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Histophysiology of Byssus Gland of Captive *Mytilus viridis* Revealed through Histochemistry

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Abstract*: Histochemical and Electron microscopic studies were carried out on byssus gland of Mytilus viridis (found along the coast of Gujarat) to get an idea of histophysiology of the gland of the animal during the conditions of chronic and acute starvation under laboratory conditions. Byssus production during chronic starvation is not impaired. During acute starvation byssus production is negligibly affected. Gland becomes paler in color during acute starvation as Tyrosine and Arginine are reduced during starvation. Tryptophan is not detected in the gland by histochemical staining during the periods of starvation. Amount of keratin and elastin does not change. The amount of mucins and sulphated sugars increase during starvation, this can be accounted to the stress condition arisen thereby. Light mucins increases and dense mucins decrease during starvation. Acid mucosubstances increase during starvation. Lipids stored at the posterior end of the foot are completely replenished during starvation however phospholipids are detected here.
Key words: histochemistry, histophysiology, Mytilus viridis, byssus gland, starvation.

I. INTRODUCTION

Mechanism of byssus formation is very complex because the byssus itself is complex in its detailed structure and chemical nature. Many methods have been employed to study the mechanism of byssus formation and much work has been done on this line [1-3] but a complete success has not yet been achieved. Scientists are trying to unveil the mystery of byssus formation so that they may produce and manipulate this valuable material in the laboratory putting it to various desired uses in the field of medicines and industries especially biotechnological applications [4]. So far much work has been done in species of *Mytilus* other than the Indian (*Mytilus viridis*). A few studies on this line have been done on Indian species by Banu et al. (1979) [5]. There are no reports on the study of this line on *Mytilus* found in the coast of Gujarat along Dwarka and Mocha so far. In this paper, attempts are made to throw some light on the byssus formation under normal conditions as well as under starvation. It was a common observation that when the animals underwent starvation during their maintenance under laboratory condition, they still continued to form byssus threads till their death. It was therefore necessary to study the histophysiology of the gland during normal condition as well as during starvation. In the present chapter histophysiology of the glands during normal times and during the times of chronic and acute starvation were done through histochemistry and electron microscopy.

II. MATERIALS AND METHOD

A. Preparation of foot for microtomy

The animals after being cleaned thoroughly in sterile artificial seawater, were narcotized by placing them in hypotonic $MgCl_2$ solution for about 30 minutes. They were dissected by opening the shell and severing the posterior retractor muscle with the help of a sharp scalpel. The foot tissue was then excised by means of sharp sterilized scissors and kept in seawater. This was then transferred immediately to 9% formalin solution and kept for a day. These fixed tissues were then embedded in paraffin wax and used for microtomy. 5µm sections were taken on a hand rotary microtome.

B. Chemicals used

The following stains:

1) Haematoxilin (Delafields) - BDH, Glaxo lab. India pr. no 38803.
2) Eosin - BDH, London, C.I. 648912.
3) Basic fuchsin - BDH, England, C.I. 42510.
4) Alcian blue - Fluka Switzerland, C.I. 439.
5) Toludine blue - E. Merk, Germany, C.I. 52040.

6) Sudan black-B - HiMedia, India, C.I. 26150.
7) Bromophenol blue - CDH, India, C.I. 20015.
8) Nutral red - HiMedia, India, C.I. 50040.

Other chemicals like $HgCl_2$, HCl, H_2SO_4, formic acid, paraldehyde etc. were of analar grade. Double distilled glass water was used during all the procedures.

C. Histochemical procedures

Various histochemical procedures were carried out by the protocol suggested in book on Histochemistry by Pearse [6-9].
Animals under three different conditions were selected for the study.

1) Normal animals collected from the sea,
2) The animals kept in aquarium for 2 months (chronic starvation) and
3) The animals kept in aquarium for 4 months (acute starvation).

D. Preparation of material for TEM

Foot was excised from mussel and fixed in cold 5% glutaraldehyde in 0.01 M phosphate buffer for one hour. After washing in buffer, the tissue was post-fixed in 1% osmium tetroxide for an hour, dehydrated in a graded ethanol series and infiltrated [10]. This was then embedded in Spurr's medium. Thin sections were stained with uranyl acetate followed by lead citrate. The sections were examined with a Zeiss (Germany) Transmission Electron Microscope.

