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REGENERATION IN MOUSE SKELETAL MUSCLE INJURED BY TRICHINELLA LARVAE

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Abstract. During the infection caused by *Trichinella pseudospiralis* serious damage to muscles is partly compensated with regeneration process. Short, thin fibrillae with central position of the nuclei — regenerating myotubes originate 20—40th day post infection in endomysial tubes remaining after damaged muscle fibres, left by migrating larva. On the 10th day post infection in the vicinity of moving larva activation of nuclei and increased origin of satellite cells under basal membrane occur. With development of the infection endothelia in the vicinity of altered fibrillae are increasing in number but there is small inflammatory and fibroproductive reaction only. During the infection caused by *Trichinella spiralis* and *T. nativa*, alteration of muscle fibres is accompanied by early inflammatory and fibroproductive reaction towards immediate surroundings of larvae. In a changed segment of muscle fibre with the larva — in pseudocyst there is altered basal lamina as well, built in increased glycocalyx and in the course of 20 days encased by connective tissue. Revascularisation and regeneration in a short damaged segment does not occur even during long-lasting infection because of separation by capsule.

It was ascertained that striated muscles of many vertebrates may regenerate if damaged in a certain way (Shafiq et al. 1967, Carlson 1978, Hay 1970). During embryonal myogenesis all myoblasts originate from cells with one nucleus derived from mesenchyme but in case of regeneration of mature muscle fibres myoblasts are created from satellite cells (Reznik 1969) originating from damaged muscle fibre. Shafiq et al. (1968) proved that muscle satellite cells found by Mauro (1961) were myoblast precursors. According to Allbrook (1981), satellite cells in normal mature muscle occur very rarely, but their multiplication increases after muscle damage.

The aim of this study was to determine if damage of muscles caused by various species of *Trichinella* results in compensation regenerative processes as other pathological states, e.g. primary myopathy (Shafiq et al. 1967), the influence of local anaesthetics (Grim et al. 1981) etc.

The process of regression and capsule creation in muscle infected by *Trichinella spiralis* larvae was studied by many authors, but regeneration of muscle fibres after infection by *Trichinella* was not observed. Despommier (1975) ascertained that during infection by *Trichinella spiralis* in the muscles there was developing "nurse-cell-infective first stage larva complex" which almost balances the host-parasite relation.

MATERIALS AND METHODS

Inbred (77) white mice, ICR-SPF i.e. 7 mice in 11 groups were perorally infected either by 400 larvae of *Trichinella pseudospiralis* (33 mice) or *T. spiralis* (22 mice) or by 400 larvae of *T. nativa* (22 mice).

On the first and third days, and next each tenth day in the period of 10—90 days post infection (p.i.) seven mice were killed and their skeletal muscles (m. tibialis anterior et m. femuralis) were studied

histologically and ultrastructurally. Colchicin (0.2 ml of 0.1 mol/l) was applied to infected mice intraperitoneally six hours before sacrifice. Muscle excisions stretched and fixed on a support were fixed with 2.4% glutaraldehyde in 0.1 mol/l cacodylate buffer, pH 7.2 for 1 hour. Small pieces from different regions of the same muscle selected by means of stereoscopic microscope were postfixed in 1% OsO_4. After dehydration and embedding muscle segments into Epon, ultrathin sections were cut by ultramicrotome Reichert, stained by uranylacetate, lead citrate (UA, LC) and observed in Jeol 100 CX II microscope. Semithin sections were stained by Azur-toluidine blue method.

RESULTS

Previous study has demonstrated the influence of three *Trichinella* species on mice muscles 10—40 days post infection (Hulínská et al. 1984a).

Muscle alteration 10—90 days p.i. by *Trichinella spiralis* or *T. nativa* is limited to a part of muscle fibres (m.f.) only (350—500 μm on average in case of *T. spiralis* infection, 400—600 μm on average for *T. nativa*) in an immediate larvae neighbourhood.

In case of *T. pseudospiralis* infection the whole muscle fibre (m.f.) is altered (Fig. 1).

