The Gland Cells in the Tails of Cercariae

Z. ŽDÁRSKÁ

Institute of Parasitology, Czechoslovak Academy of Sciences, Prague

Abstract. Gland cells were observed in the tails of the cercariae of Echinoparyphium aconiatum, Moliniella anceps, Notocotylus attenuatus and Plagiorchis laricola. These cells disappear before the cercaria emerges from the snail. They are located inside the tail of the cercaria throughout its length and persist only until their contents are expelled through fine ducts onto the surface of the tail. Thus they participate in forming the tegument of the tail. Their contents while inside the cell and also when discharged onto the surface of the tail exhibit specific histochemical properties indicating acid mucosubstances with sulpho- and carboxyl groups and a small amount of tryptophane.

The locomotory organ of the cercaria — the tail — has been studied by many authors as will be discussed later in the text. The development of the tail has mostly been studied only at a certain stage of development; never throughout the whole development of the cercaria. This has led to some disagreement in the designation of the individual cell elements especially in the usage of the term “caudal bodies”. In the literature the term “caudal bodies” usually designates in cercariae, with the exception of the furcocercariae, two very divergent types of large cells — the myoblasts and the gland cells situated inside the tail throughout its length. We have proved by histochemical reactions that these gland cells persist in the four cercaria species under consideration only for some part of their development inside the snail and perish after having expelled their secretion onto the surface of the tail. In free-swimming cercariae, of the large cells only the myoblasts remain in the tail. The fact that this secretion forms the tegument of the tail escaped attention and only Kruidenier (1953) observed it in monostome cercariae.

MATERIAL AND METHODS

The hepatopancreas of three snail species infested with four species of larval trematodes was used in our histological and histochemical studies. These were: the hepatopancreas of Limnaea stagnalis (Linné, 1758) with the cercaria of Echinoparyphium aconiatum Dietz, 1909 and Plagiorchis laricola
(Skrabin, 1924), the hepatopancreas of Limnaea palustris (Müller, 1774) with the cercaria of Moliniiella anceps (Molin, 1859), Hübner, 1939 and of Limnaea auricularia (Linné, 1758) with the cercaria of Notocotylus attenuatus (Rudolphi, 1809), Kossack, 1911. The hepatopancreas was fixed in Baker’s neutral solution (Pearse 1960) and embedded in paraffin in the ordinary way. Free-swimming cercariae were treated similarly.

For morphological studies we used sections stained with Weigert’s and Böhmer’s haematoxylin-cosin and with Goldner’s and Masson’s trichrome. Van Gieson’s modified method (Holusa 1967), Gomori’s method, aldehyde fuchsin and resorcin-fuchsin were used for demonstrating connective tissue. Transversely striated muscle fibres stained best with Weigert’s phosphowolfram haematoxylin. Mucous substances were demonstrated histochemically with the PAS method combined with acetylation, desacetylation and the saliva test. When using Pearse’s (1960) original formula we were unable to acetylate the contents of the caudal gland cells; acetylation had to be carried out for 48 hrs at 58 °C. Acid mucous substances were detected with alcian blue (AB) pH 2.6 (Quintarelli, Scott and Dellovo 1960). To differentiate them any further we used Pearse’s (1960) methylene blue extinction method, the method with alcian blue pH 2.6 (Scott, Quintarelli and Dellovo 1964, Quintarelli, Scott and Dellovo 1964) in combination with methylation according to Fisher and Lillie (1954), demethylation according to Spicer and Lillie (1959) and the critical electrolyte concentration method (CCE) (Scott and Dohling 1965, Scott and Willet 1966, Scott and Stockwell 1967) in the modification by Quintarelli and Dellovo (1965) with alcian blue pH 2.6 and MgCl₂. Mowry’s (1963) AC-PAS method was used for detecting the presence of neutral and acid mucous substances.

