Further Studies of Multicellular Organisms by Soft X-Ray Microscopy

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Biological applications of the XM-1 soft X-ray microscope, located on Beamline 6.1.2 of the Advanced Light Source Facility at the E. O. L Berkeley National Laboratory, has included the examination of a variety of cells and unicellular organisms [1-4], location of surface molecules [5], and tracing of intracellular distribution of proteins and nucleic acids [6]. These results suggested that soft X-ray microscopy could have much greater application in life sciences, and could be a very useful technique to elucidate the structure of small multicellular organisms. This was confirmed during our preliminary studies, conducted during the summers of 1997 and 1998, when soft X-ray microscopy was used to examine two model systems: glutaraldeyde-fixed newborn larvae of *Trichinella spiralis* and the microfilariae of the dog heartworm, *Dirofilaria immitis*. The results obtained indicated that soft X-ray microscopy could be used to obtain new information which could not be obtained by other microscopy techniques, and the soft X-ray images could also serve as a reference for interpretation and integration of results obtained by other electron microscopy techniques. These studies were continued in 2000, using the XM-1 soft X-ray microscope with improved new zone plate, to further elucidate the structure of these two nematodes.

The quality of images obtained with the upgraded XM-1 was greatly superior to those obtained in the preliminary studies. Two notable new observations were made on the *T. spiralis* newborn larva: it was possible to discern the secretory granules within the cytoplasm of the stichocytes, a component of the esophagus of the larva (Fig 1, left). In addition, we were able to demonstrate that the larva contains a fully formed bacillary bands which form the excretory system (Fig. 1, right). This was the first demonstration that the newborn larva possesses an bacillary band. The structure of the bacillary band and of the stichocytes of the newborn larva resembled the corresponding structures of the adult worm. These new and unexpected observations indicate that the newborn larva has attained a remarkable degree of development and differentiation that was hitherto not suspected.

Examination of several microfilariae revealed the internal architecture, arrangement of cells within the body wall, and the relationship between the cellular components of the microfilaria. Particularly impressive were the two complexes: the Excretory and the R1-Anal Vesicle complex, as shown in Figs. 2 and 3, respectively.

Figure 2 depicts the excretory cell complex that consists of the Excretory Cell (EC), its cytoplasmic arm (CA) and the excretory vesicle (EV). The Excretory Cell is much larger than the cells of the nuclear column (NCC) and contains a prominent nucleus. In addition, other discernible features include the esophageal thread (ET) and the muscle cells (M).

Figure 3 depicts two sequential components of the R1-Anal Vesicle complex. The R1 cell is the largest cell with the largest nucleus and nucleolus of all the cells of the microfilaria. Cells R2, R3 and R4 contribute to the formation of the Anal Vesicle, which apparently acts as a rudimentary intestine in this larval stage.

These studies have demonstrated that the unique capabilities of soft X-ray microscopy can be successfully applied to examine the structure of small, multicellular specimens. It provided new information about the specimens examined and was especially useful in depicting the cellular relationships within both the newborn larva and the microfilariae. Further studies are planned to use this technique to identify specific organ systems within the microfilaria, e.g. the location of the nerve cell ganglia and three dimensional reconstruction of the specimens examined.

