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Light and Transmission Electron Microscopy of the Granular Cell in the Skin Epidermis of a Cottid, *Pseudoblennius cottoides*

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Abstract The granular cell in a skin epidermis of a cottid fish, *Pseudoblennius cottoides*, is a voluminous one having coarse or finely granular contents which are acidophilic and may be regarded as containing basic and aromatic amino-acid groups from preliminary tests for protein histochemistry. This cell is mostly buried in the middle layers of the skin epidermis, and seems to attain the mature condition through graded developmental stages similar to those described by Thomson (1969) in a boxfish, *Ostracion meleagris*. Electron microscopy reveals that this cell at the developmental stage III is occupied by an enormous central vacuole containing relatively high dense granules without membrane-bounded structure. This vacuole is uniformly surrounded by several layers of larger vesicles having granules closely alike to those found in the central vacuole. The enormous central vacuole may probably be formed by successive ruptures of the vesicles. Considerably complicated interdigitations are visible between the granular cell and the filament-containing cell. The characteristics of integumentary structures common to the teleosts endowed with the granular cells remain to be solved. The function of this cell is also discussed.

The granular cell is one of voluminous unicellular glands found in the skin epidermis of some bony fishes, and is generally sac-like structure having coarse or finely granular contents. This cell has hitherto been investigated by various authors under various names: sackformige seröse Drüse (Studnicka, 1906; Pawlowsky, 1911); Körnerdrüse (Rauther, 1907); offener Kolben (Hase, 1911); granulated cell or sac-cell (Bhatti, 1938); cellule sacciforme (Bertin, 1958); eosinophilic vesicle (Satō, 1967); granular cell (Henrikson and Matoltsy, 1968); club cell (Thomson, 1969); sacciform gland (Mittal and Munshi, 1971). Recently, Mittal and Banerjee (1975) studied this cell of a murrel, *Channa striata*, histologically and histochemically, and proposed a new term "sacciform granulated cell."

The granular cell is found mainly in the skin of the fishes belonging to suborder Siluroidei. The occurrence of this cell, however, is not restricted in a special group, but is considerably widespread in fishes. Pawlowsky (1911) regarded the granular cell as belonging to the category of albumen glands containing albumen contents, and Rauther (1907) suggested that this cell provides albumen-containing secretion which may have poisonous properties. According to Mittal and Banerjee (1975), the contents of this cell are basic proteins which constitute a thick coat of slime together with mucopolysaccharides and lipids. On the other hand, Thomson (1969) suspected the production of ostracitoxin by the granular cell (club cell named by him) in a boxfish, *Ostracion meleagris*. The nature and function of this cell, however, have not been ascertained fully. Thomson (1969) performed also histological observations and divided the developmental process of this cell into five graded stages. The ultrastructure of this cell was examined by Henrikson and Matoltsy (1968), but they made no reference to the relation between the formation of granules and cytoplasmic organelles of this cell.

In the previous paper (Satō, 1967), the histological structure and staining reaction to some common histological dyes were examined on the granular cells in three cottids. The present report attempts to further characterize this cell by results obtained from light and transmission electron microscopy and from some histochemical tests, mainly protein histochemistry, on the granular cell of a littoral cottid.

Material and methods

Specimens, *Pseudoblennius cottoides* (Richard-

son), used were of about 10 cm in total length, collected from the seashore of Fukauramachi, Aomori Prefecture. For routine histological observation, skin fragments of about 5×5 mm were cut from various parts of the head and the trunk, and were fixed in Bouin's fluid, Zenker's fluid and Zenker-formol. Paraffin sections, cut at 8 μm, were stained with Delafield's haematoxylin and eosin, Heidenhain's azan triple stain, and Mallory's triple stain. The histochemical methods employed to identify general proteins and amino-acid groups were as follows: ninhydrin-Schiff method for protein-bound NH_2 (Yasuma and Ichikawa, 1953), deamination/ninhydrin-Schiff method (Lillie, 1954), chloramine-T Schiff method for protein-bound NH_2 (Burstone, 1955), deamination/chloramine-T Schiff method (Lillie, 1954), coupled tetrazonium reaction (Pearse, 1968), tryptophane method for formalin-fixed tissue (Adams, 1960), rosindole reaction for indols (Glenner, 1957), Sakaguchi oxine reaction for arginine (Caver, Brown, and Thomas, 1953), DDD reaction for SH groups (Barrnett and Seligman, 1952), and ferric ferricyanide method for SH (Chèvermont and Fréderic, 1943). These histochemical tests were done according to the instructions in Pearse (1960, 1968) and Lillie (1954). Most of these tests were performed in paraffin sections, but a few of them in frozen sections, cut at 20 μm using an American Optical Company cryostat.

