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Specialized cells in the gills of *Chaenogobius castanea* and *Ch. urotaenia*
belonging to the family of Gobiidae.

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I. Introduction

Since Smith, H.W. (1930) first suggested that the gills might be the site of an extra-renal excretory function and Keys, A. and Willmer, E.N. (1932) described a type of cell in the gill of the eel and other species which they suggested might be the site of an active chloride transport system and tentatively termed it the 'chloride-secretory' cell, many studies of chloride-secretory cells have been reported. The fish belonging to the genus of *Oncorhynchus* descend to the sea after a period of fresh water life. M.G. Zaks and M.M. Sokolova (1961) stated, on the basis of their investigation of adaptation to change of water salinity by *Oncorhynchus nerka*, that the development of the chloride-secretory cells in the gills of the sockeye salmon precedes the seaward migration, that is, that the migration of the sockeye salmon to the sea is caused by the decrease of the concentration of salt in the serum, that in turn is brought about by the development of the chloride-secretory cells. Later, it was postulated by Yu. V. Natchin and G.D. Bocharov (1962) that the chloride cells in the gills of *Oncorhynchus keta* and *O. gorbuscha* can both excrete and absorb. According to them whether the chloride cells excrete or absorb, is determined by the environment. Hamada, K. (1961) observed in the course of the ecological study of the genus of *Hypomesus* that a considerable number of pond smelt reside in fresh water, although many of them descend to the sea in the spring. Also there are land-locked forms of *Oncorhynchus masou*, *O. nerka*, *Hypomesus olidus*, *Chaenogobius castanea*, *Ch. urotaenia* and *Gasterosteus aculeatus*. These facts cause us to doubt whether the fish listed above, migrate to the sea as a necessary consequence of a physiological change in the fish itself.

Although the function of the chloride-secretory cells in electrolyte excretion has not as yet been firmly established, the chloride-secretory cells have been described in a variety of marine, euryhaline and anadromous teleosts and cyclostomi (Copeland, D.E. 1948, Getman, H.C. 1950, Morris, R. 1957, Parry, G. 1958, Zaks, M.G. and Sokolova, M.M. 1962 and the others). *Ch. castanea* and *Ch. urotaenia* are swept down the stream to the sea just after hatching, and ascend the stream from the sea in the summer. If the migration up the stream is a necessary consequence of a physiological change, the fish which leave the sea may be unable to tolerate sea water. This experiment was designed to make clear whether the

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chloride-secretory cells are always found in the gills of *Ch. castanea* and *Ch. urotaenia* without regard to the salinity of the environmental water or not, and whether the fish can tolerate sudden changes of salinity or not.

II. Materials and Methods

Ch. castanea and *Ch. urotaenia* used in this study were taken from a stream and an artificial pond in Minato-machi, Hakodate (Table 1 and 2). These fish were used for the experiments after they were kept for a week to three months in a glass tank in the laboratory containing about 10 l of fresh water.

Pieces of the gill were removed from 7 living fishes adapted to fresh water, and were fixed for observation of the chloride cells. When Bouin's fluid was used, pieces of the gills were cut off after the entire animal was fixed (Table 1). Twelve animals were transferred immediately from fresh water to the glass tank filled with 100% sea water (Table 2). The water temperature was not controlled. A little distilled water was added, once in a while, to the tank to keep the sea water from becoming too saline as a result of evaporation. One animal was put back into fresh water for 3 hr and 25 m after being kept for a day in sea water. The values of chlorinity shown in Table 2 were determined at the close of the experiment. Pieces of the gill were fixed with the same method used for the fish adapted to fresh water. The fixing agents used in this study were Bouin's, Champy's, Flemming's, Rossman's and Zenker's fluids. The best fixative was Flemming's fluid and Bouin's fluid modified by replacing the acetic acid of the original formula with the same volume of formic acid. After fixation, the tissues were dehydrated, cleared, embedded in paraffin, and sectioned, usually at 5 or 10 microns, and the sections were mounted serially. These sections were stained with Heidenhain's iron haematoxylin and light green (or orange G, eosin). Some tissues were stained with Mallory's triple stain. The PAS technique and mucicarmine staining were used to demonstrate

Table 1. *Chaenogobius castanea* and *Ch. urotaenia* kept in fresh water.

