

THE CYSTOGENIC GLAND CELLS OF THE CERCARIA OF PHILOPHTHALMUS SP.

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Dedicated to Doc. RNDr. Bohumil Ryšavý, D.Sc., Corresponding Member of the Czechoslovak Academy of Sciences, on the occasion of his 50th birthday.

Abstract. Observations have shown that there are four types of gland cells participating in the formation of the cyst wall of the cercaria of *Philophthalmus* sp. All these four types of gland cells release their secretion to the surface of the cercarial body to form its tegument while still in the intermediate host. The dorsal and suboesophageal gland cells containing acid mucosubstances, form the outer layer of the cercarial tegument and, at encystment, the outer layer of the cyst wall. The ventral gland cells containing proteins with tyrosine and SH groups and hydrophilic lipids form the inner layer of the tegument covering the entire body surface except the suckers and the caudal end. The secretion of the dorsocaudal gland cells containing neutral mucosubstances, forms the inner layer of the cyst wall on the caudal body end. During encystment, the inner layer of the cyst wall originates from the inner layer of the tegument. Hence, the cyst wall represents the separated tegument of the cercaria only.

This study of the cystogenic gland cells of the cercaria of *Philophthalmus* sp. is another of the series of earlier studies concerned with these gland cells in the cercaria of *Echinoparyphium aconiatum* (Žďárská 1968), *Plagiorchis laricola* (Žďárská 1969), *Notocotylus attenuatus* (Žďárská 1970) and *Moliniella anceps* (Žďárská 1970b). In comparison with these cercariae, the structure of the cyst wall of the cercaria *Philophthalmus* sp. is less complicated, which seems to be related with the location of the adult trematode in its definitive host.

MATERIAL AND METHODS

The cercariae and rediae of *Philophthalmus* sp. obtained from the snail species *Amphimelania holandri* Fer. were given to me by courtesy of Dr. Denev from Bulgaria, who studied the development of these larvae in the definitive host in collaboration with Dr. Vasilev. The species of the adult trematode has not been identified by these two authors (Vasilev, Denev 1963). The hepatopancreas of the snail *A. holandri* was fixed in 10% neutral formalin and the series of paraffin sections were stained with methods used in our earlier works (Žďárská 1968, 1969, 1970, 1971) for the demonstration of mucosubstances, proteins and several lipids.

RESULTS

1. THE HISTOLOGY AND HISTOCHEMISTRY OF THE GLAND CELLS

1. The dorsal gland cells (Fig. 1A — a; Plate I, Fig. 1 — a; Plate II, Figs. 1, 5 — a) are situated along the whole dorsal side of the cercarial body. Their shape is irregular measuring $21 \times 21 \mu$ in diameter and containing a nucleus of $6 \times 5 \mu$ in diameter with fine chromatin, and a nucleolus of 2μ . We detected with histochemical methods (Tables 1, 2) in the finely granulated cytoplasm of these gland cells acid mucosubstances only, which were resistant to hyaluronidase, neuraminidase and β -glucuronidase. The same reactions were obtained from the secretion released by these cells to the surface of the cercarial body.

2. The suboesophageal gland cells (Fig. A — b; Plate I, Fig. 1 — b; Plate II, Figs. 1, 5 — b) are situated in two longitudinally arranged rows below the oesophagus, but mainly radially around the oral and ventral suckers. They are club-shaped and the attenuated endings of the gland cells below the oesophagus are pointing in ventral direction, while the endings of those surrounding the sucker, are pointing towards the outer margin of the suckers. Their size is $21-24 \times 12 \mu$, the nucleus measures 5μ and contains fine granules of chromatin, their nucleolus measures 2μ in diameter. The finely granular cytoplasm inside the cells and later also on the surface of the cercarial body is PAS negative, AB positive

and resistant to β -glucuronidase, neuraminidase and hyaluronidase pretreatment. Hence, these gland cells contain acid mucosubstances (Table 1) differing from those in the dorsal gland cells only in being PAS negative.

