

## HISTOCHEMISTRY OF THE CERCARIA OF ORIENTOBILHARZIA TURKESTANICA (SKRJABIN, 1913)

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**Abstract.** Four types of gland cells — escape, head, praacetabular and postacetabular — were demonstrated by histochemical methods in the cercaria of *Orientobilharzia turkestanica*. The secretion of the head gland contains proteins with cystine and cysteine, and phospholipids. It exhibits the acid phosphatase activity. The secretion of the escape glands contains proteins with cystine and cysteine and the secretion of the postacetabular gland cells proteins with cystine, cysteine and tyrosine, and neutral and acid mucosubstances.

Adult specimens of *O. turkestanica* were found in the veins of mesentery and liver in cattle, sheep, goats, camels, horses, donkeys, pigs, and cats. Since this parasite causes considerable economical losses in cattle and sheep breeding in certain regions of Kazakhstan, the histochemistry of this cercaria was studied and compared with that of the members of the genus *Schistosoma*. The histochemistry of *O. turkestanica* larvae has not yet been studied by any author. Most recent data on the morphology of this cercaria were published by Belyakova and Tarmakhanov (1985).

### MATERIALS AND METHODS

Sporocysts with cercariae were obtained from naturally infected snails *Radix auricularia* from the Kzyl-Ordin region of the Kazakh S.S.R. The material was fixed with Baker's fluid at 4 °C for 2—24 h. Histochemical methods used for the detection of mucosubstances, proteins and enzymes were described in the papers by Ždárská and Panin (1977) and Ždárská et al. (1978).

### RESULTS

Four types of gland cells — head, escape, praacetabular, and postacetabular — were demonstrated by histochemical methods in the body of cercaria. The first two types are small, whereas the remaining two are characterized by large sizes of cell bodies filling the major part of cercaria body.

1. The head gland cell (Fig. 1) is situated dorsally in the oral sucker and its numerous openings are directed towards the tegument lining the cavity of the oral sucker. The cell contains a large nucleus, well visible as an uncoloured isle after staining with the methods for the detection of cysteine. The secretion of this cell exhibits acid phosphatase activity and gives a reaction of medium positivity for cysteine (DDD method), strongly positive reaction for cystine (thioglycolic acid and DDD, peracetic acid and aldehyde fuchsine), and positive reaction for hydrophilic lipids (Luxol Fast Blue).
2. The escape gland cells (Fig. 1) are localized on the ventral side, laterally to oesophagus. There is one pair of these glands on each side. They are small, contain a large nucleus and are situated in front of the praacetabular gland cells. The escape gland cells open into the oral sucker through thin ducts. Their secretion gives a strongly positive reaction for cystine and a reaction of medium positivity for cysteine.

3. The *praeacetabular* gland cells (Fig. 1) (two pairs) are localized in front of the ventral sucker. They are large and their ducts open into the oral sucker. They contain a large, bladder-like nucleus with a nucleolus and their cytoplasm cannot be stained by any of the used histochemical methods for the detection of proteins, mucosubstances, and enzymes. The demonstration of calcium by Kóssa's method gave also negative results. Their ducts run laterally to oesophagus together with the ducts of *postacetabular* gland cells.

4. The *postacetabular* gland cells (Fig. 1) (three pairs) are localized behind the ventral sucker. Their ducts are the longest and thickest of all other gland cell ducts in the cercaria body. These gland cells contain a large, bladder-like nucleus with a nucleolus. In the cytoplasm, there are fine granules (Plate I, Fig. 2, Plate II, Figs. 1 and 2) staining intensively by the methods for the detection of cystine (peracetic acid and aldehyde fuchsin, thioglycollic acid with DDD), cysteine (DDD method) and tyrosine (Morel—Sisley's method). The cytoplasm is strongly positive to PAS (Plate I, Fig. 1) and Best's carmine reaction even after saliva test. It is also positive to alcian blue

(pH 2.6) for the detection of acid mucosubstances, particularly in young cercariae. In older cercariae, this reaction is weak or negative. The affinity for alcian blue does not disappear after demethylation, which indicates that the acid mucosubstances with COOH groups are involved. At the simultaneous detection of neutral and acid mucosubstances by AB-PAS method, the cytoplasm of *postacetabular* gland cells stains violet in young cercariae and red in older cercariae. Consequently, these gland cells contain neutral mucosubstances, acid mucosubstances with COOH groups, and proteins with cystine, cysteine and tyrosine. A secretion of the same histochemical properties is contained also in the ducts of these cells running on the dorsal side behind the *praeacetabular* gland cells which open at the anterior end of the oral sucker.

