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Branchial Glands and the Problem of Chloride Regulation in the Gills of a Freshwater Cobitid fish, *Lepidocephalichthys guntea* (Ham).

B. R. Singh

Abstract The gills in fishes are generally recognized as the site for extra renal salt regulation, the exact cellular location being the large acidophil cells or "chloride cells". Munshi (1964) reported mucous gland cells responsible for chloride regulation in 5 species of fresh water teleosts belonging to different genera and families.

In the present study, two types of specialized cells were found: (a) the mucous gland cells which in addition of secreting mucous, give positive reaction for chloride, indicating thereby that they also play active role in chloride regulation and (b) large acidophil cells which show almost all the characterestic features of "chloride cells" but fail to give positive reaction for chloride.

It is therefore, argued that the cobitid fish in a fresh water habitat utilizes mucous gland cells as the site for chloride regulation and the acidophil cells ("chloride cells") are engaged in some other business, probably taking active part in carbonydratelipid metabolism of the gills.

The active participation of "Chloride Cells" in the extra-renal excretion of gills have been claimed by many workers in marine teleosts: (Smith, 1930, Keys, 1931, Keys & Willmer, 1932, Bevelander, 1939, Krogh, 1937, Liu, 1942, Copeland, 1948, Pettengill & Copeland, 1948, Getman, 1950, Morris, 1957, Colombo, 1961, Threadgold & Houston, 1961, Vickers, 1961, Kessel & Beams, 1962, Philpott, 1961–65, and others). Contrary to this view there are a number of workers who are of the opinion that the "Chloride Cells" do not participate in the regulation of Chloride in the gills: (Parry & Holliday, 1960, Doyle & Gorecki, 1961, Straus, 1963, Straus & Doyle, 1961, Holliday & Parry, 1962, Fleming & Kamemoto, 1963, and others).

Recently, Munshi (1964) reported the presence of actively secreting "Chloride Cells" in certain species of fresh water teleosts which were mucoid in nature. He is of the view that the variations in the hypertonicity of the blood and other body fluids in fishes necessiate the development of the "Chloride Cells" according to the need of the fish.

The present study was conducted on a fresh water cobitid, *Lepidocephalichthys guntea* which shows well developed acidophil cells ("Chloride Cells" of other authors) in addition to the actively secreting Chloride Cells (mucoid gland cells) in a fresh water habitat. Cytological and histochemical studies have been made on these cells to determine their probable role in the physiology of the gills.

Material and Methods

Living specimens of *Lepidocephalichthys guntea* were obtained from the river Burhi Gandak and the fresh water ponds in the localities of Siliguri, India. It is a small fresh water cobitid fish. Small pieces of its gills were fixed in Zenker's solution, Bouin's and Helly's fluid. The fixed materials were decalcified in 3% nitric acid in 70% alcohol. Sections were cut at 6–8 μ, stained with Haemotoxylin and Eosin to study the gross anatomy of the gills. In Zenker's solution, acetic acid replaced by equal volume of formic acid gave the best histological preparations. It also avoided decalcification of the tissue since

formic acid present in the solution decalcified the tissue while it was in the process of fixation.

Helly's fluid, followed by incubation in saturated solution of potassium dichromate for 3 days at 37°C served as a good cytological fixative. Mitochondria preserved by this method were stained with Heidenhain's iron Haemotoxylin. Some of the sections were also stained with Anilin-Fuchsin/Methyl Green method to demonstrate the presence of mitochondria.

Toluidene blue was used to demonstrate the metachromatic reactions in mucous producing cells. The Leschke silver technique as modified by Copeland (1948a, b) was employed to show the chloride reactions in the gill epithelium. Frozen sections of the gills were cut after fixing it in Formaldehyde/Calcium to demonstrate the presence of lipids.

Most of the histochemical tests were performed on the tissues fixed either in Zenker, Carnoy or Helly fluid. Ninhydrin test was applied either on the material fixed in Carnoy or on frozen sections after fixation in 5% formalin to detect the presence of free NH_2 groups in the acidophil cells. Methyl green/Pyronin method was used to demonstrate the presence of D.N.A. and R.N.A. Small pieces of gills were also stained vitally with Neutral red and Janus green B to study the secretory granules and mitochondria in the acidophil cells.