In altered m.f. during 3—10 days p.i. the tissue immediately around the larvae of *T. spiralis* or *T. nativa* is gradual changed, it is losing muscle character and pseudocyst is developing. Larva and muscle fibre segment interaction results in creation of nutritive chamber for larva quite isolated from other muscle tissue. Early changes of m.f. (10—20 days) in the larva vicinity include: swelling of muscle fibre segment (m.f.), thinning and change of composition of m.f. basal lamina segment, sarcoplasm myofibrilosis, villous sarcolemma and inrease of fibrillar glycocalyx on its surface.

These changes were not detected 3 days p.i. by *T. spiralis*. Third days some single larvae penetrated by blood capillaries in endomysium. Ten days p.i. by *T. spiralis* thinned basal lamina is built in increased segment glycocalyx (Pl. I, Fig. 1). Increase of sarcoplasmatic organellae (mitochondria, ribosomes), activation of myoblast nuclei, which have lost peripheral localization, increased and were euchromatic we observed on 20th day p.i. (Pl. I, Fig. 2).

In the period of 10—20 days p.i. by *T. spiralis* and *T. nativa* inflammatory infiltration around altered segment develops (Pl. I, Fig. 3), which on 30th day p.i. in connection with fibroproductive process closes altered segment with larva into capsule (Fig. 1). In encased sarcoplasm (40—60 days), nuclei of myoblasts gradually degenerate (Pl. I, Fig. 4). Ninety days p.i. some separated capsules with larvae are necrotizing. We observed that alteration of short segment m.f. by *T. spiralis* and *T. nativa*, its rapid encasing and separation by inflammatory infiltrate from undamaged segments of remaining fibre prevented during larvae development the revascularization and reinnervation of this segment. There are neither physiological nor morphological (change of basal lamina) conditions for regeneration of injured m.f. segment in which larva is persisting.

The infection of muscles by *T. pseudospiralis* larvae is different, 10—90 days p.i. whole m.f. are altered, through which larvae are moving all the time. Third day some fibres are swelling. Myofibrilolysis develops slowly and is always of a focular character. Ten days p.i. there are still myofibrils conserved in the majority of fibres as well as in larvae vicinity, but Z-lines are already changed. Nuclei of myoblasts in altered fibre at the beginning of invasion do not change their peripheral localisation under sarcolemma and basal lamina but they are activated (Pl. II, Fig. 1). During development of *T. pseudospiralis* (10—30 days) few changes are in basal membrane and T sarcotubular system of the m.f. There is weak inflammatory infiltration of endomysium, as well as altered m.f., similarly to fibroproductive process.

