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The phospholipids in *Enterocytozoon bieneusi*: an electron spectroscopic imaging study

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Abstract. The treatment with ferriosmium of meronts and sporonts of the microsporidan *Enterocytozoon bieneusi* has shown the lamellar organization of the membranes of the endoplasmic reticulum and of the nuclear envelope. The Electron Spectroscopy Imaging (ESI), (Ottensmeyer and Andrew 1980) of unstained ultrathin sections of meronts showed the presence of phosphorus in the lamellar structures thus confirming their phospholipidic composition. Phosphorus was also detected in the lamellar polaroplast of the spore.

Ultrastructural studies have shown the occurrence of elongated cleft like structures in the meronts and sporonts of the microsporidian *Enterocytozoon bieneusi* Desportes et al., 1985, a parasite of human patients infected with the human immunodeficiency virus (HIV). These electron lucent clefts were observed throughout the cytoplasm. They were also seen lining the flattened surface of the mitotic nuclei which were characterized by their elongated shape (Cali and Owen 1990). These clefts were bounded by a thick and electron dense membrane (Fig. 1). The utilization of a fixative containing ferriosmium has shown that the thick membranes corresponded to lamellar structures which were closely associated with the cisternae of the endoplasmic reticulum and with the envelope of the nuclei (Fig), (Desportes et al. 1991, Hilmarsdottir et al. 1993). The clefts occurred within these lamellar structures. They corresponded to the space due to the separation of two lamellae or from their alteration. Ferriosmium is known to be a fixative of cell lipids. The preservation of the lamellar structures by ferriosmium indicated that they could correspond to a storage of phospholipids. The electron spectroscopy imaging (Ottensmeyer and Andrew 1980) which allows the analysis of elemental distribution in ultrathin sections has been employed in order to detect the presence of phosphorus in the lamellar structures.

MATERIALS AND METHODS

Biopsies containing the parasite were obtained from two patients with AIDS and chronic diarrhoea. The samples obtained by upper intestinal endoscopy were processed for transmission electron microscopy as previously reported (Desportes et al. 1991, Hilmarsdottir et al. 1993). Electron spectroscopy imaging of unstained ultrathin sections (30-40 nm) was performed on a CEM 902 A electron microscope (Zeiss) equipped with an integrated electron energy filter according to Castaing-Henry-Ottensmeyer (Ottensmeyer and Andrew 1980). The instrument was operating at a voltage of 80 kV. Image analysis for the mapping of phosphorus (P), after acquisition through a TV camera, was performed with an IBAS (Kontron, Münich and Zeiss, Oberkochen) program using a "two window method". Images were taken at two different energy losses: background window at Delta E=120 eV and a P window at Delta E=155 eV (with 400 µm diameter condenser and 100 µm diameter objective apertures and a 10 eV energy selecting slit). Net phosphorus mapping images (corrected for background contribution) were thus obtained.

RESULTS

The data herein presented were obtained from the sections of meronts and spores. In the meronts, ESI showed a high concentration of phosphorus in the lamellar structures (Figs. 3, 4). A high concentration of phosphorus was also detected in the lamellar polaroplast of the spores. It was also present in the manubrium of the polar tube (Figs. 5, 6).

DISCUSSION

The presence of phosphorus in the lamellar structures confirms their phospholipidic composition. The phospholipidic composition of the lamellar polaroplast is also shown by ESI. Phospholipidic material with a lamellar periodicity has been reported in different biological materials. It is a constituent of the lamellar bodies which are lipid storage organelles found on the surface of various organs and tissues: lung, gastric parietal cells, enterocytes

