FOLIA PARASITOLOGICA (PRAHA) 22: 345–354, 1975.

# HISTOCHEMISTRY OF THE PARENCHYMA OF CYSTICERCUS BOVIS

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**Abstract.** The contents of mucosubstances, proteins and lipids, and the activity of several enzymes were studied in the parenchyma of the bladder wall and the invaginated portion of *Cysticercus bovis* aged 108 days. The parenchyma of both parts contained a large amount of glycogen and minute bodies with a high activity of acid phosphatase and non-specific esterase. By contrast to older larvae, the young larva contained a minute amount of hydrophobic lipids. The rostellum was developed and similar to the suckers contained glycogen.

The present paper is a continuation of our previous study on the tegument of *C. bovis* (Žďárská 1973); both were performed during a complex investigation of muscle cysticercosis in bovine animals. The main concern of this study was the histochemistry of the parenchymal components of the invaginated portion and the inner layers of the bladder wall of *C. bovis*.

## MATERIAL AND METHODS

Our material consisted mainly of developmental stages of *C. bovis* obtained from calves on day 83 and 108 of experimental infection. Infection of the calves was performed by Dr. B. Machnicka, Institute of Parasitology, Polish Academy of Sciences, Warsaw. The cysticerci were fixed in Baker's neutral formaldehyde and embedded in paraffin for the demonstration of proteins and mucosubstances, in gelatine for the demonstration of lipids; enzymes were demonstrated in sections cut with the freezing microtome. The methods employed were essentially those described in an earlier paper (Žďárská 1973).

## RESULTS

### I. MORPHOLOGY OF THE PARENCHYMAL COMPONENTS OF THE INDIVIDUAL PARTS OF THE CYSTICERCUS

#### A. Invaginated portion

The parts distinguished in the parenchyma of this portion were these: Invaginated scolex with rostellum and suckers (Plate I, Figs. 1, 2), excretory system, nervous system, parenchymal cells, calcareous corpuscles, muscle- and connective tissue fibres. That part of the parenchyma of the invaginated portion facing the bladder cavity was not bordered off distinctly except for a greater density of parallelly arranged connective tissue fibres and a lower incidence of calcareous corpuscles (Plate II, Fig. 1). The par-

enchyma facing the spiral canal was bounded by the tegument described in an earlier paper (Žďárská 1973). The suckers measuring $315 \times 250$ μ in diameter were composed of densely arranged circular and radial muscle fibres (Plate I, Figs. 1, 2) with an occasional myoblast and connective tissue fibres. A distinct, thin layer of connective tissue formed a sharp border between the suckers and the remaining parts of the parenchyma.

The rostellum (Plate I, Fig. 2; Plate II, Fig. 1, 2) measured 130 μ in width and 80 μ in length. It was formed by circular and longitudinal muscle fibres intermingled with connective tissue fibres. The rudimentary rostellum was surrounded by circular fibres of the rostellar sac (Plate III, Figs. 1). Its separation from the remaining parts of the parenchyma was less distinct than that of the suckers (Plate I, Fig. 2).

The excretory system consisted of two wide ventral excretory canals with thin walls extending from the rostellum, where they bent to form a wide arch, to the bladder wall (Plate I, Fig. 2), and of two narrow dorsal excretory canals. The ventral excretory canals received the fine canals terminated with a typical flame cell which was thickened in its middle with a connective tissue ring. Along the excretory canals we observed bundles of longitudinal muscle fibres. The area around the canals contained spherical to ovoid bodies measuring $0.4 \times 0.4 - 0.75 \times 0.75$ μ or $0.5 \times 0.8$ μ.

The nervous system was formed by two cerebral ganglia situated at the base of the rostellum (Plate I, Fig. 2). They contained large bodies of neurocytes. Two thick nerve trunks extending from these ganglia continued in longitudinal direction through the entire invaginated portion of the cysticercus. Numerous minute branches extended from the nerve trunks to the tegument (Plate IV, Figs. 1, 2).

**B. Bladder**

The parenchyma of the bladder wall consisted of several distinct components, i.e. a well visible excretory system, parenchymal cells, glycogen pouches, connective tissue fibres and muscle fibres. The parenchyma of the bladder was not delimited from the cavity by a special layer. Its outer side was covered with the tegument described in an earlier paper (Žďárská 1973). The excretory system of the bladder wall was very complicated. In light microscopy we distinguished two systems of canals. The first composed of wide, mashwork-like connected canals (Plate VI, Fig. 1) was situated below the tegument. The second system with its considerably narrower canals was deposited deeper down in the bladder wall and contained three types of canals. The flame cell was followed by canals of the first order appearing as cytoplasmic stripes in the light microscope. Generally, four of these canals opened together in one canal of the second order. The latter was formed by an elongate cell with a nucleus pressed towards the periphery. Canals of the second order opened into the lumen of a canal of the third order (Plate VI, Fig. 1, 2), the wall of which was thickened with fine ribs. A nervous system could not be demonstrated in the bladder. Elements of connective- and muscle tissue formed a continuous layer below the tegument composed of two systems of fibres arranged in perpendicular direction to one another. Inside the parenchyma we observed separate fibre bundles extending parallelly with, and not perpendicular to, the surface. Bodies of tegumental cells and myoblasts were situated in the parenchyma below the layer of connective- and muscle tissue. Also an occasional myoblast was found in the deeper layers of the bladder parenchyma. In addition to occasional cell elements, the parenchyma contained a number of spherical to ovoid bodies ($0.4 \times 0.4 - 0.75 \times 0.75$ μ or $0.5 \times 0.8$ μ) — (Plate V, Fig. 3), various granules and drops of lipids distinguishable by means of histochemical methods only.

