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著者	KASHIWAGI Masaaki, SATO Ryuhei
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Studies on the Osmoregulation of the Chum Salmon, *Oncorhynchus keta* (Walbaum) II. Histological Observations of the Branchial Epithelium according to the Growth of the Fish from Hatching

Masaaki KASHIWAGI and Ryuhei SATO

Department of Fisheries, Faculty of Agriculture, Tohoku University, Sendai, Japan
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Summary

1. The branchial epithelium of the chum slamon alevins and fry were observed histologically according to the development of tolerance to sea water as the fish got older.
2. The branchial epithelium of the alveins just hatched were made up mostly of undifferentiated cells and a few large cells. The large cell had fine granules and was located about the afferent branchial arteriole or cartilage in the transverse section of the gill filament.
3. The large and granular cells were elongated and approached high columnar form, and they usually had intimate contact with the blood supply and the external medium. The large and granular cell was acidophilic and was filled with mitochondria in the cytoplasm.
4. The number of these large and granular cells tended to increase in the alevins as they got older, but they tended to be stable in the fry. Their appearance in the fry was 34.9 per cent of the total cells.
5. When the fish were transfered to sea water from fresh water, the large and granular cells in the alevins at 20 days after hatching were elongated in form and increased in number. The cytoplasm were more acidophilic than in the fish left in fresh water. These changes appeared within one day after the transfer.
6. In the case of the alevins at 40 days and the fry at 60 days after hatching when transfered to sea water, no remarkable changes showed in the appearance of the large and granular cells as compared with the fish left in fresh water. For the large and granular cells of the fresh water fish also developed in shape and increased in number as a result of the growth of the fish.
7. It may be said that the large and granular cells seem to be similar to the chloride secretory cells from the location and histological appearance of the cell. They developed along with the growth of the alevins as preparations for the seaward migration.

It was mentioned by Kashiwagi and Sato (1) that the degree of salinity tolerance of the chum salmon alevins increased as they got older from hatching, and that the fry showed a high survival rate in sea water (19.04 per mill as chlorinity) when they were abruptly transfered to it from fresh water. It has been recognized that the gills of teleost fishes might be the site of the extra-renal excretory mechanisms under sea water conditions. Keys and Willmer (2) indicated, in the gill of eel, a cell type that was responsible for electrolyte excretion and they termed it the chloride secretory cell. Similar cells have been also reported by many investigators in the salmonids and they might participate the osmoregulatroy adaptation to the sea water life of fish during their seaward migration.

In the present experiments, the branchial epithelium of the chum salmon alevins and fry were observed histologicallly in relation to the development of tolerance to sea water according to the growth of the fish.

Materials and Methods

The chum salmon used in this experiment were carried to the laboratory at the stage of eyed period egg from the Tsugaruish River Salmon Hatchery, Miyako, Iwate Prefecture, and kept in a fresh water aquaria from January to May in 1966. The alevins which hatched from the eggs began feeding at about forty days after hatching then they were fed on a dry mixed ration. The yolk sac of the fish were absorbed at about sixty days after hatching.

Some stages such as the alevins and the fry of chum salmon were examined. The gills isolated from the fish were fixed in Bouin's fluids, then they were prepared as 4 or 6 microns paraffin serial sections. The sections were stained mainly with Mayer's acid hemalaun hematoxylin and eosin. Mallory's triple stain and Heidenhain's iron hematoxylin-lightgreen were also used.

In addition, the mitochondria preparation was performed in 1968 for the gill of the fry at 90 days after hatching which were carried to the laboratory from the Otsuchi River Salmon Hatchery, Iwate Prefecture. The gill was fixed in Regaud's fluid followed by postchromate then stained by Altman's acid fuchsin after preparing as paraffin sections.

Results

The Branchial Epithelium of Chum Salmon Alevins and Fry in Fresh Water

The alevins just hatched have no lamella in their gill filament and their branchial epithelium were made up mostly of undifferentiated cells and a few large cells. Some of undifferentiated cells showed a various stage of mitosis. The large cell, spindle or spherical in shape, had fine granules, but it was an immature phase because of basophilic cytoplasm and large nucleus. The nucleus had two or three nuclei and occupied a nearly central position in the cell (Fig. 1).

