Ultrastructure of *Besnoitia* cysts from reindeer (*Rangifer tarandus tarandus* L)

Mosaad Hilali[1], **Claes Rehbinder**[2] and **Tapio Nikkilä**[2]

[1] Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.
[2] The National Veterinary Institute. Uppsala, Sweden

Abstract: The ultrastructure pf *Besnoitia* cysts in reindeer is described. The observations made on *Besnoitia* cysts and merozoites indicate a form distinct enough to be placed in a new species - *Besnoitia tarandi.*

Key words: parasitology

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Introduction

The presence of *Besnoitia* cysts in reindeer is a common finding (Hawden & Palmer 1922, Nikolaevskii 1961, Nordkvist 1966, Choquette et al 1967, Skjenneberg & Slagsvold 1968, Rehbinder *et al.* 1981). Little, however, is known about the morphology of the parasite in reindeer.

The aim of the present investigation was to study the ultrastructure of the *Besnoitia* cysts and the cyst content of merozoites from reindeer.

Material and methods

Fascias and periost from metatarsal and metacarpal bones, having palpable cysts, were collected from reindeer in connection with the slaughter of the animals the 9th of March 1988 (Mausjaure, Lappland, Sweden). The specimens were cut into pieces of approximately 1 mm^3 and fixed according to Karnowsky at a temperature around 4°C for 4-8 hours and at room temperature for 48 hours.

Before embedding, the material was postfixed in 1% OsO_4 solution buffered with 0.1 M phosphate buffer. After embedding in Epon, 1 mm thick sections were cut and stained with toluidine blue for light microscopy.

Ultrathin sections were prepared on an LKB ultratome, picked up on formvar coated and uncoated copper grids, stained with uranyl acetate and lead citrate and examined in a Philips electron microscope 420, at 60 kv, at magnifications varying between 1 000 and 60 000.

Results

By light microscopy the *Besnoitia* cysts are subspherical and measuring 0,18 - 0,22 x 0,28 - 0,32 mm. The cyst wall appears to be composed of two layers, a parasitophorous vacuole of a hypertrophied multinucleated cell and a surrounding homogenous extracellular capsule. The parasitophorous vacuole contains thousands of banana shaped merozoites.

The outer cell membrane of the parasitophorous vacuole, in the light microscope, has numerous pseudopods appearing almost as microvesicles bordering the extracellular capsule. The nuclei are large and pale, some containing dark nucleoli. The extracellular capsule is mostly of a homogenous character but has scattered and clustered elongated nuclei (Fig 1).

Ultrastructurally the extracellular cyst wall consists of an electron lucid material with fibrillar structures and fibrocytes morphologically similar to the keratinocytes of the cornea.

The plasma membrane of the host cell has numerous microvilli or pseudopods protruding into the electron lucid material of the extracellular cyst wall (Fig 2).

The plasma membrane of the parasitophorous vacuole had numerous protrusions extending into the vacuole. The vacuole contains the merozoites and a ground substance which shows an obvious condensation along the plasmamembrane (Fig 3).

The nuclei of the multinucleated cell have a low electron density pattern with an evenly dispersed chromatin, while the nucleoli are com-