For electron microscopy, small pieces of head skin approximately 1.5 mm^3 were fixed for two hours in cold 1% osmium tetroxide buffered at pH 7.3 with Millonig's phosphate (Millonig, 1962) or with Benett and Luft's s-collidine (Benett and Luft, 1959). Some of the pieces fixed in s-collidine buffered osmium tetroxide solution were exposed to block staining by 3% aqueous solution of uranyl acetate for 30 minutes to one hour. Dehydration was accomplished rapidly in a series of graded ethanols, and then embedded in Epon 812, through two changes in propylene oxides. Thin sections were cut on a Porter-Blum MT-I ultramicrotome and stained first with 3% aqueous solution of uranyl acetate and then with lead hydroxide (Millonig, 1961) or lead citrate (Reynolds, 1963). Observations were made on a Hitachi HS-7D electron microscope.

Results

Histology. The skin epidermis of this fish, as with other bony fish, may be divided into three layers: the basal layer (stratum germinativum), the middle layer and the outermost layer. The granular cells are mostly buried in the middle layer of the epidermis, and are generally oval or pear-shaped, with variable sizes ranging from about 10 to 30 μm in basal diameter (Fig. 1). As reported in the previous paper (Satō, 1967), the contents of these cells are stained intensely with such acid dyes as eosin, orange G, acid fuchsin and azocarmine B. Epidermal cells being present between the granular cells are vertically elongated as a result of the lateral pressure of the latter cells. The granular cells occur in the skin epidermis of almost the whole body, but they are scanty in fins. Mucous cells are also found in the superficial and middle layer of the skin epidermis (Fig. 1, B). Between the cells of the basal layer are found irregular lymphatic spaces having small lymphocytes (Fig. 1, B and C).

Thomson (1969) described five graded stages (I～V) in the development of boxfish club cell which may be identical with the granular cell. In the present investigation, however, all of the five stages mentioned above could not be found, but the graded stages roughly corresponding to the stage II and III of Thomson were observed. A granular cell marked with Roman numerals I～II in Fig. 1, A seems to be present at a transition from the stage I to II, because this cell has a spherical nucleus and locates at the basal layer of the epidermis. All granular cells indicated by III in Fig. 1 may be present at the stage III, because they have coarse granules which are accumulated at the apical part below a thick cap of homogenous, colloidal material at the apex, and their nucleus is somewhat flattened against the basal part of the cell, owing to the accumulation of granular materials. They have generally a short and narrow neck which seems to open to the exterior. Most of the cells at this stage occupy the entire height of the epidermis. A granular cell marked with III～IV in Fig. 1. C seems to be present at a transition between the stage III and IV, because the contents of this cell is mostly composed of homogenous colloidal product.

