HISTOGENESIS OF DIENCEPHALON IN GOAT FOETUSES*

K.M. Lucy, K.R. Harshan, J.J. Chungath and N. Ashok1
Department of Veterinary Anatomy and Histology,
College of Veterinary and Animal Sciences, Mannuthy-680 651, India.

ABSTRACT
Histogenesis of diencephalon was studied using 46 goat foetuses ranging from 2.5 cm CRL (40 days of gestation) to 41.5 cm CRL (full term). By 40 days, the roof plate was converted into the tela choroidea and along with the growing blood vessels, formed the choroid plexus of the third ventricle. Proliferation of neuroblasts in localized regions of the mantle layer led to formation of nuclei in the seventh week. The nerve tracts also appeared at this stage. At 81 days the internal medullary lamina extended into the thalamus from the stratum zonale dividing it into medial and lateral portions. Supraoptic nucleus started developing towards the end of second month. Typical neurons appeared in most of the thalamic and hypothalamic nuclei during fourth month. During fifth month, supraoptic and paraventricular nuclei of hypothalamus showed bipolar cells. Histologically the diencephalon was adult-like towards term.

Key words : Diencephalon, Prenatal histogenesis, Goat.

INTRODUCTION
Diencephalon is the subdivision of embryonic prosencephalon. Gross anatomical and histological studies on the diencephalon have been made in different domestic animals (Dellmann and McClure, 1975; De Lahunta, 1983; Dellmann and Eurell, 1998; Ghosh, 2002). However, developmental changes have not been well documented in ruminants. Therefore, a comprehensive study on the histogenesis of the diencephalon in goats seems to be a relevant area of research. It is contributory to the existing anatomical knowledge and will form a basis for further physiological, pathological and neuroendocrinological studies.

MATERIAL AND METHODS
Histogenesis of diencephalon in goats was studied using 46 goat foetuses with a crown rump length (CRL) ranging from 2.5 cm (40 days of gestation) to 41.5 cm (full term). Body weight, body parameters and skull parameters of the subjects were recorded. Age of the foetuses was calculated from the formula, \( W^{1/3} = 0.096 (t-30) \) derived by Singh et al. (1979) for goat foetuses, where ‘W’ is the body weight of the foetus in gm and ‘t’ is the age of the foetus in days. Based on the age, the foetuses were divided into four groups representing 2nd, 3rd, 4th and 5th months of gestation. Brain was fixed in 10 percent neutral buffered formalin. Diencephalon was collected by incising at lamina terminalis cranially and at the mamillary body caudally. The tissue was processed conventionally and serial sections of 5 \( \mu \)m were taken. The sections were subjected to Haematoxylin and Eosin (H&E), Van Gieson’s method for collagen, Holzer’s method for glial fibres, Sevier-Munger silver impregnation method for neural tissues, Aldehyde-thionine-PAS method for central nervous system and Phosphotungstic acid haematoxylin (PTAH) method for CNS tissue, Holme’s silver nitrate luxol fast blue method for axis cylinder and myelin sheath (Luna, 1968); Periodic acid Schiff’s reaction for carbohydrates and Best’s carmine method for glycogen (Bancroft and Stevens, 1977). The data were analysed statistically (Snedecor and Cochran, 1985).

* Part of the Ph. D thesis submitted by the first author.
1 College of Veterinary and Animal Sciences, Pookot-673 576, India.
RESULTS AND DISCUSSION
Development in the second month:
Diencephalon is the rostral most division of brainstem that remained in the midline after the telencephalic vesicles grew out from the forebrain. By 40 days of gestation, the roof plate of diencephalon was converted into the tela choroidea and along with the growing blood vessels formed the choroid plexus of the third ventricle. Alar plates of diencephalon showed three zones as in the neural tube wall viz., inner ependymal, middle mantle and outer marginal layers. Width of the ependymal layer gradually reduced and these cells carried cilia on their luminal surface. Towards the middle portion of the thalamus, thickness of this layer was more. Middle or mantle zone was the thickest layer, which contributed most of the thickness of the wall. The outer marginal layer was thin. Bundles of nerve fibres divided the mantle layer especially towards the lumen. Proliferation of neuroblasts in localized regions of the mantle layer led to aggregation of cell bodies (Fig. 1). These masses or aggregations of gray substance, the nuclei, were subdivided by nerve fibres into several parts. These nuclei first appeared in the goat foetuses in the seventh week of gestation. Primitive neurons in such aggregations were small in size with dark compact nucleus and inconspicuous cytoplasm. Small scattered neurons with light staining nucleus and numerous blood channels lined with endothelial cells were also seen in this area. Arey (1957) reported that such massing of nerve cells and fibres led to regional thickenings of brain wall and was one of the chief agencies through which the brain took form and acquired its characteristic internal organisation. According to Harrison (1978) and Sadler (2004), thalamus in vertebrate embryos was a collection of nerve cells, which later became divided into separate nuclei connected with spinal cord and other parts of CNS. In general, cellular density was more towards the dorsal aspect of thalamus than in its ventral regions and in the hypothalamus. In the seventh week, the two thalami grew into approximation so that the third ventricle became a slit-like cavity. There was only roof plate along the median plane over the small dorsal portion of the third ventricle.