No. of specimens	Date of fixation	Total length
<i>Chaenogobius castanea</i>		
1	Oct. 4, 1962	58 mm
2	Nov. 5, 1962	49
3	Dec. 5, 1962	66
4	Dec. 27, 1962	72
5	Feb. 8, 1963	47
6	Feb. 16, 1963	60
<i>Chaenogobius urotaenia</i>		
7	Nov. 28, 1962	77

Table 2. *Chaenogobius castanea* and *Ch. urotaenia* kept in sea water.

No. of specimens	Date of fixation	Days of keeping	Chlorinity*	Total length
<i>Chaenogobius castanea</i>				
1	Oct. 4, 1962	16 wks and 1 d	20.955 ‰	51.5 mm
2	Dec. 5, 1962	4 2	23.510	62.0
3	Dec. 26, 1962	2 6	21.350	70.0
4	Jan. 10, 1963	for 37 minutes	21.605	65.0
5	Feb. 4, 1963	3 2	21.320	54.0
6	Feb. 16, 1963	10 2	20.535	61.0
7	Jun. 17, 1966	8 2	20.210	46.9
8	Dec. 10, 1962	4	Ringer's S.	56.0
<i>Chaenogobius urotaenia</i>				
9	Nov. 5, 1962	4	19.070	47.0
10	Nov. 5, 1962	4	19.070	62.0
11	Nov. 27, 1962	3 1	22.415	77.0
12**	Jan. 11, 1963		21.605	98.0

* The values were determined at the close of the experiment.

** This one was put back in fresh water for 3 hours and 25 minutes after being kept for 24 hours in sea water.

the mucous cells. The Leschke silver technique modified by Copeland (1948) was also used to detect the presence of chloride in chloride-secretory cells.

III. Observations

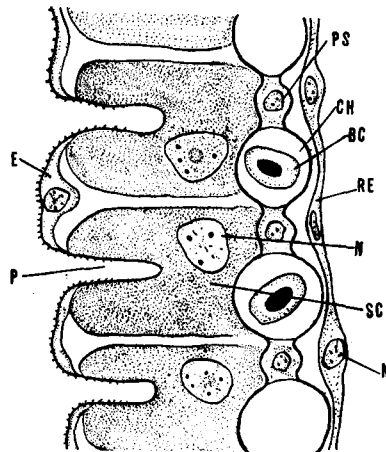
Ch. castanea and *Ch. urotaenia* exhibit a strong ability to tolerate a sudden change of salinity. No external signs of distress were noticed in the fish which were abruptly transferred to sea water, and all the fishes were alive at the time of fixation. Some of the fish were seen to feed on earthworms within about 8 hours after their transfer. The chlorinity of the sea water was about 17.6 ‰ at the beginning of the experiment, except for the keeping water of the fish listed in table 2, No. 6 (not measured). The high value of the chlorinity of sea water at the time of the fixation of the fish is a result of evaporation. The highest value was Cl 23.510 ‰ and the lowest Cl 19.070 ‰ (Table 2). One animal which was transferred to Ringer's solution also showed no sign of distress (Table 2, No. 8).

