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Microsporidia in Testicular Cells of *Acrida turrita* L. (Orthoptera: Acrididae).

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In *Acrida turrita* about one-third of the testes were infected by microsporidia. The primary site of infection is the cyst wall cell where spermatocytes and young spermatids are secondarily affected. Meronts and sporoblasts, however, are also found in very late spermatids. A pathological reaction of the host cells has not been observed. Meronts at first monokaryotic, become diplokaryotic although sporonts are again monokaryotic. The encysted spore measures 2.7 by 1.4 μm. One nucleus, rough and smooth endomembranes, a polar filament with two to three coils, and an anchoring disc are constitutive components. Besides these condensed spores, pale degenerating spores are also found in a large number.

KEY WORDS: Locust; *Acrida microsporidia;* testis; parasitism.

INTRODUCTION

Recently Afzelius *et al.* (1989) published an extensive survey on infection of sperm cells in arthropods by viruses and rickettsia. Besides four cases of their own, they found only a few additional observations in the literature where viruses or rickettsia have been described in male gametes or their preceding stages. Although intracellular parasitic or symbiotic microorganisms are frequently found in eggs of arthropods, their occurrence in male germ cells seems to be a veritable rarity (Zissler and Sander, 1982; Afzelius *et al.*, 1989). Among protozoa, microsporidia are common parasites in arthropods. Thomson (1960) listed 131 species of microsporidia found in lepidoptera and diptera. Preferential tissues for infection are fat cells and intestine. Less often, the Malpighian tubules, muscles, and silk glands are affected. The egg as a site of infection is mentioned three times, whereas the testis in this paper remains unmentioned. Microsporidia have been found in the testis (Brooks *et al.*, 1985), but this occurs very rarely.

While studying spermiogenesis of the orthopteran *Acrida turrita* we have observed an infection of the testis with microsporidia. Affected were both cyst wall cells and spermatogenetic cells. Infestation of the latter was most conspicuous in primary spermatocytes and young spermatids but less often microsporidia were also observed in late elongated spermatids. The present article gives a brief description of our findings.

MATERIALS AND METHODS

Adult *A. turrita* were collected in the vicinity of Porto-Novo, Republic of Benin, from January to March. Freshly excised testes were fixed with 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) containing 0.05% $CaCl_2$. After repeated washes, the testes were stored for several weeks at 4°C in 0.1 M cacodylate buffer. Prior to use, the material was postfixed with 2% osmiumtetroxide in 0.1 M veronal-acetate buffer, dehydrated with acetone, and embedded in araldite. Semithin sections (0.5-μm thickness) made by using an Ultracut E (Reichert) microtome were stained with methylene blue and photographed with an Olympus BH2 equipped with planapochromates. Ultrathin sections of silver to pale gold interference colors double-stained with uranyl acetate and lead citrate were studied with a Zeiss EM 109 at 80 kV. Photographs were taken with primary magnifications between 3000 and 20,000×.

RESULTS

During light microscopical examination of semithin sections, we noticed that several cells were heavily infected by oval to elongated microorganisms. One-third of the specimens (n = 30) were affected. Since sometimes only single cells per section were parasitized, the proportion of infected testes could probably be even higher. In comparison to mitochondria, the parasites are considerably larger and more intensely stained. Most frequently they are seen in cyst wall cells, often in primary spermatocytes and young spermatids with

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large nebenkern (Figs. 1 and 2), but may also occur in elongating spermatids or occasionally in the tail of very late spermatids. In cyst wall cells, different parasitic stages are found regularly within the same cell, whereas in spermatocytes mostly meronts prevail and sporoblasts may also be found in later stages. A pathological reaction of the host cell, however, is not recognizable even after massive invasion.