Observations

Branchial glands: There are only two types of branchial glands present in the gills of *Lepidocephalichthys guntea*, namely, the mucous glands and the acidophil cells.

Mucous glands: The mucous gland cells are large flask-shaped unicellular bodies, present in the epithelium covering the gill arch and the primary gill-lamellae (Fig. 1, 1). The nucleus is excentric in position and lies at the bottom of the gland. When stained in aqueous Toluidine blue, they become deeply violet bodies indicating metachromasia. These cells strongly react to PAS test. With methyl green-pyronin (Brachet's method) staining they show appreciable amount of R.N.A. The Alcian blue positive material of the cell has been identified as the acid mucopolysaccharides.

When Leschke silver test for chlorides, as modified by Copeland (1948a, b) was applied to the gills of the fish only these cells (mucous gland cells) gave the positive reactions (Fig. 1, 3 & 4). Their positive reactions with $AgNO_3$/HNO_3 indicate that these cells are responsible for ionic regulation of chlorides in the gills.

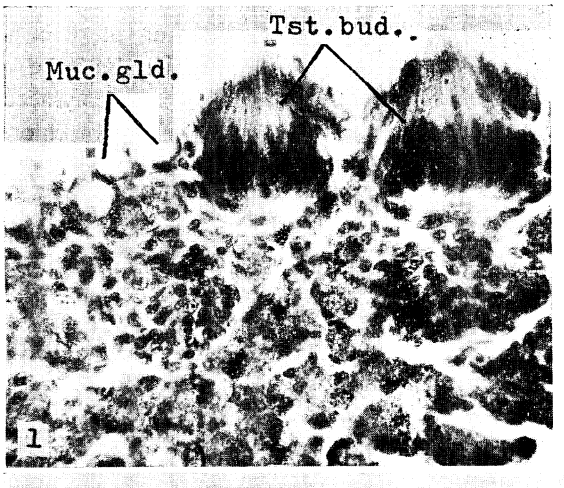
Acidophil cells:

The acidophil cells are very well developed in this fish (Fig. 1, 2). They are present in large numbers in the sub-epithelial connective tissue of the gills. They are also abundant near the primary afferent blood vessels of the primary gill-lamella. In the secondary gill-lamella they lie in the connective tissue layer just below the thin epithelium (Fig. 1, 5), close to the blood capillaries.

In the gill-head region, the acidophil cells are present at different levels in the thick epithelial coverage. Some of them grow larger than the others in size and almost reach the epithelium. This indicates that the cells are capable of migration from the sub-epithelial connective tissue layer to the epitheloid region of the gills. This is further evidenced by their occurance in the epithelial layer of the primary and secondary lamella (Fig. 1, 6).

The acidophil cells are generally oval or elliptical with a homogenous cytoplasm (Fig. 1, 2). The nucleus is small and round which is generally excentric in position. At some places the nucleus shows a central position as well. Generally, the nuclei of these cells are not distinguishable due to staining reaction but however, in some cells the nuclei are clearly marked out. The acidophil cells have a marked affinity for eosin and are easily distinguished from the mucous gland cells (Fig. 2, 3). In Mallory's triple stain for connective tissue, they take up Orange G. stain.