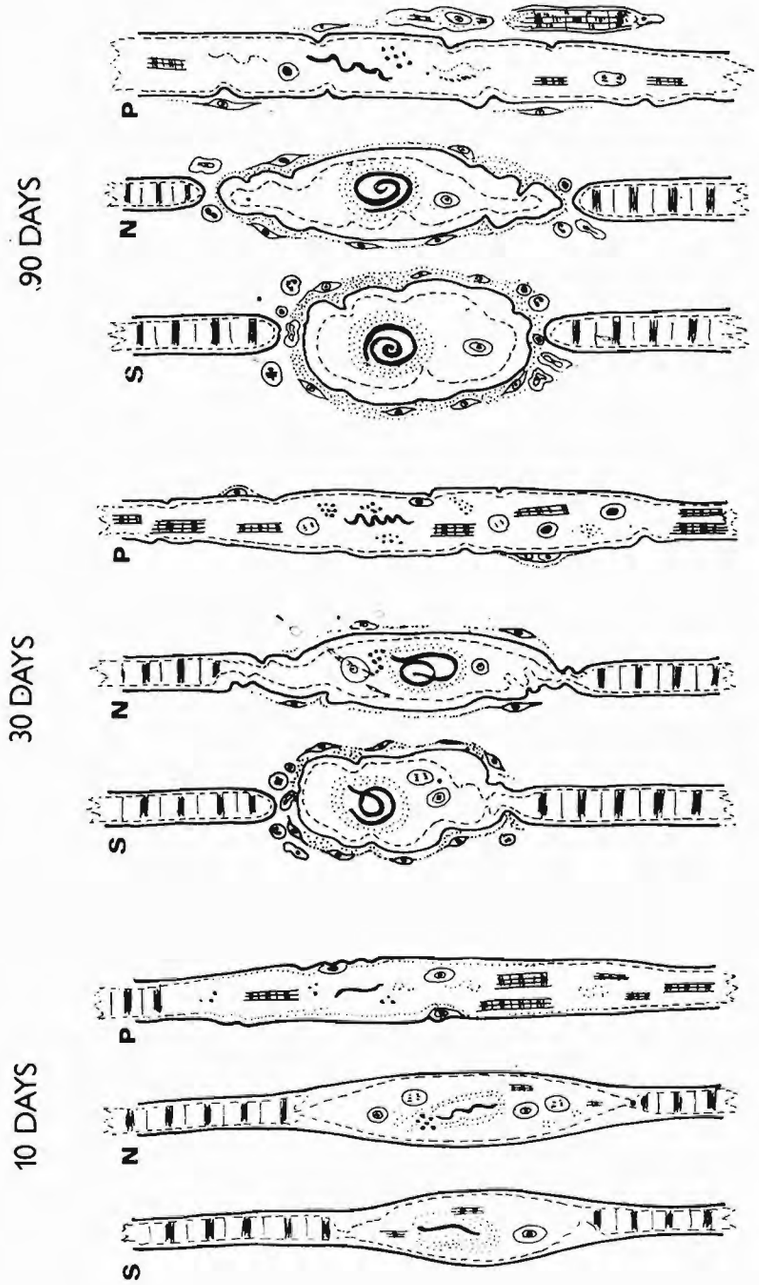

Fig. 1. Schematic illustration of the alteration in the m.f. and of capsule formation in the time course of 10—90 days of infection by larvae of *Trichinella spiralis* (S), *Trichinella nativa* (N) and *Trichinella pseudospiralis* (P). Around the larvae (S), (N) a capsule develops which consists from a reorganized segment of m.f. (gray), an amorphous glycocalyx layer embedding the changed basal lamina (white) and of collagen fibrils, fibroblast and inflammatory cells. No capsule develops around a m.f. with (P), the m.f. becomes altered throughout its length, its surface and basal lamina are infolded, its endomysial sheath thickens.

The difference from previous infection by *T. spiralis* consists in the fact that around changed fibres with movable larva of *T. pseudospiralis* endothelia multiply, and (30 to 40 days) gradually quite surround altered fibre. Also the innervation of m.f. is either conserved or being restored around infected fibres.

At the beginning of changes in m.f. there originate flat vesicles around peripheral myonuclei, which gradually border thin stripe of sarcoplasm with nucleus from the sarcoplasm, containing mitochondria and myofibrils (Pl. II, Fig. 1). Flat vesicles are fusing with invaginating sarcolemma (Pl. II, Fig. 2). Ten days p.i. unicellular cells appear in the larva vicinity, isolated from myofibrils by extracellular space and invaginating sarcolemma (Pl. II, Fig. 3).

Similar cells, presumed satellite cells, we observed under basal lamina of altered m.f. even in later period of infection (20 and 30 days p.i.) by *T. pseudospiralis*. We discovered (40 days p.i.) larvae of *T. pseudospiralis* inside the endomysium. Larvae can migrate from one damaged fibre in the new m.f.

In various infection stages we determined mitoses in areas of damaged fibres which though may belong even to multiplying endothelia (Pl. III, Fig. 1). Bi- as well as multi-nuclear myotubes with differentiating myofibrils originate by mitotic division and fusion of new myoblasts (Pl. III, Fig. 2). The nuclei of young myotubes have dispersely diffused chromatin and 1—2 nucleoli. Forty days p.i. there exist besides altered m.f. long myotubes, in which many myoblasts can be in one sarcolemma sheath, obviously in place of preexisting altered m.f. (Pl. IV. Fig. 1). In myotube sarcoplasm there are centrally localized nuclei and in their neighbourhood many small mitochondria.

Fifty days p.i. by larvae of *T. pseudospiralis* great myotubes, having still centrally, chain-like arranged nuclei dominate in foci of the greatest muscle damage (Pl. IV, Fig. 3). New myotubes are surrounded by multiplied endothelia (Pl. IV, Fig. 2). Single leucocytes, histiocytes and fibrocytes are 90 days p.i. by *T. pseudospiralis* in the vicinity of altered m.f. and in the neighbourhood of regenerating m.f. (Pl. IV, Fig. 4).