In the electron microscope various manifestations representing different stages of the life cycle are well discernible. Monokaryotic meronts, very rarely seen, represent the earliest stage (Fig. 3). They have a small and darker stained nucleus compared to the cytoplasm surrounded by a nuclear membrane with a few pores. Its darker appearance is due to the fact that at this stage the nucleus contains many particles, probably ribonucleoprotein, which are evenly distributed between fine fibrillar DNA. Ribosomes are numerous in the cytoplasm, while endoplasmic membranes, if present at all, are rare. The cell membrane is wavy, giving the parasite an irregular form. Next, the meronts become diplokaryotic, this being the most common form (Fig. 4). The two nuclei, now with fine fibrillar contents, are closely attached; their envelopes, however, remain separated from each other. In the major part the two membranes form the usual pores containing cisterna. But in the area of contact, pores and cisterna are missing, the two fused double membranes being separated by a narrow cleft. Profiles of endoplasmic cisternae become more numerous now. They seem to take their origin from the nuclear envelope, usually just next to the area of contact (Fig. 5). Some distance apart, there is a small spindle plaque. In this place, the nuclear envelope regularly forms small vesicles containing some electron-dense material (Figs. 5,6). The chromatin appears in some places more condensed but a formation comparable to a nucleolus has not been observed. A dividing meront becomes considerably elongated with two diplokarya moving to the poles (Fig. 7). Microtubules only visible in this stage are the driving force in this process.

Sporonts appear very rarely in our material. Early stages are characterized by a thicker membrane and a light cytoplasm (Fig. 8). Only monokaryotic cells have been observed. Later stages are irregularly shaped. While their cyst wall begins to form, their contents condense and appear more complex. Endomembranes increase, but most conspicuous is the formation of an electron-dense polar filament (Fig. 10). Sporonts are often found together with encysted forms preferentially within cyst wall cells. Occasionally meronts and condensed microspores occur in the tail region of elongating and even late spermatids (Figs. 11,12), thus indicating that infection does not disturb normal differentiation of the spermatid.

Mature microsporidia are round to long ovoid, measuring 2.7 by 1.4 μm (Figs. 13,14). Their envelope, measuring about 100 nm, is composed of a thin dense exosporidium and a thicker light endosporidium. The contents are highly condensed. In favorable sections one nucleus is recognizable. They possess a polar filament, wound in two to three coils. The cytoplasm contains cisternae of endoplasmic reticulum studded with numerous ribosomes and smooth membranes concentrated near the anterior pole. Besides these fully condensed microsporidia, pale forms with degenerating contents (Fig. 15) and even totally empty cysts can be found in cyst wall cells.

DISCUSSION

Microsporidia are frequently found in insects preferentially parasitizing in fat cells and in the intestinal tract (Thomson, 1960). The gonads are rarely affected and, if so, mostly as manifestation of a systemic disease. Ovaries are by far more often infected than testes. Therefore, it is surprising that no less than one-third of our specimens were infected by microsporidia. Since we had only testes for examination we could not determine the extent of the infection. In this material, microsporidia were observed in cyst wall cells at highest rate, to a lesser extent in germinal epithelium, and rarely in fat cells, occasionally adhering to the testes. From this distribution we conclude that the primary site of the infection in the testis is the cyst wall cell. This assumption corresponds with the fact that all the developmental stages of the parasite are found within this cell type.

The germinal epithelium seems to be secondarily infected by meronts since all other stages are usually missing in spermatocytes and early spermatids. Very rarely we have seen sporoblasts in these early stages. On the other hand encysted forms are often found in later spermatids. This could indicate that the whole

FIG. 1. Primary spermatocytes, some massively infected with darkly stained meronts. Compare with mitochondria. ×1225.

FIG. 2. Young spermatids with spherical nebenkern (NK). Meronts and sporoblasts within the same cell (arrowhead). ×1225.

