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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan  
Mob: +92 300 3008585, Fax: +92 41 8815544  
E-mail: editorijps@gmail.com

## Studies on the Histochemistry of the Proventriculus and Gizzard of Post-Hatch Guinea Fowl (*Numida meleagris*)

P. Senthamil Selvan, S. Ushakumary and Geetha Ramesh  
Department of Veterinary Anatomy and Histology, Madras Veterinary College,  
TANUVAS, Chennai, India-600007 (TN.)

**Abstract:** The proventricular surface epithelial cells were positive for both acid and neutral mucins and stained intensive for acid phosphatase and adenosine triphosphatase. The proprial gland cells revealed histochemical distribution and activity of various enzymes and lipids similar to that of the submucosal gland cells. However, the ductular cells of the submucosal glands showed comparatively stronger reaction for acid phosphatase, alkaline phosphatase, adenosine triphosphatase, lipase and carbonic anhydrase except for succinic dehydrogenase. In gizzard, the koilin was positive for both acid and neutral mucin. The epithelial cells of the proprial glands stained strongly for adenosine triphosphatase and moderately for acid and alkaline phosphatases. However, their response for succinic dehydrogenase and lipase was weak. The smooth muscle cells of the proventriculus and gizzard showed a moderate to strong reaction for adenosine triphosphatase.

**Key words:** Proventriculus, gizzard, mucins, enzymes

### INTRODUCTION

The proventriculus and gizzard constitute the first important site of enzyme activity. The main function of proventriculus is production of gastric juice and propulsion of juice and food in to the gizzard which is the main site of gastric proteolysis (Hill, 1971). Histochemical studies on the distribution of various enzymes of metabolic pathways in chicken (Bhattacharya *et al.*, 1994), mucins and lipids in duck (Shyla *et al.*, 1992) were available yet, the present study was performed to further unveil the distribution and localization of various histochemical components and to correlate them with the digestive/metabolic process in guinea fowl.

### MATERIALS AND METHODS

The proventriculus and gizzard for the present study were collected from guinea fowls procured from the Poultry Research Station, Nandanam, Chennai. Apparently healthy guinea fowls, six each of day-old, 1 week, 3 weeks, 5 weeks, 8 weeks and 12 weeks of age were sacrificed for the present study. Formalin fixed tissue samples were washed after complete fixation and were processed for routine paraffin embedding technique. Sections of 5-6 $\mu$ m thickness were cut. The sections were then subjected to Periodic Acid Schiff (PAS) technique (Mc Manus, 1946) for mucopolysaccharides, Hale's dialyzed iron technique (Hale, 1946) for acid and neutral mucins and Oil red 'O' method for lipids (Bancroft and Stevens, 1996). Frozen sections of tissues fixed in chilled formol calcium (4°C) were used for localization of alkaline phosphatase and acid phosphatase (Singh and Sulochana, 1996) and

lipase (Gomori, 1952). Frozen sections of fresh unfixed tissues were used for localization of succinic dehydrogenase (Singh and Sulochana, 1996), adenosine triphosphatase (Bancroft and Stevens, 1996) and carbonic anhydrase (Stoward, 1991). All the frozen sections were cut at 15-20 $\mu$ m thickness by cryostat

### RESULTS AND DISCUSSION

**Proventriculus:** The proventriculus of the guinea fowl had all the four tunics namely, tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa. The columnar cells of the superficial epithelium of the tunica mucosa possessed vesicular nucleus in varying position and a clear foamy acidophilic cytoplasm. The PAS positive mucin granules were found to occupy the supra nuclear area of the cells as reported by Hodges (1974) in domestic fowl and Shyla *et al.* (1992) in ducks. These granules were found to be abundant in the cells lining the apical part of the plica than in the cells lining the sulci (Fig. 1). The positive reaction of the surface epithelium for both acid and neutral mucin was in contrary to the finding of Pastor *et al.* (1988) in chicken who found that the neutral mucin and acid mucin positive cells occur independently in some places. The mucous coat over the plica showed more intense reaction to acid mucin, which agrees with the findings of Prasad and Kakade (1990) in duck. The presence of neutral and acid mucin protects the mucosal surface and forms a resistant mucosal barrier (Mogilnaia and Bogatyr Lia, 1983) in birds. The surface epithelium also showed a mild positive reaction to lipids (Fig. 2). The presence of fat droplets in the superficial epithelial cells was reported in fowl

