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Myofibre Changes and Capsule Formation in Mice Infected with Different Strains of Trichinella

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Evensen, Ø., E. Skjerve and B. Bratberg: Myofibre changes and capsule formation in mice infected with different strains of Trichinella. Acta vet. scand. 1989, 30, 341–346. – Mice were infected with 4 strains of Trichinella with the purpose of describing muscle changes (*M. Gastrocnemius,* diaphragma) by light and electron microscopy, paying particular attention to capsule formation in a polar bear isolate (T. no. 13).

No differences in myofibre changes or capsule formation were observed between the different strains by light microscopy at 21 and 60 days post infection. Ultrastructural studies of meat samples 60 day post infection with T. no. 13 revealed active capsule formation in muscle fibres, characterized by protrusions and invaginations of the sarcolemma. Basal lamina-like material was located close to the sarcolemma and in a lattice-like pattern in the capsule matrix.

T. no. 13 seems to have a migratory pattern similar to the other strains examined and induces muscle fibre changes undiscernible from those of the 3 other strains. Infected muscle cells are considered to be involved in capsule formation.

trichinosis; polar strain; light microscopy; ultrastructure.

Introduction

Muscular lesions caused by the invasion and encystment of Trichinella in domestic animals and man have been dealt with by several authors (*Gould et al.* 1955, *Gould* 1970, *Jones & Hunt* 1983, *Robbins et al.* 1984, *Jubb et al.* 1985).

During the invasion phase, small haemorrhages and accumulations of neutrophils, eosinophils, lymphocytes and macrophages are present in the interstitial connective tissue (*Gould* 1970). The capsulation process resulting in the formation of a cyst, causes specific alteration and adaptation of the infected muscle cells. Although most often referred to as a basophilic degenerative process, it represents a redifferentiation that probably serves a variaty of necessary functions for the larva (*Teppema et al.* 1973, *Despommier* 1976). However, there are few reports on morphological characterization of the inflammatory response, myofibre changes and capsule formation caused by infection with Trichinella in laboratory animals.

The purpose of the present investigation was to compare myofibre changes and capsule formation in mice infected with a polar bear isolate and 3 different, defined strains of Trichinella by light microscopy. Electron microscopic evaluation of capsule formation was performed on the isolate from the polar bear only.

Material and methods

A Trichinella isolate (T. no. 13), originally isolated from a polar bear (*Ursus maritimus*) from the arctic regions of Canada in 1984

Figure 1. Light microscopic myofibre changes and capsule formation caused by different strains of Trichinella.

Figure 1a. Mouse infected with *T. spiralis* II; 21 days p.i. Infiltration of inflammatory cells in infected muscle fibres (M) and interstitial connective tissue.
T = Trichinella larva. H = haemorrhage. HE. × 80.

Figure 1b. Mouse infected with *T. nativa*; 21 days p.i. Piling of nuclei in a regenerating muscle fibre. HE. × 320.

Figure 1c. Mouse infected with T. no. 13; 21 days p.i. Infected muscle fibres with sparse inflammatory reaction. HE. × 80.

Figure 1d. Mouse infected with T. no. 13; 21 days p.i. Degenerated infected muscle fibres (IMF). Neighbouring fibres (M) appear normal. HE. × 320.

Figure 1e. Mouse infected with T. no. 13; 60 days p.i. Sparse (arrow) and heavy (arrowhead) inflammatory infiltrations around Trichinella larvae in infected muscle fibres. Negligible inflammatory reaction in interstitial connective tissues. HE. × 80.

Figure 1f. Mouse infected with T. no. 13; 60 days p.i. Granuloma with multinucleate giant cells around degenerated larva (arrows). HE. × 320.

Figures 2, 3 & 4. Electron micrograph from mice infected with T. no. 13 (polar bear isolate).

Figure 2a. Schematic presentation of encysted larva (T) in a muscle cell (shown by black). The capsule is formed around the infected muscle cell and consists of an inner loosely arranged material (white zone) and an outer part with collagen fibres and fibrocytes (stippled zone). The normal muscle cell is shown to the right.