DISCUSSION

In our opinion differences in alteration of m.f. by various species of *Trichinella* genus are in agreement with differences in migratory activity of larvae (Hulínská et al. 1984a). Al Karmi and Faubert (1981) ascertained that larva *Trichinella pseudospiralis* covered distance greater than 3 mm during 30 sec., while *Trichinella spiralis* 0.25 mm during the same time. Both species of larvae, *T. spiralis* and *T. nativa*, alter short m.f. section only because they rapidly encase when stop moving (Hulínská et al. 1984b). During infection by *T. spiralis* and *T. nativa* host-parasite relations are compensated, according to *Despommier* (1975), by the origin of "nurse-cell-first stage complex" ensuring nourishment for larvae. Bordering nutritive chamber by capsule and host inflammatory cells prevent influence of metabolic products of larvae on surrounding tissue. In neighbourhood of partly or completely separated capsules neither revascularization nor restoration of innervation were detected. Basal lamina of altered segment disappeared while built into the capsule.

Difference in case of infection by *T. pseudospiralis* consists in the fact that m.f. are altered in whole length and after damage of inside milieu it is left by larva (Hulínská et al. 1984b). Thus in the tissue there remain endomysial tubes from m.f., surrounded by multiplied endothelia. Diffusion of nutritive substances and oxygen from endothelia provides in endomysial tubes conditions for creation of satellite cells.

According to Reznik (1969) and Hay (1970) regeneration of muscles occurs only when basal lamina in necrotic tissue remains conserved. Mauro (1961) observed that starting cells of regeneration were satellite cells, which are less sensitive to necrotic changes. According to Shafiq and Gorycki (1965) creation of satellite cells occurs in a limited region of muscle fibre, which is near to the damaged spot. Only in case of infection by *T. pseudospiralis* we observed creation of satellite cells from elongated nuclei localized under basal lamina near myolysis, caused by moving larva.

During transplantations of muscle grafts Carlson (1978) and Carlson et al. (1979) ascertained that satellite cells of healing tissue divided, differentiated in new myoblasts and fused in myotubes, where new fibres created. At the beginning of infection by *T. pseudospiralis* (10—30 days) in infected muscle there originate, divide and differentiate new myoblasts, fusing in myotubes.

On 90th day by *T. pseudospiralis* degenerative changes dominate, which means that infection defeated defensive and reparative activities of the host. It is caused by the fact that larvae migrate continually through m.f. and during whole infection period affect general state of the host by their metabolites. That is the explanation of differences with regeneration, observed by Grim (1981) during transplantations or after influence of anaesthetics (Grim et al. 1981) when destruction of muscles fibrils is rapidly and almost completely compensated by regeneration. Chemical influence of anaesthetics on the organism disappears after certain period, but the influence of Trichinellae is lasting throughout the whole infection period till death of the host.

РЕГЕНЕРАЦИЯ В СКЕЛЕТНЫХ МЫШЦАХ МЫШЕЙ ПОВРЕЖДЕННЫХ ЛИЧИНКАМИ ПАРАЗИТОВ РОДА *TRICHINELLA*.

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Резюме. По ходу заражения *T. pseudospiralis* тяжёлое поражение мышц частично компенсируется процессами регенерации. Короткие, тонкие фибриллы с центрально расположенными ядрами — регенерирующие миотубы — возникают с 20го по 40й день после заражения в эндомизиальных трубках, которые остались от разрушенных мышечных волокон, которые покинула мигрирующая личинка. Активация ядер и повышенное образование сателитных клеток под базальной мембраной происходит на 10й день после заражения в окружении мигрирующей личинки. По ходу заражения пролиферируют эндотелии в районе поврежденных волокон, но воспалительная и фибринпродуцирующая реакции слабо выражены. В случае заражения *T. spiralis* и *T. nativa* повреждение мышечных волоков ограничено ранней воспалительной и фибринпродуцирующей реакцией на непосредственное окружение личинок. В изменённом сегменте мышечного волокна с личинкой — в псевдоцисте — повреждена и базальная мембрана, которая встроена в разросшийся гликокаликс и в течении 20 дней после заражения окружена соединительной тканей. Даже при длительно продолжающемся заражении не происходит реваскуляризация и регенерация в коротком отсеке поражения, так как он отграничен капсулой и воспалительной реакцией от оставшихся неповрежденных отсеков мышечного волокна.