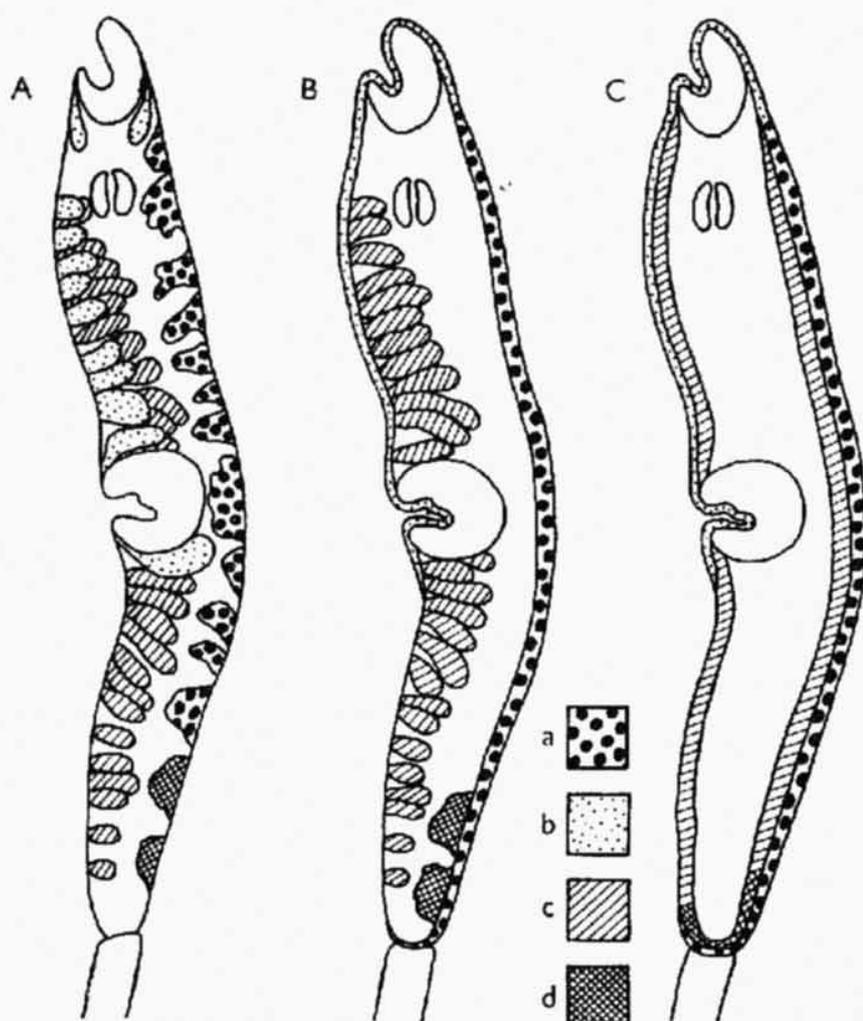


Fig. 1. Cercaria of *Philophthalmus* sp. — formation of the cercarial tegument from the secretions of the gland cells. A — longitudinal section through a cercaria with fully developed dorsal, suboesophageal, ventral and dorso-caudal gland cells; B — longitudinal section through a cercaria after release of secretion of the suboesophageal and dorsal gland cells to the body surface; C — longitudinal section through a cercaria after the release of the secretion of the ventral and dorso-caudal gland cells to the body surface; a — dorsal gland cells; b — suboesophageal gland cells; c — ventral gland cells; d — dorso-caudal gland cells.

3. The ventral gland cells (Fig. 1 A, B — c; Plate I, Fig. 1 — c; Plate II, Figs. 1, 2, 3, 5 — c) are located along the entire length of the ventral side of the cercarial body extending from the pharynx downwards to the posterior end of the body. These cells are club-shaped, $15-22 \mu$ long and $6-9 \mu$ wide. The nucleus (4μ in diameter) contains

coarser chromatine granules under the nuclear membrane, and a nucleolus. The cytoplasm is coarsely granulated. The granules stain violet with Mallory's phosphowolfram haematoxylin, red with Goldner's and Masson's trichrome and yellow in van Gieson's method. Of the histochemical methods (Tables 1, 2) they are highly positive to tyrosine and SH groups and stain with luxol blue. The gland cells differ from the foregoing two types of gland cells in containing proteins and hydrophilic lipids. The secretion of these cells covering the surface of the cercarial body, gives also the same histochemical reactions.