Figure 2b. Infected muscle cell with Trichinella larva (T), an inner and outer capsule, and adjacent normal muscle cell.

Figure 3a. Area with nucleus (Nu) of infected muscle fibre (IMF), and capsule (C) with collagen bundles (Co) in the outer capsule segment. T = Trichinella larva, F = fibrocyte, P = protrusions. × 9000.

Figure 3b. Several nuclei (Nu) in an infected muscle fibre located in a group. Prominent nucleoli (Nl) can be seen. T = Trichinella larva, M = small mitochondria. × 6000.

Figure 4a. Cytoplasm in an infected muscle fibre with prominent smooth (S) and rough (R) endoplasmic reticulum. Free polyribosomes are also demonstrated (arrows). Nu = nuclei. × 12000.

Figure 4b. Surface of an infected muscle fibre with protrusions (P) and invaginations (I). Protrusions are cut at different angles revealing smooth vesicles (arrows). Basal lamina-like material (arrowheads) is located around the protrusions and in the capsule matrix. Fibrillar material (F) without striations is also demonstrated. × 24000.

Figure 4c. Outer capsule segment demonstrating fibrocyte (F) and collagen bundles cut in longitudinal (Co) and transversal (Cot) sections. × 15000.

Figure 4d. Centrally located nuclei in neighbouring uninfected muscle fibre. Polyribosomes (P) and prominent mitochondria (M) can be seen. Nu = nucleoli. × 6000.

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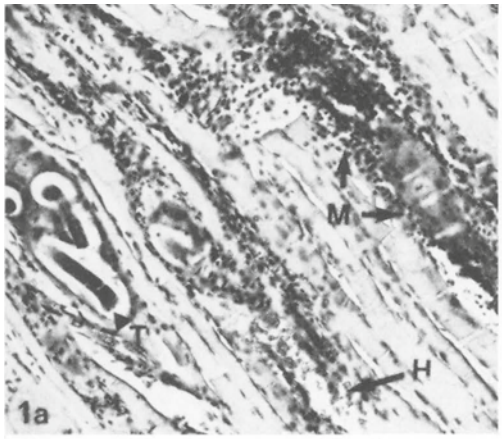

