Secretory Granules in the Renal Tubules of Male Freshwater Sculpin, *Cottus hangiongensis*, during the Spawning Period

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It has been generally recognized that the kidneys of fishes have the functions of excretion of waste materials, reabsorption and adjustment of osmotic changes in the living body (Hickman and Trump, 1969). It is further known that the kidneys of male sticklebacks, *Gasterosteus aculeatus* and *Pungitius pungitius*, become large and secrete mucous substances during the spawning period for construction of the nest (Möbius, 1885; Van Oordt, 1923; Ikeda, 1933). Secretory granules were recognized in the renal tubules in the kidneys of some marine fish such as marine catfish, *Plotosus anguillaris* (Ogawa, 1959). Recently, Goto (1974) reported that adult males of a freshwater sculpin, *Cottus hangiongensis*, had hypertrophied kidneys during the spawning period. In this case, the hypertrophied kidney was mainly composed of a large number of thick renal tubules and narrow lymphoid tissues, and in the epithelial cells of the proximal convoluted segment the presence of PAS positive substances was confirmed. He supposed that these substances might be produced in the proximal convoluted segment and secreted to the tubular lumen. The results, however, were obtained only from the histological and histochemical observations by light microscopy, therefore, detailed observations on the inner structure of the renal epithelial cells seem inevitable in order to know the significance of the substances.

In the present study, the ultrastructure of the renal epithelial cells in *C. hangiongensis* was observed by electron microscopy to clarify the morphological characteristics of the PAS positive substances.

