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Novel Structures in Secreting the Androgenic Gland Hormone

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ABSTRACT—The secretory granules in the androgenic gland of the terrestrial isopod *Armadillidium vulgare*, which have been indistinct for long time because of vulnerable structures, were revealed by using the rapid-freezing and freeze-substitution method. The fine struture of the androgenic gland is conspicuous by the distribution of numerous particular organelles in the cytoplasm consisting of the endoplasmic reticulum and the Golgi complex, and by having a number of highly organized structures developed between the androgenic gland cells. The structures connect to the intercellular space, which is seen as intercellular canaliculi for exporting the androgenic gland hormone. The plasma membranes near the particular structure of the intercellular canaliculi in the androgenic gland are often specialized to form cellular junctions. The secretory granules including the electron-dense materials, which are supposed to be peptides of androgenic gland hormone, are distributed beside the particular structure of the intercellular canaliculi. Some of the granules are seen to fuse with the plasma membranes. This observation suggests that, in the *Armadillidium vulgare*, the secretory granules containing androgenic gland hormone are transferred to the extracellular space through the intercellular canaliculi particularly developed for exporting the peptide hormone. This is the first evidence to show the secretory mechanism of the androgenic gland hormone in the Isopoda.

Key worsds: androgenic gland, secretory granule, canaliculus, isopod, Golgi complex

INTRODUCTION

The structure of the androgenic gland has been investigated in a number of species (Charniaux-Cotton and Payen, 1985, Fingerman, 1992) since the organ was first described by Charniaux-Cotton (1954), though its location in the gonad is different from species to species. In *Armadillidium vulgare*, the implantation of the androgenic glands from adult males into juvenile females induced the gonadal masculinization of the juveniles (Katakura, 1960, 1984; Katakura and Hasegawa, 1983). The chemical properties of the androgenic gland hormone (AGH) has been examined for *Armadillidium vulgare* (Hasegawa *et al*., 1987; Martin *et al*., 1990), and recently the chemical structure of the AGH was revealed to be a heat-stable protein (Okuno *et al*., 1997). The secretory granules containing the proteinacious AGH were thought to exist in the androgenic gland cells of *Armadillidium vulgare*. However, in the Isopoda, the secretory granules have not yet been detected because of the difficulties in keeping the structure of the organ intact (Martin *et al*., 1996). We have tried to preserve the ultrastructure of the androgenic gland by using the rapid-freezing and freeze-substitution method, which was found to be suitable for preserving fragile membranous structures in cells (Ichikawa *et al*., 1980, 1982; Ichikawa and Ichikawa, 1987). We could obtain relatively high electron density in the overall profile. The result indicates that the procedure is effective in reducing the damage of cytoplasmic substances occurring during the process of dehydration. As a result, we could succeed in finding the same structure in the androgenic gland of *Armadillidium vulgare* as other secretory granules containing peptide hormones (Fawcett, 1966).

The improved ultrastructure images clearly show that the cytoplasm is conspicuous by the presence of the particular structure of rough endoplasmic reticulum (RER) together with a number of Golgi complex. Moreover, secretory granules, electron-dense granules coated with the unit membrane, are also found among abundant RER. The relationship between the secretory mechanism of AGH and the ultrastructure of the cytoplasm particular in the androgenic gland cell was investigated.

MATERIALS AND METHODS

Rapid freezing and freeze-substituion

Armadillidium vulgare were reared in our laboratory at 20°C with light and dark duration regulated as a natural condition. Androgenic

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glands of male individuals at puberal stages were used. Reproductive organs which consist of testis, seminal vesicles, vas deferens and androgenic glands were dissected out into the saline solution for Crustacea. Materials were then quickly frozen in a rapid-freezing device filled with liquid nitrogen. Freeze-substitution was carried out in 4% O_sO_4 in ethanol for 20 hr at –90°C in a deep freezer. Samples were then transferred to –20°C, 0°C, and finally brought to room temperature within about 4 h. Samples were dehydrated in ethanol, embedded in Quetol-812, and sectioned by a Porter-Blum MT2 ultramicrotome with a diamond knife. Thin sections were stained with uranyl acetate and lead citrate, and viewed with a JEOL 1001 electron microscope at 80 kV.

