Association of Eosinophilic Myositis with an Unusual Species of *Sarcocystis* in a Beef Cow

A.A. Gajadhar, W.D.G. Yates and J.R. Allen*

ABSTRACT

The carcass of a mature cow had numerous, disseminated lesions typical of eosinophilic myositis. To elucidate the nature and possible cause of the lesions, histological sections were examined by light microscopy and selected areas were removed and processed for electron microscopy. The lesions were granulomatous in nature. Each granuloma contained at its centre an intact or ruptured sarcocyst associated with degenerate muscle fibers. Surrounding this was a layer of epithelioid cells and an intense accumulation of inflammatory cells, most of which were eosinophils. The primary cyst wall of the sarcocysts in these granulomas consisted of hair-like protrusions that featured many unusual electron-dense bodies. Sarcocysts with ultrastructures characteristic of *Sarcocystis cruzi* and *Sarcocystis hirsuta* were also present in muscle from the same animal, but these sarcocysts lacked any associated cellular responses. The eosinophilic myositis in this case appeared to be associated with sarcocystosis of an unknown species. Possibly, the inflammatory reaction was due to the host-parasite interaction in an unusual host.

Key words: Myositis, eosinophilic myositis, granuloma, granulomatous myositis, muscle disease, *Sarcocystis*, bovine.

RÉSUMÉ

La carcasse d'une vache de boucherie adulte présentait plusieurs lésions typiques de la myosite à éosinophiles. Afin d'élucider la nature et la cause possible des lésions précitées, on examina des coupes histologiques, au microscope photonique, et on préleva des zones choisies, en vue d'un examen au microscope électronique. Les lésions étaient de nature granulomateuse. Le centre de chaque granulome arborait un sarcocyste, intact ou rupturé, associé à des fibres musculaires dégénérées. Une couche de cellules épithélioïdes et un exsudat marqué de cellules inflammatoires, pour la plupart des éosinophiles, entouraient les granulomes. La paroi primaire des kystes des sarcocystes de ces granulomes, consistait en des protrusions chevelues qui exhibaient plusieurs corps denses inhabituels, au microscope électronique. Des sarcocystes dotés d'ultrastructures caractéristiques de *Sarcocystis cruzi* et de *Sarcocystis hirsuta* se retrouvaient aussi dans les muscles de cette vache, mais ils n'avaient provoqué aucun exsudat cellulaire. La myosite à éosinophiles de cette vache sembla attribuable à une espèce inconnue de sarcocystes. La réaction inflammatoire qui l'accompagnait résulterait probablement d'une interaction hôte-parasite, chez un hôte inhabituel.

Mots clés: myosite, myosite à éosinophiles, granulome, myosite granulomateuse, maladie musculaire, *Sarcocystis* spp., bovins.

INTRODUCTION

Eosinophilic myositis, an asymptomatic disease of unproven etiology, is diagnosed at slaughter in most regions of Canada. Well-demarcated green to grayish-green lesions are present in single muscles or groups of muscles. Some infected carcasses contain disseminated lesions and are considered unfit for human consumption.

It has been suggested that protozoa of the species *Sarcocystis*, often found in association with granulomatous myositis, are responsible for eosinophilic myositis (1,2). Based on light microscopy, it was suggested that eosinophilic myositis of cattle is caused by *S. cruzi*, *S. hirsuta* or *S. hominis* (2). However, it is believed that the ultrastructure of the sarcocyst wall is one of the few reliable criteria for identifying the species (3). Recently, results of isoenzyme electrophoretic techniques provided evidence to support this view (4). Here, we present histological and ultrastructural evidence of sarcocysts of an unknown species in granulomas of eosinophilic myositis in a beef cow. The cellular response to this parasite is compared with the lack of response to *S. cruzi* and *S. hirsuta* in the same animal.

MATERIALS AND METHODS

The carcass of a mature cow was examined by a veterinary inspector at a small abattoir in southern Saskatchewan. Samples of muscle from the hindquarters and loin areas were excised and submitted to the Animal Pathology Laboratory, Agriculture

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Canada, Saskatoon and the Western College of Veterinary Medicine for analyses. Specimens were fixed in neutral-buffered 10% formalin, processed by standard histological techniques, sectioned at 5 μm, stained with hematoxylin and eosin and examined by light microscopy. Ten to 100 sections from each tissue block were cut and examined. Selected areas of muscle sections were removed from slides, dehydrated in propylene oxide, embedded in epon, and thin sections cut (5). Thin sections were stained with lead citrate and examined with a Philips EM410LS transmission electron microscope.