## II. HISTOCHEMISTRY OF THE PARENCHYMA OF THE INDIVIDUAL PARTS OF THE CYSTICERCUS

### A. Invaginated portion

The parenchyma of this portion contained mainly glycogen (Plate III, Figs. 2, 3). Muscle fibres of the suckers gave a positive reaction for arginine, tyrosine (Plate II, Fig. 1), tryptophan and proteins with SH groups (Table 2); they contained hydrophilic lipids (Table 2) and a large amount of glycogen (Table 1). The walls of the ventral excretory canals gave a positive reaction for arginine, tyrosine, tryptophan and proteins with SH groups (Table 2), and contained hydrophilic lipids (Table 2). The walls of the excretory canals were surrounded by pouches with glycogen (Plate V, Fig. 1) and minute bodies with a high activity of acid phosphatose and non-specific esterase (Table 1). The bodies were positive for proteins with SH groups, gave a positive coupled tetraz. reaction and contained hydrophilic lipids. The flame cells were faintly positive for tyrosine and tryptophan, more strongly positive for proteins with SH groups (Table 2), and contained hydrophilic lipids (Table 2). Activity of alkaline phosphatase and cholinesterase was confirmed in the nervous system (Plate IV, Fig. 1, 2, Table 1). Histochemical methods for the detection of mucosubstances (Table 1) confirmed in the surface of older calcareous corpuscles a feebly positive reaction for AB pH 2.6, and a highly positive reaction in Best's carmine even after the saliva test (Plate III, Fig. 3). Older calcareous corpuscles gave an intensive staining with Luxol blue on the surface, and sometimes, in the centre. Young calcareous corpuscles stained throughout. The surface of an occasional calcareous corpuscle gave a positive reaction for hydrophobic and hydrophilic lipids (Table 2).

### B. Bladder

The entire parenchymal part of the bladder wall contained bodies with a high activity of acid phosphatase and non-specific esterase (Table 3; Plate V, Fig. 3). These bodies similar to those in the invaginated portion gave a positive reaction for proteins with SH groups, and a positive coupled tetrazonium reaction. In addition, a large amount of glycogen and occasional fine drops of hydrophobic lipids were deposited in the parenchymal portion. The walls of the wide excretory canals contained arginine, tyrosine, tryptophan and proteins with SH groups (Table 4). The walls of these canals were surrounded by pouches filled with glycogen (Plate V, Fig. 2; Plate VI, Fig. 3; Table 3) and bodies with an activity of acid phosphatase and non-specific esterase referred to earlier in the text. The walls of the wide excretory canals gave a highly positive reaction for hydrophilic lipids (Table 4). The walls of the canals of the first, second and third (rib-supported) order contained arginine, tyrosine and tryptophan (Table 4, Plate VI, Fig. 1), stained faintly in methods for the detection of hydrophilic lipids (Table 4) and were negative in methods for the detection of mucosubstances (Table 3). The connective tissue fibres and the muscle fibres of the bladder contained arginine, tyrosine and tryptophan and proteins with SH groups (Table 4). neutral mucosubstances (Table 3) and hydrophilic lipids (Table 4).

## DISCUSSION

In order to facilitate an understanding of histochemical finds in the parenchyma of *C. bovis*, a brief description is given of the morphology of its individual components with references to detailed descriptions in papers by Šlais (1970), and Šlais et al. (1971,

**Table 1.** Results of histochemical reactions for the demonstration of mucosubstances and several enzymes in the parenchyma of the invaginated portion of *C. bovis*

| Reaction | Parenchymal cells | Muscle fibers | Calcareous corpuscles | Ventral excretory canals | Dorsal excretory canals | Flame cells | Nerve trunk | Bodies | Suckers |
|---|---|---|---|---|---|---|---|---|---|
| PAS | +++++ | + | – | [3]) +++– | – | – | – | – | +++++ |
| Schiff | – | – | – | – | – | – | – | – | – |
| Saliva test + PAS | – | – | – | – | – | – | – | – | – |
| Acetylation 58 °C 48 hr + PAS | – | – | – | – | – | – | – | – | – |
| Desacetylation + PAS | +++++ | +–/– | +/– | [3]) +++++ | – | – | – | – | +++++ |
| AB + PAS | red | rose | [2]) blue | [3]) red | – | – | – | – | red |
| AB pH 2,6 | – | – | [2]) + | – | – | – | – | – | – |
| + methylation | – | – | | – | – | – | – | – | – |
| + demethylation | – | – | + | – | – | – | – | – | – |
| Best's carmin | +++++ | – | [2]) ++–++ | [3]) ++–++ | – | – | – | – | +++++ |
| Saliva test + Best's carmin | – | – | [2]) +–++ | – | – | – | – | – | – |
| Alkaline phosphatase (α-naphthyl phosphate + Fast Blue BB or Fast Red TR) | – | – | [1]) +++ | – | – | – | +++++ | – | – |
| Acid phosphatase (α-naphthyl phosphate + HPR) | – | – | – | – | – | – | – | +++++ | – |
| Non-specific esterase (α-naphthyl acetate + HPR) | – | – | – | – | – | – | – | +++++ | – |
| Cholinesterase (Karnowsky, Roots) | – | | – | – | – | – | +++++ | – | – |

Notes: [1]) also positive for controls, [2]) surface only, [3]) pouches around the canals