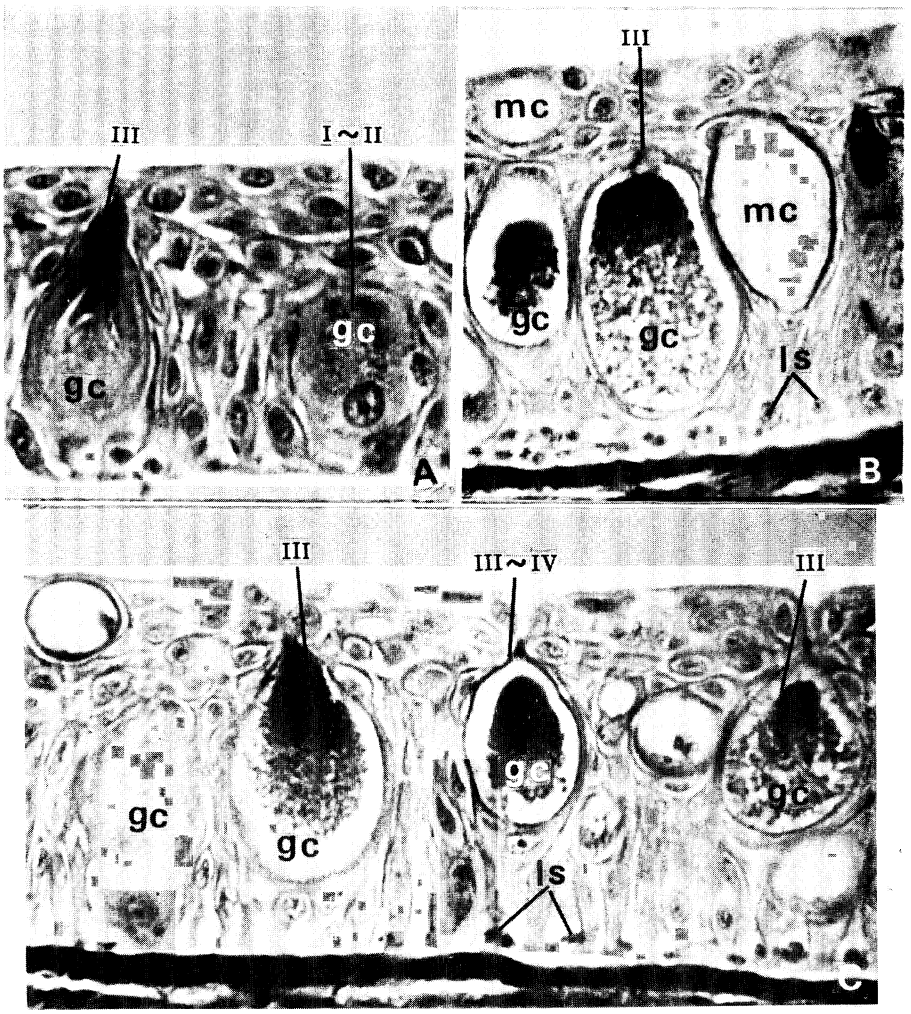

Fig. 1. A, B, and C. Cross section of the head skin of a cottid, *Pseudoblennius cottoides*, showing granular cells at various developmental stages and the structure of the epidermis. ×ca. 600. gc, granular cell; ls, lymphatic space; mc, mucous cell. Roman numerals (I~II, III, and III~IV) indicate the developmental stage of the granular cell.

Fine structures. Following descriptions are based on the granular cell at the stage III or III~IV. In these stages, major part of the cell is occupied by an enormous central vacuole within which are scattered relatively high dense granules. These granules are not bounded by membranes, and have variable sizes and forms (Fig. 2). The remainder of the protoplasm of this cell is compressed between the central vacuole and the cell membrane. The central vacuole is uniformly surrounded by some layers of larger vesicles which are buried in the compressed cytoplasm and contain granules closely resembling those found in the central vacuole (Fig. 2). Some of the larger vesicles being present in contact with the central vacuole may often be seen to rupture and to release their granules into the central vacuole (Fig. 3, A and B). From these electron micrographic figures, it may be supposed that some vesicles containing granules appear in the perinuclear cytoplasm at first, and then release their granules into spaces formed by rupture of the vesicles, followed by gradually increasing sizes of the spaces owing to successional ruptures of the vesicles, and finally occupy the major part of the cell. In the cell at the stage III~IV, upper