RESULTS

Morphology of androgenic gland

A 0.6 mm length of the androgenic gland of *Armadillidium vulgare*, which is attached to the apex of the testis, is sectioned serially from the tip to the root adjacent to the testis. The tip region of the androgenic gland is branched into a few parts like islands (Fig. 1). Each island, which is surrounded by a sheath with a thickness of 0.2 μm, seems to be connected by secretory products. The cytoplasm of each island is filled with well-developed rough endoplasmic reticulum (RER) like other cells specialized for protein secretions, and with large mitochondria with a diameter of 0.8 to 1 μm. A number of clusters of glycogen particles are seen among the RER. Some secretory granules of 100–250 nm in diameter are distributed in the peripheral region of the gland. Moreover, the androgenic gland is conspicuous by having a particular shape of intercellular canaliculi, which exhibit highly organized structures such as a knitting ball (Fig. 2a). The unique structures of the intercellular canaliculi are distributed adjacent to the cross point of the androgenic gland cells or the periphery of the cell, and connected to the intercellular space or extracellular region respectively (Fig. 2c). Sometimes the secretory granule appears to fuse with the plasma membrane making intercellular space (Fig. 2b). The particular structures of the intercellular canaliculi, which are found in the androgenic gland almost from the tip to the root region, appear as a part of the open canalicular system formed by the cell membranes. It is probable that this arrangement is of functional significance in facilitating the liberation of the androgenic gland hormone stored in the cell. This particular structure may be well-developed intercellular canaliculus characteristic of secretory cells.

A secretory granule having unit membrane is shown in the intercellular canaliculus (Fig. 3), suggesting that the secretory granule is exported via this particular structure into the extracellular space. In the periphery where the conspicuous intercellular canaliculus is extended, a sheath is separated into each membrane as the export of the androgenic gland hormone becomes possible (Fig. 4). In another part of the androgenic gland cells, typical cytoplasmic components such as the Golgi complex are well developed, and a cluster of glycogen granules is located in association with the Golgi complex (Fig. 5). A large number of RER containing low electron-dense materials are extended in the cytoplasm. Cell membranes exhibit several specializations forming intercellu-