There is little fundamental difference in the structure of the gill of *Ch. castanea*, *Ch. urotaenia* and other teleosts. If the tolerance to sudden change in salinity of both the species is maintained by an active transport of ions in the gill, the so-called 'chloride-secretory' cells must always be found in the gill. In *Ch. castanea* and *Ch. urotaenia*, the chloride-secretory cells as described by Keys and

Willmer (1932) in the common eels were not found at the proximal portion of the platelets. Copeland (1948) found columnar, acidophilic cells localized on the side of the filament supplied with afferent blood in the gills of *Fundulus heteroclitus*. In *Ch. castanea* and *Ch. urotaenia*, there were, however, numerous mucous cells in that area, and no columnar, acidophilic cells (Pl. II, Fig. 4).

In *Ch. castanea* and *Ch. urotaenia*, there is a crescent-shaped cavity at the base of the gill filament on the side of the afferent blood vessel. This cavity is covered with simple squamous epithelium. In the fish kept in sea water for 4 days, there are columnar cells which possess the tube-like pit between the epithelium and the basement membrane (Pl. I, Fig. 1 and 2, Pl. II, Fig. 3). These pitted cells are in contact with each other and are arranged in a sheet or a layer. These cells extend out on the under side of the first platelet (for convenience platelets will be called here the first, the second and the third in the order of their positions near the base of the filament). Among these cells mucous cells were very rare. The shape of the pitted cells is sometimes round or oval, especially in cells located in the platelets (Pl. III, Fig. 7 and 8). The pit opens to the exterior through the epithelium covering. The term 'chloride-secretory' cell will be used here for the pitted cell for convenience. The epithelium seems to enter into the pit of the cell as shown in Text-fig. 1 and Pl. III, Fig. 7. There are fingerprint-like patterns in the epithelium. The patterns are similar to the patterns found by Yamada, J. (1966) in the epidermis of teleosts. So far as the writer observed, the epithelium which possesses such patterns covers the whole of the gill arch, but does not exist in the respiratory epithelium except for the epithelium covering the chloride-secretory cells in the first platelet. The cytoplasm of the chloride-secretory cells adapted to sea water stain a slightly dirty violet in Mallory's triple stain. When the cells are fixed with Champy's or Flemming's fluids and are stained with iron haematoxylin

Text-fig. 1. Diagram showing the pit of the chloride-secretory cell in the platelet. BC, blood cell; CH, blood channel; E, epithelium covering the chloride-secretory cells; N, nucleus; P, pit; PS, pillar cell; RE, respiratory epithelium; SC, chloride-secretory cell



and eosin (or light green, orange G), the cells are filled with blackish granules which may be mitochondria. These cells do not stain especially heavily in eosin or orange G in comparison with other cells of the gill filament. The nucleus of the cell is situated towards the base of the cell (Text-fig. 1). Although the mucous cells situated along the edge of the gill filaments reveal a clear positive PAS and mucicarmin reaction, the chloride-secretory cells give no reaction to those methods at all. The Leschke Silver technique modified by Copeland ('48) was used to detect the presence of the chloride in these cells, but the presence was not evident.

The chloride-secretory cells were also observed in the gills of the fish adapted to fresh water. Between the fish adapted to sea water and those adapted to fresh water, there is no clear difference in the number or size of the chloride-secretory cells or in their characteristics, except for the pit. The chloride-secretory cells adapted to fresh water usually possess a vesicle instead of the pit, and sometimes possess a tube-like pit (Pl. III, Fig. 6 and 7). In the fish kept in sea water for 37 minutes, the chloride-secretory cells had vesicles (Table 2, No. 4). In the fish which was put back in fresh water for 3 hours and 25 minutes after being kept for 24 hours in sea water the cells in the gills also exhibited vesicles (Table 2, No. 12). Although the vesicle was usually opened to the exterior through the epithelium, it was sometimes closed. Moreover, there were a considerable number of cells which did not possess the vesicle. There was no clear transformation or variation of numbers in mucous cells between the fish adapted to fresh water and those adapted to sea water.