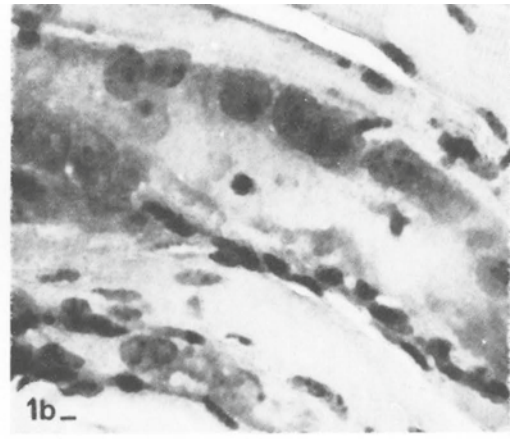

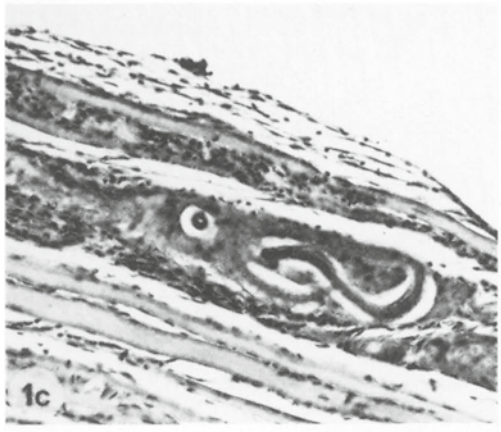

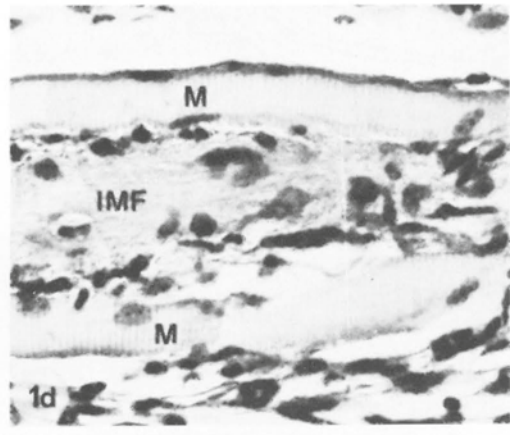

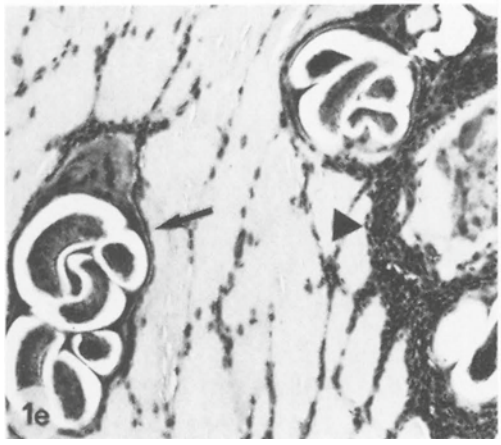

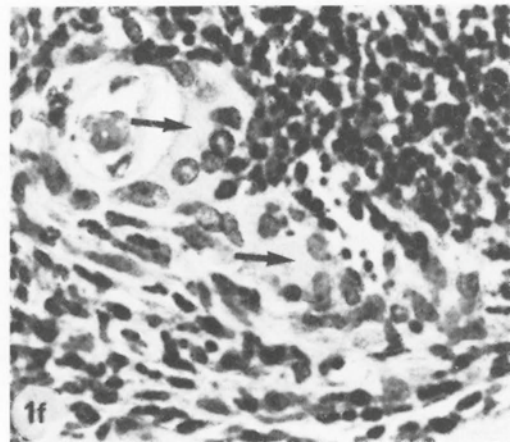

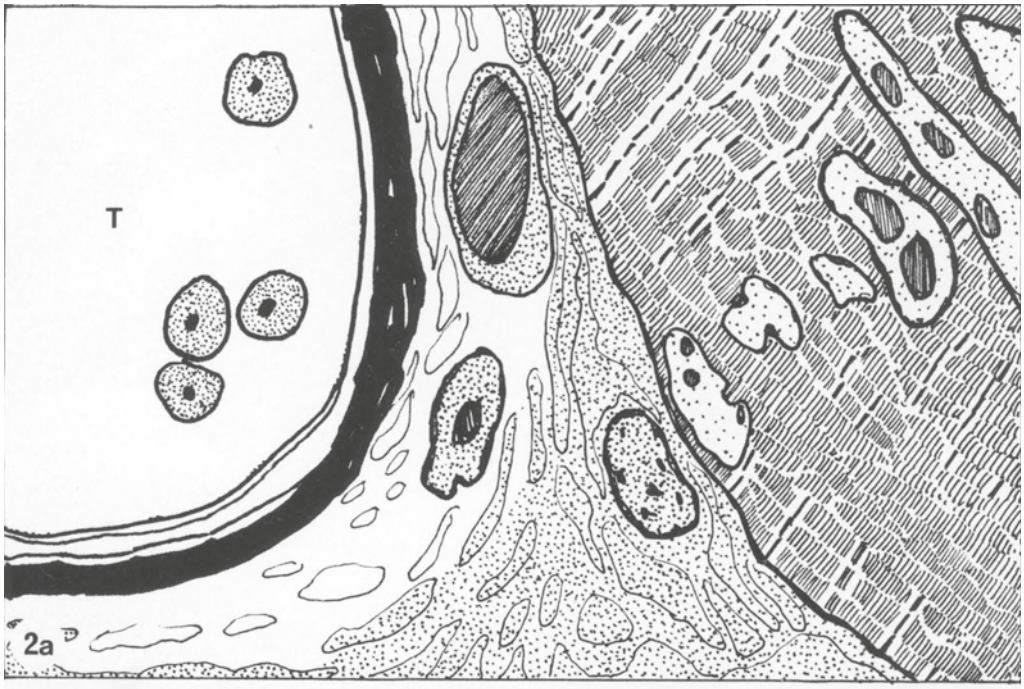
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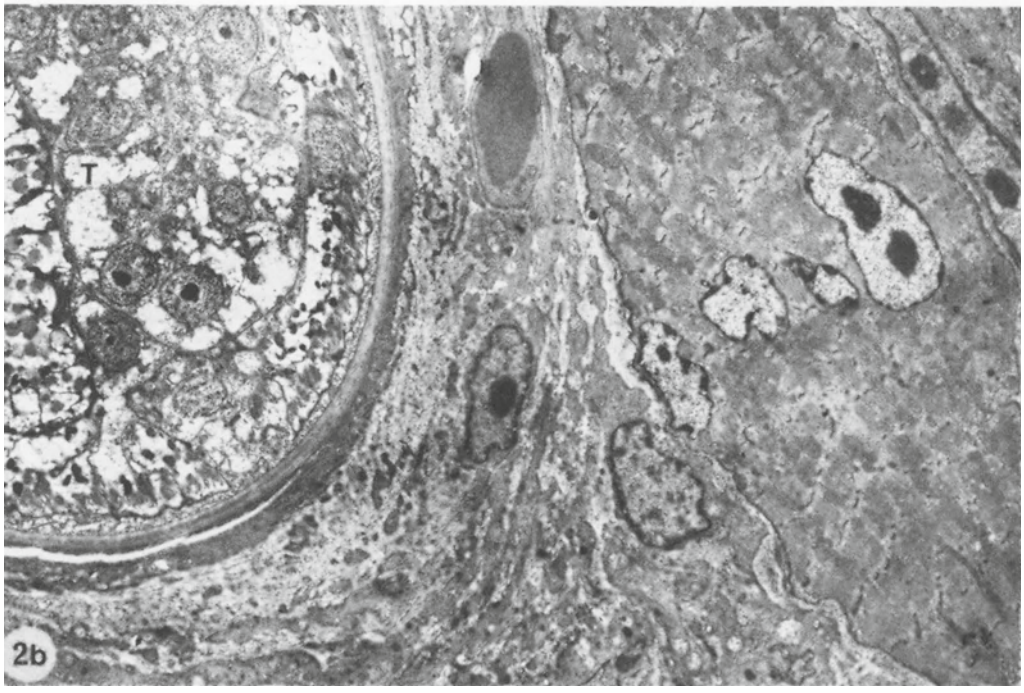