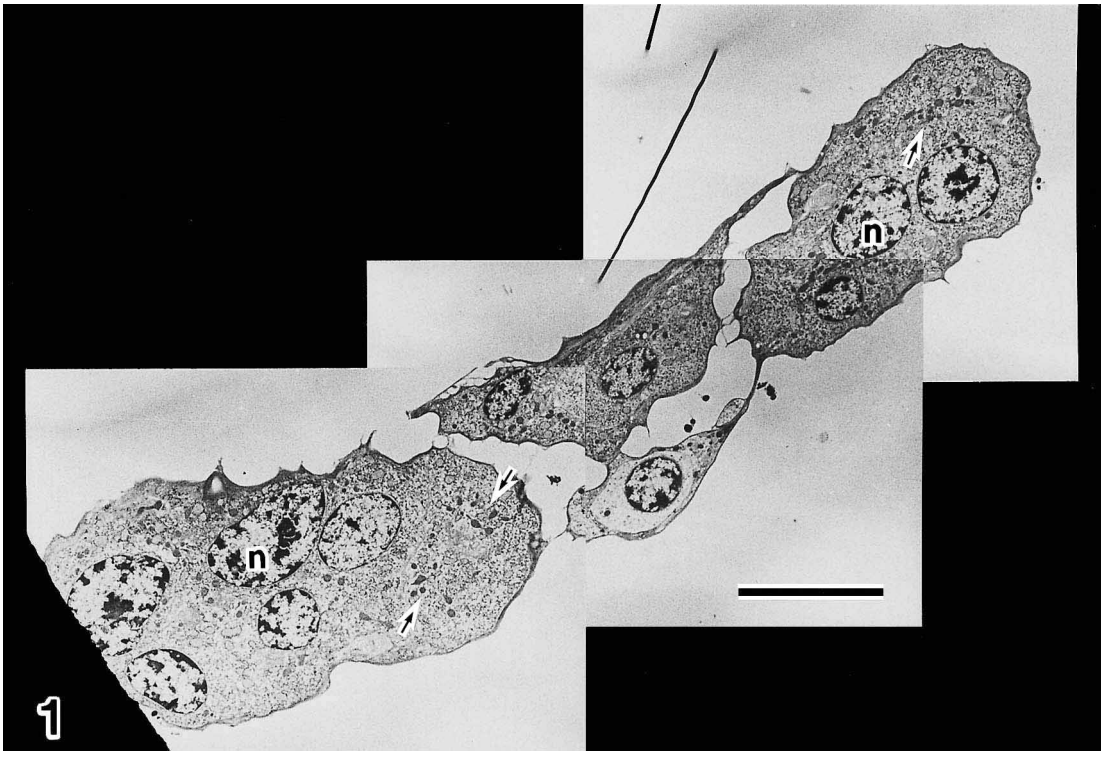

Fig. 1. The tip region of an androgenic gland. A few islands are associated with connective substances. Each cell contains large size of nucleus (n) and a large number of mitochondria (arrows). Bar=10 μm.