In the vital staining with Neutral red, these acidophil cells show large Neutral red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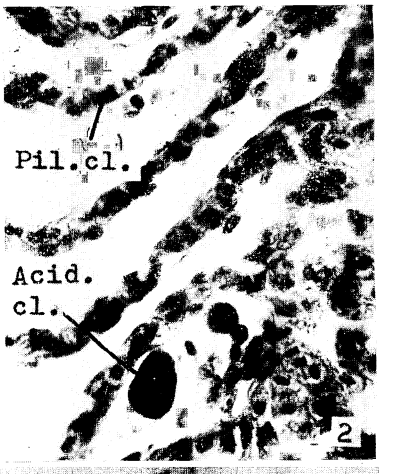
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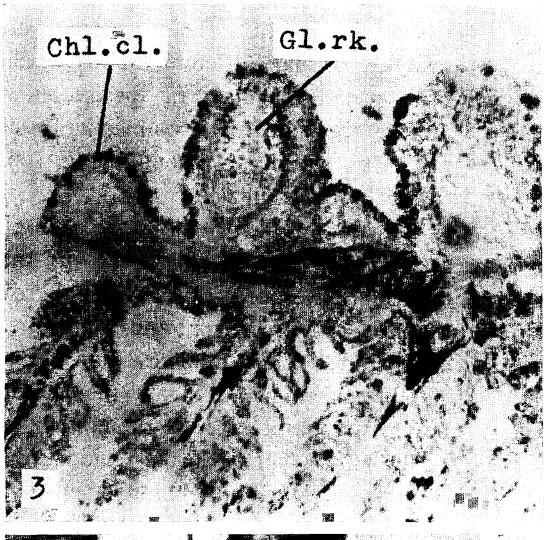
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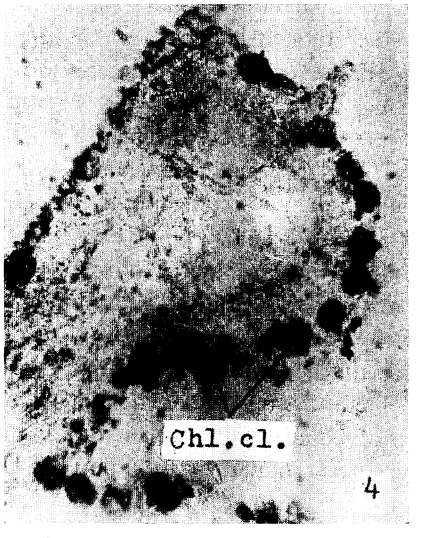
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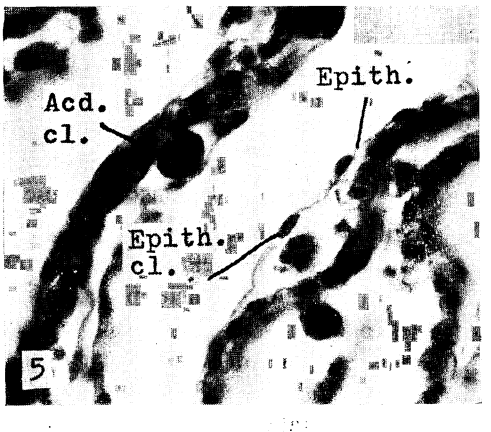
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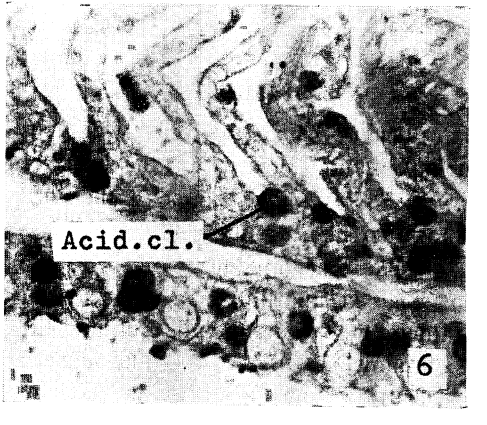
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vacuoles showing that they are secretory in nature. When stained with Janus green B, vitally, these cells show small granular mitochondria which is in accord with the results obtained by Heidenhen's Iron haemotoxylin preparations of the materials fixed in Helly's fluid. The cytological study of these cells reveals that they are either provided with small granular mitochondria (Fig. 2, 2) or with large spheroids (Fig. 2, 4). When stained with Anilinefuchsin/Methyl green, the cells show large fuchsinophil inclusions. In Iron-haemotoxylin method for mitochondria, the granules take up deep black colour and the mitochondria become indistinguishable. However, in some preparations, if the differentiation of the cells are made to the proper extent granular mitochondria become discernable.

The histochemical study of the cells shows that they are sudanophilic which enables them to be distinguished from other types of cells. When stained with Sudan Black B or Sudan IV, the nucleus is not distinguishable from the surrounding cytoplasm which takes up deep black or red colour respectively. Further differentiation of the lipid with Acid haematein method (Baker, 1946) indicates the presence of phospholipid.