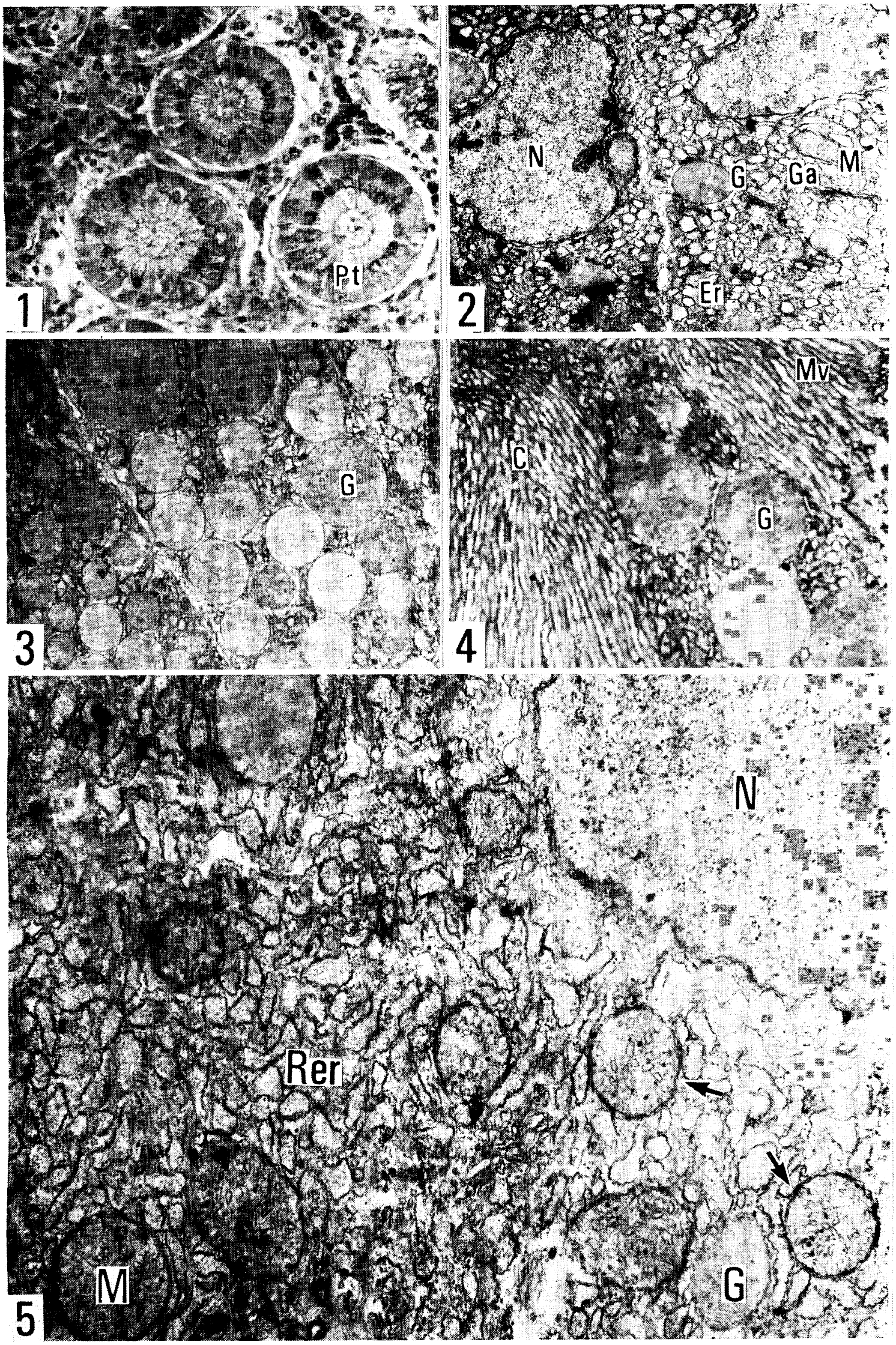
Materials and methods

Collections of the freshwater sculpin, *Cottus hangiongensis*, were made monthly from April to December in 1978, in the Daitobetsu River near Hakodate, Hokkaido. Only adult fish (27 males, 90～143 mm SL; 27 females, 82～119 mm SL) judged according to the criterion of Goto (1977) were selected from total catches caught with a dip net, and dissected to take out their kidneys. For light microscopic observations, the kidneys were fixed monthly with Helly's fluid. Serial tissuemat 7 μm sections of the kidney were made, and stained with Delafield's hematoxylin and eosin. In addition, small pieces of kidneys of fish sampled in April, the spawning period, and in September, the resting period, were fixed with 2% glutaraldehyde in phosphate buffer (pH 7.4) and postfixed with 1% osmium tetroxide for the electron microscopic observations. Ultrathin sections were stained with lead monoxide and examined with a Hitachi HS-7 electron microscope.

Observation

Light microscopic observations showed that

Fig. 1. Photomicrograph of renal tubules in a male kidney during the spawning period (April). Pt, proximal convoluted segment. ×410. Fig. 2. Electron micrograph of the basal region of the epithelial cell of the proximal convoluted segment in a male kidney during the spawning pericd (April). Some granules (G) scatter in the cytoplasm. Er, endoplasmic reticulum; Ga, Golgi apparatus; M, mitochondrion; N, nucleus. ×7000. Fig. 3. Electron micrograph of the central region of the epithelial cell of the proximal convoluted segment in a male kidney during the spawning period (April). Numerous granules (G) are present in the cytoplasm. ×8300. Fig. 4. Electron micrograph of the surface region of the epithelial cell of the proximal convoluted segment in a male kidney during the spawning period (April). A cytoplasmic protrusion in which some granules (G) are contained is observed. C, cilium; Mv, micro villus. ×8300. Fig. 5. Electron micrograph showing the perinuclear region of the epithelial cell of the proximal convoluted segment in a male kidney during the spawning period (April). Arrows indicate the intermediate between mitochondrion and granule. G, granule; M, mitochondrion; N, nucleus; Rer, rough-surfaced endoplasmic reticulum. ×35700.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the renal tubules of the kidneys of adult males became hypertrophied during the spawning period (April, Fig. 1) and atrophied during the resting period (July to September). In contrast, the kidneys of adult females scarcely changed in size and structure throughout the observation periods.

Under electron microscopy, the epithelial cells in the proximal convoluted segment in males were remarkably thick and had irregular-shaped nuclei situated at a slightly basal part of the cells. Rough endoplasmic reticula were frequently found redundantly in the cytoplasm of these cells. They were vesicular in shape and appeared mainly in the basal region of the cells (Fig. 2). Elliptic mitochondria having tubular cristae were scattered in the basal cytoplasm. The Golgi apparatus often appeared in the cytoplasm between the nucleus and the basement membrane. Many dense granules of various sizes (0.4～2.0 μm in long axis) were also observed in the cytoplasm (Fig. 3). They were oval in shape, and increased in number and size toward the tubular lumen (Fig. 4). It was observed with higher magnification that the granules were covered with a double structured membrane, and filled with numerous particulate materials. Some particles situated at the basal cytoplasm showed morphological transitions between mitochondria and granules (Fig. 5). These particles had a double structured membrane, and contained some vesicular cristae and a lot of particular materials suggesting the granules originate from the mitochondria. The largest granules were about 2 μm in long axis, and often observed as partly projected to the tubular lumen (Fig. 6). The free surface of the cell projected to the tubular lumen as cytoplasmic protrusions of papillar shape, as described by Ogawa (1959). On the free surface of the cells, there appeared a large number of micro villi and cilia, about 3.6 μm in length. On the other hand, in the epithelial cells of the distal convoluted segment the granules described above were not recognized at all, though many tubular smooth-surfaced endoplasmic reticulum and mitochondria in various shapes were found surrounding the nucleus.