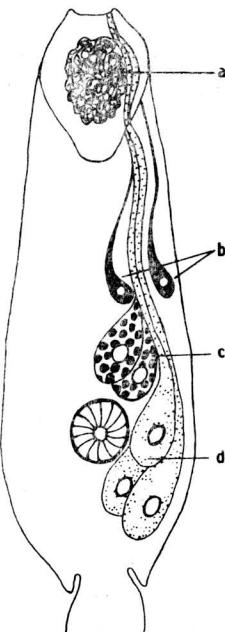


Fig. 1. Scheme of the glandular apparatus of *O. turkestanica* cercaria; a — head gland, b — escape gland cells, c — *praeacetabular* gland cells, d — *postacetabular* gland cells.

The nerve ganglion of cercaria exhibits non-specific esterase activity and the sporocyst wall alkaline phosphatase activity. Alkaline phosphatase was not demonstrated in the body of cercaria. Acid phosphatase activity was detected in the widened distal part of oesophagus representing the anlage of intestinal branches.

## DISCUSSION

The glandular apparatus of *O. turkestanica* cercaria has not yet been studied in detail. Like the cercaria of *Schistosoma mansoni*, which is the most often investigated species of the genus *Schistosoma* (Stirewalt and Kruidenier 1961, Stirewalt and Walters

1964, Ebrahimzadeh 1970, Ebrahimzadeh and Kraft 1971, Dorsey 1974, 1975, 1976, Brink et al. 1977), also *O. turkestanica* cercaria contains four types of gland cells. The largest are the *praeacetabular* and *postacetabular* gland cells. The small escape glands are situated in front of *praeacetabular* gland cells and the head gland is localized in the oral sucker. Short ducts of this gland are directed into the tegument lining the oral sucker cavity. In the cercariae which have not yet left the intermediate host, the secretion of this gland is pressed out into the tegument. The head gland is preserved even in free-swimming cercariae, whereas the escape glands cannot be demonstrated in this cercaria. In *S. mansoni* cercaria it is supposed (Ebrahimzadeh 1970, Ebrahimzadeh and Kraft 1971, Dorsey 1974) that their secretion is used up during the penetration through the intermediate host tissues. In contrast to the escape gland, according to Miyagawa (1916) and Cort (1919), the secretion of the head gland in *S. japonicum* is used up during the penetration of cercariae into the definitive host. Dorsey (1976) states that the secretion of this gland in *S. mansoni* contains phospholipids and serves for the reparation of tegument during the penetration through the skin of the definitive host. As it was demonstrated in our studies, the head gland of *O. turkestanica* cercaria contains, in addition to phospholipids, proteins with cystine and cysteine.

We have not managed to demonstrate calcium in the *praeacetabular* gland cells of *O. turkestanica* by means of Kóssa's method, though the calcium salts are supposed to be present in these glands in *S. mansoni* cercaria on the basis of the positive reaction of their secretion to vital staining by purpurin (Stirewalt and Kruidenier 1961). Stirewalt (1963) supposed that the secretion of these gland cells in *S. mansoni* is used up during the penetration through the definitive host. However, the results obtained by Brink et al. (1977) disprove his assumption.

Histochemical methods revealed only mucosubstances in the *postacetabular* gland cells of *S. mansoni* cercaria, whereas in *O. turkestanica* cercaria, we have demonstrated, in addition to neutral and acid mucosubstances, also proteins with cysteine, cystine and tyrosine. Since the ultrastructure of the secretion of *O. turkestanica* cercaria has not yet been studied, we assume that, like in *S. mansoni* cercaria (Ebrahimzadeh and Kraft 1971, Dorsey 1975), its *postacetabular* gland cells contain a secretion with two components. Light "droplets" in *S. mansoni* cercaria seem to correspond to mucosubstances in their structure, whereas the electron-dense granules contained in these "droplets" might represent protein granules with the above amino acids. The secretion of these glands is used up during the movement of cercaria on the substrate (Linder 1983) and during its penetration into the body of the definitive host (Ebrahimzadeh 1970, Stirewalt and Kruidenier 1961). In contrast to *S. mansoni* cercaria (Ebrahimzadeh 1970), the alkaline phosphatase activity was not demonstrated in the tegument of *O. turkestanica* cercaria. This enzyme was found to be present only in the sporocyst wall.

