Revista Latinoamericana de Microbiología (1999) 41:303-307
Asociación Latinoamericana de Microbiología

Role of the Calcareous Corpuscles in Cestode Physiology: A Review

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ABSTRACT. Cysticercosis is a parasitic disease caused by the larval stage or cysticercus of the cestode tapeworm *Taenia solium*. Cysticerci are able to survive in the host tissues for long periods in the presence of an immune response. Tissues of cestodes contain mineral concretions termed calcareous corpuscles. These corpuscles might serve for the focal deposition of exceeding amounts of calcium protecting the larvae against calcification. Studies on the morphology and composition of calcareous corpuscles in cestodes have resulted in a number of hypotheses on their origin and function. Calcareous corpuscles are believed to form either intracellularly or extracellularly and the cell type involved, the place of formation and the mechanism of mineral deposition seem to be also diverse. This review intends to provide an updated guide to the published literature on calcareous corpuscles in cestodes, giving emphasis on their role in larval physiology. Understanding biomineralization might lead to novel ways for the treatment of diseases caused by cestode larvae.
KEY WORDS: Calcareous corpuscles, Calcification, Cestodes.

RESUMEN. La cisticercosis es una enfermedad parasitaria causada por la forma larvaria o cisticerco del céstodo *Taenia solium*. A pesar de la respuesta inmune del huésped, los cisticercos son capaces de sobrevivir en los tejidos por largos periodos. Los céstodos contienen estructuras minerales llamadas corpúsculos calcáreos. Estos corpúsculos podrían servir para concentrar las cantidades excedentes de calcio y otros minerales, protegiendo a la larva de la calcificación. Estudios sobre la morfología y composición de los corpúsculos calcáreos de los céstodos ha llevado a la formulación de una serie de hipótesis sobre su origen y su función. Se cree que los corpúsculos calcáreos se forman de manera intracelular o extracelular, mientras que el tipo de célula involucrada, el lugar y mecanismos de formación parecen ser de origen diverso. Esta revisión pretende proveer de una guía comprensiva a la literatura publicada sobre corpúsculos calcáreos en céstodos, dando énfasis al papel de estas estructuras en la fisiología de la larva. Comprender los procesos de biomineralización en este parásito podría permitir encontrar nuevas maneras de tratar la enfermedad causada por la larva de este céstodo.
PALABRAS CLAVE: Corpúsculos calcáreos, Calcificación, Céstodos.

INTRODUCTION

Within the phylum Platyhelminthes, cestodes are one of the major groups of parasites that cause disease in humans. Human infections by adult worms usually generate moderate pathology although some problems of gut-wall damage and anaemia may occur. In contrast, two types of human larval cestodiosis can result in a severe disease: cysticercosis caused by the cysticercus of *Taenia solium*, and hydatid disease resulting from the proliferation of the hydatid cyst of *Echinococcus granulosus*.

Among parasitic platyhelminths, mineral concretions have been described both in trematodes[1,10,11,23] and cestodes.[3,4,6,7,20,40,44] Two different processes of biomineralization can be distinguished: the first is characterized by bulk extracellular or intracellular mineral formation, without involvement of organic matrices, and is widely observed in bacteria and algae.[21] The second is organic matrix-mediated and involves an organic framework onto which the ions are actively incorporated and induced to crystallize in structures of varying degrees of complexity.[21,33,34] Mineral concretions in cestodes are known generically as calcareous corpuscles and belong to the organic matrix-mediated type (Fig. 1).

Calcareous corpuscles structure and composition. The earlier reports on calcareous corpuscles in tapeworms can be traced back to the 18th century,[13,28] although the initial studies on their structure and composition started in the decade of the 30's,[8,31,39,41,43] and the first clues regarding the composition of calcareous corpuscles were provided until the end of the 50's, when by means of X-ray diffraction studies the corpuscular material from *Taenia saginata*

