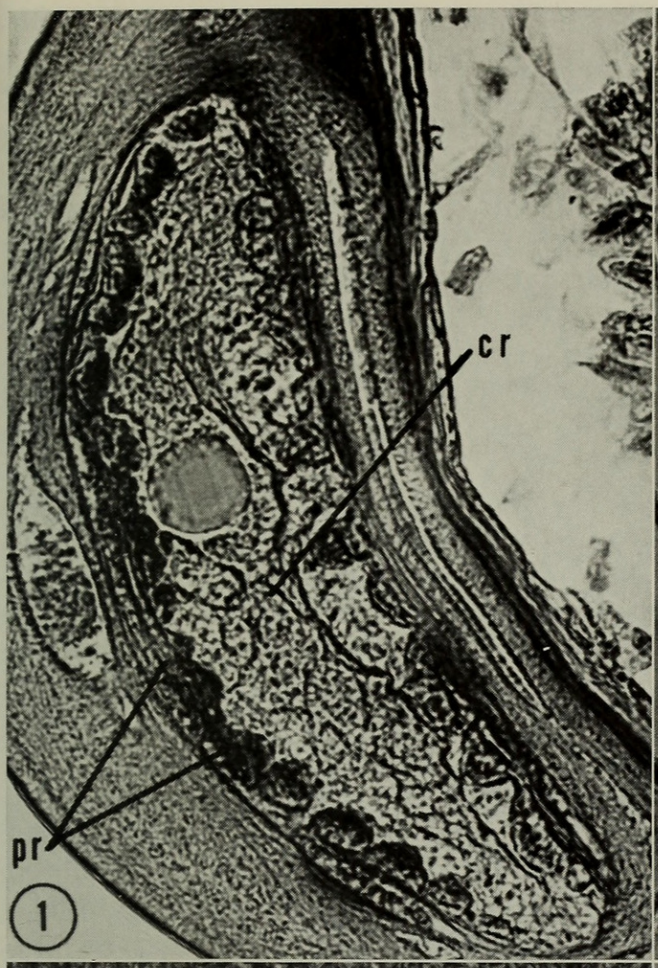
Fig. 1. Cross section through the giant nucleus stained with osmium tetroxide. The oblong nucleus appears to be divided into 2 distinct regions: a dark staining lobed peripheral region (pr), and a central region (cr) containing 1 large vesicle and an extensive fiber network. ×300.

Fig. 2. Cross section through the giant nucleus stained by the Feulgen reaction. The transverse lacunar canal below the giant nucleus bifurcated to form a lacunar reservoir (lr) on each side of the nucleus. Many tubular structures (ts) may be noted in the central region. ×400.

Fig. 3. Electron micrograph of the peripheral area and a portion of the central region. At the top is a layer of dermal material (dm) with the outer membranous structure below it. An invagination forms a canal (c) leading into the interior of the giant nucleus. These invaginating canals divide the peripheral region into peripheral lobes (pl). Below the left peripheral lobe is a tubular structure typical of those seen in the central region. ×3,000.