III. OBSERVATION

A. Histochemistry of gland from normal animals

Byssus glands of animals, captured from the sea i.e. in their natural condition, show a complex structure comprising of numerous ciliated channels and many types of glands histologically appearing similar but histochemically different. [11].

B. Histochemistry of gland from chronically starved animals

When the animals were maintained in laboratory for a period of two months. The animals did not consume food which was given to them and they were found starving under this condition. Upon dissection, they were found to have empty guts and regressed gonads. Byssus production was not impaired. The foot glands when observed under microscope after various staining procedures of histochemistry (Table-1) no significant changes in the anatomy as well as histochemistry were seen. In addition, the localization of carbohydrates, protein as well as fats was almost the same as in case of normal individuals.

C. Histochemistry of gland from acutely starved animals

The animals under acute starvation i.e. for a period of four months upon dissection showed severe regression of the gonad and almost invisible gut to a naked eye observation. The foot showed no much change in its contours but one remarkable change was that mucus secretion was more when observed by naked eye and they became paler than normal. When such glands were stained for histochemical studies it was revealed that the mucus secreting cells were more visible. The ciliated nature of the cells lining the channels was almost lost and the protein content in and along the channels of the gland was reduced so much so that some amino acids were not detected through histochemical studies (Table-1).

IV. RESULTS AND DISCUSSION

A. Histophysiology from histochemical studies

The gross anatomy of the gland does not undergo any changes during the periods of starvation. The only changes that are marked are the reduced ciliated epithelium lining the channels of the gland. Also mucus secretion is excess. The amount of proteins in the gland, especially in the channels is decreased due to starvation (Plate-1).
Histochemical staining through Millon's reaction confirmed that in the case of starved animals, tyrosine was reduced in the channels which are known to secrete byssus. Also as mentioned before the byssus gland became paler during the periods of starvation. This has a direct bearing with phenol gland [12] involving tyrosine metabolism. Similar stained regions (magenta) by Ninhydrin-Schiff's method in fed as well as starved animals confirmed that proteins containing amine group were reduced under acute starvation. Tryptophan was absent in starved animals. Sakaguchi's reaction showed that amino acid Arginine is reduced during starvation. Pink

and deep magenta stained regions in the gland (Plate-2) indicates that amount of S-S group containing amino acids as well as keratin in the byssus material under the condition of starvation does not change. No change in color reaction during Aldehyde fuchsin staining shows that elastin in byssus secreted during starvation does not change.

Intense purple color metachromasia indicates that presence of carbohydrates like mucins and sulphated sugars increases during the periods of starvation (Plate-3). This may be accounted to the stress condition created during starvation, when actually the byssus generation was not impeded. Purple red and intense blue coloration by Periodic acid Schiff"s staining reveals the presence of glycogen thereby. Also ciliated nature of the channels is obstructed by the intense coloration (Plate-4). Dense mucins decreased whilst light mucins increased, this is confirmed from Alcian blue staining techniques at different pH. Intense blue coloration due to PAS-Alcian blue positive staining confirms the increased amount of carbohydrate moieties (acid mucosubstances etc.).

Important information regarding the lack of impairment in the power of production of byssus during the periods of chronic as well as acute starvation came from the very fact that Sudan black-B and Acetone Sudan black-B staining for unbound and bound lipids respectively was negative in starved individuals. This indicates the consumption of fats from those areas (posterior regions of foot as well as gonads). Lipid utilization from these areas could be a mechanism to keep a tone with the increased crisis during the periods of starvation. Reduction in Peracetic acid Schiff's and Performic acid Schiff's positive staining localized at the posterior most portions of the glands indicates utilization of lipids from the posterior region of the gland.

B. Histophysiology from Electron Microscopic studies

Electron microscopy of the glands of normal and starved animals showed no much difference. The cells under starvation had the same number of vesicles (Plate-5). Also nucleus was well seen; some vesicles at the stages of coalescence as well as secretion were seen. This indicated that during starvation byssus production was not impeded.

V. CONCLUSION

Byssus production during chronic starvation is not impaired. During acute starvation byssus production is negligibly affected. Color of gland changes (becomes paler) during acute starvation. Tyrosine and arginine are reduced during starvation. Tryptophan is not detected in the gland by histochemical staining during the periods of starvation. Amount of keratin and elastin does not change. The amount of mucins and sulphated sugars increase during starvation, this can be accounted to the stress condition arisen thereby. Light mucins increases and dense mucins decrease during starvation. Acid mucosubstances increase during starvation. Lipids stored at the posterior end of the foot are completely replenished during starvation however phospholipids are detected here.