IV. Discussion

The pond smelt descends to the sea and some of them reside in lakes or rivers. It is, also, common knowledge that the sockeye salmon and a salmon, *Oncorhynchus masou*, are occasionally found in land-locked lakes. In Lake Onuma, in Hokkaido, there is a land-locked form of the threespine stickleback. These facts point out that these fish do not necessarily require sea water for their life. On the other hand, we have not found a land-locked form of chum salmon. This may mean that chum salmon require sea life for a certain period in their life history. A stickleback, *Pungitius pungitius* which is closely related to the threespine stickleback is unable to live in the sea, and the goldfish can not tolerate 100 % sea water. The surf-smelt related to the pond smelt and the ocean perch are unable to maintain their existence in fresh water. It may be because of certain specific physiological functions of organs or tissues of some species that enable them to live in either the sea or lakes or ponds while others are incapable of tolerating a change in the salinity of their environment.

Ch. castanea and *Ch. urotaenia* of the size used in the experiment of tolerance to sea water, are not found in the sea, since they have already ascended to streams

from the sea. If their migration to streams is a consequence of the loss of the excretory function and the development of the absorbing function of salts in the gill, they may be incapable of tolerating a sudden change from fresh water to sea water. However both of the species survived in sea water with no sign of distress despite of the abrupt transfer from fresh water. The development of the absorbing function of the chloride cell in the gill is not the cause of the anadromous migration, but there may be other reasons such as a change in the water temperature or the food supply.

It has come to be generally accepted that the chloride-secretory cells in the gill of teleost bring about some ions, principally sodium, potassium and chloride, movement. If this hypothesis is accepted, it would be expected that these fish always possess the chloride-secretory cells which are able to excrete or absorb the ions in response to changes in salinity, for they are capable of tolerating sudden changes of salinity. When they were kept in a sea water, tube-like pits were found in the chloride-secretory cells. And when they were kept in fresh water with few exceptions vesicles were found instead of pits. Moreover, there were a considerable number of cells with no vesicle or pit. The facts as stated above show that the chloride-secretory cells in the gill of *Ch. castanea* and *Ch. urotaenia* respond to changes in the salinity of their environment. Bevelander (1935, '36) concluded that there are no specialized secretory cells other than mucous cells in the gill. Recently, Vickers (1961) suggested that the so-called 'chloride-secretory' cells represent a metaphasic form of mucous cells. However, in *Ch. castanea* and *Ch. urotaenia* the mucous cells differ from the chloride-secretory cells in location and exhibited no clear variation in shape or number when they were kept in sea water. Moreover, the chloride-secretory cells never exhibited a positive PAS reaction. Therefore, the mucous cells probably are not directly concerned with extra-renal electrolyte excretion.

The hypothesis that the branchial chloride-secretory cells is associated with extra-renal excretion was supported by Copeland '48, Getman '50, Threadgold and Houston '61, Zaks and Sokolva '62. Bevelander ('35, '36) suggested that the entire gill epithelium is responsible for the salt transfer. Parry, Holliday and Blaxter ('59) have objected to the secretory hypothesis. However no author has felt that the gill is not associated with electrolyte metabolism, though there are differences of opinion as to just what part it plays.

Though the histological techniques used in this study give no direct evidence as to the functions of the pitted cells, we can conclude that the cells in the fish adapted to sea water have tube-like pits and the cells in the fish adapted to fresh water have the vesicle or nothing. And this is in light of the fact that other research has shown, that the cells are associated with extra-renal electrolyte excretion and absorption.

Here, I wish to sincerely thank Prof. H. Niiyama for his guidance and critical reading of the manuscript, and I am much indebted to Dr. A. Fuji for determining the chlorinity of the sea water.