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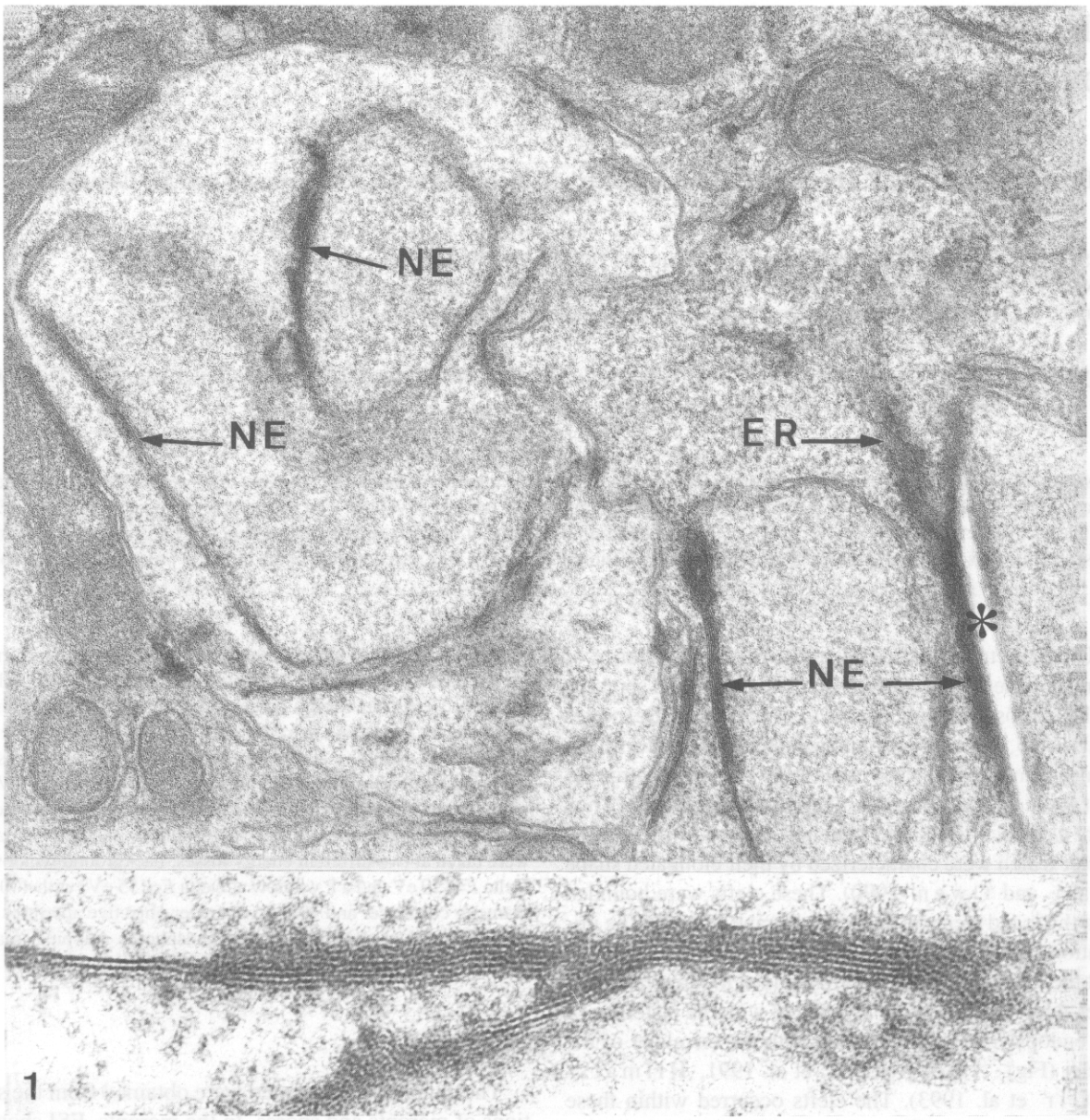
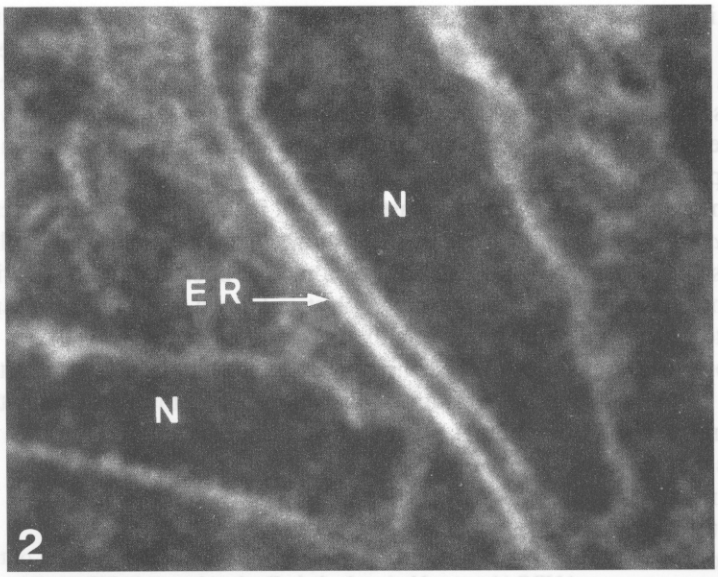


Fig. 1. -Top: Conventional transmission electron micrograph (TEM) of two adjacent meronts containing several nuclei. A cleft (asterisk) and the electron dense portions of the nuclear envelope (NE) and endoplasmic reticulum (ER) can be seen. x 60 000. -Bottom: High magnification showing the lamellar organization of the electron dense portions of NE and ER. Both structures are in continuity. The lamellar periodicity approximates 4.5 to 5 nm. x 210 000.