VI. ACKNOWLEDGEMENT

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Table-1 Histochemical localization of proteins, carbohydrates and lipids in byssus gland of *Mytilus viridis* under conditions of starvation.

METHOD	LS of foot in normally fed *Mytilus*	LS of foot in chronically starved *Mytilus*	LS of foot in acutely starved *Mytilus*	REMARKS
Proteins				
1.Bromophenol-blue method	+ +B, G	+ +B, G	B, G	Proteins reduced
2.Million's reaction	+ yR	yR	-	Tyrosine reduced
3. Ninhydrin-Schiffs method	+M	+M	M	Amine bound proteins reduced
4. Tryptophan method	mG	-	-	Tryptophan absent under starvation
5. Sakaguchi's reaction	+O	O	-	Arginine diminished
6. Performic acid Schiffs method	+Pn, ++M	+Pn, M	++Pn, M	-S-S- group and keratin present.
7.Aldehyde-Fuchsin method	++B	++B	++B	Elastin present.
Carbohydrates				
1. Toludine blue method (temporary)	+++P	+++P	+++P	(Metachromasia) Mucins, Sulphated sugars
2. Toludine blue method (permanent)	++P	++P	+++P	(Metachromasia) Mucins, Sulphated sugars increase
3.PAS method	+++B, +Pr	+B, ++Pr	+B, +Pr	Glycogen
4.Alcian blue (pH. 2.5)	+B	++B	+++B	Light mucins increase
5.Alcian blue (pH. 1.0)	+++B	++B	B	Dense mucins decrease
6.PAS-Alcian blue	++B	++B	+++B	Acid Mucosubstances
Lipids				
1.Sudan black-B	++Br	Br	-	Lipids
2.Acetone Sudan	+++Bl	Bl	-	Bound lipids
3.Peracetic acid Schiff	+R (at Post. Most portion)	R (at Post. Most portion)	R (at Post. Most portion)	Phospholipids with double bonds present
+-Positive,–- Negative, B-Blue, mG-Mauve, G-Green, yR-Yellowish-red, M-magenta, Y-Yellow, O-Orange, Pn-Pink, P-Purple, Pr-Purple red, Br-Brown, Bl-Black, R-Red., +-indicates light, ++-indicates moderate and +++- indicates intense				

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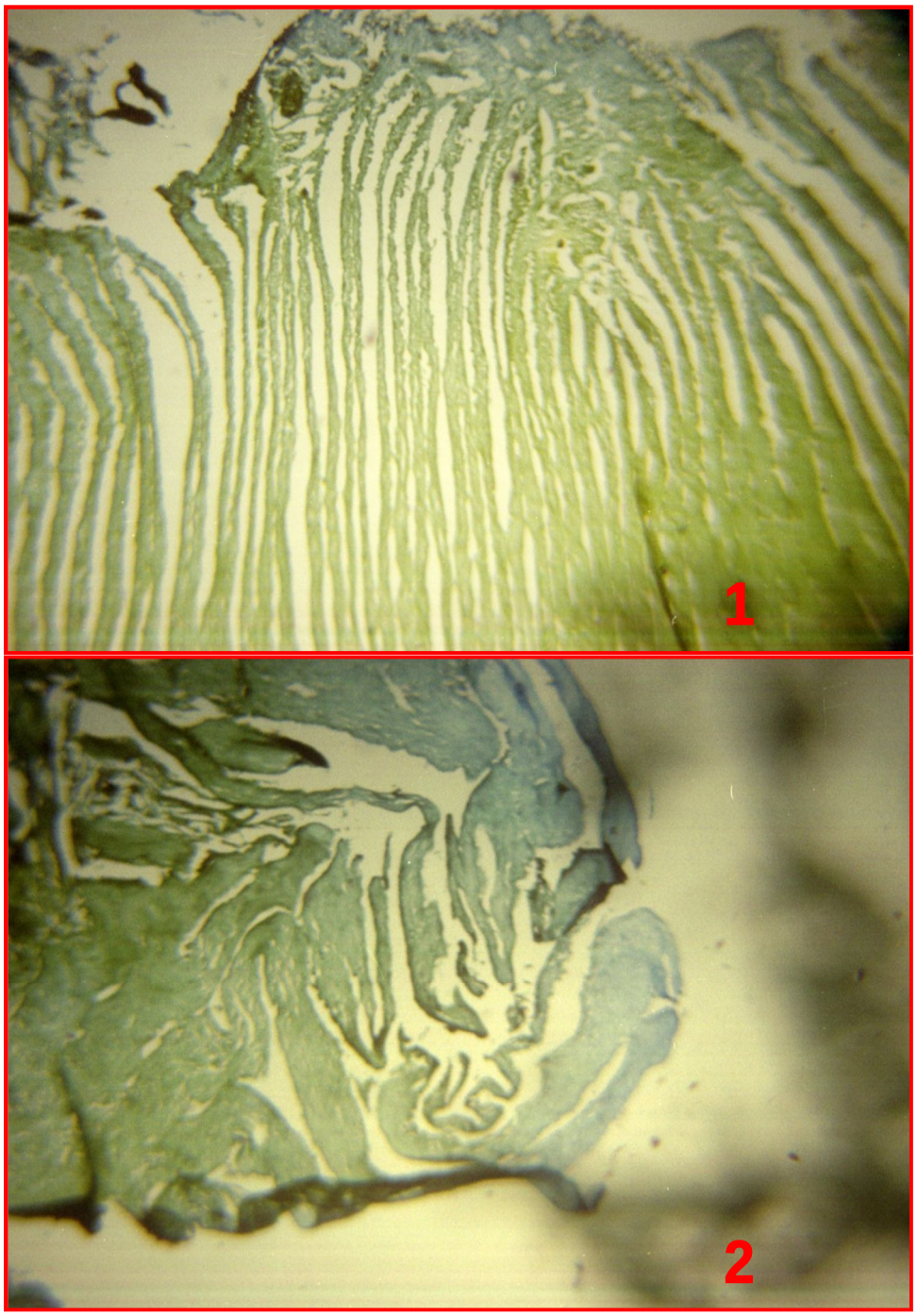