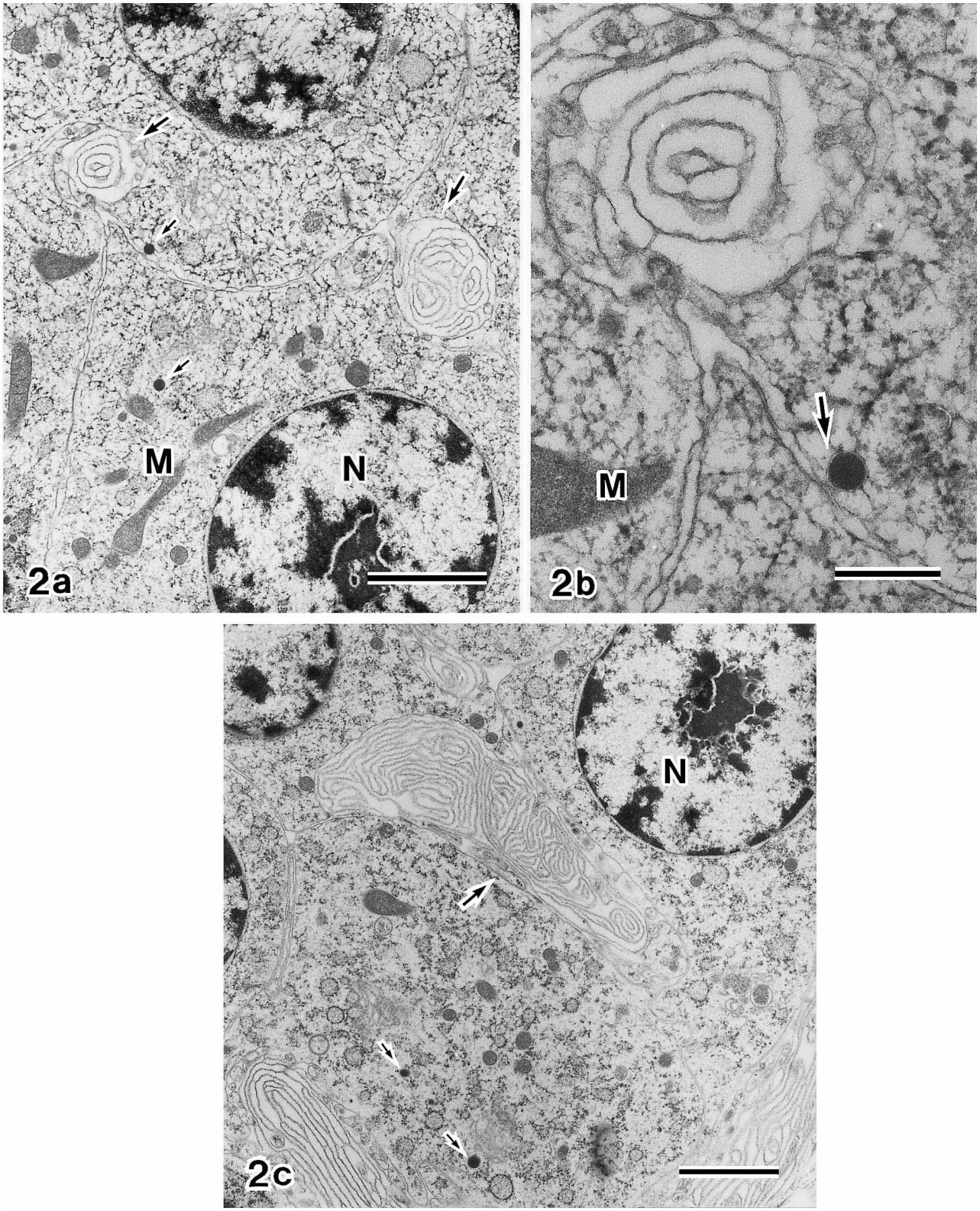

Fig. 2. Particular structure of intercellular canaliculi. (a) Central region of an androgenic gland. Knitting-ball like structures (arrows) are located in association with cell membranes. Bar=2 μm. (b) Enlarged figure of the upper-left side of 2a. A secretory granule (arrow) surrounded by a unit membrane takes a close contact with cell membrane. Bar=500 nm. (c) Peripheral region of an androgenic gland. The intercellular canaliculus exhibits a long shape (arrow). Secretory granules (small arrows) distribute besides the structure. Bar=2 μm. M. Mitochondria; N, nucleus.