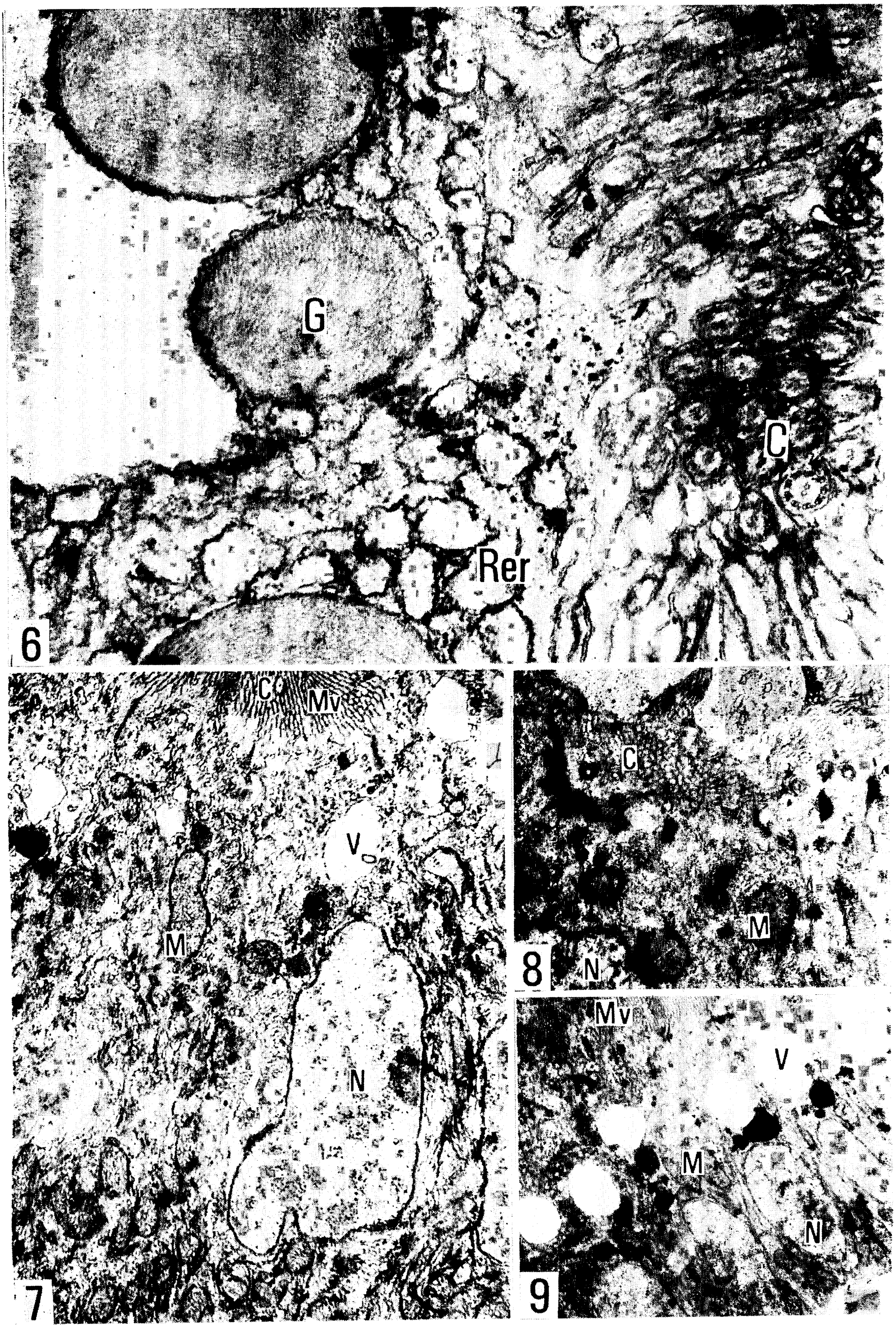
In the kidneys of adult females in April, the epithelial cells of the proximal convoluted segment were remarkably low in thickness when compared with those of males in the same month. The nuclei were elliptical in shape in many cases and situated at the basal cytoplasm. The mitochondria were scattered throughout the cytoplasm and the rough endoplasmic reticulum were remarkably small in amount. On the free surface of the cells, there appeared numerous micro villi and cilia of short length. No granules were observed in any cells of the epithelium of the proximal convoluted segments of the female kidneys (Fig. 7).

In male kidneys in September corresponding to the resting period of reproduction, the epithelial cells of the proximal convoluted segment became remarkably thin (about 17 μm) when compared with those (about 33 μm) in the male kidneys during the spawning period, and the free surface of the cells showed smooth contours without any cytoplasmic protrusions (Fig. 8). In the female kidneys during the same month, the epithelial cells of the proximal convoluted segment became atrophic and contained many numbers of vacuoles near the free surface of the cells (Fig. 9). In both sexes in September, the epithelial cells contained no granules in the cytoplasm.