Plate-1 LS of byssus gland of 1- chronically starved and 2- acutely starved *Mytilus* stained with Bromophenol blue.(4X)

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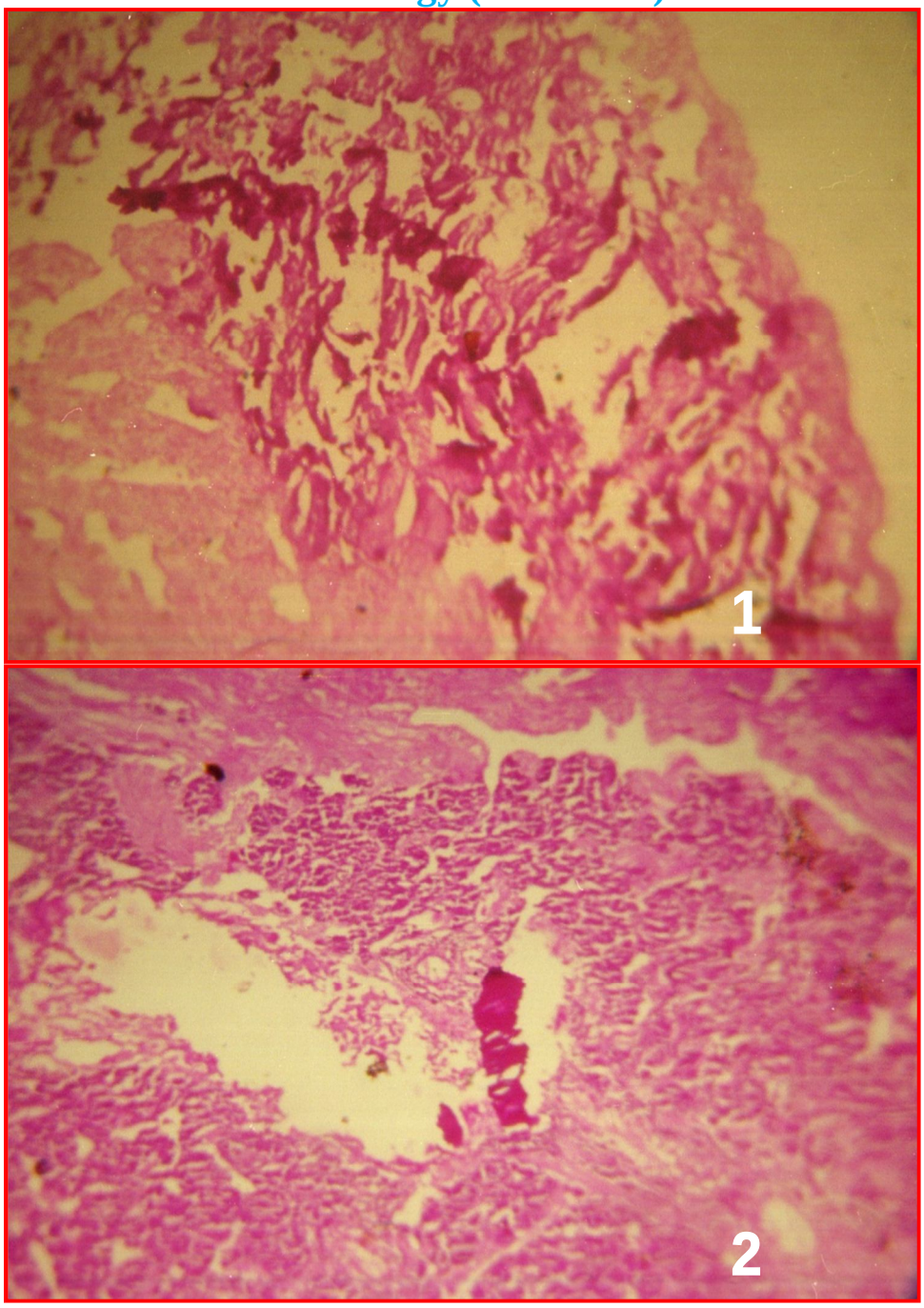