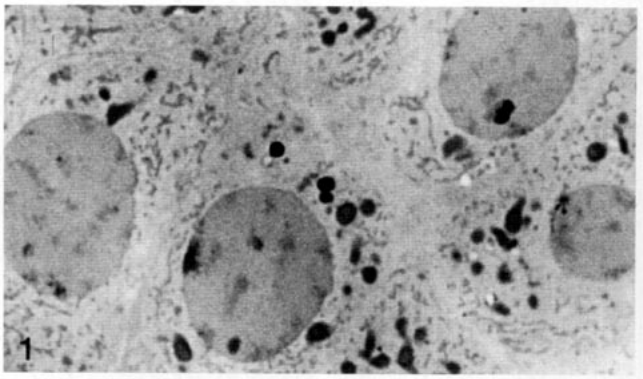
FIG. 3. Monokaryotic meronts. Small nucleus (N) contains fine fibrillar contents with numerous RNP particles. Cytoplasm with many ribosomes but no endoplasmic reticulum. ×18,500.

FIG. 4. Diplokaryotic meront. Nuclei (N) with evenly distributed fine fibrillar DNA, nuclear envelope with pores, cisternae of endoplasmic reticulum (ER), and plenty of ribosomes in the cytoplasm. ×24,500.

FIG. 5. (a,b) Diplokaryotic meronts. Nuclei (N) with partially condensed DNA. Local extension of nuclear cisterna near area of contact forming tubular endoplasmic reticulum (large arrowhead), spindle plaque with vesicle (small arrowhead). ×19,500.

FIG. 6. Diplokaryotic meront in cross section with spindle plaque and small vesicles (arrowhead). ×32,500.