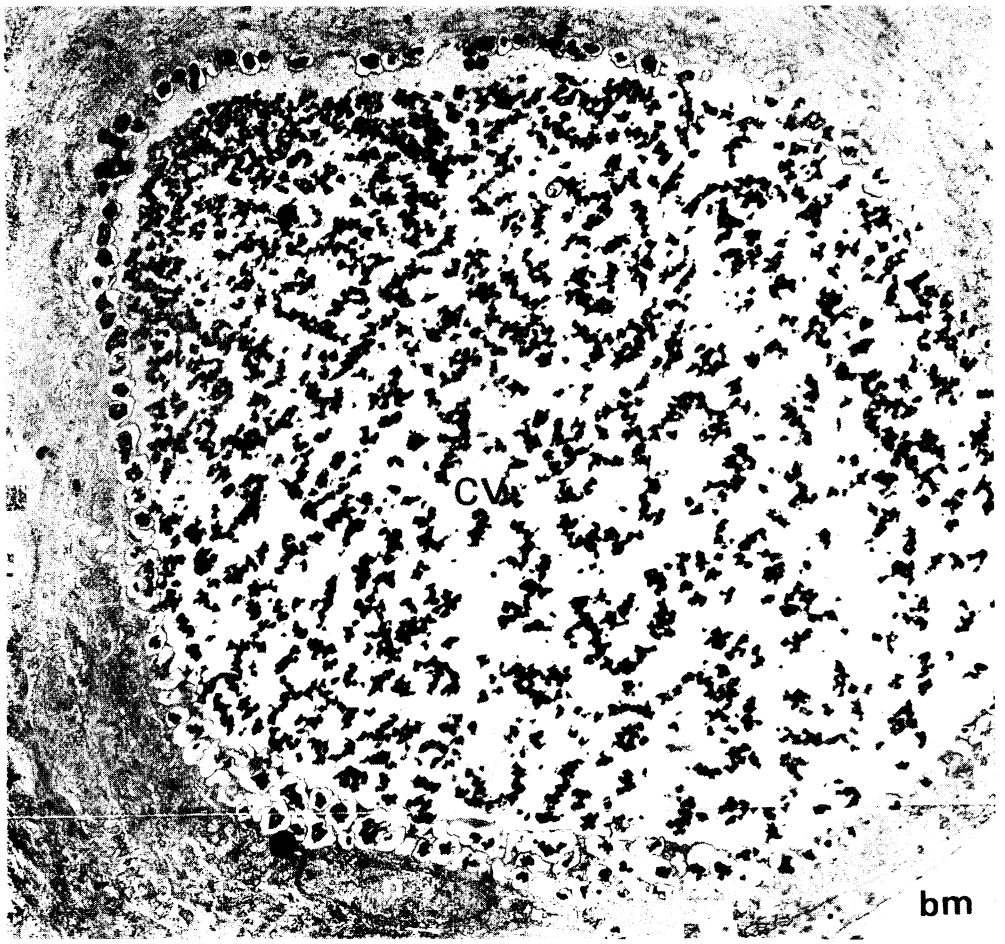

Fig. 2. Vertical section of the granular cell at the stage III, showing an enormous central vacuole (cv) surrounded by larger vesicles, and a nucleus (n) flattened against basal part of the cell. ×4,000. bm, basement membrane.

Table 1. A summary of histochemical tests showing the protein nature of the granular cell of *Pseudoblennius cottoides*. +, positive; −, negative; ±, weakly positive.

Technique employed	Fixative and section	Reaction
Ninhydrin-Schiff	Carnoy, Zenker, paraffin	+
No treatment Schiff	Carnoy, Zenker, paraffin	−
Deamination/ninhydrin-Schiff	Carnoy, Zenker, paraffin	−
Chloramine-T Schiff	Carnoy, Zenker, paraffin	+
Deamination/chloramine-T Schiff	Carnoy, Zenker, paraffin	−
Coupled tetrazonium reaction	10% neutral formalin, paraffin	+
Tryptophan method	10% neutral formalin, paraffin	+
Rosindole reaction	10% calcium acetate-formalin, paraffin	+
Sakaguchi oxine reaction	Bouin, Carnoy, paraffin	+
DDD reaction for SH group	Trichloroacetic-ethanol, paraffin	−
Ferric ferricyanide method for SH	10% formalin, frozen	±