RESULTS

GROSS PATHOLOGICAL FINDINGS

The muscles of the hindquarters and loin areas had numerous, green lesions of a few mm in diameter. These lesions contained a grayish cyst-like structure in the centre.

HISTOPATHOLOGICAL FINDINGS

Granulomas were present in various portions of the tissue (Fig. 1), with cellular infiltrations of the greatest intensity in areas adjacent to the gross lesions observed. Fourteen granulomas were examined. An intact or ruptured sarcocyst was present in each granuloma (Fig. 2), although it was sometimes necessary to cut several serial sections to demonstrate this. The sarcocysts were oval, measured up to 265 × 162 μm and had numerous hair-like protrusions extending from the outer surface. There were many distinct septa and numerous bradyzoites within each sarcocyst. Many bradyzoites were degenerate. Pale to neutral staining eosinophils were observed in sarcocysts with ruptured walls and the cytoplasmic granules of some eosinophils were extracellular. Surrounding the central sarcocyst were areas of caseation necrosis and degenerated, hyalinized muscle fiber remnants (Fig. 1) bordered by a distinct rim of elongated epithelioid cells (Fig. 3). Beyond this border was an intense accumulation of mixed inflammatory cells, most of which were eosinophils (Fig. 4), but which included macrophages, neutrophils, plasma cells, and erythrocytes.

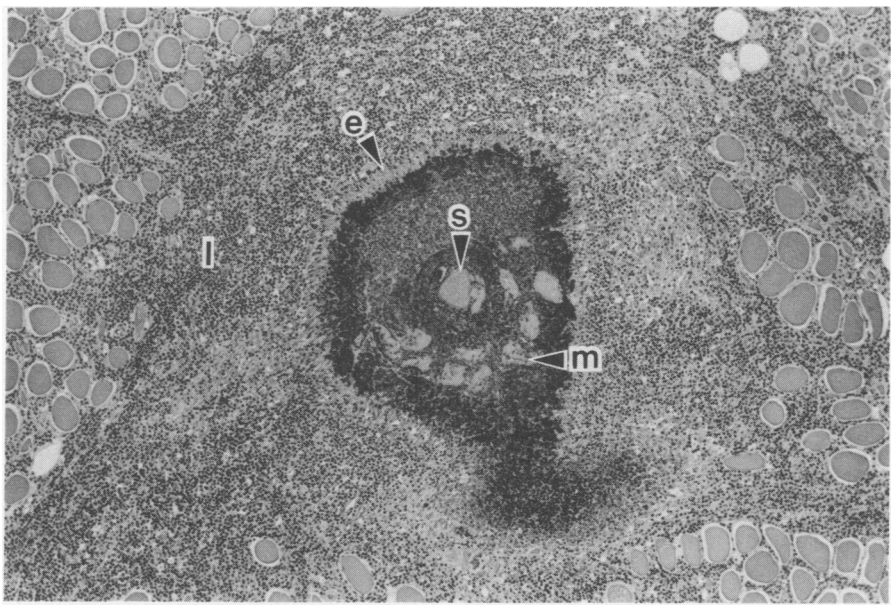

Fig. 1. Photomicrograph of granuloma in muscle. Note the central sarcocyst (s), degenerate muscle fibers (m), epithelioid cells (e) and inflammatory leukocytes (1). H & E. X64.

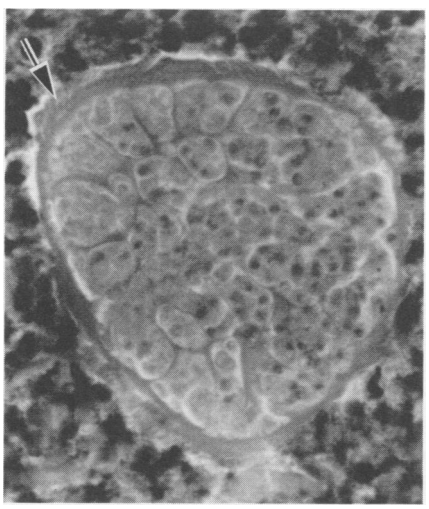

Fig. 2. Section of a sarcocyst within a granuloma. Numerous hair-like protrusions (arrow) extend from the outer surface. H & E. X675.