Fig. 4. Electron micrograph of the peripheral region of the giant nucleus and lacunar reservoir. The lacunar reservoir is separated from the dermal material by a highly convoluted membrane (cm). A lobe of the peripheral region containing large electron dense granules may be noted below the dermal material. ×5,500.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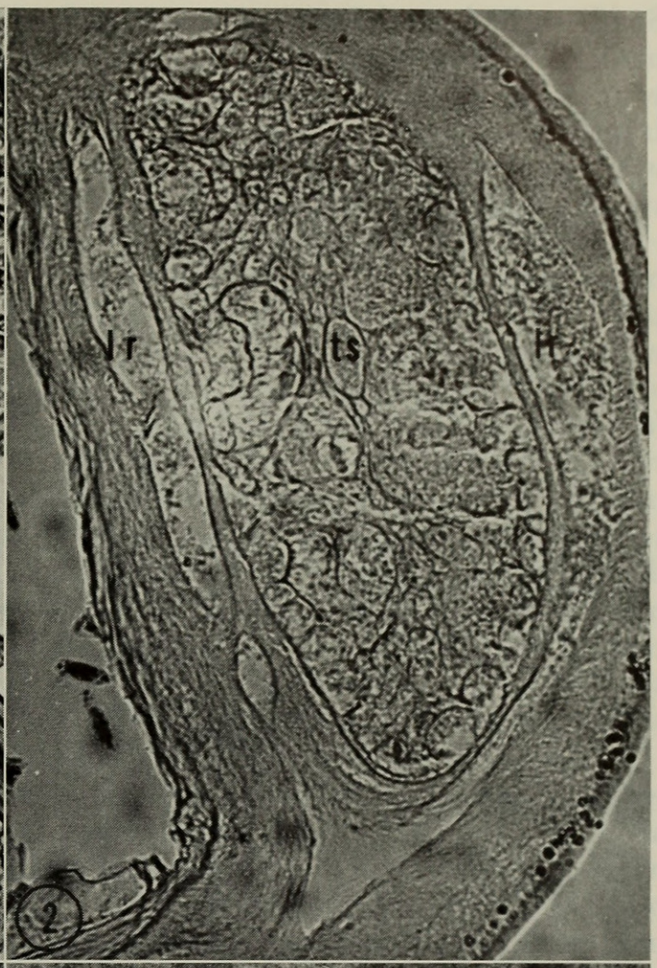
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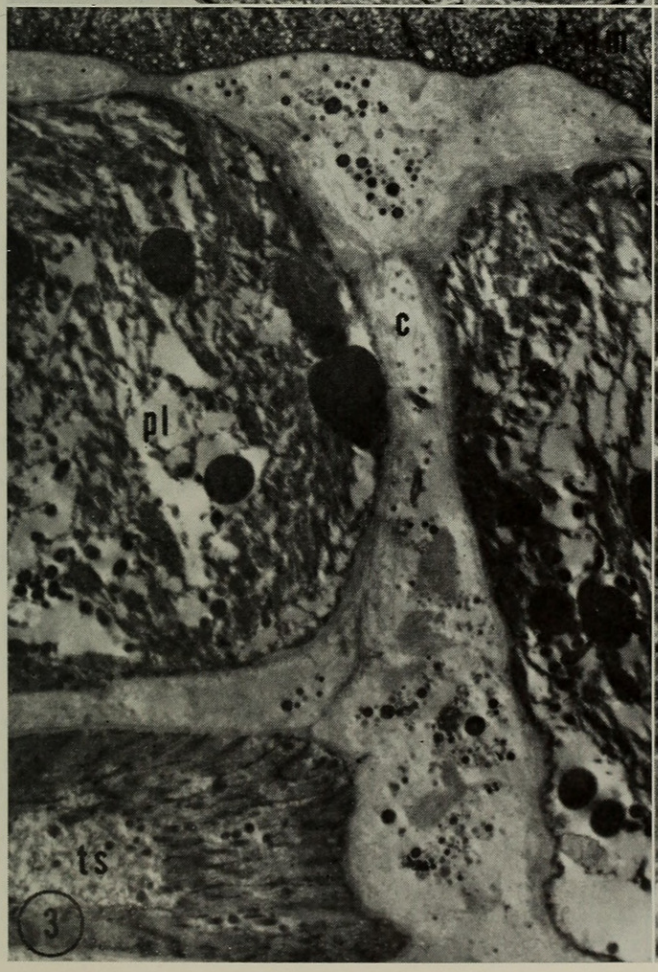
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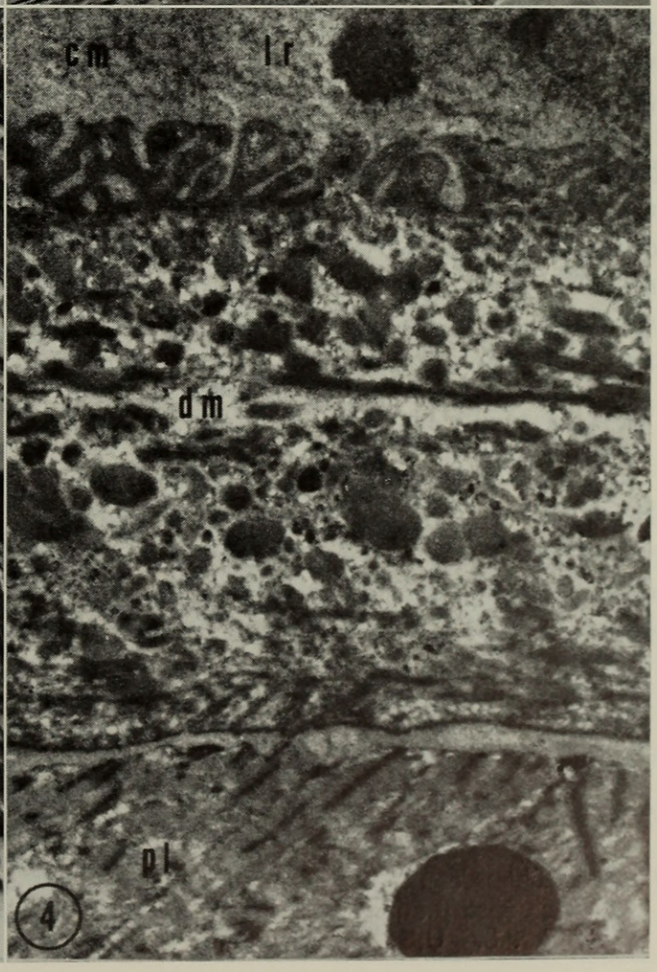
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Southern Biological Supply Company. Immediately prior to experimentation, turtles were sacrificed and the worms removed from the intestine. Disposition of the worms after removal depended upon the specific study to be conducted.

