Journal of Animal Research: v.6 n.2, p. 101-104. April 2016

DOI: 10.5958/2277-940X.2015.00175.8

Histochemical and Ultrastructural Studies on the thyroid gland of *Pati* Ducks *(Anas platyrhynchos domesticus*) of Assam

Snehangsu Sinha[1*], Munmun Sarma[1], S. Goswami [2] and K.B. Devchoudhury[1]

[1]Department of Anatomy, College of Veterinary Science, Assam Agricultural University, Guwahati, Assam, INDIA
[2]Department of Pathology, College of Veterinary Science, Assam Agricultural University, Guwahati, Assam, INDIA

**Corresponding author: S Sinha; Email: drsnehangsusinha@gmail.com*

Received: 25 January, 2016 **Accepted:** 18 February, 2016

ABSTRACT

A total of 36 ducks were utilised in this study ranging in age from 2 weeks upto 40 weeks of age. For histochemical parameters, the thyroid glands were separated out of the thoracic cavity and the required representative tissue samples were preserved in deep freeze maintained at -80ºC. For scanning electron microscopy the tissue samples were processed by SAIF, NEHU, Shillong. The colloid showed a strong PAS positive reaction in all the age groups. The activities of both acid and alkaline phosphatases were present in all the age groups.The scanning electron microscopy revealed the normal structure of the thyroid gland in all the age groups. Parenchyma of thyroid gland was composed of follicles which were separated from each other by interfollicular connective tissue made up of collagen and reticular fibers. The follicles were closely packed together and their shape varied from oval to polyhedral. Microvilli of the follicular cells were observed on the apical surface. The SEM also showed the thyroid artery penetrating the gland.

Keyword: Thyroid gland, histochemistry, scanning electron microscopy, duck

The present research programme on the thyroid gland was taken up on ducks aiming to elucidate the histochemical and ultrastructural features of the thyroid gland in *Pati* ducks during post natal growth period.

MATERIALS AND METHODS

For this study, the ducks were randomly selected according to 6 different age groups viz. 2 weeks, 4weeks, 8weeks, 20 weeks, 30 weeks and 40 weeks, with 6 ducks in each group. The experimental ducks were sacrificed according to the method of Gracy (1986). After slaughter, a mid-ventral incision was given, clavicle along with breast muscles were cut and were reflected carefully without disturbing the other organs of the region. The thoracic cavity of each bird was exposed by making a ventro-median incision and then the thoracic muscular layers and air sac of the clavicle were reflected. The thyroid glands were separated out of the thoracic cavity and the required representative tissue samples were preserved in deep freeze maintained at -80º C (except for PAS-alcian blue, where paraffin sections were utilized). The tissue samples were then shifted directly to cryostat microtome (Shandon Finesse) which was maintained at -22ºC. The frozen sections were cut at 10 µm thickness and were collected on clean slides. They were temporarily stored at -22ºC and were then treated as per the method of Chayen *et al.* (1991) for histochemical demonstration of Alkaline phosphatase and Acid phosphatase.

Representative tissue samples from the same birds were also processed as per procedure followed by Luna (1968) and paraffin sections. Sections were cut at 8 µm thickness and stained for mucopolysaccharides by Periodic Acid Schiff –alcian blue method (Luna, 1968).

For scanning electron microscopy, the tissue samples were processed as per techniques of Parsons *et al*. (1991) which were slightly modified by SAIF, NEHU, Shillong. The samples were cut into small pieces of 2 mm size and fixed in 2% glutaraldehyde solution for 4 hours at 4ºC, washed in 0.1M sodium cacodylate buffer, post-fixed in 1% osmium tetroxide in 0.1M sodium cacodylate buffer,

dehydrated by ascending grades of acetone and gold coating was applied in the tissue samples. The stubs with the tissue samples were loaded in the JMS-35CF (Joel) scanning electron microscope operated at 20KV and electron micrographs were taken.