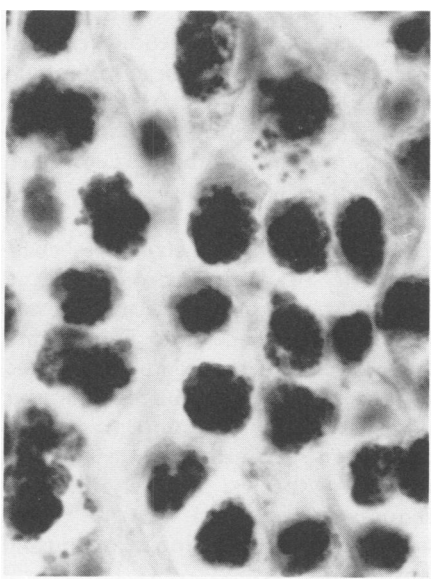

Fig. 4. Eosinophils typical of those at the periphery of lesions. H & E. X1,500.

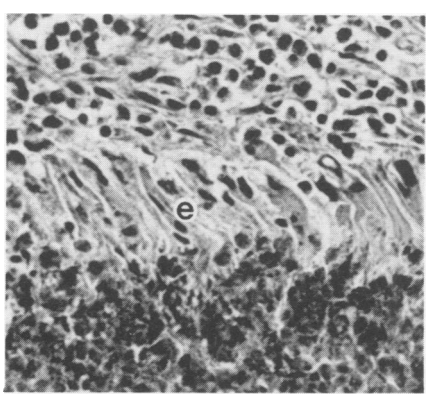

Fig. 3. Portion of the epithelioid cell layer (e) surrounding a granuloma. H & E. X320.

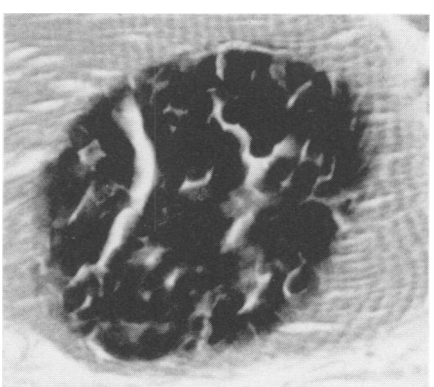

Fig. 5. Section of the thin walled sarcocyst of *S. cruzi* in a muscle fibre of normal appearance. H & E. X925.

