PRENATAL DEVELOPMENT OF SPINAL CORD IN GOATS (*Capra hircus*)

By
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Thesis submitted in partial fulfilment of the requirement for the degree of

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I hereby declare that the thesis entitled **"PRENATAL DEVELOPMENT OF SPINAL CORD IN GOATS *(Capra hircus)*"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship, associateship or other similar title, of any other University or Society.

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Certified that the thesis entitled **"PRENATAL DEVELOPMENT OF SPINAL CORD IN GOATS *(Capra hircus)*"** is a record of research work done independently by **Smt. S. Maya,** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Introduction

1. INTRODUCTION

The nervous system of vertebrates plays three vital roles: it enables the organism to acquaint itself favourably with the surrounding environment; makes possible the integrated control of the internal environment and serve as a centre for learning. These functions are accomplished by the brain, spinal cord and nerves together with the receptors (sense organs) and the effectors (muscles and glands).

The spinal cord is the major nerve tract of vertebrates, extending from the base of the brain through the vertebral canal. It is composed of nerve fibres and neuronal circuits, which mediate a variety of functions such as walking movements, withdrawal and postural reflexes and reflexes that control local blood vessels and gastro-intestinal movements. The brain never functions alone but always in association with the spinal cord. Among vertebrates, Chordata, the highest animal phylum, possess a dorsal neural tube protected by the vertebral column (Jenkins, 1978).

The first indication of the formation of the central nervous system in mammals is the appearance of a neural plate in the presomite stage. It becomes a neural groove guarded by a pair of neural folds at the first somite stage, which subsequently form a closed neural tube (Patten, 1948). The cells bordering the neurocoel or the central canal multiply very rapidly at the sides of the tube to form a thick wall, while the roof and floor remain thin. The thick lateral walls have three layers: inner germinal layer, middle mantle layer forming the gray matter and outer marginal layer forming the white matter (Huettner, 1967). The cephalic end of the neural tube (encephalon) expands into the brain and the rest of the neural tube (myelon) forms the spinal cord. The spinal cord provides the best example for the symmetric development of the neural tube by layers. The manner in which the neural tube takes the shape of adult spinal cord is related to the fate of the tissue inside the neural tube. The relationship of the gray and white matter in the adult spinal cord corresponds to that of the mantle and marginal layers in the developing neural tube (Buchanan, 1957).

The spinal cord of the adult is usually a dorsoventrally flattened rod, the flattening being more pronounced in cyclostomes. In tetrapods, the cord presents two enlargements, the cervical and the lumbar, corresponding to the limbs, which are absent in fishes, reptiles and birds. The cavity of the spinal cord is reduced to a very small central canal in adults. The gray matter occupies the central region and the

white matter forms a thick peripheral zone of the cord in amniotes. In cross-section, the gray matter has the well known 'H' or butterfly-like shape. In anamniotes, the shape varies from rounded or quandrangular to an inverted T or Y. The white matter is divided by the columns of gray matter into dorsal, lateral and ventral funiculi. The columns and funiculi differ in shape and proportions at different levels of the cord and are less defined in anamniotes (Hyman, 1942). In those vertebrates with abundant tail musculature such as lower fishes, tailed amphibians and reptiles, the cord extends to the caudal end of the vertebral column. However, in mammals, the embryonic vertebral column elongates more rapidly than the spinal cord and hence at birth the cord is shorter than the column and tapers caudally to a fine strand, the terminal filament (Kent, 1969).

Growth pattern is an important information in livestock breeding to determine the prospective potentials of an individual, as it is generally presumed that the growth trend determines the expression of future events (Jeetendra *et al.*, 1995). As the embryonic pattern foreshadows the structure of the spinal cord in the adults, to achieve an insight into the architecture of the adult nervous system, it is essential to know how the nervous system develops during embryonic life. A comprehensive study of prenatal development is essential to understand the normal variations in development and to establish a foundation for the study of various factors causing deviations from the normal development. A sound knowledge on the topographical aspect of the spinal cord and its segments will form a basis for diagnosis and treatment of neurological problems involving the lesions of the vertebral column and spinal cord. As the data on the developmental aspect of spinal cord in goats are very limited, this project proposes to study the normal structure and development of the spinal cord in goat at different stages of prenatal growth with the following objectives:

1. to trace the morphogenesis and histogenesis of spinal cord during prenatal period in goats and
2. to establish a standard growth pattern, to be used while studying the teratological factors in prenatal and postnatal periods.

Review of Literature

2. REVIEW OF LITERATURE

2.1 MORPHOLOGY

The spinal cord (medulla spinalis) of adult mammals is the elongated, cylindrical portion of the central nervous system that resides within the vertebral canal, enveloped by the meninges, bathed in the cerebrospinal fluid and anchored in the vertebral canal by ligaments (Kappers *et al.*, 1967).

According to Jenkins (1978), gross cross-sections of the spinal cord in mammals revealed peripheral white matter surrounding the central gray matter. The gray matter was in the form of a butterfly or 'H' with dorsal and ventral horns or columns and the connecting structure of gray matter, which surrounds the central canal. The connecting gray matter dorsal and ventral to the central canal constitutes the dorsal and ventral gray commissures. Within the white matter between the ventral gray commissure and the dorsal end of the ventral median fissure is the ventral white commissure. There is no dorsal white commissure. Bilaterally the white matter is divided into a dorsal funiculus, between the midline and dorsal root attachments; a ventral funiculus, between the ventral median fissure and ventral root attachments; and a lateral funiculus between dorsal and ventral root attachments. Throughout its length, the spinal cord was divided into two hemisections by a dorsal median sulcus and a ventral median fissure. Lateral to the dorsal median sulcus, there is a dorsolateral sulcus along which the dorsal rootlets of the spinal nerves attach. In the cervical and cranial thoracic regions on each side of the cord between dorsal median and dorsolateral sulci there is an intermediate sulcus. The ventral rootlets of the spinal nerves attach at the ventrolateral surface of the cord, where there is no ventrolateral sulcus.

Clark (1984) opined that the spinal cord is not a true cylinder, with a greater transverse width than its dorsoventral diameter and with two enlargements at the cervical and lumbar levels, which are related to the innervation of the extremities. Caudally the cord tapers abruptly to become the conus medullaris, which is attached by the filum terminale, a meningeal structure to the fundus of the dural sac at the level of second sacral vertebra in man.

Smuts and Bezuidenhout (1987) described two ventrolateral sulci on either side of the ventral median fissure in camel. According to Ulinski (1997) the shape of the adult brain and spinal cord was determined by the spatial and temporal pattern of cell division in the neural tube. The size and internal structure of the spinal cord segments were related to the specializations of the postcranial body. The cervical and lumbar enlargements reflected the accumulation of a greater number of neurons at these levels and the number of neurons within a cord segment varied according to the size and character of the part of the body it served.

2.2 MORPHOMETRY

2.2.1 Body parameters

The CRL (Crown Rump Length) and bodyweight showed a positive correlation with increase of foetal age, with a rapid rate of increase in last three months of gestation in ox (Roberts, 1976). Taluja and Shrivastava (1982) reported the mean CRL of goat foetuses as 9.260 ± 0.184 cm, 14.520 ± 0.539 cm and 26.320 ± 1.084 cm during first, second and third trimesters of pregnancy respectively. The CRL and bodyweight showed a positive correlation with increase of foetal age in Black Bengal goat also (Majumder *et al.*, 1986). Osuagwuh and Aire (1986) compared foetal weight, CRL, height at withers and external changes in West African dwarf goats and opined that the foetal weight and height at withers were more reliable indices of age than CRL. Seventy nine per cent of the growth in foetal body weight occurred in the last trimester and this exceeded the relative growth of all other characters during the period (Mc Donald *et al,*. 1988). In Australian foetal goat, there was little difference between the relative growth of crown-rump-straight and curved length, vertebral column length and the thoracic circumference over trimesters of gestation (Mc Donald *et al.,* 1988). Growth rate is more during late stages of gestation in buffaloes also (Jeetendra *et al.* (1995). Sivachelvan *et al.* (1996) found that at different stages of foetal growth, sheep foetuses were larger in body size i.e., had greater CRL and body weight, than goat foetuses, but both the species maintained similar and proportionate growth relationships. In sheep foetuses, the mean CRL was 6.000 ± 0.900, 10.100 ± 1.300, 35.000 ± 0.090 and 46.000 ± 1.200 cm respectively at 30 to 60 days, 61 to 90 days, 91 to120 days and 120 to term (Mufti *et al.*, 2000).

2.2.2 Vertebral Column

Sisson and Grossman (1953) reported that the greatest cross-sectional area of the vertebral canal at the level of atlas. According to Rao and Tewari (1974), in ox and buffalo apart from the atlas, the vertebral canal had the greatest cross-sectional area at the junction of C7 and T1 and at L6 vertebrae. They opined that the observations on the area of vertebral canal at different levels pointed out that the cross-sectional area correspondingly increased to accommodate the enlarged segments. They also opined that the thoracic region formed the longest region of the vertebral column in domestic animals. The cervical region contributed more significantly to the length of precoccygeal vertebral column and total length of vertebral column than lumbar region in horse, cow, bull, ox, and buffalo, but the reverse was true in cow, dog and man. In goat foetuses, the longest vertebra was C2 and the shortest in the cervical region was C7 as reported by Taluja and Shrivastava (1989). They also reported that T11, T12 and T13 were the longest vertebrae in the thoracic region, the lumbar region presented little variation between length of its component vertebrae and in the sacral region S1 was the longest and S4 was the shortest. According to them maximum vertebral width was recorded at C1 vertebra.

2.2.3 Morphometry of Spinal Cord

2.2.3.1 Spinal Cord Weight

Latimer (1938) noticed a direct relationship between the spinal cord weight and body weight in cat. The adult weight of the spinal cord was 270 g in horse, 240-250 g in ox, 42 g in pig, 30-150 g in dog and 26-29 g in fowl (Sisson and Grossman, 1953). According to Arey (1957) the central nervous system was relatively large throughout the foetal period. At birth the brain contributed 10 per cent of the body weight and in adults it was two per cent. The spinal cord overgrew the brain during the postnatal years, increasing from 0.9 per cent of the brain weight to two per cent. In mice, a linear relationship of growth of spinal cord was demonstrated by Blinderman and Brown (1966), by plotting the growth curves of various divisions of the CNS as percentage of dry weight against bodyweight at different periods of maturation, indicating a uniform growth with respect to each other, of the CNS components except cerebellum.

In the cerebellum, an early rapid increase of percent solids occurred as the animal matured. In buffalo, the cord weight did not bear any relationship to other body parameters (Sharma and Rao, 1971b). Richardson and Hebert (1978) observed a linear relationship between the cube root of organ weight and gestational age in ovine foetuses between 50 and 140 days of age and this linear relationship became sigmoid for the weights of the brain, cerebellum and spinal cord. Taluja *et al.* (1989) reported that the weight of the spinal cord in foetal goat was 0.226 g, 0.641g and 3.130 g in first, second and third trimesters of gestation respectively with a significant correlation with weight of foetus. The volume of the spinal cord ranged from 0.2 to 0.4 ml, 0.5 to 0.8 ml and 1.0 to 6.5 ml in these three trimesters.

2.2.3.2 Total Length of Spinal Cord

A direct relationship was reported between the cord length and body length in cat (Kreig and Groat, 1944).

The adult length of the spinal cord was 192.5cm in horse, 165-170cm in ox, 61cm in goat and 38 cm in dog (Sisson and Grossman, 1953). The length of the spinal cord was 310 to 370 mm in adult cat (Thomas and Combs, 1962) and 175 to 280 mm in Rhesus monkey, 180 to 227mm in Irus monkey and 311 to 334 mm in Baboon (Thomas Combs, 1965).

Sharma and Rao (1971b) also noticed a direct relationship between the cord length and body length in cat in buffalo. A highly significant positive correlation was observed between CRL and length of the spinal cord in all stages of gestation in foetal goat by Taluja and Shrivastava (1982).

Taluja and Shrivastava (1982) recorded the mean length of the cord as 6.620 ± 0.093 cm, 11.330 ± 0.547 cm and 17.800 ± 0.602 cm in first, second and third trimesters of pregnancy respectively, in foetal goat. According to Ghazi and Gholami (1993) the total length of the cord in sheep was 177 ± 18.30 mm in third month, 238 ± 16.15 mm in fourth month and 326 ± 23.00 mm in fifth month of gestation, 470 ± 34.80 mm in new born lambs and 834 ± 47.10 mm in adult sheep.

Greenaway *et al.* (2001) found that in rabbits the age, weight, sex and method of preparation did not show any correlation with the length of the spinal cord.

2.2.3.3 Regional Length

Lassek and Rasmussen (1938) repored that on the basis of regional distribution of spinal nerves, the spinal cord could be divided into cervical, thoracic, lumbar, sacral and coccygeal regions and in man, the thoracic region was the longest followed by the cervical, lumbar, sacral and coccygeal regions. Sisson and Grossman (1953) recorded the regional cord length as 41 cm in cervical region, 72 cm in thoracic region, 32 cm in lumbar region and 7 cm in sacral region in ox; 65 cm, 86 cm, 27 cm and 15cm respectively in horse and 11 cm, 17.40 cm, 7 cm and 2.6 cm respectively in dog, all showing the maximum regional length in the thoracic region. According to Malinska *et al.* (1972) the thoracic region was the longest, but the coccygeal region was longer than the sacral region in the Central European hedgehog. Taluja and Shrivastava (1982) reported that in foetal goat also the maximum regional cord length was observed in the thoracic region and the regional thoracic cord length expressed as percentage of the total length of the cord declined from 38.51 per cent in the first trimester to 36.96 per cent in the third trimester. They also observed a highly significant positive correlation between total cord length and length of the thoracic region in all age groups. As reported by Ghazi and Gholami (1993), the thoracic region was the longest in all the age groups in sheep also, followed by the cervical, lumbar, sacral and coccygeal regions.

2.2.3.4 Enlargements of Spinal Cord

There were two enlargements in the spinal cord: the cervical enlargement or intumscentia cervicalis and lumbar or lumbosacral enlargement or intumscentia lumbalis (Arey, 1957).

2.2.3.4.1 Cervical Enlargement

The cervical enlargement corresponded to the innervation of the forelimb and brachial plexus (Kappers *et al.*, 1967). The cervical enlargement was at the level of C5 to T1 in buffalo (Sharma and Rao, 1971b), C6 to T1 in goat (Sharma *et al.*, 1973), C4 to T1 in rabbit (Kozma *et al.*, 1974), C5 to T1 in dog and C6 to T2 in ox, horse and pig (Dellmann and Mc Clure, 1975). In goat foetuses, the contribution of cervical enlargement expressed as percentage of total cord length increased from 11.48 per cent in first trimester to 13.70 per cent in the last trimester of gestation (Taluja and

Shrivastava, 1982). The extend of cervical enlargement was at C6 to T1 in elephant (Mariappa, 1985) and C7 to T2 in camel (Gholami *et al.*, 1998).

2.2.3.4.2 Lumbar Enlargement

The lumbar enlargement corresponded to the innervation of the pelvic limb and lumbosacral plexus and was smaller than the cervical enlargement. The smaller size of the lumbar enlargement was due to the greater amount of white matter present at the cervical level because all ascending and descending pathways to both the limbs were present at the cervical level whereas only those related to hind limbs were present at the lumbosacral level (Kappers *et al.*1967). The lumbar enlargement extended from L3 to S2 in buffalo (Sharma and Rao, 1971b), L4 to S3 in rabbit (Kozma *et al.*, 1974), L4 to S1 in pig, L3 to S1 in dog and L4 to S2 or S3 in goat, ox and horse (Dellmann and Mc Clure, 1975). In goat foetuses, the length of lumbar enlargement showed an upward trend from first to last trimester and with the contribution of lumbar enlargement expressed as percentage of total length of the cord increasing from 12.68 per cent in 1st to 15.56 per cent in the last trimester (Taluja and Shrivastava, 1982). Mariappa (1985) reported that in elephant foetus lumbar enlargement was larger than cervical enlargement and it extended from L1 to L4 segment. The lumbar enlargement extended from L5 to S2 segment in camel (Gholami *et al.*, 1998).

2.2.3.5 Spinal Cord Segments

Barry (1956) divided the spinal cord segments in man into four regions based on changes in angulation, but these regions differed from those described for the domestic animals.

Dellmann and Mc Clure (1975) opined that in sheep and goat, the displacement of spinal cord segments in the cervical, thoracic and cranial lumbar regions was the same as in cattle. i.e., the cervical segments were displaced cranially and all the thoracic and first two lumbar segments were displaced caudally. In cattle, L3 was located at the corresponding vertebral level but rest of the segments was displaced cranially. In sheep and goat, the cranial displacement was not so marked as in cattle.

As reported by Taluja and Shrivastava (1982) in foetal goats, there was no clear demarcation between segments of the spinal cord except for the interval between root fibres of the adjacent nerves. Each spinal cord segment was numbered as per the attachment of the paired spinal nerves and the cord consisted of as many segments, as there were pairs of spinal nerves.

A spinal cord segment consisted of a portion of the spinal cord proper and all of its rootlets that joined to form the associated pair of spinal nerves. The number of rootlets that formed a spinal nerve and the length of the cord segments varied from one level of the cord to the next (Clark, 1984).

According to Ulinski (1997) the distinct differences in the number of spinal cord segments between species reflected individual morphology and suited the functional requirements of the animal. The size and internal structure of the spinal cord segments were related to the specialization of the postcranial body.

2.2.3.5.1 Segment Length

In horse, the longest spinal cord segments lay between C3 and C6 (Sisson and Grossman, 1953). Thomas and Combs (1962), confirmed the presence of these four regions in cat, with longer segments in the first and third regions and shorter segments in the second and fourth regions. They opined that in cat, the lower thoracic and upper lumbar were among the longest segments of the cord and the shortest segments were at C1, C6 to T3 and the segments caudal to L3. The length of the spinal cord segments were directly related to the root attachment and inter rootlength in cat. Thomas and Combs (1965) reported that a similar condition was found in monkey and the segment length in the Baboon were similar to those of common domestic animals, whereas Rhesus and Iris monkeys had less variation in segment length, particularly in the cervical region. Fletcher and Kitchell (1966) observed a similar condition as seen in the cat existed in dog also. They also observed that in dogs, the spinal cord–vertebral segment relationships varied depending on the size, age, breed and sex and the spinal cord exhibited shorter segments in the above mentioned second and fourth regions as in the cat.

In buffalo, the segments between C2 and C4 were the longest and the length of the spinal cord segments were directly related to the root attachment and inter root length (Sharma and Rao, 1971b).

In goat, like buffalo, C2 through C4 were the longest segments of the cord, while C3 was the longest among them (Sharma *et al.,* 1973). In calves, the longest was C3 and the shortest was C8 segment (Hussain *et al.*, 1990). Rao (1990a) reported that in sheep the longest cord segments were at C2, T13, L3 and S1 levels and the shortest were at C8, T1, L6 and S4 levels. But Ghazi and Gholami (1993) reported that C1 was the longest segment in sheep of all ages, except new- born lambs where C3 was the longest. According to them, in five- month- old foetus T2 segment was smallest in thoracic region. The length of the spinal cord segments was directly related to the root attachment and inter root length in camel also (Gholami *et al.*, 1998). In calves, C2 was the longest segment, which formed 4.04 per cent of the total cord length (Taluja *et al.,* 1999).

2.2.3.5.2 Segment Width and Height

According to Dellmann and Mc Clure (1975) in horse, the diameter of the spinal cord was the greatest in the cervical and lumbar enlargements, being approximately 20-24 mm in adult horse. In domestic animals, they found that, the cross-section of the C1 segment was flattened dorsoventrally in the cranial end and almost circular in the caudal end. C3 segment was flattened dorsoventrally and the flattening increased in the cervical enlargement where the cord was twice wider than being high. Thoracic segments were circular in cross-section, except two or three caudal ones, which were dorsoventrally flattened upto L2, and from L3 onwards the cord became gradually circular. In dogs, the spinal cord had a mean transverse diameter of 0.826 mm between 24 and 28 days of gestation (Engel and Draper, 1982a) and 1.400 mm between 41 and 44 days of gestation (Engel and Draper, 1982b). The maximum segment width and height was at C6 and C7 in first trimester and at C7 and C8 in second and third trimester in goat foetuses (Taluja *et al.*, 1983). Morgan *et al.* (1987) found that the location of maximal spinal cord width in the lumbosacral region was different between breeds, with a more caudal termination of the cord in Dachshunts compared to the German Shepherd dogs. In this region, the maximal spinal cord width and height in the latter breed were at the central portion of L4

segment (which decreased rapidly caudal to this level), whereas in the former breed, it was at the caudal portion of L4 segment. In calves, the maximum dorsoventral and transverse diameters and circumference of spinal cord were observed at the cervical enlargement (Hussain *et al.*, 1990). The greatest diameter and cross-sectional area were confined to the last cervical, first two thoracic, last lumbar and first sacral segments in sheep as per reports of Rao (1990a). In dogs, Fourie and Kirberger (1999) noticed that small breeds had a higher cervical cord to canal ratio than large breeds. In calves, the minimum dorsoventral and transverse diameters and circumference of spinal cord were observed at the coccygeal part (Parmar *et al.*, 2000).

2.2.3.6 Allometric Growth of Spinal Cord and Vertebral Column

Kappers *et al.* (1967) found that unlike the spinal cord of birds and reptiles, the mammalian spinal cord seldom extends through the entire length of the vertebral canal. But in early embryonic life, the cord occupies the whole extent of the canal. Later, due to unequal growth of the skeleton and the spinal cord, there is a lack of correspondence between the length of vertebral canal and of the spinal cord so that in carnivores the conus reached upto the end of lumbar column, while in ungulates it extended even to middle of sacral region.

In Albino mouse, Sakla (1969) found that the cervical region was the most rapidly lengthening region of the cord, followed by the sacral and lumbar region. Harrison (1978) reported that in man by the second month of foetal life, the spinal cord was well formed and had spinal nerves attached to it and extended the entire length of vertebral canal. After third month, the vertebral column grew at a faster rate than the spinal cord and at the time of birth the cord extended to the caudal border of second lumbar vertebra.

Because of the disparity in length between the spinal cord and vertebral column, the nerves from the lumbar and sacral segments had a long distance to travel before reaching their respective foramina of exit. The cervical and cranial thoracic rootlets were situated nearly at right angles to the cord, but caudal thoracic, lumbar and sacral rootlets entered and left the cord at more oblique angles. This discrepancy was so pronounced in the caudal third of the vertebral canal that the rootlets ran parallel to one another in a cranial to caudal direction, thus forming the cauda equina in mammals (Jenkins, 1978).

In the late teens, the spinal cord attained its adult position and terminated at the level of the intervertebral disc between the first and second lumbar vertebrae in man (Clark, 1984). In sheep (Ghazi and Gholami, 1994) and camel (Ghazi *et al.* 1998a), the growth of the vertebral column was greater than that of the spinal cord during postnatal life and the difference in growth was not constant throughout the cord. In the thoracic region, the growth rate of the spinal cord in relation to the vertebral column was positively allometric, in the cervical region it was isometric and in the caudal part of the spinal cord there was a decline in growth compared to the respective regions of the vertebral column. Craniocaudally, the growth rate became negatively allometric.

2.2.3.7 Correspondence between Spinal Cord Segments and Vertebrae

In domestic animals, Hopkins (1935) distinguished four regions in terms of segment- vertebra relationship: cranial cervical region, caudal cervical to cranial thoracic region, caudal thoracic to cranial lumbar region and the remainder of the cord. In the first and third regions the segments lay within the corresponding vertebrae and in second and fourth regions segments lay cranial to the corresponding vertebrae. The fact that spinal cord was not co extensive with the entire vertebral canal, and spinal cord segments did not lie within the boundaries of their corresponding vertebrae seem to be recognizable features of many mammals. All the cord segments did not correspond to the transverse and vertical planes of the corresponding vertebrae in cat (Kreig and Groat, 1944). In domestic animals, the vertebral column overgrew the cord, so that beyond the most cranial cervical region, the cord segments lay cranial to their corresponding vertebrae and the nerve roots made progressively more acute caudal angles to the cord as they proceeded to their appropriate exits (Sisson and Grossman, 1953).

In man, Barry (1956) observed that the cranial and middle thoracic vertebrae showed an increase in length over the spinal cord segments by the eighth week (36mm) of development while the lumbosacral region did not exhibit this differential growth in length until the tenth week. Upto C4 segment, the caudal slant of spinal nerves was less marked as age advanced. From C5 to T4 this trend was reversed and the spinal nerves exhibited an increasing tail ward slope. From T5 to T10 spinal nerves showed no change in angulation. Caudal to T10, spinal nerves lag behind

others in development so that in specimens younger than 11 weeks (72 mm) the caudal slope was less as coccygeal segments were reached. In older specimens, the tail ward slope caudal to T10 segment was greater because of the greater rate of elongation of vertebral column than spinal cord with the resultant formaion of the filum terminale and cauda equina.

Mc Leod (1958) stated that in ox, all regions of the spinal cord corresponded to the regions of the vertebral column except in the lumbosacral region where the lumbar region of the cord ended at the junction of L5 and L6 vertebrae. Fletcher and Kitchell (1966) found that all the cord segments did not correspond to the corresponding vertebrae in dog also and the only spinal cord segments found to lie entirely within their numerically corresponding vertebrae were C1, last two thoracic and the first two or three lumbar segments. Sharma and Rao (1971b) noticed that in buffalo also, all the cord segments did not correspond to the vertebrae and of all the spinal cord segments, only C6, last three thoracic and L1 segments were located entirely within their corresponding vertebral limits. Here the cervical portion of the cord extended from the rostral limit of C1 to the mid level of C7 vertebra, followed by the thoracic region, which extended upto the junction of T13 and L1 vertebrae and the lumbosacral portion lay between L1 and S1 vertebrae. According to Mc Clure *et al.* (1973) in cat, the cervical segments were distributed within seven cervical vertebrae but caudally, from L5, the segments were located more cranially than the corresponding vertebrae. Dellmann and Mc Clure (1975) also distinguished four regions in terms of segment-vertebra relationship and found that the same condition as seen in other domestic animals occurred in horses.

2.2.3.8 Spinal Nerves

Each spinal nerve was attached to the cord surface by dorsal and ventral roots with each containing a number of rootlets. The dorsal root presented a dorsal root (spinal) ganglion. The cervical nerve roots were short with the caudal cervical and thoracic nerve roots becoming progressively longer caudally. The dorsal and ventral roots united about 1cm from their origin in cat (Stromsten, 1947). As reported by Bradley (1948) in dog, the dorsal spinal nerve rootlets left the cord along a definite line, but those of the ventral root emerged from a narrow longitudinal area. The two roots pierced the dura separately but close together. Due to the gradual shortening of

the cord these nerve roots passed caudally to enter the corresponding foramen and thus formed cauda equina in the sacral region (Papez, 1967).

In dog, Purinton (1982) reported that there was anastomosis between fibres from one spinal root to adjacent root and emergence of rootlets in a series of small bundles rather than distinct roots. He also reported that occasionally a single spinal root was formed by rootlets from two spinal segments. It divided into two spinal nerves at the level of the dorsal root ganglion and two or more roots were bound together in a common connective tissue sheath in the lumbosacral region.

During the postnatal life in male camel, the angulation of the spinal nerves showed an inverse relation with the length of the dorsal roots (Ghazi *et al.*, 1998 b).

2.2.3.8.1 Root Emergence Length (REL)

Sharma and Rao (1971a) reported that in buffaloes the ventral roots at the point of emergence from the dura mater contained fewer but larger bundles than dorsal roots. The latter, which emerged over greater areas were fan-shaped. Hence, the dorsal root projected caudally than the corresponding ventral root. Similar findings were reported by Sharma *et al.* (1973) in goats. Greatest root emergence length was recorded at C3, T1, L4 and S1 segments in sheep (Rao, 1990a).

2.2.3.8.2 Root Attachment Length (RAL)

Thomas and Combs (1962 and 1965) in cat and monkey, reported a greater ventral root attachment as compared to the dorsal roots with the spinal cord segments contributing to brachial and lumbosacral plexuses having a longer RAL. Similar reports were made by Fletcher and Kitchell (1966) in dogs, who found a greater root attachment length at C3 in this species. Sharma and Rao (1971a) reported the same about the cord segments contributing to brachial and lumbosacral plexuses in buffalo and observed a greater root attachment length at C2 through C3 and a greater ventral root attachment as compared to the dorsal roots. Sharma *et al.* (1973) opined that the RAL contributed significantly to the segment length and found a greater ventral root attachment as compared to the dorsal roots in goats. Taluja *et al.* (1983) recorded the maximum RAL at C7 in all the age groups in goat foetuses. In sheep, RAL was greater in cranial cervical (C3), mid thoracic (T7), caudal lumbar (L5) and cranial sacral (S1) segments (Rao, 1990a). The segments contributing to brachial and

lumbosacral plexuses had longer RAL in camel (Gholami *et al.,* 1998) also. In calves, the maximum RAL was at C5, C7 and C8 segments (Taluja *et al.*, 1999).

2.2.3.8.3 Inter Root Length (IRL)

Inter root length was maximum at C2 segment and minimum at S4 segment in goat foetuses (Taluja *et al.*, 1983). It was maximum at C3, T13, L4 and S1 levels in sheep (Rao, 1990a), but was maximum at C2 and minimum at Cy5 in calves (Taluja *et al.* 1999). Parmar *et al.* (2000) observed the maximum value at the same level but the minimum IRL at S4 segment in calves in the precoccygeal cord.

2.2.3.8.4 Dorsal Root Ganglia

Purinton (1982) reported that in dog, there was confluence of dorsal root ganglia in the lumbosacral region. According to Taluja and Shrivastava (1988a) the shape of the dorsal root ganglia was ovoid, ellipsoid, or spindle shaped in goat fetuses. The size varied at different ages and in different regions of the vertebral column and resembled that of a mustard or a wheat seed. The maximum mean long diameter of dorsal root ganglia was at the cervical enlargement in last two trimesters of gestation. The minimum mean long diameter was recorded in thoracic ganglia at all age groups. The greatest mean short diameter of the dorsal root ganglia was at the lumbar enlargements in first two trimesters and at the cervical enlargement in the last trimester. The least mean short diameter was noted in the thoracic ganglia.

2.2.3.9 Level of Termination

The level of cord termination varied among species (and in early stage with age) and was recorded at S3 vertebra in calf (Habel, 1953), S2 in cattle (Mc Leod, 1958), L3 in fullterm human foetus and between L1 and L2 in adult man (Crosby *et al.*, 1962), between L4 and L6 in monkey (Thomas and Combs, 1965). Sharma and Rao (1971b) observed that in buffalo the cord ended at third sacral and the level of termination did not vary from first day to 16 years of age suggesting that the spinal cord attained its definite level of termination even at birth. In goat, Sharma *et al.* (1973) reported the termination of spinal cord at S3 but according to Dellmann and Mc Clure (1975) it was at S2 vertebra.

The caudal extremity of the spinal cord tapered as the 'conus medullaris' caudal to the lumbar region. From the conus a slender non-nervous filament of pia

mater, the 'filum terminale', extended caudally in the dural sac in domestic animals (Dellmann and Mc Clure, 1975). Breazile (1976) reported that during early foetal life the spinal cord developed at the same rate as the vertebral column and extended the full length of the vertebral canal. Afterwards, vertebral column elongated at a faster rate than the spinal cord and as a result the spinal cord was pulled rostrally within the vertebral canal. Chandna and Tyagi (1981) also reported the level of cord termination at S2 in adult goat same as in the buffalo (Dhingra *et al.*, 1976). But, in prenatal stages it varied from the caudal end of S4 vertebra to rostral end of Cy1 in the first trimester, caudal end of S3 to rostral end of S4 in the second trimester and caudal end of S2 to rostral end of S3 in the third trimester in goat foetuses (Taluja and Shrivastava, 1984).

The level of termination was recorded at S1 vertebra in sheep (King, 1987), between L7 and S2 vertebrae in cat (Kot *et al.*, 1994), within L5 or L6 vertebrae in pig, L6 or L7 vertebrae in dog, S2 vertebra in horse (Dyce *et al.*, 1996) and yak (Kulbhushan *et al.*, 2000). It varies between S1 and S3 vertebrae in rabbit (Greenaway *et al.*, 2001).

2.3 HISTOLOGY

2.3.1 Development of spinal cord

According to Waterman (1976) the major events in the morphogenesis of the central nervous system (brain and spinal cord) in all vertebrate species except certain fishes include: thickening of the dorsal ectoderm into a neural plate, elevation of the margins of the neural plate as neural folds on either side of a neural groove and fusion of the neural folds to form a neural tube.

The entire central nervous system except one type of glial cell, is formed from ectoderm in mammals. It is the first body system to begin differentiation and the last to complete the functional anatomic development. The progressive developmental stages of ectodermal proliferation and differentiation for both brain and spinal cord in sequence are: neural plate, neural groove and neural tube. The spinal cord retains the original cylindrical shape whereas the brain region of the neural tube expands and bends in a complex manner (Jenkins, 1978).

2.3.1.1 Neurulation

The process of neurulation, by which the neural tube, the primordium of the central nervous system (CNS) is formed was described by Keith (1947) in man, Patten (1948) in pig, Arey (1957) in man, Kingsley (1962), Rugh (1964), Huettner (1967), Kent (1969) and Wischnitzer (1975) in vertebrates, Langman (1981) in man, Moore *et al.* (1987) in mammals and Ghosh (2002) in animals.

The nervous system made a very early appearance, becoming evident at the embryonic disc stage as the neural plate (medullary plate), which folded into a neural groove at 13 days of gestation in pig embryos (Patten, 1948). Arey (1957) reported that in man this happened at the time of appearance of somites and the neural groove began to close at six-somite stage.