V. Summery

The histology of the gills of *Ch. castanea* and *Ch. urotaenia* were studied after the fish had been kept in fresh water or sea water. The fish tolerated a sudden change of the media from fresh water to 100‰ sea water and survived with no signs of distress. In *Ch. castanea* and *Ch. urotaenia*, there are specialized cells in the crescent-shaped cavity at the base of the gill filament of the side of the afferent blood vessel, and the cells extend out on the under side of the platelet situated nearest the base of the filament. These specialized cells are in contact with each other and arranged in a sheet. Their shape is columnar, and sometimes round or oval. A simple squamous epithelium covers them. The cells adapted to sea water possess a tube-like pit which opens to the exterior through the epithelium. In the cells adapted to fresh water, the pit is replaced with a vesicle. A considerable number of the cells of the fish adapted to fresh water did not have vesicles, and there were a few, but only a very few, pitted cells. The mucous cells are located usually along the edge of the filament supplied with afferent blood in the gill. The chloride-secretory cells are not found at the proximal position of the platelets or along the edge of the gill filament. The specialized cells found in both the species may be associated with extra-renal electrolyte excretion and absorption.

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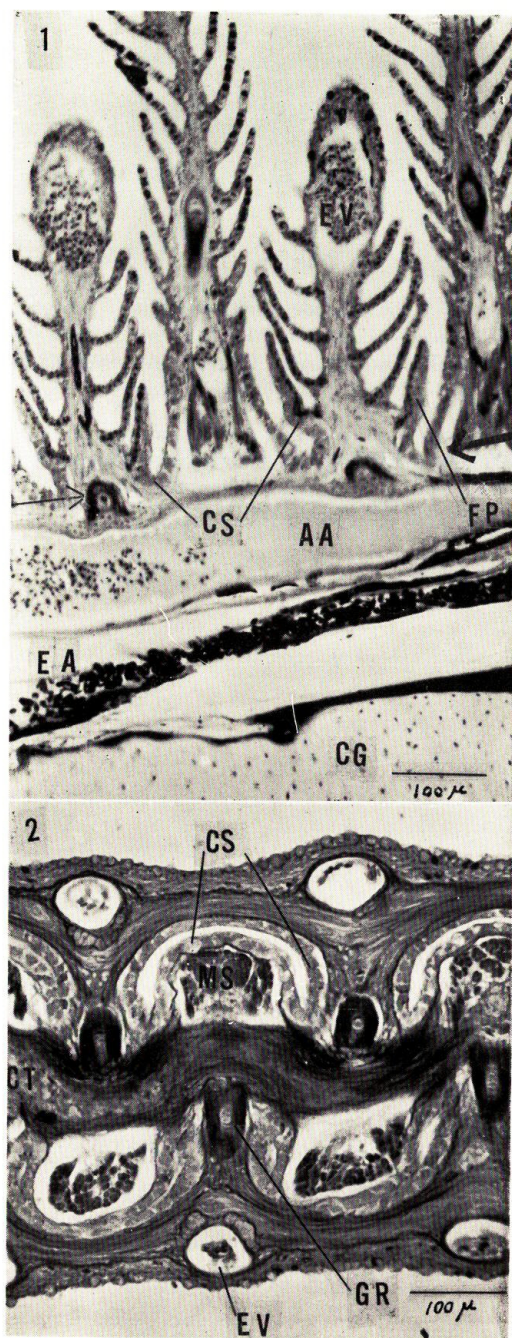
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Explanation of Plates

