

EARLY HISTOMORPHOGENESIS OF MYELENCEPHALON IN GOATS*

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ABSTRACT

Early development of the myelencephalon was studied using six goat fetuses up to one month of gestation. By 24 days of age (1.4cm CRL), the neural tube was completely fused and the brain vesicles started developing. The myelencephalon was distinguished from the anterior metencephalon by its much thinner roof and the location of the otocyst at the junction between the two. All the myelencephalic parameters showed a significant positive correlation with the parameters of other brain vesicles. Wall of the myelencephalon showed the typical arrangement of longitudinal alar plates and basal plates and the sulcus limitans. Diamond-shaped lumen of the myelencephalon in 24 days-old subjects became narrower and coffin-shaped at 26 days and slit-like in 27 days of age. Froriep's ganglion of the spinal accessory nerve was seen on either side of the myelencephalon by 24 days. Histologically, wall of the myelencephalon showed an inner ependymal, middle mantle and outer marginal layers bounded by the inner and outer limiting membranes.

Key words: Myelencephalon, early development, foetal goat

Myelencephalon is the part of the primitive brain that develops into the medulla oblongata. Gross anatomical and histological studies on the brain were undertaken in different domestic animals (De Lahunta, 1983; Dellmann and Eurell, 1998 and Ghosh, 2002). However early embryological changes have not been well documented in ruminants. A comprehension of the cellular mechanisms has significant importance in understanding the neural tube defects. Hence, this study was undertaken to trace the morphogenesis and histogenesis of myelencephalon during initial stages of prenatal life.

MATERIALS AND METHODS

Early development of the myelencephalon was studied using six goat fetuses up to one month of gestation. The age of the fetuses was calculated using the formula $W^{St} = 0.096 (t-30)$ derived by Singh *et al.* (1979) for the goat fetuses, where 'W' is the body weight of the foetus in g and 't' is the age of the foetus in days. After recording the body parameters, embryos were fixed in toto in 10% neutral buffered formalin, processed conventionally and serial sections of 5µm were taken to trace the developmental pattern of the neural tube. Standard procedures were adopted for histological and histochemical studies (Luna, 1968). Measurements

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of the layers were taken using an ocular micrometer. Total length of the myelencephalon was measured from the longitudinal sections of the foetuses. The data were analysed statistically (Snedecor and Cochran, 1985) to find out the relationship, if any between different parameters.

RESULTS AND DISCUSSION

Morphogenesis

In goat embryos of 24 days of age with a crown rump length (CRL) of 1.4cm, the neural tube was completely fused and the brain vesicles started developing. Five expansions were evident at the cranial end of the neural tube, the caudalmost one being the myelencephalon. It was distinguished from the anterior metencephalon by its much thinner roof. All the myelencephalic parameters showed a significant positive correlation with the parameters of the other brain vesicles. There was no clear demarcation between the myelencephalon and metencephalon in the 24 days-old embryos. The junction between the two was demarcated by the sharply defined otocyst located lateral to the neural tube.

The wall of the myelencephalon showed the typical arrangement of longitudinal alar plates and basal plates and the sulcus limitans (Fig. 1). Basal plate was thicker than the alar plate. Ghosh (2002) reported that in domestic animals, the myelencephalon was fairly comparable to their homologue in the spinal cord. Stretching of roof plate to form the thin roof of fourth ventricle had not commenced in the first month of gestation in goat embryos. The choroid plexus of the ventricles of the brain were also not developed. Diamond-shaped lumen of the myelencephalon in 24 days-old subjects became narrower with the growth of basal and alar plates. By 26 days of age (1.5cm CRL), the lumen became coffin-shaped (Fig. 2). By 27 days of age, the alar plates also projected into the lumen. The lumen at this stage was slit-like except at the region of sulcus limitans (Fig. 3). Height of the

myelencephalon showed considerable increase by that time.

Midway along each side of the hindbrain was the otocyst with an elongated lumen, and medial to it was the endolymph duct (Fig. 4). Caudal to the otocyst, transverse section of the glossopharyngeal nerve and the jugular ganglion of the vagus nerve were seen while the trunk of the accessory nerve was sectioned lengthwise as it curved forward from the level of the spinal cord (Fig. 5). Between the myelencephalon and the pharynx several rootlets of the hypoglossal nerve were seen on either side.

Froriep's ganglion of the spinal accessory nerve was seen on either side of the myelencephalon by 24 days itself. In pig embryos, Patten (1948) noticed that the Froriep's ganglion usually disappeared in the adult and the nerve was left without ganglia.

Jenkins (1978) studied the relation of gray and white matter and found that the spinal cord retained the original neural tube relationship of gray substance and white substance. In the medulla and the rest of the brainstem, this relationship was lost, so that there was more of an intermingling of gray and white matter.