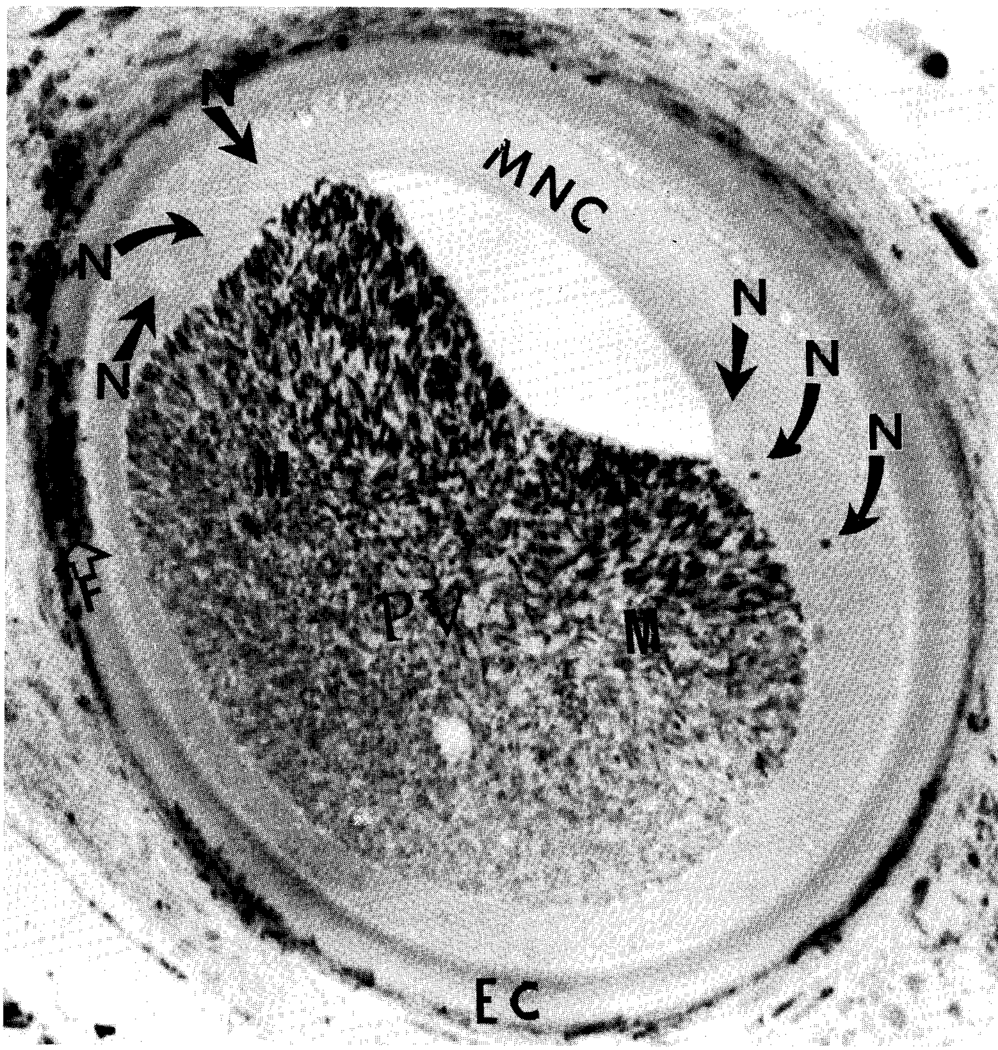

Fig. 1. *Besnoitia* cyst. Note extracellular capsule of a homogenous charcter (EC.) fibrocytes (F) several nuclei (N) of the multinucleated cell (MNC) and parasitophorus vacuole (PV) filled with merozoites (M). Light microscopy x 800.