The lamellae were observed in the gill filament of the alevins at 9 days after hatching. The large and granular cells were present at the base of lamellae as shown in the longitudinal section of gill filament (Fig. 2).

As the fish got older, such as at 22 days after hatching, some of these large and granular cells were elongated and approached the high columnar form in which the nucleus situated at the basal part of the cell (Fig. 3). The cytoplasm was stained lightly with eosin and some granules were stained with anilin blue. In older alevins, such as at 41 days after hatching, the branchial epithelium were occupied mostly with the high columnar cells which took the place of the spindle or spherical cells about the location of afferent arteriole and midway portion as shown in the transverse section (Fig. 4). These high columnar cells, specially conspicuousness in the fry, such as at 66 and 90 days after hatching, were in intimate contact with the basement membrane and external medium (Fig. 5). The cytoplasm was clearly acidophilic and packed with mitochondria oriented in the long axis of the cell (Fig. 6). Some kinds of the epithelial cells above mentioned were shown as the diagram in Fig. 7.

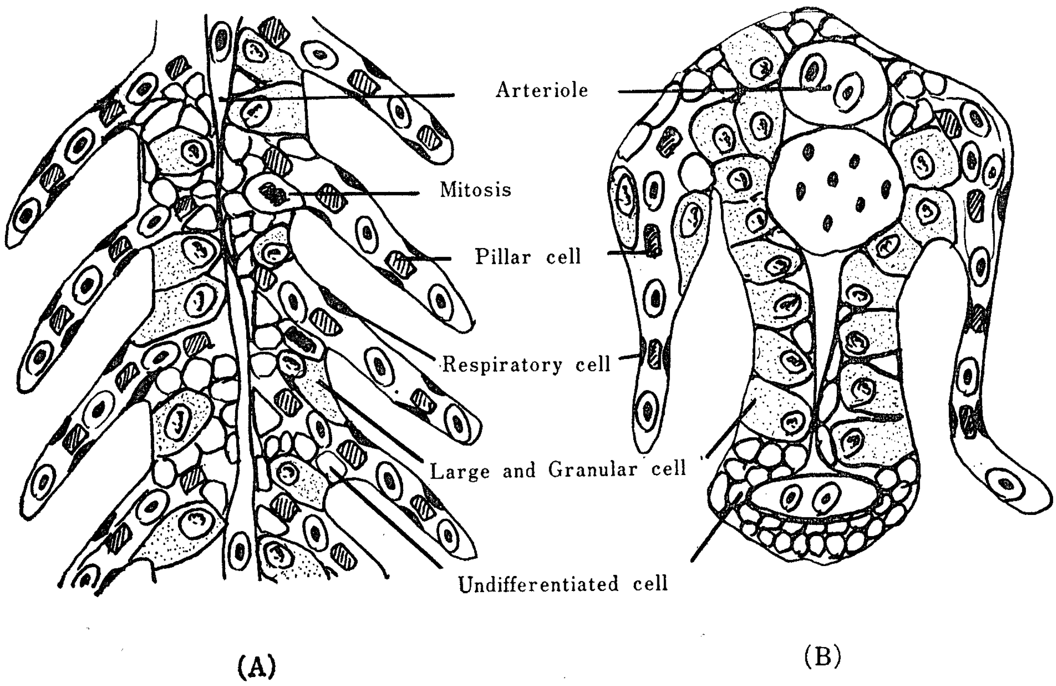

FIG. 7. The diagram showing the branchial epithelium at the longitudinal (A) and the transverse section (B) of the chum salmon alevins and fry.

