

Ultrastructural Changes in the Epithelium of the Stomach of *Aphanius dispar* (Cyprinodontidae), Due to Stress from Starvation

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ABSTRACT: Ultrastructural changes in the epithelium of the stomach of *Aphanius dispar*, a cyprinodont fish, due to starvation have been described. The changes in the epithelium after 24, 48, 72, 96, and 120 hours have been discussed. The degeneration of the epithelial cells commenced after 24 hours and steadily progressed till 96 hours at which the maximum change was observed. Changes in response to starvation include the disappearance of lipid droplets, mitochondrial damage, goblet cells degeneration, morphological aberration of the nuclei and overall abnormalities in the structural integrity of the rugae. This study confirms that stress due to starvation causes significant pathomorphological changes in the stomach in four days.

KEYWORDS: fish; *Aphanius dispar*; stomach; epithelium histology ultrastructure; stress; starvation.

Histological studies describing the structure and ultrastructure of fish digestive tracts are many (Osman and Caceci, 1991; Grau *et al.*, 1992; Gargiulo *et al.*, 1997, 1998). Most of these have also attempted to relate structures to different types of feeding, nutritional requirements, digestive functions and adaptations (Kuperman and Kuz'mina, 1994; Murray *et al.*, 1996). However, investigations exploring the histological changes imposed by physiological stress are very few. The food supply available to fish in its natural environment is not always optimum and periods of food shortage cause stress due to starvation.

Aphanius dispar (Rüppell 1828), a widely distributed cyprinodont fish in the fresh and brackish waters of the Middle East, is physiologically capable of coping with a variety of adverse environmental conditions. Recently, we described the histology of the stomach of *A. dispar* using light microscopy and specifically discussed the changes in the structure and number of goblet cells in response to starvation (Ba-Omar *et al.*, 1998). This study describes the ultrastructure of the epithelium of the stomach and its changes in response to stress caused by starvation.

Materials and Methods

Specimens of *A. dispar* with a total length (TL) range of 30.4-46.3 mm, weighing from 0.43-0.70 gm were collected from Wadi Al-Khod near the Sultan Qaboos University, Sultanate of Oman

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(Lat. 23° 30' N; Long. 58° 40'E). Fish were acclimatized for two weeks in an aerated holding tank and were fed with Tetramin flakes, *ad libitum*, twice daily. An experimental tank was prepared with dechlorinated water and held a large perspex visjar in its center containing the same water, with the same water level as that of the outside tank. After a stabilization period of seven days, 36 fish, irrespective of sex were randomly transferred from the holding tank to the outer area of the experimental tank. The feeding regimen for these fish was the same as that for those in the holding tank. The experiment was conducted after another acclimatization period of seven days.

