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Developing protoscoleces of *Echinococcus granulosus* on the outer surface of the brood capsule, detected by scanning electron microscopy

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ABSTRACT

By means of scanning electron microscopy, stalked protrusions were observed arising from the outer surface of intact brood capsules of *Echinococcus granulosus*. Histological studies showed these protrusions to be developing protoscoleces. However, complete development is not attained and the protoscoleces eventually die. It is suggested that external development is a result of overcrowding within the brood capsule.

The development of protoscoleces of *Echinococcus granulosus* on the outside of a brood capsule is a phenomenon that, according to Cameron (1927), does not occur. He concluded that all previously reported cases of external development were due to the rupture of the brood capsule wall. This is elastic and tends to turn inside out giving the appearance of external development. This theory has since been supported after observations on brood capsules from both primary (Dissanaike and Paramananthan, 1961; Gill and Rao, 1967; Reddy *et al.*, 1969) and secondary hydatid cysts (Pennoit-De Cooman and De Rycke, 1972), as well as from studies *in vitro* (Coutelen, 1927a, 1927b; Smyth, 1962).

Attention was drawn to the usefulness of using the scanning electron microscope in studying the morphology of brood capsules of *E. granulosus* by Vaněk (1970). Recently, this technique has revealed the occurrence of external protrusions arising from the outer surface of intact brood capsules. This report is concerned with the nature of these protrusions.

MATERIALS AND METHODS

All brood capsules examined were removed from fresh horse hydatid cysts.

Scanning electron microscope observations were carried out on material fixed in 10% formalin or gluteraldehyde, dehydrated through the alcohols and treated in amyl acetate for one hour prior to being placed in a Polaron critical point drier for one hour. The specimens were then secured onto aluminium stubs using Silver Dag, and transferred to a standard dessicator for six hours. Specimens were subsequently coated with gold in a Polaron E5000 Diode Sputter-Coater before transfer to a Mark 2A Cambridge Stereoscan microscope.

Brood capsules were also examined using the light microscope. Temporary wet mounts were used, as well as permanent mounts made in lactophenol, or stained in Whitlock's

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haematoxylin (Sweatman and Williams, 1963) and mounted in balsam. Sections were stained in haematoxylin and alcoholic erythrosin.

RESULTS

Scanning electron microscope observations on material from several hydatid cysts revealed bud-like protrusions attached by means of a stalk to the outer surface of many intact brood capsules (Figs. 1 and 2).

Light microscopy showed many of these protrusions to be dead or dying protoscoleces (Fig. 3). Typically, they were yellow, smaller and more shrunken than normal protoscoleces, and lacked calcareous corpuscles. Abnormal protoscoleces, with scattered rostellar hooks but without suckers, were also observed (Fig. 4).

From examining sections of brood capsules, successive stages in the development of these protrusions were observed. Initially, there is an external cupping of the brood capsule wall which gives rise to the appearance of an external bud (Fig. 5). As this bud enlarges and grows outwards, an invagination of the cup develops which is the first stage in the differentiation of the rostellum of the protoscolex. Subsequently, hooks and the rudiments of the suckers appear, and the formation of a stalk containing a lumen which apparently links the rostellar region of the protoscolex and the interior of the brood capsule takes place. However, this link eventually becomes occluded as the lumen within the protoscolex, which will accommodate the developing inverted scolex, becomes separated from that in the stalk as growth continues (Fig. 6).

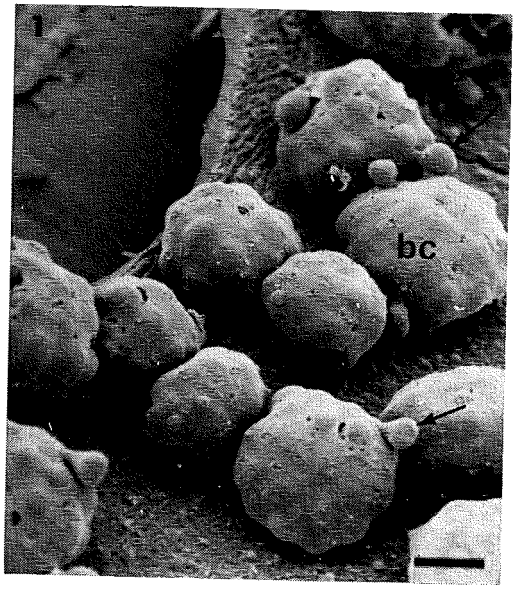
Although numerous preparations have been examined, further developmental stages have not been observed.