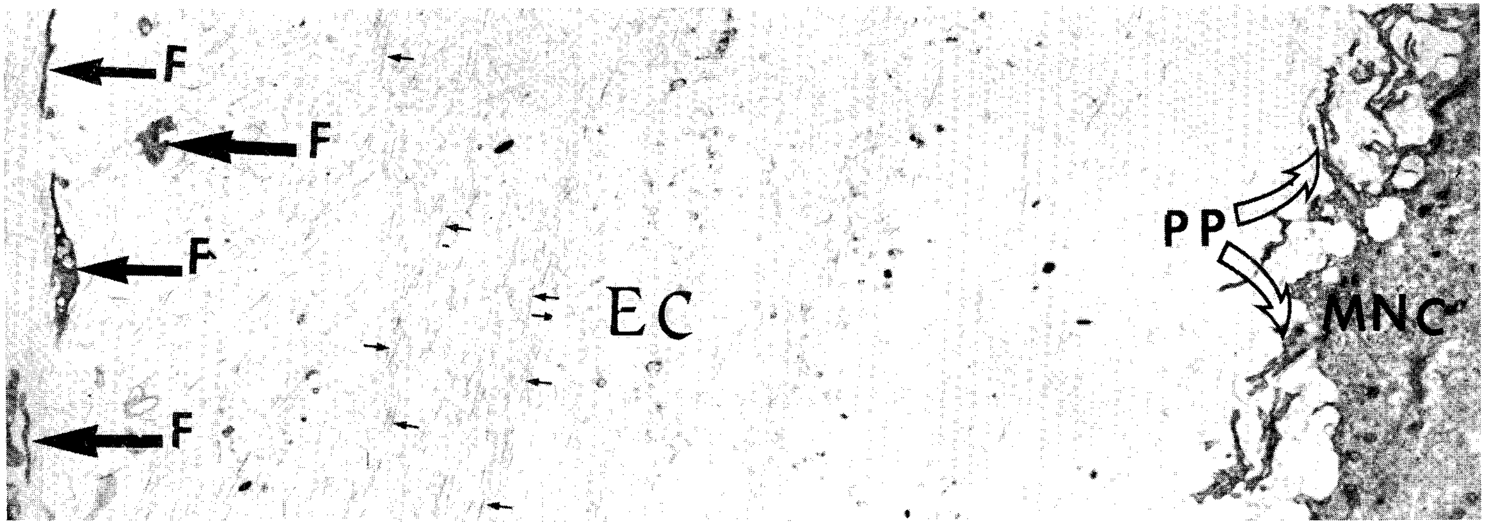

Fig. 2. Extracellular wall. Note electron lncid matrix with filbrillar structures (arrows), fibrocytes morphologically similar to the kerationcytes of the cornea (F) and nnmerous microvilli or pseudopods (P.P) of the multinucleated cell (MNC) x 5400.