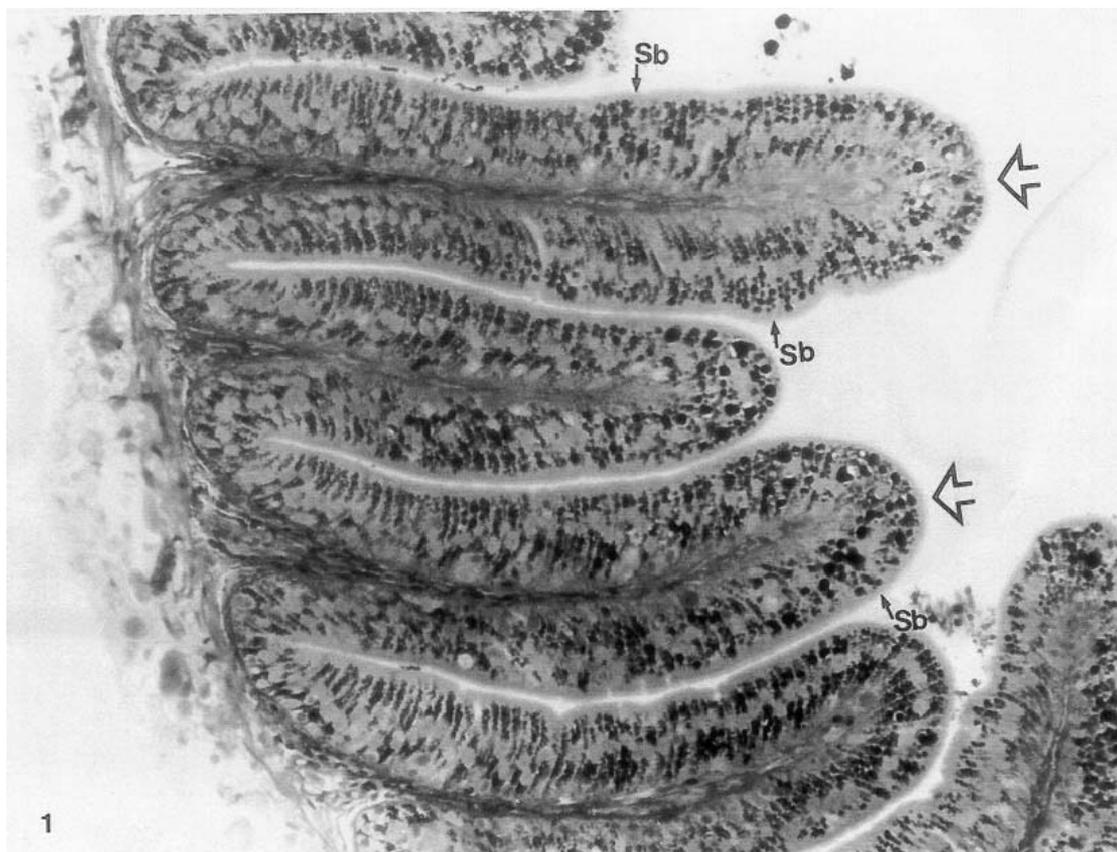


Figure 1. Light micrograph (LM) of a transverse section of the control fish showing rugae of the stomach (large open arrowheads) and the striated border (Sb) (x100).

At the start of the experiment, 30 fish were randomly transferred to the central visjar and were deprived of food. The six remaining control fish in the outer area of the experimental tank were fed *ad libitum*, twice daily as usual. The water in the central visjar was replaced with dechlorinated, pre-stabilized water at short intervals to prevent coprophagy. Six starving fish were randomly sacrificed at each interval of 24, 48, 72, 96 and 120 hr for histological studies. At the end of the experiment, the six control fish, fed throughout the experimental duration were also sacrificed for histological investigations. The fish were placed in ice bath and were immediately decapitated. The entire stomach from each fish was then removed and immediately fixed at room temperature. The methods for the preparation of specimens examined under light microscopy are given elsewhere (Ba-Omar *et al.*, 1998).

For transmission electron microscopy, the stomach was fixed in Karnovsky fixative buffered with sodium cacodylate to a pH of 7.4 for four hours and then cut into small pieces. These were washed in cacodylate buffer and then post-fixed in 1% aqueous solution of osmium tetroxide for 1 hour and dehydrated in a series of alcohol before embedding in Agar 100 resin. Semi-thin and ultra-thin sections were cut using Reichert ultramicrotome. The semi-thin sections were stained with toluidine blue and the ultra-thin sections were stained with uranyl acetate and post-stained in lead citrate. The sections were examined using a Philip109 transmission electron microscope.

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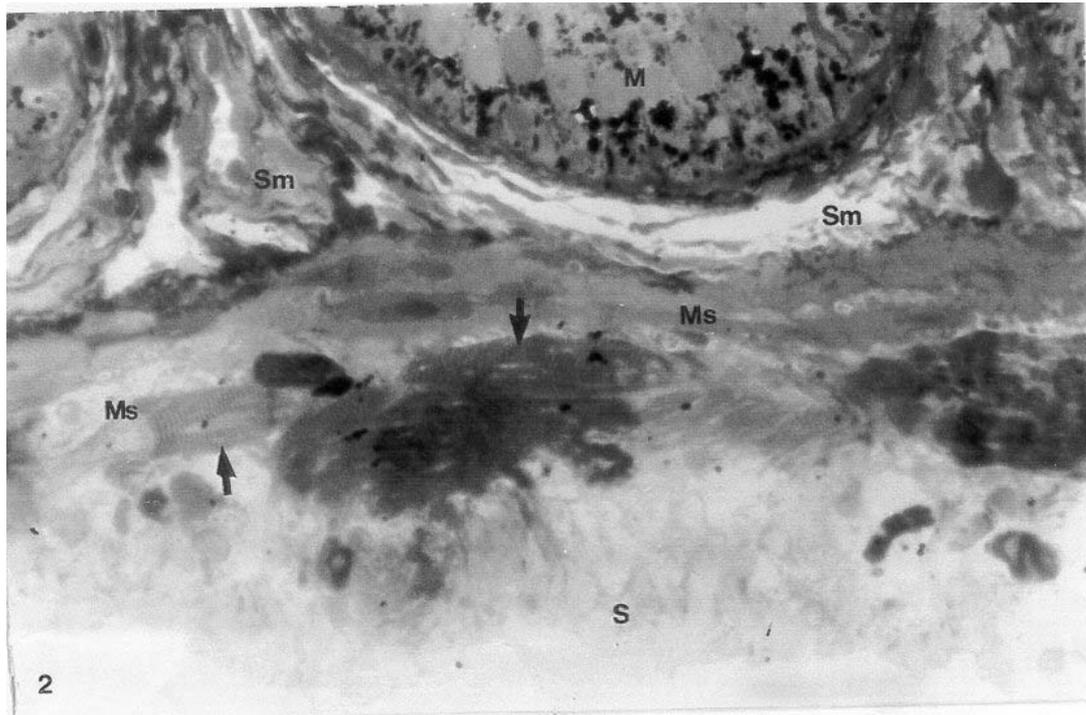