**Table 2.** Results of histochemical reactions for the demonstration of proteins and lipids in the parenchyma of the invaginated portion of *C. bovis*

| Reaction | Parenchymal cells | Muscle fibers | Calcareous corpuscles | Ventral excretory canals | Dorsal excretory canals | Flame cells | Nerve trunk | Bodies | Suckers |
|---|---|---|---|---|---|---|---|---|---|
| Sakaguchi | +/− | +++ | − | − | ++ | + | − | − | + |
| Morel—Sisley | +/− | −++ | − | − | −++ | + | − | − | −+ |
| DMAB | +/− | −+ | − | − | +−+ | + | − | − | −+ |
| Coupled tetrazonium reaction | +/− | + | − | − | ++ | + | − | +− | + |
| DDD | + | +++ | − | −++ | +++ | +++ | | +++ | ++− |
| N-ethylmaleimide + DDD | + | + | − | + | + | + | − | + | + |
| Thioglycollic acid + DDD | + | ++ | − | + | ++ | ++− | − | + | +−+ |
| PFA—AB | − | − | 1) + | − | − | − | − | − | − |
| AB pH 2,6 | − | − | 1) + | − | − | − | − | − | − |
| Sudan black B (jelly) | − | − | 2) +++ | − | +/− | + | − | ++ | + |
| Chloroform-methanol extraction + Sudan black B | − | − | − | − | − | − | − | − | − |
| Chromation + acid haematein | − | + | 2) +++ | − | +/− | +− | − | ++ | ++ |
| Chloroform-methanol extr. + acid haematein | − | − | − | | | − | − | − | − |
| Luxol blue | + | +− | −+++ | − | − | + | − | +− | −+ |
| Fettrot 7B | − | − | 2) ++ | | | − | − | − | − |
| Chloroform — methanol extr. + Fettrot 7B | − | − | − | − | | − | − | − | − |
| OTAN | − | pink | red-brown and black | pink | pink | pink | pink | pink | pink |
| Chloroform — methanol extr. + OTAN | − | − | pink | − | − | − | − | − | − |

Notes: 1) surface only, 2) on the surface of several calcareous corpuscles only

**Table 3.** Results of histochemical reactions for the demonstration of mucosubstances and several enzymes in the bladder parenchyma of *C. bovis*

| Reaction | Parenchymal cells | Muscle fibers | Wide excretory canals | Orders of excretory canals | | | Flame cells | Bodies |
|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 (rib-supported) | | |
| PAS | +++++ | +++ | [1]) ++++ | – | – | – | – | – |
| Schiff | – | – | – | – | – | – | – | – |
| Saliva test + PAS | – | – | – | – | – | – | – | – |
| Acetylation 58 °C 48 hr + PAS | – | – | – | – | – | – | – | – |
| Desacetylation + PAS | +++++ | ++– | [1]) +++++ | – | – | + | – | – |
| AB – PAS | red | pink | [1]) red | – | – | +– | – | – |
| AB pH 2,6 | – | – | – | – | – | – | – | – |
| + methylation | – | – | – | – | – | – | – | – |
| + demethylation | – | – | – | – | – | – | – | – |
| Best's carmin | +++++ | +++ | [1]) +++ | – | – | – | – | – |
| Saliva test + Best's carmin | – | – | – | – | – | – | – | – |
| Alkaline phosphatase (α-naphthyl phosphate + Fast Blue BB or Fast Red TR) | – | – | – | – | – | – | – | – |
| Acid phosphatase (α-naphthyl phosphate + HPR) | – | – | – | – | – | – | – | ++++– |
| Non-specific esterase (α-naphthyl acetate + HPR) | – | + | – | – | – | – | – | +++++ |

Notes: [1]) pouches around the canals

**Table 4.** Results of histochemical reactions for the demonstration of proteins and lipids in the bladder parenchyma of *C. bovis*

| Reaction | Parenchymal cells | Muscle fibers | Wide excretory canals | Orders of excretory canals | | | Flame cells | Bodies |
|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 (rib-supported) | | |
| Sakaguchi | + | +++ | ++ | + | - | +++ | - | - |
| Morel–Sisley | + | +++ | ++ | +++ | +-+ | -++ | ++ | - |
| DMAB | - | +-+ | +- | ++ | -- | ++ | - | - |
| Coupled tetrazonium reaction | - | ++ | +-- | +++ | +-+ | +++ | +- | ++ |
| DDD | - | +-- | ++ | - | ++ | +++ | -++ | ++ |
| N-ethylmaleimide + DDD | - | - | - | - | - | + | +/- | - |
| Thioglycollic acid + DDD | - | -- | + - | + | ++ | +++ | -++ | ++ |
| Sudan black B (jelly) | - | - | +++ | - | - | - | + | ++ |
| Chloroform–methanol extr. + Sudan black B | - | - | - | - | - | - | - | - |
| Chromation + acid haematein | - | - | +-- | - | - | - | + | ++ |
| Chloroform – methanol extr. + acid haematein | - | - | - | - | - | - | - | - |
| Luxol blue | + | ++ | ++ | - | - | + | -- | ++ |
| Fettrot 7B | - | - | - | - | - | - | - | - |
| Chloroform – methanol extr. + Fettrot 7B | - | - | - | - | - | - | - | - |
| OTAN | pink | pink | pink | pink | pink | pink | pink | pink |
| Chloroform – methanol extraction + OTAN | pink | - | - | - | | - | - | - |

1972). The morphology of those parts only for which detailed descriptions are not available in the cited papers (rostellum and several bodies), will be dealt with comprehensively in the text.

The rostellum of *Taeniarhynchus saginatus* has escaped the attention of numerous authors in view of its minute size and the absence of hooks. However, its presence in the adult cestode is undoubtable. Abuladze (1964) remarked on its rudimentary form and maintained that it was located apically in the form of a fifth sucker. Schaaf (1905) inferred that the anlage of the rostellum was formed already in early developmental stages of *C. pisiformis* and *C. cellulosae* together with the suckers and that its size was almost final. Further growth of the scolex occurred mainly in that connective tissue was layered on its surface. Although the rostellum of the adult cestode *T. saginatus* is minute in comparison with the suckers, it attains a full third of their size in a *C. bovis* aged 108 days. In spite of the fact that the rostellum of *T. saginatus* is not armed with hooks and, hence, not an attachment organ as it is in the remaining cestodes of the family Taeniidae, it is developed in a larva aged 108 days. However, its structure differs greatly from that of a rostellum armed with hooks and used for attachment in the host (Schaaf 1905, Baron 1968, Rees 1951, Bilqees and Freeman 1969, Mount 1970). In this paper, however, only the parenchyma of *C. bovis* is dealt with and therefore we described only the parenchymal part of the rostellar region. The changes of tegument in this region will be dealt with in another paper.