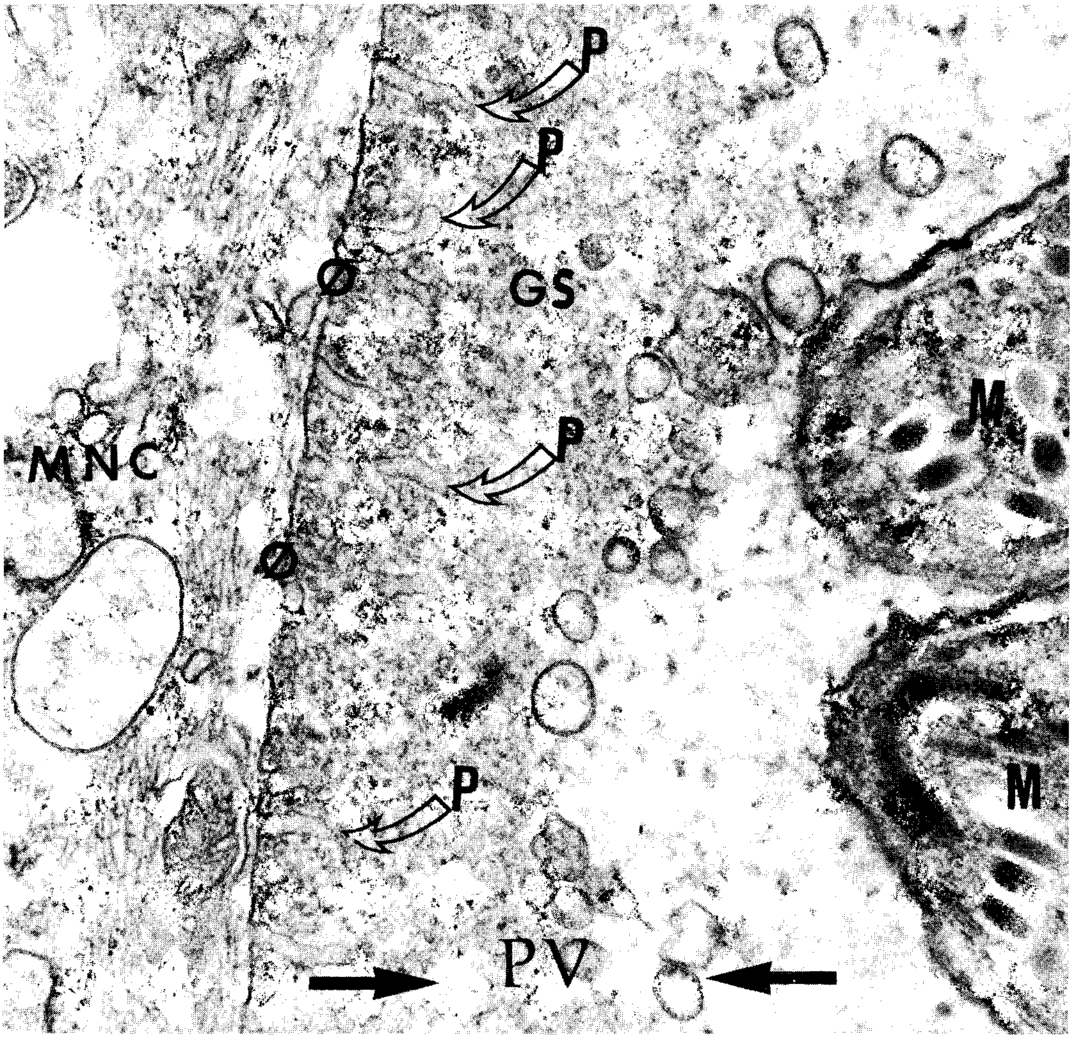

Fig. 3. The plasma membrane (Ø) of the multinucleated cell (MNC) forming a parasitophorus vacuole (PV) containing merozoites (M). Note numerous protrusions (P) and a condensation of the ground substance (GS) at the plasma membrane x 55000.

pact and markedly electron dense. The cytoplasma contains a considerable amount of mitochondria and dilated rough endoplasmic reticulum (Fig 4).

Ultrastructurally, merozsites are found inside a large parasitophorous vacuole of a multinucleated cell.

The spindle shaped merozoites are lying in an electronlucid material. They are pointed at both ends and measuring 6.5 - 11.0 μm x 1.0 - 1.5 μm (mean 8.7 x 1.2 μm). The merozoites have a typical coccidian pellicle. They contain in the anterior end a polar ring, the conoid 22 microtubules, 1-6 rhoptries and numerous elongated micronemes. The nucleus is relatively large and usually located at the beginning of or in the posterior half of the merozoite. The position of the mitochondrion varies from the region of the micronemes to the posterior end of the merozoite (Figs 5 & 6).

In the anterior part of the merozoite one or two vacuoles 1.0 x 0.5 μm are present . These vacuoles have a not very well defined wall and some contain a rounded clump of an electron dense material (Fig 7).

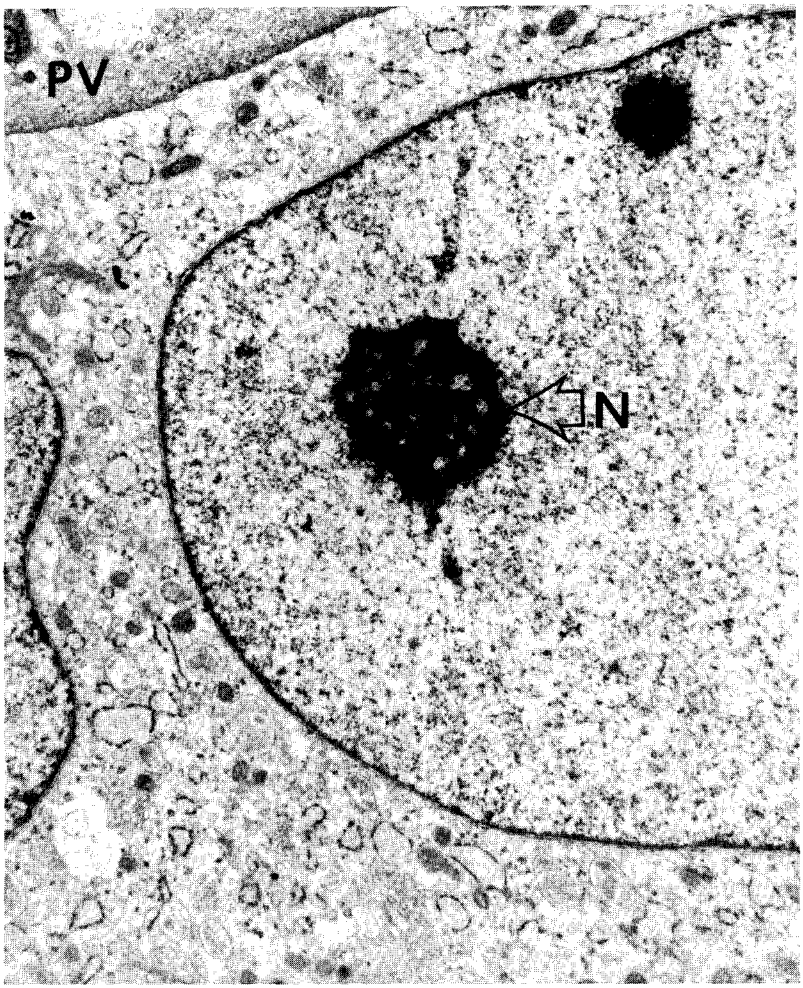

Fig. 4. Multinucleated cell. Note the electron dense nucleous (open arrow N) and the evenly dispersed chromatin of the nucleus. Note also numerous mitochondria and dilated rough endoplasmic reticulum. Pv=parasitophorus vacuole x 9000.