Portions of the worms containing giant nuclei were embedded in paraffin and sectioned at 10 μ. The sections were stained with osmium tetroxide, or the Feulgen reaction as described by Humason (1967) for study with the light microscope. Sections of female worms bearing eggs were used for the Feulgen reaction, and selected sections of the mature female worms were designated as controls. The DNA was removed by rinsing the tissue in 0.5 N perchloric acid for 25 min. The experimental sections remained in distilled water for the same time period.

For electron microscopic study, whole worms were placed in 0.85 percent saline, and the giant nuclei carefully excised, removing as little excess dermal material as possible. The giant nuclei were fixed in 5 percent glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2). Postfixation was completed in 1 percent osmium tetroxide in the same buffer for 20 min and then stained with 0.5 percent uranyl acetate for 2 hours. The giant nuclei were dehydrated in an ethanol and propylene oxide series followed by embedding in Epon 812. The nuclei were sectioned on a Sorvall MT-2 microtome and examined with a Ziess electron microscope, Model 9A.

Results and Discussion

When stained with osmium tetroxide, the nuclei appeared oblong in shape and varied in size depending upon location within the worm. A gradual reduction in size was noted from the large anteriorly located nuclei to the smaller posterior nuclei. Light microscopy indicated 2 distinct nuclear regions within the giant nuclei of *Neoechinorhynchus* sp.: a dark staining peripheral region and a lighter staining central region (Fig. 1). The peripheral region consisted of large lobes around the perimeter of the giant nucleus. The lobes were not always continuous around the entire periphery and sometimes were absent (Figs. 1, 2). This may indicate that not all of the giant nuclei in the same worm have similar structure due to the age, nutritional state of the worm, or the location of the giant nucleus in the worm. In many cases, fibrous networks of the central regions were observed and appeared to be continuous with the dark peripheral region (Fig. 1). At the level of light microscopy, numerous tubular structures, appearing vesicular in nature, were observed in the central region. An extensive fiber network was also observed extending throughout the region (Figs. 1, 2). The tubular structures of the central region varied in size, shape, and number. In most of the nuclei observed there was a large spherical vesicle containing a fine granular background substance (Fig. 1). This large vesicle may be analogous to the nucleolar vacuole Robinson (1973) reported in the giant nucleus of *Moniliformis dubius.*

Usually, there were 3 or 4 transverse lacunar canals in close proximity to a giant nucleus. Serial sectioning demonstrated that most of these transverse lacunar canals near a giant nucleus bifurcate to form lacunar reservoirs above and below the giant nucleus (Fig. 2). These extensions of the lacunar canals encompassing the giant nuclei would indicate a close association between the giant nuclei and the lacunar system.