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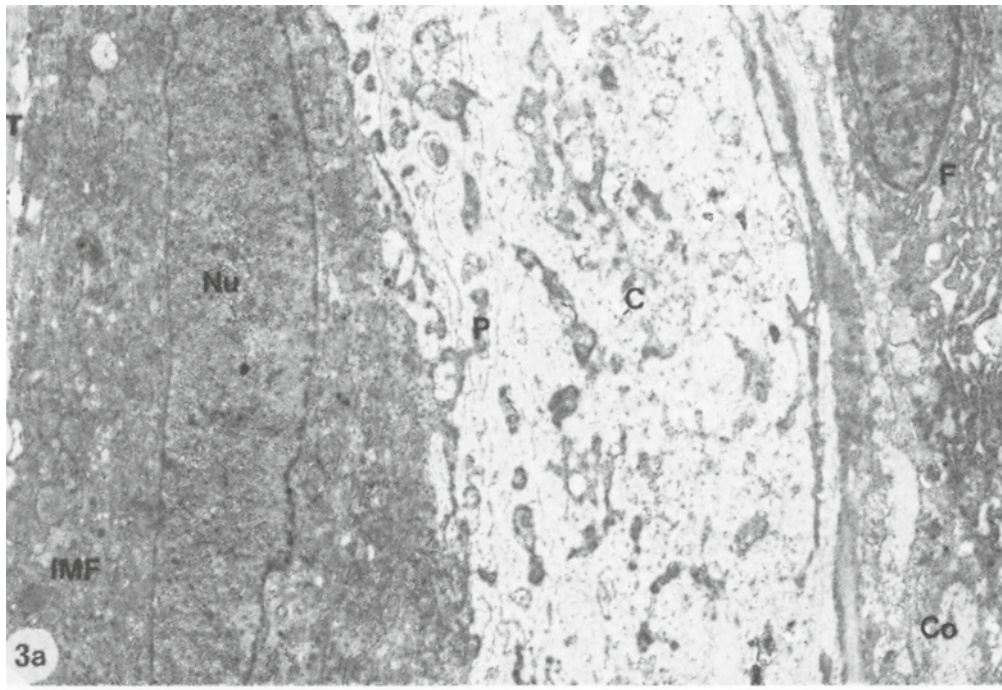
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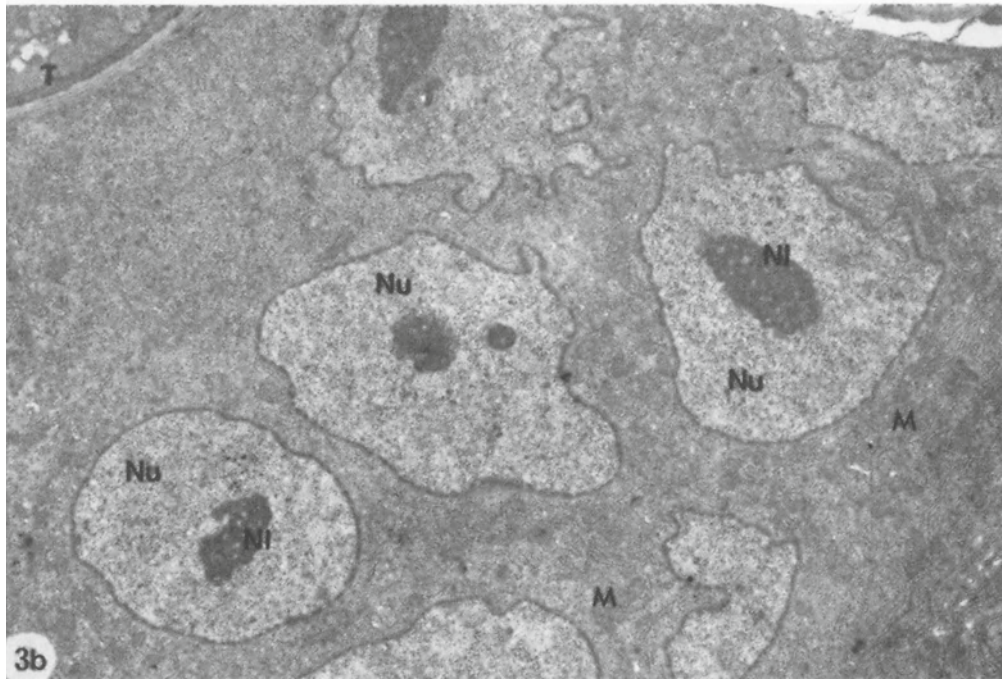
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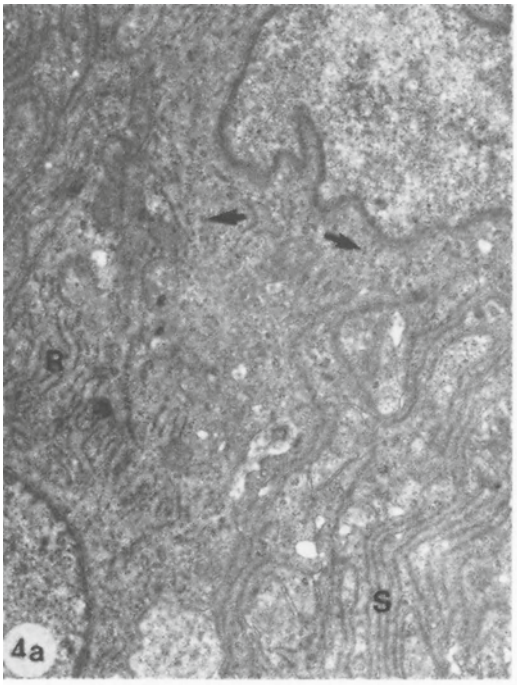
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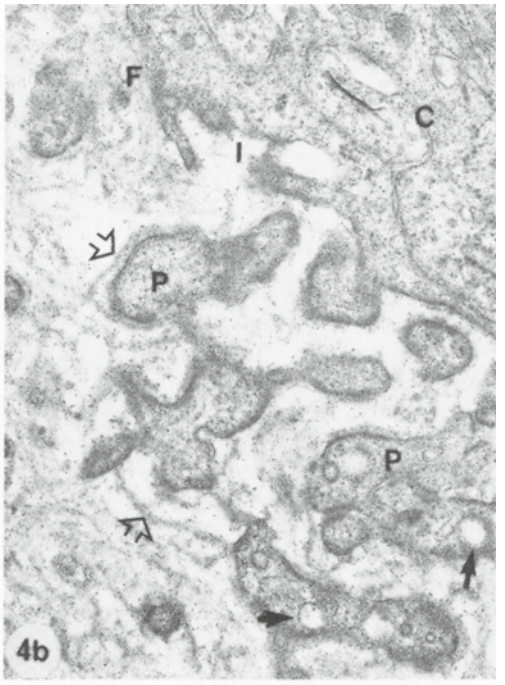