RESULTS

Depending upon the histochemical activity on the thyroid gland on Assam *Pati* ducks, it was seen that only the lining epithelium was reactive to acid phosphatase and alkaline phosphatase; while the colloid in follicles were strongly PAS positive. The colloid showed a strong PAS positive reaction in all the age groups under the present study. The follicular lining also showed moderate PAS positive reaction (Fig.1) in all the age groups. Interfollicular area and capsule showed weakly PAS positive reaction in all the age groups (Fig.2). The intensity of histochemical reaction for glycogen in the different structures of thyroid gland at various age groups is shown in table 1. Acid and alkaline phosphatases' positive activity was shown by the lining epithelium of the thyroid follicles. The activities of both acid and alkaline phosphatases were present in all the age groups with different intensities shown in table 2. The acid and alkaline phosphatase showed strong activity from day old to 20 weeks old ducks and moderate activity in 30 weeks and 40 weeks old ducks.

However day old duckling was excluded from the study as sectioning was not possible in cryotome because the gland was too small in day old duckling. The scanning electron microscopy revealed the general structure of the thyroid gland in all the groups under study. Parenchyma of thyroid gland was composed of follicles which were separated from each other by interfollicular connective tissue made up of collagen and reticular fibers. The follicles were closely packed together and their shape varied from oval to polyhedral.

The thyroid follicles were oval to elliptical in shape and were surrounded by connective tissue fibers (Fig 3). Microvilli of the follicular cells were observed on the apical surface (Fig 4). Follicular capillary network was well appreciated on the surface of the thyroid glands and this capillary anastomosis showed presence of some bead like structures (Fig 5). The SEM also showed the thyroid artery penetrating the gland (Fig 6).

Table 1. Showing the Intensity of Glycogen in the Different Structures of Thyroid Gland at Various Age Groups

Age groups	Different structures of thyroid gland			
	Capsule	Interfollicular space	Follicular lining	Colloid
2 weeks	+	+	++	+++
4 weeks	+	+	++	+++
8 weeks	+	+	++	+++
20 weeks	+	+	++	+++
30 weeks	+	+	++	+++
40 weeks	+	+	++	+++

Intensity of histochemical reaction:

Nil, absent; +, Weak; ++, Moderate; +++, Strong; ++++, Intense

Table 2. Showing the Intensity for Histochemical Activity in the Follicular Epithelium of the Thyroid Gland of Pati Ducks in Different Ages

Enzyme	Age groups					
	2 weeks	4 weeks	8 weeks	20 weeks	30 weeks	40 weeks
Acid phosphatase	+++	+++	+++	+++	++	++
Alkaline phosphatase	+++	+++	+++	+++	++	++

Intensity of histochemical reaction:

Nil, absent; +, Weak; ++, Moderate; +++, Strong; ++++, Intense

DISCUSSION

The colloid showed a strong PAS positive reaction in all the age groups under the present study. The follicular lining also showed moderate PAS positive reaction in all the age groups. Interfollicular area and capsule showed weakly PAS positive reaction in all the age groups. Prasad *et al.* (1999) in domestic ducks and Balasundaram (2005) in domestic fowl reported similar findings. Acid and alkaline phosphatases' positive activity was shown by the lining epithelium of the thyroid follicles. The activity of both acid and alkaline phosphatases was almost present in all the age groups with different intensities. The acid and alkaline phosphatase showed strong activity from day old to 20 weeks old ducks and moderate activity in 30

weeks and 40 weeks old ducks. This might be due to the reduced activity of the gland in adult stage. This was in accordance to Prasad *et al.* (1999) in domestic ducks and Balasundaram (2005) in domestic fowl.

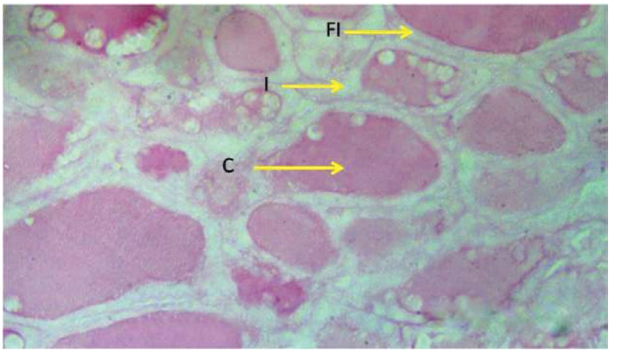

Fig 1: Photomicrograph (40 weeks) showing the follicles with colloid (C) being strongly PAS positive; follicular lining (FI) moderately PAS positive; interfollicular area (I) being weakly PAS positive. PAS, X40.