The controls for the Feulgen reaction

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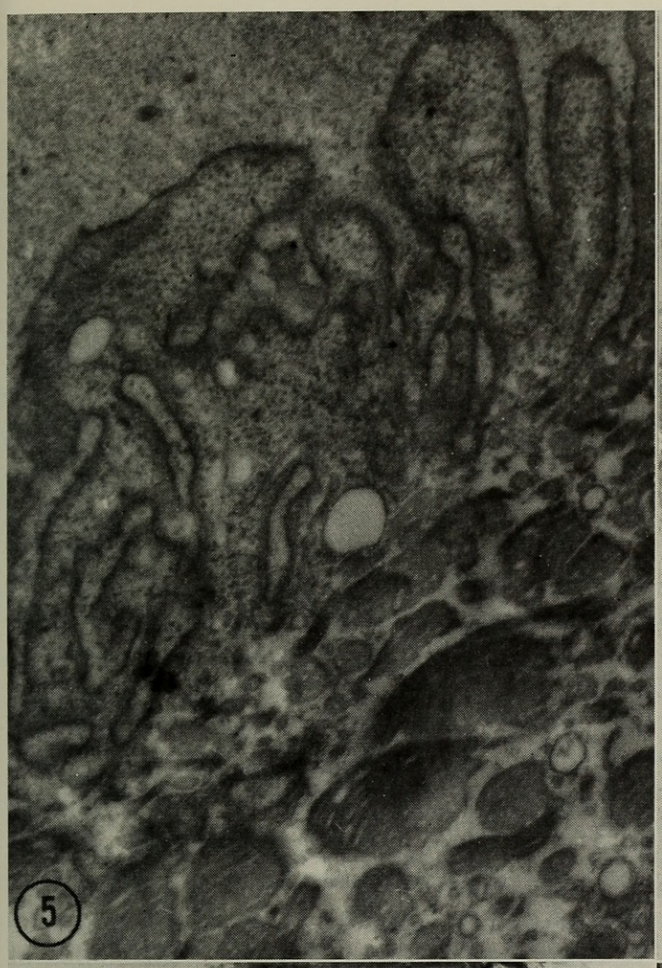
FIG. 5. The convoluted membrane that separates the lacunar reservoir and dermal material associated with the giant nucleus. ×12,000.

FIG. 6. A tubular structure within the central region. The medulla of this tubular structure appears to contain some small granules and the cortex appears fibrous. ×3,900.

FIG. 7. This second type of tubular structure within the central region appears to contain electron dense granules and a disrupted tubular system interlacing the granules. ×5,200.

