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Effect of Cadmium Chloride on Ultrastructure of Gill Filament in *Tilapia mossambica* (Peters)

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Abstract:

The present work deals with cadmium induced ultrastructural changes in the gill of Tilapia mossambica (Peters). Fishes were exposed to sub lethal concentration of 8 ppm of Cadmium chloride for 6 days. The present study shows contraction of gill rakers and devastating changes within the entire epithelial lining covering the lamellar and interlamellar regions. Primary gill filaments appear wavy and twisted and secondary gill filaments appear highly contracted and give a stumpy look. Fusion of the adjacent secondary lamellae is seen at the tips of the primary lamella. Continued exposure has been found to affect the taste buds which show a tendency to separate from each other and the free cytoplasmic processes of the cell show an increased number of cytoplasmic granules. The connective tissue fibres in the gill head region of the treated fish too are drastically reduced. Noticeable changes were seen in mucous cells, pillar cells, interlamellar cells and chloride cells of the gill filament. These changes include vacuolation of the cells, separation of cell layers, indentation of cell nuclei, and appearance of mitochondrial vacuolation. Appearance of short tuberculate processes over the free cell surface is characteristic in treated fish. Mucous cells increase with the treatment and chloride cells exhibit an increased number of villar processes. Pillar cells appear in a highly contracted state.

Key words: Cytoplasmic vacuoles, mitochondrial vacuolation, phagocytic vesicles, emarginated cell surface.

Introduction:

The careless disposal of heavy metals in the aquatic system is a cause of concern because of their toxicity and biomagnification. Cadmium is one such heavy metal which is known for its non-corrosive nature and is widely used in manufacturing batteries, paints and dyes and also in the plastic industries. Cadmium occurs naturally in the environment in insignificant amounts but its release in the recent past is steadily increasing due to anthropogenic activities causing pollution of soil and aquatic ecosystems. Biomagnification of Cadmium takes place at trophic levels and is found to be highest in algae (Ferard *et al* 1983; Authman *et al 2013*). It also accumulates in many aquatic organisms including fish which are a part of the aquatic food chain.

Since many aquatic organisms including fish form a part of the food chain this has resulted in accumulation of large amounts of toxic metals in them (Ishaq S. Eneji et al 2011, D. Kumar Babu *et al* 2009, Kumar et al 2008, Jayakumar *et al* 2006, Thopon S. *et al* 2004). Cadmium is found to be teratogenic, embryo toxic, carcinogenic, nephrotoxic in humans too. It acts as a stressor affecting enzymes, which control all the biochemical reactions of the cell in particular and the organism as a whole.

Gill epithelium of fish is a sight of gaseous exchange, ionic regulation, acid-base balance and nitrogenous waste excretion; hence various environmental pollutants like xenobiotics and heavy metals are known to affect its morphology. Thus fishes act as heavy metal indicators and assess pollution in the aquatic environment. The present study has being done to evaluate the effects of cadmium chloride on

the Ultrastructure of gill filament in *Tilapia mossambica* (Peters).

Materials and Methods:

Live fish were obtained from Masunda lake in Thane district and kept for a fortnight for laboratory acclimatization. They were fed on alternate days with live tubifex worms. During experimental exposure, to maintain the concentration of toxicant, test water was changed every 24 hours. The tanks were aerated with oil free air. Test water quality was evaluated employing standard methods (APHA, 1985).

In the present study the gill tissue of fish was treated with Sub lethal concentration of 8 ppm of cadmium chloride for a period of 6 days. A control tank was also set up. Fishes from each tank were sacrificed by decapitation and the gill tissues were fixed in 3 % glutaraldehyde for 30 mins at 4 ºC & secondarily fixed in Osmium tetraoxide for another 1 hour at 4 ºCand processed for electron microscopy. Semi thin and ultra-thin sections were taken on LKB ultramicrotome and picked up on G-200 copper grids. Semi thin sections were stained with Toluidine blue for half an hour and ultrathin sections were stained for one hour with uranyl acetate and counter stained with lead citrate. Semi thin sections were seen under the compound microscope and grids were scanned under a Ziess EM 109 electron microscope and JEM Joel 100 'S' Japan make electron microscope.