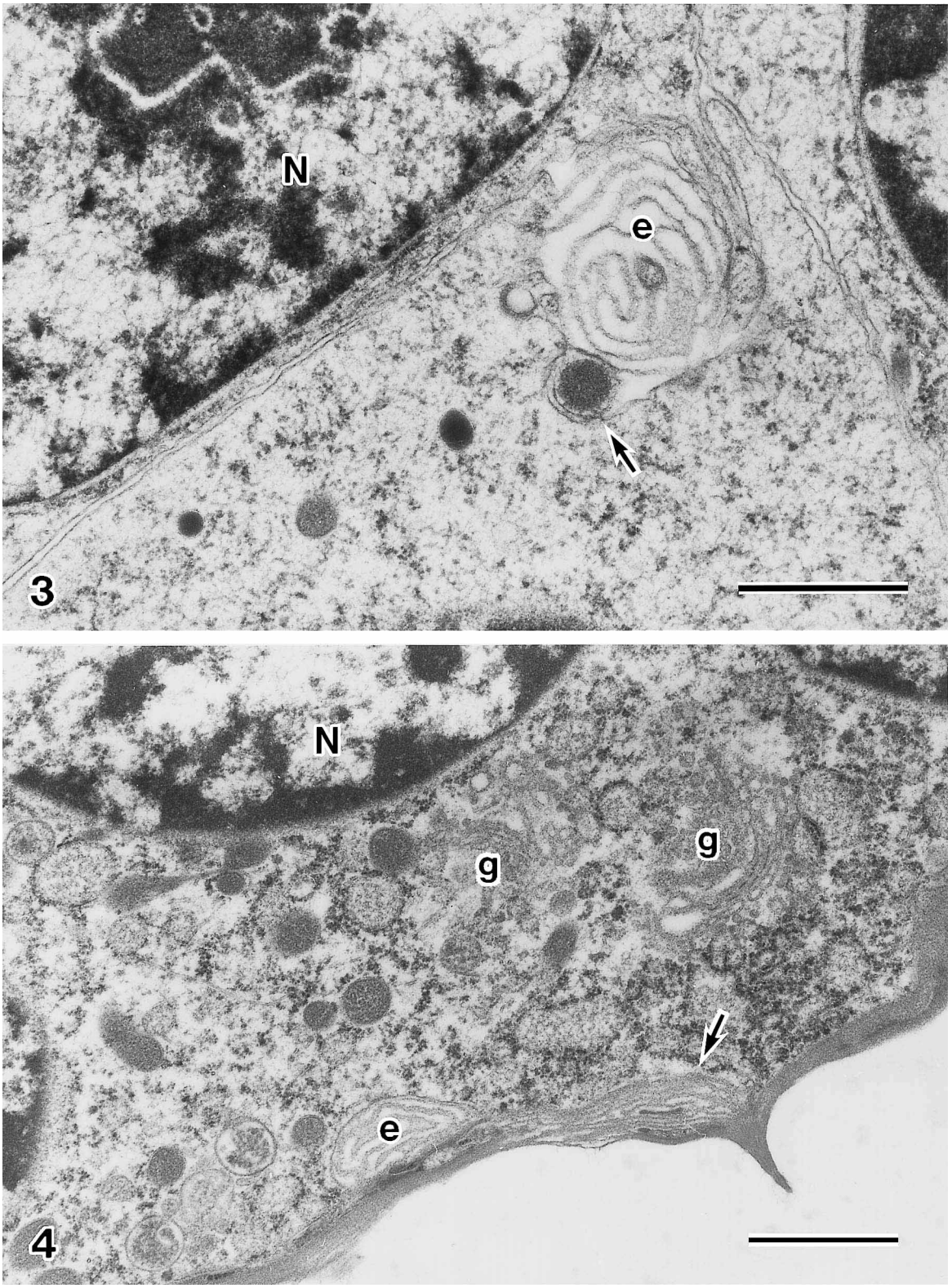

Fig. 3. Central region of an androgenic gland. A secretory granule (arrow) is found inside the intercellular canaliculus (e) located in the crossing of cells. N, Nucleus. Bar=1 μm.

Fig. 4. Peripheral region of an androgenic gland. A sheath is separated into each membranous component (arrow) near the intercellular canaliculus (e). The Golgi complex (g) is shown between nucleus (N) and a sheath. Bar=1 μm.