The neural plate overlay notochord and paraxial mesoderm immediately anterior to the primitive streak (Mc Ewen, 1957) as a thickened portion of ectoderm, which surrounded the line of closure of the blastopore (Kingsley, 1962). According to Rugh (1964), the plate appeared by 12 days in pig embryos, folded into a neural groove by 18 days and began to close by 19 days in pig.

According to Hughes and Freeman (1974) the closure of the caudal end of the neural tube occurred after the tail bud formation in animals with normal length tails (rat and sheep) and before tail bud formation in animals with tails that underwent a reduction in length (chick, pig and human baby). Wischnitzer (1975) opined that in pig embryos, the edges of the neural groove elevated to form neural folds, which at three somite stage (14 days) began to close over the neural groove to form a neural tube first at mid–body level where the fusion might be aided by somite development and then anteriorly and posteriorly. The tube was complete except at the anterior and posterior extremities (neuropores) by the 11 somite stage (at 15 days). The anterior neuropore closed first, and marked the position of the future epiphysis. In six mm embryo (at 17 to 18 days) brain developed from the portion of the neural tube anterior to the first pair of somites. In 10 mm pig embryo (at 22 days), the brain merged with the spinal cord.

Geelen and Langman (1977) noticed that in mouse embryos at 9 to 20 somites stage, the closure of the neural tube started in the cervical region, which progressed in the rhombencephalic direction. Sadler (1978) found that in mice, the pattern of initial

contact between opposing neural folds differed. In mid and hindbrain areas, contact was initiated by overlying ectoderm, whereas in the spinal cord region, it was first established by the neuroepithelial cells.

Gasser (1979) found that during the period of somite break up, the neural tube grew dorsally, away from the notochord which lay adjacent to the ventral surface of neural tube and the somite remnant moved laterally and dorsally.

In some animals, the posterior neuropore did not close at all and communicated with the subarachnoid space at the level of cauda equina (De Lahunta, 1983).

The neural plate appeared at the beginning of third week in man and the closure of the anterior neuropore occurred at 25th day (18 to 20 somite stage), followed by closure of the posterior neuropore two days later at 25 somite stage (Langman, 1981). Moore *et al.* (1987) described that the neural plate consisted of a flat plate of cells.

Gilbert (1997) opined that this neurulation process occurred by two ways: in primary neurulation, the chordamesoderm directed the ectoderm overlying it to form a hollow tube. In secondary neurulation, the neural tube arose from a solid cord of cells that sunk into the embryo and subsequently cavitated to form a neural tube. In mammals (e.g. mice and man) the head and trunk underwent primary neurulation and the region posterior to hind limb i.e.tail (at 35 somite level) followed secondary neurulation.

2.3.1.2 Morphogenesis and Histogenesis of Spinal Cord and Spinal Nerves

Due to histological differentiation, in the cellular cytoplasm, a fibrillar meshwork -a myelospongium- had been laid down and the wall of the neural tube became composed of three layers. The inner layer of epithelial cells lining the lumen (neurocoele or neural canal) formed the germinal layer or ventricular zone or the ependymal layer composed of germinal cells or medulloblasts by the fourth week of development in man (Keith, 1947).

As reported by Patten (1948) in pig, the original cells proliferated very rapidly during the neural groove stage and immediately after closure of the tube. Later, these cells lost their original clear-cut outlines and merged into a syncytium. The

ependymal cells, which lined the lumen, underwent mitosis in pig embryos of 7 mm size by 18 days and a middle mantle layer and an outer marginal layer got differentiated in 9 mm embryos by 19 days.

In vertebrates, Shumway and Adamstone (1954) found that the neural tube originated from the neural plate, which developed from cells, which could be identified in the blastula stage. Arey (1957) found that in man, these three layers could be identified in the wall of the spinal portion of the neural tube by the fourth week of gestation. The marginal layer was a fibrous mesh and lacked cells in the early months of gestation and towards the lumen, an internal limiting membrane bound the neural tube and peripherally an external limiting membrane marked its extent at sixth week.

Buchanan (1957) opined that the primitive neural tube consisted of a rostral encephalon and a caudal myelon, which developed into brain and spinal cord respectively. Kingsley (1962) noticed that in vertebrates, the neural plate was broader in front, which resulted in a large anterior portion of the tube, the brain, while the rest of the tube gave rise to spinal cord.

Rugh (1964) observed that the three layers of the neural tube were laid down by 22 days in pig and the transition from a single-layered neural groove into a multilayered spinal cord occurred with relatively great speed when compared to the differentiation of brain and other organs. The internal and external limiting membranes were formed at 22 days (10 mm embryo).

Jenkins (1978) noticed that in the adult mammals, these cells retained their embryonic shape, ciliated surface and also their position lining the central canal as the ependymal cells. By proliferation and differentiation, these cells gave rise to a thick middle mantle layer, which later developed into nerve cells and neuroglia and formed the future gray matter. The axonal processes of the nerve cells in the mantle layer extended outward and formed the outermost, marginal layer, which formed the future white matter.

Engel and Draper (1982a) observed that in dog, the cells from the ependymal layer migrated by 24 to 28 days to form the mantle layer. By 24 days, a thin marginal layer was differentiated and by 28 days it became thicker. Engel and Draper (1982b) found that by 41 to 44 days of gestation in dogs, the ventral median fissure was

distinct and reached the oval central canal and a less developed dorsal median septum was also present.

According to Jacobson and Tam (1982) there was considerable increase in volume of the neural plate during closure, and cell proliferation and enlargement of daughter cells seemed to account for this growth.

De Lahunta (1983) observed that the neural tube was the primordium for the brain and spinal cord, which in early open neural tube stage was composed of a single layer of neuroepithelial or neuroectodermal cells forming a pseudostratified columnar epithelium. The cell membrane of each cell connected to both sides of the neural tube but nuclei were at different levels depending on the stage of mitosis. At interphase, the nuclei were located on the external surface of the tube and during mitosis it migrated to the luminal surface.

As per the reports of Moore *et al.* (1987) neurulation was accompanied by changes in the shapes of the cells of the neuroepithelium. As the neural plate was formed, the cells elongated, resulting in the thickening of the neuroepithelium. Later, as the neural folds elevated, the cells became tapered at their apices.

2.3.1.2.1 Gray Matter

Keith (1947) found that at the beginning of fifth week in man, the lateral walls of the embryonic spinal cord were thick and packed with proliferating cells but, the dorsal and ventral margins were thin and formed the roof plate and floor plate respectively. Roof and floor plates did not contain neuroblasts and served as pathways for nerve fibres crossing from one side to the other. At this stage, the central canal was coffin-shaped or diamond-shaped. Later a lateral groove, the sulcus limitans, appeared at the midpoint of each lateral wall of the lumen of the neural tube and divided the mantle layer horizontally into a dorsal alar plate and a ventral basal plate. The alar plate formed the dorsal sensory horn of the spinal cord and the basal plate formed its ventral motor horn. During 11^{th} week of development in man, the mantle zone was differentiated into dorsal and ventral horns and by 13^{th} week the adult form was reached. By third month the nerve cells took up their permanent stations in the gray horns.

According to Arey (1957) in embryos of 10 to 15mm in man the mantle layer thickened on each side ventrolaterally to form the ventral horn, which in later stages supplied migrant cells that organized also into an intermediolateral horn and foetuses in the fourth month of gestation had their gray substance arranged in the permanent form with the formation of enlargements. He also reported that in pig, the enlargements were well developed in an 18 mm embryo. In 35 mm embryo of pig, since the body of the embryo elongated faster than the spinal cord, the spinal nerves at first were directed at right angles and coursed obliquely in the lumbosacral region.

Buchanan (1957) reported that the relationship between the gray and white matter in the adult spinal cord corresponded to that of the mantle and marginal layers in the developing neural tube.

The lateral walls of the embryonic spinal cord started proliferation and became thick in an 18mm embryo at 26 days in pig (Rugh, 1964; Huettner, 1967).

As reported in mammals by Jenkins (1978), the ventral horn differentiated before the dorsal horn. In each lateral plate the neuroblasts became grouped from ventral to dorsal horn into: somatic motor, splanchnic motor (both in the basal lamina), splanchnic sensory and somatic sensory (both in the alar lamina).

According to De Lahunta (1983) the vertebrate spinal cord provided the best example of the symmetric development of the neural tube by layers.

Ghosh (2002) observed that the differentiation of structures in the spinal cord proceeded from before backwards in domestic animals. The transformation of this embryonic organization into that of the mature spinal cord in domestic animals resulted from massive proliferation, asymmetric movement of immature neurons and development of neuronal processes. Thus, mantle layer was shaped like a butterfly with prominent dorsal and ventral horns, which was uniform along the entire length of the cord. As limbs developed, the cervical and lumbosacral enlargements were formed at the corresponding levels of the cord. Also in the thoracic level, immature neurons migrated out of ventral horn to form the intermediate horn and these sequence of events were almost the same in all domestic animals.

2.3.1.2.2 White Matter

Keith (1947) reported that early in the second month of gestation in man, the fibres of the dorsal root entered the marginal zone of the dorsal side of the cord and formed the rudiment of the dorsal funiculi. The dorsal funiculi had their maximum size in man, next being in the anthropoid apes. In third month, most of the ascending and descending tracts in the ventrolateral marginal zone were formed. The formation of the pyramidal tracts started only by fifth month of gestation. The myelination in man commenced at fourth month and was not finished until about 18 months after birth. The oldest tracts, viz. the ones which were first required to carry messages, were the earliest to be myelinated. The ventral roots got myelinated first, followed by the dorsal roots. By eighth month, all the tracts were myelinated with the ascending tracts preceding the descending ones. The pyramidal tracts were the last to be ensheathed ie., at 18^{th} month following birth.

Arey (1957) detected that the myelin first appeared in the spinal cord at the middle of the foetal life in man. According to him, the myelination started first in the cervical spinal cord and then extended progressively to lower levels. Fibres of the ventral roots acquired myelin before those of the dorsal roots. Kostyra (1958) distinguished four phases of myelination in cattle and opined that the myelination started in the 17^{th} week of pregnancy in cattle.

According to Clark (1965) in man, the earliest tracts in the marginal zone differentiated as intersegmental tracts by the second month. He also reported that before any fibres at all had acquired a medullary sheath, the myelin substance was already present in nervous tissues. In the brain, it could be detected in the cytoplasm of neuroglial elements, while along the peripheral nerves it was found in the Schwann cells and in the connective cells of the perineurium. The process of myelination seemed to show considerable variation in different mammalian species. In newborn rat, there was relative absence of myelinated fibres. In sheep, the degree of myelinization was more advanced at birth than in many other mammals accounting for its functional maturity. However, the order of myelinization of the various elements of the nervous system in the sheep foetus agreed in general with that of other mammals.

Majstruck (1967) found that in pig also, deposition of the lipids in the sheaths started in the cervical neuromeres, before in the thoracic, lumbar and sacral neuromeres. In each neuromere, lipid sheaths formed first in the ventral funiculi, then in the lateral and dorsal funiculi and finally in the Lissauer's terminal zone and dorsal spinocerebellar tract. From the 66th day of foetal life (active myelination) the lipid content, mainly of cerebrosides, increased in the thickening myelin sheaths.

According to Kent (1969), many of the nerve fibres that grew into the marginal layer turned upward or downward in the cord or brain for long distances and synapsed with neurons elsewhere in the CNS.

Jastrzebski (1970) was also able to identify four periods for myelination in cattle. He also found that the fourth period, in which these sheaths reached a thickness equal to the diameter of the axon cylinder, extended upto two months after birth. The first tract to undergo myelination was the pontocerebellar tract (at the beginning of 20th week of foetal life), whereas, the dorsal and ventral spino cerebellar tracts showed myelination at the end of 20th week. The olivocerebellar tract was the last to get myelinated (22nd week of foetal life).

Langman (1981) opined that the tracts in the nervous system became myelinated at about the time they started to function. Okado (1982) observed that in man, the first appearance of myelinated fibres occurred in the lateral portion of the ventral marginal layer of a 66 mm (10week) foetus. Sturrock (1982a) detected that in the developing rabbit spinal cord the phase of rapid gliogenesis, which began prior to the onset of myelination and continued during the period of fast myelination was brought about by division of glial cells already situated in the gray and presumptive white matter and not by the proliferation of a subventricular precursor pool. A quantitative study of vascularization of the prenatal spinal cord in rabbit by Sturrock (1982b) revealed that the onset of myelination coincided with the beginning of a rapid increase in vascularity in both gray and white matter. It was also reported that in the mouse, the percentage vascularity in both gray and white matter remained fairly constant from onset of myelination at 18 days of gestation to five days postpartum, when myelination was fairly well established.

Berthold *et al.* (1983) reported that in cat, the myelination started between 40th and 45th days after mating. Logan *et al.* (1983) found that in rat, the myelination

commenced at eight to nine days postpartum. While studying axon classes and internodal length in the ventral spinal root of L7 segment of adult and developing cats, Nilsson and Berthold (1988) also arrived at the same conclusion that the myelination started between 40th and 45th days after mating.

2.3.1.2.3 Spinal Nerves

Keith (1947) reported that a neuromere was that part of the neural tube and neural crest, which corresponded in position to a primitive body segment. In a human embryo with 18 somites, 11 segments were noted in the spinal cord. From the cells of a neuromere, dorsal and ventral roots of a spinal nerve originated. The cervical and lumbar enlargements appeared in the fourth month of gestation. The fibres from the cells in the ventral horn began to emerge as the ventral root in the latter part of the fourth week. The processes from the ganglion cells of the dorsal root entered the marginal zone of the alar plate a week later.

Rugh (1964) studied that in 10mm pig embryos at 22 days, the ventral motor root was formed from ventral neural ectoderm and joined with peripheral process of dorsal root ganglionic cells to form the spinal nerve.

Langman (1981) found that the axons of the basal plate neurons passed through the marginal zone at the ventral part of the spinal cord and formed the ventral root of spinal nerve. The axons of the alar plate neurons penetrated into the marginal layer where they descended or ascended to a higher or a lower level to form association neurons.

2.3.1.2.4 Central Canal

Keith (1947) reported that in the seventh week of development in man, the central canal still retained its diamond-shape, a shallow dorsal median sulcus developed in the dorsal midline and the basal plates grew ventrally creating a deep ventral median fissure. In the ninth week, the dorsal part of the central canal was obliterated by the apposition of the lateral plates; the ependymal zone reduced, the dorsal funiculi and dorsal median septum were formed. Ghosh (2002) found that gradually, the diamond shaped canal became triangular in outline during development in animals.

2.3.1.2.5 Termination of Spinal Cord

The caudal part of the cord was the last part of the neural tube to be formed. In early embryonic life, the cord occupied the whole extend of the vertebral canal. In man, the caudal region, where the tail was atrophied, became secondarily dedifferentiated and at ninth week of gestation, the caudal segment started regression, the last three coccygeal ganglia disappeared and the cephalic part of the dedifferentiated area remained as the walls of the terminal ventricle (ventriculus terminalis). By 12^{th} week, the caudal part of the cord became thin and differentiated into a distal or extra dural part, which was drawn out to form the coccygeal thread, while the intradural part formed a fibrous strand, the filum terminale (Keith, 1947) at the caudal end of which, a small vesicle (the coccygeal medullary vestige) persisted until late in embryonic period as in mammals (Kappers *et al.,* 1967).

2.3.1.2.6 Differentiation of Cells

Buchanan (1957) reported that the division of the germinal cells gave rise to neuroblasts, medulloblasts and spongioblasts in man. Neuroblasts became neurons. Medulloblasts were indifferent cells, which differentiated further into neuroblasts or spongioblasts. The spongioblasts developed into astroblasts and oligodendroblasts, which in turn became adult astrocytes and oligodendrocytes.

Neuroblasts developed large nuclei while the nuclei of the spongioblasts remained small (Rugh, 1964). Hannah and Nathaniel (1975) reported that in rat, the neuroblasts at birth could be distinguished from other cellular elements by a round or ovoid nucleus with the homogenous nuclear chromatin lacking peripheral condensation (as seen in glial cells) and by paler cytoplasm.

According to Langman (1981) after neural tube closure, the neuroepithelial cells gave rise to neuroblasts (primitive nerve cells) characterized by a large round nucleus with pale nucleoplasm and dark staining nucleolus.

According to Ghosh (2002) in animals at the beginning of development, the neural plate was composed of undifferentiated proliferative epithelium. Later, its daughter cells entered into two lines of specialization: one towards the nerve cells and the other towards neuroglial cells, the latter forming the supporting tissue of the nervous system. The embryonic nerve cell was a neuroblast passing through a bipolar

stage before reaching the multipolar stage. The spongioblast was the forerunner of neuroglia (except microglia). Some migratory spongioblasts also formed neuroblasts.

2.3.1.2.6.1 Neuroblasts

Arey (1957) found that in man, mitosis among neuroblasts ceased during the first year of postnatal life. There after the nervous system matured and enlarged, but the ability to produce new neurons was forever lost. After cessation of cell division further increase in size of the spinal cord and brain was due to the thickening of the myelin sheaths, some of which were present at birth but were thin.

According to Montagna (1960) the number of neurons in an individual species was relatively constant and did not increase after late embryonic life. In pigs, Rugh (1964) noticed that the neurons appeared first at about 14 days from the neural ectoderm as neuroblasts that later formed 'proneurons' and finally permanent neurons. These cells developed throughout the body continuously from the neurula stage until two or three weeks after birth. Harrison (1978) found that the region of the formation of these neuroblasts was mainly the ventral aspect of the neural tube, which bulged on either side so as to change the shape of the tube. According to Jenkins (1978) in mammals, the time at which a neuron had undergone its last division was called its birth date. The neurogenic cells in the ventral part of the spinal cord and hindbrain were the first to stop dividing with birth dates of dorsal and intermediate neurons following.

Sims and Vaughn (1979) found that in cervical spinal cord of mouse, the ventral motor neurons were the first neurons generated, which arose by the embryonic day 8.8. Interneurons and dorsal root ganglionic neurons arose by 9.5 days.

Ulinski (1997) found that the shape of the adult brain and spinal cord was determined by the spatial and temporal pattern of cell divisions in the neural tube. The migration of the neuronal precursors or neuroblasts resulted in diffuse distribution of neurons in the wall of the neural tube or in definable aggregations as nuclei or as sheets or layers of cells.

According to Ghosh (2002) the neuroblasts in domestic animals initially had a central process extending to the lumen- a trancient dendrite-which disappeared as they migrated into the mantle layer to form the apolar neuroblasts. Later two new

cytoplasmic processes appeared on opposite sides of the cell body, forming the bipolar neuroplast. Further, either it lost one process and became a unipolar neuroblast or, the process at one end got elongated (the primitive axon) and the other process developed a number of primitive dendrites to form a multipolar neuroblast. The latter with further development became the adult nerve cell or neuron.

2.3.1.2.6.2 Spongioblasts

According to Arey (1957), the spongioblasts (glioblasts or gliablasts), were formed from neuroepithelial cells after the production of neuroblasts had ceased and were originally radially arranged like columnar epithelium. The astrocytes appeared first in the third month and were derived from full-length primitive spongioblasts, from spongioblasts that (because of the thickness of the tube) never connected with the periphery, and from wandering spongioblasts. Those occupying the gray substance were named protoplasmic astrocytes and another type, fibrous astrocytes which developed fibrils within their cytoplasm were seen in the white substance. The oligodendroglia developed solely from migratory spongioblasts at a later period than astrocytes. Buchanan (1957) noticed that the spongioblasts passed into the mantle zone and marginal zone and got differentiated. Rugh (1964) observed that by 10 weeks, neuroglia were abundant in man.

A few 'oligodendrocyte-like cells' first appeared in the 32mm human foetus (Okado, 1980). Langman (1981) found that in the second half of development, a third type of supporting cell, microglia cell appeared in the CNS originating from the mesoderm surrounding the neural tube. When neuroepithelial cells ceased to produce neuroblasts and gliablasts those spongioblasts that retained their primitive shape and bordering relation to the neural canal formed the ependymal cells.

Sturrock (1981) reported that between 12 and 15 days postconception, large phagocytic cells, which were derived from macrophages derived from blood monocytes were present especially in the ventral horn. Astrocytes played only a very minor role in phagocytosis in the developing spinal cord. The ependymoglial cells, carried out some phagocytosis, probably of small pieces of debris.

Sturrock (1982a) found that glial cells were first found in the ventral gray matter at 12^{th} day of gestation, in ventral and lateral white matter at 14^{th} day and in dorsal columns at 20^{th} day. Astrocytes and microglia increased slowly but steadily in

all areas. Oligodendrocytes were first identified at the onset of myelination (at 24th day in ventral and lateral columns and at 26th day in dorsal column).

Sturrock (1983) reported that the most rapid and substantial phase of glial cell production in CNS was that which preceded and accompanied the initiation of myelination. The method of glial cell production differed in different parts of the CNS. In the forebrain, there was a mitotically active layer of glial precursors lying deep to the ependyma of the lateral ventricle, forming the subependymal layer. In the spinal cord, no such layer was present. He also reported that about 80 per cent of the increase in glia was by glial division in the white matter with the remaining 20 per cent occurring by migration from ependymal layer and gray matter.

Lord and Duncan (1987) found that in dogs during the first few days after birth, precursor cells made up 43 per cent of the glial cell population. Most of them developed into oligodendroglia rather than astrocytes.

In rabbit, at light microscopic level the dividing cells could be identified as oligodendrocytes, astrocytes, microglial cells or spongioblasts with the latter cell predominating and the first mitotic cell was found in a 26 days post conception spinal cord (Sturrock, 1987b).

2.3.1.2.6.3 Neural crest cells

Willier *et al.* (1955) observed that the neural crest cells giving rise to ganglia formed two columns, one between the spinal cord and myotomes and the other alongside the aorta in vertebrates.

Arey (1957) found that at regular intervals, agreeing with the position of somites, the proliferating crest cells gave rise to bead-like enlargements, the spinal (dorsal root) ganglia, interconnected for a short time by parts of the crest substance which disappeared later. According to him, the cells of the ganglionic primordia differentiated into ganglion cells and supporting cells comparable to neuroblasts and spongioblasts. Each neuroblastic forerunner of a ganglion cell developed two processes and transformed into a spindle-shaped neuron of bipolar type. Although bipolar at first, ganglion cells became unipolar in a way not surely understood. The supporting cells of the ganglia at first formed a syncytium in the meshes of which the neuroblasts were found and later differentiated into flattened capsule cells, which

invested the ganglion cells and into sheath cells, which migrated peripherally along the developing nerve fibres and enveloped the axons. According to Balinsky (1960), these cells were found as an irregular flattened mass between the neural tube and the overlying epidermis.

Rugh (1964) observed that in vertebrates, during invagination of the neural plate, a group of ectodermal cells, the neural crest cells, appeared along each edge of the neural groove and formed an intermediate zone between tube and surface ectoderm extending from procencephalon to the caudal somites. The neural crests were formed at eight- somite stage (14 ½ days) in pig. He also detected that in pigs, before 10-somite stage (before 15 days) they divide into numerous pairs that gave rise to cranial and dorsal root ganglia. He also reported that in 10 mm pig embryos at 22 days, the neural crests were metamerically distinct as dorsal root ganglia and showed peripheral and central out growths.

Reddy (1972) reported that the neural crests in the bovine embryos appeared in the form of two cords by 21 days in the areas where neurulation was incomplete and as the neural folds fused two cords united in the mid-dorsal line. He also opined that in bovine embryos, neural crest cells migrated to the lateral surface of the neural tube in embryos of 24 days and by 26 days segmented into dorsal root ganglia.

Pannese (1974) observed that the increase in the volume of the dorsal root ganglia occurred at the beginning due to the recruitment of additional migrating cells moving away from the neural crest and to the proliferation of the cells, which have reached their final position in the ganglia. Later the increase in volume of the dorsal root ganglia depended on the gradual and considerable growth of the cell body of the individual elements, which differentiated into neuroblasts and then developed into mature nerve cells. Finally, the increase in number of the satellite cells and development of the interstitial spaces and blood vessels contributed to the further increase in volume of the dorsal root ganglia. The satellite cells of the dorsal root ganglia originated from the neural crest but those of the sympathetic ganglia developed from both neural crest and neural tube. Undifferentiated cells were rounded or polyhedral cells with ovoid nucleus and short expansions and they differentiated along two lines: the ganglionic neurons and satellite cells. Later short expansions disappeared, cytoplasm increased in size, the cell elongated to become

spindle-shaped and two processes grew out from the two opposite poles of the perikaryon to form the bipolar neuroblast or primitive neuroblasts. Later, the cell became bell shaped as the two processes approached each other to form intermediate neuroblast with oval shape and eccentric nuclei. Subsequently the cell became globular or pear-shaped with a single process, the pseudounipolar cell.

As per the reports of Vermeij and Poelman (1980) at 7.3 days postcoitum stage in mouse embryos, the neural plate was not developed, but the neural crest was present between the neuroectoderm and the future surface ectoderm situated just lateral to the notochordal plate. During transformation of the neuroectoderm, via the neural plate and the neural groove, into the neural tube, the neural crest was shifted first laterally and then dorsally and medially and dropped cells. These cells proliferated immediately and differentiated.

According to Langman (1981) these cells on each side migrated to the dorsolateral aspect of the neural tube and gave rise to the dorsal root ganglia of the spinal nerves or sensory ganglia of certain cranial nerves and to sympathetic neuroblasts, Schwann cells, pigment cells, odontoblasts, meninges and cartilage cells of the branchial arches. The centrally growing process penetrated the dorsal portion of the neural tube. In the spinal cord, they either ended in the dorsal horn or ascended through the marginal layer to higher brain centers to form the dorsal sensory root of the spinal nerve. The peripheral process joined the fibres of the ventral root to form the trunk of the spinal nerve.

Engel and Draper (1982a) reported that in dogs, the dorsal root ganglia were well defined by the 24th day of gestation.

2.3.2 Microscopic Structure of the Spinal Cord

The general microscopic structure of spinal cord in animals was described by several workers (Kappers *et al.,* 1967; Papez, 1967; Dellmann and Mc Clure, 1975; Jenkins, 1978; Clark, 1984; King, 1987). They described that the spinal cord proper in mammals consisted of a peripheral white matter surrounding the central gray matter. The white matter consisted of ascending and descending pathways along with axons that made intersegmental connections. The gray matter consisted of neuronal cell bodies, nonmyelinated fibres, blood vessels and neuroglia. In addition, the neuronal

dendrites and axons plus the glial processes composed the background meshwork, the neuropile.

Goller (1962) reported that in cattle, the proportions of gray and white matter in cross-section were relatively constant but formalin fixation produced a shift in the values in favour of the white matter.

Santiago Ramon Y Cajal (1852-1932) was the greatest of all anatomists who described the fundamental structure of the spinal cord and demonstrated neuroglia in detail (Gardner, 1969).

According to Truex and Carpenter (1969), the cervical segments had bigger size, large amounts of white matter and the prominent gray matter particularly in segments of cervical enlargement. The dorsal horn was enlarged and near the base, the reticular nucleus presented a series of projections into the lateral funiculi as the reticular process. The thoracic segments had smaller size, thinner gray columns, prominent lateral horn (due to intermediolateral nucleus) and nucleus dorsalis, a rounded prominence characterized by large vesicular nucleus eccentrically placed with coarse chromophilic material distributed around the periphery of perikaryon. Of the lumbar segments, the segments corresponding to lumbar enlargement were enlarged in area. White matter was increased and gray columns were massive. The sacral segments had small diameter, thick quadrangular gray matter with pronounced substantia gelatinosa, short gray commissure and small amount of white matter.

Pearson and Sauter (1971) reported that the lower sacral and coccygeal regions in the cord of man, rabbit and monkey foetuses were diminished in the size and had smaller sized spinal nerves. According to Jenkins (1978) the size and shape of the spinal cord and gray matter and relative amount of gray and white matter varied at different levels with the variation in the size of the nerve roots. There was an increase in the white matter from lower to higher levels of the cord with the cervical segments containing the largest number of fibres in mammals.

According to Taluja *et al.* (1981) the greatest cross-sectional area of spinal cord and gray matter in foetal goat was at cervical and lumbar enlargements. They reported the lowest of these values in the thoracic region.

In dogs, the mean ratio of gray matter diameter to spinal cord diameter was 0.843 between 24 to 28 days of gestation (Engel and Draper, 1982 a) and 0.714 to 0.788 between 41 and 44 days of gestation (Engel and Draper, 1982 b).

Clark (1984) found that in mammals, the deepest of the several indentations, which extended the length of the spinal cord surface, was the ventral median fissure with an average depth of 3mm in man. It penetrated the ventral surface of the cord to the level of ventral white commissure and had the branches of the ventral spinal artery located in it. There was a shallow dorsal median sulcus, with a dorsal median septum, a thin glial structure extending ventrally to 4 to 6 mm and nearly to the central canal. From cervical to anterior thoracic regions, a dorsal intermediate sulcus overlay the dorsal intermediate septum, a structure that separated the fasciculus gracilis medially and fasciculus cuneatus laterally. Two indistinct sulci, a dorsolateral sulcus at the dorsal root entry zone and a ventrolateral sulcus at the ventral root exit zone, were also present.

In goat foetuses, Taluja *et al.* (1990) also found that the height (total vertical distance) of spinal cord was maximum at C7 and minimum at L2 segments. The width (total transverse distance) was greatest at C8 and L5 levels but least at T7 segment. The transverse distance was greater than the vertical distance at all the levels except in the cervical region in the first trimester of gestation. Distance from gray matter of one side to central canal was more at C7 and L5 and minimum at T7 segments, which might be due to the difference in size of the nerve roots and the relative quantum of gray matter. The height and other vertical distances of the spinal cord revealed 2-3 times increase in later half of gestation in all regions except at L5. Contrary to this, the width and other transverse distances of the spinal cord showed about two times increase over height in the first half of gestation with the exception of T7.

2.3.2.1 The Cells in the Spinal Cord

The nervous tissue parenchyma is composed of two main categories of cells: neurons and their supportive cells, the neuroglia (glial cells or interstitial cells) (Fletcher, 1993).

2.3.2.1.1 Neurons

Lorente de No (1953) described that the neuron consisted of a cell body (soma) and two types of processes, the dendrites and axon. He also estimated that the soma of the perykaryon formed only six per cent of the surface of a neuron and the unstained neuropile of the gray matter formed the remaining 94 per cent in man.

Peterson (1966) confirmed that there was an inverse relation between neuronal size and firing rates and between size and the rate of protein synthesis per square micron of cytoplasm with a direct relation between the rate of firing and that of protein synthesis. There was also an inverse relation between neuronal size and the ratio of the neuronal surface area to volume.

Bowsher (1967) found that in the higher animals, the largest fibres in the CNS were about 20μm and the smallest axons, about 1μm in diameter including axon sheath. The large axons originated from large nerve cells and vice versa. The largest unmyelinated fibres had a total external diameter of about 2 μm and the smallest 0.1μm.

Truex and Carpenter (1969) described the cytoplasm (perikaryon) of the neuron as granular and basophilic due to Nissl bodies, which were scattered throughout the perikaryon and cytoplasm of the dendrites but not in axon. The nervous tissue exhibited argyrophilia.

Haltia (1970) during a study on the postnatal development of the spinal ventral horn neurons in the ventrolateral nuclei of seventh cervical segment in rats, found that in young rats, undernourished from birth until after weaning, the neuronal dry mass and its RNA content were much lower than in normal rats of the same age. The first 15 days of life were critical in this respect.

According to Getty (1975) the axon or axocylinder was the process of a neuron that conducted impulses away from the cell body and was always single where as dendrites conducted impulses towards the cell body and varied in number.

Dhall (1979) noticed a positive linear correlation between the mean diameter of the nucleus and that of the cellbody without much correlation between nuclear volume and total cell volume.

In domestic animals, Banks (1981) stated that the neurons varied in size, shape and number and length of the processes. A neuron or nerve cell consisted of cell body (cyton, soma) and a variable number of cellular processes, dendrites and axon. The cell body of a neuron varied from four to 135 μm in diameter, with a centrally placed, round large vesicular nucleus (three to 18 μm in diameter) and a prominent nucleolus. According to him, most of the neurons in the CNS were multipolar possessing an axon and two or more dendrites. Those with long axons were designated as Golgi type I and those with short and freely branching axons were classified as Golgi type II. The neurons without any axon were called anaxonic neurons like amacrine cells of retina. True unipolar neurons had only one axon and were confined to the developing nervous system. Pseudounipolar neurons had only one process, which got divided into two and were seen in the cranial and dorsal root ganglia. Bipolar neurons were spindle shaped with a dendrite at one end and the axon at the other end were seen in areas of special visceral and somatic sensation.

The neurons had long neurotubules and shorter and smaller neurofibrils in their axon. The neurofilaments were characteristic of nerve cells and neuroglial cells and were formed from controversial protein constituents (King, 1987).

According to Fletcher (1993), in females of some species a sex chromatin body ('Barr body' named after Barr who along with Bertram first described it in 1949) was usually evident in the vicinity of nucleolus (cat) or of nuclear membrane (primates). He also opined that the neurons assumed a variety of shapes and sizes related to their function, whereas the size of the cell body was related to that of its processes.

2.3.2.1.2 Neuroglia

In addition to the various types of neurons, the CNS in mammals contained the interstitial or stromal cells called as the neuroglia. Similar supportive cells were present in all vertebrates and some of the invertebrates (Coggeshall and Fawcett, 1964). Within the CNS, the neuroglia outnumbered neurons in the ratio of about 10:1 (Bowsher, 1967).

Carl Weigert (1845-1904) was a German pathologist who contributed to the study of development of staining methods of myelin sheaths and neuroglia (Gardner, 1969).