The region of interthalamic adhesion did not contain any nerve fibre. Its average height was 0.130±0.013cm during second month. This is in accordance with the observations made by Harrison (1978) in vertebrate embryos. On either side of massa intermedia, bundles of nerve fibres in the mantle layer represented the internal capsule (Fig. 2). In the hypothalamus also, bundles of nerve fibres and aggregation of primitive neurons were noticed. Mammillary body formed the caudal limit of the hypothalamus. Keith (1947) reported that in the human foetus, during the second month, a scattered series of nerve cells differentiated in the hypothalamus into anterior, middle and posterior ill-defined groups. It was also reported that the earliest nerve tracts to appear in the brain arose in connection with these centres. Above the level of optic nerve, the supraoptic nucleus started developing in the floor of the hypothalamus towards the end of second month (Fig. 3). Paraventricular
nucleus was not clearly evident but a large number of neuroblasts were seen accumulated near the third ventricle at this stage.

The developing eyeball demarcated the area of diencephalon. At the junction between diencephalon and mesencephalon, immediately beneath the posterior commissure, on the dorsal surface of aqueduct of Sylvius, the ependymal cells were highly modified to form the subcommissural organ (SCO).

**Development in the Third Month:** Thin layers of white matter covered the dorsal and lateral surfaces of the thalamus by 76 days of age. That on the dorsal surface formed the stratum zonale and the white matter on the lateral surface was the external medullary lamina. At 81 days, a vertical plate of white substance, the internal medullary lamina, extended into the thalamus from the stratum zonale dividing it into medial and lateral portions. The medial nuclei occupied the dorso-medial portion of the central half of the thalamus. Wedged between this and the ventral nuclei caudally was the centromedian nucleus, the largest of the intralaminar nuclei. The internal medullary lamina partially surrounded this nucleus. Truex and Carpenter (1969) recorded similar observations in adult vertebrate diencephalon. Differentiation of neurons was not complete. Thickness of the inner ependymal layer greatly reduced and the cilia on their luminal surface measured about 7.5μm in height.

In the middle of gestation, a thin connective tissue capsule covered the pineal gland. Column of the fornix divided the hypothalamic nuclei into medial and lateral nuclear groups. In vertebrates, Truex and Carpenter (1969) reported that the fornix represented one of the largest afferent systems to the hypothalamus that arose from the hippocampal formation in the temporal lobe of the cerebral hemisphere. Width of the optic tract and diameter of the supraoptic nucleus greatly increased at 81 days of age.

By 76 days of age, the posterior commissure appeared as a rounded bundle of fibres that crossed the mid plane beneath the stalk of the pineal body at the junction between the diencephalon and mesencephalon. This was just dorsal to the opening of cerebral aqueduct into the third ventricle. Larsell (1951) reported that the posterior commissure connected the rostral colliculi with each other and fibres of the pretectal region of one side to the other side. Just beneath the posterior commissure was the subcommissural organ, which was lined by several layers of non-cliated, elongated tanycytes as seen in the second month. Talanti (1959) investigated various stages of development of SCO in the bovine foetus and the results suggested that the SCO of the bovine foetus was functionally active at least by third month of intrauterine life.

**Development in the Fourth Month:** The ependymal lining of the third ventricle was pseudostratified during fourth month. Free surface showed cilia. Rajtova (1999) noticed in the third ventricle of sheep and goat foetuses, three to four layered ependyma between 40 and 50 days of development. This changed through a pseudostratified epithelium (upto day 130) into the typical one-layered ependyma. Another feature noticed during this period was that neurons appeared in most of the thalamic and hypothalamic nuclei. During the third month, they were mostly neuroblasts with the exception of a few neurons.

Supraoptic nucleus of the hypothalamus showed long spindle shaped cells. Most of these were bipolar cells (Fig. 4). Each one measured 18.8μm with a nucleus of 11.3μm and nucleolus of 3.8μm at 101 days. Paraventricular nucleus also started developing during the fourth month.
Development in the fifth month: Anterior nucleus of the thalamus was partially flanked medially and laterally by the internal medullary lamina (Fig. 5). Dorsomedial nucleus showed a rostral magnocellular portion consisting of large polygonal deeply staining cells and a caudal parvocellular portion made up of small pale staining cells at 144 days as reported by Truex and Carpenter (1969) in adult human brain. Nissl’s granules appeared in the cytoplasm of neurons of the diencephalon at 144 days. Interthalamic adhesion showed midline nuclei consisting of small fusiform dark staining cells. Reticular nucleus could be located lateral to the external medullary lamina. Mamillothalamic tract was very distinct.

Supraoptic and paraventricular nuclei of hypothalamus were composed mostly of bipolar cells and peripherally distributed Nissl substance. Paramasivan and Sharma (2001), in their studies on the hypothalamus of Gaddi sheep found that the preoptic nucleus was composed of spindle shaped medium-sized neurons located rostrocaudal to the optic chiasma. Paraventricular nucleus was triangular with densely grouped neurons placed as a vertical plate along the wall of the third ventricle. Truex and Carpenter (1969) reported the presence of colloidal material in these cells indicating its secretory activity. Both these nuclei sent fibres to the neurohypophysis. The supraoptic and paraventricular nuclei were interconnected by scattered cells that formed an incomplete bridge between the two. Histologically the diencephalon was adult-like towards term.

REFERENCES