PLATE I

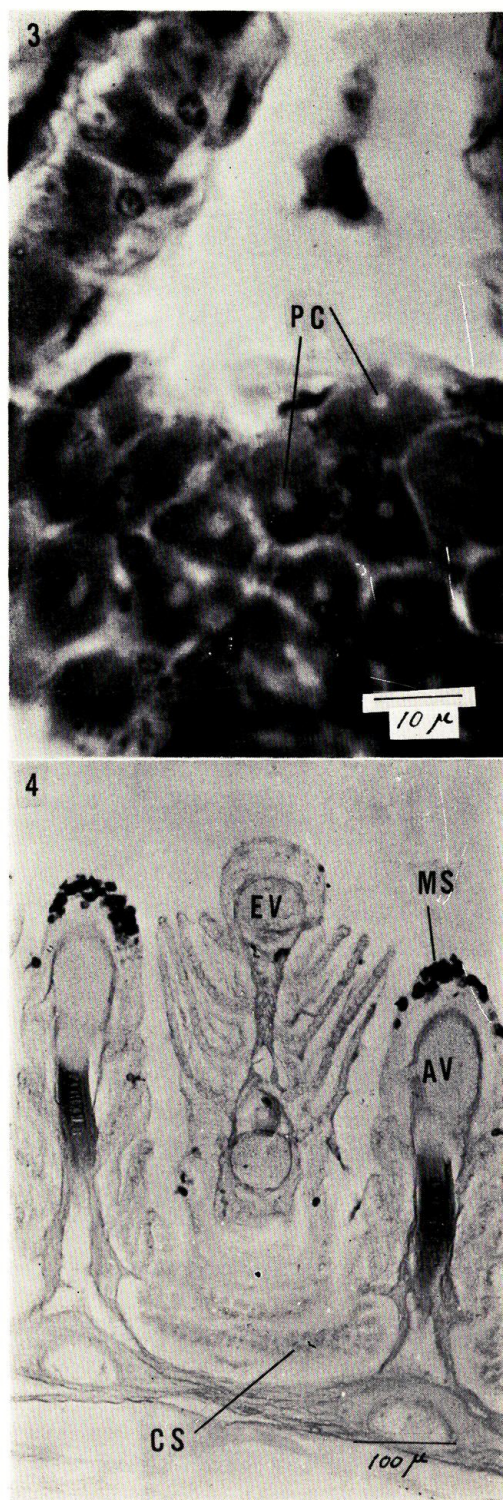
- Fig. 1. A horizontal longitudinal section of the gill lamellae of *Chaenogobius castanea*, 74 mm in total length, showing the presence of the chloride-secretory cells at the base of the gill filament and on the first platelet. Arrows indicate the position of the cross section shown in Fig. 2. AA, afferent branchial artery; CG, cartilage of the gill arch; CS, chloride-secretory cell; EA, efferent branchial artery; EV, efferent blood vessel; FP, the first platelet. Heidenhain's iron haematoxylin and light green
- Fig. 2. A cross section of the gill filament showing the chloride-secretory cells at the base of filament. Mallory's triple stain. CT, connective tissue; GR, cartilage; MS, muscle



K. HAMADA: Specialized cells in the gills

PLATE II

- Fig. 3. The surface view of the chloride-secretory cells at the base of the gill filament of *Chaenogobius castanea*, 61 mm in total length, kept in sea water for 10 weeks and 2 days. Heidenhain's iron haematoxylin and light green. PC, pit of the chloride-secretory cell
- Fig. 4. A horizontal longitudinal section of the gill lamellae of *Chaenogobius urotaenia*, 98 mm in total length, showing the position of the PAS-positive cells (mucous cells) in the gill filaments (Table 2, No. 12). AV, afferent blood vessel; MS mucous cell