(Fehrenbach et al. 1991, Schmitz and Müller 1991) and also on the surface of the frog's taste organ (Sbarbati et al. 1991). In those cases it is considered as a surfactant material playing a role in lubrication, cytoprotection and solute exchange.

Lamellar bodies also occur due to the disturbance of lipid metabolism or lipid traffic observed in human diseases such as atherosclerosis or psoriasis (Schmitz and Müller 1991).

Fig. 2. High contrast image (Delta E=250 eV). The large distribution of carbon in the cell gives a high sensitive image of the meront which facilitates the interpretation of Figs. 3, 4. N - nucleus. x 10 000. **Fig. 3.** Reference image (Delta E=150 eV) showing the distribution of phosphorus in the same meront. x 10 000. **Fig. 4.** Net distribution of phosphorus. Image obtained after subtraction of the background (Delta E=150eV-Delta E=120eV). x 10 000. **Fig. 5.** High contrast image of the spore (Delta E=250 eV). The arrow indicates the anterior part of the polar tube (manubrium) associated with the lamellar polaroplast. x 10 000. **Fig. 6.** Net distribution of phosphorus (Delta E=150eV-Delta E=120 eV). A high concentration of phosphorus is detected on the lamellar polaroplast and on the manubrium of which only the anterior part can be seen in the section (arrow). x 10 000. **Fig. 7.** TEM of the lamellar polaroplast and manubrium (arrow). x 66 000.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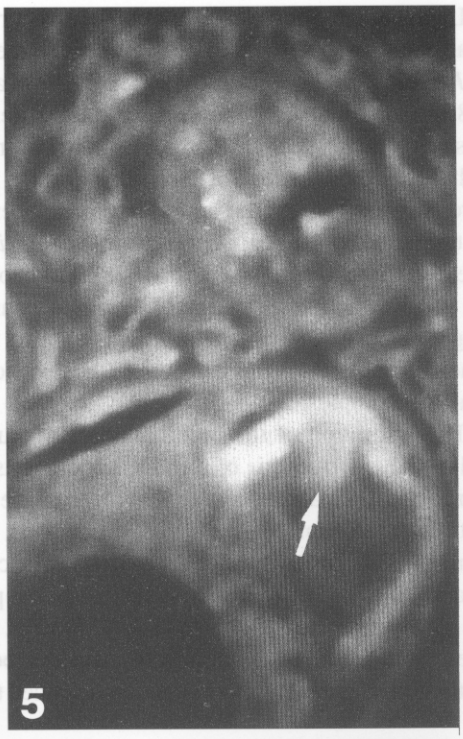
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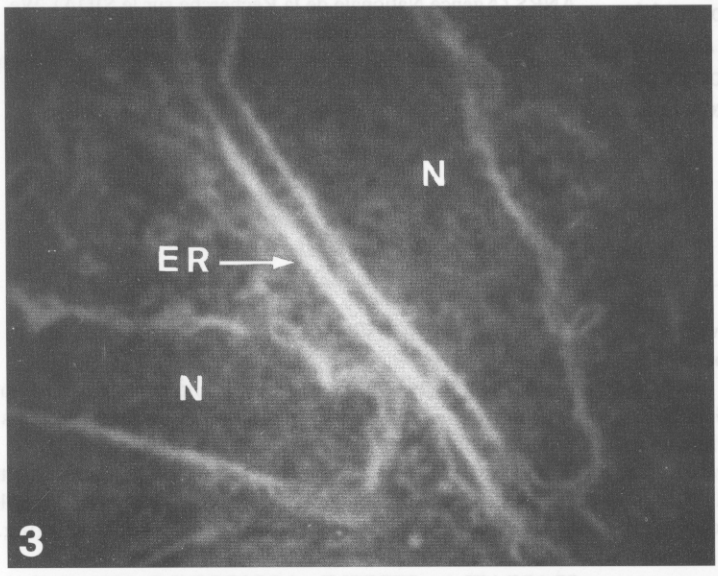
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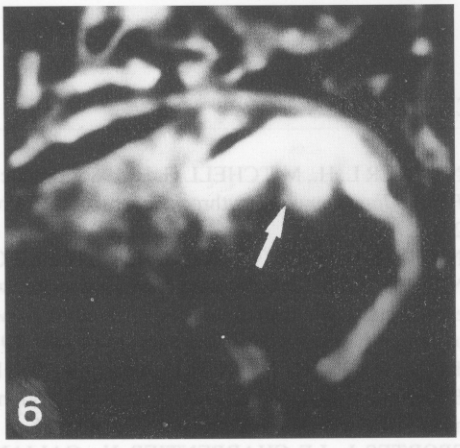
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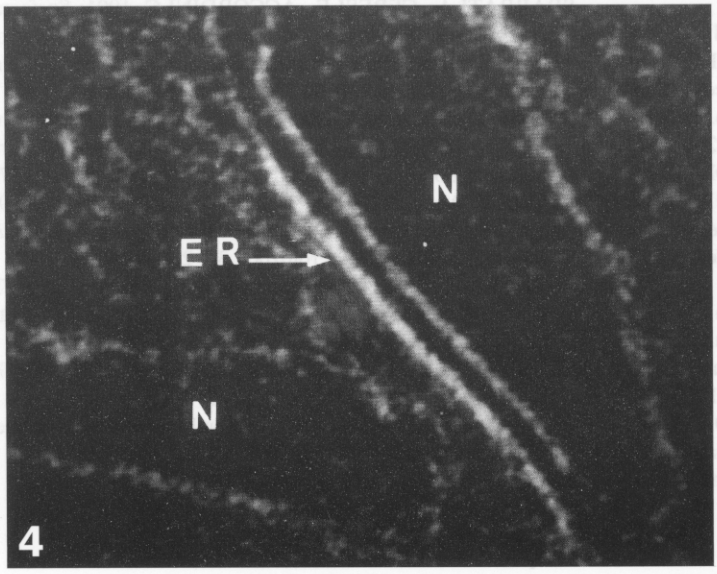
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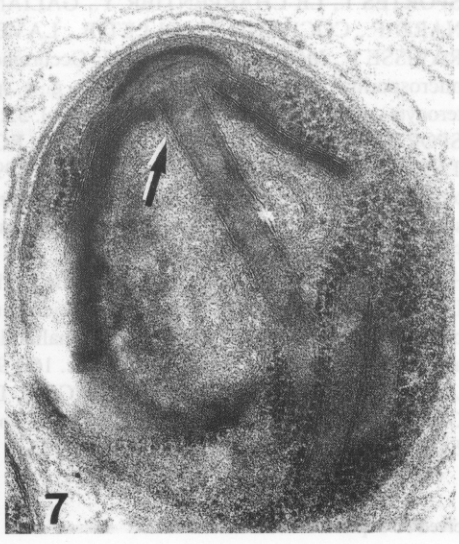