Explanations

a — myotube, b — basal lamina, c — capsule, d — cell division, e — endothelia, g — glycocalyx, h — host cell, k — myofibrils, l — larva, m — myoblast, n — nucleus, o — mitochondria, p — sarcolemma, r — reticulum, s — sarcoplasm, t — vesicles

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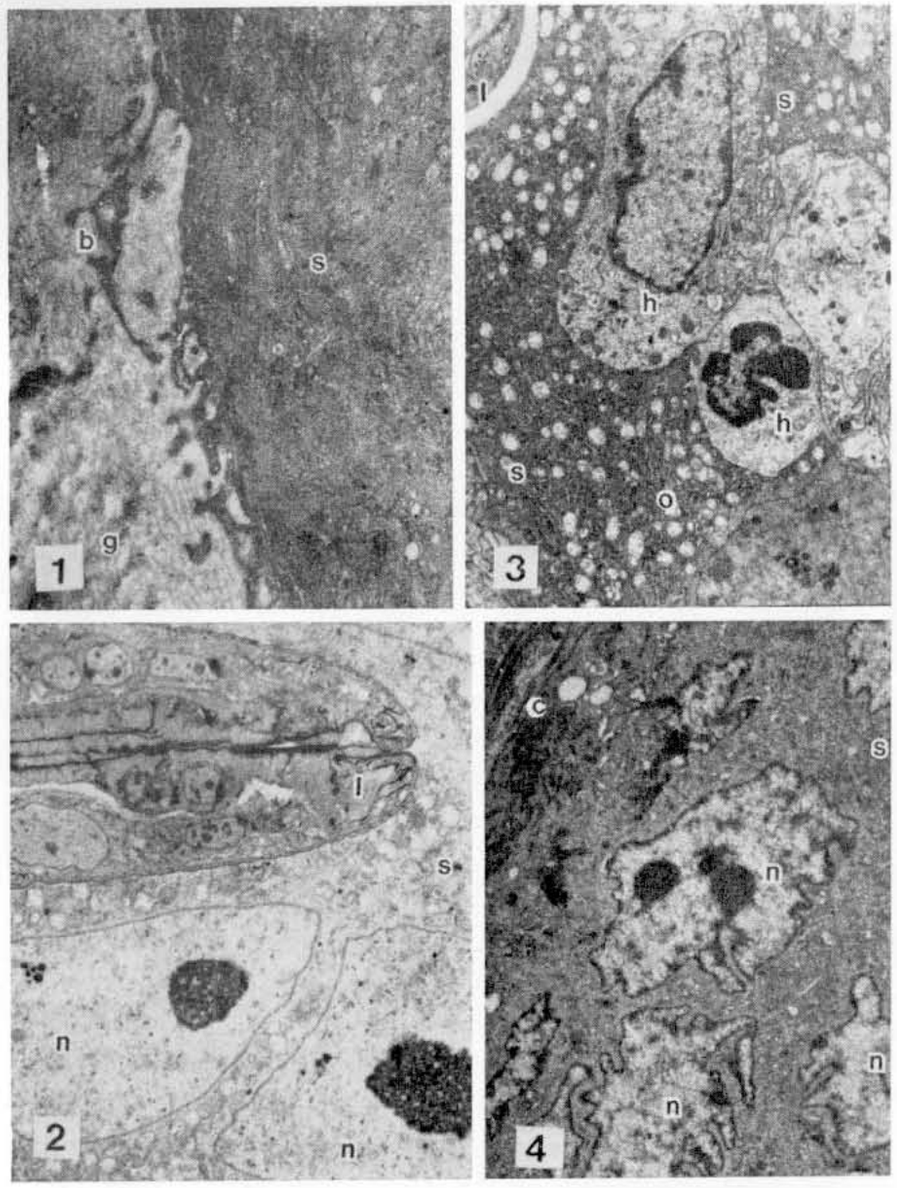

Fig. 1. Altered sarcoplasm (s) of m.f. by *T. spiralis* larva is covered by multiplied glycocalyx (g) with inbuilt changed basal lamina (b) (× 28,000). **Fig. 2.** Activated enlarged nuclei (n) in the sarcoplasm (s) are near larva (1) (× 4,500). **Fig. 3.** Inflammatory infiltration (h) around altered segment of m.f. infected by *T. spiralis*. In sarcoplasm (s) there are multiplied mitochondria (o) (× 14,900). **Fig. 4.** In encased (c) sarcoplasm (s) nuclei (n) gradually degenerate (× 16,900).