Figure 2. Light micrograph (LM) of a transverse section of the control fish showing the different layers of the stomach; mucosa (M), submucosa (Sm), Muscularis (Ms) with patches of skeletal muscle (arrowheads) and serosa (S) (x400).

Results

The stomach of *A. dispar* is a hollow organ with four different layers: mucosa, submucosa, muscularis and serosa. The mucosa, thrown into folds of variable lengths (rugae) is composed of columnar epithelium with striated border (Figure. 1).

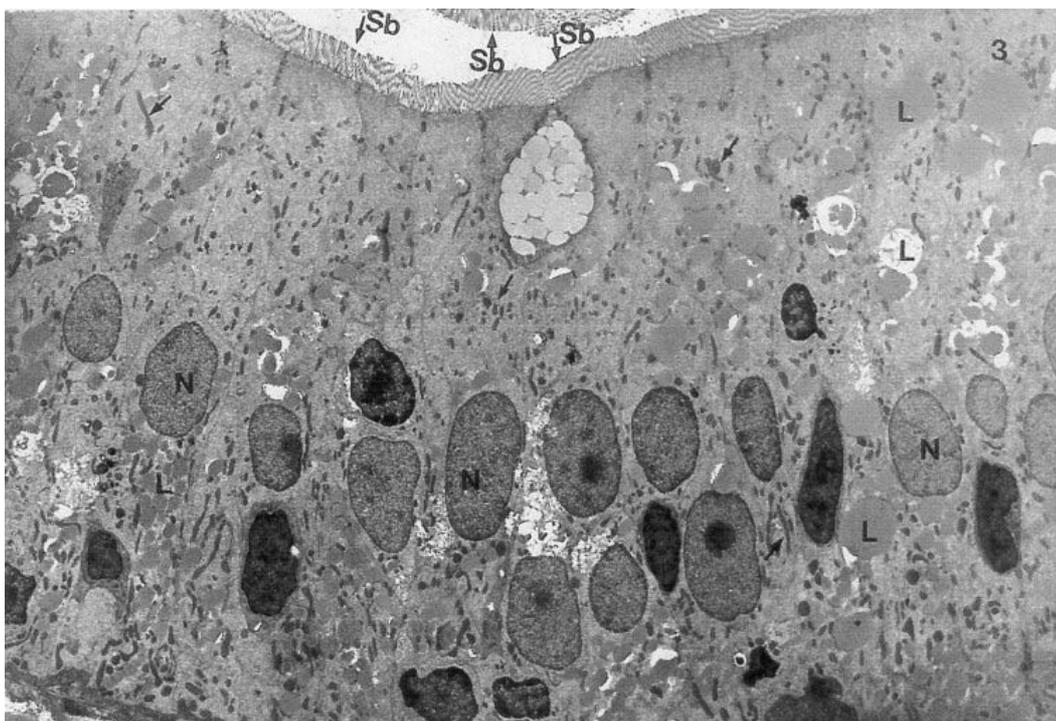


Figure 3. Transmission electron micrograph (TEM) of the control fish showing columnar cells with their nuclei (N), mitochondria (small arrowheads), lipid droplets (L) and striated borders (Sb) (x1300).