We have as yet been unable to determine which of the canal systems of the bladder receives the ventral excretory canal of the invaginated portion, and which receives the dorsal excretory canal. It might be derived from the location, histochemical reactions and the width of the canal system that the system of wide canals situated below the tegument passes into the ventral excretory canal of the scolex, and that the system of rib-supported canals deposited in the deeper layers of the bladder parenchyma is received by the dorsal excretory canal of the scolex as this was recorded for *C. fasciolaris* by Rees (1951). Similar to Pintner (1896), we did not find communications between the first and second canal system. These were found by Šlais (1970), but his text did not indicate clearly enough whether this finding referred to *C. cellulosae* or *C. bovis*. The arrangement of the excretory system in the scolex portion of *C. bovis* was found to be similar to that in adult cestodes (Howels 1962).

We should like to remark on the histochemistry of the calcareous corpuscles that the degree of intensity of their reaction depends on the age of these corpuscles. For younger calcareous corpuscles we obtained a strong positive reaction with AB for the demonstration of acid mucosubstances particularly in the centre of these corpuscles. This finding is in agreement with that of Nieland and von Brand (1969). Young calcareous corpuscles give also a strongly positive reaction with Luxol blue. In the reaction with Best's carmine combined with the saliva test, the staining remained on their surface. After the saliva test, glycogen deposited in the cytoplasm around the calcareous corpuscles disappeared, but the surface of the corpuscles remained stained. In calcareous corpuscles of *C. bovis*, the PAS reaction was negative. For these reasons we cannot agree with Chowdhury et al. (1955) and von Brand et al. (1960) in that the calcareous corpuscles contain a glycogen-like polysaccharide.

The observation of very faint reactions for proteins in calcareous corpuscles is in agreement with the finding by von Brand et al. (1960), but not with that by Chowdhury et al. (1955) who obtained a more distinct reaction. Occasional hydrophobic and hydrophilic lipids were present in either the periphery or the centre of some calcareous corpuscles. In several instances it was difficult to decide whether these lipids belonged to the calcareous corpuscles or to the surrounding cytoplasm.

The organic material of calcareous corpuscles is made up of mucosubstances, proteins

and lipids. These interchange with irregular, differently bent layers of inorganic material — calcium (Kossa-positive) as observed also by Scott et al. (1962) and Šlais (1966).

In azocoupling methods for alkaline phosphatase, mainly with Fast Red TR, the surface of the calcareous corpuscles stained unspecifically (both controls, i.e., denaturation at 100 °C and control without substrate, were positive). Similar results were obtained by Erasmus (1957) for the adult cestode *Taenia pisiformis*. Chowdhury et al. (1962) ascribed it to the activity of alkaline phosphatase, but failed to indicate clearly that a control test was performed.

Minute bodies displaying an activity of acid phosphatase and non-specific esterase were found in the parenchyma of the bladder wall and in that of the invaginated portion. We are uncertain as yet about their classification and function. They were concentrated mainly around the walls of the wide canals both in the bladder and in the invaginated portion. The elucidation of their structure necessitates further electron optic and histochemical studies.

It has been suggested by our study that the parenchyma of both the bladder and the invaginated portion of larvae of *T. saginatus* contain a large amount of glycogen up to day 108 of infection. The site of its deposition in the bladder is in accord with the electron optic finding by Baron (1968) and Šlais et al. (1971). In the invaginated portion, glycogen occupies all spaces among the calcareous corpuscles, and is present also in a large quantity in the suckers and rostellum. In these young larvae, the amount of hydrophobic lipids is minute in comparison with that present in older cysticerci.


ГИСТОХИМИЯ ПАРЕНХИМЫ *CYSTICERCUS BOVIS*

З. Ждярска

**Резюме.** Было изучено содержание мукосубстанций, белков и липидов и активность некоторых ферментов в паренхиме пузыря и инвагинированной части *Cysticercus bovis* 108 дней после заражения. Паренхима обеих частей содержит большое количество гликогена и мелких телец с высокой активностью кислой фосфатазы и неспецифической эстеразы. В отличие от более взрослых личинок, эта молодая личинка содержит только небольшое количество гидрофобных липидов. Хоботок развит и, как присоски, содержит гликоген.


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Received 3 April 1975.