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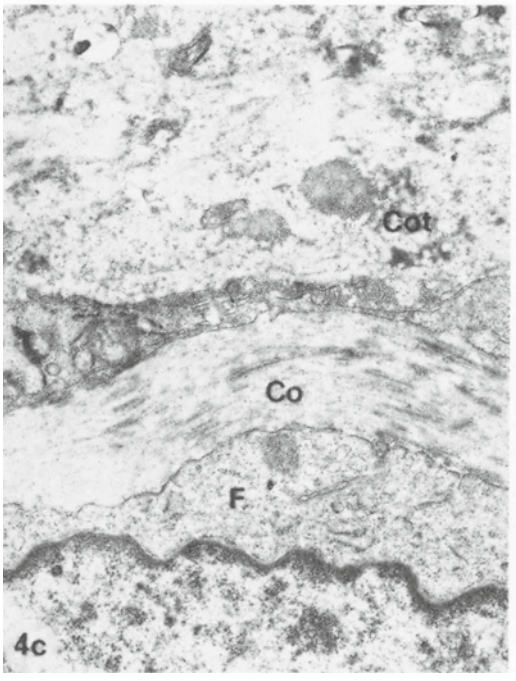
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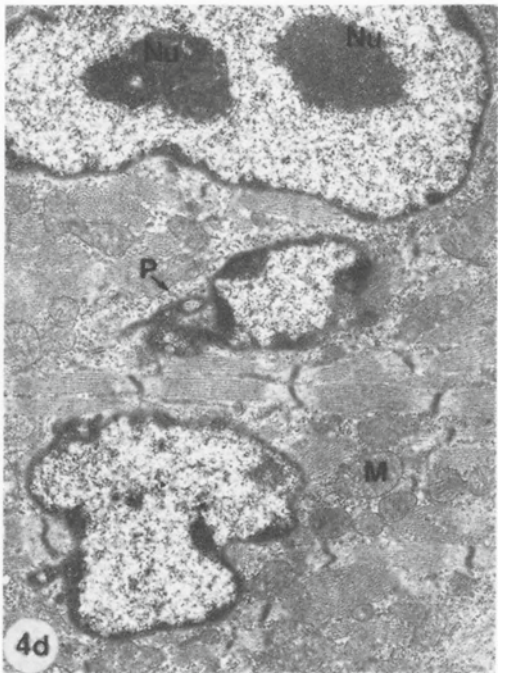
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Nu
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(*Skjerve,* in prep.), was used in the experiment. The isolate was passed 4 times in laboratory mice (BOM:NmRI) before the start of the experiment. Three other strains were also included in the experiment, 1 Norwegian strain (*T. spiralis* I) originally isolated from a red fox, 1 Dutch strain (*T. spiralis* II) isolated from a pig, and 1 polar strain (*T. nativa*) received from USSR through *Dr. Martinez-Fernandez,* Dept. of Parasitology, Universidad Complutense, Madrid, Spain.