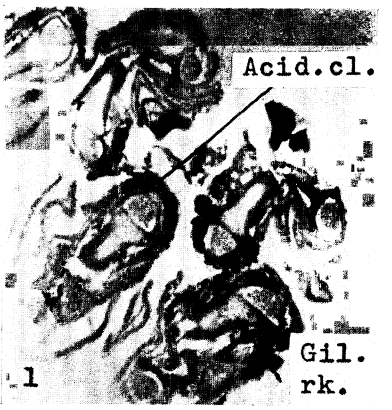
The acidophil cells do not show metachromasia with Toluidine blue. They strongly react to PAS test. The carbohydrate containing material of the cell has been identified as mucoprotein complex belonging to the group III of Pearse (1961). Pearse writes that "Mucoprotein stain more strongly with PAS than glycoproteins. This material has not been found to give strong gamma metachromasia". He further writes that "Protein materials which contain diastase fast carbohydrate in sufficient quantity to give a colour by the PAS method, but which do not exhibit gamma metachromasia with Toluidine blue are to be regarded as muco- or glycoprotein or as carbohydrate-protein complex". The positive Brom-phenol blue reaction can be taken as sufficient indication of the presence of protein (Pl. 2 fig. 1). When Ninhydrin test for NH_2 group was employed, the violet colour spread all over the sections hence it was not certain whether NH_2 group was present in the acidophil cells or elsewhere.

Discussion

From 1930 onwards, there has been much controversy regarding the nature of the "Chloride Cells" in the gill-epithelia of teleosts. The work of Munshi (1964) on the "Chloride Cells" in the gills of fresh water teleosts clearly shows that the mucous gland cells are responsible for chloride excretion in these fishes. The chloride test applied to the gill-epithelia of *L. guntea* supports the observation of Munshi (1964). In this fish also, the mucous gland cells excrete chloride along with mucous.

The presence of well developed acidophil cells in addition to the active "Chloride Cells" in a fresh water habitat is a new result reported here. It has been observed that the acidophil cells belong to the connective tissue layer, though occasionally they come up to the epithilial layer. They are rich in protein, mucopolysaccharides and phospholipids. The morphological study with light microscopy reveals that the acidophil cells resemble in many important features with the "Chloride Cells" of marine teleosts described by several authors. The cytological and histochemical properties further support the idea that they