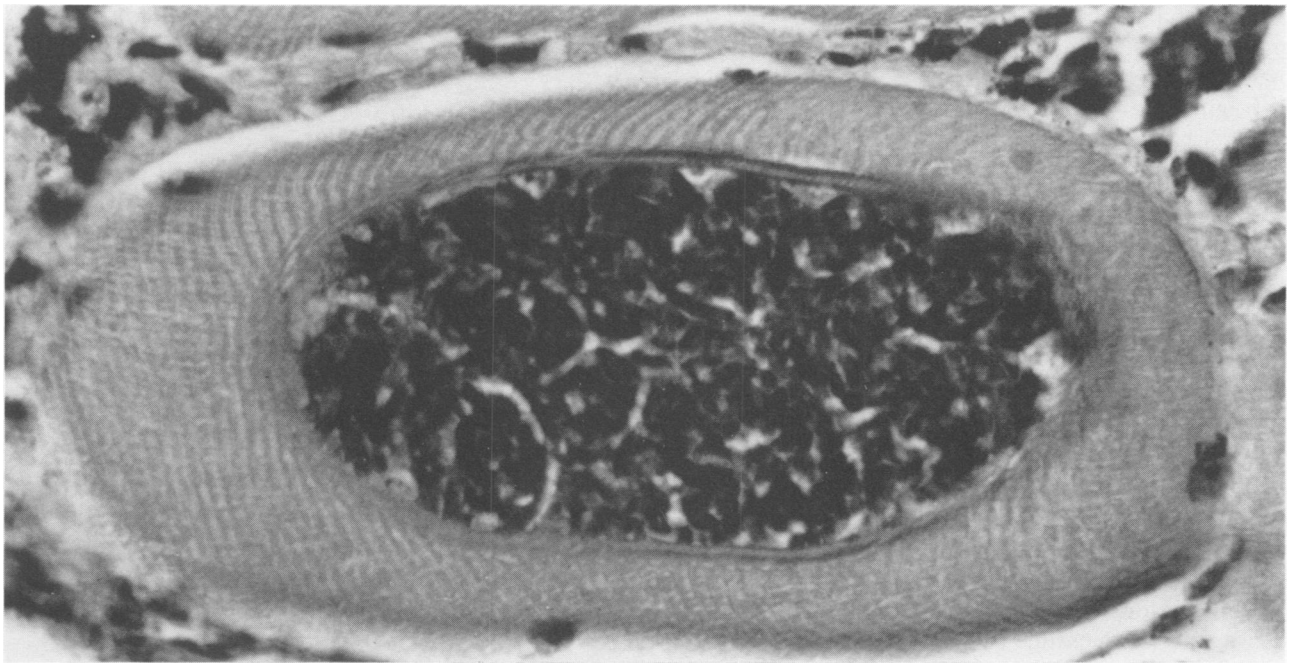

Fig. 6. Section of the thick walled carcocyst of *S. hirsuta* in a muscle fiber of normal appearance. H & E. X590.

This predominantly eosinophilic response radiated outward through fascial planes, diminishing in intensity as distance from the granuloma increased.

All sarcocysts found in granulomas were similar to one another in structure, shape and size. They were found only within granulomas and were always accompanied by an intense eosinophilic response. Occasionally two other types of sarcocysts were present in the same tissue section. Seven of one species and eight of another corresponded to mature cysts of *S. cruzi* and *S. hirsuta*, respectively (Figs. 5 and 6). These latter species were found within otherwise normal muscle fibers. They were composed of intact walls and numerous bradyzoites, typical of their respective species, and showed no associated cellular responses by the host.

ULTRASTRUCTURE

The sarcocysts that were associated with granulomas contained septa which extended inward from the cyst wall, forming many compartments that enclosed groups of spheroid to ovoid bradyzoites. These zoites had a mean measurement of 5.97 × 2.48 μm (n=15) and contained a nucleus, rhoptries and micronemes. Some sarcocysts with ruptured walls also contained several eosinophils and extracellular eosinophilic granules. The sarcocyst wall was uniformly thick, $\bar{x}$ = 4.37 μm (n=11). Many finger-like protrusions extended outward from the primary cyst wall (Fig. 7). Villar protrusions were obliquely arranged, up to 8.5 μm long and contained several longitudinally arranged fibrils. Many short, usually ovoid, electron dense bodies, approximately 64.8 nm (n=10) long, were located on each villar projection. Eosinophils were sometimes in contact with the primary cyst wall (Fig. 8). In

