

Histochemical studies on thymus of prenatal buffalo

P JAGAPATHI RAMAYYA¹, OPINDER SINGH² and K S ROY³

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab 141 004 India

Received: 5 May 2009; Accepted: 7 June 2010

Key words: Buffalo, Prenatal buffalo, Thymus

Thymus is a primary lymphoid organ and it is central site for formation of T-lymphocytes during prenatal period and plays a major role in cell mediated immunity. Therefore, a comprehensive study on the histochemical distribution of carbohydrates, lipids and phospholipids in buffalo was undertaken. This will be contributory to the existing anatomical knowledge and that will form a basis for further investigations.

Tissue samples were collected from 38 buffalo fetuses (CRL 0.9 cm to 80.0 cm, 26 days to 254 days). The approximate age of the fetuses was determined by using Soliman's (1975) formula. The buffalo fetuses were divided into three groups based on their CRL (crown rump length), viz. group 1 (0.9 cm to 20.0 cm CRL, age 26 days to 119 days), group 2 (20.1 cm to 40.0 cm CRL, age 120 days to 164 days) and group 3 (40.1 cm to 80.0 cm, age 166 days to 254 days). Fresh tissue samples were used for cryostat sections, and some samples were processed for paraffin sections by acetone benzene schedule. The cryostat and paraffin sections of 5–10 µm thick were subjected to various histochemical methods (Luna 1968).

Neutral mucopolysaccharides: In group 1 capsule and trabeculae showed weak PAS positive reaction and they were moderate to strongly PAS positive in group 2 and group 3. Lymphocytes were devoid of neutral mucopolysaccharides in all three groups of prenatal buffalo (Fig. 1). The absence of neutral mucopolysaccharides in lymphocytes of buffalo thymus were in conformity with the observations of Wislocki *et al.* (1949) in mice.

In group 1, PAS reaction was moderate in epithelial reticular cells of medulla and weak in cortex, whereas in group 2 and group 3, these cells were strongly PAS positive in medulla (Fig.1). The intense PAS reaction in reticuloepithelial cells of thymus in the thymus was in

accordance with the findings of Metcalf and Ishidate (1961) in mice, Ramakrishna *et al.* (1978) and Geetha (1989) in buffalo.

Hassall's corpuscles were strongly PAS positive in all the three groups which is in conformity with the findings of Geetha (1989) and Prakash and Chandra (1999) in buffalo fetuses. The above findings suggested that the secretory material of the Hassall's corpuscles contained neutral mucopolysaccharides.

Acid mucopolysaccharides: In the present study capsule and trabeculae were weak to moderately alcinophilic in thymus of group 1, whereas in group 2 and group 3 fetuses these were moderately positive for acid mucopolysaccharides as reported by Mainde *et al.* (2008) in thymus of goat fetuses.

Hassall's corpuscles were moderately alcinophilic in the thymus of group 1, however strong activity was observed in mid and late gestations. Similar observations were also noted in buffalo fetuses by Geetha (1989). These observations confirmed that Hassall's corpuscles also contained acid mucopolysaccharides.

Lipids: In the present study capsule and trabeculae were devoid of lipids in the prenatal thymus of all three groups. However, the cell membrane of the lymphocytes showed few lipids. Contrary to this Wislocki and Dempsey (1946) reported that lymphocytes were devoid of lipid droplets in young Rhesus monkeys peripheral blood.