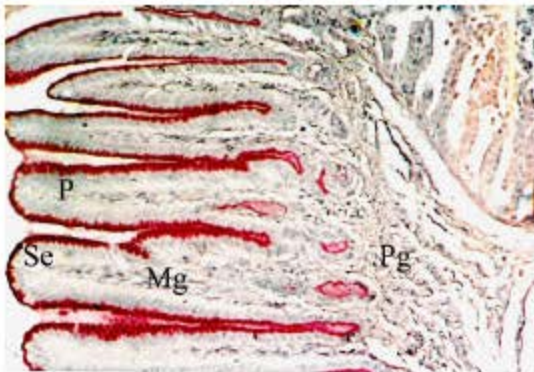


Fig. 1: Photomicrograph of the proventriculus of a 5 week old guinea fowl showing the PAS positive reaction in the surface epithelium and proprial glands. (PAS x 200), P-Plicae, Pg-Proprial glands, Mg-Mucin granules, Se-Surface epithelium.

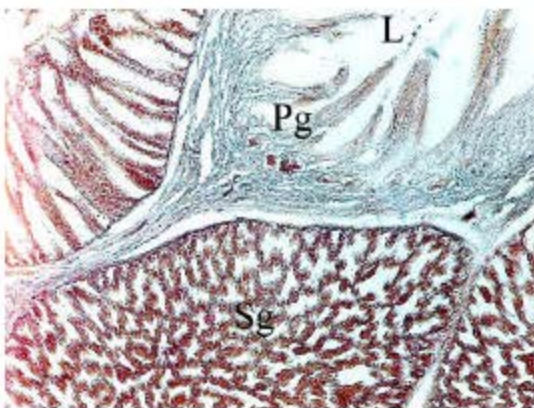


Fig. 2: Photomicrograph of the proventriculus of a 5 week old guinea fowl showing the glandular epithelium positive for lipids (Oil red 'O' x 100) L-Lumen, Pg-Proprial glands, Sg-Submucosal glands.

(Wight, 1975; McLelland, 1979) and in duck (Shyla *et al.*, 1992). Moreover, these lipids filled within the epithelial cells were found to be a source of pink stomach oil in Procellaria which is shoted in defense against predators (King and McLelland, 1975).

A mild positive reaction for alkaline phosphatase and lipase was noted in the surface epithelium. The activity for acid phosphatase was intense in higher age groups (8 and 12 weeks) than younger age groups (day-old to 5 weeks). The intense reaction for adenosine triphosphatase by the epithelial cells against any other enzymes agrees with the findings of Salem *et al.* (1992) and Bhattacharya *et al.* (1994) in birds. The activity for succinic dehydrogenase was not observed in the present study (Fig. 3). In contrary, Bhattacharya *et al.*

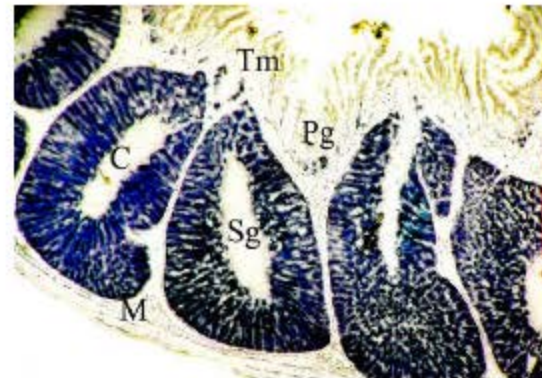


Fig. 3: Photomicrograph of the proventriculus of a 3 week old guinea fowl showing Succinic dehydrogenase activity in the proprial and submucosal glands. (SDH x 40) Tm-Tunica mucosa, Pg-Proprial glands, C-Central cavity, Sg-Submucosal glands, M-Tunica muscularis.