In view of the fact that all histochemical reactions for individual protein components (arginin, tyrosin, SH- and SS-groups) were negative in the caudal gland cells, we refer to methods described in an earlier paper (Žďárska 1968). Tryptophane was detected with the dimethyl-amino-benzzaldchyle (DMAB) method and the coupled tetrazonium reaction modified by Müller and Chytel (1962). This was performed on material fixed for 12 hrs at 4 °C in Baker’s neutral formaldehyde adjusting its pH to 6.5 with 0.1N NaOH (Lojda 1965).

RESULTS

A. Designation of the cell elements in the tail

Most investigators have given incomplete descriptions of the cell elements in the tail, describing from wholemounts only the largest elements such as gland cells and myoblasts. The latter are the bodies of the transversely striated muscle cells forming the longitudinal musculature of the tail. Most often these cell elements seem to have been confused one with the other. However, we are not in the position to give a critical view on the vast number of data recorded by various authors, because we do not even know whether only fully developed cercariae have been described. Therefore, we have considered only the more important records.

Originally, the term caudal bodies was used only for the gland cells situated inside the stem of the bifurcated tail of furcocercariae. The best description of their morphology and location among the other cell elements was given by Pearson (1962) and Dünges (1964). In all the other groups of cercariae these cells have been given different names by various authors (see Table 1). Ginetsinskaya (1962) who studied various types of free-swimming mature cercariae used the term “caudal bodies” for cells rich in glycogen. Here, the myoblasts of echinostome and monostome cercariae seem to have been confused with the caudal bodies of furcocercariae,
these being different types of cells. The tail of the furcocercaria which, however, has not been studied by us, contains in addition to the myoblasts also caudal bodies persisting in it even after its emergence from the first intermediate host. Similar cells of the same morphology and location as the caudal bodies of the furcocercariae are found in the monostome and echinostome cercariae under consideration, the difference being that these persist only until the time, when the cercaria leaves its

<table>
<thead>
<tr>
<th>Furcocercariae</th>
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<tr>
<td>Caudalkörper</td>
<td>Dönges 1964</td>
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<tr>
<td>Stellate caudal bodies</td>
<td>Pearson 1961</td>
</tr>
<tr>
<td>Grandes cellules claires</td>
<td>Vercammen-Grandjean 1960</td>
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<tr>
<td>(corps caudaux)</td>
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<tr>
<td>Kaudalnye tela</td>
<td>Ginetsinskaya 1962</td>
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<tr>
<td>Grandes cellules glandulaires</td>
<td>Dubois 1929</td>
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<tr>
<th>Other types of cercariae</th>
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<tr>
<td>Cellules glandulaires caudales</td>
<td>Dubois 1929</td>
</tr>
<tr>
<td>Kaudalnye tela (kletki)</td>
<td>Ginetsinskaya 1962</td>
</tr>
<tr>
<td>Tail glands</td>
<td>Kruidenier 1953</td>
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<tr>
<td>Grandes cellules claires</td>
<td>Vercammen-Grandjean 1960</td>
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first host. In addition these cells do not contain any glycogen. Before the cercaria emerges from its host, these cells disappear and their secretion forms the tegument of the tail. By contrast, after these cells have completed secretion, the myoblasts project freely into the lumen of the tail and contain a large quantity of glycogen.

We therefore suggest that the term "caudal bodies" should be used only with reference to the furcocercaria. For other groups of cercariae it would be more convenient to call similar cells which persist only until the time, when the cercaria leaves its first host and which participate in the formation of the tegument of the tail, the gland cells of the tail.

B. Morphology of the tail in the various cercariae

The cell components of the tail can easily be distinguished in the cercariae of *Echinoparyphium aconiatum*, *Moliniella anceps* and *Notocotylus attenuatus*. By contrast, the tail of the cercariae of *Plagiorchis laricola* contains very small cells and very thin muscle fibres and, therefore, it is difficult to distinguish them even by the highest magnification of the light microscope. The existence of only two types of cells in the tail of this cercaria has been confirmed reliably. These are
firstly the very small cells situated inside the tail on its longitudinal central axis. The second group of cells includes those which are rich in glycogen and seem to be the cells of the circular and longitudinal muscles.