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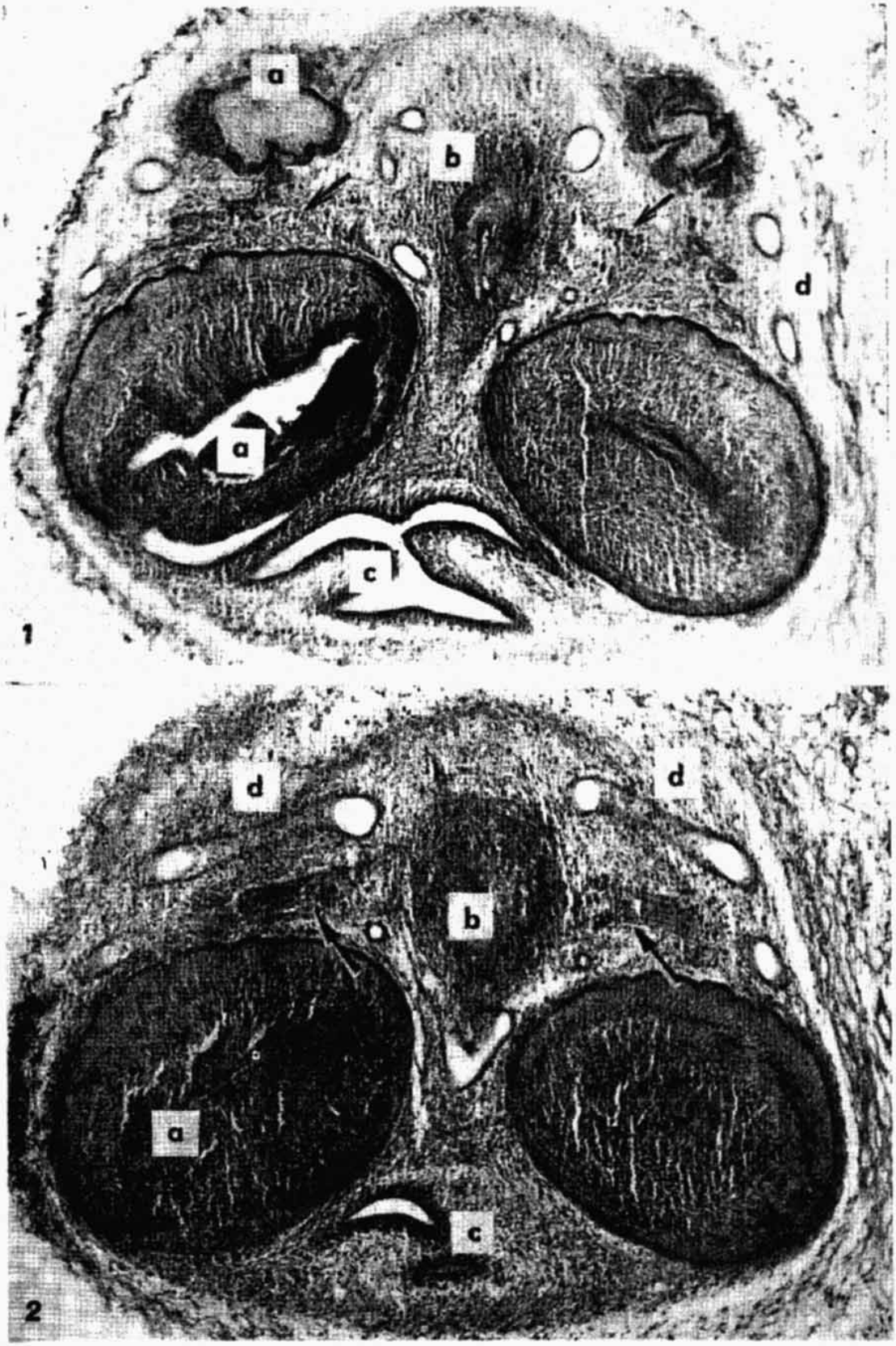



**Fig. 1.** Transverse section through the scolex of *C. bovis* at the level of the opening of the rostellum (b) into the spiral canal. a — sucker, c — spiral canal, d — ventral excretory canal; arrows — cerebral ganglion. Mallory's phosphotungstic haematoxylin (140×).

**Fig. 2.** Transverse section through the scolex of *C. bovis* at the level of the rostellum base (b). Arrows point to the two clearly visible cerebral ganglia; d — the two bending ventral excretory canals. Haematoxylin — eosin (140×).