FIG. 7. Diplokaryotic meront in division. Two diplokarya (N) near the poles. ×22,500.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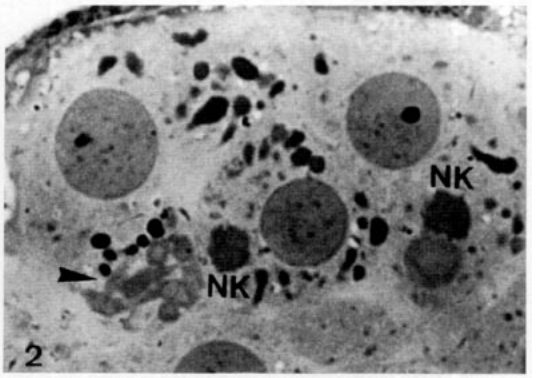
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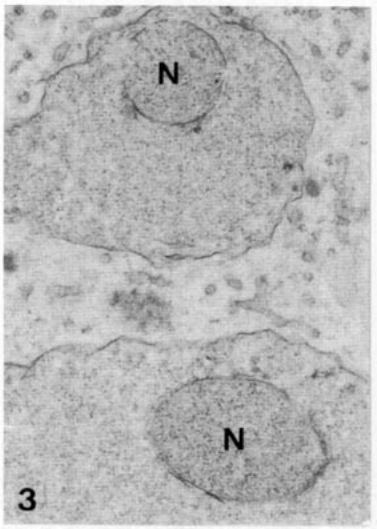
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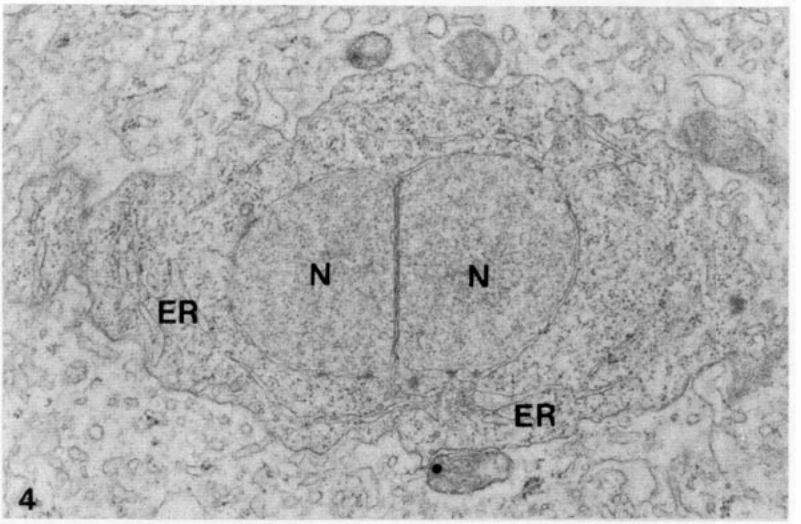
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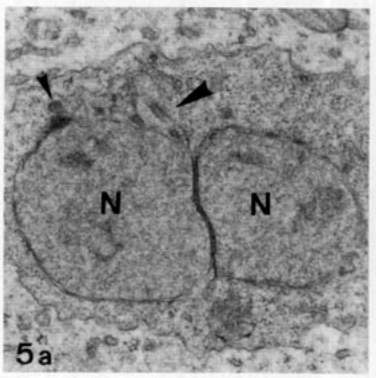
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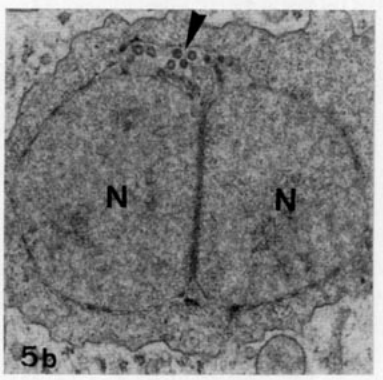
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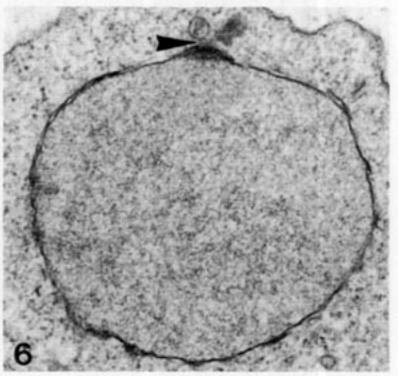
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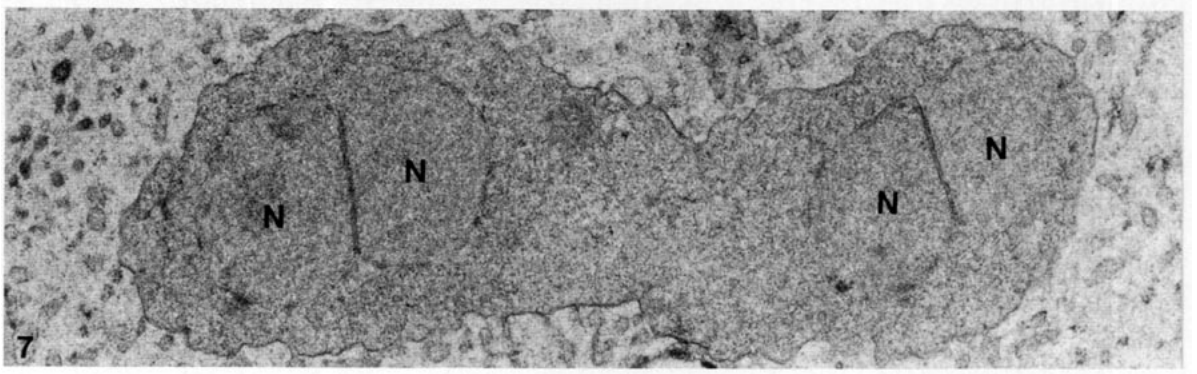
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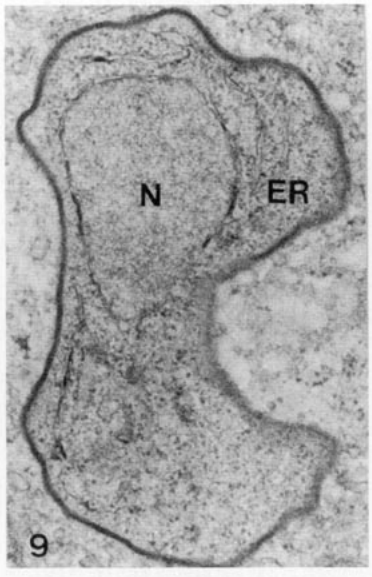
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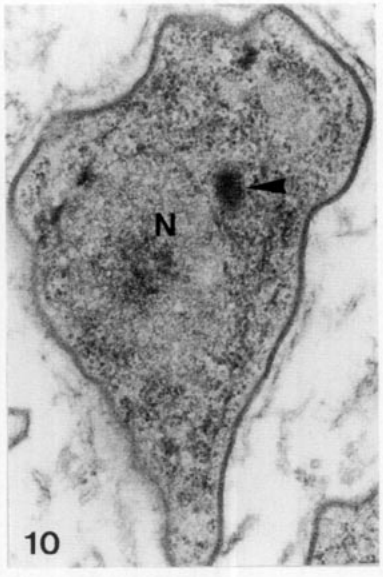
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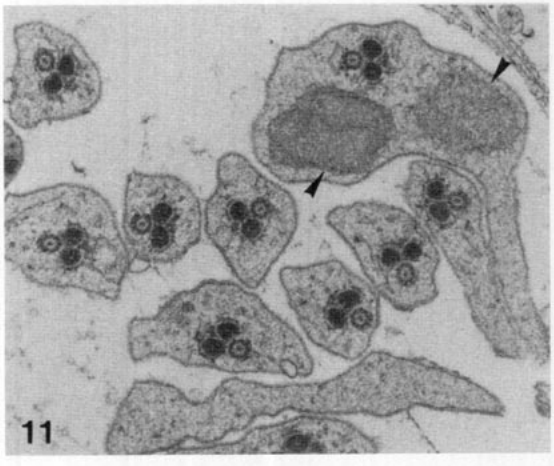
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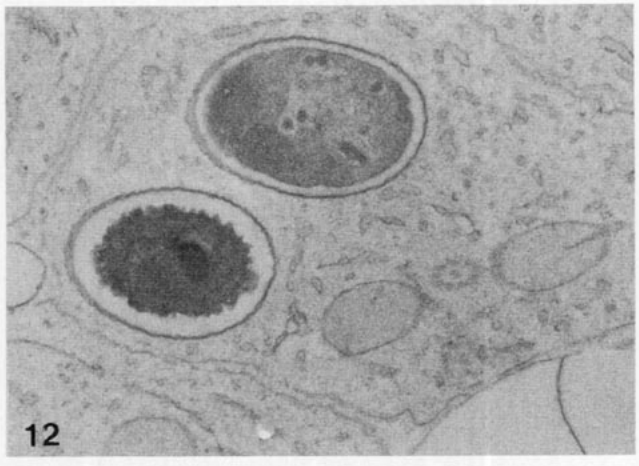
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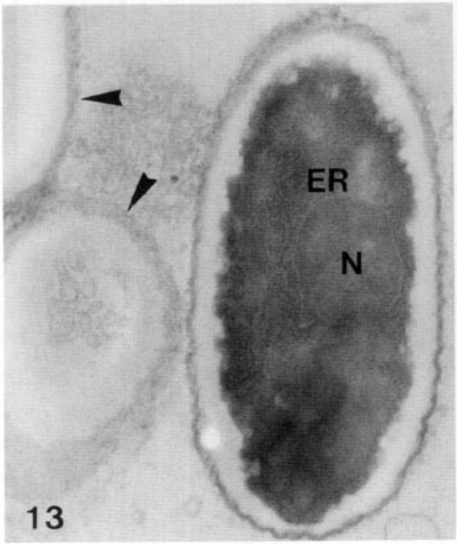
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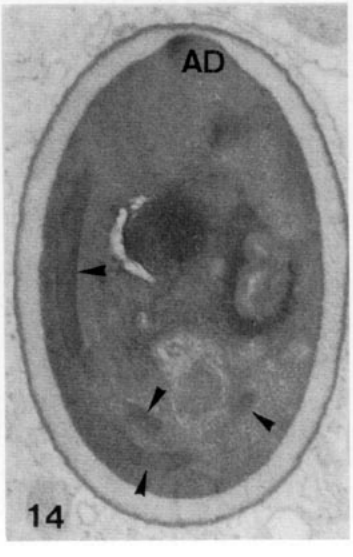
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cycle also takes place in the germinal epithelium. We do not know how much time is needed. In tissue culture cells Streett *et al.* (1980) have stated 72 hr; Hassanein (1951) found mature spores of *Nosema apis* in the queen honey bee not before 168 hr after feeding with contaminated food. If this is an average time it would certainly be too short for the differentiation from spermatocytes to late spermatids. The infection of these far advanced cells must have occurred at a later stage. Nonetheless, the presence of microsporidia in nearly all stages of the spermatogenetic cycle without apparent pathological changes indicates that the infection does not disturb this highly specialized differentiation process.