Histogenesis

By 24 days of age, wall of the neural tube was organised so that three concentric zones were distinguished. These were the inner ependymal, middle mantle and outer marginal layers, bounded by the internal and external limiting membranes (Fig. 2). Arey (1957) made similar observation in human foetus in the sixth week of development. Inner ependymal layer represented the germinal layer of proliferating neuroepithelial cells. Middle nucleated mantle layer derived from proliferation of innermost cells was distinguishable only in the ventral wall of the developing myelencephalon in

Fig. 1

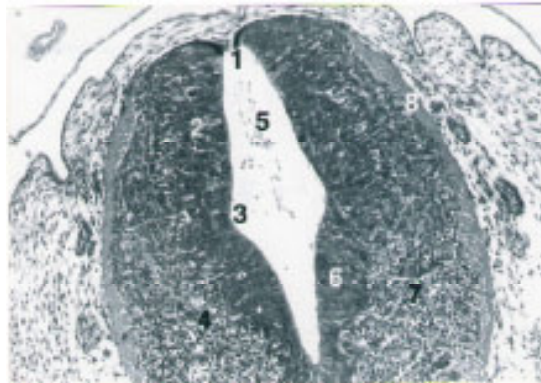
C. S. of the myelencephalon (24 days). H & E. x 100



1. Roof plate 2. Alar plate 3. Sulcus limitans 4. Basal plate
5. Floor plate 6. Lumen 7. Froriep's ganglion 8. Mantle layer

Fig. 2

C. S. of the myelencephalon (26 days). H & E. x 100



1. Roof plate 2. Alar plate 3. Sulcus limitans 4. Basal plate
5. Coffin-shaped lumen 6. Ependymal layer 7. Mantle layer 8. Marginal layer

Fig. 3

C. S. of the myelencephalon (27 days). H & E. x 100



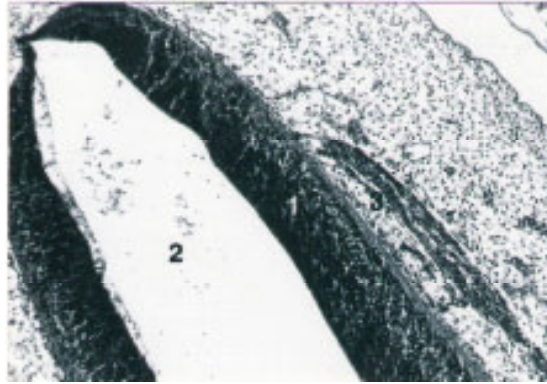
1. Roof plate 2. Alar plate 3. Sulcus limitans 4. Basal plate 5. Lumen

Fig. 4

Frontal section of neural tube (24 days) H & E. x 100



1. Neural tube wall 2. Neuromere 3. Otocyst 4. Endolymph duct 5. Lumen of hind brain

Fig. 5**L. S. of the myelencephalon (24 days). H & E. x 100**

1. Myelencephalic wall 2. Myelocoele 3. Accessory nerve

24 days-old embryos (Fig. 1). The present results confirmed the earlier observations of Harrison (1978) in vertebrates who stated that the region of the formation of neuroblasts was mainly the ventral aspect of neural tube. From 26 days onwards, the entire neural tube wall showed all the three layers (Fig. 2). In the region of myelencephalon, the mantle layer was the thickest layer in basal plate region at the age of 26 days. Arey (1957) noticed that the basal plate of the myelencephalon differentiated a little earlier than the alar plate. In vertebrates, Gilbert (1997) reported that the mantle layer became progressively thicker as more cells were added to it from the germinal neuroepithelium. In the alar plate of myelencephalon, the ependymal layer was thicker than the mantle layer. In the floor plate, only two layers were seen, the ependymal and marginal layers (Fig. 1). The mantle layer was absent. Roof plate was the thinnest, formed of a single layer of ependymal cells as observed by Sadler (2004) in mammals.

The cells of mantle zone were differentiated into two types, the neuroblasts and the spongioblasts by 24 days. Neuroblasts were first identified in the myelencephalon region by 24 days. They were less in number, possessed large pale vesicular nucleus with peripheral condensation of chromatin and a small dark staining nucleolus. Neuroblasts developed cellular processes and were converted into primitive neurons or multipolar neuroblasts by 27 days of age. Neurons developed before the formation of neuroglia. Large neurons developed earlier than the small neurons. In human embryos, Sadler (2004) reported the presence of scattered series of differentiated nerve cells in the wall of neural tube during the second month. It was concluded that the earliest nerve tract to appear in brain arose in connection with these centres.

Spongioblasts were more in number and possessed smaller and darker nucleus without a nucleolus. Their processes formed a network at 26 days. Patten (1948) noticed that in the neural tube of

pig embryos, the neuroblasts and spongioblasts could be differentiated from each other by the fact that the neuroblasts developed large nuclei while the nuclei of the spongioblasts were small. The formation of supporting tissue from the spongioblasts took place by the development of exceedingly slender and irregular cytoplasmic processes.

From 24th day onwards, scattered nucleated erythrocytes were noticed in both the ependymal and mantle layers. Blood channels lined by endothelium also appeared. The outer acellular marginal layer was the thinnest, composed of growing processes of neurons in the mantle layer. This layer was limited by the external limiting membrane.

Moore et al. (1981) noticed that the neurulation was accompanied by changes in the shape of cells of the neuroepithelium. Cells of the neuroepithelium were classified according to the shape of their profiles as rectangular, round and tapered. This method of cell shape analysis may be useful for the quantification of differences between normal and abnormal neurulation and for the analysis of cellular mechanisms, which are disturbed in neural tube malformation.

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