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Effect of penetration Gland Secretion on Cercarial Surface-coat Formation

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Introduction

A characteristic feature of the cercarial stage of the trematode is the presence of gland cells in the body. Erasmus (1972) has classified these cells into three groups by their function: mucoid gland cells, cystogenous gland cells and penetration gland cells. The nature and function of the mucoid gland cells have been described using light microscope by Kruidenier (1951, 1953 a, b, c, d) in non-virgulate xiphidiocercariae, monostome cercariae, opisthorchoid cercariae and *Paragonimus kellicotti*, Yokogawa and Yoshimura (1956, 1958) in *Paragonimus westermani* and *P. ohirai*, and Ito and Watanabe (1957, 1958 a, b) in *Pseudexorchis major*, *Metagonimus* sp. and *Centrocestus armatus*. Gymnocephalous cercariae have been investigated by Dixon (1966) and echinostome cercariae by Rees (1967). Up to date, the secretion produced by the mucoid gland has been believed to form a thin coat surrounding the outer surface of the cercarial body. Concerning the cercaria of *Metagonimus* sp., Ito and Watanabe (1958) have related the cercarial surface-coat to the secretion of the mucoid gland consisting of five pairs. On the other hand, observation of penetration gland cells of the genus *Metagonimus* (Tongu *et al.*, 1975) has indicated that these glands may participate in the formation of the surface-coat. The present study was undertaken to determine the relationship between penetration gland cells and cercarial surface-coat in *Metagonimus* sp.

Materials and Methods

Cercariae of *Metagonimus takahashii* and *M. yokogawai* were allowed to escape naturally from their molluscan host, *Semisulcospira* sp. collected in Hiroshima Prefecture.

The cercariae were fixed in 2.5% glutaraldehyde with phosphate buffer at pH 7.4 for 1 hour, followed by post-fixing in 1% osmium tetroxide solution with phosphate buffer at pH 7.4 for 2 hours. The cercariae were dehydrated in an ethanol series by routine methods and embedded in an epoxy resin mixture after passing through N-butylglycidyl ether. The specimens were thin sectioned with a Porter-Blum and Reichert ultramicrotome and stained with uranyl acetate and lead citrate. Hitachi HS-8 electron microscope was used for observations. To compare the surface-coat with the granules in the penetration gland ducts, cercariae were sectioned crossly at a level near the oral sucker. Mucoid substances in the ducts of the penetration gland cells and on the surface of the body tegument were examined in the same section.

Results

The penetration gland duct (Figs. 1, 2, 4, 6, 7; PGD) consisting of four groups ran toward the head and opened at the oral sucker (Fig. 1). The lumen of the duct was usually filled with granules of varying sizes, shapes and densities (Figs. 1, 3, 6, 7; PGD). Granules with an average-density matrix (Fig. 6), or with a dense point in the center (Figs. 3, 6) were common in the duct. Fibrous materials appearing like fuzz (Figs. 1, 3, 4, 7) and less-dense or diffused granules (Fig. 4) were also located in the duct near the opening. The major part of the cytoplasm of gland cells was occupied by many granules with average-density matrix. The outer surface of the cercarial tegument except the tail was usually covered by a surface-coat consisting of fibrous or fuzz-like materials of varying thickness (Figs. 1, 5, 7, 8; S). This coat was thickest notably around the oral sucker (Fig. 1; S), and was thinner in proportion as it stood back from the head. In some specimens the surface-coat was, however, not identified anywhere (Fig. 6), or was only observed partially (Fig. 8; S). The tail tegument was overspread with thin dense materials only. The cercarial body tegument (Figs. 1, 5, 6, 7, 8; T) contained granules consisting of four types; 1. electron dense granules, 2. less-dense granules contained fibrillar materials, 3. electron dense granules dotted with vacuoles, 4. and vesicle-like granules. The first type was located especially in a group in the tegument near the oral sucker (Figs. 1, 2). The second type occurred in the body and tail, and was often released from the tegument near the opening of the penetration gland duct (Fig. 2; arrows). The third type was laid in the tegument near the surface. The last type was distributed in the anterior part of the body and tail tegument. In the tail tegument (Fig. 3, T), only the second and the fourth types were seen; moreover, the fuzz-like surface-coat was not identified in the tail. Particular relationships between surface-coat and existence of granules in the penetration gland ducts in the present study were indicated in Table 1.