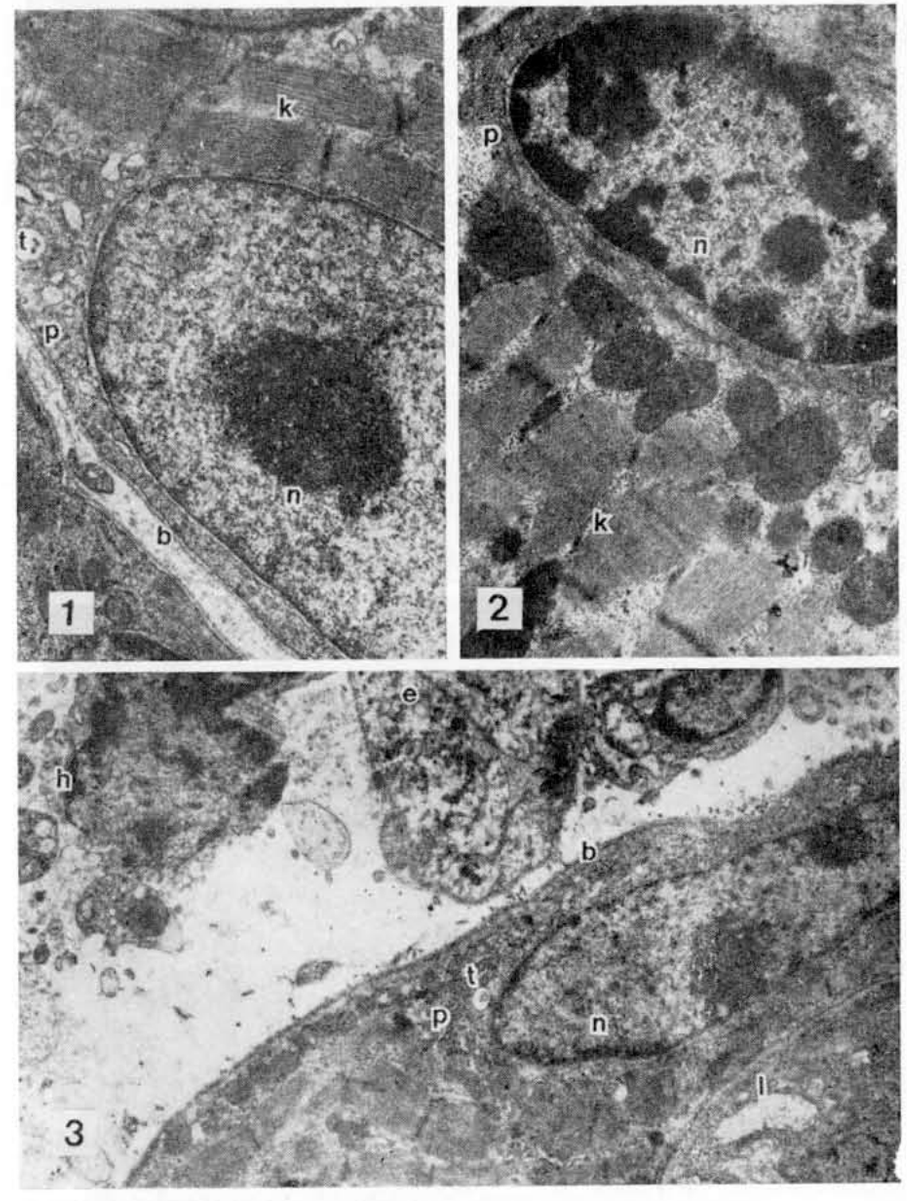

Fig. 1. Ten days p.i. by *T. pseudospiralis* myofibrils (k) and numerous vesicles (t) and activated nuclei (n) are under basal lamina (b) (× 19,000). **Fig. 2.** Invaginating sarcolemma (p) margins sarcoplasm with nucleus (n) from myofibrils (k) (× 19,000). **Fig. 3.** Satellite cell under basal lamina (b), separated from myofibrils is near larva (1) of *T. pseudospiralis* (× 25 000).