Results & Observations:

Gill of fish:

Teleost have 4 pairs of gills. The structure of gill though varies with habit, habitat and the species of the fish, its basic structural patterns remains the same in all forms (Hughes 1973). The gross structure of *Tilapia mossambica* (Peters) with

its cellular and Ultrastructural details has been described earlier (Sundaresan and Shanbhag 2009, Pai 1993, Dixit Shailaja 1979). Photograph no. 1 & 2.

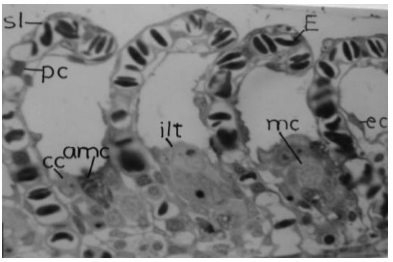


Fig 1: EM (Semi thin section) of gill filament (control)

Key: pl – primary lamellae, pc – pillar cell, ec – epithelial cell, sl – secondary lamellae, e – erythrocyte, cc – chloride cell, mc – mucous cell, amc – acidophilic mast cells, ilt – interlamallar tissue

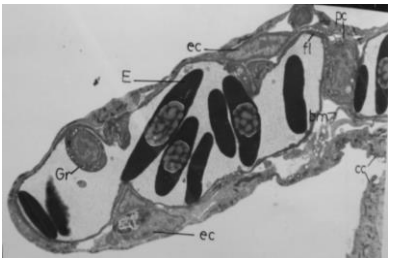


Fig 2: EM of gill (control) showing secondary lamalla

Key: pc – pillar cell, ec – epithelial cell, E – erythrocyte, cc – chloride cell, bm – basement membrane, fl – flanges, gr – granulocyte

Gill of treated fish:

Treatment of fish with 8 ppm of $CdCl_2$ for 6 days produced the following changes:

i) Gross changes that are observed in Gill:

a) Pale colouration of gill filament which is almost white at times.

b) Visible loss of secondary lamellae, especially at the bases of primary lamellae.

c) Distorted and wavy nature of both primary and secondary lamellae.

d) Fusion of the adjacent secondary lamellae at the tips of primary lamella.

ii) Ultrastructural changes that are observed in Gill:

The study indicates that the treatment with cadmium chloride brings forth vast changes in the structure of a gill lamella. Such

changes are obviously due to the effects that occur at the cellular level.

The effects of the treatment although vary with different cell types, there are few which are common to all cell types. Such generalized changes observed are:

(1) Vacuolation of cells

(2) Separation of cell layers or at times separation of individual cells

(3) Indentation of cell nuclei

(4) Appearance of short tuberculate processes over the free cell surface and

(5) The appearance of mitochondrial vacuolation

The most prominent effect of all such changes is the separation of the cell layers- the epithelial lining in particular. This has been described as "the lifting of the epithelial layer"by previous workers (Hughes G.M. and Morgan Miriam, 1973; Daterao M.S., 1989). In this condition the cells get separated from the underlying basement membrane at various levels leaving gaps in-between. This gives a bulged or swollen look to each secondary lamella. At times individual cells might get separated from each other. This type of separation which may occur at other places too is seen in the cells of the interlamellar regions.

Changes observed in individual cell types:

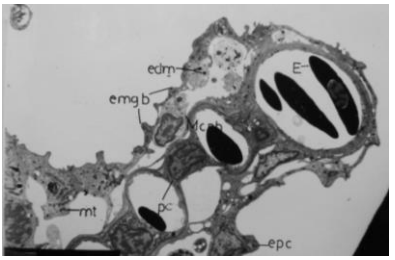
Secondary lamella

The secondary lamella proper are comprised of only two types of cells namely,

a) Epithelial cells and b) Pillar cells

(a) Epithelial Cells: The epithelial cells which line the outer extremity are the ones that are affected to a great extent. The outer margin of the cells is full of short tuberculate processes

that give an emarginated look to the outer margin. The cytoplasm of the epithelial cells appears denser owing to the increase in the number of granules. The cytoplasm is full of vacuoles. The vacuoles are of varying dimensions (Photograph no. 4). Mitochondria are few, round in shape and give a somewhat contracted look. The endoplasmic reticulum is profuse and of rough type.