The large and granular cells also increased in number according to the growth of fish. The cells were counted in the 50 interlamellae of branchial epithelium of individual fish. The site counted was at the portion closed to the cartilage in the lognitdinal section of gill filament (4 microns in thickness). The number of the large and granular cells were given 11, 107, 203, 250 and 201 in mean of three fish at 9, 22, 33, 41, 66 and 90 days after hatching, respectively. While the total cell number, which including the respiratory, mucous and undifferenciated cells but excluding the pillar and blood cells, were nearly constant with 621±58 in mean of 15 fish but except for the 9 day alevins with 489 in mean of three fish (Fig. 8-A). The differences of the number were seemed to be caused by the fact of the lack of respiratory cell on the lamellae of which were not as yet developed fully in the alevins at 9 days after hatching (Fig. 2). These results demonstrated, as shown in the proportion of the large and granular cells to the total cell number, that the index of the large and granular cells tend to increase with days after hatching but tend to be stable after about 40 days (Fig. 8-B).

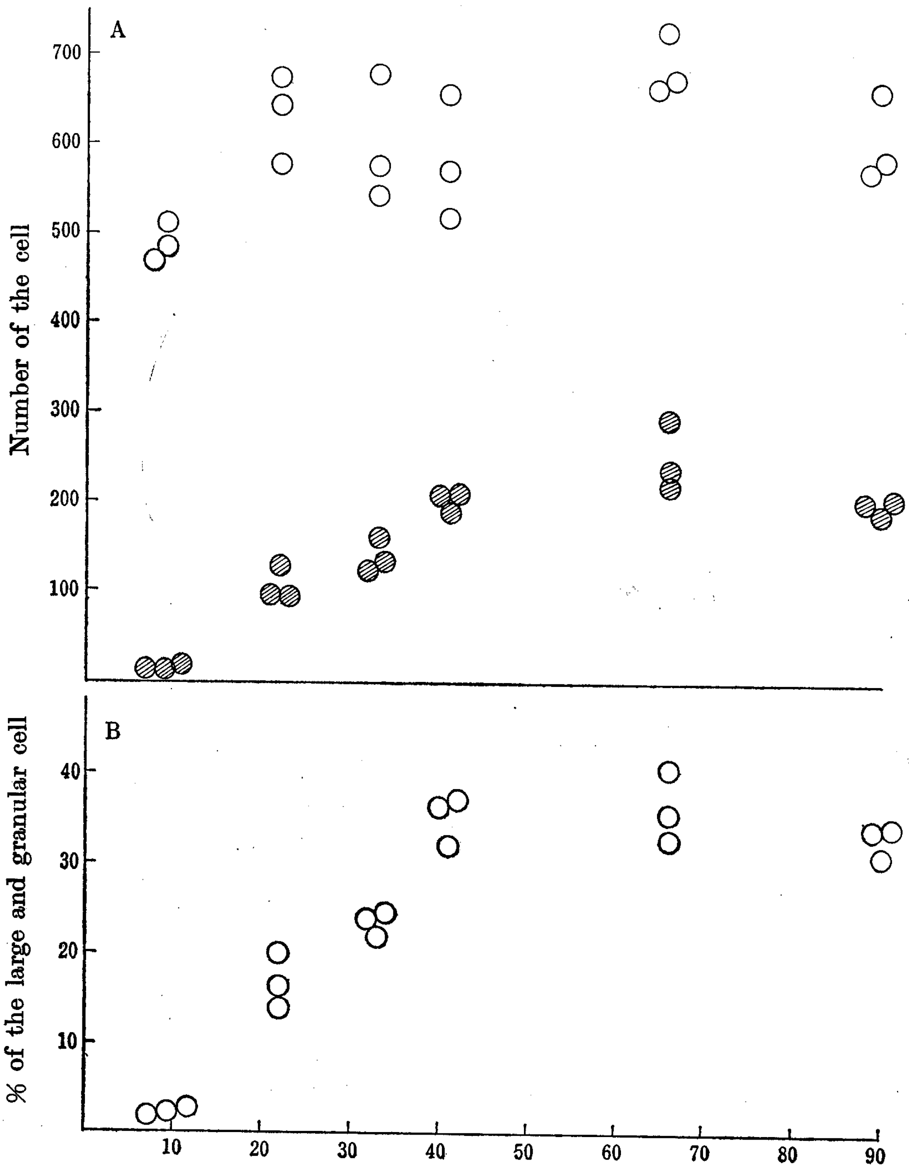

FIG. 8. Changes in cell number of branchial epithelium of the chum salmon alevins and fry in relation to their growth in fresh water. (A) showing the number of the large and granular cells (◍) and the total (○), (B) showing the proportion of the large and granular cell to total. Each points refers to an individual fish.