The cell elements in the other three cercaria species under consideration are respectively similar, whereby each type of cells is most distinct. These are: the smooth muscle cells forming the outer circular muscle layer; the myoblasts which are cellular bodies belonging to the transversely striated muscle fibres, which form the inner longitudinal muscle layer. The amorphous ground substance is deposited among the muscle fibres and forms a continuous layer on the surface of the tail. One row of central cells orientated dorsoventrally and dividing the tail in a left and right portion, is situated in the middle of the tail. Laterally, on both sides, follows a row of gland cells. These, however, persist only until the time when the cercaria becomes ready to emerge from its first host (Fig. 1). Before leaving its host, the secretion of these cells is discharged onto the surface of the tail forming an uninterrupted layer (Plate II).

![Diagram](image)

**Fig. 1.** Tail of the cercaria *Moliniella aniceps* (Molin, 1859) Hübner, 1939. A — transverse section through the proximal portion of the tail at the place which is not yet covered by the tail membrane; B — transverse section through the central portion of the tail with the tail membrane; C — transverse section through the distal portion of the tail. On the lateral sides of the central supporting cell (black) the gland cells of the tail (gray); the tail membrane is facing this cell on the dorsal and ventral side. The transversely striated muscles form four bands; (dashes = myofibrils in the cells, dots = cytoplasm); D — schematic illustration of the tail. Longitudinal vertical sections through various depths of the tail shown on a transverse section through the tail, a — through the transversely striated muscles with myoblasts and the lateral parts of the gland cells of the tail, b — through the gland cell of the tail, c — through the central supporting cell, d — horizontal longitudinal section schematically shown in two functional stages on Fig. 2.

**C. Histology and histochemistry of the gland cells of the tail**

The shape of the gland cells of the tail varies considerably in relation to the state of contraction of the tail. On cross section their shape is almost triangular. Their base lies on the central cell which points in dorsoventral direction. From this base
Table 2. Results of histochemical tests for identifying mucosubstances in the gland cells of the tail

<table>
<thead>
<tr>
<th>Test</th>
<th>Cercaria</th>
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<tr>
<td></td>
<td>N. attenuatus</td>
<td>E. aconiatum</td>
<td>M. aniceps</td>
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<tr>
<td></td>
<td>gland cells</td>
<td>gland cells</td>
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<td>of the tail</td>
<td>of the tail</td>
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<td></td>
<td>tegument of the tail</td>
<td>tegument of the tail</td>
<td>tegument of the tail</td>
</tr>
<tr>
<td>Best’s carmine</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Saliva test + Best’s carmine</td>
<td>++</td>
<td>++</td>
<td>+</td>
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<tr>
<td>PAS</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saliva test + PAS</td>
<td>++</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Acetylation 58 °C, 48 h +</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>− PAS</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Desacetylation + PAS</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aldehyde-fuchsine</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>AB + PAS</td>
<td>blue</td>
<td>violet</td>
<td>blue</td>
</tr>
<tr>
<td>AB pH 2.6</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>+ methylation</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+ demethylation</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>CEC (AB pH 2.6 + MgCl₂)</td>
<td>6%</td>
<td>12%</td>
<td>18%</td>
</tr>
<tr>
<td>Hyaluronidase + AB</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Methylene blue extinction at pH</td>
<td>2.6</td>
<td>2.6</td>
<td>4.6</td>
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fine ducts extend in ventral and dorsal direction and terminate on the dorsal and ventral side close to the median depression (Fig. 1; Plate II, Fig 1).

These gland cells resemble in shape the dorsal gland cells described from the body of the cercaria of *E. aconiatum* (Zdárská 1968). The young cells contain a large nucleus (5 μ) with a small amount of chromatin deposited under the nuclear membrane and round the nucleolus. This measure 3 μ. In fully developed cells the nucleus is shrivelled. In *M. anceps* the cytoplasm of the caudal gland cells contains small eosinophilic vacuoles.