Discussion

The ultrastructure of the *Besnoitia* cysts and merozoites from reindeer show great similaritres with *B. jellisoni* (Sheffield 1968, Senaud 1969, Scholtyseck *et al.* 1973, Scholtyseck *et al.* 1974) and *B. besnoiti* (Senaud *et al.* 1986). *Besnotia* cysts of reindeer are smaller and the merozoites are longer and more slender than those of *B. jellisoni* and *B. besnoiti* (Senaud 1969, Scholtyseck 1973, Widauer 1983). The number of rhoptries in the merozoites of *Besnoitia* cysts from reindeer (1-6 in number) differs from those reported in *B. jellisoni* (3-5 in number, Scholtyseck *et al.* 1973) or *B. besnoiti* up to 12 in number, Senaud 1969). *B. besnoiti* merozoites possess two preconoidal rings (Widauer 1983, Göbel *et al.* 1985) while *B. jellisoni* has no preconoidal ring (Scholtyseck *et al.* 1970) and it is also lacking in the merozoits from *Besnoitia* cysts of reindeer.

Also differing the merozoits found in *Besnoitia* cysts of reindeer from merozoits of *B. jellisoni* and *B. besnoiti* is the presence of vacuoles with a not very well defined wall and containing rounded clumps of an electron dense material.

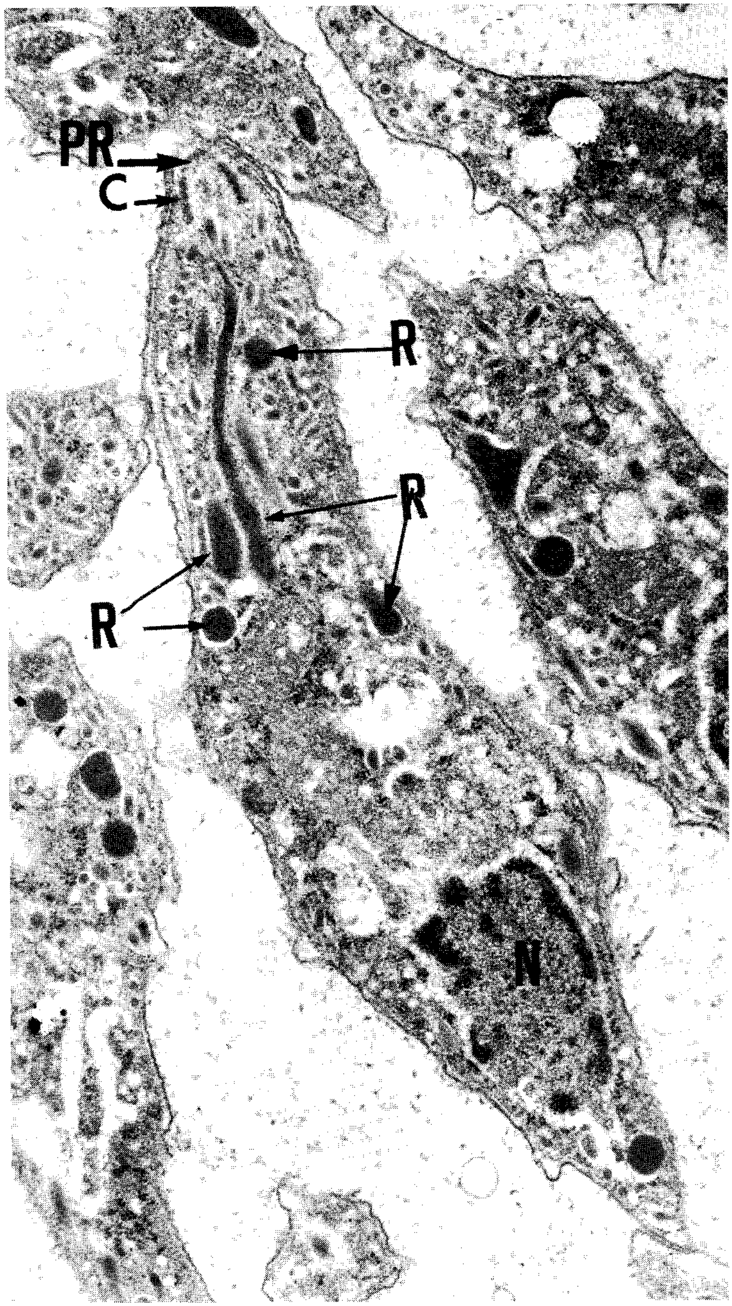

Fig. 5. Merozoites. Note polar ring (PR) conoid (C), rhoptries (R) and a relatevely large nucleus x 24000.