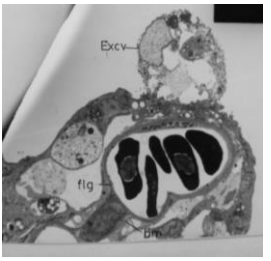
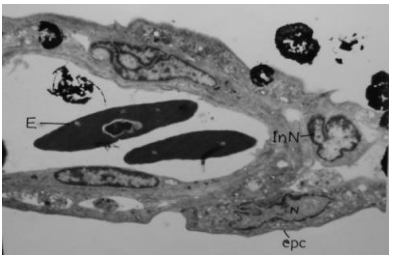


Fig 3 and 4: EM of gill treated (8 ppm 6 days) showing secondary lamalla

Key: edm – electron dense material, Excv – exocytic vesicle, Mcph - - Macrophage, pc – pillar cell, epc – epithelial cell, mt – mitochondria, E – erythrocyte, flg – flanges, Bm – basement membrane, emgb – emarginated border

The epithelial cells in treated forms develop several of membrane bound vesicles. Such vesicles are spherical or oval in outline. They may be represented within the vacuolar spaces or else may be seen bulging from the outer extremity of the epithelial cells (exocytic cells) (Photograph no. 4). These vesicles enclose a less dense matrix with few tubules, several vacuoles and at times few mitochondria. These mitochondria undergo degeneration and appear contracted and spherical in outline. The trabeculae are somewhat irregular or totally lacking. This is accompanied by mitochondrial vacuolation. Fine, radiating electron dense processes can also be seen at the peripheral extremities of these mitochondria; thus giving it a spiny look. (Photograph nos. 3 & 4). Golgi body and Lysosomes are prominent. Vesicles seen are large and at times may enclose

some dense material within. Nuclei of the epithelial cells are irregular in outline and are often indented (photograph no. 5).



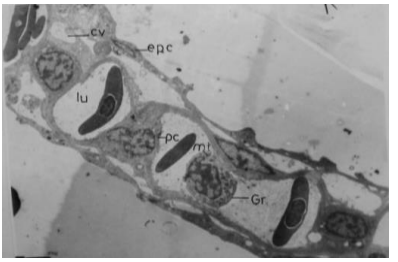


Fig 5 and 6: EM of gill filament (treated 8 ppm 6 days) showing degenerated secondary lamalla

Key: N – Nucleus, Gr – granulocyte, pc – pillar cell, epc – epithelial cell, mt – mitochondria, E – erythrocyte, InN – indented nucleus, cv – cytoplasmic vesicle, lu - lumen

Basement Membrane: The basement membrane is a continuous structure that lies between epithelial cells and row of pillar cells. It is fairly thick and gives a wavy look. This membrane is closely placed over pillar cell flanges and seen as dense band with several fibrils within.

(b) Pillar cells/ (pilaster cells): Pillar cells seem to be affected to a lesser extent with the treatment (Photograph no. 6). The changes noted are the contractions of the cell leading to the change in shape to a certain extent. Owing to the contraction, the collagen processes are deeply arched increasing the space within. The cell surface now looks more concave. The cytoplasm develops several vacuoles of varying sizes. Mitochondrial vacuolation is evident. Cristae are degenerated. Nuclei of the cells are often distorted.

Interlamellar cells: Interlamellar regions are represented by four types of cells namely, a) Interlamellar cells b) Mucous cells c) Chloride cells & d) Acidophilic mast cells

(a) Interlamellar cells: These cells lie between two adjacent lamella and at the bases of primary lamella. They are rounded or slightly oblong in shape and have a granular cytoplasm. Mitochondria are arranged peripherally in the cytoplasm. ER is of both rough and smooth types. These cells establish a contact with neighbouring cells and at times a junctional complex can be observed. They may also form associations with chloride cells (Photograph No. 7). With treatment these cells appear darker with dense granules, phagocytic vesicles and vacuolated mitochondria. (Photograph No. 8)

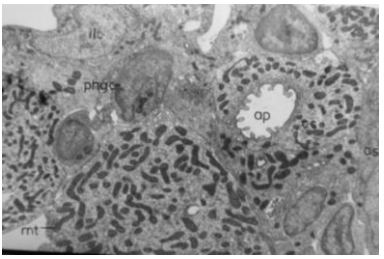


Fig 7: EM of gill (control) showing the interlamellar region

Key: ap – apical pit, ilc – interlamellar cell, cc – chloride cell, asc – associated cell, mt – mitochondria, phgc – phagocytic cell