The study dealt with 67 cercariae including 35 *M. takahashii* (Mt) and 32 *M. yokogawai* (My). Of these, 21 cases (Mt-17, My-4) without granules in the duct were completely covered by a thick surface-coat. Their penetration gland ducts were filled with fuzz-like fibrous materials only. Granules occurred in the ducts in 46 (Mt-18, My-28) of 67 cercariae. Of these, 33 (Mt-6, My-27) cases filled completely with dense granules had no surface-coat on the tegument, and 8 (Mt-7, My-1) packed with a few diffuse granules were covered by a thick surface-coat. The remaining 5 (Mt-5) cases which were completely filled with dense gran-

Table 1 Cercarial numbers showing the relationship between the surface-coat and granules in the penetration gland ducts

Granule in duct \ Surface-coat		Present		Absent
		thick coat	thin or partial coat	
Present	filled completely		Mt-5	My-27 Mt-6
	a few or diffused	My-1 Mt-7		
Absent		My-4 Mt-17		

My: *Metagonimus yokogawai*, Mt: *Metagonimus takahashii*

ules had either thin surface-coats or partial coats. No cercariae had both a thick surface-coat and completely packed dense granules. On the other hand, cercariae lacking the surface-coat were filled completely with the dense granules. As specific differences, 82% of *M. takahashii* had a surface-coat compared with 18% of *M. yokogawai*. Many cercariae lacking surface-coats were seen in *M. yokogawai* (82%) in comparison with *M. takahashii* (18%).

Discussion

Since the mucoid gland cells of cercariae were described by Kruidenier (1953 a, b, c, d), Ito and Watanabe (1957, 1958 a, b), and Yokogawa and Yoshimura (1958, 1959) using the technic of metacromatic staining, the secretion produced by these cells has been believed to form a surface-coat surrounding the outer surface of the cercarial body. Ito and Watanabe (1958 a) have presented the characteristic features on the mucoid gland cells of the cercaria of *Metagonimus* sp. as seen by light microscope. According to their observation, this cercaria possesses five pairs of mucoid glands arranged regularly in the body. And mucoid substances discharged cover the body surface, especially on the surface near the oral sucker to give a thicker coat. The surface-coat was not identified in the tail, and was not referred to the opening of the gland ducts. With light microscopy, Yokogawa and Yoshimura (1956), and Kruidenier (1953 a, b, c, d) have suggested that the duct of mucoid glands opens into the buccal cavity. In the present observation with an electron microscope, the surface-coat around the oral sucker was thicker than that of other parts. However, the duct opening of the mucoid glands was not recognized anywhere in *Metagonimus* cercariae. Furthermore, the mucoid gland cells described by Ito and Watanabe (1958 a) were not identified in the body apart from the penetration gland cells and cystgenous gland cells. Therefore a positive association between mucoid gland cell and sub-tegumental cell is suggested by the results of the present study.

The fact that the surface-coat around the oral sucker was thicker than that of other parts corresponded with the fact that the ducts of the penetration gland cells opened at the oral sucker. In addition, apart from 5 cases, the quantity and quality of the granules in the penetration gland ducts correlated well with the existance of the surface-coat as in Table 1. Although these 5 cases were completely packed with dense granules, they were surrounded by a thin or partial coat. The duct may have received a deficient supply of granules from the penetration gland cells. On the basis of the present results, it seems reasonable to assume that most of the surface-coat of *Metagonimus* cercariae is elaborated by the secretion substance from the penetration gland cells. There is, however, the possibility that the surface-coat materials were released from the dense granules in some parts of the tegument. Concerning this point, Belton and Belton (1971) and Belton and Harris (1967), have indicated that finely fibrillar vesicles were extruded from the cercarial tegument of *Acanthatrium oregonense*. Other workers, Hockley (1972), Hockley and McLaren (1973), and Stein and Lumsden (1973) have also suggested that the surface-coat of *S. mansoni* may be formed by the tegument itself. Therefore the present study dose not deny that a small part of the surface-coat may be provided by the tegument. There was a difference between the two species in the number of emerged cercaria with a surface-coat. In this connection, it seems that *M. takahashii* is slightly different from *M. yokogawai* in some physiological functions.