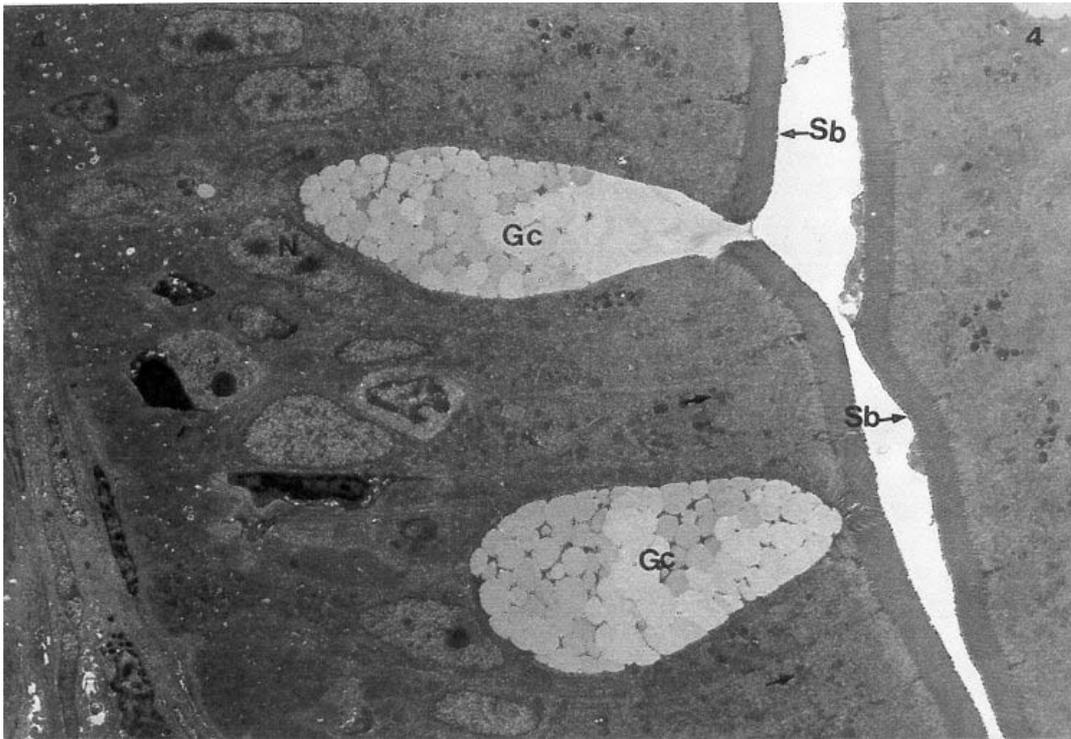


Figure 4. Transmission electron micrograph (TEM) of the control fish showing goblet cells (Gc) with nuclei (N), mitochondria (small arrowheads) and striated borders (Sb) (x1300).

The submucosa is composed of connective tissue, the muscularis is composed mainly of smooth muscle and patches of skeletal muscle and the serosa is made up of loose irregular connective tissue with mesothelial cells (Figure. 2).



Figure 5. Transmission electron micrograph (TEM) of fish after 24 hours of starvation showing the columnar cells with their nuclei (N), Goblet cells (Gc) and the striated borders (Sb) (x980).

Under transmission electron microscopy, the epithelial cells of the mucosa were columnar in shape with microvilli forming a striated border with their nuclei at the basal side (Figure. 3). Mitochondria with

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variable sizes and a number of irregular sized lipid droplets are distributed throughout the cell (Figure. 3).

Most goblet cells were seen at the apical side of the rugae. They have irregularly shaped nuclei located at the basal side, with up to two nucleoli (Figure. 4). Goblet cells possess numerous densely packed electron-translucent granules which are distributed throughout the cytoplasm (Figure. 4).