It was possible to prove with histochemical reactions that these gland cells of the tail develop simultaneously with the rest of the cell elements of the tail but, by contrast, that they persist only until the time when the cercaria leaves its intermediate host. At that time, the cells discharge their secretion onto the surface of the tail and die. It was possible to discover on the sections (Plate II, Figs. 1—3) various stages of cell discharge up to the stage when only a shrivelled remnant of these cells remained in the tail. In a cercaria emerging from its first host nothing is left of these gland cells.

Glycogen could not be demonstrated in these gland cells with histochemical reactions. The PAS reaction was positive even after digestion with saliva, the staining with Best’s carmine was negative. By contrast, the myoblasts reacted positively to the PAS-test and to Best’s carmine, but negatively after digestion with saliva. The histochemical reactions shown in Table 2 indicate that the contents of the gland cells of the tail consist of acid mucosubstances containing both sulpha- and carboxyl groups. In the proteins the amino acids were represented only by a small amount of tryptophane. The same reactions were obtained with the secretion discharged onto the surface of the tail.

D. Other cell elements of the tail

The myoblasts with the transversely striated muscle fibres are, next to the gland cells of the tail, the most easily noticeable elements of the tail. When the gland cells of the tail are present, the myoblasts are pressed to the transversely striated fibrils situated peripherally. During contraction of the tail and when the gland cells have discharged their secretion, the pear-shaped bodies of the myoblasts projecting into the tail cavity and connected with the myofibrils by an attenuated portion become more visible. The conspicuously large, bladderlike nucleus contains a large oxyphilic nucleolus. The oxyphilic cytoplasm of the myoblast contains glycogen and numerous granules which are strongly basophilic. The myofibrils — their isotropic and anisotropic bands being easily seen when stained with Mallory’s phosphowolfram-haematoxylin — are not uniformly distributed along the circumference of the tail, but form four groups — two dorsal and two ventral ones (see cross section through the tail, Fig. 1 A—D). The smooth muscle cells forming the outer circular muscle layer contain nuclei with condensed chromatin surrounded by a small quantity of cytoplasm (Fig. 2). The thin muscle fibrils are organized circularly
and always two fibrils are running close together. However, it was not possible to
detect whether these two closely adjoining muscle fibrils originate from a single
muscle cell. The fibrils are anisotropic and, in the cercaria of *M. anceps*, they pass
into the membrane of the tail. They stain
red with haematoxylin-eosin and with Gold-
ner's and Masson's trichrome, yellow with van
Gieson and violet with Mallory's phosphowol-
fram haematoxylin.

The supporting central cells are orientated
dorsoventrally and appear spindleshaped in
cross section through the tail (Fig. 1). With
one of their ends they are attached in mid-line
to the circular muscle layer on the dorsal side,
with the other to the ventral side. Their bas-
sphilic cytoplasm contains granules which
are strongly basophilic. The large bladderlike
nucleus contains a nucleolus. These cells per-
sist permanently in the tail and make a solid
connection between its ventral and dorsal side
throughout the length of the tail. In cross
section the wall of the tail is drawn inwards
at the sites where these cells are fixed to it.
In the cercaria of *M. anceps* the tail membrane, extending throughout the length
of the tail, is fixed to this site (Fig. 2 A–C). A row of these cells forms the longitu-
dinal axis of the tail.

**DISCUSSION**

WUNDER (1923) described the tail of all types of mature cercariae as a hollow clo-
sed tube filled with fluid; its wall consists of four layers. These are, starting from
the outside, the tegument, the circular muscle layer, the longitudinal muscle layer
and the layer of loose parenchymal cells. This basic structure of the tail was con-
formed in electron microscopic studies of an echinostome cercaria by CARDELL,
PHILPOTT and PHILPOTT (1960) and of a xiphidiocercaria by BELTON and HARRIS
(1967).