Summary

The relationship between surface-coat and granules in the penetration gland ducts in genus *Metagonimus* cercariae (*M. takahashii*-35, *M. yokogawai*-32) as seen by electron microscopy is described. Without exception 21 cases without granules in the duct were completely covered by a thick surface-coat. Eight cases among 29 specimens covered by

a thick surface-coat contained less-dense fused granules in the duct. Of the 46 which were positive for dense granules of the duct, 33 had no surface-coat on the tegument. Of the 38 completely filled with the dense granules, 5 were covered by a thin or partial coat. There were no cercaria lacking both the granules and the thick surface-coat. It was concluded that most of the surface-coat of *Metagonimus* cercariae is elaborated by the secretion granules from the penetration gland cells, although the surface-coat may be discharged, at least in part, from granules in the tegument. *M. takahashii* and *M. yokogawai* were shown to have slight differences in their physiological habits.

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セルカリア体表の被膜形成に及ぼす侵入腺細胞の影響について

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67個体のメタゴニムス属セルカリア（*Metagonimus takahashii*-35, *M. yokogawai*-32）の侵入腺細胞の導管の開口部近くと体表を電子顕微鏡で観察した．21個体は導管内に全て顆粒は無く，体表は厚い被膜に被われている．反対に導管内に顆粒の充満した33個体は体表被膜を持たない．5個体は顆粒が充満していたが非常に薄いか又は部分的に体表被膜を持つていた．厚い体表被膜を持つた8個体は非常に少数の融解した様な顆粒を持つていた．しかし顆粒が全々無く，しかも被膜も持つていない個体は1個体も認められなかつた．これらのことから侵入腺の分泌顆粒が大部分の体表被膜の形成に関与していると考えられる．しかし口吸盤付近では外皮(tegument)内の顆粒が放出されている像が認められ，これらの顆粒が少量ではあるが被膜形成に関与している事も否定出来ない．又横川吸虫，高橋吸虫の間に体表被膜形成に於ける差が認められた．

Explanation of Figures

Fig. 1 The orifice (arrow) of penetration gland ducts (PGD) in the oral sucker of *M. takahashii*. S: surface-coat, Sp: spine

Fig. 2 High power magnification of Fig. 1. Several granules (arrows) have been released into the lumen of the oral sucker.

Fig. 3 Cross-section of a tail of *M. takahashii*. M: muscle, T: tegument

Fig. 4 Cross-section of *M. takahashii* showing penetration gland ducts (PGD) near the orifice. Mt: microtubules