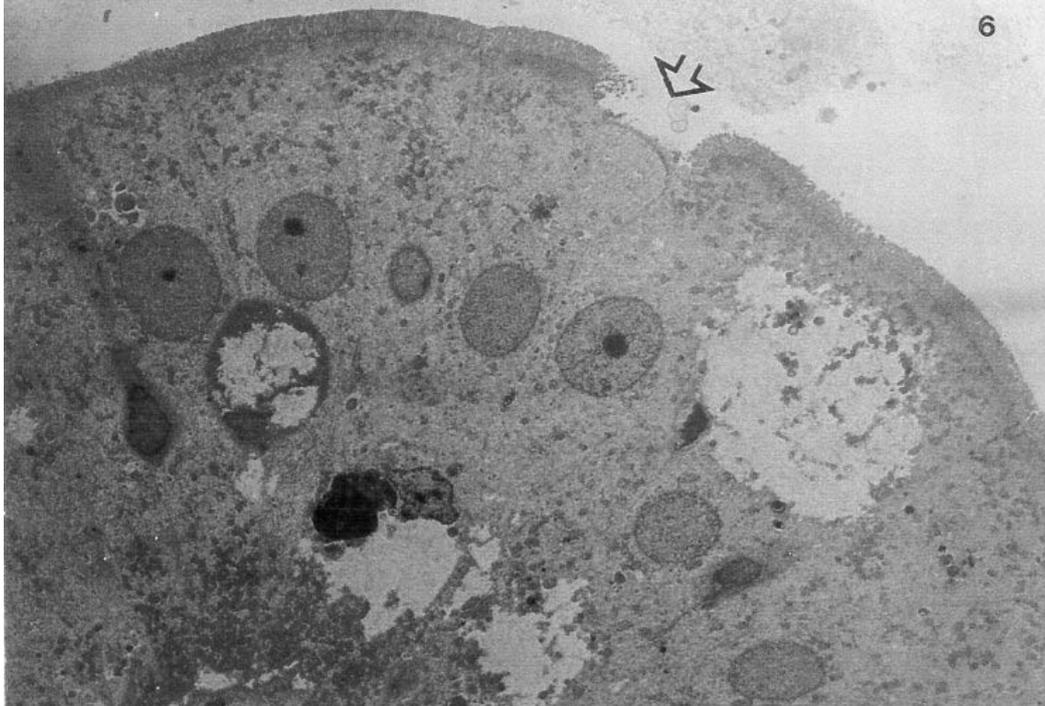


Figure 6. Transmission electron micrograph (TEM) of fish after 48 hours of starvation showing the damaged rugae (large open arrowhead) (x1300).

After 24 hours of starvation, the epithelial cells of the mucosa are columnar in shape with most of the nuclei elongated in shape (Figure. 5). Most of the mitochondria were seen toward the apical side.

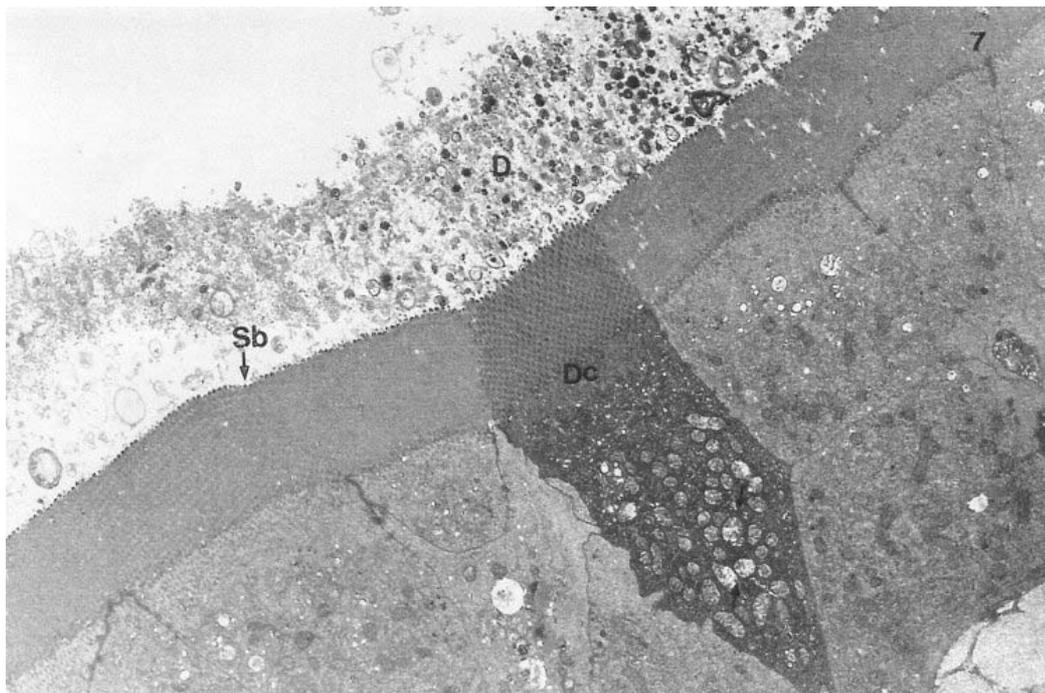


Figure 7. Transmission electron micrograph (TEM) of fish after 48 hours of starvation showing a darkly stained damaged cell (Dc), mitochondria (small arrowheads), striated borders (Sb) and debris (D) (x4800).