Truex and Carpenter (1969) opined that the neuroglial elements may comprise almost half the total volume of the human brain indicating their important and specialized role. Neuroglia may be classified as macroglia (viz. astrocytes and oligodendrocytes), ependyma and microglia. According to Jenkins (1978) it is erroneous to consider them as true connective tissue cells because all neuroglia, with the exception of one type were formed from ectoderm, whereas the connective tissue cells were derived from mesoderm. Fletcher (1993) stated that neuroglial cells (gliocytes) were relatively small; and with routine stains only their nuclei and perikarya were evident.

2.3.2.1.2.1 Astrocytes

Fielder and Drommer (1976) found that astrocytes formed a membrana limitans gliae superficialis beneath the pia mater, which in the pig varied in thickness and consisted mainly of fibrous astrocytes. On the surface of membrana limitans was a continuous basement membrane.

Cox (1977) reported that the nuclei of astrocytes were pale, round or ovoid and had a diameter of eight to ten microns. Jenkins (1978) found that these were the largest, most numerous and most elaborate of all the glial elements forming most of the 'packing tissue' of the neuropile of the nervous system. These had perivascular feet (end feet) and some of their processes formed a superficial glial membrane beneath the pia mater of the cord and the brain. There were two types: fibrous astrocytes (spider cells) seen in white matter and protoplasmic astrocytes (mossy cells) seen in gray matter. The processes of protoplasmic astrocytes were wavy and profusely branching, but those of the fibrous astrocytes were longer, straighter with less branching. These cells formed the blood- brain barrier.

According to King (1987) astrocytes formed a continuous single cell layer immediately beneath the pia mater, which covered about 85 per cent of the basal lamina of all blood vessels in the CNS by means of their perivascular feet and got fused with the ependymal cells.

By scanning electron microscopic studies in cat brain and spinal cord, Uehara and Ueshima (1988) described that this superficial glial limiting membrane of the spinal cord consisted of fibrous astocytes. Their processes were less complex than those in the parietal cortex and also contained foot processes, consisting of end-feet,

ascending processes and cylindrical processes. They also reported that the end –feet had numerous fine processes and some long, unbranched processes.

Fletcher (1993) noticed that in routine staining astrocytes were recognized by a large pale round nucleus, which was the largest among glial nuclei. With silver stains, astrocytes were seen to possess numerous processes. He also opined that in addition to forming the glial limiting membrane, the end feet of astrocytes also formed the septa in spinal cord. According to Peplow (2004) the astrocytes if bound with glutamate, were capable of affecting the communication between neurons.

2.3.2.1.2.2 Oligodendrocytes

Dekaban (1956) described the oligodendroglia as the most numerous cells in the nervous system of adult mammals.

Truex and Carpenter (1969) described these cells as the smallest among macroglia cells and as found in both the gray and white matter. These were numerous at birth but became less conspicuous in the adult brain and spinal cord. Three types were: perineuronal satellites in gray matter, inter fascicular cells in white matter and juxtavascular cells near blood vessels. The interfascicular cells were numerous in the white matter of the foetus and newborn but they rapidly diminished in number as myelination progressed. These cells had a nucleus, which was smaller with a diameter of seven microns (Cox, 1977), fewer and smaller processes than astrocytes and gave rise to myelin in the CNS where it formed myelin sheath around more than one axon at a time (Jenkins, 1978). The nucleus of these cells was more round than that of the astrocytes and contained a fair amount of heterochromatin and scanty amounts of cytoplasm (Banks, 1981). According to Fletcher (1993) a single oligodendrocyte was known to contribute myelin sheaths to about 50 myelinated axons.

2.3.2.1.2.3 Microglia

According to Truex and Carpenter (1969) microglia entered the nervous system as perivascular mesenchymal cells along with neural blood vessels. The microglia were also known as the mesoglia, Hortega cells, rod cells, gitter cells, cerebral histiocyte, scavenger cells, compound granular corpuscles and foam cells (Cox, 1977).

Harrison (1978) stated that the final type of neuroglial cell to develop in the neural tube was the microglial cell, which began to appear when blood vessels grew into the neural tube. It developed from the adventitia of blood vessels and therefore was mesodermal in origin.

They were small cells, which occurred both in gray and white matter being more in the former (Jenkins, 1978) and were characterized by scanty cytoplasm which contained a small, dark round, indented or irregularly shaped nucleus. The cell body had numerous short processes (Banks, 1981).

Sturrock (1981) identified microglia both in gray and white matter of the spinal cord of embryonic and foetal mice from 12 days postconception onwards.

Fletcher (1993) noticed that the microglial cells had antigenic and phagocytic capabilities. They were sparce and difficult to find in normal tissue. With silver impregnation, they were seen as small elongate cells with polar processes.

2.3.2.1.2.4 Ependyma

According to Kingsley (1962) the ependymal cells differed from epithelia in that processes arose from their basal ends and extended inward or through the gray matter. In vertebrates, Huettner (1967) reported that some of the germinal cells, which were left behind in the germinal layer formed the ependymal cells in the adult, constituting the non-nervous lining of the neural canal. Kent (1969) noticed that the ependyma was the only type of neuroglia found in cyclostomes. In higher vertebrates, the neuroglial cells were increasingly abundant and diversified.

According to Banks (1981), the ependymal cells were retained in the adult as the lining of central canal of spinal cord and ventricles of brain. Embryonic ependyma were ciliated low cuboidal or low columnar cells. The latter form was retained in the adult with large pale nuclei having one or more nucleoli. In the adult, the basal borders resided on a basement membrane and were separated from the nervous tissue. In younger animals, the basal modifications and cellular processes extended through the mantle layer of the developing nervous tissue.

Fletcher (1993) found that zonulae adherentes near the luminal border joined the ependymal cells and the luminal surface of each cell had microvilli and numerous motile cilia. Large molecules from the extracellular space of central nervous system

passed between the ependymal cells to reach cerebrospinal fluid (CSF). At certain sites, neuron processes extended between the ependymal cells to contact CSF serving a receptive function.

Rajtova and Kokardova (1998) during a scanning electron microscopic study of ependymal cells in sheep and goat foetuses found that the external morphology of ependymal cells was independent of their developmental stage, resembling that of postnatal ependymocytes. The surfaces of proximal apical membranes of ependymal cells were covered with cilia and microvilli.

2.3.2.2 Gray Matter

Rexed (1952) described the architectural organization of neurons in the cat spinal cord. He believed that a similar lamination or zoning of gray matter existed in all higher mammals and this lamination was useful for localizing axonal degeneration in the mammalian spinal cord.

The nuclear aggregations in the spinal cord gray matter in buffalo calf were identified by Rao (1970). According to Truex and Carpenter (1969) thick frozen sections of Weigert-Nissl stained human spinal cord showed differences in laminar configuration between various segments and regions of the cord in man. These ten laminae (nine cell layers and region ten surrounding the central canal) constituted regions with characteristic properties, but their boundaries were zones of transition. They described the lamina I as a thin layer of gray substance that capped the dorsal horn with a spongy appearance, penetrated by small and large fibre bundles. It contained small, medium and large cells including dorsomarginal nucleus. Lamina II corresponded to substantia gelatinosa and consisted of tightly packed small cells and large fibres from dorsal funiculus with very less glial cells and fibres. Lamina III formed a band across dorsal horn, consisted of less packed and larger neurons and was rich in myelinated axons. In man it had lighter appearance and less breadth compared to cat. Lamina IV was the broadest of the first four layers with diffuse borders, small to large sized cells of variable shapes and contained nucleus proprius. Lamina V was a broad zone extending across the neck of the dorsal horn and had medial and lateral parts except in thoracic region. Lateral part ('reticular nucleus') was prominent in cervical region and had large perykarya with coarse Nissl bodies. Medial zone extended to dorsal funiculi with few small perikarya, small amounts of

Nissl substance and a characteristic pale appearance. Lamina VI was a broad layer, typical only in cervical and lumbar enlargements, where it was subdivided into small medial (compact due to dark stained, medium and small sized cells) and large lateral (less compact with larger, triangular or star- shaped perikarya scattered) zones located at the base of the dorsal horn. Lamina VII formed the intermediate zone of gray matter with medium-sized and light stained perikarya. It contained nucleus dorsalis, intermediolateral and intermediomedial nuclei. Lamina VIII contained small and medium sized cells and was not delimited from lamina VII. In lumbar and cervical enlargements confined to medial part of ventral horn. Axons of these neurons were commissural fibres, which crossed the midline in ventral commissure. Lamina IX was composed of the largest cells of the spinal cord. The sharply defined lateral nuclei had large and small perikarya rich in Nissl bodies whereas less defined medial nuclei had a diffuse border with lamina VIII.

Pearson and Sauter (1971) noticed that the gray matter became reduced in size and spread dorsally in the lower sacral and coccygeal segments of the spinal cord in man, rabbit and monkey embryos and foetuses.

Dellmann and Mc Clure (1975) described the structure of spinal cord in domestic animals. According to them, the gray substance of the cord was arranged in columns, which extended the entire length of the cord. The gray matter in cross-section was in the form of the letter 'H'. The dorsal and ventral protuberances on either side were the dorsal and ventral horns respectively, which were largest in the cervical and lumbosacral enlargements. The central intermediate substance surrounded the central canal with dorsal and ventral gray commissures located dorsally and ventrally and was continuous with the lateral intermediate substance located between dorsal and ventral horns. The lateral horn projected laterally from the lateral intermediate substance and was prominent in the thoracolumbar parts of the spinal cord.

Zargar *et al.* (1975) opined that the nuclear pattern of spinal cord gray matter in albino rat was similar to that in buffalo calf. Jenkins (1978) opined that in mammals, the organization and relative positions of the laminae were not uniform throughout the cord.

Clark (1984) found that the shape and size of the horns varied from one region to the next. The ventral horns were wider in the cervical and lumbar enlargements and much narrower in anterior cervical and thoracic levels as seen in man. Taluja *et al.* (1991) also found that the nuclear pattern of spinal cord gray matter in goat foetuses during last trimester of gestation was similar to that in buffalo calf.

2.3.2.2.1 Dorsal Horn

Buchanan (1957) stated that the dorsal horn neurons were of two types, the internuncial neurons and tract cells both receiving impulses from the dorsal root fibres. The internuncial (association) neurons were distributed to other cells in the gray matter and complete spinal reflex arcs. The tract cells formed ascending tracts towards the brain. These dorsal horn neurons (column cells and Golgi type II cells) were confined to the CNS.

According to their observations in rat, Nathaneil and Nathaneil (1966) reported that the dorsal horn contained neurons, astrocytes, oligodendrocytes and blood vessels.

According to Kappers *et al.* (1967) three divisions occurred in the dorsal horns of mammals, viz. a marginal zone of Waldeyer (Lissaeur) or zona marginalis, the substantia gelatinosa Rolandi (lamina II of Rexed) and the body of the dorsal horn. They stated that beneath the marginal cells was the substantia gelatinosa (lamina II), which formed the outer caplike portion of the head of the dorsal horn. It was composed of small cell bodies and contained fine nonmyelinated fibres and was well developed in most mammals especially in ungulates where it formed convolutions. In carnivores, it joined its fellow of the opposite side.

Papez (1967) found that the dorsal horn size corresponded to the sensory field of distribution of dorsal roots and inversely to the length of cord segment. Thus, long thoracic segments have a very narrow or short dorsal horn in man.

Truex and Carpenter (1969) described the marginal zone (nucleus magnocellularis pericornualis or nucleus dorsomarginalis) as a thin layer of cells that covered the tip of the dorsal horn and as situated in the zona spongiosa or lamina I. They were tangentially arranged stellate or spindle-shaped cells reaching a diameter of over 50 μm. They were found throughout the cord and were most numerous (6 to

10) in the lumbosacral segments, less so in the cervical and least in thoracic segments (1 to 2) as reported in man. They also reported that the head and cervix of the dorsal horn was occupied by the nucleus proprius (nucleus centrodorsalis, nucleus magnocellularis centralis) corresponding to the lamina III and IV. This poorly defined cell column consisted of medium-sized spindle-shaped cells and large polygonal cells. The cells were most numerous in the lumbosacral cord. Lateral to this nucleus the small and medium sized cells found in the reticular process (lamina V) were named the nucleus reticularis. Nucleus dorsalis (nucleus thoracicus, Clark's column, nucleus magnocellularis basalis, nucleus spinocerebellaris) was the cell column with eccentrically placed nuclei, placed in the medial portion of the base of the dorsal horn with the number of cells ranging from three to four in anterior thoracic region to 10-15 in posterior thoracic with 20 cells seen in T12 and L1 segments.

As noticed by Rao (1970) in buffalo calf, the nuclei present in the dorsal horn were nucleus proprius (in all regions of the cord), nucleus thoracicus (in thoracic region) and formatio reticularis (in lumbar enlargement).

Nucleus dorsalis was well defined in C8 and extended through the thoracic and anterior lumbar regions being more prominent in T10, T12 and L1 segments in domestic animals and gave origin to dorsal spinocerebellar tract (Dellmann and Mc Clure, 1975).

In domestic animals, Dellmann and Mc Clure (1975) reported that in the cranial cervical area the dorsal gray horn and substantia gelatinosa were continuous with the spinal nucleus of the trigeminal nerve. Hannah and Nathaneil (1975) found that the cells of the substantia gelatinosa at birth were small with large oval nuclei and scanty cytoplasm. Jenkins (1978) observed that the nucleus was composed of rows of ovoid or polygonal cells with deeply staining nuclei about 6 to 20 μm in diameter. It extended the whole length of the cord and was the largest in the lumbosacral and first cervical segments in dog.

Zargar *et al.* (1979) in albino rat, also reported the presence of nucleus proprius, nucleus thoracicus and formatio reticularis in the dorsal horn.

Todd and Lewis (1986) recognized two main cell types in lamina II by Golgi-staining of the rat spinal cord, as stalked cells and islet cells similar to those found in

other species. Stalked cells were present in large numbers in the dorsal part of the lamina. Islet cells were found throughout the lamina.

2.3.2.2.2 Lateral Horn

Rao (1970) in buffalo calf, reported the presence of substantia (nuclei) intermedia lateralis and centralis in the lateral and medial aspects (respectively) of lateral (intermediate) horn of thoracic region. The visceral efferent neurons in the lateral horn were ovoid or spindle-shaped cells with thinner and shorter dendrites, vesicular nuclei and finer chromophilic bodies and were smaller than the somatic motor cells. In Weigert preparations, they were surrounded by a clear homogenous background, which resembled the gelatinous substance (Truex and Carpenter, 1969). In dog, the intermediolateral (lateral) horn extended from T1 to anterior lumbar segments (Jenkins, 1978). Rethelyi (1976) revealed a regular orientation of dendrites in the intermediate region forming a circular or elliptical gray matter in cats. Grottel (1979) found that the neurons of various centers of substantia intermedia differed more with the range of their dendrites than with the size of their perikaryons. Grottel and Teresa (1979) noticed that the dorsal dendrites of neurons of lateral horns in the thoracic segments reached lamina II and the ventral ones reached the lateral group of motor nuclei. The dendrites directed medially passed to the medial area of substantia intermedia while those directed laterally entered the lateral funiculus to run a short distance.

Hancock and Peveto (1979) reported that in rat pelvic nerve nucleus was located in the lateral intermediate gray in from L6 to S1 spinal segments.

Zargar *et al.* (1979) in albino rat and Taluja *et al.* (1991) in foetal goat in the fifth month of gestation also reported the presence of substantia intermedia lateralis and centralis in the lateral horn. The size of the cells varied from nine to 13.50 μm in goat foetuses.

Arciszewski (1999) reported the presence of a nucleus tractus spinocerebellaris dorsalis in the equine foetus as an interrupted band of cells, extending from first thoracic neuromere to third lumbar neuromere. It was situated in the gray matter of the spinal cord dorsolateral to the central canal. The nerve cells, which formed the nucleus were oval or spindle shaped and they were of small and medium size.

2.3.2.2.2.1 Intermediolateral Nucleus

Slawomirski *et al.* (1973) reported that the nucleus intermediolateralis in dog was relatively weakly developed and was an interrupted band of cells extending from C8 to L3 segments at the base of the dorsal and ventral pillars of the spinal cord between the lateral edge of the gray matter and central canal. The intermediolateral nucleus consisted of large multipolar cells, small round calls and spindle-shaped cells and extended from C8 to L4 segments in cats (Okamoto, 1977) and from T1 to L3 segments in horses both in adults (Welento *et al.*, 1979) and in prenatal period (Arciszewski *et al.*, 1999b).

2.3.2.2.2.2 Intermediomedial Nucleus

Welento *et al.* (1977) observed that the nucleus intermediomedialis of the pig lay in the pars intermedia of the cord gray matter as a long cellular band from C1 to Cy2 or Cy3 being best developed in the sacral segments, a little more weakly in the lumbar and cervical segments and showed weakest development in thoracic segments. In horses, Arciszewski *et al.* (1999a) found that this nucleus in prenatal period extended from C1 to S5 segment as an interrupted band of cells.

2.3.2.2.3 Ventral Horn

2.3.2.2.3.1 Ventral Horn Neurons

The ventral horn contained two types of neurons, viz. α and γ neurons. Additional multipolar neurons known as the spinal border cells of Sherrington were found along the lateral and medial borders of the ventral horn in the thoracic, lumbar and sacral segments of the cord and formed the nucleus pericornualis anterior in man, which contributed to the ventral spinocerebellar tract (Cooper and Sherrington, 1940). Similar neurons were also identified in the dorsal and intermediate regions of ventral horns in monkey as propriospinal cells of Sprague. In monkey, the nerve cells of the ventral horn could not be classified functionally on the basis of either their topographic position or morphology (Sprague, 1951).

Truex and Carpenter (1969) described these neurons as contributing axons to the ventral roots of the spinal nerves and had a transverse diameter of 30-70 μm and the cell body was 100 μm in length. Such elongated multipolar neurons had 3-20

dendrites and axons with diameter of 10-13 μm. These somatic efferent neurons had a large, central, vesicular nucleus and coarse Nissl bodies in the cytoplasm and were largest in the lumbar and cervical enlargements. These neurons were smaller in the thoracic segments. Scattered among the large ventral horn cells were smaller cellbodies of gamma efferent fibres.

Rafalowska (1977) found that in cat, the ventral horn neurons smaller than 30 μm were connected with oxygen metabolism, while large cells were connected with glycogen metabolism. Jenkins (1978) reported that the large multipolar neurons had a size of 80-120 μm in mammals.

In goat foetuses during last trimester of gestation, Taluja *et al.* (1991) found that the size of the neurons ranged from 19.8 to 28.8 μm in ventromedial and dorsomedial nuclei, from 30.6 to 34.2 μm in dorsolateral nucleus and from 25.2 to 30.6 μm in ventrolateral nucleus in foetal goat. According to Fletcher (1993) volume of the cellbody of the ventral horn neurons was proportional to the volume of its axon and the size of the motor unit being innervated.

2.3.2.2.3.2 Ventral Horn Nuclei

In the ventral horn of mammalian spinal cord, Larsell (1951) noticed three columns of nerve cells: as lateral, medial and central regions. The lateral nuclei supplied the musculature of the extremities and were present only at the enlargements. The medial nuclear group supplied the trunk and limb musculature and was present throughout the length of the cord.

Keswani and Hollinshed (1956) reported that the phrenic nucleus in man occupied the most medial portion of the ventromedial group of cells in the ventral gray horn of the third, fourth and fifth cervical segments.

According to Truex and Carpenter (1969), the medial group was divided into dorsomedial and ventromedial groups in man. The nucleus of hypoglossal nerve in the medulla was the continuation of this column. The dorsomedial group was smaller and most distinct in the cervical and lumbar enlargements and was missing in the sacral portions of the cord. The lateral nuclear group was smaller and undivided in thoracic segments and was large and divided in the cervical and lumbar enlargements.

Fleiger (1970) revealed that in pig the nucleus of the accessory nerve consisted of two (dorsal and ventral) bands of cells, which extended from the posterior end of the olivary nucleus to the posterior sections of the first and fifth cervical segments of the cord respectively.

In the ventral horn of spinal cord in buffalo calf, Rao (1970) identified ventromedial and dorsomedial nuclei (in all regions of the cord, to be distributed to axial musculature) and ventrolateral and dorsolateral nuclei (only in cervical and lumbar enlargements, for limb innervation).

In domestic animals, Dellmann and Mc Clure (1975) found that the accessory nerve nucleus was situated in the cervical part except in caudal two segments in the lateral aspect of lateral intermediate substance.

In albino rat, Zargar *et al.* (1975) also identified ventromedial, dorsomedial nuclei, ventrolateral and dorsolateral nuclei in the ventral horn similar to those in buffalo calf.

The phrenic nucleus in the rabbit (Ullah, 1978) extended from fourth to sixth cervical segment as a longitudinal column of cells lying between the ventromedial and ventrolateral columns at ventral margin of the ventral gray horn of the spinal cord.

Takahashi and Yamamoto (1979) reported that by ultrastructural studies the cell group X of Onuf, originally described in man was identifiable in the cat at the ventrolateral margin of ventral horn in the S1-S2 cord level.

Clark (1984) included the phrenic nucleus (from C7 to C7 segments) and the accessory nucleus (from C1 to C6 levels and give origin to spinal portion of the accessory nerve) in the central nuclear group, which was the most limited of the ventral horn neurons both being located in the cervical cord. The phrenic nucleus in the cat (Takahashi and Ninomiya, 1985) extended from fourth to sixth cervical segment at ventral margin of the ventral gray horn of the spinal cord similar to that in cat. According to Ullah and Salman (1986) in rabbit, the spinal nucleus of accessory nerve extended from the caudal part of the medulla oblongata to the cranial part of sixth cervical segment of spinal cord.

Ruhrig *et al.* (1995) observed that the caudal pole of the hypoglossal nucleus formed a uniform cell column with the ventral horn of the spinal cord at 10 mm CRL.

From 30 to 50 mm CRL onwards all nuclear groups could be identified and at 53 mm CRL these corresponded to the adult pattern in cattle.

2.3.2.3 White Matter

Sisson and Grossman (1953) reported that in domestic animals the white matter of spinal cord was divided into three white columns or funiculi by the entering dorsal and emerging ventral rootlets of the spinal nerves on each side. These were dorsal, ventral and lateral funiculi.

As reported by Buchanan (1957), the intersegmental tract or the fasciculus proprius of each funiculus acquired the name designating its location, viz. dorsal, lateral and ventral fasciculus proprius. Each was composed of ascending and descending fibres, which bordered the gray matter, originated and terminated within the spinal cord to connect the spinal neurons of intrasegmental and intersegmental levels.

Truex and Carpenter (1969) found that these funiculi were formed of numerous fibre tracts or fasciculi, which were of three types: ascending, descending and intersegmental. The fibre bundles having the same origin, general course and termination were named as the fibre tracts. Since all levels of the spinal cord were connected with the brain by long ascending and descending fibres, the white matter increased from lower to higher levels of the cord.

These tracts were developed to different degrees in different species of domestic animals (Dellmann and Mc Clure, 1975). Different levels of the cord revealed variations in the gray-white matter relationships because of variations in the size and number of fibres forming spinal nerves, which attached to the cord at different levels, and differences in the cross-sectional sizes of the spinal cord tracts at different levels. Both ascending and descending tracts were smaller in the caudal region than in the cephalic region of the cord and some tracts like fasciculus cuneatus were not present in caudal segments of the cord as reported by Jenkins (1978) in mammals.

Clark (1984) reported that even though the white matter was white in fresh state because of myelin, in mammals there were more unmyelinated than myelinated axons. In the rat sacral cord there were approximately 177,000 myelinated and

263,000 unmyelinated axons in the white matter. The white matter also contained blood vessels and neuroglia. Collagen, except in blood vessels and pia mater, was absent in the spinal cord tissue.

Fletcher (1993) found that in domestic animals while ascending tracts terminate in the brain, the axons of descending tracts came from brain and synapse on interneurons within the gray matter.

2.3.2.3.1 Dorsal Funiculus

According to Kappers *et al.* (1967), the dorsal white column in mammals showed a characteristic accumulation of fibres from lower to higher levels. They also reported that in cat, dorsal funiculus made 22 per cent of the total white substance, in monkeys 26 per cent and in man 39 per cent. Pearson and Sauter (1971) noticed that in the lower sacral and coccygeal segments of the spinal cord of human, rabbit and monkey foetuses the dorsal funiculus disappeared gradually. As reported by Brown (1973), all the myelinated fibres in the dorsal funiculus did not ascend the full length of the cord in all animals and thus only 25 per cent of the fibres of the dorsal funiculus present at the lumbar level of spinal cord reached the nucleus gracilis in medulla in cat.

Dellmann and Mc Clure (1975) reported that in domestic animals the dorsal funiculus contained fasciculus gracilis (gracile fasciculus), fasciculus cuneatus (cuneate fasciculus) and dorsal fasciculus proprius. The medially located fasciculus gracilis extended from the caudal part of the spinal cord to the medulla oblongata, and the lateral one, fasciculus cuneatus, extended from the mid- thoracic portion of the spinal cord to the medulla. Jenkins (1978) reported that the lateral cuneate nucleus in the medulla and the cuneocerebellar tract were the forelimb equivalents of the thoracic nucleus (of Clark) and the dorsal spinocerebellar tract of the hindlimbs. De Lahunta (1983) reported the presence of a cuneocerebellar tract also in the dorsal funiculus.

According to King (1987), almost all tracts were mixed with other tracts in the spinal cord and were not separated into well-defined bundles. The only general exception was the gracile and cuneate fascicles, but in several domestic species (eg. sheep) even these tracts were blended with other tracts. He also reported a small dorsal corticospinal tract in the dorsal funiculus of ungulates upto C5 segment level

and that in most rodents, majority of the pyramidal fibres travel in the dorsal funiculus.

2.3.2.3.2 Lateral Funiculus

Lateral white column contained both ascending and descending fasciculi and lateral fasciculus proprius. The ascending tracts in the lateral funiculus were: the dorsolateral fasciculus of Lissauer, the dorsal and ventral spinocerebellar tracts, the spinotectal tract and the lateral spinothalamic tract. The descending tracts in the lateral funiculus were the lateral corticospinal (pyramidal), rubrospinal and lateral (medullary) reticulospinal tracts in mammals (Jenkins, 1978). According to King (1987), the dorsal and ventral spinocerebellar tracts were also called as direct and indirect spinocerebellar tracts respectively. The lateral white columns were the largest in the sacral region in dog.

2.3.2.3.2.1 Ascending Tracts in the Lateral Funiculus

2.3.2.3.2.1.1 Dorsolateral Fasciculus of Lissauer

Kappers *et al.* (1967) in mammals reported that the dorsolateral fasciculus of Lissauer was located deep to the dorsolateral sulcus at the peak of the dorsal horn and it contained both descending and ascending fibres. They also described the Lissauer's tract and the ventrolateral or ventral spinothalamic tract in mammals as the mammalian representatives of the spinomesencephalic and spino-tectal system of the lower forms. Papez (1967) described the dorsolateral fasciculus of Lissauer as a part of the dorsal funiculus in vertebrates.

According to Jenkins (1978), the dorsolateral fasciculus of Lissauer was an ascending tract composed of thinly myelinated and nonmyelinated fibres of the dorsal root, which ascended only one segment on the same side before either terminating within the substantia gelatinosa or passing through it to terminate in the dorsal horn gray matter.

2.3.2.3.2.1.2 Spinocerebellar Tracts

The dorsal spinocerebellar tract (Flechsig's fasciculus) was composed of myelinated fibres, which had their cellbodies within the dorsal nucleus of Clark ascending from L3 or L4 segments in cat to enter cerebellum via restiform body (Grant and Rexed, 1958). The ventral spinocerebellar tract originated from the base

and neck of the dorsal horn in lumbar segments in the cat and ascended through the brain stem and rostral cerebellar peduncle into the cerebellum (Hubbard and Oscarsson, 1962). A rostral (cranial) spinocerebellar tract was identified in cat (Oscarsson and Uddenberg, 1964).

The spinocerebellar tracts were also described in sheep, goat and ox (Dellmann and Mc Clure, 1975). In man also, Noback and Demarest (1977) noticed a rostral spinocerebellar tract. As reported by Jenkins (1978) in mammals, the ventral spinocerebellar tract was located at the periphery of the lateral funiculus, ventral to the dorsal spinocerebellar tract and was bordered on its medial side by the lateral spinothalamic tract. The spinotectal tract extended from dorsal horn of opposite side to the rostral colliculus and was situated medial to the ventral spinocerebellar and the lateral spinothalamic tract. The lateral spinothalamic tract was more dorsal in the cat spinal cord. King (1987) opined that the rostral spinocerebellar tract was the forelimb equivalent of the ventral spinocerebellar tract.

2.3.2.3.2.1.3 Spinocervical Tract

Peele (1977) described a spinocervical (spinocervicothalamic) tract located in the dorsolateral portion of the lateral funiculus, adjacent to the dorsal spinocerebellar tract. This system was prominent in cat and dog among domestic animals. According to him, it originated ipsilaterally from cellbodies in laminae IV and V of Rexed in the dorsal horn of the lumbosacral spinal cord.

2.3.2.3.2.2 Descending Tracts in the Lateral Funiculus

According to Barone and Braque (1980), the descending tracts in the spinal cord of ox were similar to those described for horse, sheep, and goat with a very narrow corticospinal tract and well developed rubrospinal tract.

2.3.2.3.2.2.1 Corticospinal Tract

Kappers *et al.* (1967) reported that this tract extended to the sacral segment in primates, where it had the greatest development. In man, it extended the whole length of the cord. The percentage of cervical spinal cord occupied by the corticospinal tract in mouse was .1.14 per cent, in cat 7.00 per cent, in rabbit 5.00 per cent and in man 11.00 per cent. In marsupials and ungulates, the lateral corticospinal tract descended only upto the cervical region; in carnivores and rodents it reached the lumbar cord.

The cortical dominance of the corticospinal fibres became evident from lower to higher animals, viz. in dogs these formed 10 per cent of the total white matter of the cord, in monkeys 20 per cent and in man 30 per cent.

According to Dellmann and Mc Clure (1975), the pyramidal tract disappeared caudal to third or fourth cervical spinal cord segment in sheep and in goat, it reached only to the first cervical segment. As reported by Jenkins (1978) in mammals, the lateral corticospinal (crossed pyramidal) tract occupied the central area of the upper quadrant of the lateral funiculus, medial to the dorsal spinocerebellar tract dorsal to the rubrospinal tract, lateral to the lateral fasciculus proprius and ventral to the dorsolateral fasciculus of Lissauer. It originated in the cerebral cortex and terminated in the ventral horn of the spinal cord gray matter. The pyramidal system was reported to be much more important in man than in animals whereas, rubrospinal and vestibulospinal tracts were of greater importance in animals than in man.

King (1987) opined that only the mammals possessed the pyramidal system. It was absent in birds, reptiles, amphibia and fishes. Among mammals, the pyramidal pathways varied greatly probably due to the relatively recent phylogenetic origin of the system. He also found that in sheep and goat there was physiological evidence that the pyramidal fibres continued down the cord as non-specific neurons.

According to Dyce *et al.* (1996) the pyramidal fibres reached all levels of the cord; in the dog about 50 per cent terminated in cervical segments, 20 per cent in thoracic segments and 30 per cent in lumbo-sacro-caudal segments. In contrast, the pyramidal system in ungulates terminated at the level of origin of brachial plexus although there were hints of diffuse continuation to lower levels within the dorsal funiculus.

2.3.2.3.2.2.2 Rubrospinal Tract

In animals the rubrospinal tract represented the crossed pyramidal tract of man (Sisson and Grossman, 1953). It was inconspicuous in man (Austin, 1961). In cat, it was traced upto the sacral segments (Nyberg, 1966). It was much larger in most mammals than in birds and reptiles and extended farther caudally (Kappers *et al.*, 1967). The rubrospinal tract was demonstrated in goat and ox (Dellmann and Mc Clure, 1975) and reached upto the most caudal part of the cord.

The rubrospinal tract arose from red nucleus and was located ventrolateral to and partially intermingled with fibres of the lateral corticospinal tract, being medial to the dorsal spinocerebellar tract in mammals (Jenkins, 1978). It was an important tract in carnivores and was the best developed of all motor pathways in ungulates (Dyce *et al.,* 1996).

2.3.2.3.2.2.3 Reticulospinal Tract

The lateral reticulospinal tract originated from the medullary reticular formation and descended in the ventromedial portion of the lateral funiculus (Jenkins, 1978). As per the reports of Dyce *et al.* (1996) the reticulospinal system consisted of dorsal and ventral tracts located within the lateral funiculus and a third (pontine reticulospinal) tract within the ventral funiculus.

2.3.2.3.3 Ventral Funiculus

According to Nyberg (1966), in animals the ventral funiculus was larger than the lateral funiculus, which contained phylogenetically younger rubrospinal and corticospinal fibres. In man the sequence of myelination of these tracts paralleled the order in which they differentiated phylogenetically, viz. the vestibulospinal fibres became myelinated first and the corticospinal fibres last.

Jenkins (1978) also found that in mammals the ventral funiculus primarily contained descending phylogenetically older tracts and these were, ventral corticospinal (pyramidal), vestibulospinal, tectospinal, ventral reticulospinal and the spinothalamic tracts, medial longitudinal fasciculus and fasciculus proprius.