Fig. 1. Cytelogical microphographs of gill-lamellae of *Lepidocephalichthys guntea:* symbols, Acd. cl . . . Acidophil cell, Chl. cl . . . Chloride cell, Epith . . . Epithelium, Epith. cl . . . Epitherial cell, Gl. rk . . . Gill raker, Muc. gld . . . Mucous gland cell, Nuc . . . Nucleus, Pil. cl . . . Pilaster cell, Tst. bud . . . Taste bud. (1) Mucous gland cells and taste buds at the margin of the primary gill-amella ×430, (2) Large acidophil cells in secondary lamella, note the difference in size ×1200, (3) Chloride reactions in the epithelium covering the glli arch, gill-rakers and the gill--lamellae. ×140, (4) A part of the same under H.P. ×450, (5) Acidophil cells just below the thin epithelium in the connective tissue layer of secondary lamellae. ×1200, (6) Acidophil cells (PAS positive) coming up to the epithilial layer in primary and secondary lamellae ×450.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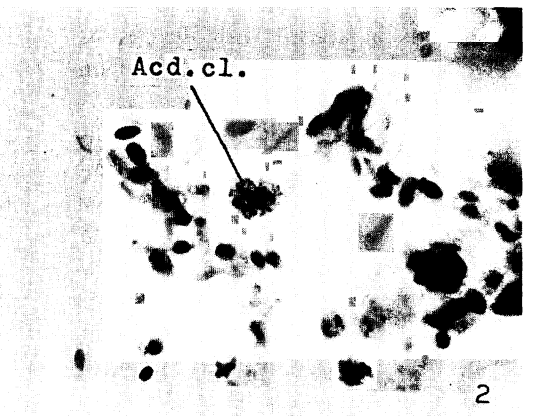
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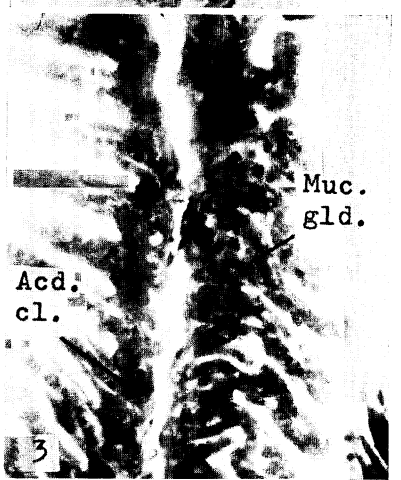
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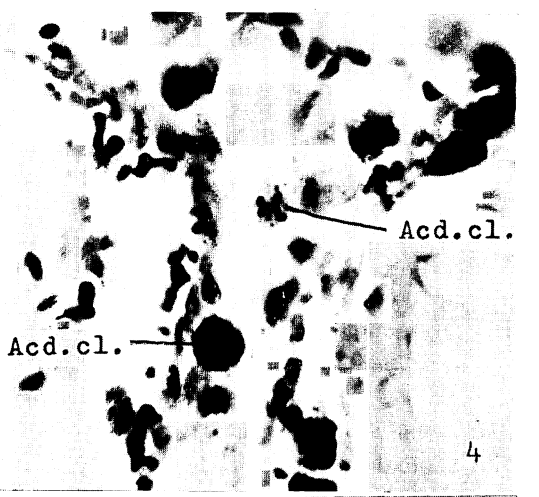
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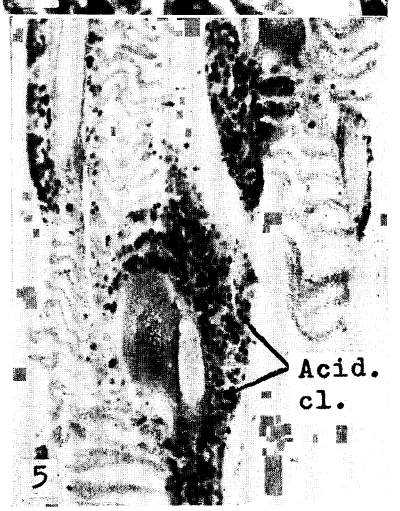
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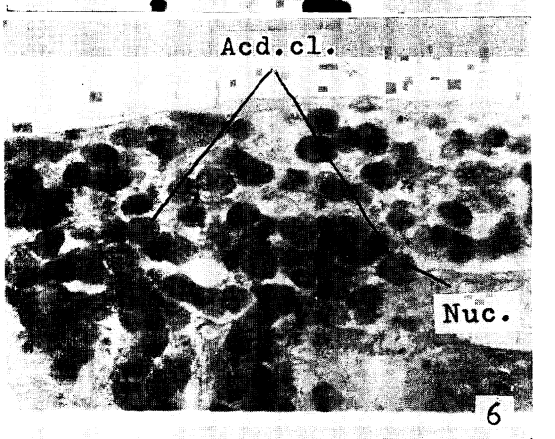
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are the same type of cells described by other workers in different teleosts. A list of the properties as recorded by earlier workers on the "Chloride Cells" with which these acidophil cells tally is given below:-

(1) The chloride secreting cells have been described as large, ovoid or columnar; the cytoplasm is granular and they have a marked affinity for eosin (Copeland, 1948 and 1950; Keys and Willmer, 1962).

(2) The nucleus is nearly spherical and may be excentric in position (Liu, 1942); the cytoplasm may be clear and gives the acidophilic reactions (Morris, 1957).

(3) They belong to the gill-epithelia of the primary and secondary lamellae and may even reach the sub-epithelial connective tissue (Getman, 1950).

(4) There is correlation between the number of the chloride cells present and the degree of vascularity (Burns & Copeand, 1950).

(5) Alkaline phosphatase has been shown to be present in the excretory vesicle of the chloride cells (Pettengill & Copeland, 1948) but however, Vickers (1961) could not get this reaction.

(6) They show no metachromasia with Toluidene blue but strongly react to Periodic acid/Schiff (PAS) reactions (Vickers, 1961); the cytoplasm and inclusions are sudanophilic (Morris, 1957).

(7) The cytological study on the "Chloride Cells" revealed that the mitochondria may be in the form of large spheroids, filaments or granules and large number of fuchsniophil inclusions occupy the cells (Morris, 1957).