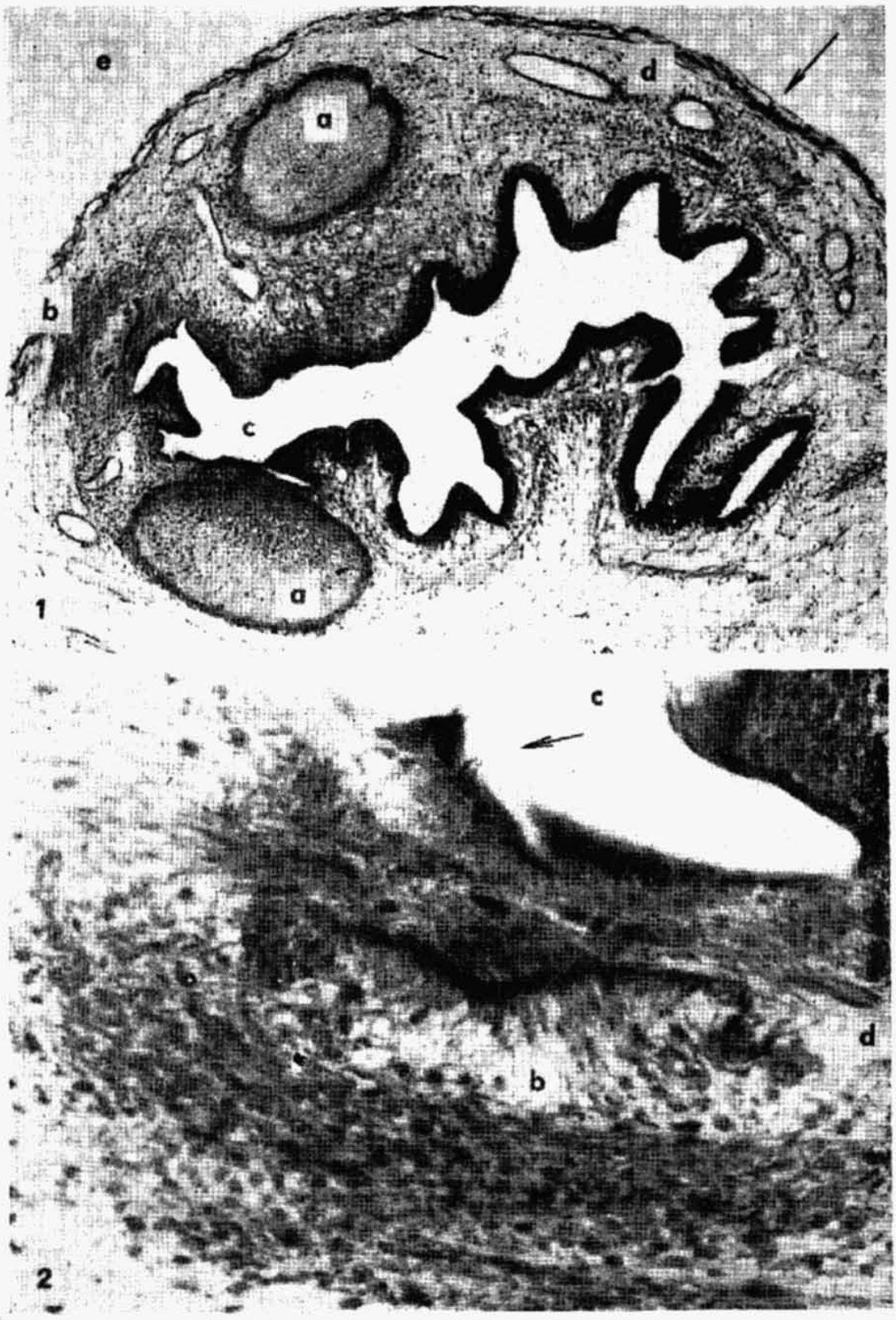
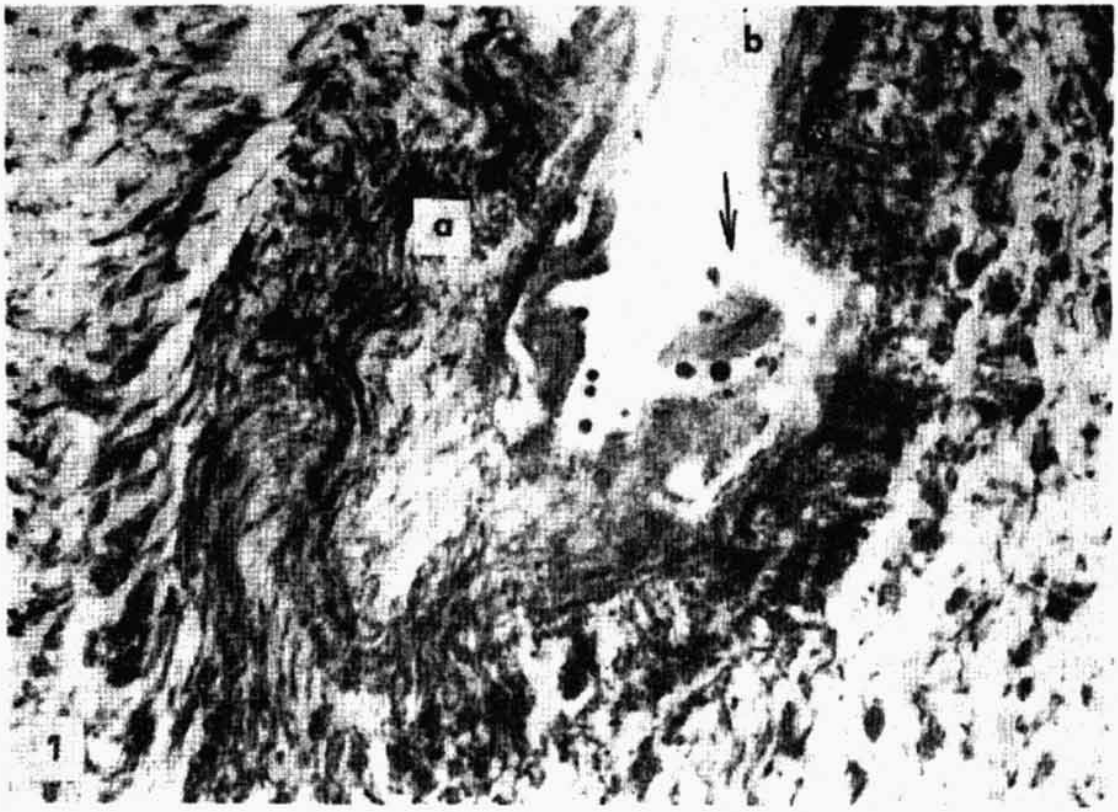



**Fig. 1.** Longitudinal section through the invaginated portion of a 108 day-old *C. bovis* with a clearly visible rostellum (b) at the end of the spiral canal (c) and an increased density of connective tissue fibres (arrow) at the surface facing the bladder cavity (e). a — sucker, d — ventral excretory canal. Morel Sisley's diazotization method (120×).

**Fig. 2.** Analogous to fig. 1 except for a higher magnification of the rostellar region. b — rostellum, c — spiral canal, arrow — hooks, d — fibres of the rostellar sac (850×).





