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MORPHOLOGY OF CYSTICERCUS BOVIS DURING ITS DEVELOPMENT

J. SCHRAMLOVÁ and K. BLAŽEK

Institute of Parasitology, Czechoslovak Academy of Sciences, Prague

Abstract. On day 14 p.i., *C. bovis* is a spherical formation without cavity (in neonatally infected calves, the cavity already starts to form). On day 21 p.i., a conical anlage of the scolex is formed and on day 23 p.i., it bears a conspicuous invagination of the tegument. On day 28 p.i., the scolex with spiral canal, sucker anlages and single calcareous bodies is developed. On day 42 p.i., the suckers are fully differentiated and the calcareous bodies in scolex parenchyma are distributed up to below the level of suckers. On day 55 p.i., the calcareous bodies are present in the morphologically differentiated scolex even below the suckers. The authors assume that at that time the larva is already infective and if its infectivity is assessed on the basis of morphological criteria, the presence, number and distribution of calcareous bodies should be considered.

The morphology of *Cysticercus bovis* during its development has been only little studied (McIntosh and Miller 1960, Šlais and Machnicka 1976). We are therefore presenting the results of our own observations after experimental peroral infection of calves with eggs of *Taenia saginata* and assume that some of the results might be useful for a more exact determination of the duration of infection when cysticerci are found in slaughtered cattle.

MATERIAL AND METHODS

The material was obtained from 8 calves at the age of 3—4 months and 2 calves at the age of 4 days perorally infected with *T. saginata* eggs. The method is described in detail in the papers by Blažek et al. (1980, 1982).

Cysticerci for histological examination were taken from skeletal muscles on days 14, 21, 23, 28, 34, 42, 55 and 112 after infection. The material was fixed in neutral formol and in Baker's solution. After fixation it was embedded in paraffin and histological sections were stained with haematoxylin-eosin using the methods of Weigert and Kossa. A part of the material was fixed in 3 % glutaraldehyde in 0.1 M cacodylate buffer and postfixed in 2 % OsO_4 in 0.1 M cacodylate buffer. The material was then dehydrated, embedded in Epon 812, sectioned at 1 μm and stained with toluidine blue.

RESULTS

T. saginata larvae were studied on days 14, 21, 23, 28, 34, 42, 55 and 112 after experimental peroral infection of 4-day- and 3-4-month-old calves.

Cysticerci at various stages of development were found in each host (intermediate host), which was more marked with increasing time after infection. The morphology of those forms, which were most numerous at a certain period, is described in this paper (Fig. 1).

On day 14 p.i., the cysticerci were spherical and transparent and measured 0.4—0.6 m in diameter (Plate I, Fig. 1). They were covered with a smooth, thin tegument se-

parated from the inner, cellular part by a thin layer of muscles. The cells were of irregular, lobular shape, they were freely arranged and filled the whole space of the inner part. A large nucleus with a conspicuous nucleolus was visible in the cytoplasm. The cells often contained two or three nuclei.

In neonatal infections, the cysticerci found on day 14 p.i. possessed already a small cavity in the centre (Plate I, Fig. 2). The larva had a form of spherical, thin-walled bladder measuring 1.5 mm in diameter. Its wall was 7.6 μm thick and consisted of a smooth thin tegument and muscle layer. The cells possessed a large nucleus and nucleolus, like those in the cysticerci from older calves. In contrast to them, however, the cysticerci from 4-day-old calves contained also cells with darker cytoplasm in which lighter vacuoles with homogeneous contents were visible.

On day 21 p.i., the larvae were small, thin-walled bladders measuring less than 1 mm in diameter, with outlines of beginning polarity (the site of future scolex was marked). At these sites the bladder tegument was thickened (Plate II, Fig. 4) and folded at the periphery, whereas on the remaining part of bladder it was thinner (Plate II, Fig. 6) and smooth.

