EFFECTS OF LOW TEMPERATURES ON LARVAE OF THE GENUS TRICHINELLA

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Abstract. We examined the effect of an exposure to —25 °C (for 8 days) on the histochemistry and the fine structure of 30-day-old Trichinella larvae from muscle fibres of the diaphragm. The larvae of T. pseudospiralis and T. nelsoni were either destroyed in the muscle fibres, dead, cosinophils, or were not found. The structureless mass of a degenerating changed sarcoplasm was highly A1P-active, and gave a weak positive reaction for SS-groups of proteins. The wall of the deformed capsule around T. nelsoni, and the outside of the larva, stained diffusely; it did not contain AM. In a few muscle fibres exposed to —25 °C, histochemical reactions of the capsule surrounding larva of T. nativa and sometimes of larva of T. spiralis, and reaction of the changed sarcoplasm, were similar to those of the controls. A few mobile larvae were isolated by digestion only from a diaphragm infected with T. nativa. Detergent to a prolonged survival of larvae were the formation of ice crystals and a denaturation of proteins by which the sarcoplasm of the infected muscle fibre was changed gradually into both a plasmolytically and karyolytically altered mass. Degenerative changes in the fine structure of infected muscle fibres were demonstrated by the presence of "spheromembranous bodies" in the sarcoplasm resembling myelina formations observed after exposure to poisonous substances, e.g., colchicine.

So far, changes in the fine structure and histochemistry both of muscle fibres and Trichinella larvae exposed over a prolonged period to low temperatures have not been examined. On the other hand, experimental data are available on the resistance of T. nativa from the muscles of dog, bear and rat to low temperatures (Miroshnichenko 1976, Sokolova 1978, Britov 1982). Ransom (1916) stated that the longevity of larvae of T. spiralis was shorter at a temperature of —4 °C, and observed a complete loss of infectiveness after a 10-day-exposure to —12 °C. Larvae encysted in arctic muscles were little calcified even after several years, and the wall of their cyst was thicker (Rausch 1970). Sukhdeo and Meercovitch (1979) maintained that solely arctic isolates of T. spiralis var. nativa (senso Dick 1983) could be regarded as an independent species different from T. spiralis.

The scope of our present study was to determine whether T. nativa larvae reported in the literature to have been maintained even after a passage to white mice, in comparison with the remaining Trichinella species under identical conditions, i.e., at the infectious stage, could be maintained also in the muscles of mice (very light-weight animals) after an eight-day-exposure to —25 °C, the temperature of the arctic region. According to Campbell (1983), an infection was more frequent in rodents from the arctic region than in those from temperate and tropical zones. He added that an infection of arctic carnivores was most frequently acquired by feeding on an infected rodent.

MATERIALS AND METHODS

The effect of —25 °C was studied on 30-day-old larvae of T. spiralis, T. nelsoni, T. pseudospiralis and T. nativa, using histochemical and biological methods, and electron microscopy. We infested experimentally eight inbred male white mice with 200 larvae each using two mice
controls, the number of larve recovered from frozen diagrams was minimal and none resembled a larva life. Although we failed to recover larvae of T. pseudo spiralis from muscle fibres of a frozen diagram, a local, strong, AIP-active substance was detected in various parts of the muscle fibre. In the muscle fibres substance, more AIP-active than in the control, negative areas of the nuclei could not be distinguished (Plate I, Fig. 4). While infected muscle fibres were dilated throughout their length in the controls, those from frozen diagrams were dilated in sites only occupied by the AIP-positive substance. Reactions were negative both for SS and SH groups of proteins, and for acid mucopolysaccharides (AM) infected with T. natae, the capsules containing either dead larvae or their remnants, were not yet closed at the poles. Neither the altered segment nor the larva differed histochemically from data describing the situation in a frozen diagram infected with T. spiralis. Other muscle fibres contained a thick capsule with a coiled larva inside it. The capsule was not closely adjoined by an AIP-active sarcoplasm in which were present activated, unswollen nuclei (Plate II, Fig. 1). In their reaction for SH groups (DDD) and acid mucopolysaccharides (AM), staining was obtained for the capsule, the cuticle and the larval stichosome (Plate II, Figs. 2, 3). The structure of several larva and that of a few capsules was similar to the control.