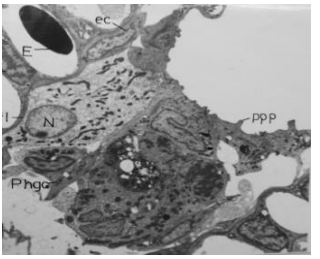


Fig 8: EM of gill (treated 8 ppm 6 days) showing basal part of a secondary lamella together with the interlamellar tissue

Key: N – Nucleus, pc – pillar cell, E – erythrocyte, fl – flanges, ppp – papillary process, ec – epithelial cell, phgc – phagocytic cell, mtv – mitochondrial vacuolation

(b) Mucous Cells: Mucous cells increase with treatment of Cadmium chloride. The cell is large and flask shaped in outline and encloses a cavity at the center. This cavity is full of membrane bound vesicles – The mucoid bodies. These vesicles are spherical in outline and are seen in two shades, dark and light. The mucoid substance within the vesicles attributes a white colouration to the vesicles. The presence of mucous filled cavity pushes the cytoplasm towards the base and peripheral extremities. The cytoplasmic portion is dense and full of granules. Endoplasmic Reticulum is of rough type.

Mitochondria are comparatively small and peripherally arranged. Nucleus is roughly spherical and is placed eccentrically at the basal extremity. Chromatin material is dense and is clumped at various places. (Photograph No. 9)

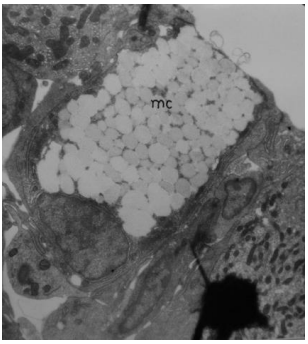


Fig 9: EM of gill (treated 8 ppm 6 days) showing interlamallar region with single mucous cell

Key: mc – mucous cell

(c) Chloride cells: With treatment, the free cell surface of chloride cells exhibit increased number of villar processes. Though the endoplasmic reticulum appears to be less prominent, both the smooth and rough type of tubules are seen. The lumen of the tubule may exhibit some dense granules. The cytoplasm undergoes vacuolation and the cell therefore appears pale. Mitochondrial vacuolation is another prominent characteristic feature. The chloride cells are also full of vesicles containing some amorphous material. In some cases, the cells of the treated forms have been found to have ahighly irregular border. No apical pit has been observed but the region is occupied by a prominent cytoplasmic evagination. Such evaginated vesicles have dense granules, few mitochondria and degenerated tubules of ER (Photograph no. 10 & 11).

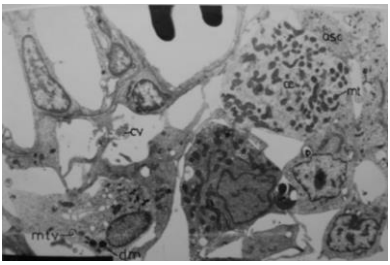


Fig 10: EM of gill (treated 8 ppm 6 days) showing interlamellar region between two lamallae

Key: asc – associated cells, mt – mitochondria, cc – chloride cell, cv – cytoplasmic vesicle, mtv – mitochondrial vacuolation, dm – dense material

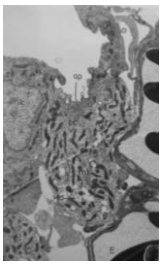


Fig 11: EM of gill (treated 8 ppm 6 days) showing chloride cell with apical pit

Key: cc – chloride cell, E – erythrocyte, ves – vesicles, V – vacuoles, mt – mitochondria, ilc – interlamaller cell

(d) Acidophilic mast cells: The change observed includes the appearance of cytoplasmic granules, loss of mitochondria and degeneration of endoplasmic reticulum (photograph no. 12).