(1994) found that the luminal border of the surface epithelium were positive for this enzymic activity in pullets. The reaction for carbonic anhydrase activity was mild however, Pochhammer *et al.* (1979) reported that its activity was restricted to the apical cytoplasm of the surface epithelial cells in chicken. The diverse role of carbonic anhydrase includes formation of acid, bicarbonate and protection of gastric mucosa (Anderson *et al.*, 1982).

The lumen and luminal border of the proprial glands showed periodic acid schiff positive material (Fig. 1). In Hale's technique, neutral mucins were observed in the apical portion of the cell while, the luminal border showed more intense reaction to acid mucin. However, Imai *et al.* (1991) demonstrated both neutral and acid mucins in the glandular cells of the lamina propria in fowl. These cells form the undifferentiated gastric glands similar to that of the gastric glands in mammals. These glandular cells also showed a moderate reaction for lipids (Fig. 2), alkaline phosphatase and acid phosphatase. However, an intense reaction was noticed for succinic dehydrogenase (Fig. 3) and adenosine triphosphatase.

In the present study, the submucosal glandular epithelium showed a negative reaction for mucins. The reaction for lipids was moderate (Fig. 2). However, Wight (1975) stated that large amount of lipids occur in the oxyntico-peptic cells of chicken. In contrary, Shyla *et al.* (1992) opined that no fat vacuoles were present in duck. The reaction of the deep glandular cells for succinic dehydrogenase is intense (Fig. 3) as reported by Bhattacharya *et al.* (1994) in fowl. The strong reaction for adenosine triphosphatase was supported by the findings of Salem *et al.* (1992) in fowl who reported its presence in these cells. The role of the adenosine



triphosphatase is to drive the acid secreting process in the oxyntic cells of the mammalian stomach and are the most active cells of the body (Wight, 1975). The reaction for acid phosphatase was intense whereas, for alkaline phosphatase and lipase were mild. A mild to moderate reaction for carbonic anhydrase coincides with the study of Palatroni *et al.* (1980) in fowl. The presence of various enzymes in these glandular cells was due to the involvement of the glands in various metabolic pathways (Bhattacharya *et al.*, 1994).

The ductular cells that lined the ducts of the submucosal glands showed PAS positive reaction in their apical ends. The reaction for acid mucin was noticed on the apical border of the cells whereas, their apical parts showed neutral mucins. Inforzato de Lima and Sasso Wda (1985) observed more neutral glycoprotein than acid mucin in the ductular epithelium of the proventricular glands in pigeon. A mild reaction for lipid and succinic dehydrogenase was observed. The reaction for alkaline phosphatase, acid phosphatase, lipase, carbonic anhydrase and adenosine triphosphatase was more intense in the ductular epithelium than the glandular epithelium in guinea fowl. Moreover, Bhattacharya *et al.* (1994) reported the presence of alkaline phosphatase and adenosine triphosphatase in domestic fowl suggesting that the reactivity for phosphatases was more conspicuous in the luminal border of the epithelium due to its association with the plasmalemma. The tunica muscularis showed a positive reaction for adenosine triphosphatase activity.

**Gizzard:** The gizzard also possessed all the four tunics in addition to an internal lining/ koilin, a secretory layer above the mucosa. In the present study, the koilin showed a positive reaction to PAS (Fig. 4) and with Hale's technique, the vertical koilin trapped in between the glandular epithelium was positive for neutral mucin while, the rest was positive for acid mucin and was predominant (Fig. 5). But, Sukanuma *et al.* (1981) reported that the internal lining contained only neutral mucopolysaccharides in some birds. The reaction of the membrane for protein by ninhydrin-schiff was mild and contained cysteine a sulphur containing amino acid in accordance with the finding of Akester (1986) in fowl. Banks (1981) opined that this membrane protects the underlying glands from proteolytic and hydrolytic activity of proteases and hydrochloric acid. The activity of alkaline phosphatase and acid phosphatase was negative and concurred with the findings of Gaikwad *et al.* (2002) in domestic fowl. The reaction of the membrane for succinic dehydrogenase and adenosine triphosphatase (Fig. 6) was also negative.