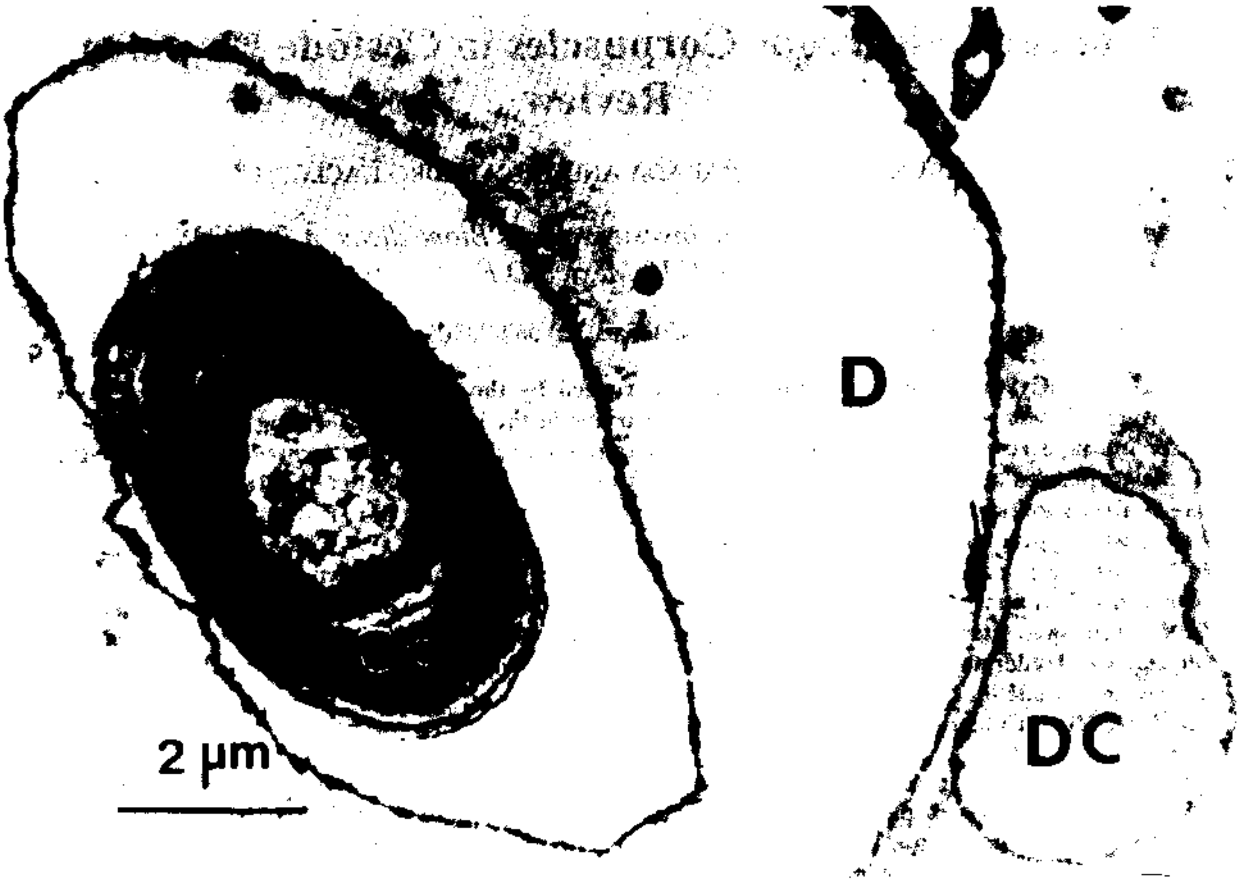

Figure 1. *Taenia solium* mature calcareous corpuscle showing the typical pattern of granular concentric layers (Cc Calcareous corpuscle, D protonephridial duct, DC duct cell). Courtesy of Springer-Verlag.

was analyzed.[9,44] In the following years, several species of taeniids were studied and found to contain calcium, magnesium, phosphate and carbon dioxide.[16,17,32,36,48,49] In *Mesocestoides corti* silicon and zinc have also been reported to form part of the calcareous corpuscles.[2,12,18]

The organic matrix is organized in concentric rings and a double outer envelope; it contains proteins, lipids, polysaccharides, mucopolysaccharides, RNA, and DNA.[3,37,38] The organic matrix provides the structural framework or substrate for deposition of minerals.[26,47]

There is much variation in size of calcareous corpuscles in different species but they usually range from 7 up to 34 μm in diameter.[8,12,17,22,24] The calcareous corpuscles of *T. solium* cysticerci are smaller in size, varying between 1.5 to 6 μm in diameter.[42] Calcareous corpuscles can be irregularly spherical or ovoid, and variations have been described both in the same and between different species.[22,51] The bodies always contain minor amounts of other inorganic elements, and these, as well as the amount of phosphate are affected by the diet of the host and may constitute as much as 41% of the dry weight of the organism.[22,31] The number and chemical composition of calcareous corpuscles can also vary between species as well as within the same species[26,32,48,49,50,51] and the latter appears to be related to the composition of the environment.[2,18,19] Calcareous corpuscles have been reported in vast numbers in both juvenile and adult stages of cestodes.[26]

Calcareous corpuscles formation. The formation of calcareous corpuscles in cestodes has been studied almost exclusively in cyclophyllidean species. Available information suggests that they may be formed through diverse processes. Earlier reports described the formation of corpuscles in intracellular compartments of several cell types. It has been described that calcareous corpuscles are formed in the nuclei of parenchymal or mesenchymal cells in *Thysaniezia ovilla*,[20] *Hymenolepis microstoma*,[5] *Echinococcus granulosus* and *Taenia hydatigena*.[29] The nucleus enlarges by continuous accretion of minerals, whereas the cytoplasm degenerates concomitantly producing the mature corpuscle. Formation of calcareous corpuscles can also occur in the cytoplasm of the corpuscle forming cells, as described in *H. microstoma*,[5] *E. granulosus*, and *T. hydatigena*.[29] In the case of *T. saginata*, the calcareous corpuscle appears to form by mineral deposition around the plasma membrane of a mesenchymal cell that is supposed to be specially avid for calcium.[3] In *Taenia taeniaeformis*, the