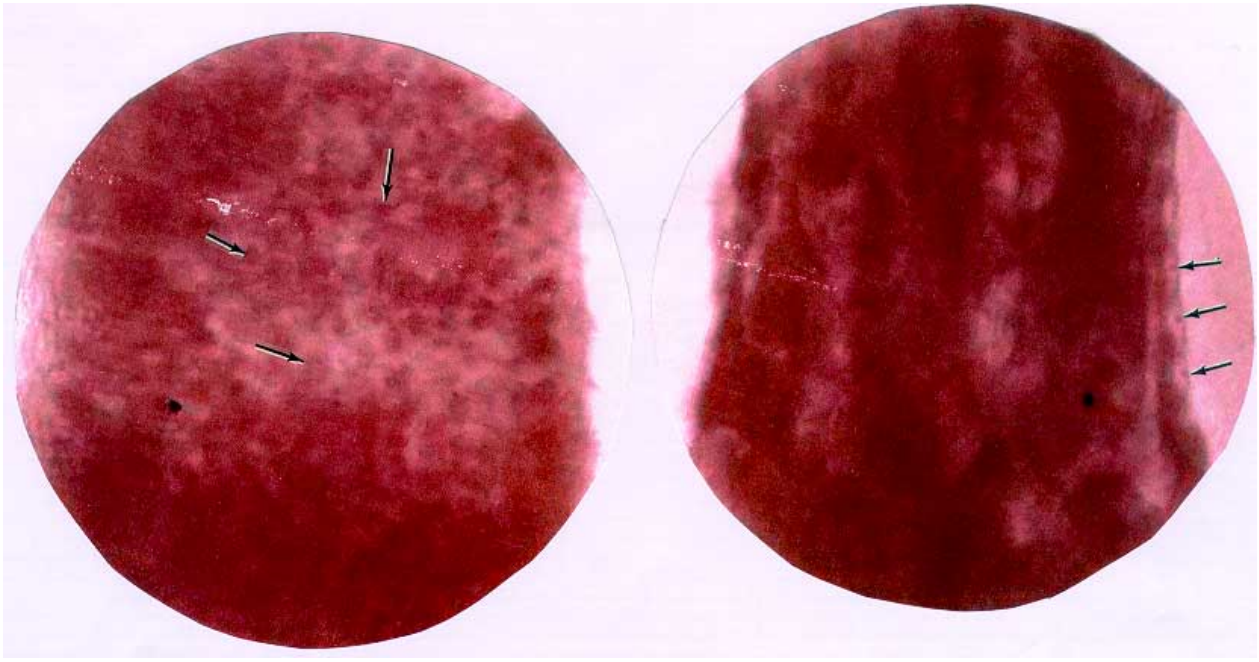

Fig. 1. Micrographs of *T. spiralis* newborn larva. Left – secretory granules (arrows) within a stichocyte. Right – pores (arrows) of the bacillary band.

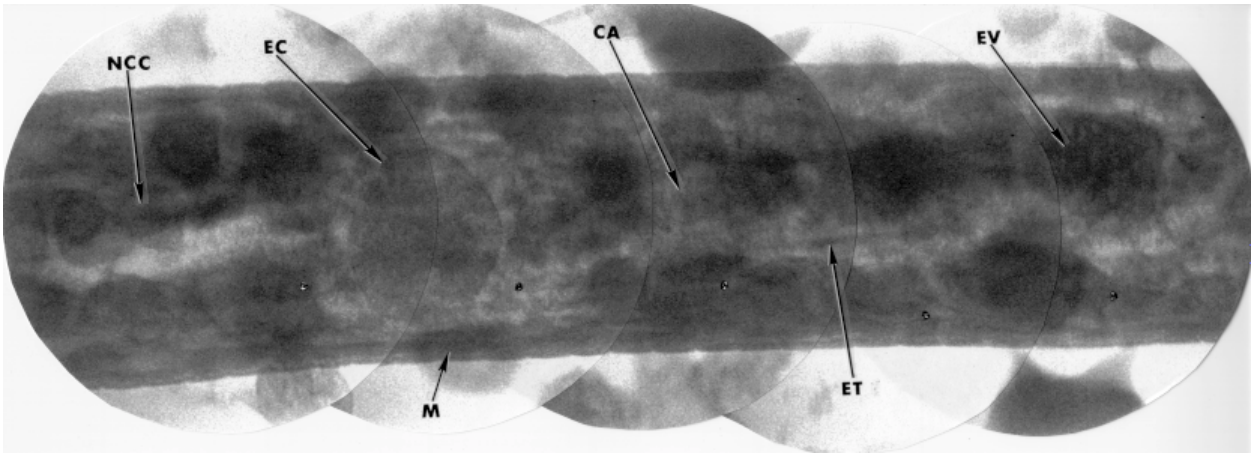

Fig. 2. The excretory cell complex that consists of the Excretory Cell (EC), its cytoplasmic arm (CA) and the excretory vesicle (EV)