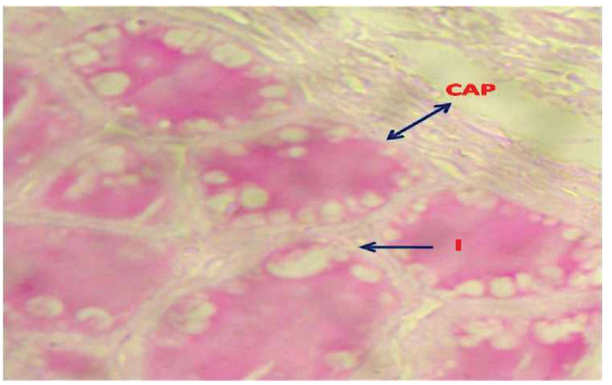

Fig. 2. Photomicrograph showing the capsule (CAP) and interfollicular space (I) being weakly PAS positive. X40.

Parenchyma of thyroid gland was composed of follicles which were separated from each other by interfollicular connective tissue made up of collagen and reticular fibers. The follicles were closely packed together and their shape varied from oval to polyhedral. This was similar to the findings observed by Lucy *et al*. (2009) in Kuttanad ducks. The thyroid follicles were oval to elliptical in shape and were surrounded by connective tissue fibers which were similar to the observations of Morita *et al*. (1994). Microvilli of the follicular cells were observed on the apical surface. This was similar to the observations of Briet *et al.* (1998).

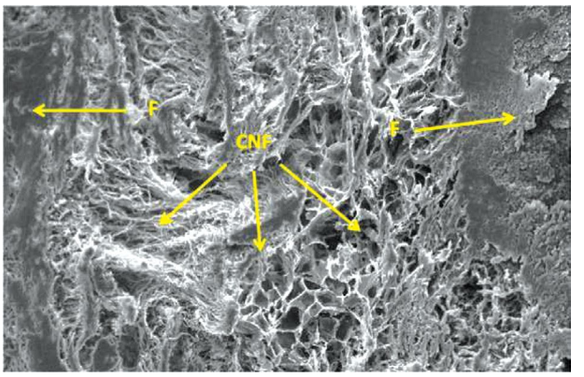

Fig. 3. Scanning electron micrograph showing the follicles (F) surrounded by collagen fibers (CNF) in 40 weeks old *Pati* ducks bar= 100µm, 100x

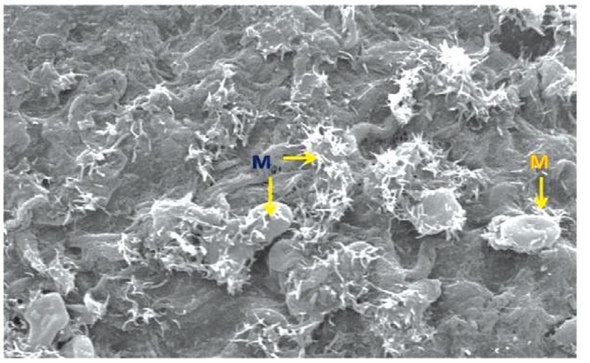

Fig. 4. Scanning electron micrograph showing the microvilli (M) of follicular cells in 4 weeks old *Pati* ducks bar= 10µm, 1,600x

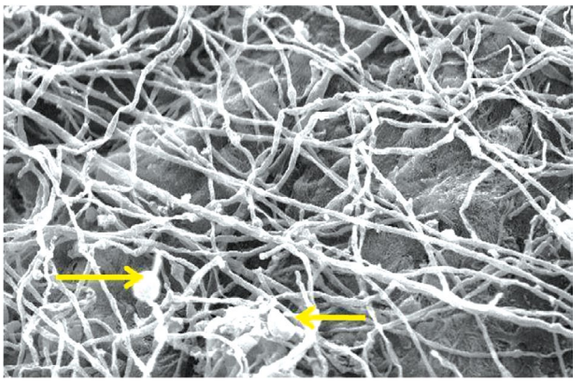

Fig. 5. Scanning electron micrograph showing the capillary network of thyroid and anastomosis forming ovoid (arrows) structures in 8 weeks old *Pati* ducks bar= 10µm, 1,100x

Follicular capillary network was well appreciated on the surface of the thyroid glands and this capillary anastomosis showed presence of some bead like structures which was also reported by Cozzolino (2005). The SEM micrograph also showed the thyroid artery penetrating the gland. Similar finding was also found by Janthap *et al.* (2013).