Table 1. Results of histochemical reactions for identification of mucosubstances

| Reactions | Gland cells | | | Tegument | | | |
|-----------------------------------------------------|-------------|---------------------|---------|----------------|----------------|---------------------------------------------------|----------------|
| | dorsal | suboeso- phageal | ventral | dorsal side | | ventral side (praenacetabular portion only) | |
| | | | | outer layer | inner layer | outer layer | inner layer |
| Best's carmine | - | - | - | - | - | - | - |
| Saliva test -- Best's carmine | - | - | - | - | - | - | - |
| PAS | +++++ | - | - | +++++ | - | - | - |
| Schiff | - | - | - | - | - | - | - |
| Saliva test + PAS | +++++ | - | - | +++++ | - | - | - |
| Acetylation 58 °C 48 h + PAS | - | - | - | - | - | - | - |
| Desacetylation -- PAS | +++++ | - | - | +++++ | - | - | - |
| AB PAS | violet | blue | - | violet | - | blue | - |
| AB pH 2.6 - methylation + demethylation | +++++ | +++++ | - | +++++ | - | +++++ | - |
| CEC (AB pH 2.6 -- + MgCl ₂) | 18 % | 18 % | - | 18 % | - | 18 % | - |
| Hyaluronidase + AB | +++++ | +++++ | - | +++++ | - | +++++ | - |
| Neuraminidase + AB | +++++ | +++++ | - | +++++ | - | +++++ | - |
| β -glucuronidase + AB | +++++ | +++++ | - | +++++ | - | +++++ | - |
| Aldehyde-fuchsin | +++++ | +++++ | - | +++++ | - | +++++ | - |

Table 2. Results of histochemical reactions for identification of proteins

| Reactions | Gland cells | | | Tegument | | | |
|----------------------------------------|-------------|---------------------|----------------------|----------------|----------------------|--------------------------------------------------|----------------------|
| | dorsal | suboeso- phageal | ventral | dorsal side | | ventral side (pracacetabular portion only) | |
| | | | | outer layer | inner layer | outer layer | inner layer |
| Sakaguchi | | | + | - | - | - | - |
| Morel-Sisley | | | ++++ | | ++++ | | ++++ |
| DMAB | - | | | | - | | |
| DDD | | | violet | | violet | | violet |
| N-ethylmaleimide + DDD | | | slightly positive | | slightly positive | | slightly positive |
| Thioglyc. acid + DDD | - | | violet | | violet | | violet |
| PFA-AB | ++++ | ++++ | | ++++ | | ++++ | - |
| AB pH 0.2 | ++++ | ++++ | | ++++ | | ++++ | |
| Periodic acid + aldehyde fuchsin | ++++ | ++++ | - | ++++ | | ++++ | |
| Aldehyde fuchsin | ++++ | ++++ | | ++++ | | ++++ | - |
| Coupled totrazo- nium reaction | - | | ++ | | ++ | | ++ |

4. The dorsocaudal gland cells (Fig. 1 A, B — d) are situated in two pairs at the utmost end of the dorsal side of the cercarial body. They are similar to the dorsal gland cells in their irregular shape but are slightly bigger in size ($25-30 \times 18 \mu$). Histochemically, these gland cells differ from the dorsal gland cells in their only feeble PAS positivity and in containing occasional AB positive granules. The inner layer of the tegument on the caudal end of the cercarial body originating from the secretion of these cells, gives the same histochemical reactions as this secretion.

II. THE EMPTYING OF THE GLAND CELLS AND THE FORMATION OF THE TEGUMENT OF THE CERCARIA

All four types of gland cells (Fig. 1 A — a, b, c, d; Plate I, Fig. 1 — a, b, c; Plate II, Figs. 1, 5 — a, b, c, d) develop simultaneously in the body cavity of the young cercariae in the redia. The first gland cells to start releasing their secretion in cercariae with completely developed suckers and pharynx are the suboesophageal and dorsal gland cells

(Fig. 1 B; Plate II, Figs. 1, 5, 6). Their secretion is pressed through the body wall to the surface of the body. The secretion of the dorsal gland cells covers only the dorsal and caudal side of the cercaria avoiding the area of the oral sucker. The secretion of the suboesophageal gland cells covers the ventral and oral sucker and the ventral side of the anterior portion of the body (Fig. 1B, C). After the complete emptying of these two types of gland cells, the ventral and dorsocaudal gland cells start to empty theirs. The secretion of the ventral gland cells spreads over the whole surface of the cercarial body except the area of the ventral and oral sucker and the caudal end (Fig. 1C). The secretion of the dorsocaudal gland cells covers only the caudal portion of the body under the secretion of the dorsal gland cells. This means that the secretion of the ventral gland cells forms a second, i.e. inner, layer on the complete dorsal side and, on the ventral side, on its anterior portion only. Thus the tegument of the cercaria is not bilayered throughout. On the ventral side one layer is formed on the oral and the ventral sucker and on the posterior end of the body. This one-layered tegument covering the suckers is formed by the secretion of the suboesophageal gland cells; that covering the posterior end on the ventral side, by the secretion of the ventral gland cells. In view of the fact that no other gland cells are present in the cercarial body we assume that the wall of the cyst of this adolescaria originates by the mere separation of the tegument of the cercaria which has been formed by the secretion of these four types of gland cells.