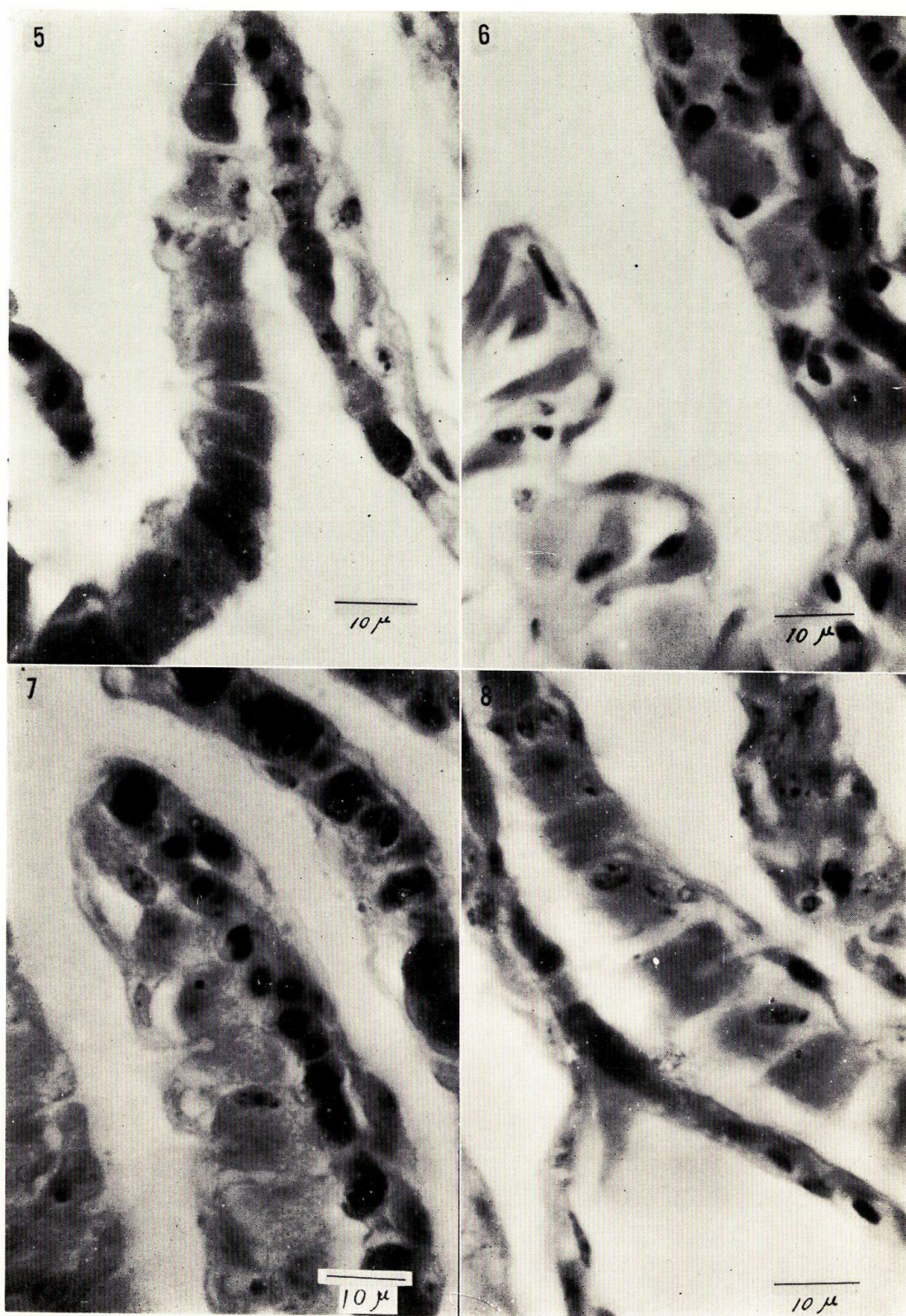
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PLATE III

Fig. 5, 6, 7 and 8 indicate the chloride-secretory cells in the first platelet

Fig 5 and 6 are the platelet of *Chaenogobius castanea*, 47 mm and 74 mm in total length respectively. Both of the fishes were kept in fresh water. The former possesses the chloride-secretory cells with tube-like pits and the latter has vesicles in the cells. Heidenhain's iron haematoxylin and light green

Fig. 7 and 8 are the platelets of *Chaenogobius castanea*, 70 mm and 61 mm in total length respectively. Fig. 7 indicates the chloride-secretory cells of the fish kept in sea water (Cl. 21.350‰) for 2 weeks and 6 days, and Fig. 8 indicates the cells of the fish kept for 10 weeks and 2 days in sea water (Cl. 20.535‰). Heidenhain's iron haematoxylin and light green



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