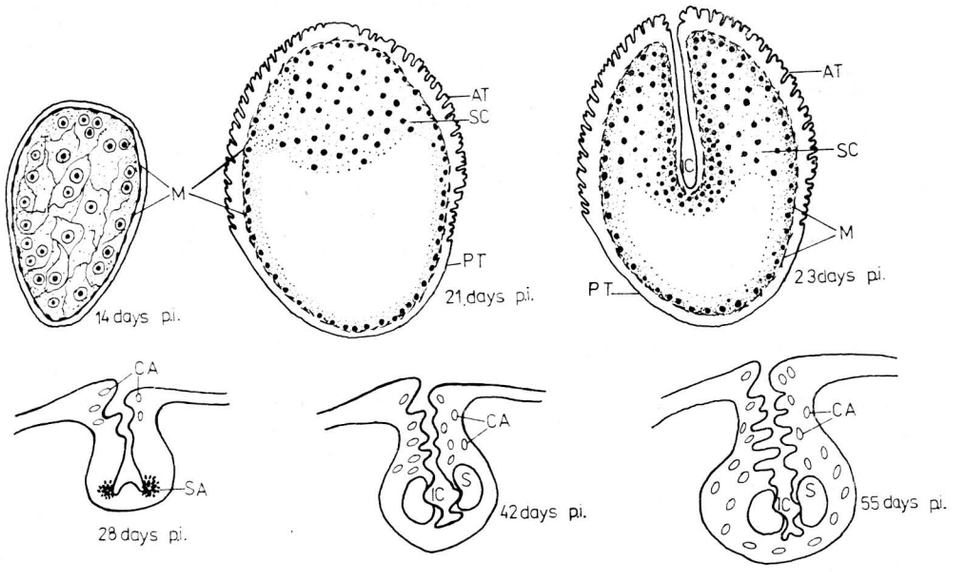

Fig. 1. Scheme of the development of *C. bovis* larva.

AT — anterior tegument
CA — calcareous corpuscle
IC — invaginated canal
M — muscle
PT — posterior tegument
S — sucker
SA — sucker anlage
SC — scolex anlage

The part of bladder with thickened tegument was termed anterior and that with thin tegument posterior. The anterior part of bladder contained a cluster of non-differentiated, strongly basophilic cells forming a cellular base of the future scolex. These cells differed from the other cells bordering the tegument in greater size and number and more close arrangement. Compared to the previous stage, the muscle layer was more developed, the cells were more numerous and spaces filled with glycogen already occurred among them.

On day 23 p.i., oval bladders measuring 1 mm in diameter were found. In the anterior part of bladder, the tegument was buried into the cellular scolex anlage which increased and occupied approximately two thirds of the bladder cavity (Plate II, Fig. 3). The tegument bordered a canal which was slightly widened at its end and formed a conical cavity. The cells of the scolex anlage were accumulated around the invaginated tegument. No other differences from the previous stage were observed.

On day 28 p.i., all organs of mature scolex were apparent, though they were not yet fully differentiated. The cysticercus had the form of an oval bladder measuring 2.2× ×1.9 mm. The invaginated scolex was pyriform, its wider base was directed towards the bladder cavity and the spiral canal opened at the bladder surface. The scolex measured 0.6 mm. The spiral canal possessed fine folds and opened into four sucker anlages (Plate III, Fig. 7). The rostellar cone rose from the bottom of spiral canal. The sucker anlages consisted of basophilic cells densely arranged in several circles and measured 0.99 mm in diameter.

Calcareous bodies occurred for the first time at this stage. Single bodies were visible in the parenchymal part of scolex, close to the site where the invaginated canal passed to bladder surface, but they were absent in the remaining parts of scolex. The calcareous bodies were only slightly basophilic.

On day 34 p.i., the cysticerci resembled those found on day 28 p.i., only the bladder was somewhat larger (2.5—3.0 × 3.0—4.0 mm, and the scolex was longer (0.07 mm). The diameter of sucker anlage was 0.127 mm. The localization of calcareous bodies was similar to that in cysticerci on day 28 p.i., but their number was slightly higher. Hooklets were visible in the tegument of rostellar cone.

On day 42 p.i., the bladder of cysticerci measured 4×3 mm and the invaginated scolex part was 1 mm long. The scolex possessed fully differentiated suckers measuring 0.18—0.2 mm in diameter, which consisted of circular and radial fibrils (Plate IV, Fig. 9). A conspicuous thin layer of connective tissue separated them from the remaining part of parenchyma. The spiral canal became longer and formed numerous folds. The rostellar cone was divided into two parts: a bulb consisting of muscle fibrils and a little distinct prebulb. Hooks occurred in the tegument of the rostellar region. They resembled in their morphology strobilar microtriches (Plate III, Fig. 8). The calcareous bodies were more numerous and they were found even in middle parts of scolex above suckers. The receptacle started to differentiate on the surface of scolex.