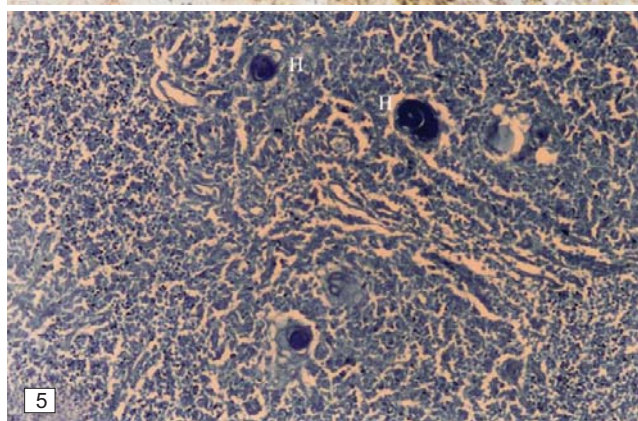
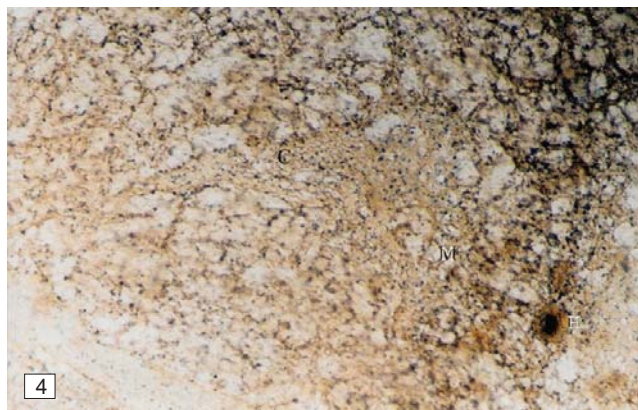
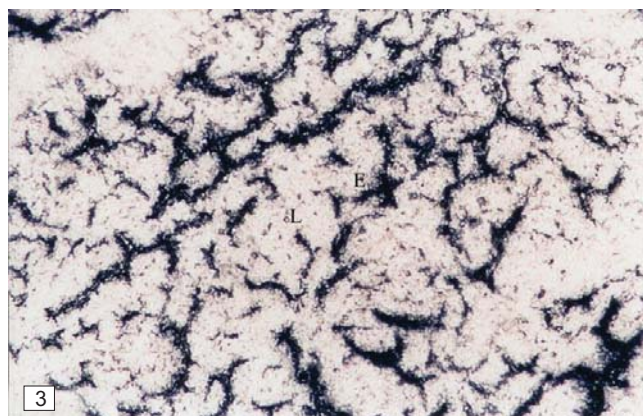
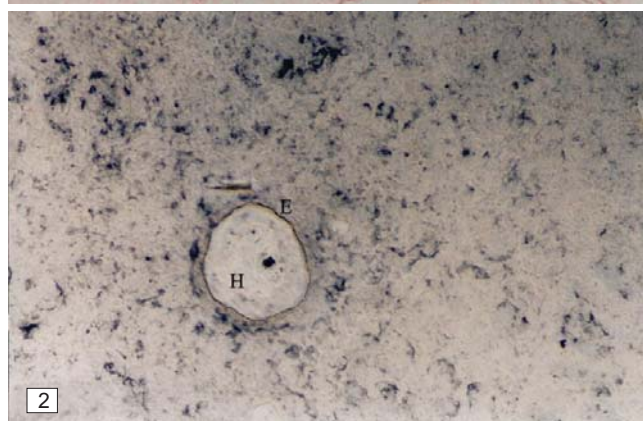
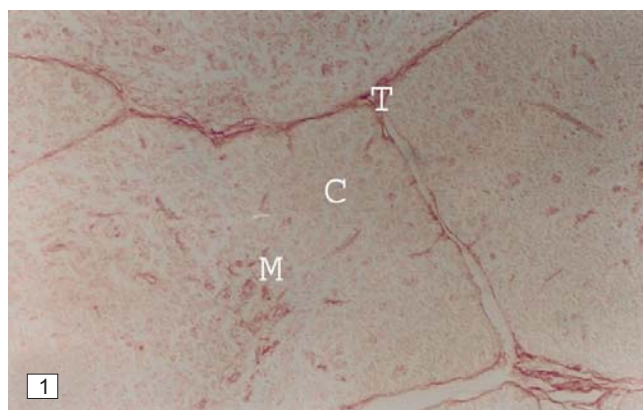
The epithelial reticular cells of the thymus of group 1 contained little amount of lipids, whereas in groups 2 and 3 (Fig. 3) these cells contained abundant lipids. Due to high content of lipids in epithelial reticular cells these cells were termed as lipopigment cells in the mice (Smith *et al.* 1952, Smith 1969).

In the present study lipids droplets were localized in peripheral cells of Hassall's corpuscles of group 2 (Fig. 2) and group 3. According to Smith and Holt (1953) epithelial reticular cells and Hassall's corpuscles might have lysosomal activity in the thymus that led to formation of lipid pigment as end product in phagocytosis.

Phospholipids: In the present study phospholipids were noted in the thymus of all age groups of prenatal buffalo. In

Present address: ¹Associate Professor and Head, (e mail: drjagapathi_palla@yahoo.com). College of Veterinary Science, Proddatur, Andhra Pradesh 516 360.

²Associate Professor, ³Professor Emeritus, Department of Veterinary Anatomy and Histology, College of Veterinary Science.