Discussion

In a freshwater sculpin, *Cottus hangiongensis*,

Fig. 6. Electron micrograph showing secretory granules on the free surface of the epithelial cell of the proximal convoluted segment in a male kidney during the spawning period (April). The granules (G) project into the tubular lumen. C, cilium; Rer, rough-surfaced endoplasmic reticulum. ×31400. Fig. 7. Electron micrograph of the epithelial cell of the proximal convoluted segment in a female kidney during the spawning period (April). C, cilium; M, mitochondrion; Mv, micro villus; N, nucleus; V, vacuole. ×6300. Fig. 8. Electron micrograph of the epithelial cell of the proximal convoluted segment in a male kidney during the resting period (September). C, cilium; M, mitochondrion; N, nucleus. ×9300. Fig. 9. Electron micrograph of the epithelial cell of the proximal convoluted segment in a female kidney during the resting period (September). M, mitochondrion; Mv, micro villus; N, nuclues; V, vacuole. ×3600.


G
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6
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Mv
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7
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9

the granules were found in the kidney during their spawning period. These granules appeared only in males in the epithelial cells of the proximal convoluted segment. Goto (1974) already reported that in males of the same species the epithelial cells of the segment contained PAS positive materials only during the spawning period. Therefore, the granules observed in the present study are considered to be corresponding to PAS positive materials.

The granules were small in size in the basal cytoplasm and became larger toward the inner free surface of the epithelial cells. The largest granules were often observed on the most inner surface as partly projected into the tubular lumen. These features suggest that the granules originate in the basal region of the epithelial cell, growing in size as going up toward the free surface of the cells, and finally they were secreted into the tubular lumen. Grafflin (1937) first reported that in fish there were a large number of granules in the epithelial cells of the proximal convoluted segment in the eel, *Anguilla rostrata*. Ogawa (1959) also observed in the kidneys of marine catfish that the epithelial cells in the proximal convoluted segment contained a lot of large granules stained with iron-hematoxylin and the free surface of the cells often remarkably projected as a papillar shape. As to the origin of the granules, he supposed that they seem to be derived from mitochondria. The present results based on the electron micrographs of the formation of the granules clearly indicate mitochondria origins.

Up to date, it has been demonstrated that the segments of nephron are provided with cilia in some fish, such as marine catfish (Ogawa, 1959), English sole (Bulger and Trump, 1968), bluegill and northern squawfish (Hickman and Trump, 1969). The brush border in the proximal convoluted segment of *C. hangiongensis* was constituted with micro villi and cilia. The presence of the cilia may suggest the transportation of granular substances secreted from the epithelial cells, because it is generally recognized that cilia propel a constant stream of mucus or other secretory materials in the living body. It is noteworthy in relation to this function that the most developed cilia were observed in the male kidneys during the spawning period.

The biological function of the granular substances produced in the kidneys of *C. hangiongensis* is yet unknown. Ogawa (1959) considered that the granules in the kidneys of marine catfish might be correlated with absorptive functions of the epithelium. In the freshwater sculpin, however, it may be unreasonable to consider that the granules take part in reabsorption, because the granules are contained only in the kidneys of the male fish during the spawning period. Preferably, the granules secreted may have some roles with relation to their spawning behavior, as in sticklebacks, where mucous substances produced in the male kidneys are used to construct the spawning nests. The granule function may be common in the freshwater sculpins of the genus *Cottus*, because it has been confirmed that adult males of two types of *C. nozawae* have hypertrophied kidneys during their spawning periods (Goto, unpublished).

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淡水産カジカの産卵期の雄成魚の腎細尿管にみられる顆粒について

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電子顕微鏡観察によって，カンキョウカジカ *Cottus hangiongensis* の産卵期の雄成魚の腎臓の近位細尿管上皮細胞中に，卵形をした顆粒が多数存在することが認められた．顆粒は，細胞基底部では小形（約 0.4 μm），で数も少ないが，細尿管内腔に向かうに従って大形（最大約 2 μm）になり，数も増加した．内腔に面した上皮細胞の自由面は，絨毛 (micro villi) と繊毛 (cilia) から構成された刷子縁によって縁どられていた．刷子縁には数多くの舌状突起がみられ，突起中には，しばしば大形の顆粒が内腔に突出した状態で存在するのが観察された．

以上の結果から，これらの顆粒は，近位細尿管上皮細胞の基底部で産生され，内腔に移行しつつ生長し，最終的には分泌顆粒となって内腔に排出されるものと推測される．顆粒の起源については，細胞基底部でミトコンドリアからの移行像が観察されたことから，この顆粒はミトコンドリアに由来するものと推察される．また，その機能に関しては，カジカ属魚類の産卵習性に何らかの役割を有している可能性がある．

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