Changes of Branchial Epithelium of Chum Salmon Alevins and Fry when Transfered to Sea Water from Fresh Water

The alevins at 20 days aftr hatching could survive in 50 per cent sea water but died in 100 per cent sea water (19.04 per mill as chlorinity). Of the Branchial epithelium in the 50 per cent sea water, the large and granular cells were elongated and showed more acidophilic cytoplasm and its base were remarkably contact with the basement membrane (Fig. 9, contrast with Fig. 3). These changes appeared within one day in the sea water after the transfer from fresh water. The cell number increased within one day after the transfer; 180 in mean of three fish

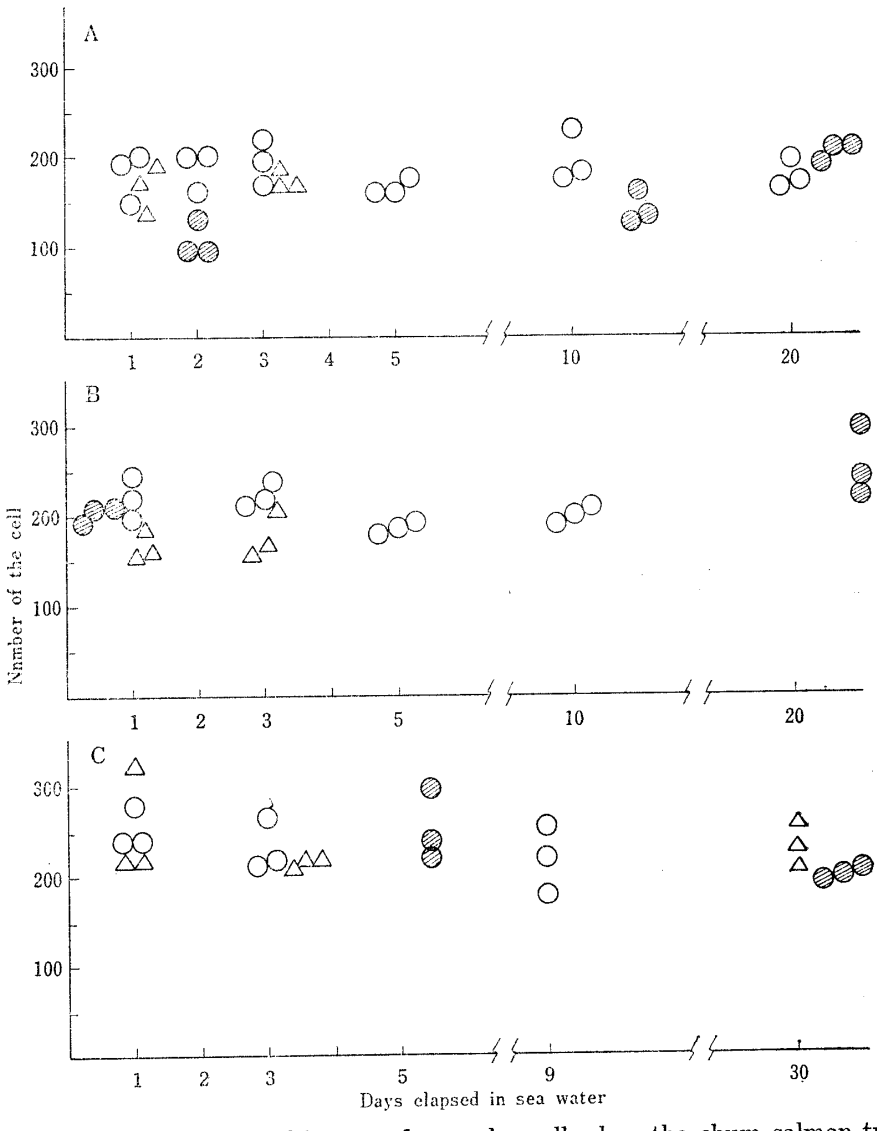

FIG. 10. Changes in number of large and granular cell when the chum salmon transfered abruptly to the 50 per cent sea water (○) and 100 per cent sea water (△) from fresh water (◍). (A) showing the results of the experiments of the alevins at 20 days after hatching, (B) the alevins at 40 days, and (C) the fry at 60 days. Each point refers to an individual fish.