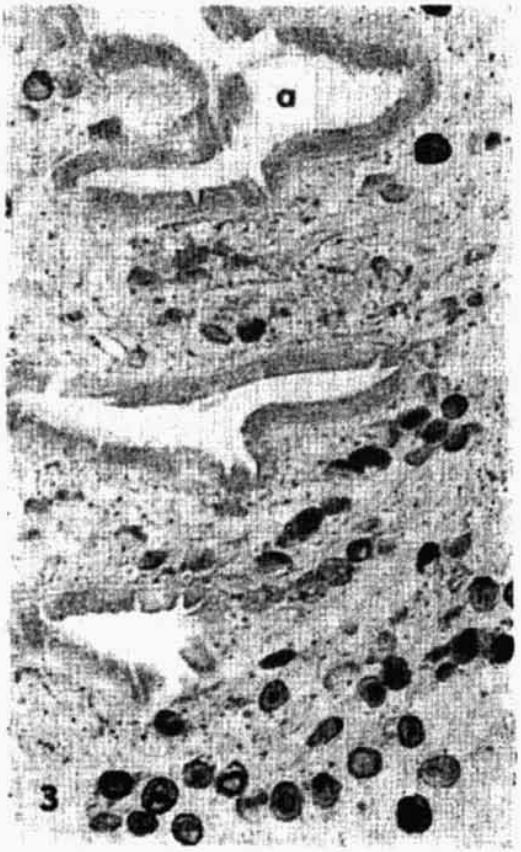


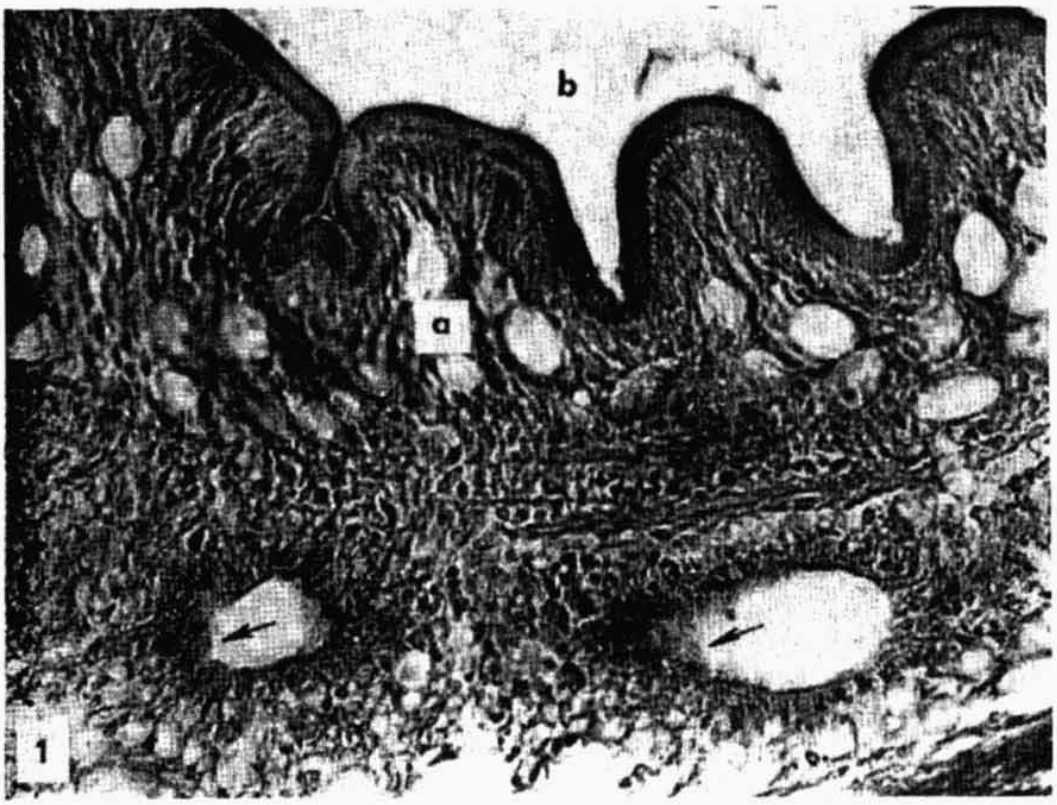

**Fig. 1.** Oblique section through the rostellar region of *C. boris* showing clearly the fibres of the rostellar sac (a) in the parenchyma, and a hook (arrow) in the tegument of the rostellar region (b). Mallory's phosphotungstic haematoxylin (1,200×).

**Fig. 2.** In the parenchyma of the invaginated portion, a large amount of glycogen is deposited among the calcareous corpuscles. a — spiral canal. Best's carmine (160×).

**Fig. 3.** Control section for fig. 2. After the saliva test, calcareous corpuscles only were stained. Saliva test + Best's carmine (160×).


**Fig. 1.** In longitudinal section through the invaginated portion of *C. bovis*, the main nerve trunk (a) and a sensory nerve cell (b) with its cellulipetal process and sensory endings (arrow) in the tegument of the spiral canal (c) show a high activity of alkaline phosphatase. α-naphthylphosphate + Fast blue BB (310×).

**Fig. 2.** The main nerve trunk (a) in transvere section sending off nerve fibres (b) and the bodies of two nerve cells (arrows) with their processes displaying a high activity of alkaline phosphatase. α-naphthylphosphate + Fast blue BB (410×).