FIG. 8. Ultrastructure of the packed fiber network within the central region. ×7,000.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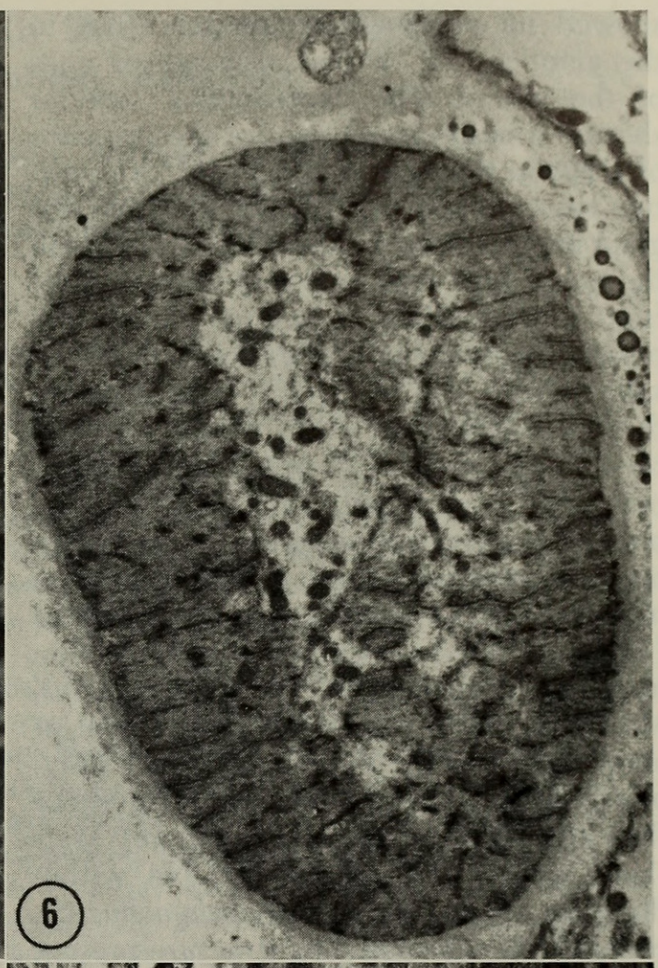
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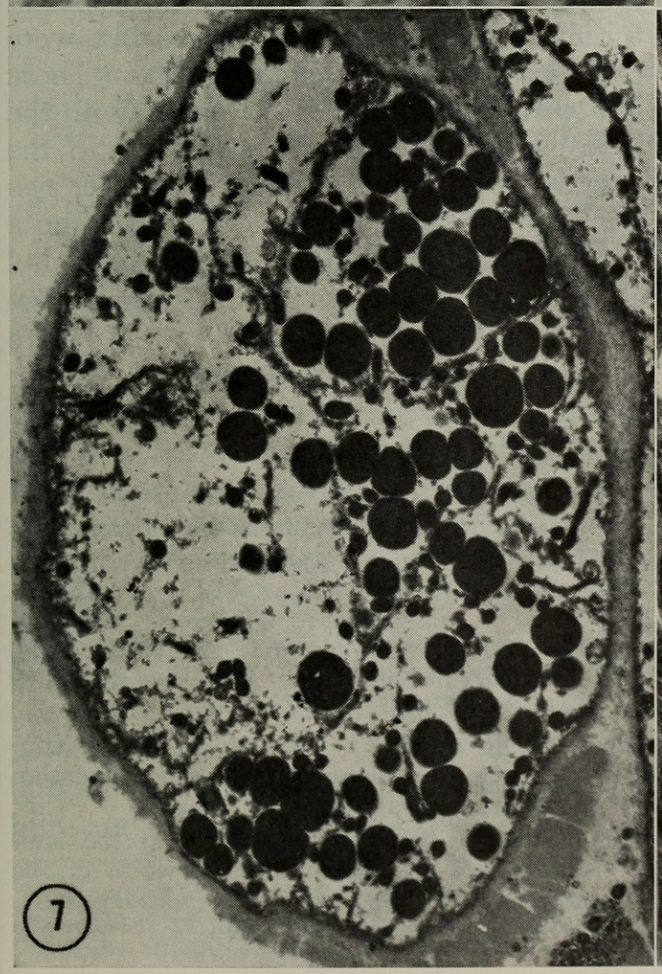
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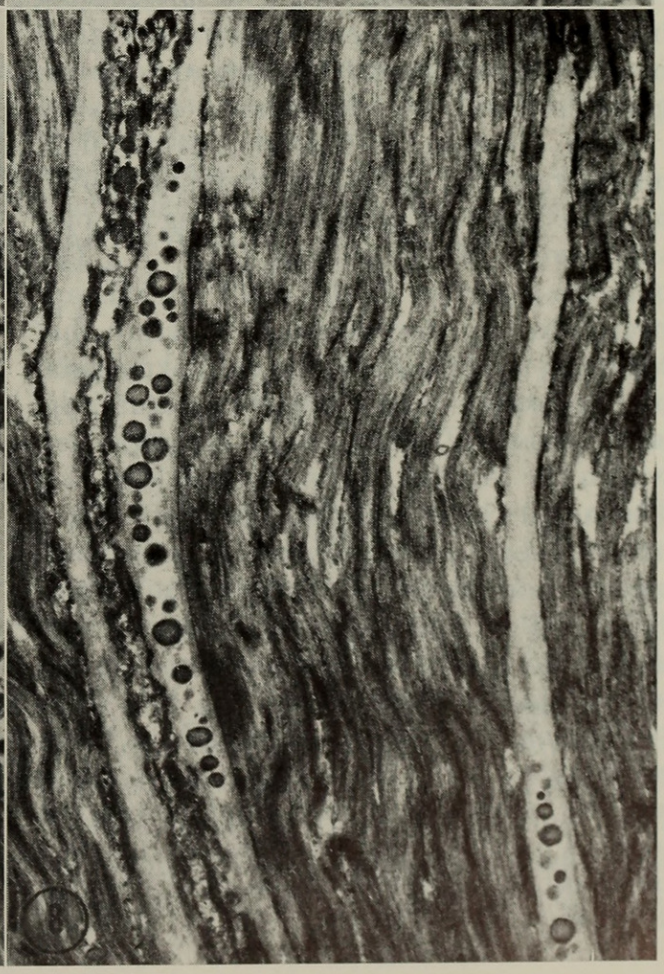
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showed no positive reaction for DNA in the giant nuclei, dermal material, or eggs of the worm. The experimental sections showed a positive reaction for the presence of DNA in the eggs of the worm. No positive reaction could be detected within the giant nuclei or dermal material even though the procedure was repeated using nuclei from several different worms. Under the present experimental conditions, these results indicate that no DNA is present within the giant nucleus of adult *Neoechinorhynchus* sp. This does not preclude the possibility that small quantities of DNA may be present at a concentration too low to be detected by this procedure. These results are in contrast to previous reports. Van Cleave (1951) reported a positive Feulgen reaction in the giant nuclei of *Neoechinorhynchus*. Marshall (1973) found the giant nuclei of larval *Moniliformis dubius* to be in a polyploidy state, and Robinson's (1973) study of the early stages of the worm support Marshall's work. The absence of DNA within the giant nuclei of *Neoechinorhynchus* sp. coupled with the complex structure present indicate that in the adult worm the giant nuclei have differentiated into unique organelles of unknown function.