2.3.2.3.3.1 Ventral Corticospinal Tract

Verhaart and Kramer (1958) described a ventral uncrossed pyramidal tract in elephants. The ventral corticospinal tract was smaller than the lateral corticospinal tract and was traced upto lumbar levels in higher mammals (Kappers *et al.,* 1967) but was rare in the cat spinal cord. Jenkins (1978) found that the ventral cotricospinal tract bordered the ventral median fissure and consisted of those corticospinal fibres, which did not cross at the medullary level in mammals. King (1987) found that the pyramidal tract was best developed in primates and carnivores, but the latter had a less developed ventral tract than that of man. Decussation of pyramid was 100per cent in dogs so that there was no ventral tract at all. In cat, there was a small ventral tract,

reaching only upto anterior segments of the neck. In man, 85 per cent of the pyramidal fibres decussated; the rest 15 per cent formed ventral tract, which ended in the first thoracic segments. Therefore, in both primates and carnivores the ventral tract was of relatively little importance. In ungulates, the entire pyramidal system was small and ended at the cervical region. The fibres decussated in the pyramids and mainly descended as the ventral tract alongside the ventral median fissure.

2.3.2.3.3.2 Vestibulospinal Tract

Nyberg and Mascitti (1964) detected that the lateral vestibulospinal tract in the cat contained descending fibres from the lateral vestibular nucleus (of Dieter) in the medulla and extended the whole length of the cord. As reported by Dellmann and Mc Clure (1975) the vestibulospinal tracts were demonstrated in goat and ox. Jenkins (1978) opined that the medial vestibulospinal tract originated from the medial vestibular nucleus, descended within the medial part of the ventral funiculus in the area of the medial longitudinal fasciculus and extended upto the mid-thoracic segments of the cord. King (1987) reported that there were two vestibulospinal tracts: lateral and medial.

2.3.2.3.3.3 Other Tracts in the Ventral Funiculus

Verhaart (1953) opined that the ventral spinothalamic tract in animals was of no clinical significance. But he was unable to locate this tract in the cat spinal cord.

Verhaart and Beusekom (1958) described the medial longitudinal fasciculus as a very old tract that extended from the rostral midbrain upto lumbar levels in dog and second thoracic segment in cat.

According to Nyberg (1966), reticulospinal tract was the only pathway by which spinal cord received impulses from higher centers in cyclostomes and was the first to possess the earliest phylogenetic signs of cephalization. Added to this, in passing to higher animals were the vestibulospinal, tectospinal and the corticospinal systems.

Jenkins (1978) reported that the ventral (pontine) reticulospinal fibres extended from the caudal pontine reticular area to the lumbosacral segments.

De Lahunta (1983) described the tectotegmentospinal tract as a part of the lateral funiculus in animals. Tectospinal tract arose from the rostral colliculus,

descended in the periphery of the ventral funiculus bordering the ventral median fissure and terminated in the upper four cervical segments.

2.3.2.4 Central Canal

According to Stromsten (1947) the small central canal extended throughout the cord and measured 0.5 mm in diameter in cat. In mammals, the small central canal extended as a continuation of the fourth ventricle of the brain (Leach, 1952). Arey (1957) observed that for a time during early development, the central canal was diamond-shaped in transverse section. Later the sidewalls united dorsally and by about nine weeks of gestation, fusion obliterated the dorsal part of the neural cavity. In a foetus of three months, the persisting ventral portion rounded into the definitive central canal.

The position of the central canal was believed to be central in the spinal cord (Trautmann and Fiebiger, 1957).

Goller (1962) opined that the shape of the cross-section and of the gray matter and the position of the central canal showed only very slight variations in cattle. According to Kappers *et al.* (1967) the central canal widened to form the terminal ventricle in lower sacral and coccygeal regions of the spinal cord in mammals.

A sharply defined central canal was seen only in foetal and new born spinal cords. In the adult, the ependymal lining was discontinuous and lumen contained a variety of debris including round cells, macrophages and neuroglial processes (Truex and Carpenter, 1969).

Similar to earlier reports, Pearson and Sauter (1971) also noticed that in lower sacral and coccygeal regions of the spinal cord in man, rabbit and monkey foetuses the central canal widened to form the terminal ventricle.

Sarnat *et al.* (1975) found that in the spinal cords of vertebrates (including cats, dogs and monkeys) the central canal was filled with diffuse, fine granules stained with PAS. Granular deposites of glycogen were found in ependymal cells (including the proximal segments of ependymal cell processes) of all species examined.

Seitz *et al.* (1981) could not observe any zonulae occludentes in the spinal cord ependyma of mouse. Sturrock (1981) reported that the central canal was

collapsed in the lower segments of the spinal cord and assumed a characteristic dorsoventrally elongated shape in mouse. According to him the material, which filled the lumen below the cervical cord was caused by inadequate fixation.

In the rat also, Bruni and Reddy (1987) observed a collapsed central canal in the lower segments of the spinal cord, which assumed an elongated shape. Zonulae occludentes were not observed in the spinal cord ependyma of rat also.

Bjugn *et al.* (1988) reported that in mice, the spinal cord ependyma was found to consist of simple, cuboidal and unspecialized epithelial cells with no regional variation along the length or circumference of the central canal. It was also stated that in mice, all segments of the spinal cord presented a central canal with round or ovoid cross-section and a partially collapsed central canal was associated with inadequate tissue preservation. With good tissue preservation, the central canal was always round to ovoid in cross-section at all levels. Unlike other levels, the central canal of the filum terminale was the most variable in shape and occupied the largest cross-sectional area of the cord. It was reported that the zonula occludens like apical junctions were present among mouse ependyma. A considerable number of gap junctions at the lateral aspects of the ependymocytes were also reported and apart from luminal/apical protrusions (interpreted as apocrine secretion observed on spinal cord ependyma) the central canal was free of amorphous material in mice.

Bohme (1988) reported that the development of the neural tube resulted in a relative reduction of its lumen accompanied by an increasing thickness of its wall. The central canal measured only about $1/5^{th}$ of the former neural canal as a result of obliteration or fusion of a part of the lumen. This occurred between 28 and 34 foetal days in cat and was characterized by an elongation and shifting of the dorsal ependymal matrix cells and by the apposition of lateral walls in the same region. The increase in the size of the dorsal funiculi caused the elongation of the ependymal cells, the basal processes of which remained to form the dorsal glial septum. The proliferation of neurons and the resultant growth of the dorsal gray horn was believed to be responsible for the narrowing of the lumen. The lumen-contacting matrix cells were displaced from the former surface. These 'blast cells' developed into neurons or glial cells. Until two or three months after birth there was a small wedge-shaped area in the dorsal wall of the central canal, which consisted of foetal matrix cells with long

tapering basal processes extending into the glial septum. After this date, the matrix was exhausted and the ependyma formed the complete lining of the surface of the central canal.

According to Taluja *et al.* (1990) in goat foetuses, the percentage ratio between different distances revealed location of the central canal slightly dorsal, ventral and approximately central in cervical, thoracic and lumbar regions respectively, probably to maintain a caudal flow of the cerebrospinal fluid (CSF).

2.3.3 Spinal Nerves

As reported by Moog (1950) along most of its length, the spinal cord bore a segmental series of paired spinal nerves, in each of which the neurons were arranged according to a definite pattern.

Mc Cabe and Low (1969) studied the subarachnoid angle in rats, which was the lateral limit of subarachnoid space, where nerve roots entered and left and which was an important site of transition for nerve sheaths. Here the perineurium of peripheral nerve left the surface of the nerve and extended between the dura and arachnoid. The endoneurium acted as a pathway for transmission of infection from periphery to the CNS.

Fraher and Rossiter (1983) found that prior to the onset of myelination, in the rat ventral rootlet, pedunculated clusters of cells were seen projecting from the surface of its most proximal part, immediately adjascent to its attachment to the spinal cord. This part of the rootlet was reffered to as the proximal rootlet segment.

Clark (1984) observed that approximately 40 per cent of the dorsal root fibres contained small unmyelinated fibres. According to Radek *et al.* (1986), each dorsal and ventral spinal nerve root was attached to the cord surface by a number of rootlets. A sheath composed of cellular and fibrous laminae surrounded the spinal roots but the structural arrangements differed from those of the peripheral nerve trunks in rat. Fraher and Sheehan (1987) found under electron microscope that an outgrowth of central nervous tissue termed 'central tissue projection' extended distally into the proximal part of those sensory rootlets and was surrounded by peripheral tissue. That segment of rootlet, which contained both central and peripheral tissue was termed the 'transitional zone'. Light and electron microscopic studies by Bristol and Fraher

(1987) revealed that the number of fibres per rootlet averaged 29 in rat with 60 per cent of the fibres having a diameter of 6.95 μm. Mean transitional zone length was 166 μm and its cross-sectional area increased in a proximo-distal direction. This was proportional to the number of fibres in the rootlet. All the parameters varied between rootlets and were highly correlated with one another, indicating a dimentional similarity of transitional zones. Smith and Bennett (1987) found that near the entry zone of a lumbar dorsal root the associated fibres were aggregated near the lateral margin of the dorsal column and become displaced medially at progressively more rostral levels by the entry of additional dorsal roots.

2.3.3.1 Dorsal Root Ganglia

Ultraviolet absorption studies on living spinal ganglion cells by Deitch and Moses (1957) revealed the presence of descrete aggregations of cytoplasm containing a high concentration of nucleoprotein. These structures were identical with Nissl bodies of the same cells seen after staining with basic dyes. Therefore they opined that Nissl granules were not staining artifacts.

Pannese (1974) noticed that the nucleus-cytoplasmic ratio was very high in undifferentiated cell of the ganglionc cell and the ratio decreased later.

In buffalo, Rao (1978) found that the greatest cross-sectional area of the ganglion was at C7 and L5. He also found that the smallest ganglionic neurons were located in T5 ganglion and the largest were at C3 in buffalo.

Orendacova and Anna (1979) found that in the axis of the cervical sensory ganglia of dog, there were axons of nerve cells, which were equally distributed all along the circumference of the ganglion. It was also found by electron microscopic studies that normal structure of dorsal root ganglia of the dog agreed with that of other species.

According to Clark (1984) the neuronal cell bodies for all somatic and visceral sensory input from the body to the cord were located in the dorsal root ganglia, situated in the intervertebral foramina.

Adam *et al.* (1987) opined that the nucleus-cytoplasmic ratio was a good index of the functional activity of cells in life and in the ganglionic neurons in sheep this ranged from 1.00: 11.00 to 1.00: 17.00.

Taluja and Shrivastava (1988a) reported that dorsal root ganglia were aggregations of unipolar nerve cells on the roots of the spinal nerves with a distinct connective tissue capsule in goat foetuses. The fibrous capsule was 13 to 23 μm in thickness in the last trimester of gestation. Two types of unipolar cells were seen in dorsal root ganglia of foetal goat: large clear cells and small obscure cells. The ganglion cells were ovoid or spheroid in shape and varied in size from 7.70 to 9.90 μm, 11.82 to 15.95 μm and 21.72 to 30.80 μm in first, second and third trimesters respectively.

In sheep, Rao (1990b) reported the presence of cortical and central regions for the ganglia and found that the greatest cross-sectional area was at C7 segment with the largest neurons and the smallest at T3 segment with the smallest neurons. The greatest nuclear cross-sectional area in sheep was at T3 and T12 segments and the smallest at L4 segment and the neurons were densely packed (56 per square mm).

2.3.4 Meninges

According to Hyman (1942), in vertebrates, the central nervous system did not fill the bony canal in which it lay, but was separated from it and protected by fluid and by connective tissue membranes, the meninges. In cyclostomes and fishes there was only one layer, the primitive meninx. Amphibians, reptiles and birds had two layers, an outer dura and an inner secondary meninx. In mammals, there were three meninges, which consisted of from without to within: the tough dura mater and the delicate arachnoid and pia mater both differentiations of the secondary meninx.

The meninges are sheet-like connective tissue coverings of the central nervous system and are named from outward to inward as dura mater, arachnoid and pia mater. A large epidural space separated the dura from the vertebral canal and contained fatty areolar tissue and blood vessels. Arachnoid is very delicate and thin and pia mater is vascular. Between the arachnoid and pia mater is the subarachnoid space, which contains cerebrospinal spinal fluid and is crossed by arachnoid trabeculations. The pia mater presented a denticulate or dentate ligament along its lateral aspect, which suspended the cord in the dural sheath. The pia extends caudally in the dural sac, as a long thread, the filum terminale (Sisson and Grossman, 1953).

All the three layers were of mesenchymal origin in man, with a contribution from neural crest cells towards arachnoid and pia mater (Arey, 1957). The spinal

meninges are continuous at the foramen magnum with the meninges of the brain in domestic animals. Although dura extends the whole length of vertebral column throughout life, the other meninges do not (Dellmann and Mc Clure, 1975).

In dog, between 24 and 28 days of gestation, the primitive meninx differentiated into an endomeninx and an ectomeninx (Engel and Draper, 1982a) and by 41 to 44 days of gestation the ectomeninx (the future dura) was separated from the mesenchyme that formed the periosteum of the vertebral arch. The endomeninx was also differentiated into the arachnoid and pia mater with a subarachnoid space containing numerous arachnoid trabeculae. These findings were compatible with human foetus during the 14^{th} week of gestation (Engel and Draper, 1982b). In lower vertebrates, the dura developed from the surrounding mesenchyme and the arachnoid and pia were derived from the neural crest (Clark, 1984; Ghosh, 2002).

2.3.4.1 Dura Mater

As reported by Bradley (1948) in dog, venous sinuses were absent in the spinal dura mater. Arey (1957) reported that the dura was a distinct membrane at eight weeks of gestation in man.

As per the reports of Ramsey (1959a) in cat, the epidural space contained fat, which was well vascularized and consisted of uniform fat cells closely arranged, with minimum of supportive elements. It was almost without fibrous elements; thus well adapted to lubrication of the moving structures about it. This was in contrast with other fat deposits, which were modified with increased amounts of connective tissue to withstand pressure. Ramsey (1959b) reported that the distribution of epidural fat was characteristic for each species with more fat being found dorsally than ventrally and more in the caudal half of the epidural space than in the dorsal half in general, being disposed metamerically at the intervertebral junctions.

Jenkins (1978) found that in mammals the spinal dura was a thick fibrous tube. Venous sinuses were present in the cranial dura but the spinal dura was devoid of them. Caudally the dura terminated by surrounding the filum terminale at the level of S1 vertebra and extended caudally as the coccygeal ligament, to attach to the periosteum of the vertebral canal at the Cy 7 or Cy 8 vertebra in dog.

According to Chandna and Tyagi (1981) in goat, the thickness of dura varied in different regions of the vertebral column. It was the thickest in the lumbar region and the dural sac extended upto the sacrum. The filum terminale extended upto Cy 2 vertebra. De Lahunta and Habel (1986) reported that the dural sac extended upto S1 in dog, S3 in pig and horse, S4 or S5 in ox and Cy1 vertebra in cat.

Parkin and Harrison (1985) reported that in man, the dura mater in lumbar region possessed a midline fold in a very few cases and was apposed to the walls of the vertebral canal by connective tissue which was sufficient to allow for displacement of the dural sac during movement of the spine and venous engorgement.

Fletcher (1993) observed that in domestic animals the dura consisted mainly of collagen fibres that formed a thick, strong membrane along with elastic fibres, fibroblasts, nerves and lymph and blood vessels. The inner surface of dura was composed of multiple layers of flattened fibroblasts, to which outer cells of arachnoid fused.

2.3.4.2 Arachnoid

Morse and Low (1972a) demonstrated during electron microscopic studies that the differentiation of pia and arachnoid cells was difficult in any location but a clear irregular line demarcated arachnoid and dura. Under normal circumstances, a small number of macrophages was present in the mammalian subarachnoid space and within the pial connective tissue space (Morse and Low, 1972b).

Fielder and Drommer (1976) stated that the structure of the arachniod in pig was similar to that in mammals. The subarachnoid space was lined with a continuous layer of mesothelial cells, which in the region of the pia had a discontinuous basement membrane. Gaps and pores were not observed in this cell layer and this layer prevented direct contact between CSF and connective tissue of the leptomeninx.

By 14^{th} day postconception in mouse and 16^{th} day postconception in rabbit, meningeal macrophages were well differentiated with short processes (Sturrock, 1987c). Sturrock (1988) identified the macrophages in the leptomeninges even at 11 and 12 days postconception in mouse and rabbit spinal cords respectively.

According to Fletcher (1993), the arachnoid in domestic animals consisted of an outer layer of flattened leptomeningeal cells and an inner layer of loosely arranged

cells with collagen fibres occupying the space between the cell processes. Arachnoid trabeculae traversed subarachnoid space and joined pia mater. A subarachnoid space containing CSF separated arachnoid and pia and was lined by leptomeningeal fibroblasts with macrophages seen sporadically.

2.3.4.3 Pia Mater

According to Bowsher (1967) the lower end of the spinal cord in man (opposite the second lumbar vertebra) was tethered to the back of the coccyx by a strand of primitive neuroectoderm, the filum terminale. It was through this caudal end that the neuraxis was invaginated into its membranes, which were called the leptomeninges. The visceral layer was closely adherant to the nervous substance, and formed the adventitial coat of its blood vessels; it was called the pia mater. Around the middle part of the filum terminale (opposite the second piece of the sacrum) the pia mater became continuous with the parietal layer, which was called the arachnoidmater. The portion of the subarachnoid space, which lay caudal to the lower end of the spinal cord was known as the lumbar sac or theca; it is from here that the cerebrospinal fluid is withdrawn for sampling by lumbar puncture.

Haller and Low (1971) by electron microscopic studies in laboratory animals (rat, mouse, dog and chinchilla) noticed a sheath of flattened cells ('root sheath', 'pia-arachnoid', 'pial sheath', 'root arachnoid') arranged in several layers over spinal nerve rootlets as they passed through the subarachnoid space. The elements of the rootsheath were in intimate association with pia and arachnoid. In rat, Haller *et al.* (1972) found that the outer layers of the root sheath were homologous and continuous with the outer layer of pia. The inner layers of rootsheath, which were a structurally modified and centrally directed continuation of the perineurium across the subarachnoid space, terminated as an open-ended tube near the junction of the peripheral and central nervous systems. There was a direct continuity between the endoneurial connective tissue spaces. The latter communicated directly with the subarachnoid space through fenestrations between pial cells.

Morse and Low (1972a) observed that pia was a complete cell layer, which formed a barrier between the CNS and the subarachnoid space and consisted of pial cells, extracellular elements and macrophages. Pial cells were the most numerous cell types, possessing long, flattened cytoplasmic processes, which contained few

inclusions. Collagen fibrils made up most of the extracellular material of the pia. Macrophages were rounded in shape and contained various cytoplasmic inclusions.

Mc Clure *et al.* (1973) reported that in cat, the denticulate ligament extended from the foramen magnum to the L7 segment of the spinal cord with the spinal cord terminating at the level of L7 vertebra. The filum terminale extended caudal to the base of the tail in rabbit (Kozma *et al.* 1974).

According to Fiedler and Drommer (1976) in pig, the spinal pia mater was fibrous, composed mainly of collagenous fibres. Pia mater is intimately connected with the cord and is continuous with the ventral median fissure and dorsal median septum of the spinal cord (Harrison, 1978).

Truex and Carpenter (1969), observed that the arteries of the brain and spinal cord are invested with connective tissue of the pia-arachnoid as they lie in the subarachnoid space. They observed that the pia and subjacent glial membrane blend with the vessel wall before it penetrates into the substance of the brain or spinal cord.

In mice, Nawar (1980) reported that the pia- arachnoid lying in relation to the dorsal extremity of the dorsal median septum formed two prolongations delimited laterally by veins that drained into the dorsal spinal vein. A similar arrangement was met with the ventral spinal veins. These arrachnoid prolongations with the cells they contain might constitute a route connecting the spinal CSF with the venous blood stream.

Free cells containing large dense granules (granular pial cells) first appeared in the leptomeninges of the spinal cord at 14 days postconception in mouse and 16 days postconception in rabbit. Granular perithelial (perivascular) cells first appeared at five days postpartum in mouse and 28 days postcoception in rabbit and it coincided with the development of large vessels in the spinal cord and had the same relation with the onset of myelination (Sturrock, 1987a).

Fletcher (1993) found that in domestic animals, the pia mater coated the entire CNS surface, lining every sulcus and fissure. A basal lamina separated pia from underlying limiting membrane. Pia mater consisted of loosely arranged collagen fibres covered by flattened leptomeningeal fibroblasts. Bilaterally along the mid-

lateral surface of the spinal cord, pial collagen was greatly thickened to form the dendiculate ligament.

2.4 HISTOCHEMISTRY

Patterson *et al.* (1971) observed that during foetal and postnatal development of sheep, the chemical growth (accumulation of DNA, protein and lipids) proceeded linearly in the spinal cord. They also reported that the spinal cord differed from the remainder of the CNS in growing more after the period studied (i.e. 50-day-old-foetus to five-week-old lambs) than before it. This was attributed to lipid accumulation.

According to Klemm (1996) the nervous system contained 70 to 85 per cent of water, most of which (85 per cent) was intracellular; 82 per cent in gray matter and 72 per cent in white matter. Most nervous system solids (40 to 65 per cent) were complex lipids, which were different from lipids of other body regions.

2.4.1 Carbohydrates

Kappers *et al.* (1967) pointed out that glycogen is known to be an oxygen storer and its presence points to a role in the metabolism of the nervous system. Truex and Carpenter (1969) opined that glycogen disappeared after birth or at the beginning of aerobic respiration, suggesting that the developing nervous tissue uses the energy released by the anerobic metabolism of glycogen. According to Sahu and Rao (1975), glycogen was present in the spinal meningeal layers and all components of the spinal cord except the neuronal nucleus in buffalo. It was depleted from all the sites following injury due to trauma.

The neuropile of gray matter composed mostly of neuroglia was rich in glycogen as reported by Sarnat *et al.* (1975) in various vertebrates including cat, dog and monkey. Jenkins (1978) reported that glycogen was not found in the nervous system of the adult, but within the embryo, it was found in the ependymal cells and choroids plexuses and neurons.

Sadler (1978) studied the distribution of the extracellular macromolecules (surface coats i.e. carbohydrates) in mice prior to and at the time of closure and found that there was a consistent increase in this Ruthenium red positive material immediately prior to the closure. Heavy deposits of the positive staining material were present along apical neural fold borders and overlying ectoderm cells. Once contact

between opposing neural folds was initiated, a decrease in the stainable material was observed.

Intense PAS positive material was localized in ependyma and dura mater of spinal cord in goat foetuses, whereas it was weak to moderate in nucleus and cytoplasm of multipolar neurons. The basement membrane of endothelium of blood vessels showed moderate PAS positive activity (Taluja and Shrivastava, 1988b).

2.4.2 Proteins

Bowsher (1967) stated that the nerve cell is the most delicate cell in the whole body and the amount of Nissl substance in a cell diminished after prolonged activity. The Nissl bodies were very high in RNA (ribonucleic acid) with RNA granules varying in size between 10 and 30 millimicrons.

Nissl bodies were named after Franz Nissl (1860-1919), a German neuropathologist and psychiatrist who developed a method for staining nerve cells with aniline dyes and discovered this chromidial substance in the cytoplasm of nerve cells (Gardner, 1969).

Jenkins (1978) described the Nissl bodies as the counterparts of the granular endoplasmic reticulum in other cells and decreased in number as a result of physiological changes i.e., in old age, overexertion, disease and metabolic disturbances.

Taluja and Shrivastava (1988b) stated that deep purplish Nissl substance was characteristic of neurons in all regions of the cord in last trimester of gestation of goat foetuses when stained with Toludine blue at pH 4.

According to Klemm (1996), proteins in the nervous system generally occurred in complex with lipids with most of the protein synthesis occurring in Nissl granules.

2.4.3 Lipids

According to Majno and Karnovsky (1958) the lipogenic activity of the Schwann cell was the lowest in the newborn animal and reached its peak about 20 days in rats.

Majstruk (1967) identified phospholipids and cerebrosides in the spinal cord of pigs even in the premyelination period (36^{th} to 60^{th} day of foetal life), sphingomyelins being the most prominent and the cerebrosides least so. Phospholipids were scattered over the white matter and partly over the gray matter.

If initial fixatives containing solvents like alcohol, ether, chloroform, etc. were avoided, the normal myelin sheath was stained by osmic acid and luxol fast blue (Truex and Carpenter, 1969). Chemically myelin is composed of protein and several lipids such as cholesterol, phospholipids and cerebrosides (Cox, 1977).

2.4.4 Oxidative Enzymes

Truex and Carpenter (1969) reported that the ependymal epithelium exhibited high oxidative activity as reflected by its high enzyme content, viz. acid and alkaline phosphatases.

Cox (1977) opined that the normal astrocytes had a low activity of oxidative enzymes but a vigorous activity of glycogen-synthesizing enzymes whereas reactive astrocytes showed considerable activity of oxidative metabolism.

2.4.4.1 Alkaline Phosphatase

According to Manocha and Shantha (1969) in rabbit spinal cord, alkaline phosphatase was localized in blood vessels, synapses around neurons, nucleoli of neurons and neuropile, with the cytoplasm of neurons showing a negative reaction. They had also observed a 10-fold increase in alkaline phosphatase from ventral tracts to the ventral column, which decreased at the lateral column. It showed a greater increase at the dorsal, compared with the ventral column.

Najpande and Shrivastava (1974) noticed a high concentration of alkaline phosphatase in the neural tube of 30-day-old sheep foetuses. A high activity was present in the dorsal aspect of neural axis. The activity was weak on the ventral part.

Sahu and Rao (1975) noticed that in buffalo, alkaline phosphatase was present in the nucleus and on the neuronal surface but was not traceable after spinal cord injury. In goat foetuses, Taluja and Shrivastava (1988b) observed a weak alkaline phosphatase activity in multipolar neurons of the spinal cord.

2.4.4.2 Acid Phosphatase

Manocha and Shantha (1969) noticed that in rabbit, the acid phosphatase activity was higher in the dorsal column whereas, the ventral column contained double the activity seen in the ventral tracts. Acid phosphatase was present in the cytoplasm of neurons, glial cells and synapses around the neuronal cell bodies and their processes (mainly ventral horn cells). Substantia gelatinosa and the rest of gray matter showed diffuse staining.

Sahu and Rao (1975) found that the acid phosphatase activity was seen intracytoplasmically and tended to increase in injured neurons in spinal cord of buffalo. Taluja and Shrivastava (1988b) noticed a high acid phosphatase activity in ependyma, dura mater and a moderate activity in gray and white matter of spinal cord in goat foetuses.

Materials and Methods

3. MATERIALS AND METHODS

Prenatal development of spinal cord in goat was studied using foetuses at different ages. The study was conducted on 52 foetuses, comprising of ten sexually indifferent, 22 male and 20 female foetuses. Most of the embryos and foetuses were freshly collected from slaughter house. Full term foetuses were obtained from cases of threatened accidental abortion and neonatal death.

3.1 MORPHOLOGICAL STUDIES

Immediately after collection, the foetuses were cleaned and their body weight and sex were recorded (Table 1). To get an idea about the growth trend of the foetuses, the following parameters were recorded:

1. CRL straight (Crown Rump Length) - from the forehead (highest point of the skull) to the tail head.
2. CRL curved - from midway between eyes to the tail head.
3. Total body length - straight distance from the tuber scapulae to the tuber ischii.
4. Vertebral column length - anterior to ear to tail base.
5. Vertebral column tail length (VCTL) - from anterior to the ear to tip of the tail.
6. Total bend length of the foetus- premaxilla to tail tip.
7. Length of forelimb - from the upper end of scapula to the lower end of the hoof.
8. Length of hindlimb - from the hip joint to the lower end of hoof.
9. Length of tail- from the base of the tail to its tip.

The age of the foetuses was calculated using the formula derived by Singh *et al.* (1979), for goat foetuses, $W^{1/3} = 0.096 (t - 30)$, where,

W = Body weight of the foetus in g

t = Age of the foetus in days.

Based on the age, the foetuses were divided into five groups corresponding to five months of gestation as shown in table 2.

Embryos upto 50 days of age, were fixed as such for histological and histochemical investigations. In still older embryos above 50 days, the vertebral column was separated, freed of its musculature and kept submerged in 10 per cent

neutral buffered formalin (NBF), in normal anatomical position for 48 hours. The following biometric data of vertebral column were recorded:

1. Vertebral length (longitudinal distance between intervertebral discs).
2. Width and height of vertebrae.

Thereafter, the spinal cord was exposed by cutting the dorsal side of the vertebrae by laminectomy, the lateral sides by paramedian section of the vertebrae and by clipping off the pedicles so as to remove the walls of the vertebral canal. Intervertebral discs and their relationship to the cord segments were recorded. Gross features such as distribution of epidural fat, length of filum terminale and the level of termination of spinal cord were recorded. Then the whole of the spinal cord along with the spinal nerve roots and the dorsal root ganglia was carefully dissected out and detached from the vertebral column. In larger foetuses having 120 days of age or more, the dura mater was slit along the dorsal median sulcus and ventral median fissure and reflected laterally. The arachnoid was either lifted off or gently pushed back from the dorsal and ventral roots. In those specimens, which were meant for histological and histochemical studies, the meninges were left undisturbed.

To determine the growth of the spinal cord and to show age-related changes, the weight, and the total, regional and segmental length of the spinal cord and vertebral column were measured in each foetus above 50 days of age. The length of each spinal cord segment was determined by measuring the distance from the most rostral rootlet of the dorsal root of each spinal nerve to the same point on the succeeding nerve. The length of each region and that of the entire cord was determined by adding together the length of appropriate segments. The width of the vertebral canal, width and height of the spinal cord and the length and width of dorsal root ganglia were also measured. In foetuses having more than 120 days of age, after detaching the meninges, gross observations like root attachment length (RAL), root emergence length (REL) and the inter root length of spinal nerves at each spinal cord segment were noted. The measurements like length, width and height were recorded in millimeters with meter tape, Vernier calipers, scale or graph paper. The weight was measured in grams with single pan balance or physical balance. The volume of the spinal cord was calculated by water displacement method using a measuring cylinder.

Data on gross observations were recorded as:

1. The length of spinal cord from rostral end (cranial limit of attachment of the first pair of cervical spinal nerve roots) to its caudal end (termination of the conus medullaris).
2. Segment length from the rostral attachment of each spinal nerve root to the rostral attachment of the succeeding nerve root.
3. Segment width as the greatest transverse distance.
4. Segment height as the greatest dorsoventral distance.
5. Root emergence length (REL): The extend of the dural surface occupied by the respective spinal nerve roots at the point of their emergence.
6. Root attachment length (RAL): Length of the cord to which the rootlets of each spinal nerve were attached, viz. distance from most rostral to the most caudal rootlet of both the dorsal and ventral roots of each spinal nerve.
7. Inter root length (IRL): Length of the cord surface devoid of rootlets, lying between the attachment of the roots of adjacent spinal nerves, viz. the distance between the most caudal and the most rostral rootlet of adjacent spinal nerves.
8. Vertebral level of termination was located with reference to rostral, middle or caudal third of the body of particular vertebra.
9. Length of filum terminale was measured from the conus medullaris upto its termination.

Allometric growth rate was calculated by dividing the length of the spinal cord by the length of its related vertebrae for its total length and for the regional and segmental levels.

3.2 MICROSCOPICAL STUDIES

For microscopical investigations, embryos upto 50 days were fixed as such. For histological procedures the embryos and small foetuses were fixed in 10 per cent NBF for 48 to 96 hours. These specimens were washed, dehydrated and embedded in high melting paraffin (MP 58-60^{o}C). Serial sections of 5µm thickness were cut either longitudinally or transversely.

In the embryos of first age group, the regions of neural tube were identified from the position of the underlying structures. The cervical region extended from the

caudal end of the rhombencephalic vesicle to the region of anterior limb buds. The latter region in turn corresponded to the cervical enlargement. The section through the lungs, heart, oesophagus and trachea corresponded the thoracic region, while the section through the stomach and intestinal loops, to the lumbar region. The area of posterior limb buds represented the lumbar enlargement. The portion posterior to the lumbar enlargement upto the tail corresponded to the sacral region. The coccygeal region was located in the tail.

From 50 days to 90 days of age, the spinal cord within the vertebral column was processed as such after cutting into region-wise pieces and serial sections were made. In foetuses above 90 days of age, the spinal cord along with dorsal root ganglion was cut into several pieces of two to three segments each and processed.

Depending on the requirements, 10% NBF and chilled acetone (4^{o}C) were used as fixatives. After fixation in the appropriate fixatives, the material was processed for paraffin embedding. Tissues for histological techniques were processed in high melting paraffin (MP 58 - 60^{o}C) and sections of 5 μm thickness were made. For histochemical studies of lipids and phosphatases frozen sections of 10 to 20 μm thickness were also prepared.

A. Histological staining techniques employed for paraffin sections were:

1. Ehrlich's haematoxylin and eosin (H & E) staining technique for routine histological studies (Luna, 1968).
2. Holzer's method for glial fibres (Luna, 1968).
3. Sevier - Munger method for neural tissues (Luna, 1968).
4. Van Gieson's method for collagen (Luna, 1968).
5. Holmes silver nitrate method for axis cylinders and myelin sheaths (Humason, 1972).

B. Cytological techniques employed were:

1. Aldehyde - Thionine - PAS method for central nervous system (Luna, 1968).
2. Phosphotungstic acid haematoxylin (PTAH) method for CNS tissue (Luna, 1968).

C. Histochemical methods employed were:

1. Best's Carmine method for glycogen (Bancroft and Stevens, 1977).
2. Periodic Acid Schiff's (PAS) reaction for carbohydrates (Bancroft and Stevens, 1977).
3. Gomori's alkaline phosphatase cobalt method (Singh and Sulochana, 1996).
4. Gomori's method for acid phosphatase activity (Singh and Sulochana, 1996).
5. Oil red 'O' in propylene glycol method for lipids (Luna, 1968).

3.2.1 Micrometrical studies

To measure changes in the height and width of different layers of neural tube and spinal cord and the proportion of gray and white matter of spinal cord during different stages of prenatal growth, the following data were recorded using an ocular micrometer.