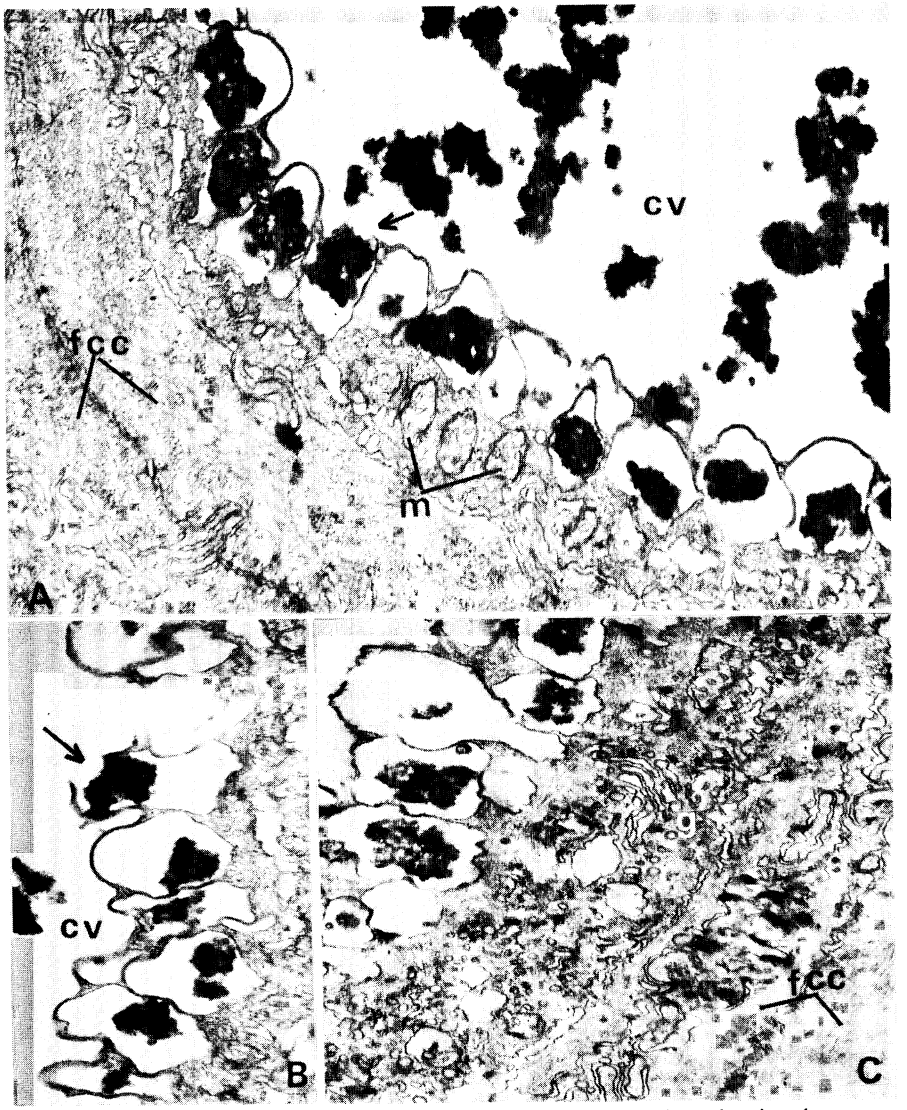

Fig. 3. A. Portions of the granular cell and the filament-containing cell (fcc), showing the rupture of the larger vesicle (arrow), mitochondria (m) and other cytoplasmic organelles of the granular cell. Considerably complicated interdigitations are visible between these two cells. ×20,000. B. Portion of the granular cell, showing the rupture of the larger vesicle (arrow). ×20,000. C. Portions of the granular cell and the filament-containing cell, showing Golgi complex (g) and other cytoplasmic organelles of the granular cell. ×20,000. cv, vide Fig. 2.

half of the central vacuole is occupied by fine fibrils which seem to correspond to the homogenous colloidal product observed by light microscopy. Numerous mitochondria, a few of Golgi complexes, rough endoplasmic reticula, and free ribosomes are discernible in the compressed cytoplasm (Fig. 3, A and C). A considerable number of the same cytoplasmic filaments as those found in filament-containing cells are also visible. As stated already, the flattened nucleus is situated at the basal part of the granular cell (Fig. 2). Considerably complicated interdigitations are seen between the granular cell and the filament-containing cell (Fig. 3, A). A few of desmosomes are also found in junction of these two cells, where no interdigitations are

observed.

Protein histochemistry. Since the contents of the granular cell do not give histochemical reactions for mucopolysaccharides (Satō, 1967; Mittal and Banerjee, 1975), protein histochemistry was performed as a preliminary test in this investigation. The results obtained are summarized in Table 1.