at one day after the transfer to sea water but 106 in fresh water. Hereafter the number were nearly constant with 50 per cent sea water (Fig. 10-A). In the 100 per cent sea water, the changes of the cell were similar both in morphology and number to the case with 50 per cent sea water at the early days in the experiments. However, all epithelium showed the condition of picnosis of nucleus on account of dehydration at the time of death. These symptoms were also found in the other alevins which could not adapt to the sea water, such as the just hatched alevins in the 50 per cent and 100 per cent sea water and the 40 day alevins in the 100 per cent sea water.

In the experiment of older fish such as the alevins at 40 days and the fry at 60 days after hatching, they could adapt to the 50 and 100 per cent sea water respectively. The conditions of the large and granular cells both in form and in number showed the same as the case of the 20 days alevins in the 50 per cent sea water (Fig. 10-B, C and Fig. 11). However, no remarkable changes were found in the aspects of the cells of the adapted fish in these sea water as compare with that of the fresh water fish of the same age (Fig. 11 and Fig. 5). For, the large and granular cells of the fish developed individually according to their growth in fresh water as mentioned in the previous chapter.

Discussion

Large and granular cells of the branchial epithelium were found in the chum salmon alevins just hatched, though they showed an immature phase. They developed acidophilic in stainning, elongation in form and increased in number as the fish got older in fresh water. While the ability of the chum salmon to tolerate to sea water gradually developed through the alevins and fry period prior to the seaward migration as reported by Kashiwagi and Sato (1), it is clear that the development of the large and granular cell maintained the increase of tolerance to sea water with growth of the chum salmon.

According to Hoar (3), the large and acidophilic cells are typically found at the basal part of the gills of fry, chum and pink salmon which migrate to the sea in their first year whereas similar cells do not appear in the gills of coho salmon until the beginning of the second year when it migrates to the sea. Nishida (4) reported that there are histological differences in the similar cell of masou salmon between the two types, the sea-run form and the land-locked form.

On the other hand, the changes in staining, form and number of the large and granular cells of the chum salmon alevins at 20 days after hatching appeared shortly after the transfer to sea water. It seems probable, therefore, that the large, granular and acidophilic cell found in the chum salmon alevins and fry is largely responsible for the ability of the fish to adjust themselves to sea water. Threadgold and Houston (5) demonstrated by the electron microscopic examination that the similar cell of the Atlantic salmon gave evidence of the secretory function in relation of electrolyte regulation in the process of adaptation to sea water.

It has been well demonstrated by Smith (6) and Keys (7) that teleost fishes are capable of elctrolyte excretion by their gills as a method of maintaining water balance of the body under sea water conditions. Keys and Willmer (2) described that the large, granular and acidophilic cells located at the base of the lamellae of tne gill filament of the eel, and suspected it to be a chloride secretory cell. The function of extra-renal excretion of the chloride secretory cell has not as yet been firmly established. Nevertheles, the hypothesis is supported by the facts that the adaptive responses of the cell observed after the transfer from fresh water to sea water and the reverse (8,9,10), histochemical demonstration of high cellular chloride content in fish adapted to sea water (8), and correlations between ability to tolerate sea water and the occurence of these cells (11-14).

The large, granular and acidophilic cells found in the chum salmon alevins and fry seemed to be similar to the chloride secretory cell, because they occurred chiefly at the basal portion of the lamellae and they were usually in intimate contact with the blood supply and the external medium. Also the hypothesis of the chloride secretory cell is supported indirectly by the close correlation between the ontogenetical appearence of the cell and the development of tolerance to sea water.