I. Vertical distance

1. Height of spinal cord, gray matter, dorsal horn and ventral horn
2. Height of central canal
3. Dorsal median sulcus - central canal distance
4. Ventral median fissure - central canal distance

II Transverse distance

1. Width of spinal cord, gray matter, dorsal horn and ventral horn
2. Width of funiculi of white matter
3. Central canal width
4. Between central canal and spinal cord of left margin
5. Between central canal and gray matter of left margin

In addition to these data, thickness of capsule of dorsal root ganglia and thickness of meninges were also noted. The total size of nuclear aggregations and the size of cells and their nuclei were also measured.

The data collected were analysed statistically following Snedecor and Cochran (1985) to find out the mean and relation if any, with age and body weight of the

foetuses. To find out the relation of spinal cord parameters with body parameters Karl Pearson's coefficient of correlation was calculated using the formula,

$$r = \frac{\sum(x-\bar{x})(y-\bar{y})}{\sqrt{\sum(x-\bar{x})^2}\sqrt{\sum(y-\bar{y})^2}}$$

For finding the rate of growth of spinal cord parameters in relation to body parameters simple linear regression was calculated using the formula,

$$b_{yx} = \frac{\sum(x-\bar{x})(y-\bar{y})}{\sum(x-\bar{x})^2}$$

where y was the dependant variable and x was the independant variable.

Results

4. RESULTS

4.1 MORPHOLOGY

The spinal cord maintained an elongated and cylindrical shape throughout the gestation period and was located within the vertebral canal, enclosed by meninges by the middle of the second month (Fig. 1). Microscopic observations in this study revealed that eventhough a vertebral body was formed by 40 days of gestation, vertebral arches appeared only by 48 days. The meninges also took their form by 48 days. The spinal cord had a greater transverse diameter than dorsoventral diameter in most of its segments, with internally placed gray matter and external white matter (Fig. 2). It presented two enlargements, at cervical and lumbar levels (Fig. 3). The enlargements were characterized by the laterally bayed out horns, whereas, a narrow horn was present in cervical, thoracic and lumbar levels. The amount of white matter was found to increase towards the cervical segments. The sacral region was characterized by its quadrangular shape (Fig. 2). Caudally, by the latter half of second month, the cord tapered abruptly to become conus medullaris (Fig. 3), from which a non-nervous filament of pia mater extended as filum terminale (Fig. 4) in the vertebral canal.

By fourth month of gestation, a grossly distinguishable dorsal median sulcus and ventral median fissure could be seen all along the length of spinal cord, eventhough this could be microscopically observed from 48 days of gestation. These two structures divided the spinal cord into two symmetrical halves. In the fifth month of gestation, a dorsolateral groove was evident to be observed (Fig. 3) along the attachment of dorsal roots of spinal nerves. No similar groove was visible on the ventral aspect. The dorsal intermediate groove could not be distinguished grossly even towards the end of gestation except in the cervical region, but microscopically this groove was evident by 48 days of gestation.

Spinal cord exhibited well developed plexus of blood vessels in the pia mater by the fifth month of gestation (Fig. 4). From second month onwards, the pia mater extended into all sulci and carried branches of blood vessels also along with it. The blood supply was from the large ventral spinal artery in the ventral median fissure and paired dorsolateral spinal arteries on the respective aspects. These vessels extended upto the caudal end of the cord. Tooth-like lateral projections of pia mater, the dentate

ligament, fixed the cord in the dural tube upto S4 segment. Epidural fat could be seen around the nerve roots as brown deposits towards the end of fifth month of gestation, both on dorsal and ventral aspects especially at the intervertebral junctions in full term foetuses (Fig. 4).

4.2 MORPHOMETRY

4.2.1 Body Parameters

The body parameters of goat foetuses in this study are detailed in tables 3 to 7. The mean body weight showed a progressive increase (Table 3) and had a positive and highly significant correlation with age, crown rump length (CRL) straight and CRL curved during the entire gestation period (Table 4). Body weight was highly dependant on age and its rate of increase was progressive with 0.066 $\pm$ 0.012 g in first month, 2.165 $\pm$ 0.053 g in the second, 4.865 $\pm$ 0.219 g during third, 12.885 $\pm$ 0.348 g during fourth and 67.817$\pm$ 5.180 g during the fifth month of gestation, with the greatest rate at the fifth month (Fig. 5). For a unit change of age, the change in body weight was 21.571 $\pm$ 1.810 g during the total gestation period (Table 5).

The mean values of CRL straight increased 22.571 times and CRL curved increased 13.410 times progressively from the first to the fifth month (Table 3) and both were highly dependant on age. The rate of increase according to age was 0.621$\pm$ 0.840, 3.444 $\pm$ 0.801, 2.780 $\pm$ 0.416, 1.687 $\pm$ 0.577 and 5.233 $\pm$ 0.568 mm for CRL straight during first, second, third, fourth and fifth month respectively. CRL straight and curved showed positive and highly significant correlation with each other and with body weight and age (Table 4). CRL straight and curved changed significantly with unit change in age and the changes were 3.505 $\pm$ 0.088 and 3.810 $\pm$ 0.087 mm respectively during the whole gestation. CRL straight changed significantly with a unit change in CRL curved and it was calculated as 0.916 $\pm$ 0.016 mm (Table 5). The rate of increase of these two parameters with increase of age were identical, with a slightly greater rate of growth during the fifth month (Fig. 6). The relative increase of these two parameters with body weight were 0.134 $\pm$ 0.009 mm for CRL straight and 0.141$\pm$ 0.011 mm for CRL curved (Table 5).

All other body parameters also increased progressively during the entire gestation period (Table 6) and exhibited a positive and highly significant correlation

with body weight, age, CRL straight and CRL curved (Table 4) and changed significantly with age, body weight, CRL straight and curved (Table 7).

4. 2. 2 Vertebral Column

The vertebral column consisted of 7 cervical, 13 thoracic, 6 lumbar, 4 sacral and 12 to 15 coccygeal vertebrae.

4.2. 2. 1 Total Length of Vertebral Column

The mean total length of vertebral column was 62.750 ± 4.267, 108.833 ± 7.581, 188.200 ± 2.435 and 349.643 ± 19.067 mm during second, third, fourth and fifth month of gestation respectively (Table 8). The correlation of body weight, age and CRL straight and curved with total length of vertebral column was positive and highly significant (Table 9) during the entire gestation period. The regression analysis showed that, for unit change of vertebral column length, the change in CRL straight was 0.918 ± 0.021. The vertebral column length changed significantly with age (b = 3.755 ± 0.118 mm), body weight (b = 0.130 ± 0.007 mm), CRL straight (b = 1.068 ± 0.024 mm) and CRL curved (b = 0.976 ± 0.030 mm) (Table 5), with a greater dependence on the age than on the other parameters.

The rate of growth of vertebral column length in relation to age was 3.556 ± 0.401, 2.752 ± 0.132, 1.747 ± 0.449 and 6.860 ± 0.419 mm during second, third, fourth and fifth month of gestation respectively. The maximum rate was at the fifth month. The rate of growth of vertebral column length in relation to body weight was maximum at second month and it was calculated to be 1.634 ± 0.199 mm. During third, fourth and fifth month the corresponding values were 0.559 ± 0.029 mm, 0.113 ± 0.036 mm and 0.099 ± 0.005 mm showing a decreasing trend.

The rate of growth of vertebral column in relation to CRL straight varied at different stages of gestation. It measured 0.849 ± 0.224, 0.982 ± 0.035, 0.614 ± 0.242 and 1.154 ± 0.142 mm during second, third, fourth, and fifth month, with the maximum rate at the fifth month.

4.2. 2. 2 Regional Length of Vertebral Column

The mean regional length of vertebral column and its percentage of total length of vertebral column and of length of precoccygeal column are presented in tables 8 and 10 respectively. Thoracic region was the longest region in the vertebral column

(Fig. 7). This region formed an average of 29.282 ± 0.204 per cent of the total length of vertebral column, followed by cervical (22.836 ± 0.208 per cent), coccygeal (21.302 ± 0.254 per cent), lumbar (18.322 ± 0.167 per cent) and sacral (8.508 ± 0.105 per cent) regions for the total gestation period (Table 10).

Thoracic region was the longest region in the precoccygeal vertebral column also, forming 37.110 ± 0.218 per cent during the entire gestation, followed by cervical (29.029 ± 0.284 per cent), lumbar (23.218 ± 0.186 per cent) and sacral (10.779 ± 0.120 per cent) regions.

The percentage contribution of thoracic region to total length showed a progressive increase during second to fifth month. Cervical and coccygeal regions showed a decreasing trend. Lumbar and sacral regions' contribution remained variable.

All these regions of vertebral column exhibited positive and highly significant correlation between their length and the total and precoccygeal length of vertebral column (Table 10). The regional length of vertebral column also showed positive and highly significant correlation with body weight, age, CRL straight and CRL curved (Table 9).

4.2. 2.3 Vertebral Length

The mean length of the vertebrae is shown in table 11. The average vertebral length, in the cervical region was 2.381 mm in the second month. It formed 3.790 per cent of the total vertebral column length. During third, fourth and fifth month, average length were 3.488 (3.205 per cent), 6.100 (3.241 per cent) and 11.235 mm (3.210 per cent), respectively. The second cervical vertebra was the longest in the cervical region and in the whole vertebral column. Its length increased progressively from 3.417 ± 0.083, 5.058 ± 0.421, 8.450 ± 0.411 to 16.929 ± 0.905 mm during second, third, fourth and fifth month of gestation respectively. It contributed 5.445, 4.647, 4.490 and 4.851 per cent, to the total vertebral column length during these respective months.

The average vertebral length in the thoracic region was 1.474 mm in the second month, forming 2.349 per cent of the total vertebral column length, 2.455 mm in third month forming 2.256 per cent, 4.246 mm in fourth month forming 2.256 per cent and 8.033 mm forming 2.297 per cent of the total vertebral column length, in the fifth

month. The longest thoracic vertebra was T13 with a length of 1.750 ± 0.050, 2.808 ± 0.231, 4.950 ± 0.138 and 9.179 ± 0.534 mm in second, third, fourth and fifth month of gestation respectively. The contribution of T13 to the total vertebral column length was 2.390, 2.580, 2.630 and 2.625 per cent during these months. The thoracic vertebrae showed very little variation in length between them in all these four age groups.

The average vertebral length in the lumbar region was 1.945, 3.347, 5.833 and 10.822 mm forming 3.100, 3.075, 3.099 and 3.095 per cent of the total vertebral column length during second, third, fourth, and fifth month of gestation respectively. Very little variation was found between length of vertebrae of lumbar region in all the age groups.

The sacrum of foetal goat consisted of four vertebrae, which were fused in all these age groups. First sacral vertebra was the longest in this region and measured 2.000 ± 0.200, 3.283 ± 0.340, 5.300 ± 0.213 and 10.429 ± 0.761 mm during second, third, fourth, and fifth month. It formed 3.187, 3.017, 2.816 and 2.983 per cent of the total vertebral column length during these respective months. The fourth sacral vertebra was the shortest in this region and measured 1.000 ± 0.020, 1.717 ± 0.114, 3.100 ± 0.100 and 6.357 ± 0.414 mm in second to fifth month. Its length formed 1.593 per cent of the total length of vertebral column during second month followed by 1.578, 1.647 and 1.818 per cent during third, fourth and fifth month of gestation respectively. The average vertebral length in the sacral region was 1.458 mm forming 2.320 per cent, 2.229 mm forming 2.048 per cent, 3.925 mm forming 2.086 per cent and 7.786 mm forming 2.227 per cent of the total length of vertebral column during these months.

The average length of coccygeal vertebra was 1.095 mm in second month forming 1.745 per cent of total length of vertebral column. It measured 1.679 mm forming 1.542 per cent in third month, 2.829 mm forming 1.502 per cent during fourth month and 5.204 mm forming 1.488 per cent of total length during fifth month of gestation.

4.2. 2. 4 Vertebral Canal Length

The vertebral canal extended from the first cervical to fourth coccygeal vertebra. Out of the 12 to 15 coccygeal segments, only first four had a vertebral canal.

The vertebral canal length increased progressively 5.786 times from 58.833 ± 2.358 mm in the second month to 304.429 ± 16.023 mm in fifth month gestation (Table 8). It showed highly significant positive correlation with age, body weight, CRL straight, CRL curved, spinal cord length and vertebral column length (Table 12), but it did not show any correlation with the length of the coccygeal part of the spinal cord.

The vertebral canal length was highly dependant on age (b = 3.278 ±0.101 mm), body weight (b = 0.130 ± 0.007 mm), CRL straight (b = 0. 931 ± 0.021 mm) and CRL curved (b = 0.852 ± 0.026 mm) (Table. 5). For unit change in age the change in vertebral canal length was 3.444 ± 0.351, 2.365 ± 0.097, 1.453 ± 0.317 and 5.680 ± 0.454 mm during second, third, fourth and fifth month, with the maximum growth rate recorded at the fifth month. The relative increase of vertebral canal length in relation to body weight was 1.582 ± 0.178, 0.479 ± 0.026, 0.113 ± 0.024 and 0.083 ± 0.003 mm during the second, third, fourth and fifth month, showing a decreasing trend, but the relative increase in relation to CRL straight was maximum at the fifth month gestation. The growth rates recorded were 0.843 ± 0.191, 0.841 ± 0.029, 0.503 ± 0.186 and 0.957 ± 0.127 mm during these respective months, with the maximum rate at the fifth month.

4.2. 2. 5 Vertebral Canal Width

The mean values for vertebral canal width for different months of gestation are shown in table 13. In all age groups, the maximum vertebral canal width was found at the atlas. It had a mean width of 3.167 ± 0.021 in second, 3.933 ± 0.170 in third, 6.700 ± 0.597 in fourth and 9.571 ± 0.429 mm in fifth month. Apart from atlas, greater width of vertebral canal was found at C6 (3.100 ± 0.010 mm) and C7 (3.000 ± 0.063 mm) and at L5 (3.000 ± 0.025 mm) in second month. In third month, it was at C7 (3.533 ± 0.183 mm) followed by C6 (3.500 ± 0.151 mm) at cervical region and at L6 (3.420 ± 0.169 mm) followed by S1 (3.367 ± 0.227 mm) at the lumbosacral region. At fourth and fifth month the maximum was at C7, the width being 5.530 ± 0.226 and 9.286 ± 0.201 mm respectively, closely followed by T1 having a width of 5.380 ± 0.302 and 9.000 ± 0.216 mm respectively. In lumbar region, the maximum width was at L6, which measured 4.730 ± 0.221 mm in fourth month and 8.286 ± 0.221 mm by fifth month.

4.2. 3 Morphometry of Spinal Cord

4.2. 3.1 Spinal Cord Weight

The mean weight of the spinal cord in foetal goat was recorded as 0.160 ± 0.003 g in second month, 0.379 ± 0.052 g in third month, 1.126 ± 0.071 g in fourth month and 7.859 ± 0.801 g in fifth month (Table 14), showing a progressive increase. It contributed 0.584 ± 0.031 per cent to the body weight during the total gestation period. The contribution was 0.980 ± 0.075 per cent at second month, 0.569 ± 0.037 per cent at third, 0.411 ± 0.008 per cent at fourth and 0.552 ± 0.200 per cent at fifth month. So, it contributed more at the initial stages, then decreased upto fourth month and then again increased at the fifth month. The maximum weight of 11.520 g was recorded at 150 days of gestation. The correlation between spinal cord weight and body weight, age, CRL straight, CRL curved and vertebral column length was positive and highly significant. It also showed a similar relationship with spinal cord length and volume (Table 9). The spinal cord weight also had a positive and highly significant correlation with all body parameters measured (Table 15). The spinal cord weight was highly dependant an age, body weight and spinal cord length, and for unit change of these parameters the weight of spinal cord changed by 0.111± 0.009, 0.005 ± 0.002, and 0.042 ± 0.002g respectively, with a higher dependence on age, than on other parameters. It was also highly dependant on its volume and the relative change was 1.039 ± 0.008 g.

Spinal cord weight was highly dependant on all the other body parameters studied (Table 15). The relation between spinal cord weight and weight of other organs like liver, was linear (Fig. 8). But, as the cube root of liver weight exhibited a linear relation with age upto 145 days of gestation (Fig. 9), the cube root of spinal cord weight showed a sigmoid relationship during fifth month (Fig. 10), indicating greater variability than liver weight. The relation of spinal cord weight with CRL, body weight, total body length, total bend length and tail length also remained linear (Figs. 11 to 13).

The rate of increase of spinal cord weight according to age was 0.005 ± 0.001 g during second month, 0.018 ± 0.002 g in third, 0.060 ± 0.006 g in fourth and 0.281± 0.026 g during fifth month, showing a progressive increase during the gestation with

the greatest increase occurring at fifth month (Fig. 14). The relative growth rate of spinal cord weight in relation to body weight was 0.002, 0.004, 0.005 and 0.004g during second, third, fourth and fifth month respectively. It was less variable during last three months. The relative growth rate of spinal cord weight in relation to spinal cord volume was the highest during second month but was lowest in the fifth month. It was 1.706 ± 0.440, 1.043 ± 0.501, 1.591 ± 0.595 and 0.987 ± 0.023 g during second, third, fourth and fifth month respectively.

4.2.3.2 Volume of Spinal Cord

The mean volume of spinal cord increased progressively from 0.200 ml to 7.450 ± 0.809 ml from second to fifth month (Table 14). A highly significant positive correlation occurred between volume of spinal cord and age, body weight, CRL straight, CRL curved, vertebral column length, spinal cord length and spinal cord weight (Table 9) and all body parameters (Table 15). It was highly dependant on age (b = 0.105 ± 0.009 ml), body weight (b = 0.005 ± 0.000 ml) and spinal cord length (b = 0.040 ± 0.002 ml) and changed accordingly (Table 5). It was more dependant on age than on other parameters. A comparison of spinal cord weight and volume at different ages is shown in fig. 15, exhibiting a similar pattern of increase. The increase of spinal cord volume in relation to body weight was more during the fifth month (Fig. 16).

The rate of growth increased progressively during gestation and it was 0.002 ± 0.001 ml during second, 0.017 ± 0.002 ml during third, 0.017 ± 0.007 ml during fourth and 0.279 ± 0.030 ml during fifth month. Its relative growth rate with spinal cord length also exhibited a progressive increase and it was 0.001 ± 0.001, 0.007 ± 0.001, 0.014 ± 0.001 and 0.062 ± 0.007 ml during these months.

4.2. 3. 3 Total Length of Spinal Cord

The mean length of the spinal cord increased 5.020 times progressively from second to fifth month of gestation. It measured 52.625 ± 3.495, 90.917 ± 6.290, 150.800 ± 2.164 and 264.179 ± 12.308 mm during second, third, fourth and fifth month, respectively (Table. 14). The spinal cord length showed a highly significant and positive correlation with body weight, age, CRL straight, CRL curved, vertebral column length, spinal cord weight and spinal cord volume (Table 9). It also exhibited highly significant positive correlation with vertebral canal length (Table 12) and other

body parameters measured (Table 15). Regression analysis (Table 5) showed that the spinal cord length was highly dependant on and changed significantly with age (b = 2.293 ± 0.034), CRL straight (b = 0.768 ± 0.017), CRL curved (b = 0.705 ± 0.019) and body weight (b = 0.106 ± 0.006). The spinal cord length exhibited a linear increase with age (Fig. 17), body weight (Fig. 18), CRL (Fig. 19) and vertebral column length and vertebral canal length (Fig. 20). The increase in relation to spinal cord weight (Fig. 21) and spinal cord volume (Fig. 22) was greater during fifth month.

The spinal cord length was highly dependant on and changed accordingly with other body parameters studied (Table 15). Total body length, total bend length and tail length also exhibited linear relation with spinal cord length (Fig. 23). The growth rate of spinal cord length showed a decreasing trend upto fourth month, but during the fifth month it exhibited the maximum rate of 4.408 ± 0.297 mm. During second, third and fourth month, the growth rates were 2.852 ± 0.262, 2.279 ± 0.119 and 1.278 ± 0.506 mm respectively. The relative growth rate of spinal cord length in relation to body weight showed a decreasing trend. It was 1.310 ± 0.135 mm during second month and decreased to 0.064 ± 0.003 mm during fifth month. Comparative length of spinal cord, vertebral column, vertebral canal and precoccygeal vertebral column are shown in fig. 24. All exhibited the maximum rate of increase at the fifth month. At all stages, the rate of growth of vertebral column and vertebral canal in relation to age exceeded that of spinal cord.

4.2. 3. 4 Regional Length of Spinal Cord

There was no clear demarcation between segments of the spinal cord in goat foetuses except for the interval between root fibres of adjacent nerves. Each spinal cord segment was numbered as per the attachment of the paired spinal nerves and the cord consisted of as many segments as there were pairs of spinal nerves. On the basis of regional distribution of spinal nerves, the spinal cord was divided into cervical, thoracic, lumbar, sacral and coccygeal regions.

The maximum mean regional cord length was measured in the thoracic region in all age groups studied (Fig. 25). It measured 19.833 ± 0.477, 31.917 ± 2.482, 53.900 ± 1.197 and 101.821 ± 5.055 mm during second, third, fourth and fifth month respectively (Table 14). The percentage contribution of thoracic cord length to the

total length of spinal cord increased from 34.561 ± 0.475 per cent during second month to 38.474 ± 0.304 per cent in the fifth month of gestation (Table 16).

Minimum mean regional cord length was recorded in the coccygeal region during all months of gestation. It measured 4.500 mm in second month, 7.250 ± 1.008 mm in third, 6.900 ± 0.482 mm in fourth and 5.607 ± 0.952 mm in fifth month of gestation (Table 14). The regional coccygeal cord length expressed as percentage of total cord length increased from 6.996 ± 0.250 in the second month to 7.811± 0.887 per cent in the third month. Thereafter it declined to 4.570 ± 0.298 and 2.217 ± 0.308 per cent in fourth and fifth month respectively (Table 16).

The various regional length of the spinal cord except coccygeal region, exhibited highly significant positive correlation with age, body weight, CRL curved, CRL straight, vertebral column length, total spinal cord length, spinal cord weight and spinal cord volume (Table 9). A highly significant positive correlation was observed between the total cord length and length of the different cord regions except coccygeal region during the gestation period (Table 9). Among the regions of the spinal cord, the thoracic and cervical regions exhibited highly significant positive correlation with corresponding regions of vertebral column, during all months of gestation (Table 17).

Regression analysis showed that the changes in the length of various regions of vertebral column were highly dependant on the changes in the corresponding regions of spinal cord except coccygeal region. The changes were 1.024 ± 0.021 mm in cervical region, 1.038 ± 0.009 mm in thoracic region, 1.049 ± 0.020 mm in lumbar region and 1.930 ± 0.156 mm in sacral region.

4.2. 3. 4. 1 Cervical and Lumbar Enlargements of the Spinal Cord

The last three cervical and first two thoracic segments formed the cervical enlargement of the spinal cord. The mean length of cervical enlargement showed a progressive growth rate and was measured as 7.583 ± 0.898 mm in second month 12.400 ± 0.744 mm in third month, 18.250 ± 0.629 mm in fourth month and 36.107 ± 1.581 mm in the fifth month of gestation (Table 14). The average contribution of length of cervical enlargement to the total cord length was 13.234 ± 0.221 per cent during the total gestation period (Table 16). The cervical enlargement extended from

C6 to T3 vertebrae during the second month and from C6 to T2 vertebrae towards the end of gestation.

The lumbar enlargement in foetal goat was formed by the last three lumbar and first two sacral spinal cord segments. The mean length of the lumbar enlargement increased from 9.167 ± 0.247 mm in second month, 15.225 ± 1.117 mm in third month, 26.400 ± 0.718 mm in fourth month and 39.786 ± 2.278 mm in fifth month (Table 14). It formed 16.230 ± 0.250 per cent of the total length of spinal cord during the total gestation period (Table 16). The lumbar enlargement extended from L4 to S2 vertebrae at second month. It was from L4 vertebra to the caudal end of L6 vertebra towards the end of gestation.

The length (Fig. 26) and percentage contribution to total length of spinal cord by the lumbar enlargement were always more than those of the cervical enlargement at different ages during this study. The length of cervical enlargement and lumbar enlargement exhibited positive and highly significant correlation with age, body weight, CRL straight, CRL curved, vertebral column length and spinal cord length, weight and volume (Table 9).

4.2. 3. 5 Spinal Cord Segments

The foetal goat had 36 pairs of spinal nerves viz. 8 cervical, 13 thoracic, 6 lumbar, 4 sacral and 5 coccygeal. In eight animals studied this number was exceeded because of the increased number of coccygeal nerves upto 7 or 8 pairs.

4.2. 3. 5. 1 Segment Length

The average length of spinal cord segments is shown in table 18. In the cervical part the segment C2 was the longest, closely followed by C3 in last three months of gestation. C2 formed the longest spinal cord segment in all age groups. Its length increased from 3.000 ± 0.010 mm in second month, to 3.533 ± 0.142, 7.000 ± 0.211, and 13.143 ± 0.864 mm in third, fourth and fifth month of gestation respectively. It contributed 5.700, 3.800, 4.640 and 4.975 per cent of total length of spinal cord in these respective months.

In the cervical region, the shortest spinal cord segments were C6 and C7 in second month with a length of 1.667 ± 0.211 mm forming 3.170 per cent of total spinal cord length. In the last three months of gestation, the shortest segment was C8

closely followed by C7. The length of C8 was 2.267 ± 0.143, 2.700 ± 0.238 and 6.465 ± 0.401 mm during third, fourth and fifth month and formed 2.490, 1.790 and 2.450 per cent of total length of spinal cord.

In the thoracic region T13 was longest in the second, fourth and fifth month of gestation with a mean length of 1.700 ± 0.630 mm forming 3.200 per cent of the total spinal cord length at second month. The average length of T13 were 5.200 ± 0.111 and 10.000 ± 0.419 mm during fourth and fifth month respectively, forming 3.448 and 3.785 per cent of the total length respectively. T12 was the longest thoracic segment during third month with a length of 2.783 ± 0.242 mm, contributing 3.060 per cent of the total spinal cord length.

The shortest of thoracic segments were T1, T2 and T3 in second month with an average length of 1.333 ± 0.105 mm forming 2.530 per cent of the total spinal cord length. During third month, T3 formed the shortest segment with a length of 2.292 ± 0.189 mm forming 2.520 per cent of total length and was closely followed by T2. First thoracic segment was the shortest in fourth and fifth month with length of 3.400 ± 0.180 mm and 6.786 ± 0.281 mm forming 2.354 and 2.569 per cent of total length respectively.

In the second month, segments L2 to L5 were equally long and were longer than the other segments in the lumbar region. They had an average length of 1.958 ± 0.042 mm with each forming 3.720 per cent of the total length of spinal cord. In third, fourth and fifth month L3 was the longest with length of 3.517 ± 0.277, 6.200 ± 0.200 and 11.179 ± 0.624 mm respectively forming 3.870, 4.110 and 4.230 per cent of total length with an increasing trend. The shortest lumbar segment was L6, with a length of 1.917 ± 0.083, 3.150 ± 0.273, 5.200 ± 0.200 and 8.643 ± 0.651mm during second, third, fourth and fifth month respectively. Its percentage contribution to total spinal cord length formed 3.640, 3.460, 3.450 and 3.270 per cent during the respective period showing a decreasing trend from second to fifth month.

In the sacral region, first sacral segment was the longest, and fourth was the shortest in all age groups. S1 had an average length of 2.000 ± 0.015, 3.333 ± 0.216, 5.500 ± 0.307 and 6.286 ± 0.450 mm forming 3.800, 3.670, 3.045 and 2.380 per cent of total length respectively showing a decreasing trend in the percentage contribution during second to fifth month of gestation. The percentage contribution of S4 also

showed a decreasing trend from second to fifth month from 1.900, 1.740, 1.525 to 1.067 per cent. Its length was 1.000 ± 0.030, 1.583 ± 0.203, 2.300 ± 0.153 and 2.821± 0.384 mm during these respective periods. The segment length gradually decreased after S1 in all age groups towards the coccygeal segments.

Comparison between segment length of spinal cord and vertebral column, showed a decrease in segment length at the cervical enlargement due to the additional C8 segment of the spinal cord. All other segments except coccygeal ones showed a similar pattern.

4.2. 3. 5. 2 Segment Width

The difference in size between the segments of cervical and lumbar enlargements was less distinguishable at the second month of gestation. The mean width of spinal cord segments is presented in table 19. The maximum width at second month was recorded among the segments of cervical enlargement followed by segments of lumbar enlargement. C6, C7, C8 and T1 segments had a width of 2.037 ± 0.147 mm whereas, L5, L6, and S1 had 2.003 ± 0.125 mm.

Comparison between vertebral canal width and spinal cord segment width at the fifth month of gestation showed that both the parameters followed a similar pattern (Fig. 27).

The mean maximum segment width was recorded at C8 segment during third, fourth and fifth month of gestation. It had 2.783 ± 0.205, 4.200 ± 0.133 and 7.521± 0.273 mm width during these respective months.

At lumbar enlargement, the L6 segment presented the maximum width for third, fourth and fifth month with 2.525 ± 0.150, 4.160 ± 0.130 and 6.750 ± 0.193 mm width respectively.

After S1 segment, the width of spinal cord segment decreased gradually towards the coccygeal region (Fig. 28).

4.2.3.5. 3 Segment Height

The average values for height of spinal cord segments are shown in table 20. The maximum segment height was noticed in segments of cervical and lumbar enlargements in the second month with a height of 1.610 ± 0.004 mm. It varied between C6, C7 and C8 during later months with C8 being the segment with

maximum height of 2.058 ± 0.186 mm during third month, C6 with 3.900 ± 0.313 mm at fourth month and C7 with 6.100 ± 0.167 mm at fifth month.

The lumbar region, during third month at L5 and L6 exhibited the maximum height of 1.908 ± 0.173 mm, at L5 in the fourth month with 3.180 ± 0.089 mm and at L6 in the fifth month with 5.371 ± 0.217 mm height. The segment height decreased through the sacral region to coccygeal region caudally.

Comparison between length, width and height of spinal cord segments at the fifth month of gestation (Fig. 29) showed that eventhough width and height increased, length decreased at the segments of cervical enlargement.

4.2. 4 Allometric Growth of Spinal Cord and Vertebral Column

The allometric growth rate for the total length of spinal cord and vertebral column decreased from second to fifth month. It was 0.839 in second month, 0.835 in third, 0.801 in fourth and 0.750 in fifth month of gestation. For the total gestation period, the value was 0.803 ± 0.006.

The allometric growth rate of the different spinal cord regions in relation to the respective region of the vertebral column varied between regions within the same group, and also between certain regions of all the four groups (Table 21). The allometric growth rate was the highest in the thoracic region in the second and third month, where as in the last two groups the cervical region had a little greater allometric growth rate than the thoracic region. In the second and third month, the allometric growth rate of the thoracic region was a little more than one, implying that during these stages the thoracic spinal cord grew faster than the corresponding vertebral region. In the cervical and lumbar regions, the growth rate was almost isometric during all these periods. The sacral part was almost isometric upto fourth month but there was a marked decline in the growth rate of sacral region of spinal cord in the fifth month of gestation.

The allometric growth rate of spinal cord segments in relation to the respective vertebrae was also different (Table 22). A drastic change in the growth rate of the spinal cord segments in relation to the vertebral segments occurred in the caudal part of the spinal cord.

4.2. 5 Correspondence between Spinal Cord Segments and Vertebrae

Upto 54 days in second month, the spinal cord extended the entire length of the vertebral canal with the conus medullaris at fourth coccygeal vertebra. So, the regions of the spinal cord corresponded to the respective regions of the vertebral column (Fig. 28).

In the third month, a slight ascend of the lumbar region was noticed as it extended only upto the middle of L6 vertebra. So a corresponding ascend was noticed in the sacral and coccygeal regions with the caudal most end of spinal cord reaching only upto the second coccygeal vertebra.

In the fourth month, the lumbar region reached upto the middle of L6, but the sacral region extended only upto middle of S3, with the conus reaching upto first coccygeal vertebra.

In the fifth month, the lumbar region extended only upto the caudal end of L5 vertebra, with the sacral region extending upto the middle of S1 and conus upto caudal end of S2 (Fig. 28).

In all age groups, C1 to C7 spinal cord segments lay within the corresponding vertebra. Due to the presence of the C8 spinal cord segment, the rest of the spinal cord segments in the cranial and middle thoracic regions lay within the next vertebra. As the middle thoracic segments ascended cranially due to their shorter length compared with the caudal thoracic segments the caudal thoracic and cranial lumbar segments came to lie within their corresponding vertebra again, which was seen more pronounced in the last two months of gestation in the present study.

4.2. 6 Level of Termination of the Spinal Cord

The caudal extremity of the spinal cord tapered as the conus medullaris. From the conus a slender, non-nervous filament of pia mater, the filum terminale, extended caudally in the dural sac. The mean length of the filum was measured 1.333 ± 1.033, 2.092 ± 0.424, 11.000 ± 1.155 and 40.250 ± 3.988 mm during second, third, fourth or fifth month of gestation respectively (Table 14).

By 54 days, no filum was measurable since the spinal cord extended the entire length of the vertebral canal. So the level of termination was at fourth coccygeal vertebra at that age. Towards the termination of second month, by 57 days of age, a

very short filum was visible with a mean length of 1.333 mm and the level of termination was at the third coccygeal at its anterior end. During the third month, the level of conus varied between first and second coccygeal vertebra. By the beginning of the fourth month, the level of termination was at first coccygeal vertebra, but towards the latter half, it was at fourth sacral vertebra. During fifth month, the level of termination varied from cranial end of S4 vertebra at the beginning, through middle of S3 to caudal end of S2 vertebra by the end of gestation (Fig. 28).