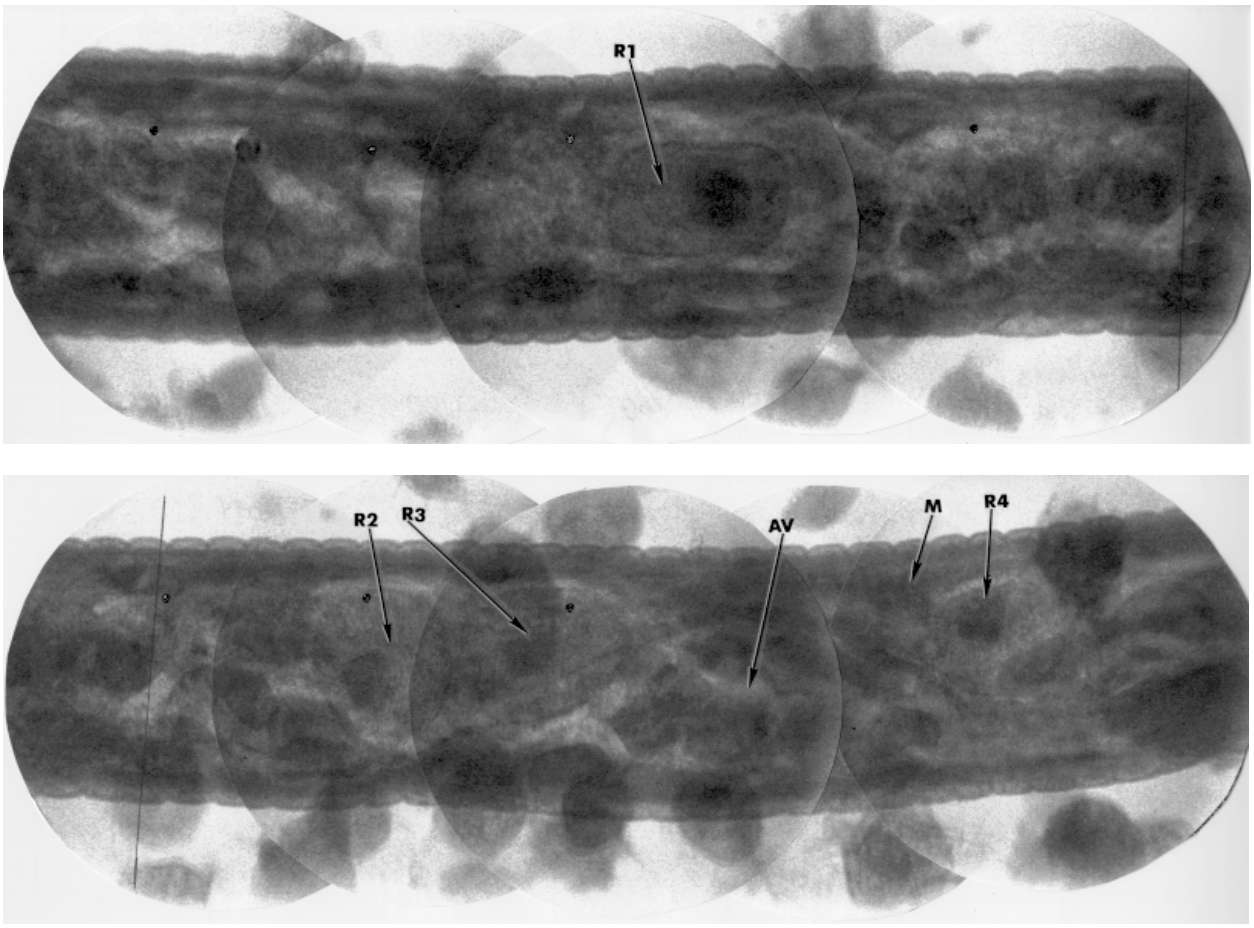

Fig. 3. Two sequential components of the R1-Anal Vesicle complex.

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