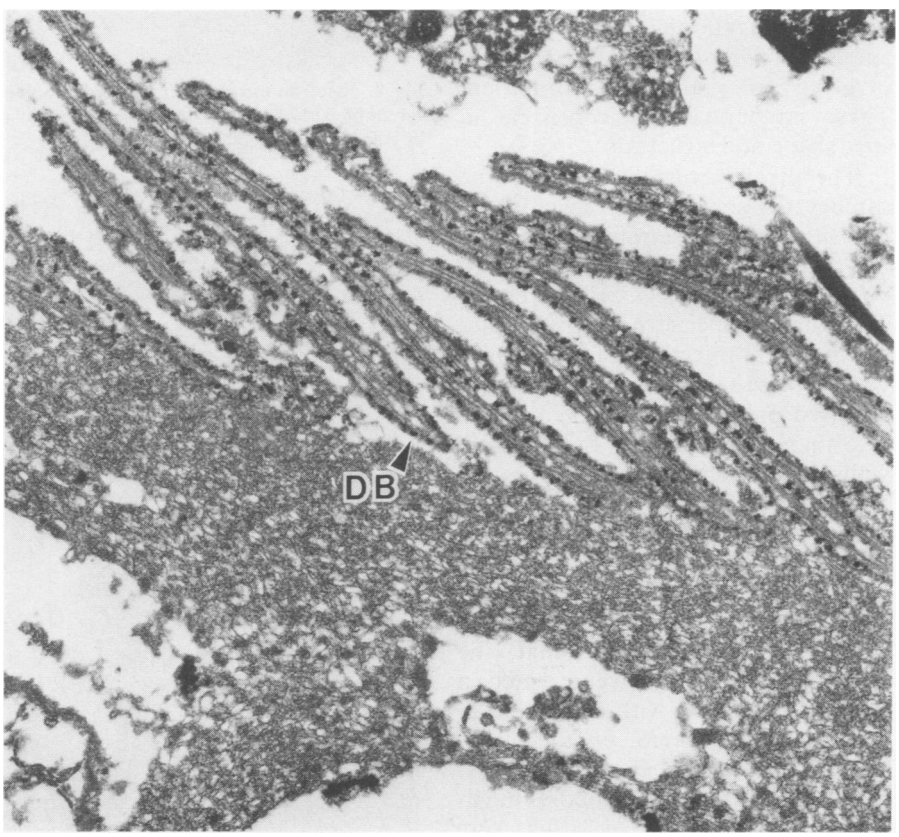

Fig. 7. Electron micrograph of the wall of a sarcocyst in the center of a granuloma. The finger-like villar projections extend outward and have many electron-dense bodies (DB). X18,100.

these areas the villar projections were thinner. Adhering eosinophils as well as those adjacent to sarcocysts contained fragmented granules. Other granules were extracellular. Cyst walls of *S. cruzi* were thin and had few, small villar protrusions, whereas those of *S. hirsuta* characteristically featured prominent palisade-like protrusions (Figs. 9 and 10).

DISCUSSION

The numerous small grayish-green lesions were typical of a form of eosinophilic myositis which results in carcass condemnation. This study confirms other findings (1,2) that sarcocystosis can be a feature of granulomas of this particular condition. Because sarcocysts within lesions were relatively small it was often impossible to demonstrate their presence in a single histological section, and many serial sections were sometimes required to demonstrate the parasite. A single species of *Sarcocystis* appeared to be involved in granuloma formation. These sarcocysts were consistent in size, shape and structure.

The ultrastructure of the wall of the sarcocysts associated with eosinophilic myositis in this case was singular and not typical of that described for *S. cruzi*, *S. hirsuta* or *S. hominis* in cattle. The numerous electron-dense bodies on the villar projections of the primary wall of these sarcocysts have not been reported previously. However, larger disc-shaped plaques in the primary walls of sarcocysts of *S. hemionilatrantis* and *S. odocoileocanis* have been reported (6). Inadequate tissue fixation prevented more detailed ultrastructural characterization and comparison with the reported disc-shaped plaques. The possibility that the electron-dense bodies represented a destructive process within the walls of disintegrating older *S. hirsuta* sarcocysts is unlikely. Speer and Dubey (6) described electron-dense bodies in villar projections of sarcocysts that were not associated with host lesions. Also, the structure of primary cyst walls and size of the bradyzoites differed from those of *S. hirsuta*. Because the structure of sarcocyst walls is considered a reliable criterion for identifying species of *Sarcocystis* (3,4), it was concluded that the sarcocysts found in this study