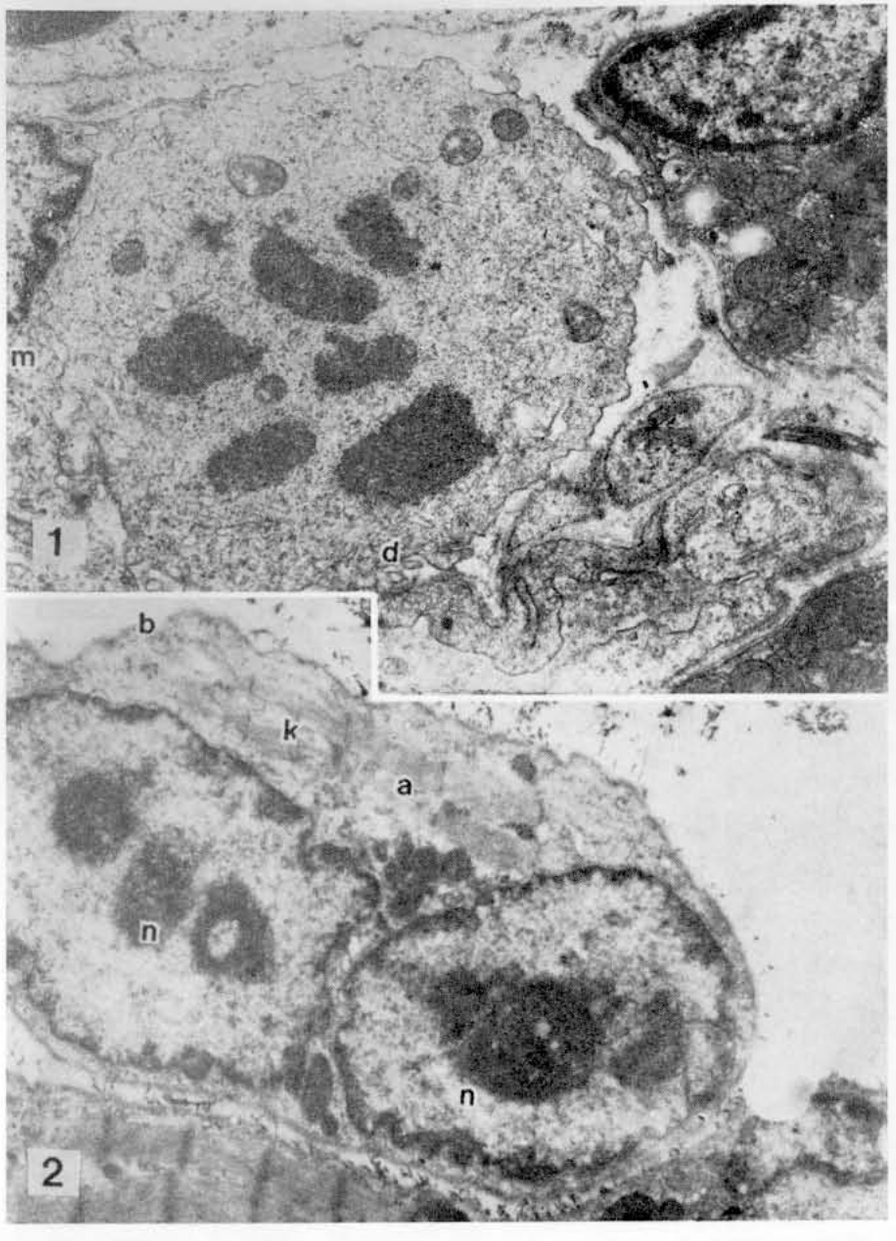

Fig. 1. Mitotically dividing nucleus (d) on the spot of altered m.f. by larva of *T. pseudospiralis* (× 25,000). **Fig. 2.** Binuclear myotube (a) surrounded by basal lamina (b) contains differentiating myofibrils (k) (× 25,000).