Figure 8. Transmission electron micrograph (TEM) of fish after 72 hours of starvation showing darkly stained cells and their degeneration characteristics such as the mitochondria (small arrowheads). It shows also a round Goblet cell (Gc) and the striated borders (Sb) (x2800).

The lipid droplets were absent. The apical side possesses striated border, but some sections of the striated border were missing. The goblet cells contained numerous, densely packed electron-translucent granules and the nuclei are located at the base (Figure. 5).

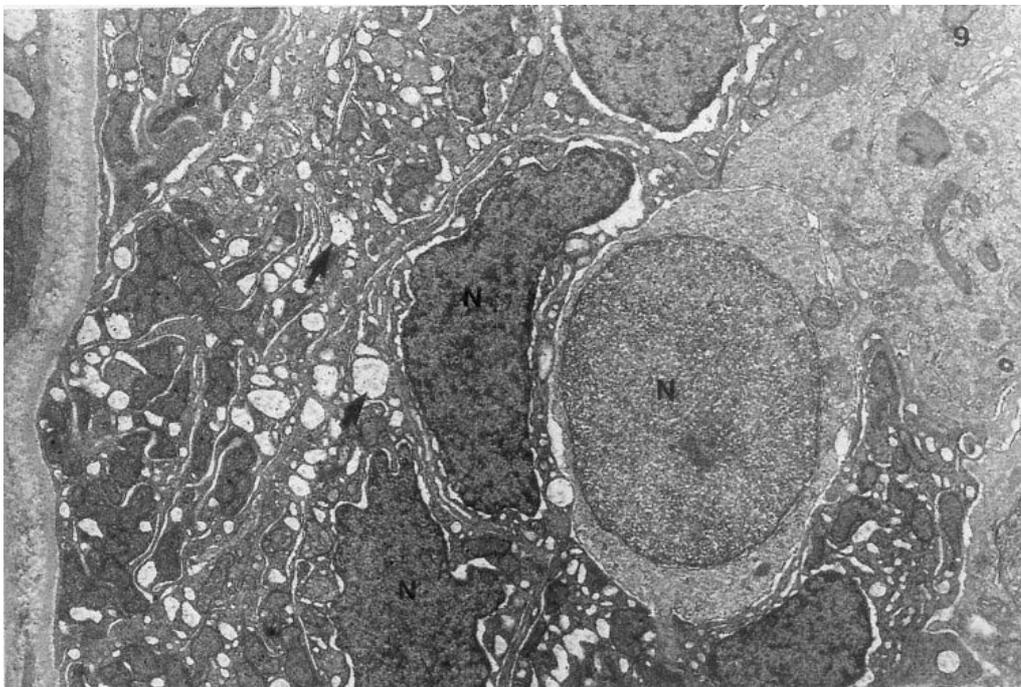


Figure 9. Transmission electron micrograph (TEM) of fish after 72 hours of starvation showing a basal part of a rugae with darkly stained cells showing degeneration characteristics such as empty spaces (arrowheads) and irregularly shaped nuclei (N) (x7500).

After 48 hours of starvation, the apical part of the rugae showed some degree of damage. (Figure. 6). The damaged cells were darkly stained with numerous empty vacuoles (Figure. 7). Mitochondria were

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abnormal in appearance with cristae in various stages of disintegration (Figure.7). The lipid droplets seen in the control were absent here.