Plate -2 Performic Acid-Schiff's staining of LS of foot gland of
1- chronically starved and 2- acutely starved *Mytilus*.(4X)

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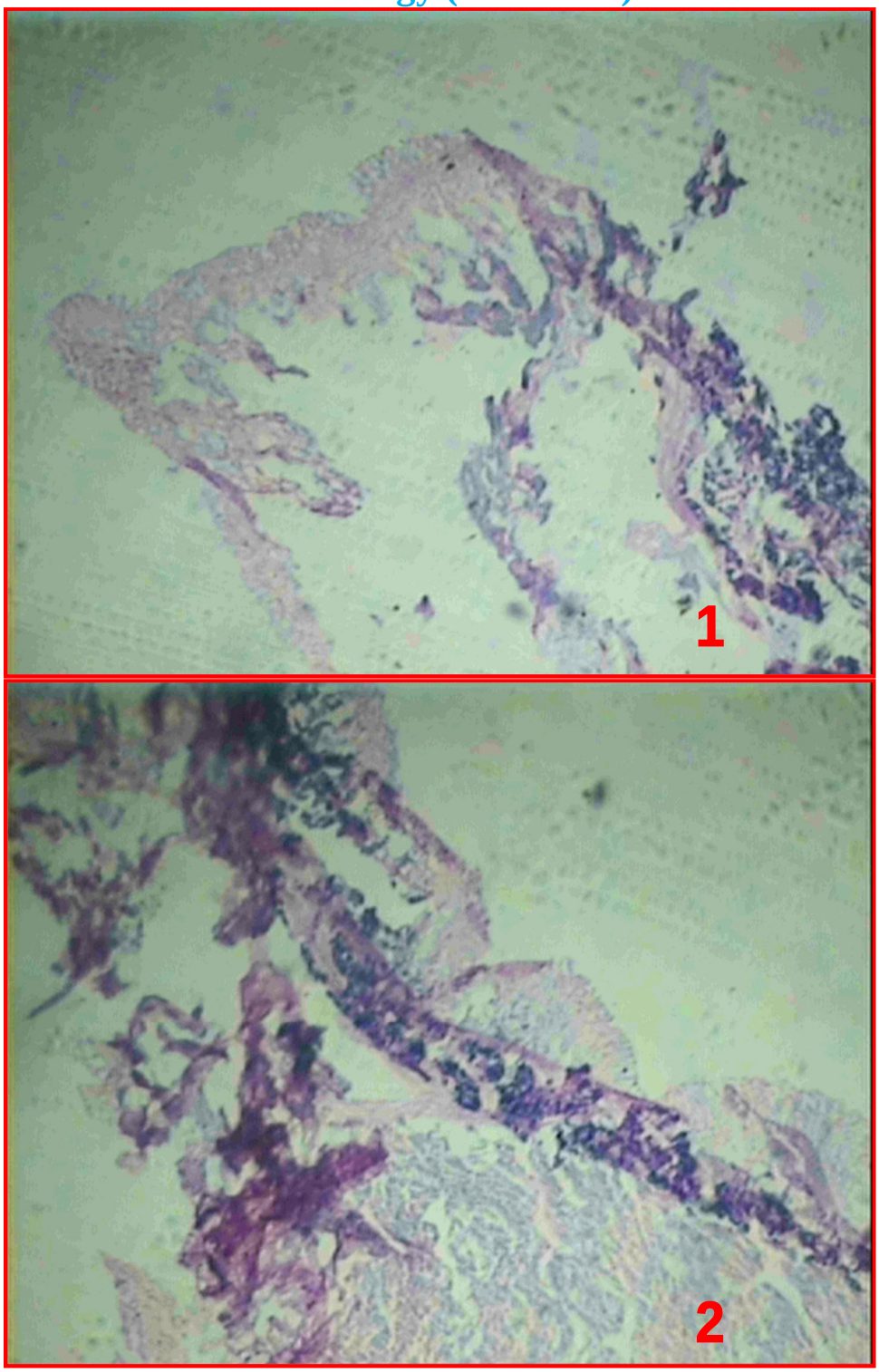