The authors have not found any reports on such vacuoles in previous studies.

The observations presented on *Besnoitia* cysts and merozoits from reindeer indicate a form distinct enough to be placed in a new species - *Besnoitia tarandi*.

References

Choquette, L.P.E., Broughton, E., Miller, F.L., Gibbs, H.C., & Cousineau, J.G. 1967. Besnoitiosis in barren ground caribou in northern Canada. - *Can. Vet. J.* 8:282-287.

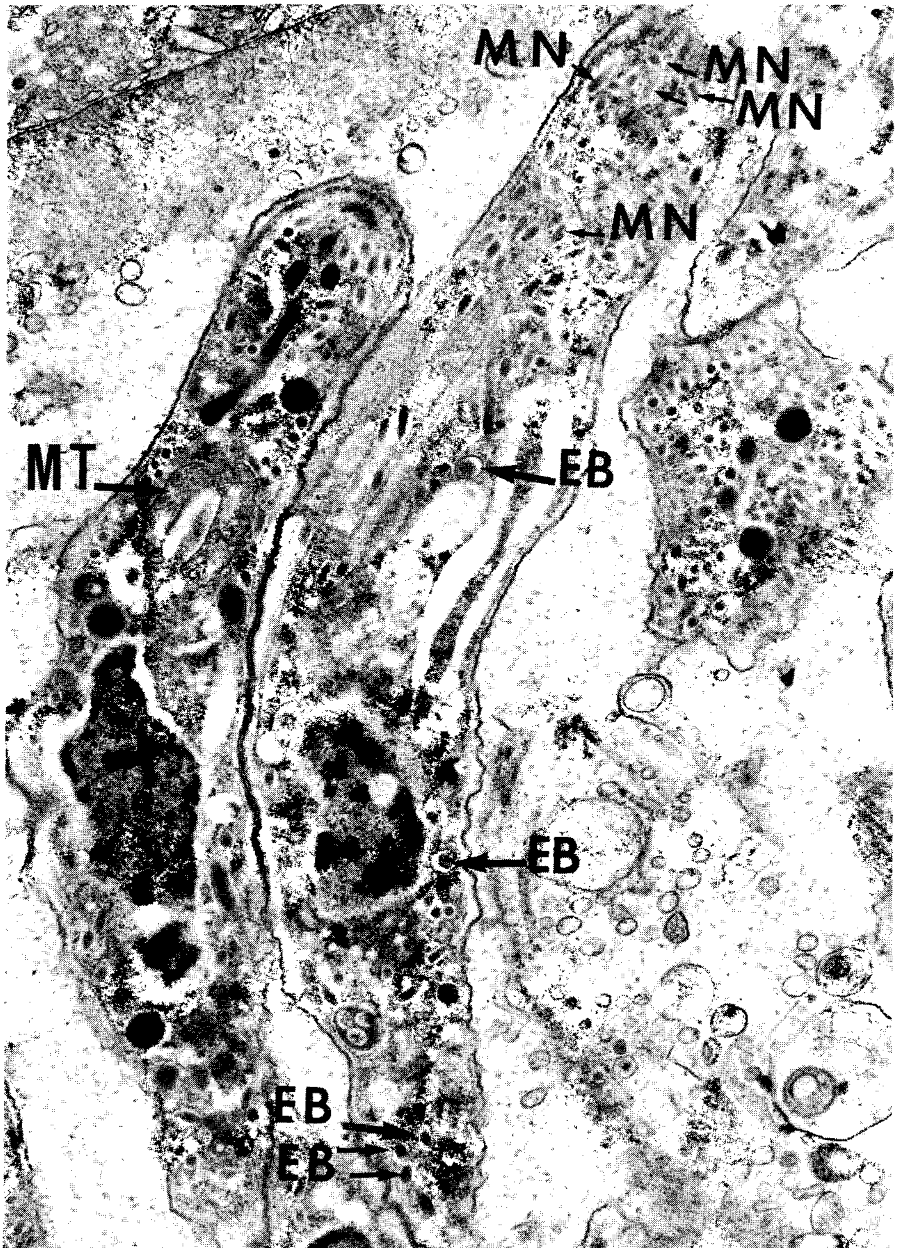

Fig. 6. Merozoites. Note elongated micronemes (MN), enigmatic bodies (EB), and mitochondrion (MT) x 24000.