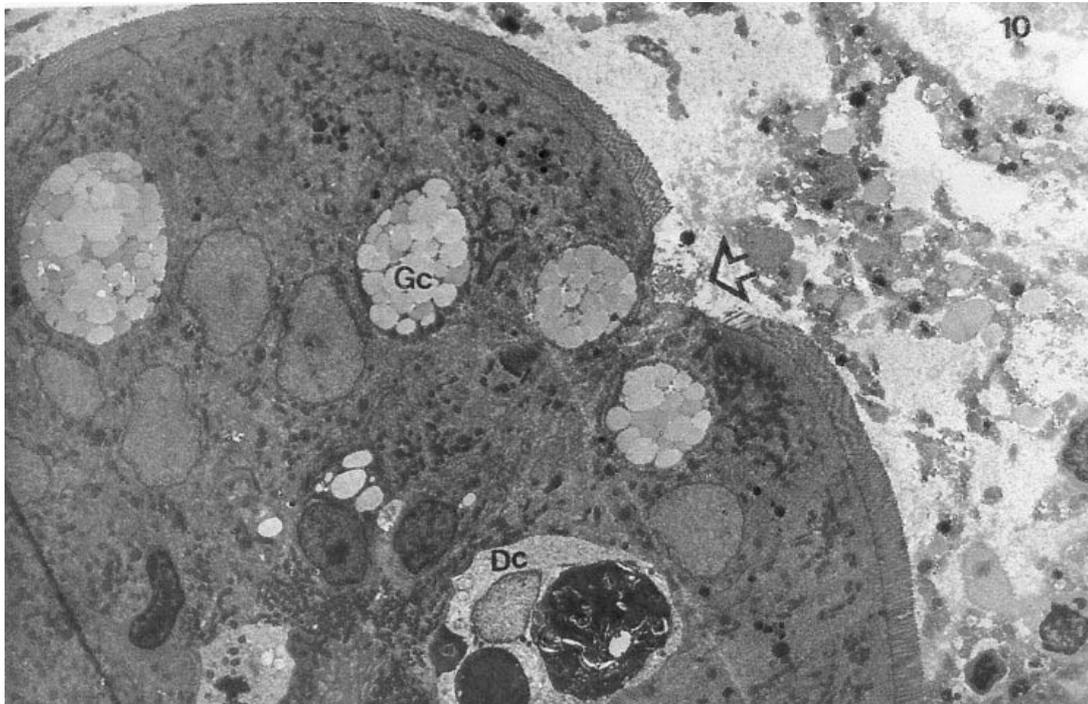


Figure 10. Transmission electron micrograph (TEM) of fish after 96 hours of starvation showing a damaged apical part of a rugae (large open arrowhead), round Goblet cells (Gc) and degenerated part of the rugae (Dc) (X1300).

In fish subjected to 72 hours of starvation, there was an increase in the number of damaged cells (Figure. 8). Goblet cells were also degenerating. These cells were irregularly shaped with electron-