Extensive degenerative changes in the fine structure occurred after freezing in most muscle fibres infected with different Trichinella species. The sarcoplasm changed by the presence of infective larvae, contained after freezing numerous "sphero membranous bodies" which differed in sizes and resembled myelid formations. Also present were dilated vesicles, sacs and canals (Plate II, Fig. 4). In muscle fibres infected with T. spiralis, unchanged in their structure, sarcoplasm contained collagen fibrils only on the surface of cytoplasmic fibres of the capsule and the sarcoplasm contained vacuoles and "membrane bodies" also visible in the endomysium (Plate III, Fig. 2). In some rare instances, the structure of larvae of T. spiralis was similar to that of controls (Plate IV, Fig. 3). At day 30 p.i., larvae from an unfrozen diagram were surrounded by mitochondria, a proliferating endoplasmatic reticulum, active nucleoli were present in the nuclei, the wall of the capsule, and the red blood cells were infected with T. pseudo spiralis contained "membrane bodies" made up of a small number of concentric membranes between which were seen cytolic compo components such as osmiophile granules (Plate III, Fig. 4), small vesicles or mitochondria (Plate IV, Fig. 1).

Thirty-day-old larvae of T. pseudo spiralis from muscles of the diagrams were surrounded by scattered remnants of myofilament, endoplasmatic reticulum and mitochondria (Plate III, Fig. 8). In some cases, the altered sarcoplasm inside the capsule contained minute vesicles, mitochondria, ribosomes and an occasional nucleus with an active nucleolus (Plate IV, Fig. 2). However, most muscle fibres in which the sheath surrounding the larva was not yet closed, were affected by degenerative changes similar to those described for an infection of muscle fibres with T. spiralis (Plate IV, Fig. 4).

In the larval stage, we isolated by digestion from frozen muscles of diagrams infected with larvae of T. spiralis and T. natae, a small number of straight, immobile larva. In addition, we isolated three larvae of T. natae from the
mature muscles of a diaphragm which, after a change in temperature cooled slowly into a spiral. The conditioned movement stopped after 30 min. Smears of an isolate from all muscles, centrifuged and stained with Giemsa, contained remnants of the larval cuticle unaffected as yet by the digestive solution.

**DISCUSSION**

Boev et al. (1979) regarded the resistance of larvae of T. nativa to low temperatures as one of the criteria by which these larvae could be distinguished from the remaining Trichinella species. According to Britov (1982) larve of T. nativa from dog and bear muscles survived and remained infective for forty days in temperatures from -20 to -30°C. Larvae of the remaining Trichinella species were unable to survive in these temperatures in the muscles of rat and mouse for more than 35 hr. Two-month-old larvae of T. nativa from the muscles of rat did not survive at a temperature of -23°C for more than two months (98%), while those from the muscles of dog remained infective throughout this period. Larvae from the meat of bear survived for more than one year at temperatures from -11 to -15°C (Miroshnichenko 1976, Sokolova 1978a, Britov 1982). We considered the use of experimental infection, because various laboratories had used these animals for reproduction and passage of strains isolated from various feral mammals and birds. We selected intentionally the diaphragm for determining the effect of low temperatures on the larvae partly because the larval load is generally high in this organ and partly because this organ is of equal width and has a similar length in contrast to skeletal muscles. Although we knew that the pattern of our experiments would not perfectly be comparable with those obtained by other authors who experimented mostly with larger animals and hence with larger muscles, we also knew that comparable conditions would be obtained for various Trichinella species in addition to information on the infectiousness of experimental material stored frequently over a prolonged period in the freezer.