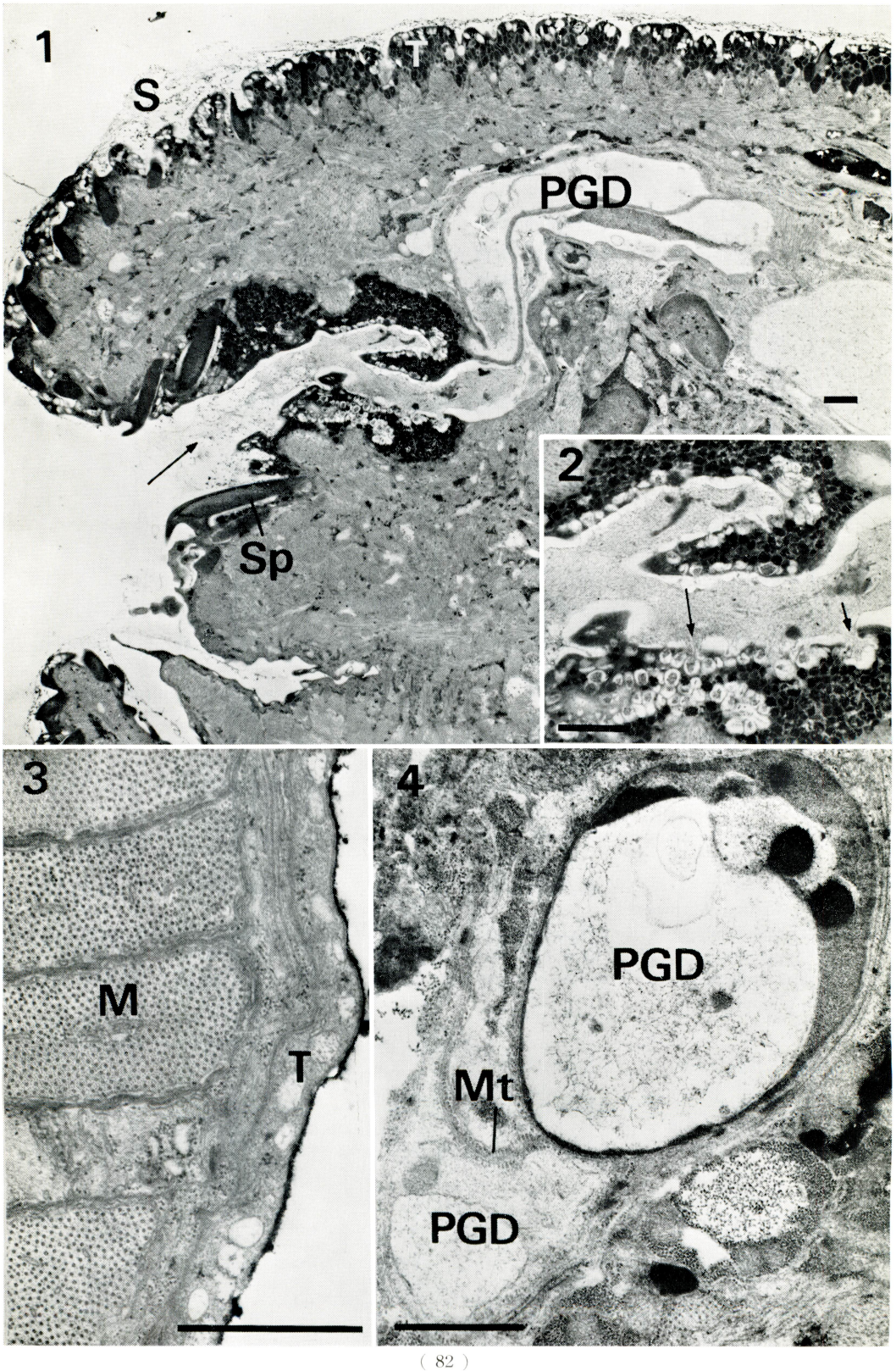
Fig. 5 *M. takahashii* showing the body tegument (T) covered by a thick surface-coat (S). M: muscle

Fig. 6 Cross-section of *M. yokogawai* showing penetration gland ducts (PGD) and the tegument (T) without surface-coat.

Fig. 7 *M. takahashii* showing penetration gland ducts (PGD) filled with fine fibrous materials and a tegument (T) covered by a thin surface-coat (S).

Fig. 8 *M. takahashii* showing the body tegument (T) covered by partial surface-coat (S). Sp: spine