4.2. 7 Spinal Nerves

There were 36 pairs of spinal nerves in the foetuses under study: 8 cervical, 13 thoracic, 6 lumbar, 4 sacral and 5 coccygeal. These spinal nerves had a common pattern of structure and were segmentally arranged. Each arose from the spinal cord by a dorsal root and a ventral root. The cervical nerve roots were shorter but nerve roots further became longer caudally. Due to the cranial ascend of the conus medullaris after second month of gestation, the spinal nerves which ran caudally in the vertebral canal before passing out through the intervertebral foramen formed the cauda equina in the sacral and coccygeal regions during fourth and fifth month with the filum terminale in the centre.

4.2.7.1 *Spinal Nerve Roots*

The ventral roots consisted of numerous thin rootlets, whereas the dorsal roots had a small number of large, coarse rootlets. The ventral rootlets usually had less tensile strength than dorsal ones.

4.2. 7. 1. 1 Root Emergence Length (REL)

The emergence length of the dorsal and ventral roots of spinal nerves were measured in the last two months of gestation. The root emergence of the dorsal roots were always greater than that of the ventral roots. So, the dorsal roots pierced the dura mater over a larger area compared to the ventral roots.

The greatest root emergence length was measured dorsally at C8 as 1.980 ± 0.020 mm at fourth and as 4.662 ± 0.078 mm fifth month of gestation. Ventrally, the greatest root emergence length was observed at C1, C2, C3 and C8 as 1.050 ± 0.085 mm at fourth month and at C3 as 3.554 ± 0.258 mm at fifth month of gestation. The root emergence length was slightly greater in cervical, rostral thoracic and lumbar

regions of the cord. It decreased rapidly through the sacral region. Ventral root emergence length remained less variable in the thoracic region.

In the thoracic region, dorsal root emergence length was greatest at T1 in fourth and fifth month. In lumbar region, the length was less variable at fourth month but in fifth month, L5 had the greatest length. In the sacral region, S1 had the greatest length in both the age groups. Ventrally, the same was true with T1 and S1, but it varied at the lumbar region.

4. 2. 7. 1. 2 Root Attachment Length (RAL)

The maximum dorsal root attachment length was measured at C2 segment at fourth and fifth month. It measured 2.444 ± 0.176 mm and 7.443 ± 1.060 mm respectively at fourth and fifth month. After lumbar region, the dorsal root attachment length decreased towards coccygeal region. A comparison between dorsal root attachment length and segment length at fifth month of gestation showed that both these parameters followed a similar pattern showing a relation between the two (Fig. 30).

The maximum ventral RAL was recorded as 2.600 ± 0.191mm at C3 segment at fourth and 7.571± 1.295 mm at C2 at fifth month.

The minimum dorsal and ventral root attachment length were observed in the coccygeal region in both the age groups studied. In general, the ventral roots originated over greater area compared with corresponding dorsal roots.

4. 2. 7. 1. 3 Inter Root Length (IRL)

The maximum IRL was noticed at C2 in the fourth and fifth month as 4.500 ± 0.129 and 6.490 ± 0.643 mm respectively. The cervical enlargement region showed a decrease in inter root length (Fig. 30). In two animals studied at the fifth month, no measurable distance was noticed at C8 segment. In the fifth month, the lumbar enlargement region also showed a decrease in inter root length after the level of L6 segment.

4. 2. 7. 2 Dorsal Root Ganglia

The dorsal root of each spinal nerve had a swelling made up of unipolar nerve cells and fibres known as the dorsal root ganglion, from 24 days of gestation onwards in the present study. Grossly, the shape of dorsal root ganglion was ovoid, ellipsoid or

spindle shaped. It was elongated in the caudal lumbar region and levels distal to it. The size varied amongst the groups and in different regions of the cord. The size resembled that of the mustard seed at second month and that of a wheat seed at the fourth month. These ganglia were situated at proximity to the dorsolateral aspect of the cord at variable distance from the intervertebral foramina. The ganglia could be noticed only upto second or third coccygeal segment.

The dorsal root ganglia did not present any grossly distinguishable difference in size and shape between regions upto the level of S3 segment during second month of gestation. The length of ganglia during second month of gestation averaged 0.515 ± 0.002 mm upto the level of S3 segment. After this level, the length decreased to 0.315 ± 0.002 mm. The length of dorsal root ganglia in the last three months of gestation are shown in table 23. In the third, fourth and fifth month, the maximum length of dorsal root ganglia was recorded at C8 segment which measured 1.517 ± 0.244 mm, 2.190 ± 0.468 mm and 5.143 ± 0.237 mm respectively.

The mean width of ganglia during second and third month were 0.500 mm and 0.750 mm respectively upto S3 segment, without any grossly distinguishable difference in size between regions. After S3 segment, the average width decreased to 0.300 mm and 0.650 ± 0.366 mm during these months respectively. The mean width of ganglia for fourth and fifth month of gestation is shown in table 24. It was more at segments of cervical enlargement with maximum width at C7 segment with 2.560 ± 0.133 mm and 3.885 ± 0.197 mm at fourth and fifth month of gestation respectively. After the level of L6 segment, the mean width of ganglia also decreased towards coccygeal region. Eventhough it was observed upto third coccygeal segment in the present study, in most cases, the gross enlargement as the dorsal root ganglia was less detectable after the last sacral segment.

4.3 HISTOLOGY

The present microscopic observations showed that during the first month, the primordium of the spinal cord had the shape of a neural tube, with three layers. The study started with a goat embryo having CRL 14 mm (with a calculated age of 24 days of gestation). The beginning of the spinal cord portion of the neural tube was demarcated by the structure being flanked by paired dorsal root ganglia. The neural

tube lay between the body wall dorsally (Fig. 31) and the notochord ventrally (Fig. 32) during the first month. In the longitudinal section of the curved back, on the sides of the neural tube wall, in addition to ganglia, somites could be identified in 14 mm embryo (Fig. 33).

4.3.1 Morphogenesis and Histogenesis of Spinal Cord in the First Month of Gestation

Three layers could be distinguished in the neural tube during the first month of gestation by 24 days. These were: an inner ependymal (germinal) layer, not distinctly demarcated, but characterized by the presence of actively dividing neuroepithelial cells, a middle mantle layer and an outer marginal layer, made up of myelospongium, into which the axonal processes of developing neurons of the cord grew later (Fig. 31).

The lumen of the neural tube was diamond-shaped in cross-section (Fig. 34). There was a lateral sulcus, the sulcus limitans, at the lateral aspect of the lumen. It divided the sidewalls or lateral plates into two zones. The zone dorsal to the sulcus, was the alar plate, into which the fibres of the dorsal root grew. The ventral zone was the basal plate, from which the ventral root fibres emerged. The walls of the neural tube at the dorsal and ventral aspects of the lumen formed the roof plate and floor plate respectively. The roof plate and alar plate were thinner than the floor plate and basal plate respectively in all regions (Table 25).

In each lateral plate, all the three layers were distinct. Towards the lumen, the neural tube was bounded by an internal limiting membrane and peripherally its extend was marked by an external limiting membrane (Fig. 35). The limiting membranes had a thickness of 2 μm by the end of first month of gestation.

The micrometrical data (Table 25) showed that the maximum height and width of the neural tube in this age was recorded at the regions, which corresponded to the anterior limb buds (which represented the cervical enlargement) followed by the region of posterior limb buds (lumbar enlargement). The minimum height and width of the neural tube were recorded at the coccygeal region.

In this first month, height of neural tube exceeded its width at the lower aspect at cervical, thoracic, sacral and coccygeal regions. The basal plate height was

maximum at cervical enlargement, but the maximum alar plate height was at thoracic region. The maximum mean values for the transverse distances like neural tube width, distance from lumen to neural tube left margin and distance from lumen to mantle layer left margin were recorded at cervical enlargement. The widest alar and basal plates were also seen at cervical enlargement followed by lumbar enlargement.

4.3.1.1 *Ependymal Layer*

By 24 days of gestation, the ependymal layer was a stratified layer composed of neuroepithelial cells. The layer was thin at the basal plate region but was thick in the alar plate region all along the length of the neural tube except at the coccygeal region, where it was equal (Table 25). The neural epithelium was thin over the roof and floor plates. The ependymal layer was the prominent component of the roof plate and floor plate. The roof plate contained only the ependymal layer whereas, the floor plate consisted of ependymal and marginal layers but no mantle layer (Fig. 34).

The neuroepithelial cells, which remained nearest to the internal limiting membrane were elongated with indistinct cell boundaries and were radially arranged about the lumen of the neural tube. Away from the lumen, the cell nuclei were longer with lesser nuclear width. Among these cells, towards the internal limiting membrane, some pale-staining cells with spherical nuclei were also seen, which represented the germinal cells undergoing mitosis (Fig. 36).

Vascularity was more towards the ventral half of the neural tube. The blood vessels were small and round in cross-section at 24 days. In embryos with CRL 15 mm (26 days of age), the blood vessels in the ependymal layer became more channel-like (Fig. 31), with more number of erythrocytes inside. In 16 mm embryo (27 days of age) vascularity around spinal cord increased and the blood channels inside the neural tube were longer (Fig. 37). The blood vessels were enveloped by an endothelial layer and contained nucleated erythrocytes.

4.3.1.2 *Mantle Layer*

The mantle layer was thin in the alar plate (Fig. 31) but was thicker in the basal plate (Table 25). Therefore, the ventral aspect of the neural tube bulged on either side. The mantle layer extended dorsally to the site of entry of the dorsal root. The layer

presented densely packed cells, which were of two types, viz. the neuroblasts and spongioblasts. The neuroblasts were larger cells with round or ovoid vesicular nucleus with homogenous chromatin and faint nucleolus. These cells had paler cytoplasm than the spongioblasts. In this layer, smaller cells with round nuclei, which represented spongioblasts were more (Fig. 38).

The mantle layer became much thicker by 26 days (Fig. 31) than at 24 days (Fig. 34). The processes of spongioblasts became more visible. More number of cells were seen per unit area of alar plate than in the basal plate in all regions. Neuroblasts were clearly seen and formed a deeper layer beneath the spongioblasts. The size of the nucleus ranged from 5.6 to 9.5 μm in large neuroblasts and was only 4 μm in small neuroblasts.

By 27 days, the mantle layer was much thicker than that at 26 days. Alar and basal plates bulged prominently to the lateral side especially at enlargements. The basal plate region showed beginning of differentiation of neurons and two types of neuroblasts could be identified: one was a multipolar neuroblast with a polygonal cell body, whereas the other was a bipolar neuroblast with elongated cell body and indistinct processes (Fig. 39). These proneurons, which formed a second layer beneath the spongioblasts, showed eosinophilic, pale cytoplasm and vesicular nucleus with faint nucleolus. The size varied from 5.6 to 11.4 μm with a nuclear diameter of 3.8 to 9.5 μm. In the alar plate, the neuroblasts were smaller with 3.8 to 7.6 μm diameter. There was a slight increase in the proportion of cytoplasm in neuroblasts. The average number of neuroblasts per unit area ranged between 93 and 105 in the first month of gestation.

4.3.1.3 *Marginal Layer*

The marginal layer formed the outermost zone and contained the processes or fibres arising from the neuroblasts in the mantle layer. Eventhough, this peripheral region lacked cells in the first month, occasionally spongioblasts were observed especially at the floor plate (Fig. 40) and at the entry and exit (Fig. 31) of nerve roots, representing the cells, which accompanied the nerve processes. In the first month, the marginal layer was present ventral to region of dorsal root entry, including dorsal root entry area. This layer was absent at the dorsal aspect of the neural tube. The dorsal, lateral and ventral funiculi started to form by 24 days (Fig. 34).

The marginal layer became thicker but was of loose consistency by 26 days. The dorsal funiculus was still in the rudimentary form, but the marginal layer representing the lateral and ventral funiculi was more developed (Fig. 31). Blood vessels entered the neural tube through marginal layer to reach the mantle and ependymal layers (Fig. 41).

By 27 days, a fibrillar meshwork, the myelospongium, was laid down by the better developed cell processes. The marginal layer was still absent dorsally. The thickness of this layer was more when compared with that of the previous embryos. The average thickness of the ventral funiculus was more at all regions (Table 25).

4.3.1.4 Central Canal

By 24 days, the lumen of the neural tube was diamond-shaped in cross-section with a distinct sulcus limitans in the anterior half of the spinal portion of the neural tube (Fig. 34). Thereafter the walls in the ventral one-fourth of the lumen was in close apposition or even fused (Fig. 42) except in the lumbar enlargement region where the lumen again became wider without any ventral fusion. In the regions caudal to lumbar enlargement, the lumen was again fused in the ventral one-fourth. In the caudal sacral region, the lumen showed a wide upper and narrow middle and lower portions (Fig. 43). In coccygeal region, the lumen was narrow and extended dorsoventrally at the cranial segments (Fig. 44). The caudal most portion of the tail showed an oval neural tube with a rounded lumen.

By 26 days, in regions caudal to cervical enlargement, the sulcus limitans became very shallow or absent. In these regions, the ependymal layer at the ventral half of the neural tube approached each other (Fig. 31) and fused either completely or incompletely leaving gaps in between (Fig. 45).

By 27 days, in addition to the ventral part, the dorsal part of the neural tube also presented a narrow lumen (Fig. 46) so that a diamond-shaped canal could be seen only in the anterior half of the cervical region.

4.3.1.5 Regional Differences

In the cervical region, the dorsal, lateral and ventral funiculi were very thin by 24 days. Alar plate was thin and did not bulge laterally. In regions, which corresponded to the limb buds, the diameter of the neural tube was more due to an

increase in thickness of the mantle layer. In the sacral region, neural tube was smaller with small basal plate (Fig. 47). The mantle layer became reduced in the caudal sacral region and was absent in the coccygeal region (Fig. 44). In the coccygeal region, the cross-section of the neural tube was simpler in structure, and the neural tube wall mainly consisted of external and internal limiting membranes, a very thin marginal layer and thick ependymal layer (Fig. 48). Here thickness of the wall was essentially due to the ependymal zone. The roof plate and floor plate were also thicker. Dorsal and ventral funiculi were very thin. Lateral funiculus was not seen. No distinguishable alar or basal plates and mantle layer could be identified. At the caudal most end, the neural tube presented a round lumen. The wall consisted of only the internal and external limiting membranes with the ependymal zone in between.

4.3.2 Morphogenesis and Histogenesis during Second to Fifth Month of Gestation

4.3.2.1 Morphogenesis

Similar to the first month, the spinal cord varied in size at different levels being enlarged at the cervical and lumbar enlargements and reduced at the conus, but its pattern of structure was uniform throughout its length.

4.3.2.1.1 Morphogenesis in the Second Month

By 40 days of gestation in foetuses with 25 mm CRL, the primordium of spinal cord had the form of a neural tube. From this age onwards, the lumen contained an eosinophilic secretion. The marginal layer was thicker, but still it did not appear at the dorsal aspect of the neural tube.

By 48 days of gestation, in foetuses of CRL 40 mm, the changes were in progress. The spinal cord primordium became positioned in the vertebral canal (Fig. 49). The vertebral body and laminae were cartilaginous, but the dorsal arch was made up of fibrous tissue. The dorsal and ventral parts of the lumen of the neural tube were being obliterated by the apposition of the lateral plates (Fig. 50), thus reducing the height of the lumen of the central canal. In the cervical region, the lumen was elongated (Fig. 51) than in other regions (Fig.52). Processes arose from the basal ends of ependymal cells and extended through the gray matter. The alar plate of both sides grew medially and obliterated the dorsal part of the central canal. The basal plates had grown ventrolaterally and a ventral median fissure was formed into which the pia mater extended. From the ventral median fissure a tract of white fibres extended upto

the central canal. It was the ventral commissure or ventral median raphae (remnant of floor plate), which connected the two halves of the spinal cord ventrally (Fig. 52). The persistent floor plate contained fibres, which crossed from one side to the other. The floor plate had an ependymal zone and a marginal zone. From the ventral end of this floor plate began the upper end of ventral median fissure. The ependymal fibres of the persistent floor plate extended from the central canal to the surface and thus retained their primitive relations. The stage of the neural tube terminated between 40 and 48 days of gestation in the present study.

The spinal cord appeared oval in shape, slightly more flattened on its dorsal surface. The outer white matter and inner gray matter could be distinguished by the middle of the second month. A longitudinal column of nerve cells constituted the gray matter and nerve cell processes constituted the white matter. The gray matter included numerous nerve fibres also originating from its cells or terminating in relation to them. On each side of the cord, the gray matter was divided into dorsal and ventral horns. The mantle layer of alar plate developed into dorsal horns and that of the basal plates formed the ventral horns. In the thoracic, anterior lumbar and middle sacral levels of the cord there was in addition, a lateral projection of gray substance on each side known as the lateral horn. In the groove between the dorsal and ventral or lateral horns, there were extensions of gray matter into the adjacent white matter, the two being intermingled as the reticular formation (Fig. 52).

The white matter occurred in three longitudinal columns, viz. dorsal, ventral and lateral funiculi of nerve fibres, on each side, extending the whole length of the cord. The dorsal funiculi were being formed in the dorsal marginal zone. Dorsal halves of either side fused just above the central canal (Fig. 50), and above this there was an unfused area, consisting of parts of both dorsal horn and dorsal funiculi (Fig. 50). A dorsal median sulcus was seen in the midline dorsally between the two dorsal funiculi. A faint dorsal intermediate groove was seen on either side of dorsal median sulcus dividing the dorsal funiculus into a medial fasciculus gracilis and lateral fasciculus cuneatus. A dorsal median septum started to extend downwards from the dorsal median sulcus between the right and left dorsal funiculi developing on each side of the original roof plate, but still there was a gap between the two dorsal halves of the spinal cord indicating that the fusion between the dorsal halves of spinal cord was not complete. Cellular aggregations were also seen on either side of dorsal midline above

the central canal. This represented the neuroepithelial cells in the ependymal zone of approaching walls of the lumen (Fig. 50). Above the central canal, where the beginning of formation of dorsal median septum was seen, a clustering of astrocytes, star-shaped cells with round or ovoid nuclei could be seen (Fig. 53). The septum was formed mostly of astrocytes and a few oligodendrocytes.

Towards the end of the second month, by 54 days of gestation in foetuses of 60 mm CRL and by 58 days of gestation in foetuses of 71 mm CRL, the length and width of central canal were still reduced (Fig. 54) and the ependymal zone formed a mere lining of the central canal. The wall of the neural tube became thicker. At this age, the dorsal median sulcus and septum had increased in depth due to the development of dorsal funiculi. Cell processes extended laterally from the dorsal median septum. The dorsal intermediate groove and fasciculus gracilis became better developed at this age (Fig. 55). In the region between the two dorsal funiculi, the dorsal median septum still presented fibrous astrocytes and small neuroblasts or neuroepithelial cells (Fig. 56). The ventral median fissure and ventral commissure were better developed and the pia mater, which descended into it presented well developed blood vessels including the ventral spinal artery (Fig. 57). The pia mater extended into the dorsal median sulcus and septum.

By the end of second month, the spinal cord was limited externally by an external (superficial glial) limiting membrane (Fig. 58) formed of astrocytic processes. Internally the spinal cord was lined by an internal limiting membrane, which lined the central canal (Fig. 57). The former had a thickness of 7.6μm whereas the latter was 1.8 μm thick by this age. From this age onwards, the central canal became wide and elongated at the caudal sacral region (Fig. 59), more elongated at the coccygeal region (Fig. 60) and enlarged at the conus medullaris to present the terminal ventricle (Fig. 61).

4.3.2.1.2 Morphogenesis in the Third Month

At the beginning of third month, by 62 days of gestation (in foetuses with 87 mm CRL), the cross-section of central canal presented an elongated shape (Fig. 62) and tapered towards the upper end but it was wide at enlargements (Fig. 63). The dorsal median sulcus and septum became clearer than the previous age. The sulcus became shallow at the caudal lumbar and sacral region and was absent at the

coccygeal region. The clustering of the neuroepithelial cells towards dorsal median septum became less evident (Fig. 64) but was still seen (Fig. 65). In addition to the cells, the septum exhibited thin fibres (Fig. 65), which in turn had thickness of 3.6 to 5.4 μm. Over the dorsal median septum, the pia mater passed with minimal amount of connective tissue actually entering. At the caudal sacral region, the dorsal horns were close together so that they were not separated by the dorsal median septum. The remnant of the floor plate was elongated and was thicker at its upper end near the central canal and was thinner towards its lower end with an average thickness of 36 μm and 14.4 μm respectively, where the pia mater was attached to it as the linea splendens. The glial limiting membrane was still seen closely adherant to piameter at the periphery of the spinal cord.

Towards the end of third month, by 81 days (in foetuses of 130 mm CRL), the central canal became oblong (Fig. 66) and showed stratified columnar epithelium and luminal content. The dorsal median septum had a thickness of 7.2 to 10.8 μm above and 18 to 19 μm below and contained neuroepithelial cells.

4.3.2.1.3 Morphogenesis in the Fourth Month

By 102 days of age, in a foetus with 198 mm CRL, the central canal was oval in shape. Immediately surrounding the central canal was a light granular area composed mainly of neuroglia, the central gelatinous substance (substantia gliosa or area gliosa). At this age, a wedge-shaped area also could be observed above the central canal, containing neuroepithelial cells (matrix cells) (Fig. 67). The internal limiting membrane was 3.6 μm thick.

The dorsal median sulcus became deeper (Fig. 68) and the dorsal median septum was well developed and 18 μm thick, but no dorsal median septum was seen in the caudal sacral region as the dorsal horns closely approached each other (Fig. 69) similar to earlier age groups. The dorsal median septum extended from ventral part of the dorsal median sulcus to the dorsal part of the central canal. Blood vessels from pia mater descended into the dorsal median septum and sulcus (Fig. 70). The astrocytes in the dorsal median septum were with a nuclear size of 5.4 μm. Gray and white commissures connecting the two halves of the spinal cord could be identified from this stage onwards. The gray commissure was divided by the central canal into dorsal and ventral gray commissures (Fig. 71).

A deep dorsal intermediate groove was seen with an average depth of 90 μm (Fig. 70). From this month onwards, a dorsolateral groove with an average depth of 150 μm was identified at the entry of dorsal root fibres (Fig. 72).

4.3.2.1.4 Morphogenesis in the Fifth Month

By 124 days of age in a foetus with 275 mm CRL and by 142 days of age in a foetus with 390 mm CRL, the central canal varied in shape from round (Fig. 73) to ovoid (Fig. 74). The dorsal median septum and the commissures became clearly distinguishable. The septum was well organized with an average thickness of 50.4 μm and extended into the gray commissure (Fig. 75). The dorsal median sulcus was shallow and blood vessels descended into dorsal median septum (Fig. 76). The dorsolateral groove was well developed (Fig. 77). The gray commissure was thick dorsally (175 μm) and thin ventrally (7.2 μm) (Fig. 73). The white commissure was 500 μm thick ventrally. The internal limiting membrane was still seen (Fig. 74). The external glial limiting membrane was well demonstrated towards the end of gestation with a continuous basement membrane at its surface (Fig. 77).

4.3.2.1.5 General Micrometrical Observations

The segment-wise and region-wise average of height and width of precoccygeal spinal cord from second to fifth month of gestation are presented in tables 26 to 31. Among the segments studied, the C8 segment exhibited maximum spinal cord height at all ages (Tables 26 and 27). The spinal cord width was maximum at L6 at second and third month but at C8 at fourth and fifth month (Tables 28 and 29). The cervical enlargement had the maximum height during the entire gestation period. It was followed by the lumbar enlargement in second and third month but in the fourth and fifth month it was immediately followed by the cervical region then by the lumbar enlargement (Tables 30 and 31).

The gray matter height and dorsal horn height were maximum at cervical enlargement at all age groups except during the fifth month of gestation, where it was at lumbar enlargement. The maximum ventral horn height was also seen at the enlargements with the lumbar enlargement predominating during the major part of the gestation. The minimum values for spinal cord height, gray matter height, dorsal horn and ventral horn height in the prococcygeal cord were at the sacral region at all age

groups, since all these values decreased towards the caudal end of spinal cord. All the regions showed a progressive increase in height during the entire gestation.

Sparing a few exceptions at thoracic, lumbar and lumbar enlargement regions between second and third month, the percentage contribution of gray matter height to spinal cord height showed a gradual decrease in all age groups (Tables 30 and 31). The gray matter height expressed as percentage of spinal cord height was maximum at lumbar enlargement from third to fifth month. During the second month, it was at the sacral region. The percentage contribution of dorsal horn to spinal cord height was greatest at the sacral region upto third month, but for last two months the maximum value was at lumbar enlargement, the sacral region occupying the second position (Tables 32 and 33). The highest percentage contribution of ventral horn height to spinal cord height was in lumbar enlargement at all ages. Sparing exceptions at the cervical region and lumbar enlargement region in third month, the dorsal horn height expressed as percentage of spinal cord height showed a decreasing trend. Similarly, except the thoracic and lumbar regions in third month and cervical enlargement and sacral regions in fourth month, all regions showed a decreasing trend in the ventral horn height expressed as percentage of spinal cord height. The dorsal horn and ventral horn height expressed as percentage of gray matter total height did not show any particular trend. During major part of gestation, the ventral horn height expressed as percentage of gray matter total height was more than that of dorsal horn at lumbar enlargement. But at the cervical enlargement, the dorsal horn contribution was more during the major part of gestation.

The greatest values for total width of spinal cord and distance from central canal to spinal cord left margin varied between cervical and lumbar enlargements at different stages of gestation but at fourth and fifth month, the values at cervical enlargement exceeded that of lumbar enlargement, indicating the dominance of cervical enlargement during later stages of gestation. The distance from central canal to gray matter left margin was also more at lumbar and cervical enlargements. Minimum values at the precoccygeal spinal cord were in the sacral region and next minimum being at the thoracic region during last three months of gestation. The total width of spinal cord was greater than the height all levels studied during second to fifth month of gestation. Dorsal and ventral horns were widest at lumbar enlargement followed by cervical enlargement in all age groups from second to fifth month of

gestation. It indicated that eventhough cervical enlargement was having greater width than lumbar enlargement in the last two months of gestation, its increased width was mostly due to increased amount of white matter at cranial levels of the spinal cord.

The percentage of gray matter width decreased after third month of gestation at all regions indicating an increase in the growth of lateral funiculus at later stages of gestation. The percentage of gray matter height started to decrease especially at the cervical and cervical enlargement regions even by the second month, indicating an early growth of ventral funiculus (Tables 30 and 31). A greater rate of growth in height of spinal cord, gray matter, dorsal horn and ventral horn were seen during the latter half of gestation (Table 34). The spinal cord width at all regions showed greater percentage of increase between third and fifth month except the sacral region, which showed a decreasing growth rate towards the end of gestation (Table 35).

4.3.2.2 Histogenesis

4.3.2.2.1 Neurons

By the beginning of second month, the neuroblasts in the alar plate were smaller with an average size of 3.8 to 5.6 μm with 1.9 to 3.8 μm nuclear size. The basal plate exhibited larger neuroblasts with 11.4 to 15.2μm size and with a nuclear size of 7.6 μm. Rarely, the basal plate region of the neural tube also exhibited differentiating multipolar neuroblasts sized 19.0 to 22.8 μm, with large vesicular nucleus and distinct nucleolus (Fig. 78). By the middle of second month, the cell bodies of the neurons in the dorsal horn had a size of 7.6 μm with indistinct cell boundaries (Fig. 53). The ventral horn neurons, which formed aggregations were with eosinophilic cytoplasm and spherical vesicular nucleus (Fig. 51). At enlargements, the ventral horn neurons were with a diameter of 11.4 to 19.0 μm and distinct nucleolus. At this age, it was very difficult to differentiate the smaller neurons from astrocytes.

By the beginning of third month, the neurons of both dorsal (Fig. 65) and ventral (Fig. 79) horns were better differentiated. Eventhough these neurons did not show clear cell boundaries the vesicular nucleus and nucleolous were seen clearly, especially in the neurons of the ventral horn of enlargement regions. No noticable difference was exhibited in the cell size between regions since the cell boundaries could not be identified. Towards the end of third month also, eventhough the cells were better differenciated (Fig. 80), the cell boundaries of neurons were not clear.

By fourth month (102 days), the nerve cell boundaries became clear (Fig. 81). The Nissl bodies appeared, but were not demonstrable with the usual histological staining methods using haematoxylin and eosin, but needed special staining methods like PTAH (Fig. 82).

The neurons became very large with increased cytoplasmic content by the beginning of the fifth month than at the earlier age groups (Fig. 83). The Nissl bodies became better developed and were demonstrated in all neurons of the spinal cord including both the large alpha and small gamma neurons of the ventral horn (Fig. 84). Eventhough the nucleus was centrally positioned at this stage generally in all neurons of different spinal cord regions, a characteristic eccentric nucleus was found in the Clark's column (Fig. 85). Towards the end of gestation, typical multipolar neurons with well-developed Nissl bodies, which were demonstrable even with haematoxylin and eosin were seen (Fig. 86). The neurons in the present study had a size of 3.8 μm at the neuroblastic stage by the second month and attained a maximum width of 75 μm at the cervical and lumbar enlargements during the fifth month. The shape of the neurons varied according to their specific location. Most of the neurons were multipolar. But some were spindle-shaped or spherical in shape as those observed in the lateral horn.

As the age advanced, the number of neuroblastic cells per unit area decreased. It was 53 to 60 by second month, 13 to 52 by third month and 9 to13 by fourth month. By the beginning of fifth month, there were 9 to 10 alpha neurons and 5 to 6 gamma neurons per unit area. Towards the end of gestation, it decreased to 5 to 6 alpha neurons and 2 to 3 gamma neurons per unit area owing to a gradual increase in size of the neurons.

4.3.2.2.2 Neuroglia

In addition to the various types of neurons, the spinal cord contained the interstitial cells, the neuroglia. Four types of neuroglia could be identified towards the end of gestation as astrocytes, oligodendrocytes, microglia and ependyma.

By 40 days, astrocytes and oligodendrocytes with a size of 3.6 to 7.2 μm could be seen among the differentiating neuroblasts and spongioblasts (glioblasts) in the mantle layer. The nuclei of these cells were smaller than those of the neuroblasts. The astrocytes had a larger and paler nucleus than oligodendrocytes. There were two

types of astrocytes: protoplasmic astrocytes predominantly seen in gray matter and fibrous astrocytes predominant in white matter. Protoplasmic astrocytes were seen with star-shape, oval nucleus and numerous wavy and profusely branching cytoplasmic processes. Fibrous astrocytes were also seen towards the periphery of the gray matter with elongated nuclei and long, straight less branching processes (Fig. 78). Oligodendrocytes were with more rounded nucleus and scanty cytoplasm and were found in both the gray and white matter. Processes of all these developing cells made up a background meshwork, the neuropile in the mantle layer. By the middle of the second month, astrocytic nuclei could be seen as pale stained and ovoid. Among neuroglia, oligodendrocytes predominated and resembled the developing spongioblasts at this stage, had smaller, dark and rounded nuclei, than those of astrocytes with a fair amount of heterochromatin and scanty cytoplasm (Fig. 53). Occasionally cell nuclei were also seen in the marginal layer. Among these, the flat, oval nuclei belonged to the fibrous astrocytes and round nuclei to oligodendrocytes (Fig. 87). The dorsal median septum was formed mostly of astrocytes and a few oligodendrocytes.

By third month, the glial cells in the white matter were also better differentiated (Fig. 88) with clear distinction between fibrous and protoplasmic astrocytes. Oligodendrocytes (3.6 to 5.4 μm) were similar with round nucleus and scanty cytoplasm and occurred both as perineuronal satellites in gray matter (Fig. 79) interfascicular cells in white matter and juxtavascular cells near blood vessels (Fig. 65). The fibrous astrocytes were with elongated nuclei and long processes. The astrocytes had a size of 1.8 μm in white matter and 3.6 μm in gray matter.

By fourth month, the histoarchitecture was similar but the cell density increased and microglia started to appear (Fig. 89). The neuroglia became clearly distinguishable by fifth month. Astrocytes presented pale, oval or round nucleus in the gray matter (Fig. 85) with a nuclear size of 5.4 to 7.2 μm. The astrocytes were the largest among neuroglia. Elongated nuclei and processes of fibrous astrocytes could be clearly distinguished towards the end of gestation in the white matter (Fig. 90). These had perivascular feet and some of their processes formed the superficial glial limiting membrane beneath the pia mater of the cord, which presented a continuous basement membrane at the cord surface towards the end of gestation.

Oligodendrocytes were similar, had fewer and smaller processes than astrocytes and were numerous by the beginning of the fifth month. The microglia were smaller and similar to oligodendrocytes (Fig. 91) with a size of 1.9 μm and were seen both in white and gray matter being more in the latter. As the vascularity increased according to advancement of age, more neuroglia were seen as perivascular satellites (Fig. 92). Oligodendrocytes started to curve around axons to form myelin sheath towards the end of gestation (Fig. 93). These cells forming myelin sheath attained a size of 11.4 to 15.2 μm at this stage. At all ages, the number of neuroglia were more than that of the developing neurons.

By the middle of second month also, the ependymal cells around the central canal formed a stratified layer (Fig. 49) but with decreased thickness. Ependymal cells developed cilia from this age onwards and were with round and pale nucleus with homogenous chromatin and distinct nucleoli. From fourth month onwards, the ependymal cells formed a pseudostratified layer (Fig. 71) and had nuclei with a size of 3.8 to 5.4 μm. The cell boundaries were not clear and the nucleolus was absent. The cilia had a thickness of 1.0 μm where as their height varied from 7.2 through 10.8 to 25.2 μm. By the beginning of the fifth month, the lumen was lined by columnar cells, which presented cilia and had width of 4.5 μm. The cilia were very short or almost absent upto lumbar region at the end of gestation (Fig. 73), but were present at sacral and coccygeal levels.