The surface epithelium was PAS positive (Fig. 4). They showed predominance of neutral mucin whereas, Sukanuma *et al.* (1981) reported that it was

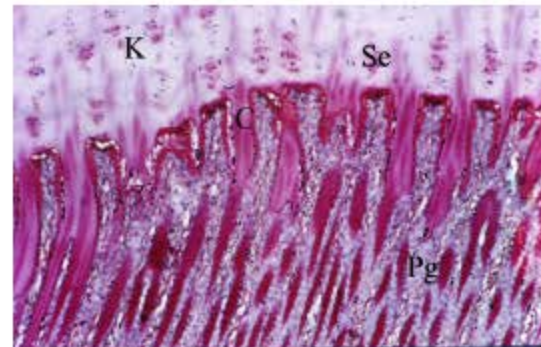


Fig. 4: Photomicrograph of the gizzard of a 8 week old guinea fowl showing PAS positive reaction. (PAS x 200) K-Koilin, Se-Surface epithelium, C-crypt, Pg-Proprial gland.

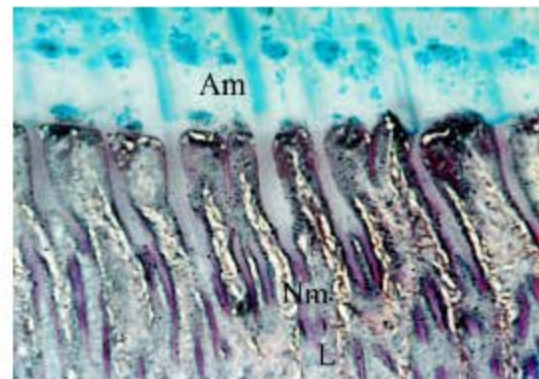


Fig. 5: Photomicrograph of the gizzard of a 8 week old guinea fowl showing its reaction for acid and neutral mucin. (Hale's x 200) Am-Acid mucin, Nm-Neutral mucin, L-Lumen of proprial glands

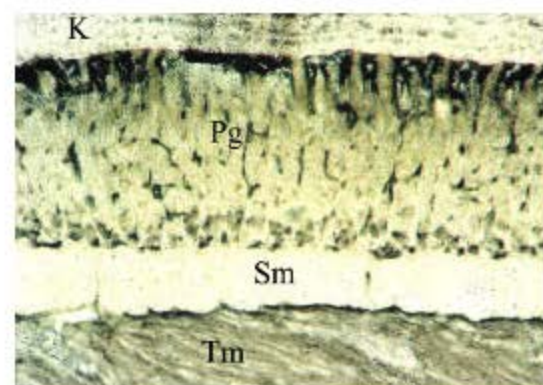


Fig. 6: Photomicrograph of the gizzard of a 3 week old guinea fowl showing adenosine triphosphatase activity (indicated by arrows). (ATP ase activity x 100) K-Koilin, Pg-Proprial glands, Sm-Submucosa, Tm-tunica muscularis.

predominated by acid mucins in birds. These cells on their apical portion also showed a positive reaction for ninhydrin-schiff. The activity of the cells for acid phosphatase was more pronounced in birds of 8-12 weeks of age. However, the activity for alkaline phosphatase and adenosine triphosphatase (Fig. 6) was found to be intense in all the age groups. Mohan *et al.* (1977) reported that the surface epithelium was positive for acid phosphatase, alkaline phosphatase and lipase in parrot, whereas the later was not observed in the present study.

PAS positive material was present in the lumen of the glands and in the cells lining the surface and crypts. However, the deep glandular cells were found to lack mucin (Fig. 4). This finding is in contrary with the findings of Gaikwad *et al.* (2002) who reported that in chicken all the components of the gizzard were periodic acid schiff positive. With Hale's staining, the lumen of the glands showed only neutral mucin (Fig. 5). A mild reaction for ninhydrin-schiff was also observed in the secretory material within the lumen of the crypt and glands. The activity for acid phosphatase was more intense in the cryptic cells and glandular cells of higher age groups (8 and 12 weeks). The glandular cells also showed a positive reaction for adenosine triphosphatase (Fig. 6). However, the reaction for succinic dehydrogenase and alkaline phosphatase was mild. The muscle fibers of the tunica muscularis showed a positive reaction for adenosine triphosphatase activity (Fig. 6).

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