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calcareous corpuscles are also formed in the cytoplasm and the organic core is provided by the remnants of the cytoplasm itself or by the Golgi membranes. This corpuscle forming cell appears to expend itself in the production of one corpuscle.[26] In the tetraphyllidean *Trilocularia acanthiaevulgaris* the formation of intracellular corpuscles in certain parenchymal and mesenchymal cells, occurs concomitantly to the autophagic degradation of the cytoplasm,[25] while in *Schistocephalus pungitii*,[40] a large intracellular cavity is formed by coalescence of cytoplamic vacuoles. Formation of calcareous corpuscles has been reported on the brood capsule and on the germinal layer in metacestodes of *Echinococcus multilocularis*.[27] The osmoregulatory system also known as protonephridium has also been involved in the formation of calcareous corpuscles in *H. microstoma* where the early stages are found in the cytoplasm of "vesiculated cells" that are connected to, or that are merely dilations of capillaries of the flame cells on their way to the nephridial ducts.[5] Transmission electron microscopic studies have shown that the calcareous corpuscles are surrounded by a double layer envelope; the outer and less dense layer consisting probably of the compressed remnants of the corpuscle-forming cell whereas the inner and denser layer may come from the membranes involved in the corpuscle development.[47]

While the formation of calcareous corpuscles has been reported as an intracellular process in taeniid cestodes, their formation in cysticerci of *T. solium* appears to be extracellular, occurring in the lumen of protonephridial ducts where the spherical microprojections of the duct wall, not mitochondria, appear to provide the organic support for the deposition of inorganic material.[42] This mechanism appears to be related to that proposed for *H. microstoma*, where corpuscle formation occurs inside capillary dilations that are connected with the osmoregulatory ducts.[5]

Two types of morphologically different calcareous corpuscles have been described in *E. granulosus*, *T. hidatigena* and *T. solium*.[29,42] Both types show the typical pattern described for tetraphyllidean[25] and cyclophyllidean species.[26,32,47]

Calcareous corpuscles function. The role of the calcareous corpuscles in the physiology of tapeworms has been the subject of much speculation. For example, it has been proposed that corpuscles may also act as reservoirs of carbonate that protect the tissues of the worm against organic acids from intermediate metabolism or during its passage through the stomach of the definitive host.[25,26,35] Support for this idea comes from the observation that a significant decrease in the number of corpuscles is observed when *H. diminuta* cysts are exposed to an acid excystment medium.[15] The calcareous corpuscles might also serve as reservoirs of phosphate; experiments on *T. taeniaeformis* show that phosphate enters the body of the larvae by passive diffusion and that phosphate salts can be deposited or released according to the environmental conditions.[45] The observation that the amount of phosphate in the corpuscle is altered by changes in the nutritional state of the host seems to support this proposal.[51] Formation of calcareous corpuscles in the protonephridial ducts in *T. solium*, also suggests that they relate with roles ascribed to the protonephridia such as regulation of the tissue fluids and elimination of metabolic wastes.[52,53] This idea is supported by the observation that *M. corti* has the ability to concentrate several distinct cations into the calcareous corpuscles, when these are introduced into the host drinking water.[18,19] Furthermore, scanning electron microscope studies on *M. corti*, show that corpuscles are emitted through the tegument. This emission could serve to remove metabolic wastes, therefore suggesting that the calcareous corpuscles may represent excretory dumps.[12]

In contrast to the cestode adult worm, the larval, juvenile stages or metacestodes contain large numbers of corpuscles.[24,37] The focal deposition of exceeding amounts of calcium in the corpuscles could protect the larval stage against calcification.[7,44] Radiolabelled calcium can be demonstrated in the corpuscles of *T. taeniaeformis* six months after a single dose of $^{45}Ca^{++}$ was administered to the host, suggesting that little calcium is removed after being deposited in the corpuscle.[46] However, it is not clear if the dissolution of some or all corpuscles, observed during development of *H. microstoma* might also serve to meet its requirements for calcium in soluble form.[5] Recently, the coding cDNA sequence of an intracellular protein of *E. granulosus* showing calcium-binding motifs has been isolated and the recombinant fusion protein (EgCaBP1-GST), expressed in bacteria, has been shown to bind calcium *in vitro*. This protein is strongly associated with the calcareous corpuscles[30] and opens new possibilities for the study of calcium deposition in larval cestodes.

In the human being, cysticerci can develop in a number of sites including the central nervous system.[14] One possible outcome for the infection is the gradual calcification of the parasite until complete substitution of the larval tissues by mineral salts. The driving factors for the calcification of the cysts are still poorly understood. Calcification appears to be mainly controlled by the host after the parasite looses its ability to maintain calcium levels within physiological limits. Understanding the mechanism involved in the process of calcification may lead to novel strategies for the treatment of the disease in humans and in domestic animals.

ACKNOWLEDGMENTS

This paper was partially supported by grants No. IN 207195 from DGAPA-UNAM and No. L0042M from CONACYT. L Vargas-Parada was supported by a fellowship from DGAPA-UNAM and CONACYT.

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