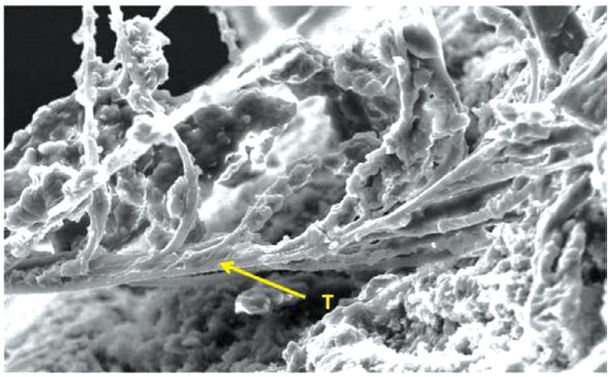

Fig. 6. Scanning electron micrograph showing the thyroid artery (T) penetrating the gland in 40 weeks old *Pati* ducks bar= 50µm, 400x

CONCLUSION

The histochemical study on the thyroid gland of Assam *Pati* ducks, showed that the lining epithelium was reactive to both acid and alkaline phosphatase. On scanning electron microscopy the thyroid follicles were oval to elliptical in shape and surrounded by connective tissue fibers. Microvilli of the follicular cells were observed on the apical surface.

ACKOWLEDGEMENTS

The author conveys heartiest thanks to Dr. Kabita Sarma, Prof. and Head, Dept. Anatomy and Histology for her support and fruitful advices during the research programme.

REFERENCES

Balasundaram, K. 2005. Histogenesis, histochemistry and histometry of the embryonic thyroid gland in the domestic fowl (*Gallus domesticus*). *Indian J of Vet Anatomy.,* **17**: 1-5.

Breit, S., Konig, H.E. and Stoger, E. 1998. The morphology of the thyroid gland in poultry with special regard to seasonal variations. *Anat. Histol. Embryol*., **27**(4): 271-276.

Chayen, O.J., Bitensky, I., Butcher, R.C. and Poulter, I.W. 1991. A guide to practical histochemistry. 2nd edition, Wiley.

Cozzolino, M.F. and Chopard, R.P. 2005. Analysis of thyroid gland microvascularization in rats induced by ingestion of potassium bromide: a scanning electron microscopy study.. *Annals of A.- Anatom Anzer*., **187**(1): 71–76.

Janthap, P., Sricharoenvej, S., Lanlua, P., Niyomchan, A. and Baimai, S. 2013. Microcirculation of thyroid gland in the Lyle's flying fox (*Pteropuslylei)*. *Sci. Res. and Essays.,* **8**(2): 95-98.

Lucy, K.M., Maya, S., Indu, V.R., Joseph, L. and Patki, H.S. 2009. Age related changes in the histomorphology of thyroid gland in Kuttanad ducks (*Anasplatyrhynchosdomesticus*). IV World Waterfowl Conference, Thrissur, India, 244-249.

Luna, L.G. 1968. *Manual of Histological Staining Methods of the Armed Forces Institute of Pathology*. Third edition, McGraw-Hill Book Company, New York, 258.

Morita, M., Ogata, T. and Araki, K. 1994. Scanning electron microscopic study of the collagen sheath of the human thyroid gland and its disorders. *Scanning Microsc.,* **8**(3): 695-704.

Parsons, K.R., Bland, A.P. and Hall, G.A. 1991. Follicle associated epithelium of the gut associated lymphoid tissue of cattle. *Vet. Pathology.,* **28**(1): 22-29.

Prasad, R.V., Chandrasekharan, S.R. and Vijayaragavan, C. 1999. Histology and histochemistry of the thyroid gland of domestic duck. *Indian J. Poult. Sci*., **34**: 2.