On day 55 p.i., the bladders measured 5 mm in length and 3 mm in width. The invaginated scolex was 1 mm long and a fully formed receptacle on its surface separated it from bladder cavity. The rostellar hooks could not be found at this stage. The calcareous bodies filled the whole region of scolex (Plate IV, Fig. 10). They were only slightly basophilic near the suckers and rudimentary rostellum, whereas in the remaining parts of scolex parenchyma, they were markedly basophilic.

On day 112 p.i., the cysticerci resembled those occurring on day 55 p.i., only the neck region has grown and the shape of the spiral canal thus became more complicated, with numerous folds.

DISCUSSION

The cysticerci *C. bovis* found in calves after a single peroral infection may be of various size and at various stage of morphological differentiation at the same time after infection and in the same intermediate host. This fact has already been pointed out by McIntosh and Miller (1960), Silvermann and Hulland (1961) and Šlais

and Machnicka (1976). The different course of development of larvae was observed particularly in massive infections, where the development of some of the cysticerci was retarded. However, it was observed also in neonatal infections and infections of older hosts, as it was reported previously (Blažek et al. 1982). Most of the larvae retarded in development die and the portion of distrophically changed or dead cysticerci markedly increases from day 28 p.i. (Blažek et al. 1981). In our experiments, larvae with scoleces possessing sucker anlages were found on day 28 p.i., like those developing subcutaneously, as reported by Šlais and Machnicka (1976). On the other hand, the cysticercus found by McIntosh and Miller (1960) four weeks after infection was only a bladder with scolex anlage and no suckers were developed still on day 35 p.i. Consequently, in order to assess more exactly the duration of the infection, it is necessary to examine a greater number of cysticerci, particularly those from skeletal muscles and those at the highest stage of morphological differentiation.

As it is now generally recognized, the decisive criterion of the age of *C. bovis* is not the size of bladder, but the stage of scolex differentiation, since it is related with the ability of the parasite to attach to intestinal mucosa of the definitive host. In the opinion of some authors, 2.5—3 months is the shortest period during which *C. bovis* cysticercus can reach the infective stage (McIntosh and Miller 1960, Silverman and Hulland 1961), since they found fully differentiated suckers at that time. In our material fully differentiated suckers occurred already on day 42 p.i. We assume, however, that also the occurrence and distribution of calcareous bodies should be considered while evaluating the morphological criteria of infectivity of larvae. Our results indicate that the calcareous bodies start to appear at the same time as sucker anlages (on day 28 p.i.) and that they gradually increase in number. On day 28 p.i., they occur only around the opening of spiral canal on bladder surface; on day 42 p.i., they are distributed in scolex parenchyma from the opening of spiral canal up to the level of suckers, and on day 55 p.i., they fill the parenchymal part of the whole scolex, even under the suckers in the vicinity of rudimentary rostellum.

Although many papers dealing with calcareous bodies have been published, the opinions on their significance are contradictory and hypothetical at the present time. Chowdhury (1977) studied the development of calcareous bodies in larvae of *Hymenolepis microstoma* and in his opinion, they belong to a complex of defense mechanism of the parasite during the passage of the larvae in the definitive host, protecting them against the effect of muriatic acid in the gastric juice. If this hypothesis is accepted, then it may be concluded that the larva is capable of a successful infection only at the time when there is a sufficient number of calcareous bodies in its scolex. According to our studies, it would be at the age of approximately 8 weeks.

The finding of hooklets on day 34 p.i. and rudimentary hooks on day 42 p.i. in the tegument of rostellum corresponds to the records of Šlais and Machnicka (1976).

МОРФОЛОГИЯ ЦИСТИЦЕРКА *CYSTICERCUS BOVIS* В ТЕЧЕНИЕ ЕГО РАЗВИТИЯ

Я. Шрамлова и К. Блажек

Резюме. На 14-й день после заражения *C. bovis* является сферической клеточной формой без полости (у цистицерка из зараженных новорожденных телят полость уже начинает образоваться). На 21-й день после заражения образован конический зачаток сколекса и впячивание тегумента видимо на 23-й день. На 28-й день после заражения развит сколекс со спиральным каналом, зачатками присосок и отдельными известковыми тельцами. На 42-й день после заражения присоски сколекса уже вполне дифференцированы и известковые тельца распределены в паренхиме до уровня присосок. На 55-й день после заражения известковые тельца в морфологически дифференцированном сколексе находятся даже и под присосками. По мнению авторов в это время личинка уже инфекционная. При оценке инфекционности на основе морфологических критерий нужно учитывать также присутствие, количество и распределение известковых телец.