Plate -3 Intense purple coloration metachromasia by Toludine blue staining in LS of 1- chronically starved and 2- acutely starved *Mytilus'* foot gland.(10X)

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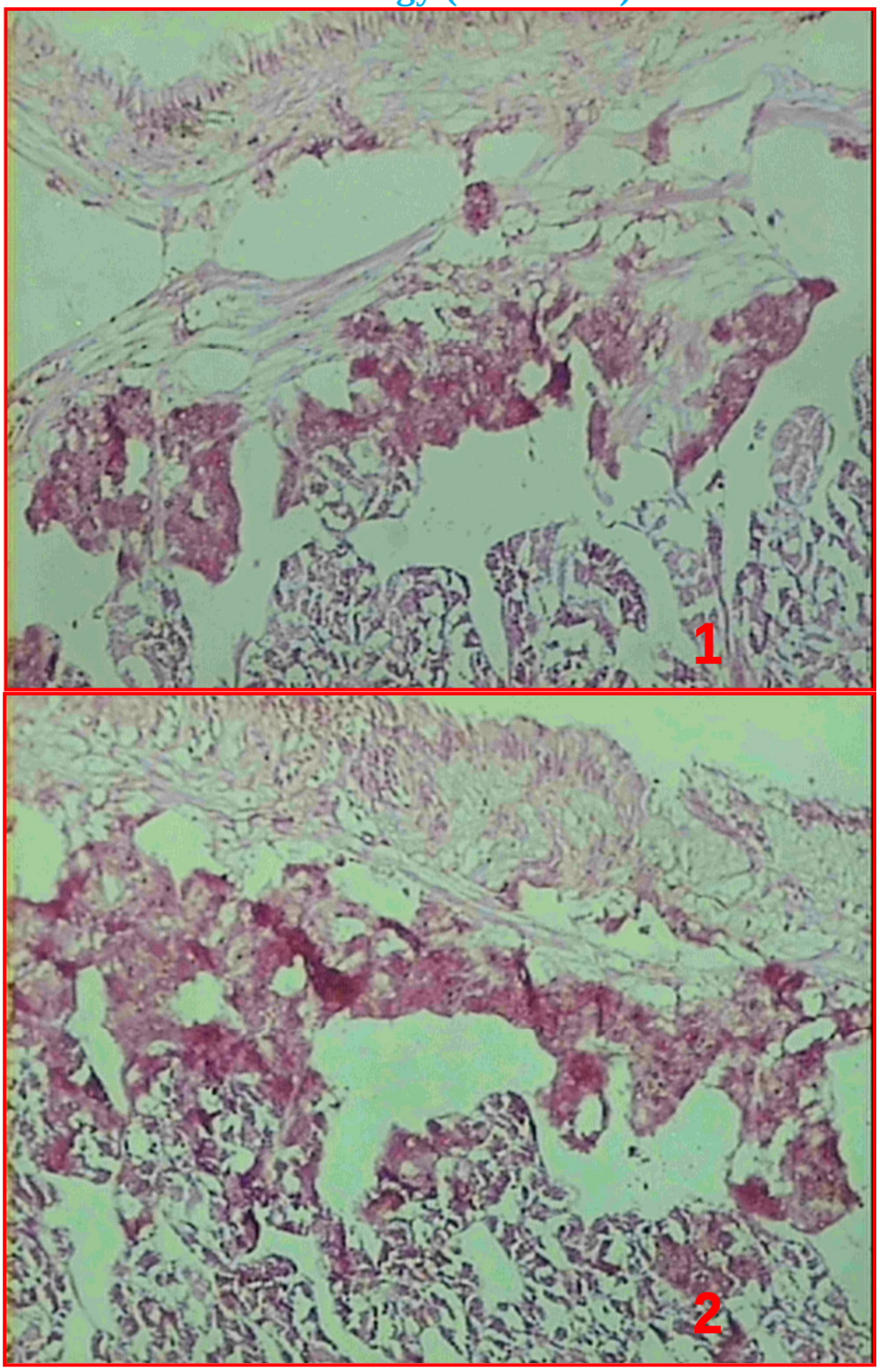