The positive colour reactions of the granular cells to ninhydrin-Schiff and chloramine-T Schiff methods are blocked completely by deamination. This shows the presence of protein-bound NH_2 groups in their contents. The colour reaction mentioned above is not due to free aldehyde. The granular cells show also positive reactions to the coupled tetrazonium reaction, the tryptophan reaction, the rosindole reaction, and the Sakaguchi oxine reaction, suggesting the presence of aromatic and basic amino-acid groups. The existence of sulphur-containing amino-acid groups, however, could not be ascertained, because of unstable reactions for sulphydral groups.

Discussion

According to results obtained by Studnicka (1906), Rauther (1907), and several other authors, the granular cells are detected in species from such diverse orders as Cypriniformes, Channiformes, Mastacembeliformes, Synbranchiformes, Tetraodontiformes, Cottiformes, and Gobiesociformes. Viewed from the integumental structures, it is not clear whether or not characteristics common to the bony fishes endowed with the granular cells are present, because these fishes show considerable variations in their integument, viz., some of them are covered with dermal scutes, spineless carapase or scales, whereas others are naked. Phylogenitical significance of this cell also cannot be discussed until the fishes provided or unprovided with this cell have been examined more fully. So far as cottid fishes concerned, an inverse relationship between the occurrence of the granular cells and the abundance of the mucous cells, even though there is such an exception as *Cottus nozawae*, was suggested in the previous paper (Satō, 1967). *P. cottoides*, however, is endowed with the granular cells and with a considerable number of small and large mucous cell. Mittal and Munshi (1971), on the other hand, reported that *Amphipnous cuchia* has both a good number of granular cells and numerous elongated mucous cells. Thus, the inverse relationship mentioned above does not seem to be tenable.

According to Bhatti (1938), the contents of the granular cell of *Otocinchus nigricauda* are almost wholly homogenous in early stages, but as soon as the cell opens to the exterior, the plasma is transformed into granular substance, the nucleus shrinks towards the basal wall of the cell, and whole cell seems to decrease in size. Thomson (1969), however, observed that the granular cell (club cell named by him) of the boxfish, *Ostracion meleagris*, passes through I～V stages of development and its coarse granular contents transform into homogenous colloidal material which finally may be extruded to the exterior. In the granular cell of *P. cottoides*, all of I～V stages of the development were not observed, but II, III, and III～IV stages were most frequently encountered. The granular cell at the stage IV was observed in a cottid, *Blepsias cirrhosus draciscus* (Satō, 1967). Accordingly, the granular cell of the cottid fishes, at least, presumably passes through graded developmental stages similar to those described by Thomson (1969).

Electron microscopy of the granular cell of *P. cottoides* shows that the fine structure of this cell is very similar to that observed by Henrikson and Matoltsy (1968) in the cell of *Corydoras*, and furthermore suggests that an enormous central vacuole occupying the major part of this cell is formed by successive ruptures of larger vesicles which are buried in the cytoplasm of the cell and contain granules. Where do the granules originate? Since the stage corresponding to the stage I of Thomson has not been investigated in *P. cottoides*, the granular formation in very early stage is unknown. However, it is expected that the formation of granules may be closely related to Golgi complex of this cell.

Mittal and Banerjee (1975) reported that the contents of the granular cell (sacciform granulated cell named by them) of *Channa striata* give strong reaction to basic protein test. This result cannot be directly supported by the present histochemical tests for proteins. The present examination shows that the contents of this cell of *P. cottoides* are acidophilic and may probably contain basic and aromatic amino-acid