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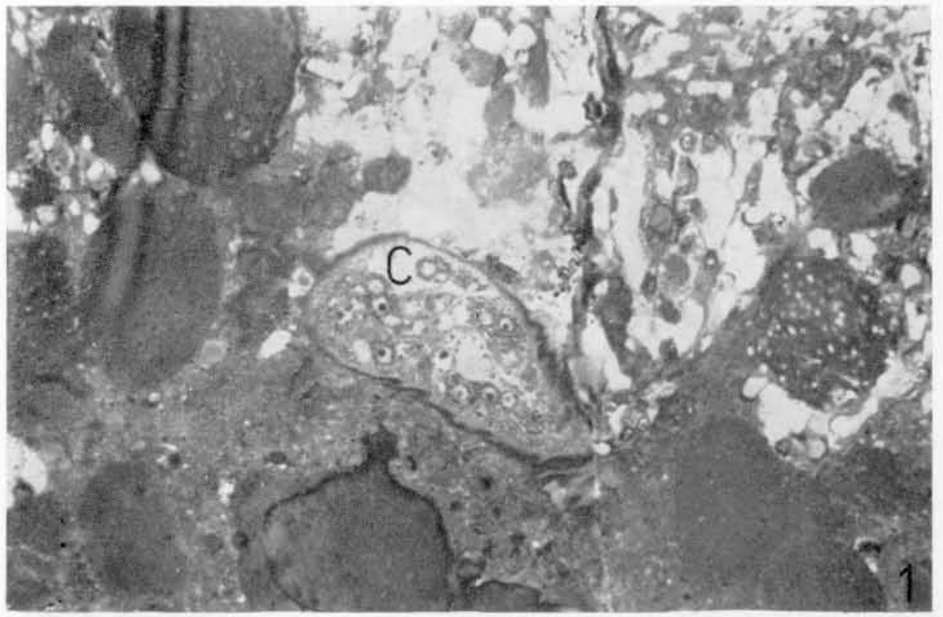
J. Sch., Parasitologický ústav ČSAV,
Flemingovo n. 2, 166 32 Praha 6,
ČSSR