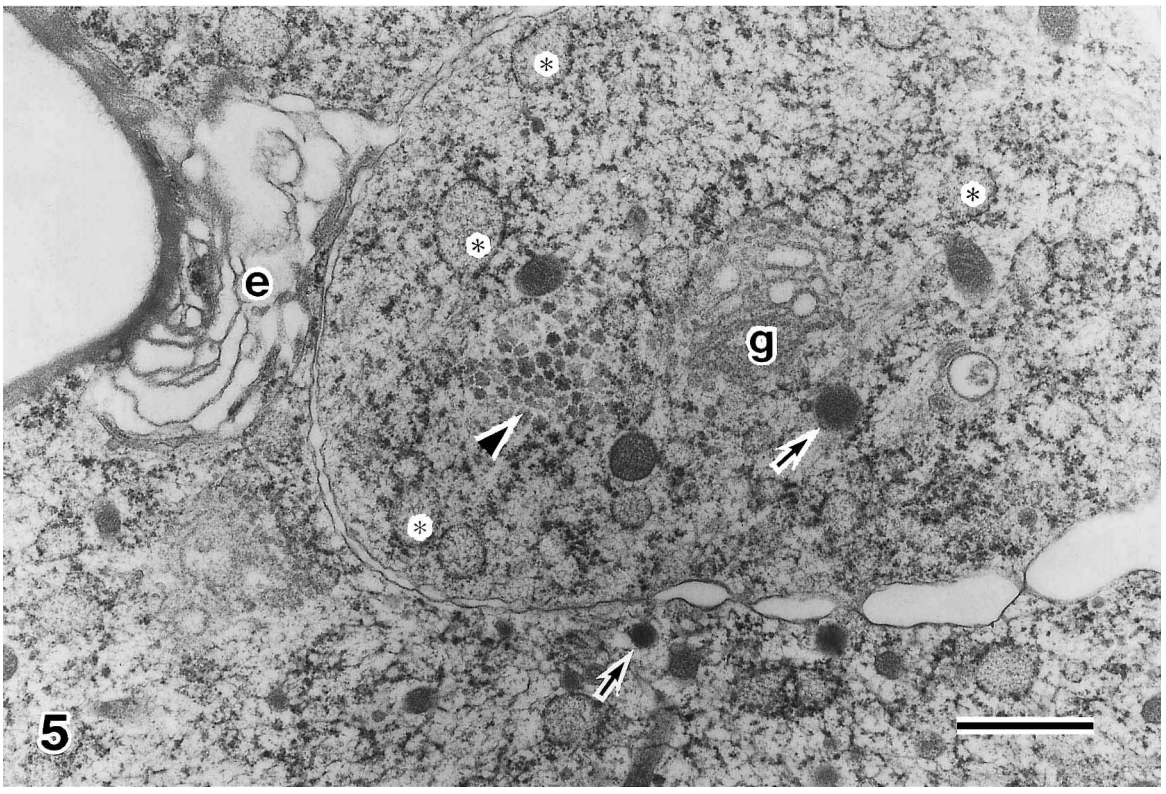

Fig. 5. Typical intercellular structures of an androgenic gland. Particular structure of intercellular canaliculus (e) beside a sheath is connecting with cell membranes forming a variety of intercellular junctions. Secretory granules (arrows), a cluster of glycogen granules (arrowhead) associated with the Golgi complex (g) are found among a large number of rough endoplasmic reticulum (asterisks). Bar=1 μm.

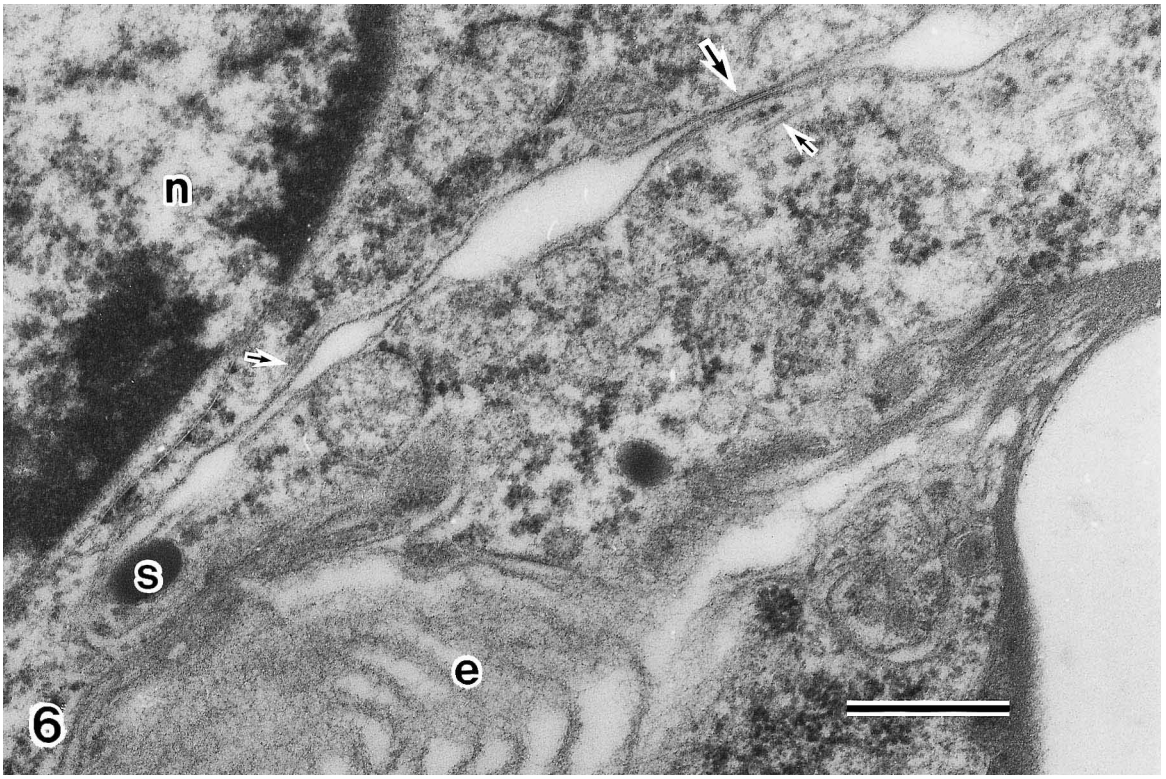

Fig. 6. Gap junction of intercellular membranes. A gap junction (arrow) is found as a part of intercellular structures. Microtubules (small arrows) distribute along the junction. A secretory granule (s) is between particular structure of intercellular canaliculus (e) and intercellular membranes. n, Nucleus. Bar=500 nm.