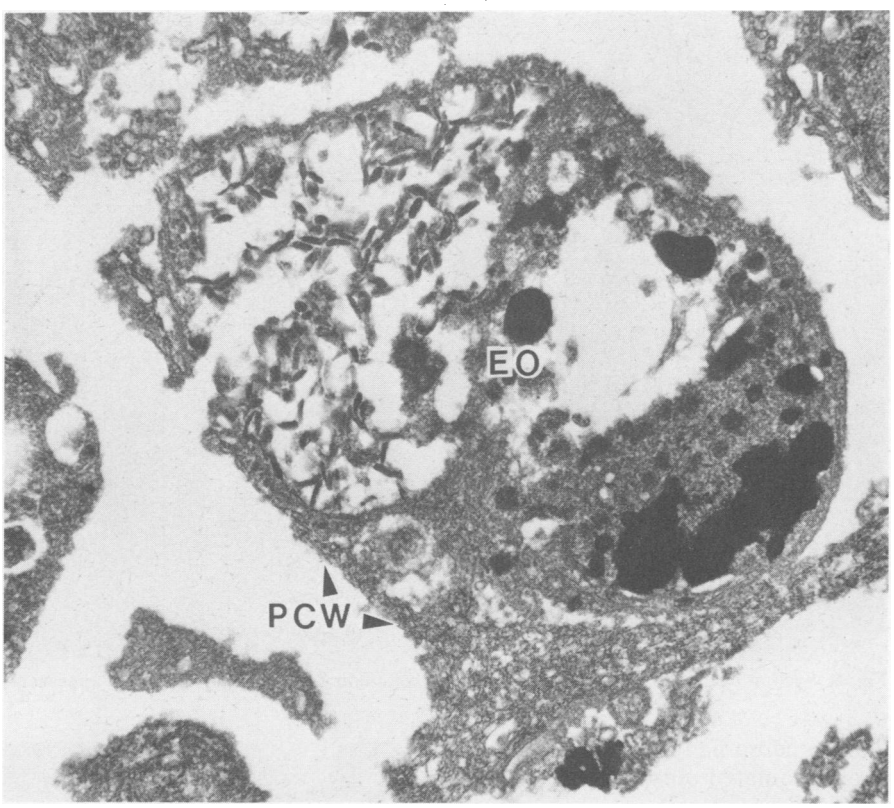

Fig. 8. Electron micrograph of an eosinophil (EO) adherent to the primary cyst wall (PCW) of a sarcocyst within a granuloma. The eosinophil appears to be degranulating, although artifact cannot be ruled out. X22,500.

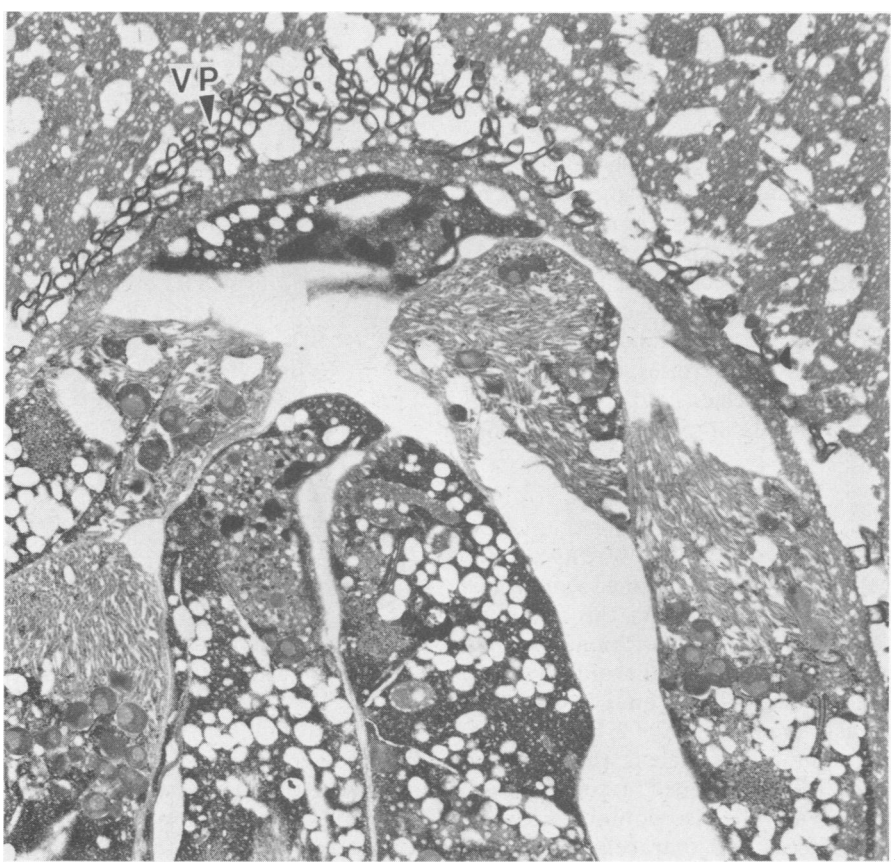

Fig. 9. Electron micrograph of *S. cruzi* with small villar projections (VP). X5,200.