In the four cercaria species under consideration (*E. aconiatum, M. anceps,
N. attenuatus* and *P. loricola*) the structure of the tail was very similar except
that of the tail of the xiphidiocercaria of *P. loricola*, which was slightly different.
The two outer layers, i.e. the tegument and the circular smooth muscle fibres, were
found to be the same in all four cercaria species, while the third layer is the same
only in the species *E. aconiatum, M. anceps* and *N. attenuatus*. In these, the longitu-
dinal transversely striated muscle fibres form four muscle bands extending through-

![Fig. 2. Tail of the cercaria Moliniella ancesps (Molin, 1859) Hübner, 1939. Scheme of a longitudinal horizontal section in the axis of the tail. A — contracted, B — expanded.](image-url)
out the length of the tail; two of them being dorsal, two ventral. (In *N. attenuatus*,
these muscle bands coalesce on the lateral side.) In *P. laricola*, the longitudinal
muscle fibres are not transversely striated, but are of the same structure as the
circular muscle fibres. They do not form bands, but are evenly dispersed along the
circumference of the tail. Selinheimo (1956) described the same structure in
xiphidiocercariae and this can be seen also in drawings by Ginetsinskaya and
Dobrovolsky (1962) and Engelsbrecht and Palm (1964).

Before the cercaria emerged from its first host, two rows of gland cells were
observed inside the tails of all four cercaria species under consideration. These were
particularly large in the cercaria of *E. aconiatum*, *M. anceps* and *N. attenuatus*,
but were very small in the cercaria of *P. laricola*. In this species, these cells could
be detected only with certain histochemical methods. When using normal histological
methods these cells were not noticed.

The morphology of the gland cells of the tail, some of their histochemical prop-
erties and their participation in forming the tegument of the tail has been described
so far only by Kruidenier (1957) and Kruidenier and Mehra (1957) in two mono-
stome cercariae — *C. urbanensis* Cort., 1914 and *Macrostilbulum eversum* Häü., 1937,
while these cells have not been studied yet in echinostome cercariae. Kruidenier and
Mehra (1957) using toluidine blue and thionin of a different pH showed that these
cells and also the tegument of the tail originating from their secretion contain
sulphated mucosubstances. According to our histochemical results the gland cells
of the tails of monostome and echinostome cercariae contain acid mucosubstances
not only with sulpho-groups, but also with carboxyl groups. Amino acids are
represented only by a small amount of tryptophane.

**CONCLUSIONS**

We proved from histological and histochemical studies of the tail of some cercariae
that the contents of the gland cells of the tail form the tegument of the tail and that
they do not contain glycogen. The cells called caudal bodies by Ginetsinskaya
and Dobrovolsky (1962) seem to be myoblasts (except in furcocercariae). These,
as confirmed by us, contain glycogen and are distributed in the same way as depicted
by Ginetsinskaya and Dobrovolsky. The size of the myoblasts in the different
types of cercariae is in keeping with the data given by these two authors. They
are large in monostome and echinostome cercariae, small and numerous in xiphidiocercariae.

The central supporting cells orientated dorsoventrally, which we found in
echinostome and monostome cercariae, have not previously been observed. They
are not mentioned by Cardell, Philpott and Philpott (1960) although they are visible on their electron micrograph.

We conclude from the histochemical and histological studies that the structure
of the tail of echinostome and monostome cercariae is analogous, but differs greatly
in the xiphidiocercariae.
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Z. Z., Parasitologický ústav
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Longitudinal section through almost the entire tail of the cercaria *Notocotylus attenuatus* (Rudolfi, 1809) Kossack, 1911, showing the paired gland cells of the tail and on a tangential section through the body of the cercaria, two dorsal gland cells. Aldehyde-fuchsin ($\times 600$).
Tail of the cercaria *Notocotylus attenuatus* (Rudolphi, 1809) Kossack, 1911. Fig. 1. Transverse section through the tail with maximum development of gland cells of the tail just before secretion starts. Fig. 2. When most of the secretion has been expelled to the surface of the tail, little secretion remains inside the cells. Fig. 3. Completely discharged secretion of the gland cells of the tail shown on the surface of the tail. Aldehyde-fuchsin. Figs. 4. and 5. Tangential section through the gland cells of the tail and through their extensions. AB-PAS (x 1150).