Figs 1–5. **1.** Photomicrograph of thymus of 70.0 cm CRL (232 days) buffalo fetus showing weak activity of PAS in cells of cortex (C), strong activity in cells of medulla (M) and trabeculae (T) Periodic acid Schiff×100; **2.** Photomicrograph of thymus of 40.0 cm CRL (164 days) buffalo fetus showing lipids in peripheral cells of Hassall's Corpuscles (H). Sudan Black×200; **3.** Photomicrograph of thymus of 70.0 cm CRL (232 days) buffalo fetus showing lipids in epithelial reticular cells (R) and lymphocytes (L). Sudan Black×200; **4.** Photomicrograph of thymus of 70.0 cm CRL (232 days) buffalo fetus showing phospholipids in cortex (C) medulla (M) and Hassall's corpuscles (H). Baker's acid haematin method×400; **5.** Photomicrograph of thymus of 40.0 cm CRL (164 days) buffalo fetus showing strong bromophenol reaction in Hassall's corpuscles (H). Bromophenol blue method×200.

group 1 moderate amount of phospholipids were observed in cortex and medulla, but in group 2 and group 3 the phospholipids content was high in medulla (Fig. 4). According to Weakley *et al.* (1964) in hamster thymus, the phospholipids were either synthesized or accumulated in cells as vacuoles, within which a system of concentric membranes formed spontaneously.

Basic protein: Strong bromophenol blue reaction was noted in the capsule and trabeculae of thymus in prenatal buffalo. The Hassall's Corpuscles showed strong reaction for bromophenol blue in group 2 and group 3 (Fig. 5). This indicated that there was a proteinacious material in their secretions, probably gamma globulins as opined by Geetha

(1989) in buffalo fetuses. The presence of gamma globulins and antigenic localization was also noted in Hassall's corpuscles of guinea pig thymus (Blau 1967).

SUMMARY

In thymus of prenatal buffalo Periodic Acid Schiff reaction (PAS) was weak in cells of cortex, whereas in trabeculae, epithelial reticular cells and Hassall's corpuscles PAS reaction was strong. Lymphocytes were negative to PAS in all age groups. Phospholipid content was high in the medulla and Hassall's corpuscles and less in cortex of thymus. Hassall's corpuscles showed strong bromophenol reaction in thymus of buffalo during pre natal life.

REFERENCES

- Blau J N. 1967. Antigen and antibody localization in Hassall's corpuscles. *Nature* **215**: 1073–75.
- Geetha S. 1989. 'Histology and histochemistry of the thymus in Indian buffaloes.' M.V.Sc. Thesis, Tamilnadu Agricultural University, Coimbatore.
- Luna L G. 1968. *Manual of Histological Staining Methods of Armed Forces Institute of Pathology*. 3rd edn. McGraw Hill Book Co., New York.
- Mainde U P, Nandeshwar N C and Zade B A. 2008. Histochemical characterization of prenatal thymus in goat foetus. Abstract. *23rd Annual convention and National symposium of Indian Association of Veterinary Anatomists* at Hisar.
- Metcalf D and Ishidate M. 1961. PAS positive giant cells in the mouse thymus cortex. *Nature* **191**: 305.
- Prakash A and Chandra G. 1999. Histomorphological observations on the medulla of prenatal thymus of buffalo. *Indian Journal of Veterinary Anatomy* **11**: 19–23.
- Ramakrishna S, Salam A and Rao K T. 1978. Studies on the thymus of infant murrah buffalo calves. *Mysore Journal of Agricultural Science*. **12**: 300–04.
- Smith C. 1969. Studies on the thymus of mammals XVI. Lipopigment cells in the cortex of the thymus of the mouse. *American Journal of Anatomy* **124**: 389–410.
- Smith C and Holt F A. 1953. Studies on the thymus of the mammals. Lipids in the thymus of irradiated mice. *Anatomical Record* **2**: 123.
- Smith C, Samson E H, Padykula H A, Laskey M G and Loewenthal L A. 1952. Studies on the thymus of the mammal V. On the origin of adipose cells replacing the thymus. *Journal of Morphology* **90**: 103–11.
- Soliman M K. 1975. Studies on the physiological chemistry of the allantoic and amniotic fluid of buffaloes at various periods of pregnancy. *Indian Veterinary Journal* **52**: 106–11.
- Weakley B S, Patt D I and Shepro D. 1964. Ultrastructure of the fetal thymus in the golden hamster. *Anatomical Record* **148**: 348–49.
- Wislocki G B and Dempsey E W. 1946. Observations on the chemical cytology of normal blood and hemopoietic tissue. *Anatomical Record* **96**: 249–77.
- Wislocki G B, Rheingold J J and Dempsey E W. 1949. The occurrence of the periodic acid schiff reaction in various normal cells of blood and connective tissue. *Blood* **4**: 562–68.