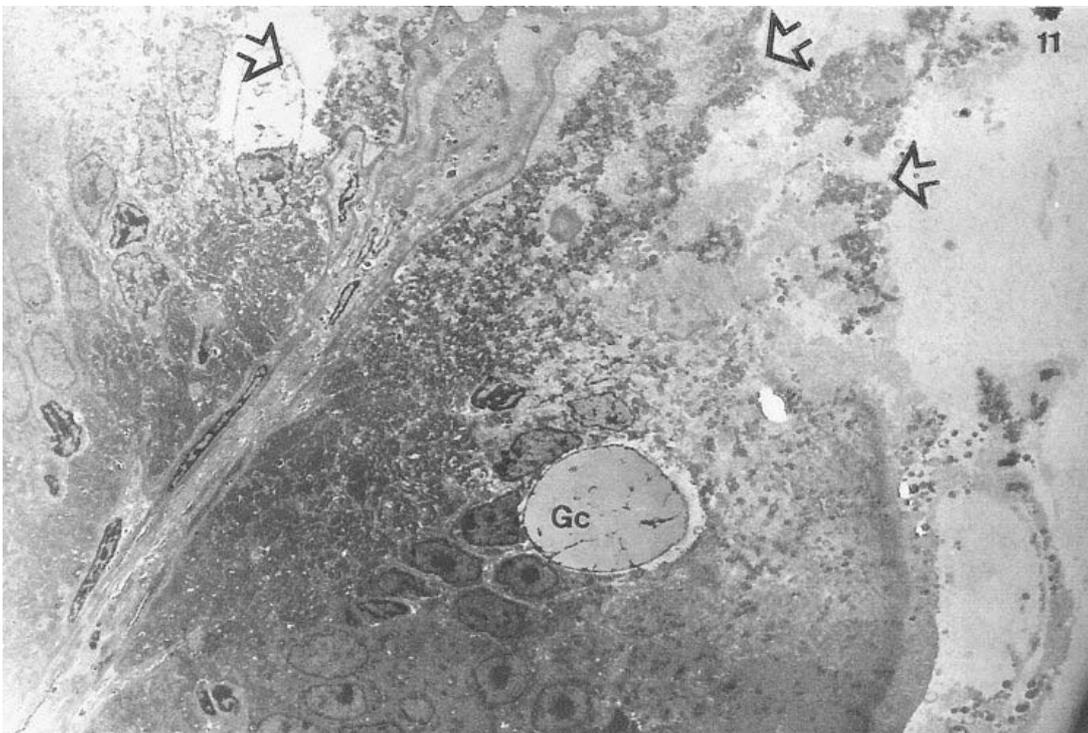


Figure 11. Transmission electron micrograph (TEM) of fish after 120 hours of starvation showing an extensive damage of the apical part of a rugae (large open arrowheads) and a round Goblet cell (Gc) (x980).

dense nuclei and the nuclei were also irregular in shape with deep indented margins (Figure. 8). As in 48

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hours of starvation, mitochondria were abnormal in appearance with cristae in various stages of disintegration. The degenerating cells at the basal part of the rugae had various sized empty spaces (Figure. 9).

The damage caused to the mucosa was similar in fish starved for 96 and 120 hours (Figures. 10, 11). The damage to the apical part of the rugae was severe characterised by extensive cell degeneration and the disappearance of microvilli (Figures. 10, 11). Most of the goblet cells were round and seen away from the surface and close to the basal part of the rugae (Figure. 11).

Discussion

The general histology of the stomach of *A. dispar* and its similarities to that of other teleosts was earlier discussed by Ba-Omar *et al.* (1998). What is referred to as stomach in this work is the morphologically distinct enlarged, sac-like portion of the gut separated from intestine by a constriction. The ultrastructure of the stomach as revealed by electron microscopy here, also confirms striking similarities between *A. dispar* and other freshwater teleosts (Gargiulo *et al.* 1997, 1998). The stomachs of *A. dispar* with large number of well defined goblet cells in the apical region of the mucosa seem typical of omnivorous and herbivorous feeders. Osman and Caceci (1991) suggested that the gastric epithelial cells specialized for the secretion of neutral mucin and the increase in surface area provided by the dense striated (= brush) border were adaptations for the surface absorption of nutrients in *Oreochromis niloticus*. Although *A. dispar* has similar morphology of the mucosa, its role in nutrient absorption needs to be verified by histochemical techniques. The large number of lipid droplets seen in well fed *A. dispar*, are probably absorbed from the commercial food with at least 5% lipid content. The turnover time for lipid in the intestinal epithelium is only a few hours. Therefore, it is not surprising that the epithelia of fish starved for 24 hours were devoid of lipid droplets.

The ability to cope with stress is an important parameter determining the survival of fish. Stress is known to induce physiological and pathomorphological changes (Boddingius, 1976; 1993; Pottinger and Pickering, 1992, Pottinger *et al.* 1994, Szakolczai, 1997). In the European eel, stress caused the atrophy of the glandular tissue layer of the stomach (Peters, 1982). Stress during harvest and transport of common carp resulted in the reduction of the number of goblet cells and the detachment of columnar epithelial cells from the basement membrane (Szakolczai, 1997).

Studies on pathological changes caused by stress due to starvation are only a few. In several teleosts including *A. dispar*, the responses to starvation include the reduction in mucosal mass and the decline in the number of goblet cells (Boge *et al.*, 1981; McLeese and Moon, 1981; Ba-Omar *et al.*, 1998). It is well known that goblet cells secrete mucous which is used for lubrication and for protection of the mucosa against chemical and physical damage. Since there is a reduction in the number of goblet cells in the stomach then this will result in the damage of the epithelial cells and other tissues (Ba-Omar *et al.* 1998). This study documents, at the ultrastructural level, the process of gastric erosion caused by starvation. The significant sequence of events from 24 - 120 hours of starvation are, the disappearance of lipid droplets, withdrawal of goblet cells from the apical surface, damage to the striated border, a steady degeneration of mitochondria, degeneration of goblet cells and the disappearance of microvilli.

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