Conclusively, it may be said that the chloride secretory cell of the gill of the chum salmon developes gradually as the fish gets older in fresh water, and prepares for the function of hyperosmoregulation at seaward migration. This idea may be supported by the fact that the number of the large, granular and acidophilic cells of the alevins increases as they get older, and tends to be stable at about 40 days after hatching.

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Explanation of Figures

FIG. 1. Transverse section of the gill filament of chum salmon alevins just hatched in fresh water, showing the large and granular cells.

FIG. 2. Longitudinal section of the gill filament of alevins in fresh water at 9 days after hatching, showing the lamellae and large and granular cells at the site of the interlamellae.

FIG. 3. Longitudinal section of the gill filament of the alveins in fresh water at 22 days after hatching, showing some large and granular cells which have elongated in form and increased in number.

FIG. 4. Transverse section of the gill filament of the alevins in fresh water at 41 days after hatching, showing the branchial epithelium which is occupied mostly with the high columnar cells.

FIG. 5. Longitudinal section of the gill filament of the fry in fresh water at 66 days after hatching, showing the base of the high columnar cells which were in contact with the basement membrane.

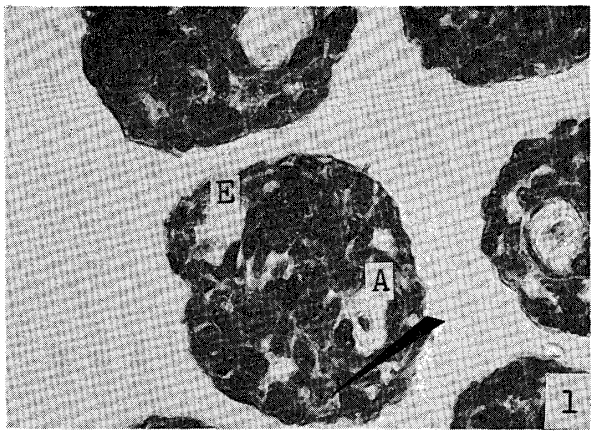
FIG. 6. Transverse section of the gill filament of the fry in fresh wster at 90 days after hatching, showing the high columnar cells possessing a mass of mitochondria which fill the cytoplasm.

FIG. 9. Longitudinal section of the gill filament of the alevins (21 days) in the 50 per cent sea water at one day after the transfer from fresh water, showing the changes to the high columnar form from spherical form. Compare with the Fig. 3.

FIG. 11. Longitudinal section of the gill filament of the fry (61 days) in the 100 per cent sea water at one day after the transfer from fresh water, showing the large granular cells which were nearly the same as the control (Fig. 5).

Magnification is 1,000× for Fig. 6 and 400× for the rest.