Our histochemical results indicated that the number of larvae of T. nativa capable of surviving for as long as eight days in a frozen diaphragm was minimal. However, no reliable evidence could be obtained on the duration of viability determinable in vitro only by digestion at which the effect of a slow freezing of the muscle at 37°C (necessary for digestion) for 6 hr is drastic. We isolated a straight, completely immobile larva which could well have been viable in the frozen muscle because, either dead or disturbed, it would have been quickly digested by pepsin, and the only evidence of its presence would have been a few remnants of its cuticle. In our material, three straight larvae of T. nativa only responded to a change in temperature by coilng, for a short time, into a spiral. It has been suggested both by our results and literary data that the resistance of larvae of T. nativa is influenced by two factors: the host species and the age of infection (the older the infection, the thicker the cuticle surrounding the larva). Britov (1982) stated that the wall of the capsule of a larva T. nativa in the muscles of dog attained a thickness of 42 μm within two years, and up to 100 μm in bear muscles. If the infection is introduced by oral routes, the larvae do not enter the muscles at the same time and, therefore, when recorded at day 30 p.i., all are not yet thick. The thickness of the capsule might be different as well as seen in histological sections of a diaphragm infected with T. spiralis. Comparison with histochemical methods and electron microscopy of degenerative changes occurred in the structure and chemistry of infected muscle fibres after exposure to low temperatures. The changes were obviously brought about by the presence of ice crystals and an increased concentration of salts resulting in a denaturation of proteins. In a recent study by Stewart (1983), the central part of the muscle fibre was regarded as a “nurse cell” of Trichinella larvae. Britov (1982) maintained that a “life” sarcoplasm inside the sheath was the only condition for larval survival. Degenerative, myopathic changes in the sarcoplasm of a majority of frozen muscle fibres infected with Trichinella larvae were not found in the frozen muscle fibres of the controls. A similarity in the fine structure and the capsule to those of the control was observed occasionally in fibres infected with T. spiralis, more frequently than in those with T. nativa.

Evidence on the effect of a low temperature were degenerative changes in the frozen, infected muscle fibres, i.e., an accumulation of large, sarcoplasmic, membranous bodies changeable both in size and structure. Fibres with T. pseudospiralis were the only ones in which these “membranous bodies” were outlined by a small number of concentrated membranes among which were cytoplasmic components similar to osmiophilic granules and small vesicles. Anderson et al. (1967) reported the presence of similar changes in the skeletal muscles of rat after an intraperitoneal injection with vincristine sulphate. Markand and Agostino (1971) found these changes after an injection with colchicine. The former authors suggested that “spherolemembranous bodies” originated from a proliferation of the sarcoplasmic reticulum, in the opinion of the latter authors, “membranous bodies” were produced by proliferating membranes of the sarcotubular reticulum. In our larval material, as suggested by a comparison with the controls, a proliferation of the sarcotubular reticulum could be induced by the presence of Trichinella larvae in the infected muscle fibres. According to Stewart (1985), the proliferation of the sarcotubular reticulum is provided as a transport medium for nutritive substances at a later stage at which the larvae had ceased to feed actively (Hulinska and Grim 1984). Spiro et al. (1966) described degenerative changes in the membranes as a demonstration of “myotubal myopathy” which evidently brought forth such changes in the inner environment of the capsule that it became deterrent to larval survival.

**ВЛИЯНИЕ НИЗКОЙ ТЕМПЕРАТУРЫ НА ЛИЧИНКИ РОДА TRICHINELLA**

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Резюме. Изучали гистохимию и ультраструктуру личинок рода Trichinella в возрасте 30 дней после закрымания при температуре -25°С в течение 8 дней. Личинки видов T. pseudospiralis и T. nativa, содержащиеся в мышечных волокнах диафрагмы, были раздвоены в межверетененном пространстве. Гистохимическая реакция, содержание саркоплазматического ретикулям, изменение строения мышцы и волокна отражали процесс инвазии. Наличие гиалиновых капель в мышечных волокнах, изменения количества и структуры саркоплазматического ретикуляма, проявлялись в виде образования «сфероэлеменсных тел». Саркоплазма, напоминающая миелобластическое образование, обнаружена после введения инъекций, напр. колхицин.