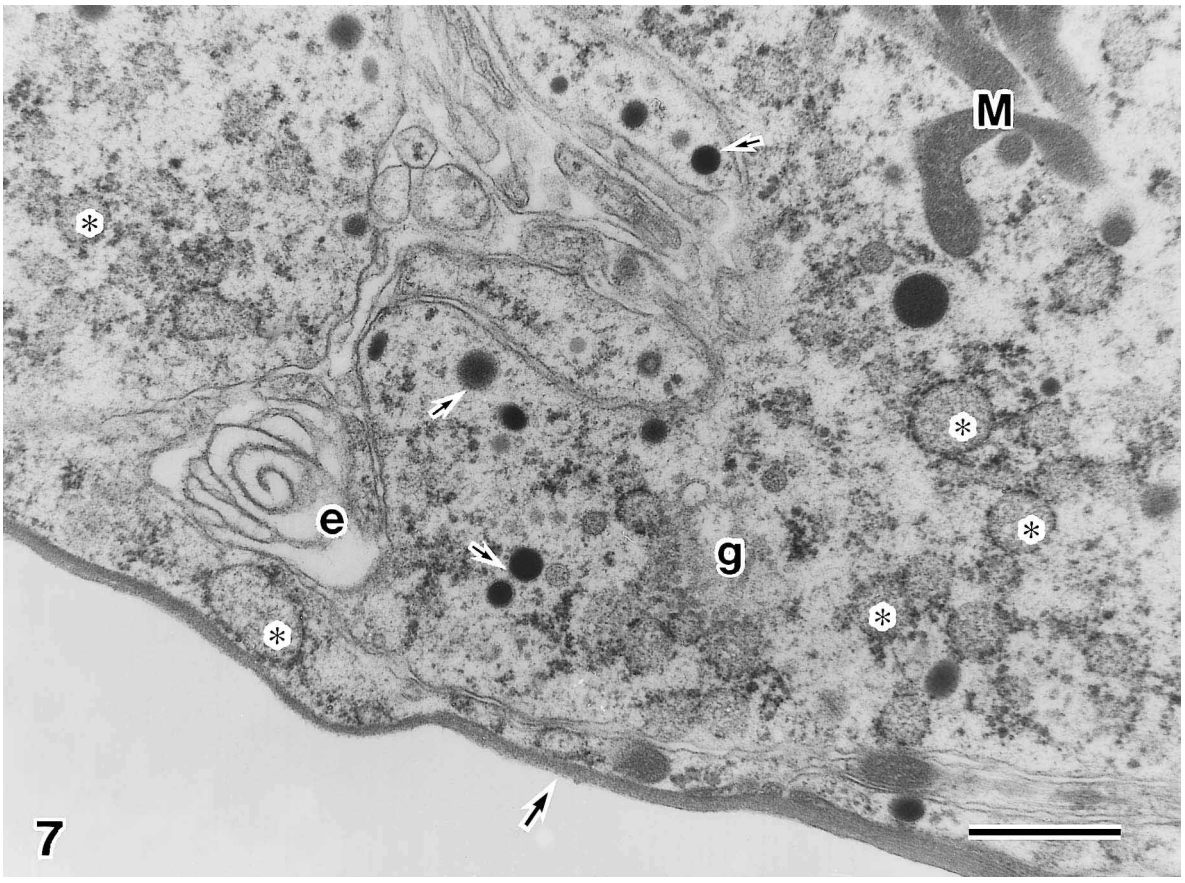

Fig. 7. Periphery of the middle region of an androgenic gland. Numerous secretory granules (small arrows) distribute inside or outside the interdigitated structure connecting with particular structure of intercellular canaliculus (e) beside a sheath (arrow). Large mitochondria (M) and a number of rough endoplasmic reticulum (asterisks) are found besides the Golgi complex (g). Bar=1 μm.

lar junctions. Gap junctions are found in many intercellular adhesions, which may serve to transfer the molecules or provide the spread of contractile depolarization (Fig. 6). The marginal bundle of microtubules, that lies around the periphery of the cell, may be involved in the secretory activity.