With electron microscopy, as in light microscopy, the peripheral region appeared to consist of a series of lobes along the outside border of the nucleus (Fig. 3). The outermost portion of the giant nucleus appeared to be a membranous structure that separates the lobes from the dermal material of the worm. Invaginations separate the lobes forming canals that extend deep within the interior of the nucleus (Fig. 3). The outer lobes contain electron dense masses ranging from 0.7 to 3.6 μ in diameter and a fibrous background substance (Figs. 3, 4). The canals often appeared to contain small electron dense masses similar to those in the outer lobes.

Electron micrographs revealed an interesting relationship between the giant nuclei and the lacunar reservoirs. There appeared to be a layer of dermal material between the giant nucleus and the lacunar reservoir approximately 6.0 to 10.0 μ thick. Between the edge of the dermal material and the lacunar reservoir is a highly convoluted membrane (Figs. 4, 5). The lacunar reservoirs appeared to be filled with a clear background substance and a few large electron dense amorphous masses. The presence of this convoluted membrane between the lacunar reservoir and the giant nucleus indicates the possibility of a highly developed transport system involving both structures. This close relationship between the lacunar system and the giant nuclei suggests that they may act as storage depots for nutriments.

Electron microscopy of the central region revealed 2 types of structures tubular in nature, and a packed fibrous network. One type of tubular structure appeared to have a fibrous cortex and a loosely arranged medulla (Figs. 3, 6). These tubular structures contained dispersed electron dense masses 0.2 to 0.6 μ in diameter. A second type of tubular structure consisted of electron dense masses ranging from 0.2 to 1.0 μ in diameter, apparently interlaced by a network of tubules (Fig. 7). Both types of tubular structures varied in size but were consistent in their oblong shape. These structures seemed to be associated with the internal canal system that extends to the exterior of the giant nucleus. The fiber system of the central region appeared to be a packed network of fibers running parallel along a common axis (Fig. 8). Small electron dense masses and small canals were noted among the fiber network.

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