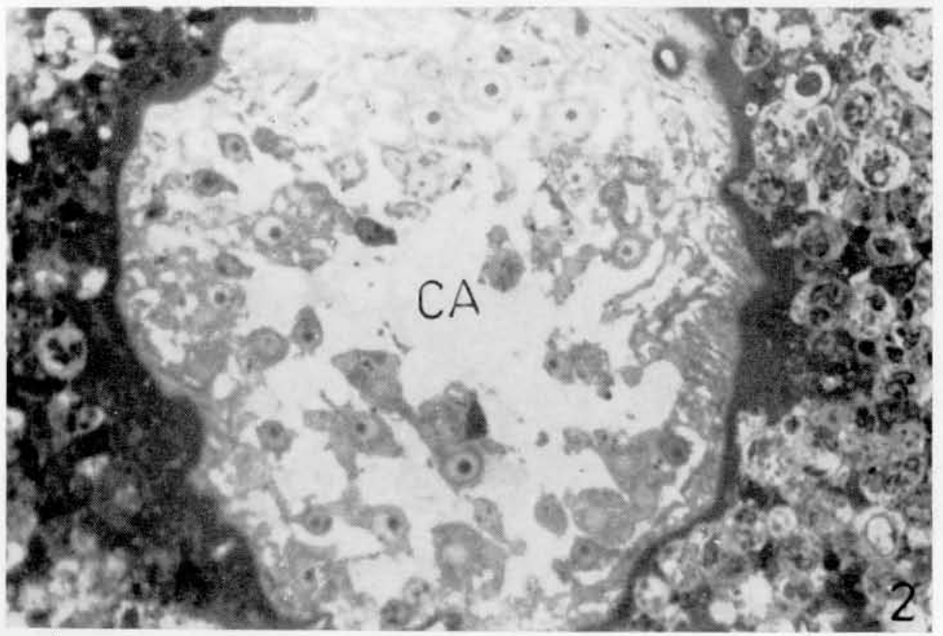
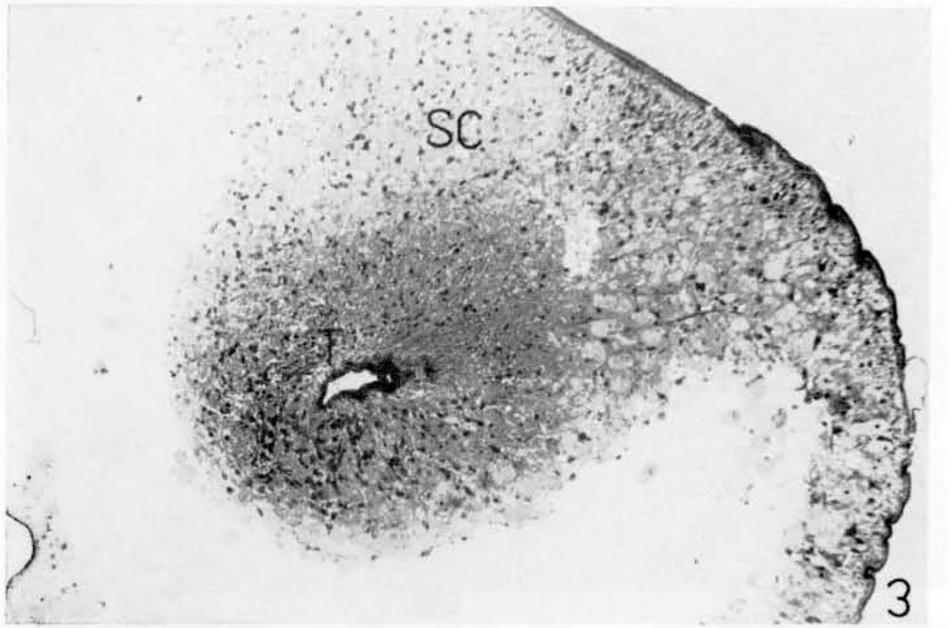
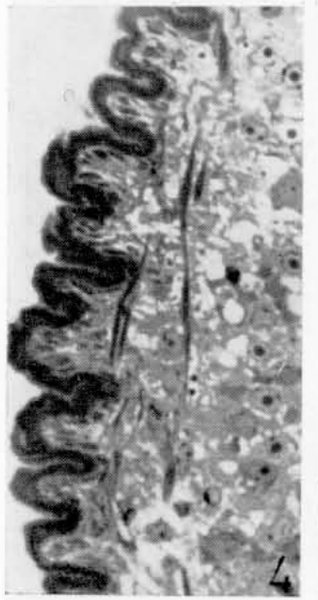
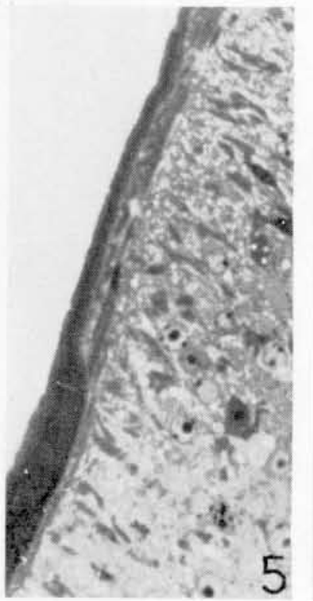


Fig. 1. *Cysticercus bovis* (C) on day 14 p.i. Toluidine blue (450×). **Fig. 2.** Neonatal infection with *C. bovis* on day 14 p.i. Toluidine blue (500×).







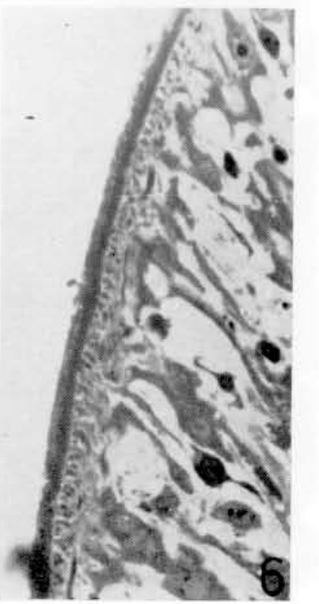
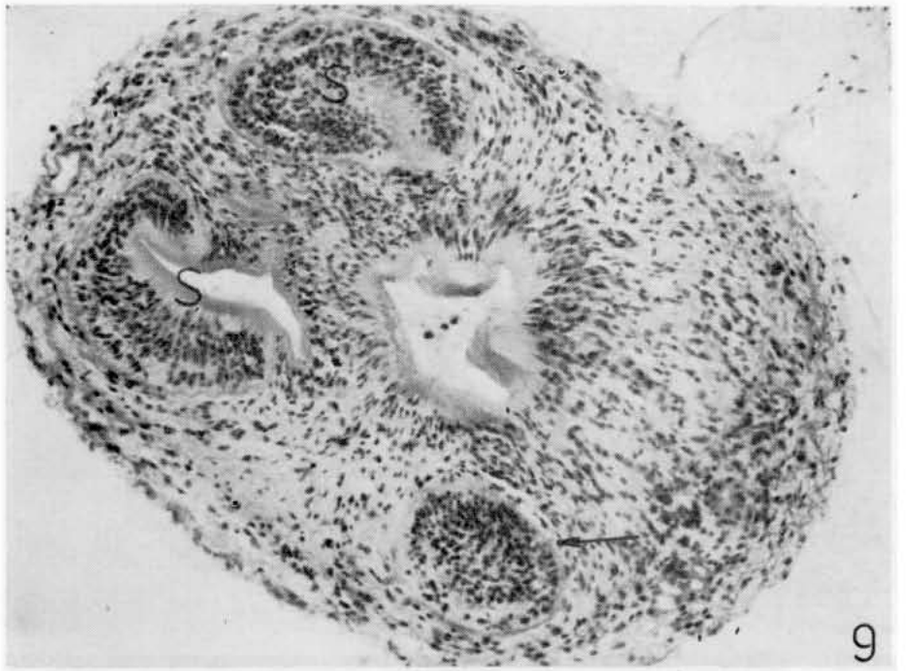