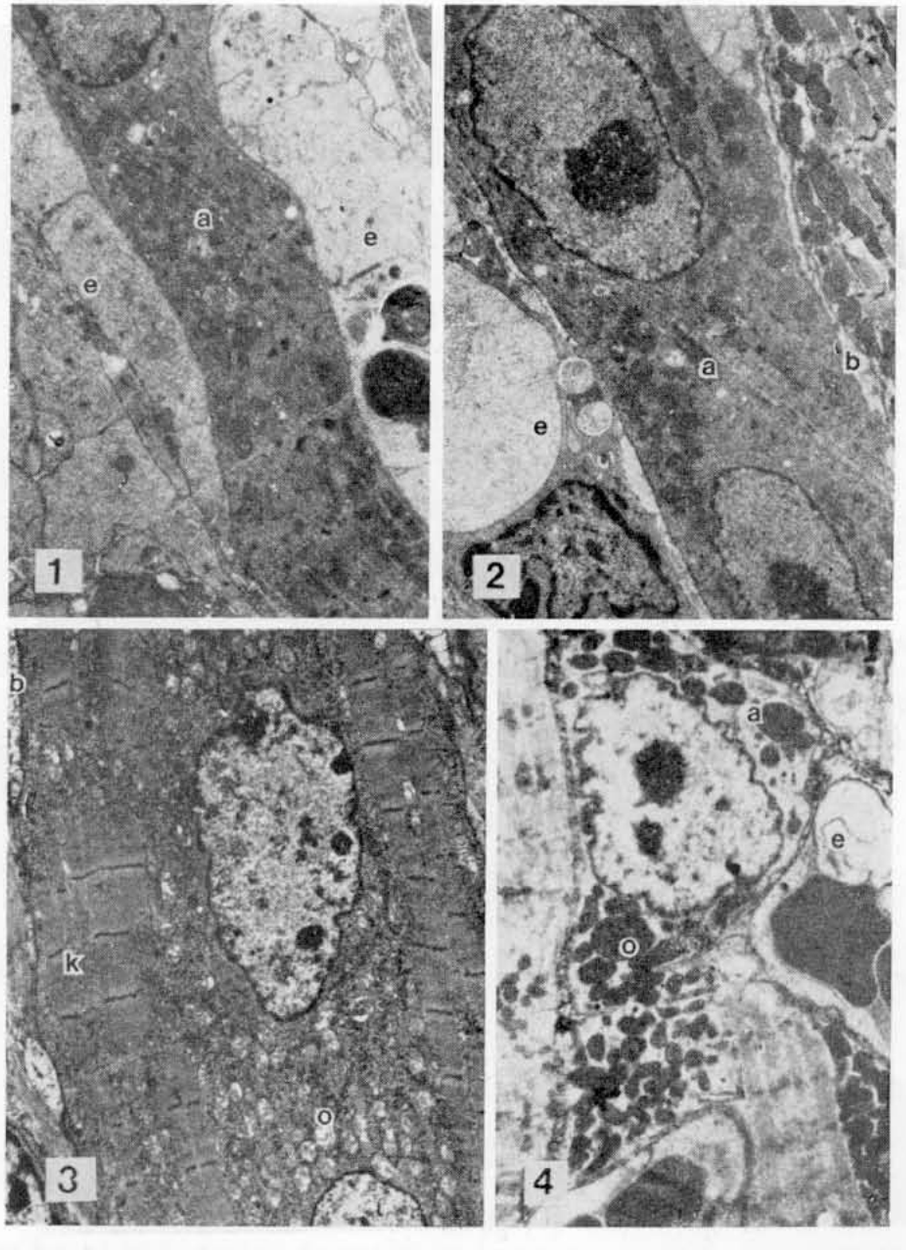

Fig. 1. Myotube contain multiplied mitochondria and great active nucleus (× 7,000). **Fig. 2.** New myotube surrounded by endothelia (e) (× 7,000). **Fig. 3.** Fifty days p.i. by *T. pseudospiralis* great myotube with centrally localized nuclei (×9,500). **Fig. 4.** Host cells and endothelia (e) in neighbourhood of new myotube ninety days p.i. by *Trichinella pseudospiralis* (× 7,000).