A; Afferent branchial arteriole
E; Efferent branchial arteriole
C; Cartilage
P; Pillar cell
M; Mitosis
R; Respiratory cell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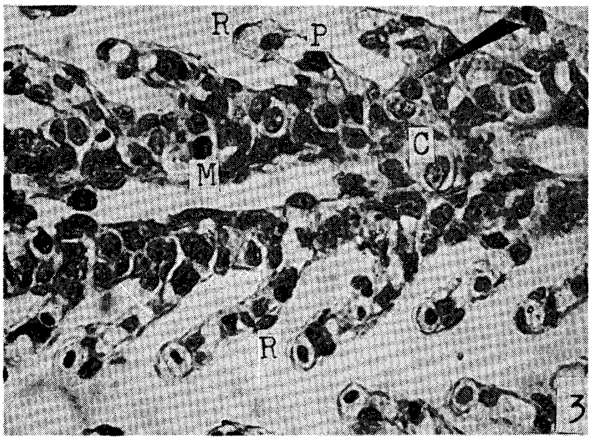
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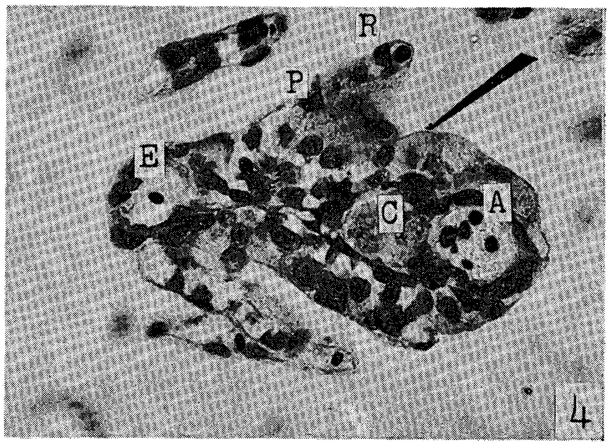
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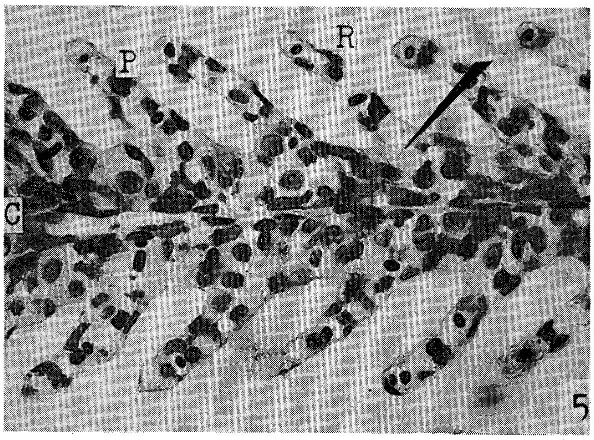
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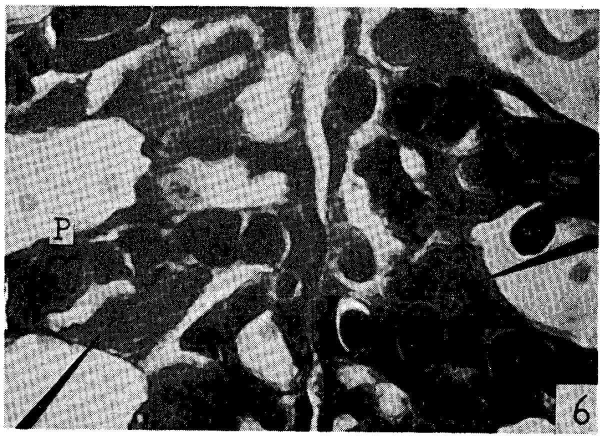
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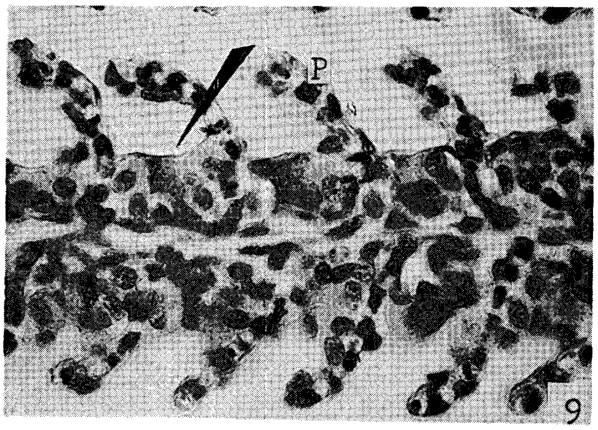
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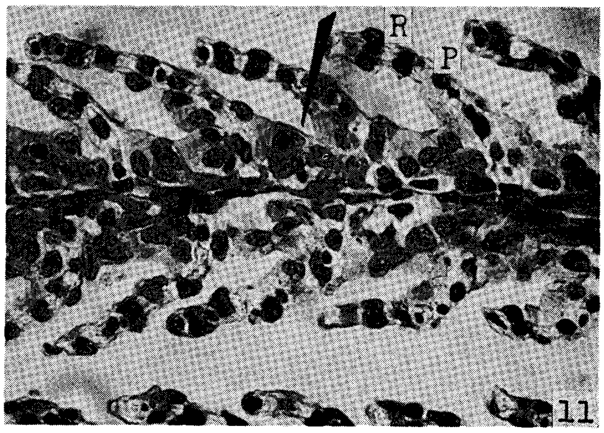
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