Göbel, E., Widauer, R., Reinmann, M & Munz, E 1985. Ultra structure of asexual multiplication of *Besnoitia besnoiti* (Marotel 1912) in Vero- and CRFK-cell cultures *J. - Vet. Med B.* 32:202-212.

Hadwen, S. & Palmer, L.J. 1922. Reindeer in Alaska. - U.S. *Department of Agriculture. Bulletin no 1089, Washington, p. 68.*

Nikolaevskii, L.D. 1961. Diseases of reindeer. - *In: Reindeer husbandry.* P.S. Zhigunov ed. Moscow. Israel Program for Scientific Translations pp 266-268.

Nordkvist, M. 1966 Renens sjukdomar (Diseases of Reindeer) - *In: Ekonomisk renskötsel,* LTs Förlag, Borås, Sweden p. 127.

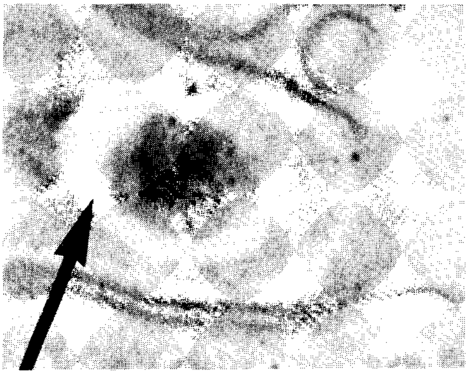

Fig. 7. Vacuole (arrow) without well defined wall containing rounded clumps of elctron dense material x 90000.

Rehbinder, C., Elvander, E. & Nordkvist, M., 1981. Cutaneous Besnoitiosis in a Swedish Reindeer (*Rangifer tarandus* L.). - *Nord, Vet.-Med.* 33:270-272.

Scholtyseck, E., Mehlhorn, H. & Friedhoff, K. 1970. The fine structure of the conoid of sporozoa and related organisms. - *Parasitenk.* 34:68-94.

Scholtyseck, E., Mehlhorn, H. & Muller, B.E.G. 1973. Identifikation von Merozoiten der vier cystenbilden Coccidien *(Sarcosystis, Toxoplasma, Besnoitia, Frenkelia)* auf Grund feinsturktureller Kriteriern. - *Z. Parasitenk.* pp. 185-206.

Scholtysek, E., Melhorn, H & Muller, B.E.G. 1974. Feinstruktur der Cysts und Cystenwand von *Sarcocystis tenella, Besnoitia jellisoni, Frenkelia sp.* und *Toxoplasma gondii.* - *Z. Protozool.* 21:284-294.

Senaud, J. 1969. Ultrastructure des formations kystiques de *Besnoitia jellisoni* (Frenkel 1953), Protozoare, *Toxoplasma,* parasite de la souris (Mus musculus). - *Protistologica.* 5:413-430.

Senaud, J., Mehlhorn, H., Heydorn, A.O. & Matuschka, F.R. 1968. Étude ultrastructurale des kystes de *Besnoitia besnoiti* chez le baeuf - *Protistologica* 22:287-290.

Sheffield, H.G. 1968. Observations on the fine structure of the cyst-stage of *Besnoitia jellisoni.* - *J. Protozool,* 15:685-693.

Skjenneberg, S. & Slagsvold, L. 1968. *Reindriften og dens naturgrunnlag.* (Reindeer Husbandry and its Ecological Principles). - Universitetsforlaget. Oslo p 142.

Widauer, R.M. 1983. *Licht- und Elektronenmikroskopiche untersuchungen zur Ungeschlechtlichen Vermehrung von Besnoiti besnoiti (Sporozoa, Apikomplexa) in Zellkulturen.* Inaugural - Dissertation zur Erlaugung der Tiermedizinischen Doktorwürde der Tierärztlichen Fakultät der Ludwig-Maximilians-Universität München. München pp 58.