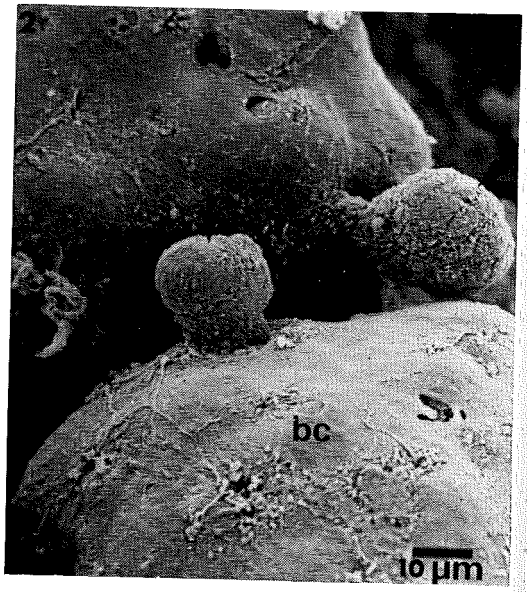
DISCUSSION

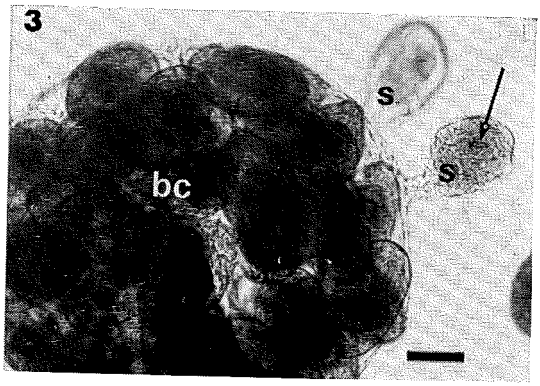
An external cupping of the wall of brood capsules from primary hydatid cysts was reported by Dew (1922). He considered that subsequent development took place after the cup or bud reverted to a normal internal position by its own contractile force.

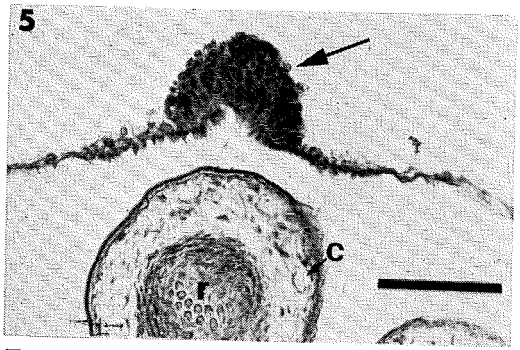
The only report of stalked protoscoleces arising from the outer surface of brood capsules concerns observations on secondary hydatid cysts by Pennoit-De Cooman and De Rycke (1972). These workers never observed fully developed protoscoleces on the outer brood capsule surface, and considered final development would take place once they had reverted by contractile and elastic forces to a normal position within the brood capsule. However, this reversion process was never observed during the course of extensive *in vitro* studies.

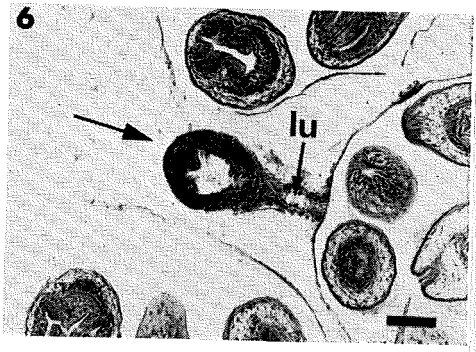
It seems unlikely that the reversion process envisaged by Dew (1922) could apply once the externally-developing protoscolex becomes stalked. Possibly, overcrowding within a brood capsule, perhaps accompanied by a local weakening in its wall, results in the formation of an external cup or bud. At this stage the developing protoscolex may revert back to within the brood capsule by contractile force. If the brood capsule is overcrowded, development may proceed externally accompanied by the formation of a stalk. However, full development does not occur and ceases shortly after the differentiation of hooks and suckers. These protoscoleces eventually become abnormal and die.











FIGURES 1—6.

The internal environment of the brood capsule must be necessary for normal development. Protoscoleces forming externally are in contact with this environment for only part of their development, via the lumen of the stalk which can be regarded as an extension of the brood capsule lumen. Once this vital link between the lumen of the protoscolex and that of the brood capsule is broken, development is arrested and death ensues. These dead protoscoleces presumably lose their connection with the brood capsule and eventually lie freely within the hydatid cysts.

The process of external development described here is unlikely to be related to endogenous daughter cyst formation. Dévé (1927) concluded that brood capsules do not participate in daughter cyst formation, a view supported by Fairley and Wright-Smith (1929) after detailed observations on hydatid cysts from several host species. Furthermore, endogenous daughter cysts have never been observed in hydatid cysts from British horses (Thompson, 1975; Ph.D. Thesis of London University).

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