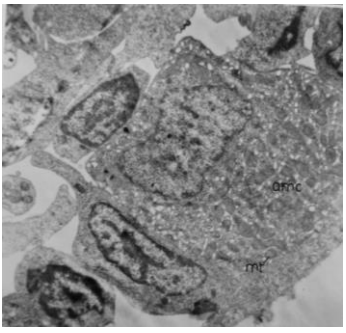


Fig 12: EM of gill (treated 8 ppm 6 days)showing acidophilic mast cell in the interlamellar region

Key: amc – acidophilic mast cell, mt – mitochondria

Discussion:

Light and electron microscopic studies on the gill of fishes have been studied by various authors. However there is no unanimity regarding its structure and the different types of cells that are associated with it. From the literature available,

one can conclude that structure of gill lamella and the cell types associated with it differ with the different species of fish.

Though the studies on the effects of heavy metals and toxicant on the gills of fishes have been carried out by many, all have concentrated mainly to note the changes that are observed in the gill filament region (Baker Jeremy T.P. 1969, Daterao M.S. 1989, Jagoe C.H. et al 1987, Gill T.S. et al 1988, Rajbanshi V.K and Gupta A.K. 1988) and practically no attention has been paid to find out changes that are observed in the gill head region, the epithelial lining in particular. The only change reported so far refers to the thickening of the epithelial lining (Hughes G.M. and Morgan M. 1973). The present observation has revealed that like the gill filament, the gill head region too exhibits certain changes with treatment. The immediate changes that are observed are contraction of gill rakers and disruption of epithelial lining. Continued exposure has been found to affect the taste buds, which show a tendency to separate from each other. The free cytoplasmic processes of the cell show an increase in cytoplasmic granules.

Cells of the central connective tissue core too shows a lot of inter spaces in between. Connective tissue fibers in treated fish are drastically decreased. Vesicular cells also termed as large acidophilic gland cells by Datta Munshi J.S. 1964, in the fresh water fish *Heteropneustes fossilis* (Bloch) are also found to increase in number and size with treatment.

Proliferation of the epithelial cells leads to thickening of epithelial lining of the gill head region after exposure to heavy metals but the present observation has not indicated any cell proliferation leading to thickening of the layer. On the contrary, there is an indication of degeneration and disruption of the epithelial lining. Mucous cells however appear in large numbers and with their increased cellular content; these cells are enlarged to a considerable extent.

Gill filament portion is affected to a great extent. The immediate changes observed are contraction of the primary

lamella and loss of gill ray skeletal axis, thus affecting the normal architecture of the primary lamella, giving it a wavy look. This is accompanied by hypertrophied nature of secondary lamella and knobbed condition of their tips. This has been reported earlier by Versteeg D. J. and Giesy J. P. (1986)

Further treatment results in sloughing of epithelial lining from the central gill rake, contraction of secondary lamella resulting in reduction of their size and accumulation of blood cells within the primary lamella. All these effects have been reported by earlier workers although different terminologies have been used to designate different changes. Sloughing of epithelial lining for e.g. has been referred to as lifting of the outer epithelium by various authors. (Hughes G.M. and Morgan M 1973; Daterao M.S. 1989) Similarly accumulation of blood cell has been referred to as blood stasis. (Hughes G.M. and Morgan M 1973) Next comes loss of secondary lamella and hypertrophic nature of inter lamellar tissue. This occurs mainly at the basal extremities of the primary lamella. Secondary lamella may be lost either on one side or both the sides of the primary lamella. Because of loss of tips of primary lamella and secondary lamella from the basal regions, the secondary lamellas are restricted only to the central region of the primary lamella. These are represented as highly curled if long enough otherwise are represented as short stumpy projections with swollen tips. This condition has been reported by earlier workers. (Gaikwad Snehalata A. 1981, Awari Subhash B. 1985, 1991). It has been noted however that sequential changes are not represented uniformly and simultaneously over all gill filaments.

Fusion of adjacent gill lamella has also been considered to be an effect of treatment by many workers. (Baker Jeremy T.P. 1969, Hughes G.M. & Morgan M. 1973, Daterao M.S. 1989 and PaiVinaya l. 1993). Fusion of lamellar tips has been reported to be a normal feature in certain fishes such as *Labeorohita* (DattaMunshi J.S. 1960). Possibly the chances of

fusion of lamellar tips may be increased owing to the disruption of cells caused due to degeneration of lamellar tips. The presence of blood clots and the abundant production of mucous might further enhance the condition. The basal region of primary lamella and the outer margins of gill septa are lined by two types of cells, basal cuboidal cells and mucous cells. Loosening of the basal cuboidal cells and an increased number of mucous cells are the only changes which are evident in the treated fish.