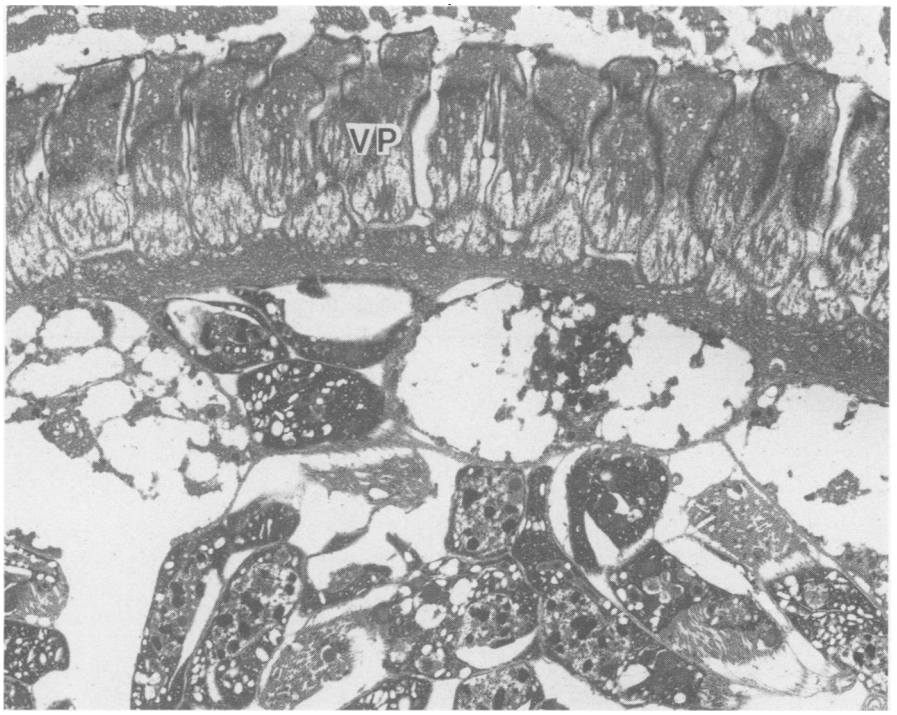

Fig. 10. Electron micrograph of *S. hirsuta* with palisade-like villar projections (VP). X10,050.

within granulomas did not represent any of the three known *Sarcocystis* species (*S. cruzi*, *S. hirsuta*, *S. hominis*) of cattle.

The function of electron dense bodies in the villar projections is not known. They may be important for villar projection rigidity, and for sites of molecular exchange between parasite and host (6). The latter role may explain the intense cellular reaction adjacent to the sarcocysts. *Sarcocystis cruzi* and *S. hirsuta* were present in the same animal, but lacked the remarkable host response and did not have electron-dense bodies on villar projections. This type of host-parasite interaction has not been reported for the three known species of bovine *Sarcocystis*.

Based on light microscope examination of sarcocysts associated with granulomatous myositis, *S. cruzi*, *S. hirsuta* and *S. hominis* were considered by Jensen *et al* to be responsible for the granulomatous reaction (2). However, the fine structure of the cyst walls was not confirmed by electron microscopy and it is possible that another species was involved. Although *S. cruzi* and *S. hirsuta* can cause focal myositis (7-9) generalized eosinophilic myositis in cattle was not seen in studies where cattle were experimentally inoculated with either of these two species (7-9). Furthermore, in North America raw human sewage is not generally available for consumption by cattle and *S. hominis* is probably rare or nonexistent. Nevertheless, *S. cruzi* and *S. hirsuta* must be considered as possible etiological agents of eosinophilic myositis in cattle. Our observations indicated that a previously undescribed species of *Sarcocystis* was involved in eosinophilic myositis.

Perhaps the normal intermediate host of this undefined species of *Sarcocystis* is not cattle but another ruminant. *Sarcocystis* spp. from bison *(Bison bison)*, elk *(Cervus canadensis)* and moose *(Alces alces)* can be infective for cattle (10). Also, cattle may graze on pastures that are contaminated with the feces of wild carnivores which as definitive hosts may shed infective sporocysts. The common composition of the granulomas and the similarity of the sarcocysts within them suggest that the infection probably occurred at a single exposure.

Although not unique, the severe eosinophilic reaction to a protozoon observed in this study is unusual. The destruction of helminths, such as *Schistosoma mansoni* and *Fasciola hepatica*, by activated eosinophils has been described (11,12). Our ultrastructural findings indicated that eosinophils were closely associated with damaged primary walls of this sarcocyst and damage to adjacent muscle fibers. Similar apposition was essential for eosinophil-mediated damage to helminths (11,12).

The intense inflammatory reaction of eosinophilic myositis in this case is associated with a species of *Sarcocystis* previously undescribed. The initial cellular response may have been induced by substances released from the sarcocyst during its normal metabolic functions. Perhaps, because this species of *Sarcocystis* is unusual in cattle, the more harmonious host-parasite relationship of a well adapted species was replaced by a severe host response. Ultimately the primary cyst wall was ruptured; the sarcocyst was invaded, and massive cellular infiltration occurred with development of granulomatous lesions.

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