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EXPLANATION OF LETTERING IN THE FIGURES

A — life larvae; B — membranous body; C — capsule; D — dead larva; E — endomyosium; F — myofibrils; G — granules; H — hypodermal larvae; I — larval cuticle; J — chromatik; K — collagen; L — basilar lamina; M — mitochondria; N — nucleus; O — larval gut; P — larval stichosome; Q — altered muscle fibre; R — coarse reticulum; S — sarcoplasm; T — tubules;
U — vacuoles; V — vesicles.

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Fig. 1. Muscle fibres infected with *T. spiralis*, after freezing. Remnants of capsules or collapsed capsules without larvae are occupied by a structureless substance displaying AIP activity. One capsule (arrow) contains an unchanged larva. (Alpha-naphthylphosphate, Fast blue BB, ×100). Fig. 2. Viable larvae of *T. spiralis* in capsules from an unfrozen diaphragm of the controls. AIP activity in the sarcoplasm outside the area of the nucleus. (Alpha-naphthylphosphate, Fast blue BB, ×100). Fig. 3. Frozen muscle fibre infected with *T. netesii*. An AIP-active substance fills either remnants of capsules without larvae or surrounds closely and enters the disturbed larval bodies in the capsule. (Alpha-naphthylphosphate, Fast blue BB, ×100). Fig. 4. Larvae are not present in frozen muscle fibres infected with *T. pseudospiralis*. Altered sections of fibres are filled with a structureless, AIP-active substance. (Alpha-naphthylphosphate, Fast blue BB, ×100).
Fig. 1. Transverse section through a 30-day-old larva of *T. spiralis* inside its capsule, in an unfrozen control diaphragm. The sarcoplasm contains mitochondria, a sarcoplasmic reticulum and a nucleus with an active nucleolus. (UA, LC × 6,000).

Fig. 2. *T. spiralis* in the muscle fibre of a frozen diaphragm is surrounded by a degenerating sarcoplasm which contains vacuoles, "membranous bodies" and a degenerating nucleus. The wall of the capsule is vacuolized (arrow), unchanged in its structure is only the collagen on the surface of the capsule. (UA, LC × 9,000).

Fig. 3. A 30-day-old larva of *T. pseudospiralis* in a not frozen diaphragm of the control. The larva is surrounded by an altered sarcoplasm which contains remnants of myofibrils, vesicles, mitochondria and endoplasmic reticulum. (UA, LC × 7,200). Fig. 4. Frozen muscle fibres altered by the presence of *T. pseudospiralis* larvae, contain vesicles with dense granules which also are present inside the membranous bodies. Sometimes, we found cisternae of the endoplasmic reticulum, and spheromembranous bodies without granules. (UA, LC × 15,000).

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Fig. 1. In muscle fibres infected with *T. pseudospiralis*, we could not distinguish, after freezing, the basal lamina in the altered section, but only the adjoining collagen. The subsarcolemmal area contains remnants of T-tubules filled with a dense substance. (UA, LC × 15,000).

Fig. 2. The structure of a life larva of *T. nattered* that had not been affected by freezing. The surrounding sarcoplasm contains vesicles, mitochondria, a coarse endoplasmic reticulum and a nucleus with an active nucleolus. (UA, LC × 12,000).

Fig. 3. Larva of *T. spiralis* inside its capsule. The structure of the larva has not been affected by freezing. Present in the sarcoplasm is an occasional large body composed of numerous membranes which contain in their centre a dense substance. Also present are cisternae of a coarse endoplasmic reticulum, and an occasional mitochondrion. (UA, LC × 15,000).

Fig. 4. In several muscle fibres infected with *T. nattered*, we also found degenerative changes in the sarcoplasm — "membranous bodies" — chromatin is released into the sarcoplasm. (UA, LC × 15,000).