

# POSSIBLE FORMATION OF NEW BROOD CAPSULE BY THE PREVIOUSLY FORMED BROOD CAPSULE IN *ECHINOCOCCUS MULTILOCULARIS* METACESTODES

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**Abstract.** In *Echinococcus multilocularis* metacestodes obtained from the peritoneal cavity of an experimentally infected jird, cellular accumulations were found not only on the inner surfaces of germinal layers but also on the outer surfaces of brood capsules. These cellular accumulations are believed to represent at incipient form of the brood capsule. It has been thought that brood capsules are produced by the germinal layer, but we speculate that the brood capsule itself, as well as the germinal layer, may have the potential to produce new brood capsules.

## INTRODUCTION

Brood capsules are the one of the main components of metacestodes of *E. multilocularis* in an appropriate intermediate host, and they are very important for maintaining the life cycle of *E. multilocularis* because brood capsules produce protoscolices which infect final hosts. The initial formation of brood capsule begins with the accumulation of cells on the inner surface of the germinal layer, the accumulation then enlarges and a lumen is formed in the accumulated cells. Thereafter, the lumen increases in capacity with the passage of time and the origin of the protoscolex protrudes into the lumen from the inner surface of the brood capsule in the metacestode of *E. multilocularis* (Ohbayashi, 1960; Sakamoto and Sugimura, 1970) and *E. granulosus* (Bortoletti and Ferretti, 1973; Thompson, 1976). However, we detected similar cellular accumulations on the outer surfaces of previously formed brood capsules. This report deals with the possibility of brood capsule formation by the brood capsule itself in metacestodes of *E. multilocularis*.

## MATERIALS AND METHODS

### Parasite

The larval stage of *E. multilocularis* used for the experimental secondary infection in this study was isolated from the infected liver of a red-backed vole (*Clethrionomys rufocanus bedfordiae*) caught in Akkeshi Town, Hokkaido, Japan in 1985 and has been maintained in the Department of Parasitology, Asahikawa Medical College, by intraperitoneal passage of the larval suspension into jirds (*Meriones unguiculatus*) (Nakao *et al.*, 1990).

### Experimental design

A female 6-month-old jird was implanted intraperitoneally with small pieces of metacestodes of *E. multilocularis* which had been obtained from the peritoneal cavity of a jird which had been intraperitoneally inoculated with a larval suspension 3 months previously. Autopsy was performed 94 days post-infection, the metacestode obtained from the peritoneal cavity was fixed in 10% formalin, dehydrated in a graded series of methanols and embedded in paraffin. Sections were cut on a microtome for 3.5  $\mu$ m thickness and examined microscopically following periodic acid-Schiff (PAS) staining.

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## RESULTS

Laminated and germinal layers, calcareous corpuscles, brood capsules and protoscolices were identified in the metacestodes obtained from the experimentally infected jird. Furthermore, many cellular accumulations which adhered to the inner surfaces of germinal layers or to the outer surfaces of the brood capsules (Fig 1) were identified. Some of the cellular accumulations adherent to the outer surfaces of brood capsules had lumina (Figs 2, 3).



Fig 1—Cellular accumulation on outer surface of brood capsule and inner surface of germinal layer ( $\times 100$ ).



Figs 2, 3—Cellular accumulation, on outer surface of brood capsule, forming a lumen ( $\times 500$ ).

B = brood capsule; C = cellular accumulation;  
G = germinal layer; L = lumen; P = protoscolex.

## DISCUSSION

It has been thought that the brood capsule is produced by the germinal layer (Ohbayashi, 1960; Sakamoto and Sugimura, 1970; Bortoletti and Ferretti, 1973; Thompson, 1976). However, our observation that cellular accumulations, some having lumina, are also identifiable on the outer surfaces of brood capsules indicates that the brood capsule itself may have the ability to produce new brood capsules. Based on our observation that the number of cellular accumulations on the inner surface of the germinal layer is greater than that on the outer surface of the brood capsule, most brood capsules of larval *Echinococcus* are produced by the germinal layer and formation of a brood capsule by a previously formed brood capsule may be an alternative way of producing brood capsules. The cellular accumulations identified on the outer surface of the brood capsule may have grown in size and formed lumina, and protoscolices would then be made with the passage of time. It has been reported that undifferentiated cells of the germinal layer are the origin of brood capsule and that the lumen of the brood capsule is lined with brood capsule-wall lining cells resembling syncytial cyst-wall forming cells, which Lascano *et al* (1975) described as tegumental cells of the germinal layer of larval *E. granulosus*, of the germinal layer of *E. multilocularis* (Sakamoto and Sugimura, 1970). These investigators also reported that muscle cells and glycogen-storing cells appear on the outer surface of the brood capsule wall where a small number of undifferentiated cells are recognizable. But, unfortunately, very little study has been done on the classification or function of brood capsule cells, and information about the brood capsule is very limited. Even now, there is no consensus as to the classification of cells of the brood capsule or of the germinal layer of *Echinococcus*, or the origin of the accumulated cells. Further investigation into the classification and function of brood capsule cells is awaited.

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