As mentioned before, the infection of mature male gametes contrary to eggs is an extraordinary rare event in insects (Afzelius *et al.*, 1989). The few cases hitherto known are exclusively caused by viruses and rickettsia. Also, microsporidia can be passed on to the next generation via the egg (Becnel *et al.*, 1986). The transmission by spermatozoa, however, should be excluded merely because of the size of the parasites. This does not exclude the possibility that venereal transmission by infected males might occur. Indeed Kellen and Lindegren (1971) found this means of infection to be a rare event for *N. plodiae* in the Indian meal moth *Plodia interpunctella,* and Thomson (1958) observed spores of *Perezia fumiferanae* among bundles of sperm in the male spruce budworm *Choristoneura fumiferana.* Solter *et al.* (1991) have studied different means of transmission of *N. pyrausta* in adult European corn borers. Although the highest rate was found when the insects were offered contaminated water, an infection of females by infected males was also observed after mating. However, the authors believe the possibility of true venereal transmission to be negligible or nonexistent. Infection only by contact seems to be much more probable. The number of cells per stage varies considerably. Thus, monokaryotic cells have been seen only very rarely. They differ in size, in the complexity of the cytoplasm, and in the presence of particles (RNP?) within the nucleus from the following diplokaryotic stage which is seen by far most frequently. Sporonts are again very rare. In the few cells which we have found only one nucleus was observed and the same holds for the sporoblasts. Therefore, the diplokaryotic state is confined to later meronts only.