It may be concluded that the gland system of *O. turkestanica* cercaria is almost identical with that of the cercariae of the genus *Schistosoma*, except that it contains four instead of two escape gland cells. The gland apparatus of this cercaria cannot be compared with that of the cercariae of the genus *Orientobilharzia*, e.g. of *O. dattai* (Yamaguti 1975), in which the smaller types of glands, i.e. the head gland and escape glands, escaped the attention.

**Резюме.** Четыре типа железистых клеток — железы выхода, головная железа, преацетабулярные и постаацетабулярные железы — были обнаружены у церкарии *Orientobilharzia turkestanica* с помощью гистохимических методов. Секрет головной железы содержит белки с цистином и цистеином и фосфолипиды и проявляет активность кислой фосфатазы. Секрет желез выхода содержит белки с цистином и цистеином и секрет постаацетабулярных железистых клеток белки с цистином, цистеином и тирозином и нейтральные и кислые мукосубстанции.

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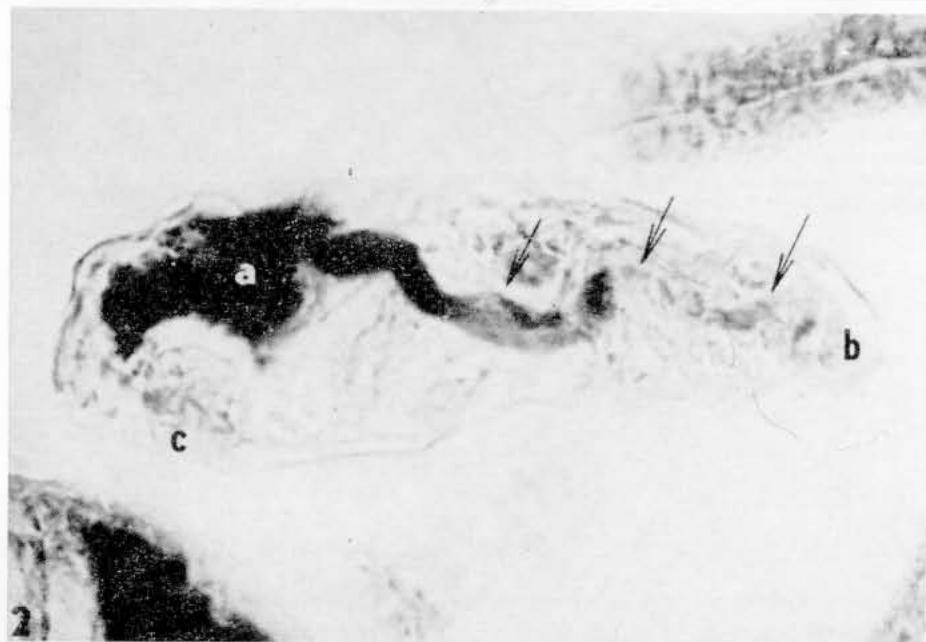