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Lamellar structures are also known to occur in unicellular parasites. They have been reported in the rhoptries of *Plasmodium berghei* sporozoites (Stewart et al. 1985) and in those of *P. knowlesi* merozoites (Bannister et al. 1986). In both cases, they correspond to a phospholipidic material which will form the parasitophorous vacuole. Lamellar bodies have also been reported at the apex of the sporozoites of *Eimeria magna* (Jenssen and Hammond 1975). The phospholipids are involved in the formation of membranes and, according to Bannister et al. (1986), the ability to generate new membranes is fundamental to the invasion process in all Apicomplexa.

The resorption of the multilamellar structures at the end of the sporogenesis of *E. bieneusi* suggests that they represent a normal lipid storage involved in the rapid formation of the sporoblasts. In this occurrence, the development of this microsporidan species may be considered as a schizogonic process similar to that reported by Bannister and Mitchell (1986) in *P. knowlesi*. These authors have shown that the lamellar bodies present in the schizont of this species corresponded to a lipidic material which contributed to the rapid formation of the plasma membrane and other various cytoplasmic membranes of the merozoites. The generation of new membranes appears to be determinant for the production of *Plasmodium* merozoites as well as for the formation of *Enterocytozoon* sporoblasts.

The polaroplast is present in the spores of most microsporidan species. ESI shows that the lamellar polaroplast corresponds also to a storage of phospholipidic material. The lamellar polaroplast is known to form the plasma membrane of the extruded sporoplasm (Weidner et al. 1984). The phosphorus detected in the anterior part of the manubrium is likely located in the two concentric membranes surrounding the polar tube (Weidner 1986), (Figs. 5–7).

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