Secondary lamella are considered to have mainly two types of cells namely, the epithelial cells and the pilaster cells. Besides these, blood cells, especially erythrocytes are seen within the central lymphatic space of the secondary lamella. The epithelial cells are flat elongated cells with centrally lodged nucleus. Cytoplasm concentrated around nucleus and each cell is provided with two cytoplasmic processes, one along each pole. It is with these cytoplasmic processes that the cells are capable of lining the secondary lamella quite efficiently. The terminal end of a lamella is represented by a single epithelial cell, the cytoplasmic processes of which are arched over to give a proper shape to the tip. When treated with cadmium chloride, these cells are affected to a great extent. Changes observed are: separation of cells, appearance of cytoplasmic vacuoles and at times deposition of some frothy material. This is especially evident at tips of secondary lamella. In extreme cases the epithelial lining gives away, leaving the lumen of the lamella open. It is through this that the blood cells are passed to the exterior. Often erythrocytes in agglutinated condition are observed at such sites.

Pillar cells are also affected with treatment. Higher dosage with long period of exposure leaves them in a highly contracted state. The processes of such cells appear slender and occasionally have a few vacuoles. The contractile nature of processes seems to get affected to a great extent. Contractibility of the pillar cell processes has been attributed to the presence of

fibrillar material in it. (Hughes G.M. and Weibel E.R. 1972; Hughes G.M. & Morgan M. 1973) the loss of contractile nature in treated fish appears to be due to the effect on the fibrillar material.

At the basal extremities of secondary lamellae and in the inter-lamellar region, the cells seen are inter-lamellar mucous cells, acidophilic mast cells and the chloride cells. These interlamellar cells are small, spherical or squarish in outline having a centrally lodged nucleus and cytoplasm full of granules .Treatment with low concentration of cadmium salt leads to an increase in the cell size initially, however, with high dosage these cells get degenerated and large vacuoles can be seen within.

Mucous cells are represented usually at the bases of primary lamellae in normal untreated fishes and occasionally may also be present in the interlamellar region. After treatment with cadmium, sure signs of their increased numbers can be visualized. They can also be observed at various levels of a primary lamella. The fact that the cells are increased in number with the treatment of heavy metal salts has also been reported by earlier workers (Hughes G.M. and Morgan M., 1973). The present study further establishes the fact.

Existence of chloride cells and their probable function has been a matter of dispute right from the time they were observed in the gills of eels by Keys and Willmer (1932) who termed the cells as eosinophilic chloride secreting cells. Liu 1942 has also suggested that the occurrence of the supposedly dormant chloride secreting cells in the gills of fresh water teleotes probably indicate that the progenitors of fresh water fishes once inhabited the seas. Keys (1931) has demonstrated that branchial chloride is excreted by animals adapted to sea water. Bevelander Gerrit (1935) on the other hand questions the very existence of specialized cells for the purpose of salt excretion. According to him it is the mucous cells which function in salt regulation. Histochemical tests have disclosed

the presence of only a limited number of epithelial cells concerned in this electrolyte excretion. Krogh (1939) too doubts the existence of chloride cells. Parry G.H. (1966) and Doyle W.L. (1960) are of the opinion that the cells are not concerned with salt exchange. Vickers T. (1961) believes that chloride cells are modified mucous cells. Threadgold and Houston (1964) also believe that the cells are concerned with electrolyte balance. Baker Jereny T.P. (1969) who studied the effects of copper poisoning on the fish winter flounder, Pseudopleuronectes americanas opines that with treatment many mucous cells get transformed into chloride cells. In the present study chloride cells are found in fairly good numbers in *Tilapia mossambica (Peters)*. Each cell has been found to possess a distinct apical vesicle- a characteristic which has been so far considered to be related to the marine fishes. In the treated fish it has been observed that the lining of the apical pits pinch of cytoplasmic vesicles in large numbers. This speaks of the possible excretory role of the cells. Of the various changes observes after treatment mitochondrial vaculation and formation of vesicles near the margin of apical pits are noteworthy.

Electron micrograph does not present any noticeable changes within erythrocytes of treated fish. The leucocytes however exhibit changes. The changes noted are deshaping of the cell, vaculation of cytoplasm and mitochondria and even degeneration of nuclei (photograph no. 5 & 6).

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