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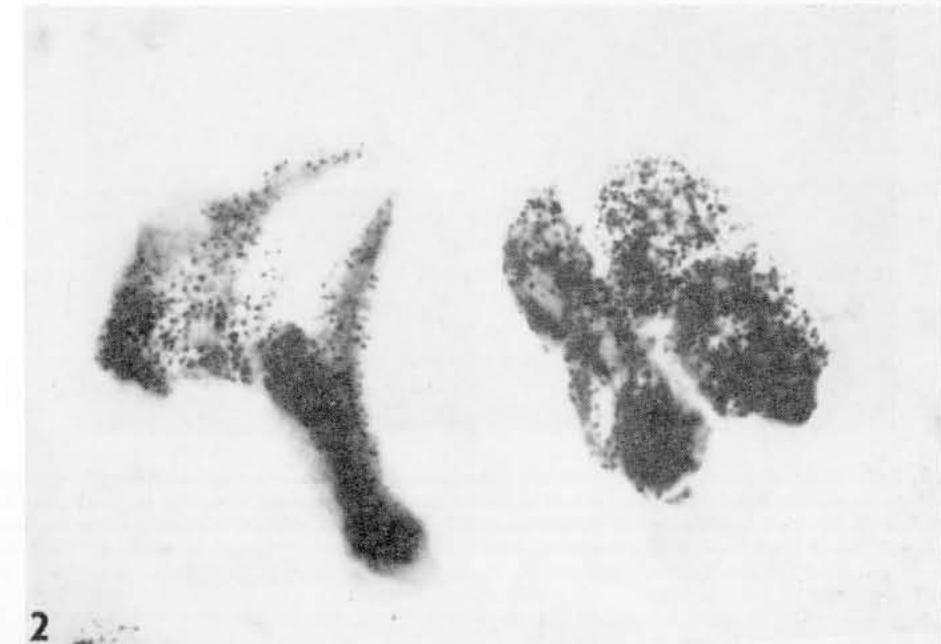
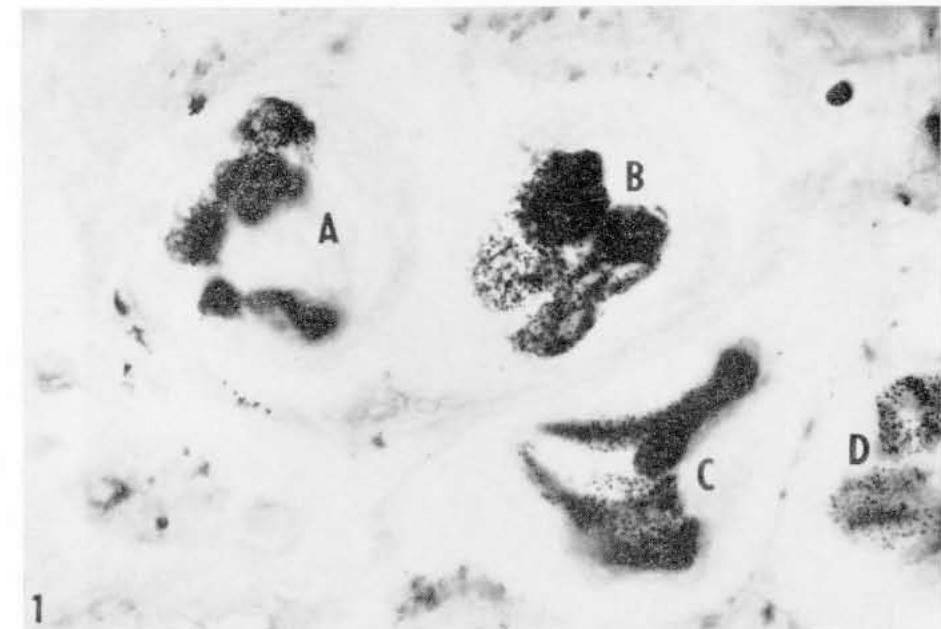
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**Fig. 1.** Longitudinal horizontal section through the body of *O. turkestanica* cercaria with intensively stained secretion of postacetabular gland cells containing neutral mucosubstances in the bodies (a) and ducts (arrows); b — oral sucker, c — tail, d — praacetabular gland cells. PAS ( $\times 770$ ). **Fig. 2.** Longitudinal vertical section through the body of *O. turkestanica* cercaria with the body contents (a) of postacetabular gland cells intensively stained with PAA-AF method for the detection of cystine. The duct contents (arrows) remain unstained; b — oral sucker, c — ventral sucker ( $\times 1200$ ).



**Fig. 1.** Oblique sections (A, B, C, D) through the posterior part of bodies of *O. turkestanica* cercariae with intensively stained contents of postacetabular gland cells. PAA-AF ( $\times 900$ ). **Fig. 2.** Detail of secretory granules of postacetabular gland cells from Fig. 1 containing cystine. PAA-AF ( $\times 1500$ ).