groups. The presence of basic proteins may be expected from the above-mentioned results, though further investigations are needed to fully elucidate proteins of this cell of this fish. Rauther (1907) conjectured that the proteinous contents of the granular cells of *Callichthys punctatus* may have poisonous properties. Pfeiffer and Pletcher (1964) reported that a distasteful secretion from granular cells of the skin of the lamprey is suspected of being biologically significant in protecting this cyclostome from predacious fish. Though it is questionable whether the granular cells of the lamprey are identical with those of teleosts, the results obtained on the lamprey seem to be very helpful to make clear the function of the granular cells of teleosts. The result similar to that of Pfeiffer and Pletcher has been obtained by the writer in preliminary tests using *Corydoras* which is provided with the granular cells (unpublished). According to Thomson (1969), the granular cells of the box-fish is suspected to produce ostracitoxin, a powerful poisonous material, which is far different from that contained in the granular cell of other teleosts. Mittal and Banerjee (1975) stated that slime on the skin of *Channa striata* is mainly composed of mucus secreted from the mucous glands and surface polygonal cells, along with basic proteins from the granular cells (sacciform granulated cells) and lipids from the surface polygonal cells. The contribution of the granular cells to formation of slime on the skin could not be ascertained in the present specimen. Whether or not the granular cell participates the formation of thick covering layer to overlie the external surface of the epidermis of cutaneous flaps of a cottid, *Hemitripterus villosus*, remained questionable in the latest paper (Satō, 1977). From the results obtained by the previous (Satō, 1967) and the present observations, it is obvious that three cottids of *Blepsias cirrhosus draciscus, Cottus nozawae* and *P. cottoides* are endowed with a considerable number of the granular cells, but they lack the thick covering layer. Since the existence of the granular cells does not always correlate with the presence of the covering layer, these cells do not seem to play the leading role in the formation of this layer.

Mittal and Banerjee (1975) proposed a new term "sacciform granulated cells" for the cells which are invariably sac-like in outline having coarse or finely granular contents. The writer expresses his approval for their proposal and wishes to adopt this new term in future publications. As the eosinophilic vesicle designated by the writer (Satō, 1967) does not imply definitely cellular structure, this term is not appropriate and should be replaced by the above-mentioned new term. "Schlauchzelle" described by Lucowicz (1966) in the skin epidermis of *Bagrus bayad*, a siluroid fish of the Nile, is a bag-like cell having granular materials, and may be included among the sacciform granulated cells. Roberts et al. (1971) found a previously undescribed cell in the basal layer of the skin epidermis of a plaice, *Pleuronectes platessa*, and named it eosinophilic granular cell. This specialized cell is similar to the granular cell in light microscopic structure and eosinophil. Electron microscopy performed by Roberts et al. (1971), however, suggested that this specialized cell has dendritic processes extending between higher epithelial cells. Accordingly, this specialized cell seems to fall under the category other than that of the sacciform granulated cell. According to Roberts et al. (1971), this specialized cell is considered to show the closest resemblance to the Paneth cell of the mammalian intestinal epithelium.

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Literature cited

Bennett, H. S. and J. H. Luft. 1959. s-Collidine as a basis for buffering fixatives. J. Biophys. Biochem. Cytol., 6: 113～114.

Bertin, L. 1958. Glandes cutanées et organes lumineux. *In* Traité de Zoologie, 13: 459～481, figs. 268～288. Ed. by Grassé, P. P., Masson et Cie, Paris.

Bhatti, H. K. 1938. The integument and dermalskeleton of siluroids. Trans. Zool. Soc. Lond., 24: 1～102, pls. 1～9.

Hase, A. 1911. Studien über das Integument von *Cyclopterus lumpus* L. (beiträge zur Kenntniss der Entwicklung der Haut und des Hautskelettes von Knochen Fischen.). Jena. Zeitschr., 47: 217～342, figs. 1～37, pls. 1～8.