Besides condensed spores we have seen a considerable number of "pale" cysts with various degrees of disorganized contents ending with empty envelopes. Apparently many of the microsporidia degenerate within their host cells, though the cause for this phenomenon remains obscure.

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FIG. 8. Cyst wall cell with different stages of the microsporidian cycle. Early sporonts with thickened membrane, light cytoplasm, and single nucleus (asterisks). Darkly stained mature spores (large arrowheads) and pale degenerating spores (small arrowheads). ×7000.

FIG. 9. Sporoblast, younger stage. Single nucleus (N), endoplasmic reticulum (ER), and ribosomes surrounded by a thin cyst wall. ×32,000.

FIG. 10. Sporoblast, later stage with the beginning of condensation. Nucleus (N), plenty of ribosomes, and cross section of polar filament (arrowhead). ×34,000.

FIG. 11. Tails of advanced spermatids with two meronts (arrowheads). ×12,000.

FIG. 12. Tail of elongating spermatid with two spores. ×19,000.

FIG. 13. Condensed spore with single nucleus (N), endoplasmic reticulum (ER) with numerous ribosomes. Pale spores containing only some vesicular material (arrowheads). ×32,000.

FIG. 14. Condensed spore with polar filament (arrowheads) and anchoring disc (AD). Envelope with dense exosporidium and broad lighter endosporidium. ×32,000.

FIG. 15. Pale spore with degenerating contents. Large vacuoles and remnants of polar filament (asterisk). ×32,000.