The mice were given about 1000 larvae from acid-pepsin digested meat through a stomach tube. The experimental design is shown in Table 1.

Table 1. Experimental design. All mice were examined by light microscopy (*M. gastrocnemius*) according to the following set up.

Isolate/ strain	No. of mice	No. of mice sacrificed at	
		21 days	60 days
T. no. 13	4	2	2*
T. spiralis I	4	2	2
T. spiralis II	4	2	2
T. nativa	4	2	2

* Electron microscopic examination (diaphragma) of mice infected with T. no. 13 at 60 days p.i.

Muscle samples from diaphragma and from *M. gastrocnemius* were obtained during ether anaesthesia, and the mice were killed immediately after. Specimens for light microscopy were fixed in 10 % neutral buffered formalin, processed routinely, sectioned at 5–6 μm, stained with haematoxylin and eosin (HE) and van Gieson (vG), and examined in a Zeiss light microscope. Specimens for electron microscopy were fixed by immersion in Karnowsky fixative with cacodylate buffer, rinsed in the same buffer, post-fixed in osmiumtetroxide, dehydrated in alcohol series, embedded in Epon LX112, sectioned on a Nova ultrotome (LKB), stained with uranyl acetate and lead citrate, and examined in a JEOL 100S transmission electron microscope.

Results

Light microscopic observations

There were no obvious differences in myofibre changes or capsule formation between the different strains of Trichinella on 21 and 60 days p.i. (Figs. 1a-f).

At 21 days p.i., variable numbers of Trichinella larvae could be demonstrated in muscle fibres from all animals. Histological changes were characterized by small haemorrhages and varying degrees of infiltration with neutrophils, eosinophils and lymphocytes around Trichinella cysts and in the interstitial connective tissue (Fig. 1a).

Regenerating nuclei in infected muscle cells was a common feature (Fig. 1b).

Trichinella cysts were surrounded by inflammatory cells and had a moderately developed capsule (Fig. 1c). Infected muscle fibres showed an eosinophilic sarcoplasm and striations were absent (Fig. 1d). Perivascular aggregates of inflammatory cells could be found, but were not a prominent feature.

At 60 days p.i. there were indications of a slight decline in infiltration of inflammatory cells in the connective tissue. In all but a few of the animals, cysts with a well developed capsule were surrounded by varying degrees of inflammatory reaction (Fig. 1e). Also, several granulomas with a central, degenerating larvae, often surrounded by multinucleate giant cells were observed (Fig. 1f).

Electron-microscopic observations

Based on light microscopy showing no obvious morphological differences between

strains, only the polar bear isolate will be described.

At 60 days p.i., the larvae were encysted in dedifferentiated muscle cells and surrounded by a well developed capsule (Figs. 2a, 2b & 3a). Enlarged, euchromatic nuclei, some with prominent nucleoli and nucleolonema formations, were characteristic findings, the nuclei often being located in groups (Fig. 3b). The cytoplasm had no contractile elements and a moderately developed rough and smooth endoplasmic reticulum. Ribosomes were also found free in the cytoplasm (Fig. 4a). There was an increase in the number of mitochondria as compared to non-infected cells, and the mitochondria appeared smaller (Fig. 3b).

Infected muscle cells showed invaginations and protrusions into the extracellular matrix, covered with a basal lamina-like material located in a lattice-like pattern in the capsule matrix. The capsule matrix was composed of an amorphous substance, often with a filamentous material (Fig. 4b). No periodicity was observed.

The outer segment of the capsule was composed of collagen, with fibrocytes localized in the same area (Fig. 4c).

Muscle fibres adjacent to infected cells showed patterns of regeneration, with centrally located, enlarged euchromatic nuclei with prominent nucleoli, sometimes with nucleolonemas. Mitochondria and free polyribosomes were frequently demonstrated (Fig. 4d).