Henrikson, R. C. and A. G. Matoltsy. 1968. The

fine structure of teleost epidermis. III. Club cells and other cell types. J. Ultrastruct. Res., 21: 222～232, figs. 1～6.
Lillie, R. D. 1954. Histopathologic Technic and Practical Histochemistry. 2nd Ed., Blakiston Co. Inc., New York, ix+501 pp.
Lukowicz, v. M. 1966. Über die Barteln und die Lippenepidermis verschiedener ägyptische Süßwasserfische mit einigen Versuchen zum Geschmackssinn. Zool. Anz., 176: 396～413, figs. 1～10.
Millonig, G. 1961. A modified procedure for lead staining of thin sections. J. Biophys. Biochem Cytol., 11: 736～739, figs. 1～2.
Millonig, G. 1962. Further observations on a phosphate buffer for osmium solutions. *In* Electron Microscopy. Vol. 2: 1～2. Ed. by Breese, S. S. Jr., Academic Press, New York and London.
Mittal, A. K. and J. S. D. Munshi. 1971. A comparative study of the structure of the skin of certain air-breathing fresh-water teleosts. J. Zool., Lond., 163: 515～532, figs. 1～3, pls. 1～2.
Mittal, A. K. and T. K. Banerjee. 1975. Histochemistry and structure of the skin of a murrel, *Channa striata* (Bloch, 1797) (Channiformes, Channidae). I. Epidermis. Canad. J. Zool., 53: 833～843, fig. 1～8.
Pawlowsky, E. 1911. Uber den Bau der Epidermis von Haut und Lippen bei *Schizothorax intermedius* und *Capoeta heratensis*. Zool. Jahrb. Abt. Anat. Ontog. Tiere, 31: 289～316, pls. 1～3.
Pearse, A. G. E. 1960. Histochemistry, Theoretical and Applied. 2nd Ed., J. & A. Churchill Ltd., London, x+998 pp., 245 figs., 10 pls.
Pearse, A. G. E. 1968. Histochemistry, Theoretical and Applied. Vol. 1, 3rd Ed., J. & A. Churchill Ltd., London, vii+759 pp., 161 figs., 14 pls.
Pfeiffer, W. and T. F. Pletcher. 1964. Club cells and granular cells in the skin of lamprey. J. Fish. Res. Bd. Canada, 21: 1083～1088, figs. 1～5.
Rauther, M. 1907. Einige Beobachtungen über die Hautdrüsen von Siluriden. Ber. Oberhess. Ges. Natur-Heilk. Giessen, Naturwiss. Abt., N. F. 1: 88～98, figs. 1～7.
Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol., 17: 208～212, figs. 1～3.
Roberts, R. J., H. Young, and J. A. Milne. 1971. Studies on the skin of plaice (*Pleuronectes platessa* L.). 1. The structure and ultrastructure of normal plaice skin. J. Fish Biol., 4: 87～98, pls. 1～15.
Satō, M. 1967. Histological and histochemical observations on the eosinophelic vesicles found in the head epidermis of *Blepsias cirrhosus* (Pallas). A preliminary report. Arch. Histol. Jap., 28: 377～382, figs. 1～9.
Satō, M. 1977. Histological observations on the cutaneous processes on the head of *Azuma emmnion* and *Hemitripterus villosus*. Japan. J. Ichthyol., 24: 12～16, figs. 1～3.
Studnicka, F. K. 1906. Drüsenzellen und Cuticulargebilde der Epidermis von Lepadogaster. Anat. Anz., 29: 132～144, figs. 1～12.
Thomson, D. A. 1969. Toxic stress secretions of the boxfish *Ostracion meleagris* Shaw. Copeia, 1969 (2): 335～352, figs. 1～13.

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アサヒアナハゼ（カジカ科）の表皮にみられる顆粒細胞の光学ならびに電子顕微鏡による観察

佐藤　光雄

アサヒアナハゼの顆粒細胞は，主として皮膚表皮の中層に存在する大型細胞である．本細胞は，Thomson (1969) がハコフグの一種 *Ostracion meleagris* で記載した棍状細胞 (club cell) の発達段階に，類似した発達経過をたどるものと考えられる．発達段階 II～III の本細胞は，多数の酸好性顆粒を含み，予備的な組織化学検索から，芳香族および塩基性アミノ酸を含むたんぱく質の存在が予想される．前記の段階にある本細胞の電顕像をみると，細胞の主要部は1個の中心空胞 (central vacuole) によって占められ，この空胞中には比較的電子密度の高い顆粒が多数散在している．空胞周辺の細胞質には，かなり大きい胞状体の層があり，これらの胞状体中にも上記同様の顆粒が含まれる．この胞状体が破れ，顆粒を中心胞内へ放出していると考えられる電顕像がみられることから，胞状体の相次ぐ破裂と含有顆粒の放出とによって，中心胞が形成されるものと推定される．本細胞をもつ硬骨魚類の皮膚組織に共通する特徴の有無，ならびに本細胞の系統的意義については，今後の検討が必要である．なお，本細胞の機能についても若干の考察を試みた．

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