The middle region of the androgenic gland, which is 200–300 μm from the apex region, is rich with secretory granules of 100–300 nm in diameter (Fig. 7). Mature secretary granules are seen in association with condensing vacuoles and the Golgi complex. Cell membranes are often interdigitated especially in the peripheral region of the gland. Some of the secretory granules are frequently found in the interdigitated area.

DISCUSSION

In the present study, the rapid-freezing and freeze-substitution method was found to be effective in the preservation of the fragile structures within the androgenic gland. The secretory granules containing electron-dense materials are shown to be distributed in a wide area of the androgenic gland cells. As these granules have the same structure as the secretory granules containing proteinous substances (Fawcett, 1966), they are very likely to be the secretory granules containing the androgenic gland hormone, which was recently revealed to be a peptide hormone (Hasegawa *et al.*, 1987; Martin *et al.*, 1990; Okuno *et al.*, 1997), and reported to possess a glycosylation site in the molecule (Okuno *et al.*, 1999). Some secretory granules are found close to the Golgi complex in association with abundant rough endoplasmic reticulum containing low electron-dense materials, which are supposed to be secretory substances such as the androgenic gland hormone. A large number of small vesicles are distributed around the Golgi cisternae, and glycogen clusters are also found near the Golgi complex. These cytoplasmic structures are partly similar to the figures on *Rhithropanopeus harrisii* (Payen *et al.*, 1971) and common in secretory cells (Fawcett, 1994). These constitutions in the cytoplasm of the androgenic gland of the *Armadillidium vulgare* probably serve for the biosynthesis of the androgenic gland hormone having a glycosylation site (Okuno *et al.*, 1999).

The most notable structures in the androgenic gland are a particular arrangement of highly developed intercellular canaliculi. In secretory organs, it is generally observed that intercellular or intracellular canaliculi are involved in the secretion of hormones (Fawcett, 1994). These structures, in the androgenic gland of *Armadillidium vulgare*, are found to be located between the cells and connected with the cell mem-

branes. The particular structure of intercellular canaliculi are therefore likely to take part in transporting the secretory granules containing the androgenic gland hormone into the extracellular space in association with the canaliculi formed by the cell membranes. The particular structures exhibit a round shape in the cross point of the cells, and a long extended shape in the periphery of the cell. The latter seems to be consistent with the result (King, 1964) that the cell membranes were frequently highly interdigitated in the peripheral region. The intercellular space, which is specialized to form cell junctions as described on the shrimp *Penaeus japonicus* (Payen *et al*., 1982), is presumably the duct for secreting materials. When the role of the intercellular junctions is considered in comparison with that of other secretory cells, the space may be suitable to be called the secretory canaliculi (Junquiera *et al*., 1992).

The secretory mechanism of androgenic gland hormone in the terrestrial isopod *Armadillidium vulgare* has been unclear for a long time, because the cytoplasmic structure characteristic of secretory organs was rarely found in the *Armadillidium vulgare*. In the present study, however, the highly organized structures composed of rough endoplasmic reticulum, Golgi complex and particularly developed intercellular canaliculi, are revealed to be involved in exporting the secretory granules in association with the intercellular junction. This result suggests that, in the *Armadillidium vulgare,* the particular structure of intercellular canaliculi connecting the cellular junctions in the androgenic glands function as secretory canaliculi to transport the secretory granules into the extracellular space. The androgenic gland hormone has been recently clarified to be a peptide (Martin *et al*., 1999; Okuno *et al*., 1997, 1999), and the particular structure was expected to exist in the organ to secrete the hormone. The present study has revealed that the same cytoplasmic structure as shown in other secretory cells containing peptide hormones (Fawcett, 1994) is possibly involved in the exocytotic secretion of the androgenic gland hormone of *Armadillidium vulgare.* This result is consistent with the result suggested in the *Sphaeroma serratum* (a sea water isopod) that the androgenic hormone may be released by exocytosis (Martin *et al*., 1996). Thus, the present result reveals that the androgenic gland hormone of *Armadillidium vulgare* is probably released into extracellular space via the same secretory mechanism as other peptide hormones enclosed in secretory granules in association with rough endoplasmic reticulum and Golgi complex (Fawcett, 1994).

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