NOTE: scale is one micrometer in each figure.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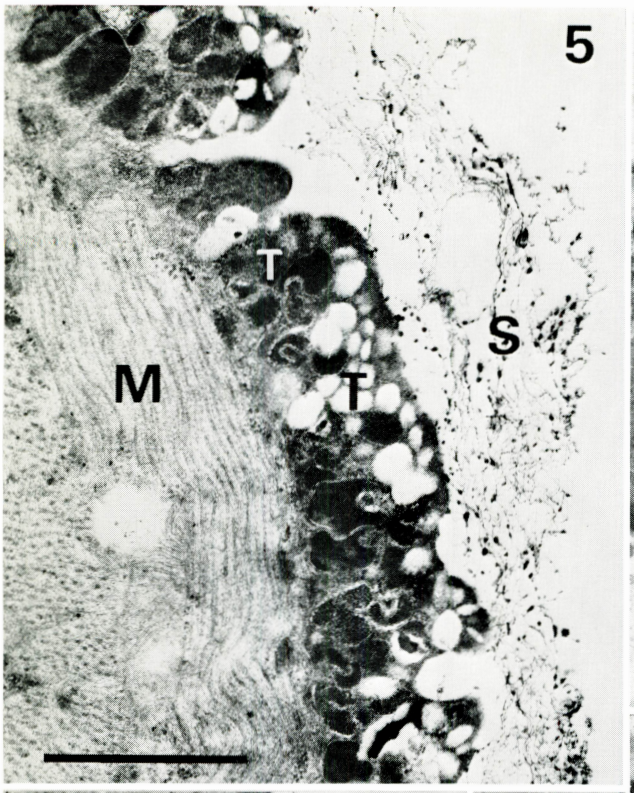
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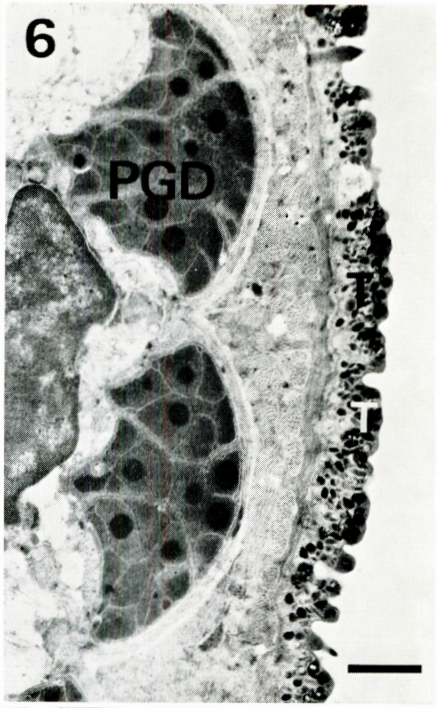
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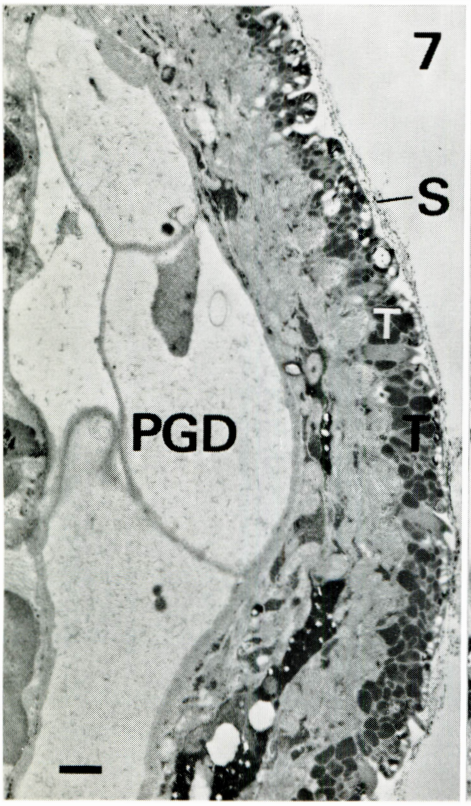
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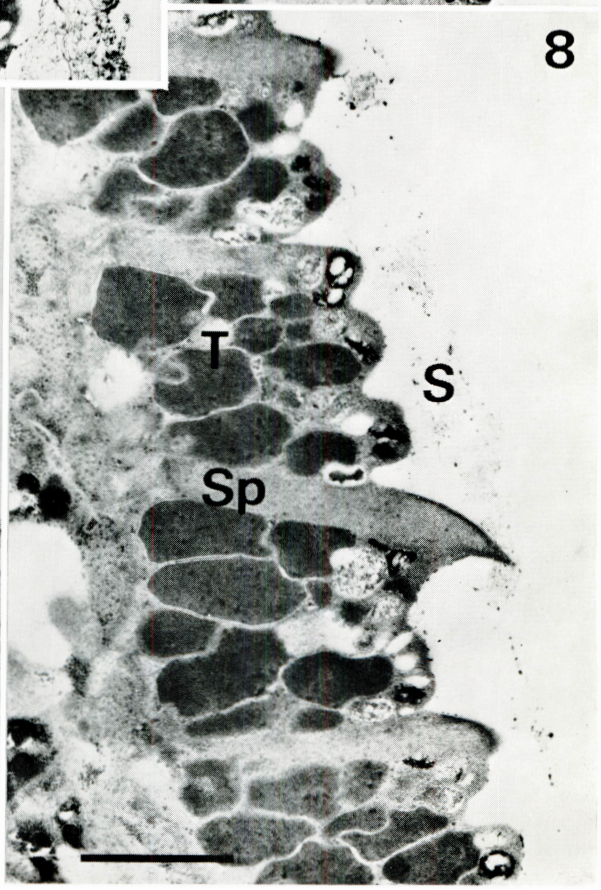
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