Discussion

No differences in myofibre changes or capsule formation between the different strains were observed by light microscopy.

Hulinska et al. (1985) reported marked differences in inflammatory reaction and capsule formation in laboratory mice between *T. nativa* and *T. spiralis* on the one hand and *T. pseudospiralis* on the other. These differences were assumed to be due to the continuous larval migration of *T. pseudospiralis*. The present investigation demonstrates no such findings, and T. no. 13 seems to have a migratory pattern similar to that of the three other strains used in this experiment.

Electron microscopy revealed active capsule formation 60 days after infection with T. no. 13, and infected muscle cells seemed to play a central role in the encapsulation process.

The ultrastructural changes of infected muscle fibres have been described as a dedifferentiation with loss of typical organization (*Hulinska et al.* 1985). According to *Backwinkel & Themann* (1972), these changes were observed from 7 days p.i., evolving gradually to result in a completely dedifferentiated muscle cell. Nuclear and cytoplasmic changes of infected muscle cells observed in the electron microscope at 60 days p.i., support the idea of adaptive changes aimed at supplying nutrients for the larvae. Enlarged nuclei, prominent nucleoli, and developed rough endoplasmatic reticulum, suggest an increased level of protein synthesis. However, these changes might as well indicate a reparative process induced by the migrating Trichinella larvae.

Capsule formation in mice starts from 20–21 days p.i. (*Walls et al.* 1973), and the largest increase has been observed between 20 and 50 days p.i. (*Teppema et al.* 1973). There is some controversy as to which cells are responsible for the capsule formation (*Gould* 1970). According to *Bruce* (1970), the early capsule is formed by fibrils of connective tissue surrounding the infected muscle fibre. *Teppema et al.* (1973), however, suggested capsule formation by the infected muscle cells, and an amorphous to fibrillar material, resembling basal lamina material, was observed around the infected muscle fibres.

There are analogous findings in the present investigation. A basal lamina-like material was located around the infected muscle cells, and also in a lattice-like pattern in the capsule matrix. Protrusions extended into the capsule, and a basal lamina-like material seemed to be deposited around these extensions. Smooth vesicles as described by *Teppema et al.* (1973), were located both adjacent to the sarcolemma of the infected cells and in transversely cut projections. It is probable that these vesicles discharge their content beneath the basal lamina.

The definite basal lamina-like material in the capsule matrix observed at 60 days p.i. dispute the findings of *Teppema et al.* (1973), who found the basal lamina to disappear gradually from 20 day p.i. and could no longer be demonstrated 45–50 days p.i. Differences in mode of infection (newborn larvae injected intramuscularly), different strains of Trichinella as well as dissimilar animal species (rats versus mice) might cause variation in larval and cyst development and could thus explain these discrepancies in cyst development.

In summary, the light microscopic investigation revealed no differences concerning the encapsulation process. Salient features 60 days p.i. with T. no. 13 were ultrastructurally characterized by active capsule formation by the infected muscle cells, with deposition of basal lamina-like material around cellular protrusions.

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Sammendrag

Muskelfiberforandringer og kapseldannelse hos mus infisert med fire stammer av Trichinella.

Lysmikropisk undersøkelse av muskelprøver (*M. gastrocnemius*) fra mus 21 og 60 dager p.i. med 3 kjente stammer av Trichinella, samt et isolat fra isbjørn (T. no. 13), viste ingen forskjeller i muskelfiberforandringer eller kapseldannelse.

Elektronmikroskopiske studier av Diaphragma fra mus viste at det fremdeles var aktiv kapseldannelse i muskelceller 60 dager etter infeksjon med T. no. 13. Disse var karakterisert av utvekster og invaginasjoner på sarcolemma-membranen. Basallamina-liknende materiale var lokalisert nær sarcolemma og i et gitterformet mønster i kapselmatrix.

T. no. 13 synes å ha et felles intracellulært vandringsmønster med de andre stammene i denne undersøkelsen og forårsaker muskelfiber-endringer som ikke kan skilles fra disse. De infiserte muskelcellene deltar i stor grad i kapseldannelsen.

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