Fig. 3. *C. bovis* larva on day 23 p.i. Scolex anlage (SC) with invaginated tegument (T) is formed in anterior part of bladder. Toluidine blue (450×). **Fig. 4.** *C. bovis* on day 21 p.i. Thickened tegument of anterior part of bladder. Toluidine blue (900×). **Fig. 5.** *C. bovis* on day 21 p.i. Tegument of posterior part of bladder passes to thickened tegument of anterior part. Toluidine blue (900×). **Fig. 6.** *C. bovis* on day 21 p.i. Tegument of posterior part of bladder. Toluidine blue (900×).

Fig. 7. *C. bovis* on day 28 p.i. Sucker anlage is formed in scolex. HE (200×). **Fig. 8.** *C. bovis* on day 42 p.i. Hooks (arrows) are visible in the tegument of rostellar region. HE (800×).



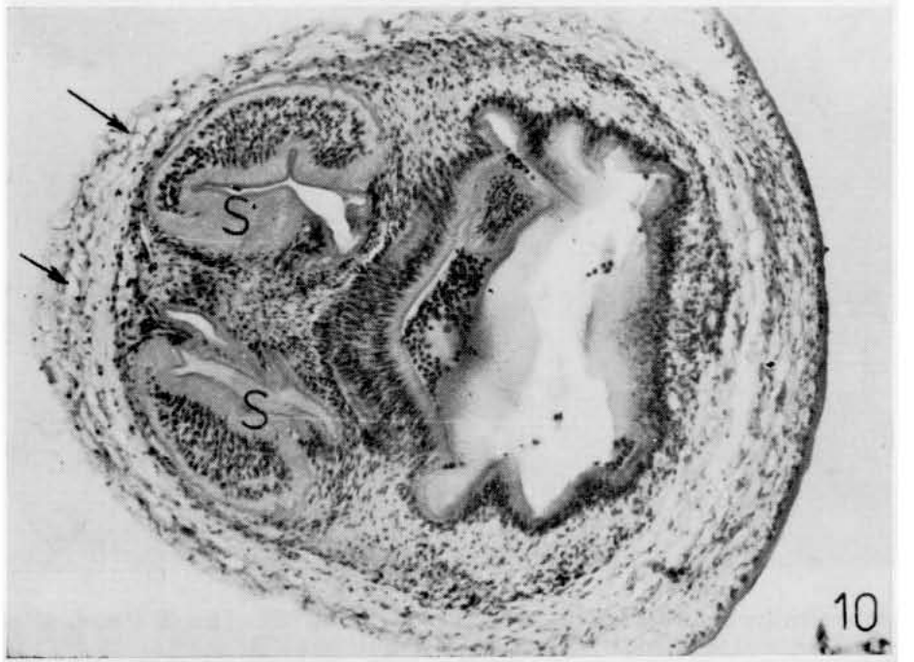


Fig. 9. *C. bovis* on day 42 p.i. Scolex with fully differentiated suckers (S) separated from the remaining part of parenchyma by connective tissue (arrow). HE (150×). **Fig. 10.** *C. bovis* on day 55 p.i. Receptacle is formed on the surface of cysticercus. Calcareous bodies (arrows) are visible below the suckers (S). HE (150×).