4.3.2.2.3 Gray Matter

By the middle of second month, the mantle layer differentiated into the dorsal and ventral horns of gray matter. The intermediate gray matter between dorsal and ventral horns, which projected laterally as the lateral horn contained the intermediolateral nucleus (Fig. 52). The gray matter in cross-section was in the form of the letter 'H' or a butterfly. The dorsal and ventral protuberances on either side were the dorsal and ventral horns respectively, which were the largest in the cervical and lumbar enlargements (Fig. 94). The central intermediate substance surrounded the central canal with dorsal and ventral gray commissures located dorsally and ventrally and was continuous with the lateral intermediate substance located between dorsal and ventral horns. The cells of the gray matter were arranged in distinct groups, the nuclei.

The different layers or laminae of gray matter could not be differentiated by the second month, but in the ventral horn clustering of nerve cells were seen at this age mainly towards the ventrolateral aspect (Fig. 51), which marked the beginning of formation of nuclear aggregations in the lamina IX. These aggregations became better defined towards the end of second month at cervical enlargement (Fig. 95) and lumbar enlargement (Fig. 96). By the beginning of the third month, the lamination of gray matter was not clear except at lamina II representing the substantia gelatinosa, lamina VII forming the intermediolateral nucleus and lamina IX constituting the nuclear aggregations in the ventral horn. But towards the end of this month, other laminae of gray matter started to differentiate which became better differentiated by the fourth month. The gray matter presented ten laminae towards the end of gestation. The average cell size of different laminae from I to X except IX at fifth month of gestation are shown in table 36.

The gray substance was a mass of nerve cells and fibres with neuroglial elements of astrocytes and oligodendrocytes and blood vessels extending the length of the cord. By the fifth month, the neuropile network, which supported the cell bodies consisted of non myelinated axons, oval or round dark nuclei of oligodendrocytes, pale oval muclei of astrocytes and the fibrous network compiled by the neuronal and neuroglial processes (Fig. 97).

4.3.2.2.3.1 Dorsal Horn

The dorsal horns started to show better differentiation towards the latter half of third month and by the end of this month, the dorsal horn presented a pointed apex, an expanded caput or head and a somewhat constricted cervix (Fig. 98). By fourth and fifth month, the dorsal horn presented a marginal zone, the substantia gelatinosa and the body of the dorsal horn, which contained the nucleus proprius and nucleus dorsalis. The marginal zone of Waldeyer (Lissaeur) was represented by lamina I. It formed a thin layer of scattered gray substance as a cap on the surface of dorsal horn and was situated in the zona spongiosa or lamina I. Beneath the marginal cells was the substantia gelatinosa, which formed the outer cap-like portion of the head of the dorsal horn and was situated in the lamina II. The head and cervix of the dorsal horn was occupied by the nucleus proprius corresponding to lamina III and IV. This was an ill-defined cell column except in fifth month at lumbar region. Lateral to this nucleus,

the small and medium sized cells found in the reticular process belonging to lamina V constituted the nucleus reticularis. The Clark's column was placed in the medial portion of the base of the dorsal horn along thoracic and lumbar regions. By fourth month (Fig. 71) gray commissural fibres connecting the two dorsal horns could be seen.

4.3.2.2.3.1.1 Lamina I

The lamina I, which represented the future marginal zone could not be distinguished upto the end of the second month (Fig. 99). By the beginning of third month of gestation, the layer presented stellate cells with vesicular nucleus, nucleolus, eosinophilic cytoplasm and 5.4 μm size (Fig. 88). Towards the end of third month, lamina I contained loose fibres and very few cells (Fig. 98). The layer covered the surface of the dorsal column especially the apex as a cap with a thin layer of cells of spindle shape, pyramidal or polygonal form with varying sizes. These cells were scattered and were arranged tangential to the surface of the dorsal gray column. On an average one or two cells were seen in each region with 5.4 to 7.2 μm width. Lumbar region presented large (14.4 μm) cells with 7.2 μm sized nucleus. This zone was found in all the segments studied and so extended throughout the length of the cord.

By fourth month, this lamina became measurable as a layer with a thickners of 54.0 to 72.0 μm (Fig. 81). The tangentially arranged cells presented an elongated body. The cell size varied from a minimum of 7.2 μm at thoracic region through 14.4 μm at other regions to a maximum of 28.8 μm at lumbar enlargement.

By the fifth month, the cells were better differentiated (Fig.100). The number of cells per unit area was more at lumbar enlargement. The thickness of this layer varied from 54 to 79.2 μm. The maximum cell size of 14.4 μm width and 46.8 μm length was recorded towards the end of gestation.

4.3.2.2.3.1.2 Lamina II

Lamina II corresponded to the substantia gelatinosa. It extended the entire length of the cord and was located beneath the lamina I. This layer was not organized upto the middle of second month, but forerunners of lamina II were seen as clustering of cells forming the outer cap of the head of the dorsal horn at this age (Fig. 50). It consisted of tightly packed small cells on the dorsolateral aspect of dorsal horn. These

neurons were with 3.6μm size and had round, vesicular nuclei with one or rarely two nucleoli (Fig. 101). The lamina II was not fully organized by 54 days and contained large fibres from dorsal funiculus and blood vessels but had very few glial cells (Fig. 102). By 58 days, this layer became better organized (Fig. 99). The cells were seen with ovoid or rounded nuclei. Two types of cells were seen, viz. one with dark nuclei and the other with pale nuclei. The cells with pale nuclei formed a second layer beneath the former.

No convolutions were seen in the substantia gelatinosa by the beginning of third month. It presented only the cellular aggregations, which were the forerunners of substantia gelatinosa. The cells were tightly packed with blood vessels and erythrocytes among them. These neurons were very small with only a rim of perinuclear cytoplasm. Of the two types of cells present, one type possessed rounded and deeply stained nuclei with a size of 3.6 μm. In between these cells the second type of cells were seen with pale nucleus and evident nucleolus (Fig. 103). The cells with pale nuclei were seen more towards the dorsal part where as the second type were seen throughout the substantia gelatinosa. Towards the end of third month, the lamina II was organized into form and presented prominent fibres (Fig. 80). The cells were small with nuclei of the same size, 3.6 μm. Erythrocytes, small blood vessels and astrocytes were seen among the cells. It presented convolutions from this age onwards but convolutions were not seen at all segments. Only T9 and L3 segments presented convolutions. At this age, the glial cells in lamina II had the similar appearance of as that of lamina I.

By fourth month of gestation, the frequency of occurrence of convolutions increased among segments. It was seen at C1, C4, C6, T2, T3, T5, L2 and S2 segments. The lamina II varied in shape at different segments, viz. conical at cervical region (Fig. 81), large and rounded at regions of enlargements (Fig. 104), small, conical or rounded at thoracic region (Fig. 105) and flattened at lumbar region (Fig. 106). From this age onwards, at the caudal thoracic region from T9 to L1 segments, the substantia gelatinosa was flattened (Fig. 106) and approached the same of the other side so that the dorsal horns were at close proximity (Fig. 107). The layer consisted of small cell bodies, which had a size of 3.6 to 5.4 μm and were arranged in

vertical or radial orientation in clumps or miniature palisades and the layer was penetrated by fine nonmyelinated fibre bundles (Fig. 104).

By the beginning of fifth month, lamina II presented a similar appearance with small cell bodies and fine nonmyelinated fibres (Fig. 108). It was convoluted (Fig. 77) in most of the segments (C1, C2, C4, C8, T2, T3, T6, T9, T10, L1, L2, L3, L5 and L6). At this age, the cells were not tightly packed and were alligned in vertical or radial orientation in clumps or miniature palisades. The cells were small with ovoid or polygonal in form. One type of cells were triangular or rounded with 5.4 to 7.2 μm size and vesicular nucleus having 3.6 μm size and nucleolus. The second type of cells resembled oligodendrocytes and predominated in this layer with a size of 3.6 to 5.4 μm with scanty cytoplasm and dark condensed nucleus. Rarely astrocytes were seen with 7.2 μm size and comparatively larger and pale nucleus. Cytoplasm was scanty. Towards the end of gestation, the cells of this layer had a maximum size of 25.2 μm with 7.2 μm sized nucleus. The cells were not tightly packed. Between and beneath these cells fibres arising from collateral branches of substantia gelatinosa were found (Fig. 100). Eventhough, in most of the segments, it exhibited convolutions, at the sacral level, substantia gelatinosa was prominent with rounded periphery (Fig. 109).

The segment-wise and region-wise average of width and height of the substantia gelatinosa are presented in the tables 37 and 38 respectively. The width and height showed progressive increase during the gestation. The width was always more than the height. The lumbar enlargement presented the maximum width at all ages. Among the segments of lumbar enlargement, L6 presented the maximum size.

4.3.2.2.3.1.3 Lamina III

The lamina III was less distinguishable during second month. By the beginning of the third month, the cell boundaries were not clear, but still a less packed area could be seen at the base of lamina II forming a band across the dorsal horn (Fig. 64) representing the lamina III. Towards the end of third month, this layer consisted of less packed neurons with a size of 10.8 to14.4 μm and with a nuclear size of 5.4 μm. The lamina was rich in axons, which were nonmyelinated at this age (Fig. 80). By fourth month, this lamina presented an average height of 54 μm and width of 288 μm. Neurons were less in number and had a length of 10.8 to 18.0 μm (Fig. 104).

At this age, this zone entered into the ventral part of the lamina II and together constituted the substantia gelatinosa (Fig. 81). By fifth month, this zone had a light appearance, was rich in nonmyelinated axons (Fig. 110) and had 360 μm height and 500 μm width. The size of neurons varied with a maximum 46.8 μm towards the end of gestation.

4.3.2.2.3.1.4 Lamina IV

The head and cervix of dorsal horn formed the lamina III and IV. These regions corresponded to the nucleus proprius. The clustering of polygonal cells seen at the base of dorsal horn on the dorsolateral aspect of central canal towards the middle of second month marked the histogenesis of nucleus proprius (Fig. 52). This nucleus could be seen as a poorly defined cell column towards the end of second month (Fig. 102) with an approximate size of 60 μm and consisted of polygonal and spindle shaped cells, which had a size of 5.4 to 11.4 μm with a nuclear size of 3.8 to 7.6 μm.

Eventhough lamina IV was not clear even by the beginning of the third month, occasionally neurons representing nucleus proprius were seen at the base of the dorsal horn at this age (Fig. 111). Towards the end of the third month, by 81 days, this poorly defined cell column with diffuse borders became much clear and was represented by polygonal cells with a size of 15.2 to 19.0 μm, located in the central part of the head and cervix of dorsal horn (Fig. 66, 80). It extended throughout the length of the cord and corresponded to lamina III and IV. It was mixed with bundles of fibres on either side of the lumen. By this age, in the anterior thoracic segments it presented only one or two cells, but in the caudal thoracic and lumbar segments it was well developed and the cell number was more. In the lumbar enlargement, it was large and had 90 μm height and 54μm width.

By fourth month, this lamina consisted of cells, which varied in size from 14.4 μm at cervical region, through 18.0 μm at other regions to 21.6 μm at cervical enlargement with a nuclear size of 10.8 μm. The cells representing nucleus proprius were less in number in most of the regions (Fig. 71), but was more in the lumbosacral region (Fig. 104). At the lumbar enlargement, it was larger and had 250 μm height and 187 μm width. It presented large neurons with 21.6 μm size and small neurons with 10.8 μm size.

By the beginning of fifth month, the layer was the broadest of the first four layers with 937.5 μm width and 375 μm height. The size of the cells varied from 10.8 to 32.4 μm. Their nuclear size varied from 7.2 to 14.4 μm respectively. Spindle-shaped and polygonal cells were scattered throughout this zone and gave a heterogenous, less compact appearance (Fig. 112). The nucleus proprius was still a poorly defined cell column with diffuse cell borders. At this stage, this cell column presented an average of 375 μm width and 275 μm height. By the end of fifth month, the size of this nucleus varied between regions. It was well developed in the lumbar and lumbosacral regions. A fibrous capsule started to form around this cell column at this age (Fig. 113). It had a size of 325, 187 and 337.5 μm at cervical region, cervical enlargement and lumbar enlargement respectively. The cells varied in size with the maximum width of 43.2μm in lumbar and lumbar enlargement regions towards the end of gestation.

4.3.2.2.3.1.5 Lamina V

The reticular nucleus representing the lamina V started to form by the beginning of the second month (Fig. 52) but was more clear towards the end of this month and was seen at the neck of the dorsal horn. Near the base of the dorsal horn, in the groove between the dorsal and ventral or lateral horns this nucleus was seen as presenting a series of projections of gray matter as the reticular process into the adjacent white matter of the lateral funiculus. Here the gray and white matter intermingled as the reticular formation (Fig. 114), which extended along the whole length of the spinal cord.

By third month, the neurons in the reticular nucleus were with indistinct cell boundaries and an average nuclear size of 3.6 μm (Fig. 115). The cells were most numerous in the zone between the ventral part of the dorsal gray horn and the lateral funiculus of white matter. It was with an average width and height of 87.5 μm each by this age in most regions, which increased to 95 μm width and 133 μm in height at the cervical enlargement.

The reticular nucleus contained polygonal cells (Fig.116) with 6.0 to 18.0 μm size and an average nuclear size of 10.8 μm by fourth month. This lateral part had an average size of 162 μm as width and 225 μm height. The smaller neurons in the

medial part had a size of 10.8 μm. The reticular formation measured 125 μm in height and width at thoracic region.

By the beginning of fifth month, lamina V found consisted of lateral and medial parts. The lateral part represented the reticular nucleus (Fig. 112) was with spindle shaped or polygonal neurons having a maximum 32.4 μm size and centrally positioned, 14.4 μm sized nucleus. Nucleolus and Nissl bodies were distinct. The medial part consisted of smaller neurons having a size of 10.8 to 14.4 μm. By the end of gestation, reticular nucleus had a size of 375 μm. The maximum cell size was 39.6 μm. It showed bundles of myelinated fibres (Fig.117), which varied in size from 37 μm to 64.8 μm. The total size varied between 187 and 212 μm. Reticular formation was seen in all regions but was better developed at cervical level with well developed fibre tracts. The axons of these cells passed in part to the lateral funiculus of the same side. The reticular formation was less developed in other regions (Fig. 118).

4.3.2.2.3.1.6 Lamina VI

Upto fourth month, lamina VI was less distinguishable with cells ranging in size from 10.8 to 25.2 μm (Fig.71). But by the beginning of the fifth month, it was identified as a broad layer with indistinguishable boundaries, located lateral to central canal. It consisted of a compact medial zone, which presented numerous dark stained, medium to small sized cells, and a lateral less compact zone with larger triangular or star-shaped scattered perikarya (Fig. 119). It presented cells with 10.8 to 36 μm in size and 7.2 μm sized nucleus. The medial (Fig. 74) and lateral (Fig. 120) zones showed similar features towards the end of gestation, by 142 days.

4.3.2.2.3.2 Lateral Horn

4.3.2.2.3.2.1 Lamina VII

This layer was represented by most of the intermediate zone of gray matter, which presented intermediolateral nucleus, intermediomedial nucleus, cervical nucleus of Stilling and Clark's column. The neurons in this intermediate gray matter ranged from 14.4 to 25.2 μm at fourth month, 14.4 to 36.0 μm by the beginning of fifth month and were larger reaching upto 54 μm width towards the end of gestation.

4.3.2.2.3.2.1.1 Intermediolateral Nucleus

The lateral horn and the intermediolateral nucleus appeared by the middle of second month (Fig. 52) and extended from C8 or T1 to L2 segments (Fig. 115). It was also seen at the middle sacral region (Fig. 121). This nucleus was better organized towards the end of second month (Fig.114).

By the beginning of third month, the neurons in this nucleus were not as differentiated as those of the ventral horn. The nucleus and the cell boundaries of neurons were not clear with approximately 5.4 μm size. Towards the end of third month, this nucleus was better differentiated, and measured 75 μm size with spindle-shaped cells (Fig. 80). The neurons were with 7.6 to 11.4 μm in size with acidophilic and scanty cytoplasm with condensed nucleus. At L2, lateral horn was almost missing but was represented with only one neuron of 7.2 μm size.

By fourth month, the intermediate gray matter included two dispersed nuclear groups. The medial part was medioposterior column and the lateral part was intercornual column. The medioposterior column bordered the Clark's column dorsolateral to central canal and extended into dorsal gray commissure. By this age, this cell column had a height and width of 150 μm each. It consisted of small to medium-sized, spindle-shaped and polymorphic cells (Fig. 122).

The intermediolateral nucleus appeared as a clear cell column in lateral horn. The horn was surrounded by fibres (Fig. 116). The cells in this nucleus were less differentiated by fourth month (Fig. 123) than those towards the end of gestation (Fig. 124).

The lateral horn with the intermediolateral nucleus was well developed by the beginning of fifth month also (Fig. 116). The medioposterior column presented 25.2 μm sized neurons with 7.2 μm nuclear size. The intercornual column was with 36 μm sized neurons with 7.2 μm nuclear size. Towards the end of this month, the lateral horn presented a size of 87.5 to 125 μm in width. Typically spindle-shaped neurons were seen (Fig. 118) with vesicular nucleus and 36 μm length and 7.2 μm width. The processes extended from these neurons at the lateral horn towards the neurons of intermediomedial nuclei (Fig. 125).

4.3.2.2.3.2.1.2 Intermediomedial Nucleus

The intermediomedial nucleus appeared for the first time towards the end of third month in the thoracic (Fig. 98) and lumbar (Fig. 80) regions. This nucleus was seen with small sized neurons lateral to central canal and ventral to Clark's column. The intermediomedial nucleus was seen ventral to Clark's column at lumbar enlargement (Fig. 122). Towards the end of fifth month, the intermediomedial nuclei had an average size of 275 μm in anterior and middle thoracic region. But this nucleus became small with a size of 50 μm at caudal thoracic segments (Fig. 126). The Nissl bodies were finer and uniformly distributed in the intermediomedial and intermediolateral nuclei. Towards the fifth month, this nucleus was small at T1 and had 87.5 μm size with neurons of 14.4 μm width and 25.2 μm length with an average nuclear size of 10.8 μm.

4.3.2.2.3.2.1.3 Cervical Nucleus of Stilling

Towards the end of third month by 81 days, this nucleus was noticed at the anterior cervical segments, at the medial part of base of dorsal column. It presented neurons of 19.0 μm size with a nuclear size of 5.4 μm (Fig. 66). It was ill-developed in regions other than the cervical region.

In the fourth month, by 102 days, the cervical nucleus of Stilling was seen with multipolar neurons of 21.6 to 25.2 μm size and height and width of 250 μm each (Fig. 67). At the same position at sacral region cells representing sacral nucleus of Stilling was also seen (Fig. 127).

By the beginning of fifth month, it was noticed with a size of 25.2 μm by 124 days (Fig. 119). Towards the end of fifth month, at C1 and C4 it was larger with 187.5 μm in size and the size decreased to 50 μm at C5. The cells were elongated with 43.2 to 79.2 μm length and 18 to 28.8 μm width.

4.3.2.2.3.2.1.4 Clark's Column

In the thoracic region, lateral to central canal in the intermediate gray matter clustering of cells could be seen from middle of the second month onwards, which marked the beginning of Clark's column (Fig. 52). It became more differentiated towards the end of second month with a maximum of 150 μm height and 60 μm width at cervical enlargement. By 81 days, Clark's column had neurons of 7.2 μm in size

and nonmyelinated fibres. The column had 100 μm height and 87.5 μm width at T9, 112.5 μm height and width at T10 and T11 and was scattered with 75 μm height and 125 μm width at T12.

By the fourth month, Clark's column (Figs. 71, 122) presented large neurons with 18 to 28.8 μm size and smaller neurons with 7.2 μm size. The size of this nucleus at thoracic region increased to 312.5 in width and 162 μm in height. It presented nonmyelinated fibres, blood vessels and oligodendrocytes and neurons. The nucleus of neurons had a size of 7.2 μm and was eccentrically placed from this age onwards (Fig. 128). At L4 segment, the Clark's column was oval in shape and measured 375 μm in width and 187 μm in height. At L5 segment, the size was reduced to 250 and 125 μm in width and height respectively. At L6, it was dispersed and consisted of only a few scattered neurons.

By fifth month of gestation, Clark's column had 187 μm width and 225 μm height at T3 and T6 segments. The neuron size was 36 μm with a nuclear size of 18 μm. At T9, Clark's column was very prominent and was surrounded by fibres, with 162 μm height and 212.5 μm width. The neurons had a size of 28.8 μm with nuclear size of 14.4 μm. At L3, it was with a neuron size of 43.2 μm and nuclear size of 21.6 μm. The small neurons had 18 μm size with nuclear size of 14.4 μm. In the lumbar region, this column was large (Figs. 113, 129) and reached a maximum size of 375 to 437 μm and was even divided at L1 and L2 segment with four to five neurons, which were oval or rounded in shape with 19.8 to 28.8 μm size. At L3 also, it was large with a cell size of 31.2 to 37.5 μm, but at L4 it was small and at L5 it was dispersed and consisted only of a few scattered cells. The cells were better differentiated, had eccentric nuclei and presented elongated shape by this age (Fig. 130).

4.3.2.2.3.3 Ventral Horn

The lamina VIII and IX together constituted the ventral horn. The ventral horn presented multipolar neurous with 22.8 μm size, large vesicular nucleus and eosinophilic cytoplasm by the beginning of third month (Fig. 79). The nucleus was eccentrically placed in most cases. Towards the end of third month, neuronal size was 19.0 to 22.8 μm with the size of nucleus varying from 5.7 to 7.6 μm.

4.3.2.2.3.3.1 Lamina VIII

The smaller neurons which constituted lamina VIII could not be differentiated by the beginning of third month (Fig. 115), but were seen by the end of third month (Fig. 80). By fourth month, this lamina became a heterogenous mixture of small and medium sized cells with scattered occasional large perikarya (Fig. 131). This lamina was not sharply differentiated from lamina VII. Neurons were with a size of 10.8 to 18 μm by fourth and fifth month. The nuclear size varied from 7.2 to 10.8 μm. By fifth month, occasionally large neurons with 43.2 μm size and nuclear size of 25.2 μm were also seen.

4.3.2.2.3.3.2 Lamina IX

This layer was composed of the largest cells of the spinal cord, namely the alpha motor neurons. The alpha motor neurons formed nuclear aggregations in the ventral horn. Eventhough clustering of cells started in the ventral horn as forerunners of the nuclear aggregations by the middle of the second month (Fig. 94), they became better defined by the end of the second month (Fig. 95, 96). Initially upto the middle of the second month, only four groups of nuclear aggregations were seen in the ventral horn. The medial nuclear group consisted of dorsomedial and ventromedial nuclei and the lateral group consisted of ventrolateral and dorsolateral nuclei. Of these, dorsomedial and ventromedial nuclei occurred in all regions of the spinal cord but all the four nuclei occurred only at enlargements. In addition to these four nuclei, by 58 days of gestation (Fig. 96), central and retrodorsolateral nuclei were observed in the ventral horn. The neurons of these nuclei had an approximate size of 7.6 μm by the end of second month.

At the beginning of third month, by 62 days, nuclear aggregations were seen but the cells were still with indistinct boundaries. These aggregations were well developed at cervical (Fig. 132) and lumbar (Fig. 133) enlargements but not so at other regions (Fig. 134) during this age. In the cranial segments of cervical enlargement a phrenic nerve nucleus was also seen (Fig. 132). The cells were better differenciated (Fig. 80) and a similar condition occurred towards the end of third month also.

The size of neurons and total size of the nuclear aggregations at fourth and fifth month are shown in table 39. The cell size was more at the enlargements especially towards the end of gestation. By fourth and fifth month, the lateral nuclear masses were always sharply delimited at enlargements where as medial nuclear masses were often less defined and had diffused borders with the lamina VIII. Within each nuclear group, two types of neurons were seen: large alpha motor neurons and small gamma interneurons (Figs. 135). By fourth and fifth month, the small perikarya had 10.8 to 14.4 μm size and the size of the large ones varied between different nuclear aggregations (Table 39). The large motor neurons of ventral horn had a large, central, vesicular nucleus and coarse Nissl bodies in the cytoplasm and were largest in the lumbar and cervical enlargements. These neurons were smaller in other segments. Scattered among the large ventral horn cells were smaller cell bodies of gamma neurons. Nissl bodies appeared by 102 days (Fig. 82) and the cell boundaries became clear. By this age, the cell columns could be clearly distinguished as the medial (Figs. 136) and lateral nuclear groups (Fig. 137) at the enlargements which became more differentiated towards the end of gestation (Figs. 138, 139). But at the other regions mainly dorsomedial and ventromedial nuclei were seen. In caudal thoracic and anterior lumbar regions, in addition to these, a ventrolateral nucleus was also seen (Fig. 140). A spinal accessory nucleus was also seen from C1 to C5 segment level (Fig. 141). The total size of the nuclear aggregations increased according to age (Table 39).

4.3.2.2.3.3.3 Lamina X

This layer was represented by gray matter surrounding the central canal (Fig. 71). By fourth month, the cells of lamina had an average size of 10.8 μm without any regional variation. By the fifth month, the neurons in lamina X could be clearly identified (Fig. 142).

4.3.2.2.4 White Matter

It was in the middle of the second month by 48 days, the outer white matter and inner gray matter became differentiated first (Fig. 52). The white matter occurred in three longitudinal columns, viz. dorsal, ventral and lateral funiculi of nerve fibres, on each side, extending the whole length of the cord. The thickness of the three funiculi in different regions at different stages of gestation are presented in table 40. All the

funiculi showed a progressive increase during the gestation period and a tremendous increase in the amount of white matter was noticed towards the latter half of gestation especially between fourth and fifth month of gestation. A remarkable increase was noticed in the amount of white matter from the sacral region towards the cervical region in the fifth month of gestation. Eventhough the ventral funiculus had maximum width at first month, the lateral funiculus exceeded other funiculi in thickness in all regions at all ages from second to fifth month. The tracts in the white matter were very difficult to be identified, but at their respective area of location clustering of fibres and cells could be seen. The definite levels of origin and termination were also very difficult to identify.

In the white matter of the spinal cord by 40 days of gestation, in addition to fibres the darkly stained spherical nuclei of oligodendrocytes and elongated nuclei of fibrous astrocytes could be seen. Later, the nerve fibres passing through the white matter had blood vessels and fibrous astrocytes on their sides. From the middle of the second month onwards, occasionally blood vessels could be seen in the white matter. By fourth month, blood vessels presented perivascular satellites with the astrocytic processes extending towards blood vessels (Fig. 143). The blood supply increased as age advanced to the end of gestation (Fig.144). The number of cells in the white matter also increased as the gestation advanced (Figs. 133, 89, 144).

The processes of multipolar neurons extending through the white matter could be well demonstrated towards the end of gestation as both bundles and tracts (Fig. 145). Oligodendrocytes were seen forming myelin sheaths around axons towards the end of gestation (Fig. 146). Other oligodendrocytes with definite cell boundaries without forming the myelin sheath were also seen associated with nerve tracts as interfascicular cells and free cells in the white matter (Fig. 93). The white matter became a mixture of myelinated and nonmyelinated axons, blood vessels and neuroglia (Fig. 147). Except in the blood vessels, collagen was absent in the spinal cord tissue. Eventhough a typical myelin sheath was observed only towards the end of gestation, clear and vacant spaces occurred around axons by fourth month itself representing sites of myelination (Figs. 143, 144). Rarely, nerve cell bodies were also seen in white matter (Fig. 93). With silver stains, astrocytes were seen to possess numerous processes and microglia were seen as small elongate cells with polar processes.

The white matter increased from sacral to cervical level (Table 40). A decreasing trend of the gray matter percentage, during the progress of gestation indicated a gradual increase in the white matter percentage. Sparing a few exceptions between second and third month, the percentage contributions of gray matter height to spinal cord height showed a gradual decrease from second month itself (Tables 30 and 31), indicating the development of ventral funiculus. The percentage of gray matter width decreased after the third month of gestation. It indicated an increase in the growth of lateral funiculus at a later stage of gestation when compared with the ventral funiculus.

4.3.2.2.4.1 Dorsal Funiculus

The dorsal funiculus of spinal cord between C1 and T13 segments presented fasciculus gracilis and fasciculus cuneatus separated by the dorsal intermediate groove from the middle of second month onwards by 48 days (Figs. 50, 68, 111). In the lumbar (Fig. 63) and sacral (Figs. 69, 148) regions the dorsal funiculus was undivided. The fasciculus gracilis was ill-defined at the middle of the second month (Fig. 50) and was very narrow towards the end of the second month (Fig. 55). Fasciculus gracilis was better developed from third month onwards (Fig. 111) but was narrower than fasciculus cuneatus (Table 41). Eventhough the height of fasciculus gracilis and fasciculus cuneatus were the same in the second month, as the dorsal median septum and dorsal funiculus developed later, the former exhibited an increase in height.

By fourth month, in addition, an intersegmental tract, the dorsal fasciculus proprius was seen as bundles of axons at the dorsal aspect of gray commissure bordering the dorsal funiculus (Fig. 68). The dorsal intermediate groove was still not well defined even upto the end of gestation. From S1 segment and caudally, no dorsal median septum was present to separate the dorsal funiculi of either side. At sacral region, dorsal commissure could be seen as fibres crossing from one side to the other and connecting the dorsal part of two halves of the spinal cord (Fig. 69).

4.3.2.2.4.2 Lateral Funiculus

At the dorsolateral aspect of the dorsal funiculus, a dark stained area could be seen from middle of second month onwards, which formed the beginning of formation of dorsolateral fasciculus or fasciculus of Lissauer (Fig. 50). By third

month, it was located at the entrance of dorsal root fibres (Fig. 98). It became better developed by fourth month (Fig. 149) and later (Fig. 150).

By the beginning of third month, a clustering of axons were seen at the periphery of the lateral aspect of the lateral funiculus which might be the forerunners of the dorsal and ventral spinocerebellar tracts (Fig. 151). Since the boundaries of these tracts were indistinguishable, extend of these tracts also could not be clearly understood but these could be seen at different stages of gestation (Figs. 152, 153). Towards the end of third month, vacant spaces around axons representing lateral corticospinal tract were seen on either side in the lateral funiculus lateral to reticular formation (Fig. 152). Fibre bundles having 21.6 μm width constituting the corticospinal tract were clearly seen at the base of the dorsal horn towards the end of gestation in the cervical region.

Towards the end of third month, the lateral reticulospinal tract appeared lateral to the ventral horn and lateral fasciculus proprius on the medial aspect of lateral funiculus as clusters of axons and oligodendrocytes (Fig. 152). It was seen as bundles of fibres by the fifth month (Fig. 154).

By fourth month, in addition to these tracts, the lateral funiculus presented an aggregation of fibres, representing the rubrospinal tract near the corticospinal tract (Fig. 72). It became better developed later (Fig. 155).

Lateral fasciculus proprius was seen by the end of third month (Fig. 152) as a pale-staining area due to axons bordering the gray matter. This tract became well distinguishable by fourth month (Fig. 156).

4.3.2.2.4.3 Ventral Funiculus

All tracts of spinal cord except medial longitudinal fasciculus and medial vestibulospinal tract were mixed with other tracts and were not seperated into well-defined bundles. Medial longitudinal fasciculus occurred as a fibre bundle located on either side of the ventrolateral aspect of central canal, on either side of the base of ventral median raphae (Fig. 52). The size of the tract varied from 15.2 μm width and 38 μm height by the middle of the second month to 62.5 μm width and 125 μm height towards the end of the fifth month (Fig. 157). It became well defined from the end of third month onwards (Fig. 158).

The medial vestibulospinal tract was seen at the base of ventral median fissure ventral to medial longitudinal fasciculus from middle of the second month onwards (Fig. 52), which became well developed by the end of third month (Fig. 158). It had a size ranging from 75 to 187 μm at the last two months.

The ventral fasciculus proprius, which was seen bordering the gray matter, was ill developed during the second month (Fig. 159). From the third month onwards it became better developed (Figs. 158, 140, 160).

Other tracts in the ventral funiculus became more defined towards the end of third month. By fourth (Fig. 160) and fifth month (Fig. 161), aggregation of axons were seen towards the periphery of the ventral funiculus near the ventral median fissure. These represented other tracts near the ventral median fissure, like the ventral corticospinal tract and the ventral reticulospinal tract (Fig. 160), which had a thickness of 112.5 μm and 212.5 μm respectively by the fifth month. The other tracts bordering the ventral median fissure, like tectospinal and lateral vestibulospinal tracts were also seen as mixtures of axons and oligodendrocytes and vacant spaces (Fig. 161).

4.3.2.2.5 Central Canal

The central canal extended throughout the entire length of the spinal cord in goat. It continued cranially through the lower part of the medulla oblongata, into the fourth ventricle. It was diamond-shaped in the middle of second month with a secretion seen inside the lumen. In the lumen, cell debris was seen along with clusters of denuded cells and erythrocytes (Fig. 162). A shallow sulcus limitans was also observed during this age. From the level of sulcus limitans, the processes of ependymal cells extended outwards towards the periphery (Fig. 51). Clustering of ependymal cell processes were seen at the ventral aspect of the central canal (Fig. 163). The extend of ependymal zone was reduced from the middle of second month onwards, but still the ependymal cells around the central canal formed a stratified layer (Fig. 49). At the centre, this stratified layer was thick. At the lower portion of central canal, the thickness of ependymal layer decreased to 15.2 μm. At the upper half of central canal, the ependymal cells got intermingled with other neuroglial cells and formed a thicker layer having a thickness of 57 μm. Ependymal cells developed cilia from middle of second month onwards (Fig. 164). Some neuroepithelial cells

were still seen on the outer aspect of these ependymal cells. The number of cells increased towards ventral aspect. Towards the end of the second month, the central canal decreased in height in cross-section, than that at the beginning of second month. The central canal became wide and elongated at the caudal sacral region (Fig. 59), more elongated at the coccygeal region (Fig. 60) and enlarged at the conus medullaris to form the terminal ventricle (Fig. 61) by second month.