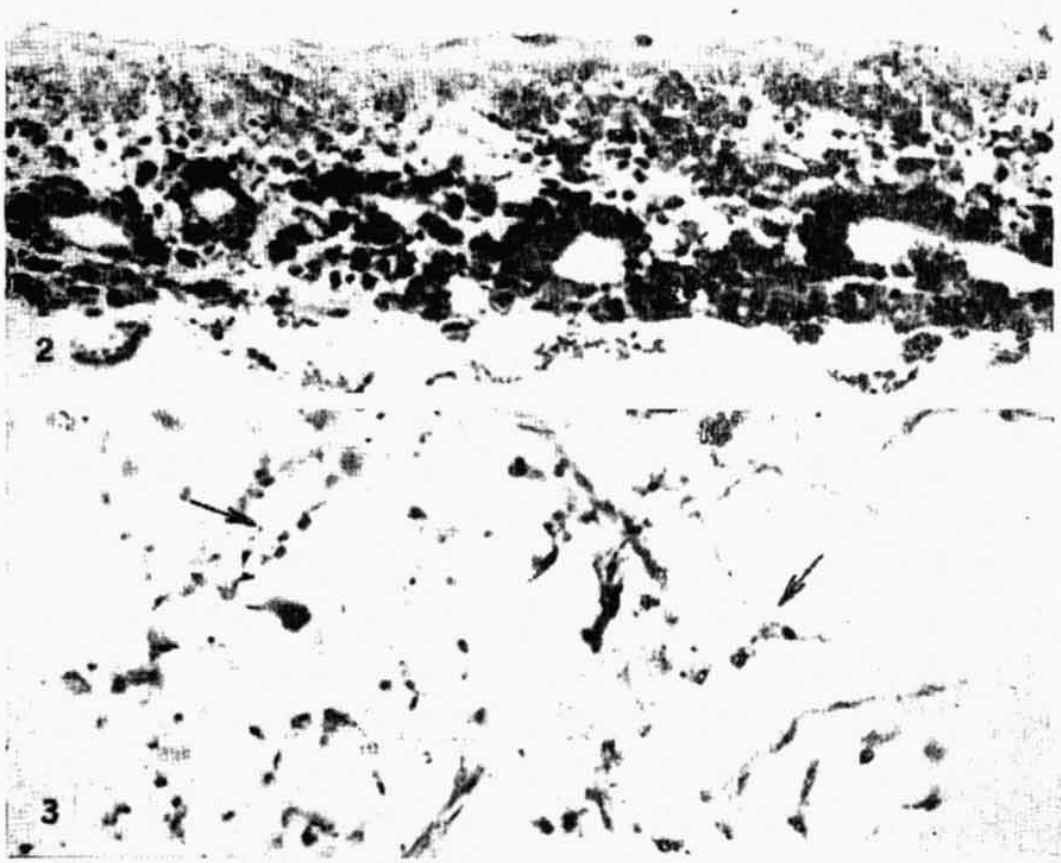




**Fig. 1.** The amount of glycogen is larger around the ventral excretory canals (arrows) than in the remaining parenchyma of the invaginated portion of *C. bovis*. a — calcareous corpuscles, b — spiral canal. AB-PAS (570×).

**Fig. 2.** Also in the bladder, a large amount of glycogen is deposited in pouches around the wide excretory canals. Best's carmine — haematoxylin (410×).

**Fig. 3.** Minute bodies (arrows) in the parenchyma of the bladder wall display a high activity of non-specific esterase. α-naphthylacetate + HPR (1,800×).

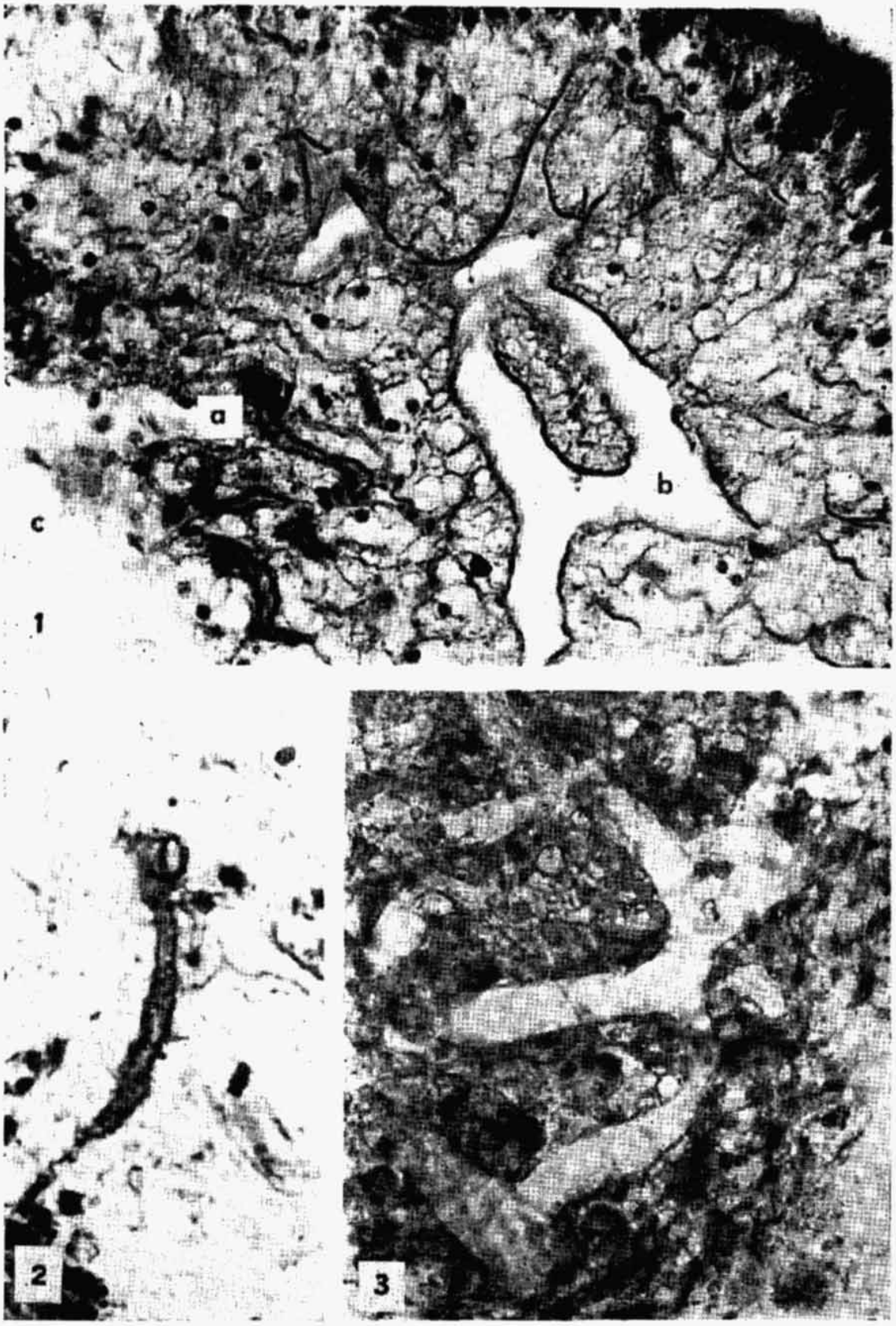



**Fig. 1.** Tangential section through the bladder wall with the well visible canals of the third order-ribbed (a), which stain intensively in the coupled tetrazonium reaction. b — wide excretory canals, c — bladder cavity (520×).

**Fig. 2.** Detailed view of a canal of the third order (rib-supported). Mallory's phosphotungstic haematoxylin (1,000×).

**Fig. 3.** Tangential section through the bladder wall at the level of the wide excretory canals, around which a large amount of glycogen is concentrated. PAS (520×).