DISCUSSION

Contrary to the cercariae of *Echinoparyphium aconiatum*, *Plagiorchis laricola*, *Notocotylus attenuatus* and *Moliniella anceps* (Žďárská 1968, 1969, 1970, 1971) no proper cystogenic gland cells, which in the four foregoing species form the thick inner layer of the cyst wall, are present in the body of the cercaria of *Philophthalmus* sp. This indicates that the cyst wall of *Philophthalmus* sp. is more simple in its structure. Buša (1965) stated that the cysts of *Ph. hovorkai* Buša, 1956 rupture upon touch. Considering the fact that the adult trematode *Philophthalmus* sp. is located in the conjunctival sac of the goose (Vasilev and Denev 1963) reaching it via the lacrimal duct, the adolescaria must have emerged from the cyst mechanically in the proximal portion of the digestive tract already; and this can be accomplished only if the structure of the cyst wall is less complicated. The cyst wall of this adolescaria originates practically from the separated tegument of the cercaria, whereby the shape of the cyst is similar to that of an unexsheathed cercaria. The photographs of adolescariae of *Ph. gralli* (Alicata 1962, Plate III, Fig. 3) show that the shape of the oral sucker is retained on the cyst in its attenuated and empty terminal portion and that the cercaria is situated in the posterior portion of the exsheathed tegument. It is also possible to observe that part of the cyst which, before encystment, had formed the covering of the oral sucker, is thinner; this is in accord with our finding that the tegument of this sucker consists of one layer only, while the remaining part of the body is covered by two layers.

The layers of the cyst wall of the adolescaria of *Philophthalmus* sp. are identical with the outer layers of the cyst wall of *N. attenuatus*. The layer originating from the secretion of the dorsal gland cells covers in both cercaria species the dorsal side of the body; later, during the origin of the cyst, it forms the outer layer of the convex portion. A difference, however, has been observed in the mode in which the ventral gland cells release their secretion. In the cercaria of *Philophthalmus* sp., the ventral gland cells empty completely and their secretion covers the whole surface of the body except the suckers and the caudal end. In the cercaria of *N. attenuatus*, these cells release only part of their content which covers the ventral portion of the body only. The remaining secretion is

released during encystment and forms, similar to *Philophthalmus* sp., the continuous second layer of the cyst wall. While in *Philophthalmus* sp. this second layer is the layer covering the cyst cavity, in *N. attenuatus* it is the middle layer, because there an additional layer is formed from the secretion of the proper cystogenic gland cells.

Histochemical reactions of the secretion of the dorsal gland cells are very similar in both species, reacting positively to acid mucosubstances. Also the ventral gland cells of both species contain proteins rich in tyrosine and SH groups and also hydrophilic lipids.

The main difference between both cercaria species is in the absence of the proper cystogenic gland cells in *Philophthalmus* sp.: this means that the cyst wall of this species has one layer less. This difference seems to be related to the location of the adult trematode in the definitive host. While the cyst of *N. attenuatus* has to pass undamaged through the digestive tract on its way to the caeca, the adolescaria of *Philophthalmus* sp. whose adults are located in the conjunctival sac is liberated from the cyst already in the proximal part of the digestive tract, passes through the nasal cavity and reaches the conjunctival sac through the lacrimal duct. Therefore, this cyst is not attacked by enzymes as are the cysts of other adolescaria species which mature in the middle or distal portion of the digestive system of their definitive host. Our concept that the structure of the cyst wall of the adolescaria depends on the location of the trematode in the definitive host, has been confirmed in another member of the family *Philophthalmidae*, i.e. in *Parorchis acanthus* Nicoll, described by Rees (1967). The adult of this species, similarly to that of *N. attenuatus*, is located in the distal portion of the digestive tract. The cercaria of this species has proper cystogenic gland cells and also the structure of the cyst wall of these adolescariae is more complicated.