It may be argued therefore, that as regards the position, shape, cytology and histochemistry, the acidophil cells of *L. guntea* resemble the "Chloride Cells" of other authors in marine teleosts. However, there are two important features in which they differ from the "Chloride Cells": (a) that they do not show "apical pits" and (b) they do not react to $AgNO_3/NHO_3$ test for chloride indicating thereby that they do not participate in ion regulation.

The "apical pits" are said to be characteristic of only marine teleosts. However, Straus and Doyle (1961) found the "apical pits" in guppies adapted to both fresh and salt water. Since the acidophil cells do not react with Leschke silver test but show other characteristics of the chloride cells, the question arises as to their probable role in the physiology of the gills.

Parry & Holliday (1959) have disclaimed the chloride secretary function of the acidophil cells in the pseudobranch of fish. In the electron microscopic study on the gills and pseudobranch of *Pleuronectes flesces*, they found the acidophil cells in the gills and pseudobranch to be identical in appearance and resembling the "Chloride Cells" of earlier workers. Similar cells have been reported by Doyle & Gorecki (1961) in the two species of elasmobranchs. On these grounds, Parry & Holliday (1962) concluded that the acidophil cells of the gills were not ion-regulatory.

Holliday & Parry (1962) further reported that the acidophil cells of the pseudobranchs have an endocrine function related to colour change. Furthermore, they suggested that the morphologically identical cells in the gills might have a similar function. The acidophil cells as such do not take active part in chloride regulation when the fish is in its natural habitat.

Fig. 2. Hitochemical microphotographs of gill-lamellae of *Lepidocephalichthys guntea*. Symbols are same as in Fig. 1. (1) Brom-phenol blue preparation showing acidophil cells rich in protein ×140, (2) Mitochondrial preparation (Iron-haemotoxylin method) in the acidophil cells, note the granular mitochondria ×1200, (3) Mucous gland cells and eosinophilic acidophil cells, note the difference in staining. (Haemotoxylin-Eosin preparation) ×100, (4) Mitochondrial preparation in the acidophil cells (Iron-haemotoxylin method), note the large spheroids ×1200, (5) PAS positive acidophil cells near the afferent blood vessel in large numbers ×100, (6) A part of the same under H.P. ×450.

They may be responsible for the general lipid carbohydrate metabolism of the gills. But when the fish is subjected to experimental conditions by intraperitoneal injections of sodium chloride of different concentrations varying from isotonic to sea-water salinity, these cells give strong positive $AgNO_3/HNO_3$ reactions for chlorides indicating their active participation in chloride regulation.

Summary

(1) Two types of specialised cells are found in the gills of *L. guntea* namely, (a) typical mucous gland cells and (b) large eosinophilic glandular cells (acidophil cells).

(2) The mucous gland cells are responsible for excreting chloride as well as mucous in a fresh water habitat.

(3) The acidophil cells resembling in a large array of characters with the "Chloride Cells" of marine teleosts described by earlier workers, fail to give positive reactions for chlorides.

(4) These cells (acidophil cells) which are rich in protein mucopolysaccharides and phospholipids seem to have an active role in lipid-carbohydrate metabolism of the gills.

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(Department of Zoology, University of Bihar, Muzaffarpur, Bihar, India)

インド産淡水ドジョウ *Lepidocephalichthys guntea* における鰓腺と塩化物調節の問題　B. R. シング

魚類において鰓が過剰塩化物調節を行うことは一般に認められており，その実際の作用の行う細胞は大形の好酸性細胞，つまり"塩化物調節細胞"である．Munshi (1967) は異科異属の5種の淡水魚において粘液腺細胞が塩化物調節を行う事を見出した．今回，インド産ドジョウ *L. guntea* の鰓を研究し，筆者は2種の特化した細胞を見出した，つまり，(a) 粘液腺細胞—これらの細胞は粘液を分泌するのみならず，塩化物に対しても正に反応，したがって，その調節にも活発な働きをしていることが示される，(b) 大形の好酸性細胞—これらの細胞は"塩化物調節細胞"のもつ特性を殆んど完全に示したが，塩化物に対して反応を示さない．したがって，淡水性のこのドジョウでは粘液腺細胞が塩化物の調節を行う場所であり，好酸性細胞（塩化物調節細胞）は恐らく他の働き，鰓における炭水化物・脂質代謝に大きな役割を演じているものと解された．

（ビハール大学動物学部　ムザファルプール，インド）