Plate-4 Periodic Acid-Schiff's staining in LS of foot gland of 1- chronically starved and 2- acutely starved *Mytilus* showing intense coloration.(10X)

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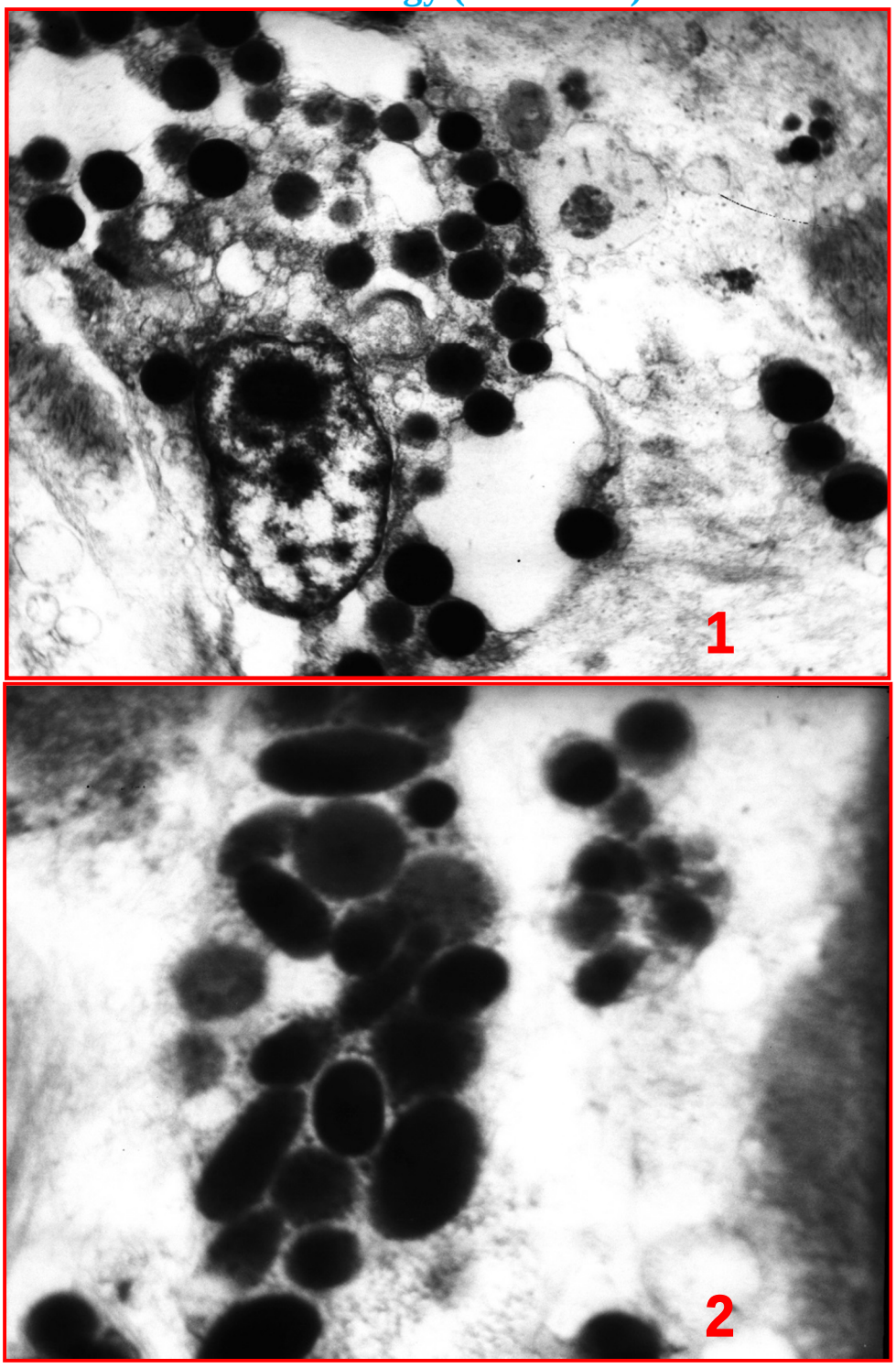