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Fig. 1



Fig. 2

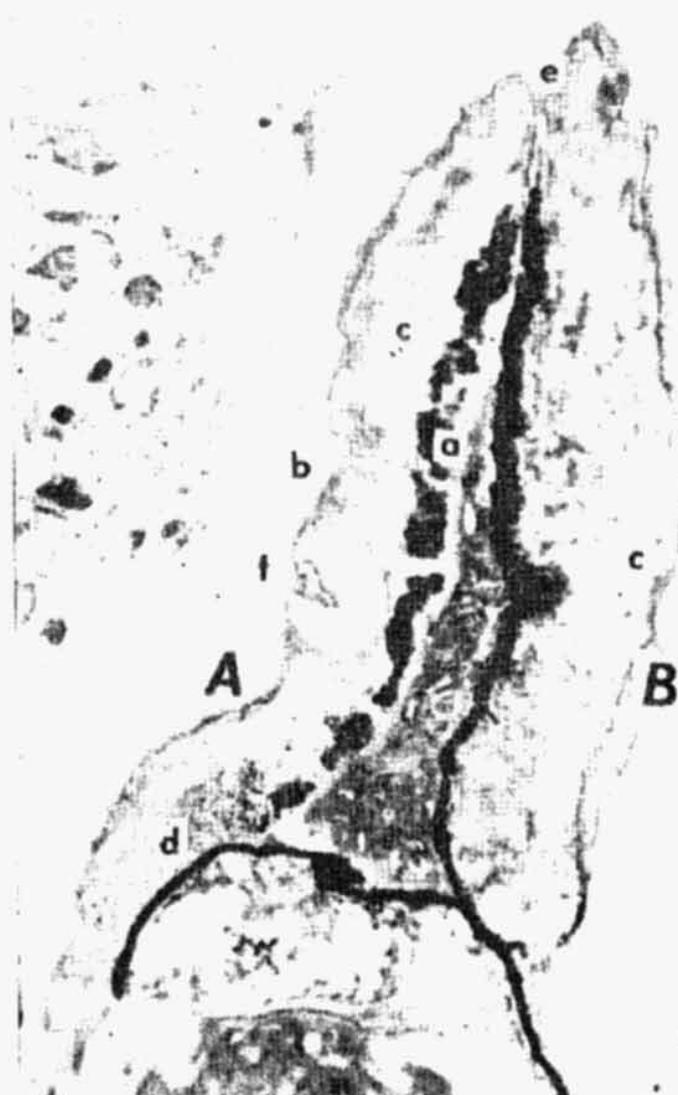


Fig. 1



Fig. 2

Fig. 3

Fig. 4



Fig. 5

Fig. 6

EXPLANATION OF THE PLATES

Plate I

Fig. 1. Longitudinal (A) and transverse (B) section through the cercaria of *Philophthalmus* sp. with developed dorsal (a), suboesophageal (b) and ventral (c) gland cells. Note the ventral sucker (d) and the excretory canal (e) on the longitudinal section.

Fig. 2. Longitudinal section through cercariae after all four types of gland cells have released their secretion. Observe the layer originating from the secretion of the ventral gland cells covering the whole body except the oral (a) and ventral (b) sucker and the caudal body end (c). The layer formed by the secretion of the dorsal and suboesophageal gland cells is not visible. Goldner's trichrome ($\times 270$).

Plate II

Fig. 1. Longitudinal section through a cercaria of *Philophthalmus* sp. with unemptied gland cells (A) — dorsal (a), suboesophageal (b), ventral (c) and dorsocaudal (d) — and through a cercaria with emptied dorsal and suboesophageal gland cells (B). The highly stained layer of secretion of the dorsal gland cells covers the dorsal side of the cercarial body except the oral sucker (e) and the caudal body end; the feebly stained layer of secretion of the suboesophageal gland cells covers the ventral side of the anterior portion of the cercarial body including the oral sucker, f-ventral sucker. AB-PAS ($\times 270$).

Figs. 2-4 (from the top to the bottom). Transverse section through cercariae illustrating various phases of release of secretion from the ventral gland cells which contain a high amount of tyrosine. **Fig. 2** — Completely developed ventral gland cells. **Fig. 3** — Part of the secretion of these cells released to the surface of the body. **Fig. 4** — Completely emptied ventral gland cells; their secretion forms the inner layer of the tegument except that of the ventral sucker. The outer layer of the tegument is not visible (compare with **Fig. 6**). Morel-Sisley's diazotization method ($\times 270$).

Fig. 5. Longitudinal section through a cercaria with developed gland cells — dorsal (a), suboesophageal (b), ventral (c), and dorsocaudal (d); ventral sucker (e). The dorsal and suboesophageal gland cells containing acid mucosubstances are stained intensively. The nuclei of the cells appear like unstained islets. Aldehyde fuchsine ($\times 270$).

Fig. 6. Transverse section through the posterior portion of the cercarial body after release of secretion of the dorsal and ventral gland cells. Intensive staining of the secretion of the dorsal gland cells containing acid mucosubstances and forming the outer layer of the tegument on the dorsal side of the cercarial body only. Unstained secretion of the ventral gland cells covers the whole body and forms the inner layer of the tegument on the dorsal side. Aldehyde fuchsine ($\times 270$).