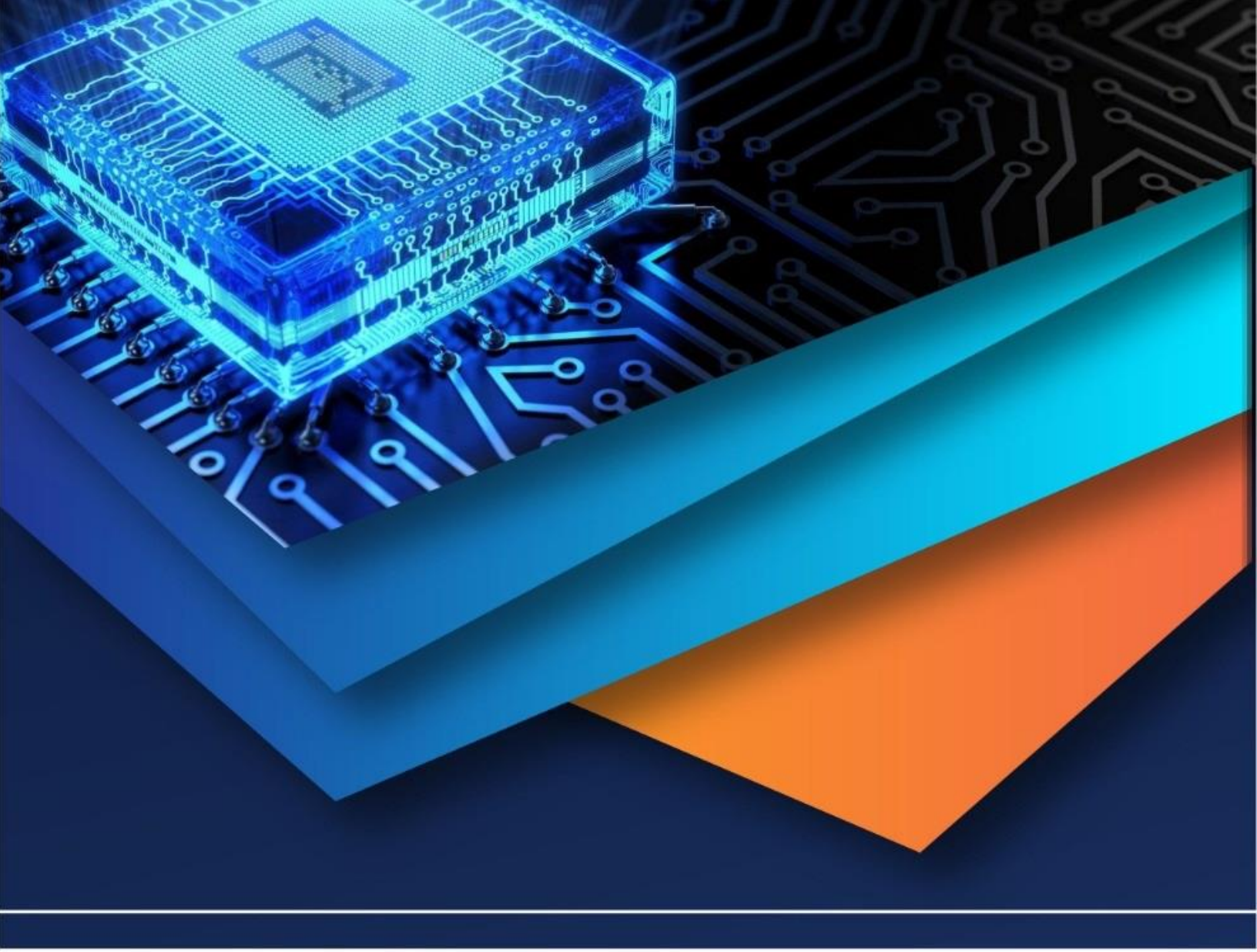


Plate-5 TEM of byssus gland cell of 1- chronically starved and 2- acutely starved *Mytilus*.(7000X)



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