By the beginning of third month, the central canal was elongated and narrow at cervical (Fig. 62), thoracic and lumbar (Fig. 111) segments, but at segments of enlargements the central canal was wider (Fig. 63). The ependymal cells were arranged in two to four layers on the lateral side. The nuclei of neighbouring cells were situated at various distances from the lumen. This was especially evident along the dorsoventral axis, where the ependyma had pseudostratified appearance. In a few instances, blood capillaries were located within the ependymal epithelium. At the ventral aspect the thickness was more, viz. 68.4 μm. At the upper end, the ependymal layer consisted of more number of cells. At the lower end, the layer was thinner with smaller number of cells. Caudally, at S4 segment the spinal cord presented a narrow and dorsoventrally elongated central canal, lined by a thinner ependymal layer. The central canal was located in the ventral half of the spinal cord, starting from the middle of the spinal cord and extending upto the ventral side. At caudal sacral (S4) and coccygeal regions, the central canal was dorsoventrally elongated into the terminal ventricle with an irregular shape (Fig. 165). It was wider above and narrow below. The ependymal layer was thick above and thinner below. Unlike other levels, the central canal of the conus medullaris varied in shape with folded lumen and occupied a large area of the cross-section of the spinal cord (Fig. 166).

From fourth month onwards, the central canal was placed eccentrically in the gray commissure at all levels (Fig. 127). The ependymal layer surrounding the central canal formed a pseudostratified ciliated columnar layer (Fig. 167). Upto five layers of nuclei could be identified in the ependymal layer. In regions of enlargements, the lumen was round in cross-section, but in other regions it was oval. From S1 onwards, the central canal was elongated and appeared narrow. At S4 level, it was located at a ventral level (Fig. 168) as in the previous age groups and presented terminal ventricle towards the caudal end.

In the fifth month of gestation also, the shape of the central canal varied from round to oval (Figs. 73, 74) upto the sacral region where the central canal was elongated and located at ventral level. The lumen was lined by columnar cells, which presented cilia at the beginning of fifth month. The cilia were very short or almost absent upto lumbar region towards the end of gestation (Fig. 73), but were present at sacral and coccygeal levels (Fig. 169). The central canal was eccentrically placed in the gray commissure so that the gray commissure was thicker dorsal to the central canal and was very thin ventral to the central canal. Ventrally, a thick white commissure was seen. The central canal was surrounded by area gliosa, which consisted of only nonmyelinated fibres (Fig. 169).

The lumen contained an eosinophilic material from the beginning of second month onwards. The luminal secretion was not seen towards the end of gestation upto caudal part of sacral region (Figs. 73, 74), but the luminal secretion was demonstrated at the caudal sacral region at the end of gestation (Fig. 169). The internal limiting membrane was still seen clearly.

Average height, width and ependymal layer thickness of central canal are presented segment-wise and region-wise in tables 42 and 43. By second month, the cross-section of central canal was elongated and narrow especially in the cervical region. It was wider and longer at the first cervical segment, but decreased in size at C2 and C4 segments. The C1 segment followed the same pattern during later stages of gestation also. The ependymal layer thickness showed a regional variation during second month, with the minimum thickness at the sacral region in the precoccygeal cord. During later stages, it did not show much variation in thickness between regions. By the fifth month, a reduction was noticed in the ependymal thickness at all regions (Table 43).

The reduction in the size of central canal was marked between third and fourth month. The height of the central canal decreased from 15.097 per cent at third month through 5.380 at fourth, to 3.780 per cent at fifth month at the cervical region. The corresponding values were 13.235, 6.351 and 4.611 per cent at cervical enlargement; 15.476, 7.267 and 5.110 per cent at thoracic; 16.030, 7.286 and 5.680 per cent at lumbar; 15.502, 7.391 and 5.483 per cent at lumbar enlargement; 20.080, 16.190 and

13.321 per cent at sacral and 49.020, 29.676 and 25.470 per cent at coccygeal region. It indicated a size reduction at all regions towards the end of gestation.

The region-wise average for distances of dorsal median sulcus at dorsal surface and ventral median fissure at ventral surface to central canal are shown in table 44. The percentage of these distances to total spinal cord height are shown in table 45. The percentage of dorsal median sulcus to central canal distance (DMS to CC distance) was more than the percentage of ventral median fissure central canal distance (VMF to CC distance) during second and third month of gestation. The maximum DMS to CC distance percentage was at the sacral region during fourth and fifth month, but it was minimum at sacral region at second and third month. It indicated a more ventral shifting of the central canal towards the end of gestation in the sacral region. In other regions, the upper end of central canal started approximately from the central point itself. Later, the VMF to CC percentage exceeded DMS to CC percentage except at lumbar enlargement and sacral regions during the fourth month and except in the sacral region during the fifth month.

4.3.2.2.6 Regional Differences

4.3.2.2.6.1 Cervical Region

Gray matter total height decreased from C1 to C4 level at all ages except at fifth month. Ventral horn height was maximum at C1 at all ages. The height and width decreased from C1 to C4 level during the greater part of gestation (Tables 26 to 29). The cervical region presented the maximum vertical thickness of dorsal funiculus. The region also exhibited the second highest values for thickness of ventral and lateral funiculi during the fifth month, indicating an increased amount of white matter in the cervical region (Table 40).

Gray matter was characterized by the presence of well developed reticular formation (Fig. 66), cervical nucleus of Stilling and spinal accessory nucleus. At the latter half of third month, the cervical nucleus of Stilling appeared on the medial part of the dorsal horn. The nucleus proprius was also seen.

The dorsal horn was wider than the ventral horn (Tables 30 and 31). The C1 segment presented the maximum value for width and height at all ages for substantia gelatinosa in this region (Table 37). Substantia gelatinosa did not present any

convolutions at the cervical region but was semicircular in shape at second month. The convolutions started to appear towards the termination of third month.

The ventral horn was with pointed ventral ends at cervical region (Fig. 170) starting from second month but at C4 segment, it became wider at the ventral end towards the end of gestation. From second month onwards, the ventral horn presented an indistinct ventromedial nucleus. Towards caudal cervical segments, vascularity was increased and ventrolateral nucleus also started to appear. In addition to this, above the ventrolateral nucleus the spinal accessory nucleus was seen. Ventromedial nucleus was still indistinct. By third month, an indistinct dorsomedial nucleus and a ventromedial nucleus with an average width and height of 195 μm and 150 μm respectively were seen in the cervical region. At this age, the maximum size of the multipolar neurons in the cervical region was 39.6 μm. At C3 dorsomedial nucleus was larger.

The spinal accessory nucleus was seen from C1 level to C4 and C5 segments on the ventrolateral aspect of ventral horn (Fig. 170) from the end of second month onwards. It had a size of and 150 μm width and 75 μm height at this age. By the fifth month, this nucleus was seen in the middle of ventral horn (Fig. 141) with a size of 175 μm and cell size of 39.6 μm.

In between ventrolateral and spinal accessory nucleus, a phrenic nerve nucleus was also seen at caudal part of cervical region (Fig. 170) from the latter half of second month onwards. By the fifth month, the phrenic nerve nucleus had 250 μm total size and 10.8 to 25.2 μm cell size at C5 segment (Fig. 132).

4.3.2.2.6.2 Cervical Enlargement

The cervical enlargement had the maximum height during the entire gestation period (Tables 30 and 31). The gray matter height and dorsal horn height was maximum at cervical enlargement at all age groups except during the fifth month of gestation. The total width of spinal cord and distance from central canal to spinal cord left margin was maximium in the cervical enlargement during later stages of gestation, viz. at fourth and fifth month, eventhough at second and third month it occupied the second position behind lumbar enlargement. Eventhough cervical enlargement was having greater width than lumbar enlargement in the last two months

of gestation, its increased width was mostly due to increased amount of white matter at cranial levels of the spinal cord. This region presented the maximum values for the vertical thickness of ventral and lateral funiculi at the fifth month. The thickness of all funiculi, except transverse thickness of dorsal funiculus at the fifth month were more at the cervical enlargement than the lumbar enlargement in the last two months.

Maximum spinal cord height and gray matter total height was at C8 segment at all ages. The maximum value for dorsal horn height varied between C8 and C6. The ventral horn height and other vertical distances also varied between the three segments studied (Tables 26 and 27).

The cervical enlargement had prominent appearance of gray matter (Fig. 171). From second month onwards at C6, the ventral horn bulged laterally. Fibre content and vascularity were increased (Fig. 172). Blood vessels were seen all over the spinal cord but were seen more in ventral horn. Size of multipolar neurons was greater (11.0 to 19.0 μm) by second month in the cervical enlargement when compared to cervical region.

From the level of C8 and T1 onwards intermediolateral nucleus, reticular formation, intermediomedial nucleus and Clark's column were also visible by the middle of second month.

Lateral funiculus was well developed and therefore the intermediate gray matter medial to lateral funiculus appeared narrower from second month onwards (Fig. 171).

In the ventral horn at the second month, neurons with dark stained nuclei with indistinct nucleoli (Fig. 172) were clustered together to indicate the formation of the nuclear aggregations at the cervical enlargement. From C6 segment onwards, these nuclear aggregations were well developed in the ventral horn (Fig. 173). Dorsolateral, dorsomedial, ventrolateral and ventromedial nuclei measured 150 x 60 μm, 195 x 195 μm, 90 x 120 μm and 150 x 150 μm respectively at this stage of gestation.

By the beginning of third month, the ventral horns became wider. The lateral nuclei were more organized than the medial nuclei (Fig. 132). Dorsomedial nucleus was better organized all along the cervical enlargement at this age. From the end of third month onwards, the ventral horns bulged laterally with nuclear groups well

developed but at T1 segment the ventral horn again became narrow on the ventral side.

The phrenic nerve nucleus was located at C6 segment between ventromedial and ventrolateral nuclei at the ventral margin of the ventral horn (Fig. 171).

The size of neurons and total size of the nuclear aggregations in the last two months are shown in table 39, exhibiting an increased size in this region.

4.3.2.2.6.3 Thoracic Region

This region presented the second lowest value for the total width behind sacral region in the precoccygeal region during last three months of gestation. At thoracic region, the height of the spinal cord was maximum at T5 than the other two segments at all ages. The maximum values for gray matter total height and dorsal and ventral horn height varied between the segments (Tables 26 and 27). Width was more at T12 in all age groups (Tables 28 and 29). By the fifth month, at T12 segment the dorsal and ventral horn width was less compared to that of lumbar cord, but it was more prominent than ventral gray column observed in T5 and T8 segments.

The thoracic region was characterized by narrow ventral horn, Clark's column and prominent lateral horn with intermediolateral nucleus (Fig. 140). From second month onwards, in addition to all these reticular formation and nucleus proprius were also seen in all thoracic segments.

By second month, the thoracic segments became narrow and towards the caudal aspect of thoracic region, the segments became narrower and smaller but at third month, upto T9 spinal cord was smaller and at T12, it became wider.

The dorsal horn presented reticular formation. Substantia gelatinosa was conical from fourth month onwards, but by fifth month it became wider.

The lateral horn presented intermediolateral nucleus from second month onwards. The height and width of the nucleus was 90 μm each at 48 days. The neurons had a size of 7.6 to 11.4 μm. This nucleus became faint at the middle thoracic region but became more prominent at more caudal levels. Towards the end of second month, the size of this nucleus increased upto 120 μm at T4 level but decreased to 30 μm at T9 level. The size increased again towards 105 μm at T12 segment.

By third month, the measurements of this nucleus ranged between 125 x 90 μm and 150 μm x 180 μm. During fourth month, it ranged from 150 to 165 μm. By fifth month, the lateral horn attained a height of 187 μm at T2 to T6 segments but the width decreased from 250 μm at T2 to 125 μm at T6 segment.

The ventral horn became narrow at the ventral part and presented the ventromedial and dorsomedial nuclei. The ventromedial nucleus had a width of 135 μm at second month. Towards the end of third month, the dorsomedial nucleus was dispersed with 105 μm height and 75 μm width. Ventromedial nucleus had 150 μm height and 75 μm width. From T9 onwards, a ventrolateral nucleus was also observed for supply towards abdominal musculature (Fig. 140).

From the beginning of third month at thoracic levels, fasciculus gracilis was more elongated and clear. A dorsal intermediate septum was present at levels cranial to T13 segment and divided the dorsal funiculus into fasciculus gracilis and fasciculus cuneatus. Towards the end of third month, fasciculus gracilis, fasciculus cuneatus, medial longitudinal fasciculus and vestibulospinal tract were seen in this region.

4.3.2.2.6.4 Lumbar Region

The spinal cord height and gray matter total height increased from L1 towards L3 segment at all ages (Tables 26 and 27). Mostly, the maximum values for dorsal and ventral horn heights and all width were at L3 but at times it varied between the other segments also. The appearance at L1 was similar to the T12 segment. A rounded Clark's column was present in this region. This prominent group of neurons formed a continuous column of cells from lumbar region upwards upto C8 segment.

From fourth month onwards, the substantia gelatinosa became laterally elongated. Nucleus proprius and reticular formation were seen at second month, but even towards the end of third month nucleus proprius was not well developed (Fig. 80). By the fifth month, this nucleus was large and had larger sized neurons.

The lateral horn disappeared at the level of L2 and reappeared again at L4. By the fifth month, Clark's column became well developed (Fig. 129) but correspondingly, the intermediomedial nucleus located ventral to Clark's column became small.

From the end of second month onwards, at lumbar segments, ventral horn became wider than that at the thoracic region. From L2 segment onwards blood supply was increased. The lumbar region presented ventromedial and dorsomedial nuclei in the ventral horn. Towards the end of third month, each had a size of 75μm. L1 segment also presented an ill-developed dorsolateral and a well developed ventrolateral nucleus (Fig. 80) to supply abdominal musculature.

4.3.2.2.6.5 Lumbar Enlargement

The spinal cord height was more at L6 segment compared with L4 and S2 at all ages, whereas the maximum value for height varied between L4 and L6 eventhough L6 had the maximum values for most of the age groups (Tables 26 and 27). The segments of this region were enlarged in area and had a greater width. The total width of spinal cord, distance from central canal to spinal cord left margin and ventral horn width were more at L6 segment at all ages, but the maximum values for distance from central canal to gray matter left margin and dorsal horn width varied between L4 and L6 at different ages (Tables 28 and 29).

The gray horns were massive and the gray matter height expressed as percentage of spinal cord height was maximum at lumbar enlargement in all age groups from third to fifth month (Tables 32 and 33). The maximum mean value for the distance from central canal to left gray matter margin was recorded at lumbar enlargement during the period from second to fifth month of gestation (Tables 30 and 31). Dorsal and ventral horns were widest at lumbar enlargement followed by cervical enlargement during this period indicating that eventhough cervical enlargement was having greater width than lumbar enlargement at last two months of gestation, its increased width was mostly due to increased amount of white matter at cranial levels of the spinal cord.

Lamina I presented more number of cells per unit area at the lumbar enlargement during fifth month of gestation. The substantia gelatinosa started to show convolutions during third month at L5, was laterally elongated by fourth month and was convoluted and prominent by fifth month (Fig. 174). From the latter half of third month onwards, nucleus proprius was larger when compared with other regions.

The lateral horn reappeared at L4 after its disappearance at L2 segment. The nucleus became smaller at L5, with dispersed neurons.

At this region, Clark's column was well developed and reticular formation and intermediomedial nucleus were also seen.

The ventral horn height expressed as percentage of gray matter total height exceeded that of dorsal horn during the major part of gestation at lumbar enlargement (Tables 32 and 33). The ventral horn bayed out laterally and presented prominent nuclear aggregations. By the middle of second month, the ventral horn became wider and exhibited ventromedial, ventrolateral, dorsomedial and dorsolateral nuclei but the dorsomedial nucleus was dispersed in form. Towards the end of second month, the nuclear groups were larger. Towards the end of third month, the fibre content of the gray matter increased at lumbar enlargement. The nuclear aggregations were well developed. All the nuclei were with an average of 125 μm width and 25.2 μm sized neurons. Dorsal funiculus was undivided. Ventral cotricospinal tract was seen at the ventrolateral aspect of the ventral funiculus on either side of the ventral median fissure at L5 segment. By fifth month, lateral reticulospinal tract and rubrospinal tract were also detected at L6 level.

4.3.2.2.6.6 Sacral Region

The height and width were less at the sacral region when compared with other regions located at its cranial level (Tables 30 and 31). The percentage contribution of the dorsal horn to spinal cord height was the greatest at the sacral region upto the third month, but for last two months, this region acquired the second position, where the maximum value was at the lumbar enlargement (Tables 32 and 33). The percentage increase of spinal cord width, central canal to spinal cord left margin and central canal to gray matter left margin showed a decreasing trend towards the end of gestation at the sacral region (Table 35).

Central canal became narrower at S1, was elongated and located more ventrally at S2 (Fig. 175) and was wider at S4 segment (Fig. 59).

Eventhough a dispersed substantia gelatinosa and undivided dorsal funiculus were seen at S1 segment at second month, from fourth month onwards S1 showed a large substantia gelatinosa (Fig. 109) with rounded periphery. S2 segment was with a fused dorsal horn and substantia gelatinosa at all ages (Fig. 69), but between the dorsal funiculus still a gap existed, which represented a dispersed dorsal median septum by the end of third month.

Nucleus proprius and reticular formation were seen at S4 level. The sacral nucleus of Stilling was seen at S1 but from S2 onwards it was dispersed. The lateral horn and intermediolateral nucleus was seen upto S2 segment. It was dispersed thereafter.

The ventral part of the spinal cord and the ventral horn were wider upto S2 (Fig. 59). The ventral horn projected laterally and had well developed nuclear aggregations at S1 and S2, which formed a part of the lumbar enlargement (Fig. 137). At S4, the nuclear aggregations were dispersed, but the ventromedial and dorsomedial nuclei were seen.

From S4 segment onwards the white matter was decreased in amount (Fig. 168). At the fifth month, at S1 segment the dorsal white commissure could be seen as white fibres crossing from one side to the other and connecting the dorsal part of the two halves of the spinal cord. At S4, the gray commissure was shorter and the distance between the two substantia gelatinosa and the dorsal horn was reduced.

Sacral region exhibited an undivided dorsal funiculus and a ventral funiculus with medial longitudinal fasciculus and other tracts (Fig. 59) from second month onwards. Other fibre tracts bordering the ventral median fissure like corticospinal, tectospinal, lateral vestibulospinal and reticulospinal tracts were also seen in the ventral funiculus from fourth month onwards (Fig. 175). In addition, the dorsal and ventral spinocerebellar tracts were seen in the lateral funiculus at S1 segment (Fig. 176).

4.3.2.2.6.7 Coccygeal Region

The micrometrical data of the coccygeal region are presented in table 46. The anterior segments of the coccygeal region was similar to the caudal sacral segments. The medial longitudinal fasciculus was still seen. The dorsal median septum was dispersed. The substantia gelatinosa of either side communicated. The dorsal horn was large but ventral horn was smaller. The central canal started from about the middle of the spinal cord and extended upto the ventral side. The dorsal and ventral funiculi were smaller. The lateral funiculus was wider.

Still caudally, the structure was similar but spinal cord became smaller in size, the gray matter was reduced and the region presented the terminal ventricle

(Fig. 165). At the conus medullaris, the structure was simple and was similar to the neural tube (Fig. 61). The ependymal layer was extensive and was composed of neuroepithelial cells. The mantle layer or the gray matter was thin and was composed of neuroblasts and spongioblasts. Only very few cells were seen next to the ependymal zone in the gray matter, which resembled the mantle layer. White matter or marginal layer was more extensive. The central canal extended dorsoventrally and formed the terminal ventricle. The lumen was wider above. The roof plate and floor plate were composed of ependymal and marginal layers. The roof plate was thicker than the floor plate. Vessels were seen on the ventral and dorsolateral aspects. At distal coccygeal region nerve bundles were seen inside the dura mater.

4.3.3 Spinal Nerves

In the first month of gestation itself by 24 days, the dorsal and ventral roots of spinal nerves were formed. The fibres from the neuroblasts in the basal plate emerged out as the ventral root of spinal nerve. The processes from the ganglionic cells of dorsal root entered the marginal layer of the alar plate. The dorsal roots formed a compact bundle (Fig. 177) where as the ventral root consisted of separate fibre bundles (Fig. 37). The dorsal roots were covered by a connective tissue sheath except at the base (Fig. 177). Therefore dorsal roots had an uncovered portion at the base and a covered portion for the rest of its length. Between the individual fibre bundles of the ventral root, connective tissue cells and erythrocytes (representing future blood vessels forming vasa nervorum) were seen (Fig. 37). So each of these fibre bundles had separate connective tissue coverings and blood supply. Spindle-shaped ganglia were associated with the dorsal root (Fig. 178), whose fibres grew out from its cells. The fibres of the ventral root arose from the mantle layer of the cord and joined the dorsal root in the common nerve trunk (Fig. 179). The common nerve trunk divided into a dorsal ramus and a ventral ramus. The dorsal ramus supplied the dorsal mass close to the ganglion and the ventral ramus coursed ventrally for supplying limbs and ventral musculature (Fig. 179). At all levels the dorsal roots joing its respective ganglia could be seen.

During later stages also, capillaries were seen along the course of the nerves representing vasa nervorum , with a thickness of 5.7 μm (Fig. 180). Along the course of nerves, elongated nuclei (of 1.9 μm size) of the cells forming perineurium were

also seen (Fig. 180). Near the spinal cord, the number of cells covering the nerves were more. The dorsal root formed a compact bundle (Fig. 181) and was thicker near ganglia and thin near spinal cord. Ventral root had several bundles. The dorsal root fibres passed through dorsal funiculus (Fig. 182) and ventral roots through ventral funiculus (Fig. 183).

4.3.3.1 Dorsal Root Ganglia

In the first month, the longitudinal section of curved back exhibited the dorsal root ganglia as lying in a series like bead-like enlargements, one behind the other (Fig. 33) at regular intervals agreeing with the position of somites. It indicated that the segmentation of the neural crest had taken place by 24 days of gestation. Any histological evidence of the ganglionic capsule could not be detected during the first month of gestation. The supporting elements of ganglia formed a syncytium, in the meshes of which the neuroblasts were found. The neuroblasts in the ganglia presented light stained nuclei, where as the supporting (satellite) cells were with small, round and darkly stained nuclei (Fig. 184). Occasionally nucleated erythrocytes and thin-walled blood vessels were also seen. By 26 days, the ganglionic neuroblasts had a pale nucleus with a very faint nucleolus. The nucleus was proportionately very large in satellite cells and the cytoplasm was scanty, but the neuroblasts exhibited cytoplasm and the cytoplasmic processes. By 27 days, blood vessels were seen inside the ganglia. Nuclei in the ganglionic neurons were oval or spherical in shape with a size of 6 to 8 μm. The cell boundaries were indistict at this stage also.

By the middle of the second month, ganglia were located inside the vertebral canal (Fig. 52). By the end of second month by 58 days, from cervical to caudal sacral region, the ganglia were at the intervertebral foramen (Fig. 185) but at the caudal most end at coccygeal region the ganglia were in the vertebral canal (Fig. 61). The ganglia were located extradurally (Fig. 186). The cell boundaries of ganglionic cells became clearly distinguishable. There were two types of ganglionic cells: small cells with a size of 3.6 μm to 7.2 μm by second month and large cells with a size of 7.2 to 10.8 μm by the middle of the second month and 14.4 to 18 μm by the end of second month. Ganglionic cells were spherical with pale staining, round vesicular eccentric nuclei with nucleolus (intermediate neuroblasts) and scanty acidophilic cytoplasm (Fig. 187). Towards the end of second month, the cytoplasmic content of the cell

increased. Satellite cells, which surrounded neuronal cell bodies were small, elongated, oval or polygonal and were with dark oval nuclei and eosinophilic cytoplasm. These cells had a size of 3.6 to 5.4 μm and resembled oligodendrocytes.

By third month of gestation (Fig. 188), the ganglionic cells and satellite cells presented similar features as those of the second month. The size of ganglionic neurons from third to fifth month of gestation is shown in table 47.

By fourth month, large and small ovoid or spherical neurons were seen. These cell bodies were characterized by their smooth surface. The nuclei of these cells were eccentrically placed, but some cells started to show centrally placed nuclei (Fig. 189). The satellite cells became arranged as a row on the periphery of the neurons from this age onwards. The cell boundaries of satellite cells were still not clear and they had the same nuclear size of 3.6 to 5.4 μm. Blood vessels were seen in the ganglion (Fig. 189) and capsule (Fig. 190). Nissl bodies could be seen in the ganglionic cells from 102 days onwards (Fig. 190).

By the beginning of fifth month, most of the ganglionic neurons had central nucleus but still a few had eccentric nucleus (Fig. 191). Towards the end of gestation, the nucleus became centrally positioned in ganglionic cells. Satellite cells surrounded ganglionic cells in one or more rows. The layers of satellite cells were more around larger neurons. In addition to satellite cells, which presented round nuclei, a second type of supporting cell, the capsule cell, could be identified from 124 days onwards. The capsule cells had fusiform, darkly stained nuclei and formed an outer layer covering the inner layer of satellite cells (Figs. 191, 192). Definite Nissl bodies could be seen in the ganglionic cells by the fifth month of gestation (Fig. 193). When stained with Aldehyde Thionine, the cytoplasm appeared granular and presented vesicles (Fig. 191). The nucleus-cytoplasmic ratio of ganglionic neurons decreased as the age advanced (Table 48).

By second month, a thin capsule of ganglia was seen, which was composed of a single layer of flattened connective tissue cells (Fig. 180). It had a thickness of 3.6 μm. Medially, dura was adherent to the ganglionic capsule. Towards the outer aspect of ganglia, connective tissue and blood vessels were attached (Fig. 180). The ganglionic capsule was continuous with perineurium and epineurium of peripheral nerves (Fig. 181). Towards the end of second month, connective tissue trabeculae

extended from the capsule into the ganglion and the neurons were seperated into irregular groups by strands of connective tissue and by bundles of nerve fibres (Fig. 181). Thus each ganglionic cell was invested by both cellular and fibrous connective tissue membranes.

By third month, the capsule was made up of wavy collagen fibres with a thickness of 3.6 to 5.4 μm. Nerve fibre bundles and blood vessels with a thickness of 38 μm and 19 μm respectively were seen in the capsule (Fig. 194). Ganglia had rich blood supply as the interstitial space and capsule presented rich blood network and bood vessels from the capsule penetrated into the ganglionic substance. Small capillaries of 10.8 μm in size were also seen in the interstitial space.

The nonmyelinated nerve fibre bundles, penetrated the ganglion dividing it into different compartments (Fig. 190) by fourth month. The capsule thickness did not increase from the previous age (Table 47).

The capsule of ganglia was of a similar structure (Fig. 195) by fifth month and the thickness varied from 5.4 to 7.2 μm by the beginning of the month to 10.8 to 43.2 μm towards the end of gestation. In the interstitial space, nerve fibre bundles composed of axons having 5.4 μm thickness and blood vessels were seen. Blood vessels in capsule were 18 μm in size. Thicker nerve fibres entered into the ganglion (Fig. 196). Blood vessels were seen on both the outer and inner aspects of ganglion. Some of the nerve fibres inside the ganglion were myelinated. The myelin sheath was formed by coiling of the satellite cells around the axons (Fig. 197). Some nerve fibres possessed thick myelin sheath.

The ganglia at coccygeal region were very small and located extradurally in the vertebral canal (Fig. 61) even from second month onwards. The large neurons had a size of 36 μm and the small ones had 18 μm. These neurous had 10.8 μm sized nucleus and nucleoli. Satellite cells had 3.6 μm width and 7.2 μm height. At coccygeal region, the capsule was thick and presented a thickness of 10.8 μm by the fifth month.

4.3.4 Meninges

By the first month, only a vascular layer, which represented the pia mater of the primitive meninx, was seen on the outer aspect of the neural tube by 24 days. This

layer was composed of mesenchymal cells and capillaries (Fig. 35). By 26 days, the surrounding mesenchyme of the neural tube was organized into a 3.6 μm thick simple squamous cell layer, making the primitive piamater of the meninx, closely adherant to the neural tube. Capillaries were seen among the mesenchymal cells (Fig. 198). By 27 days, the condensed mesenchyme, which represented the meninx, showed more than one (usually two) layer of flattened cells with a thickness of 3.6 μm (Fig. 37). Still it did not form a continuous layer especially at the dorsal aspect. Blood channels were seen in spaces surrounding the meninx. On the dorsolateral aspect of the neural tube, increased number of vascular channels were seen. On the ventromedial aspect also, a blood channel representing ventral spinal artery was observed at this age.

Surrounding the neural tube, by 40 days, meninges were seen with no epidural space except at the intervertebral foramina. The meninges presented an inner vascular layer, which represented the leptomeninges including pia mater and arachnoid and an outer fibrous layer, which represented dura mater. The pia mater presented blood channels and could be seen as a layer of flattened cells with capillaries in between and was of 3.6 μm thickness. The arachnoid could not be identified as a separate layer. Dura mater was thicker with four to five cell layers. Blood sinuses were seen between dura mater and pia mater. Channels filled with blood were seen at the dorsolateral and ventral aspects outside the spinal cord.

By 48 days, the outer layer of meninges representing the dura mater was closely adherent to the internal surface of vertebral canal dorsally, where no space was seen between the meninges (Fig. 199). But ventrally, the vertebral body and laterally vertebral laminae were seen and the meninges were separated from the spinal canal by an epidural space containing blood channels. The vertebral arches were not united. At the ventrolateral aspect, the three layers of meninges could be identified from 48 days onwards. Trabeculae of arachnoid could be clearly recognized (Fig. 200).

4.3.4.1 *Dura Mater*

Dura mater was differentiated by 40 days and consisted of a connective tissue layer with three cell layer thickness (3.6 to 5.4 μm). Flattened nuclei of cells could be identified (Fig. 200). An epidural space containing anastomosing venous channels and areolar connective tissue was seen between vertebral canal and dura mater (Fig. 51). This space had a width of 38 μm by the middle of the second month. Between dura

and the arachnoid was the capillary subdural space. The dura was attached to the outer surface of the arachnoid by thread-like trabeculae. By fourth and fifth month of gestation, dura became very thick and contained blood vessels, collagen fibres, nerves (Fig. 201) and elastic fibres (Fig. 202). Both inner and outer surfaces of the dura mater were covered by a single layer of flattened cells (Fig. 202). The dura presented a thickness of 14.4 to 18 μm and the subdural space was 150 μm wide by the fifth month. By this age, the meninges presented blood vessels ventrally, dorsally and laterally. At coccygeal region, dura was thinner with a thickness of 7.2 μm. After the level of conus medullaris, the dura formed an investment around the filum terminale to form a thin fibrous cord, the coccygeal ligament. The latter extended caudally to the dorsal surface of vertebral canal at the fourth coccygeal vertebra, so that the caudal part of the dural sac was occupied by the filum terminale and cauda equina.

4.3.4.2 *Arachnoid*

The arachnoid was distinguishable by 48 days and consisted of several thin connective tissue trabeculae extending between dura mater and pia mater and contained polygonal cells (Fig. 200) by the middle of the second month. It was a delicate nonvascular membrane, which passed over the grooves without dipping into them. It was partly separated from the pia mater by fluid spaces and by trabeculae, which passed from pia mater to arachnoid. Collectively these spaces constituted the subarachnoid space, which existed between arachnoid and pia mater and had a width of 30.4 μm by 48 days. The arachnoid trabeculae were covered with flattened cells.

The arachnoid presented a thickness of 3.6 μm by fifth month of gestation. The subarachnoid space contained arachnoid trabeculae of 3.6 μm thickness, blood vessels and nerve bundles and presented a width of 287.5 μm dorsally and laterally, but ventrally it was wider, measuring 312.5 μm. At S4 segment, the loose connective tissue trabeculae of arachnoid were very rare in the subarachnoid space.

4.3.4.3 Pia Mater

The pia mater could be identified as a vascular layer even by 24 days of gestation. By 48 days, it was adherent to and followed the contour of the cord, covered all sulci and fissures. It was a thin membrane composed of flattened cells and intermittent blood vessels. It was 3.6 to 5.2 μm thick by 48 days (Fig. 200). This investing vascular membrane consisted of two layers, viz. an inner membranous layer,

the intima pia and the superficial layer of epipial tissue. The intima pia was adherent to the underlying nervous tissue and sent fibrous septa into the spinal cord (Fig. 70). At points where blood vessels entered the spinal cord, the intima pia invaginated to form the outer wall of the perivascular space (Fig. 89).

The epipial layer was continuous with the arachnoid trabeculae (Fig. 200). This layer was well developed and at its attachment to the floor plate, it presented a fold, the linea splendens, which extended into the ventral median fissure with a thickness of 7.6 μm (Fig. 52). The blood vessels, including the ventral spinal artery, lay within this layer (Fig. 57). At the ventral midline, the linea splendens was with a thickness of 38 μm.

The spinal cord was anchored to the dura mater by two lateral series of flattened collagenous bands of epipial tissue, the dentate ligament (Fig. 153). Each ligament attached medially to the lateral aspect of the cord, midway between the dorsal and ventral roots, along the whole length of the spinal cord from the medulla to S4 segment. The lateral border presented a series of pointed processes, which were firmly attached to the arachnoid and inner surface of dura mater. The pointed dural attachments of the dentate ligament, alternate with the dural evaginations, which marked the exit of spinal nerves. In the pia mater, collagen fibres, flattened nuclei of fibroblasts and capillaries could be seen. At fourth month, the dentate ligament was 14.4 μm thick. Pia mater was 3.6 to 10.2 μm thick and contained blood vessels with 7.2 to 36 μm calibre at the coccygeal region.

The epipial layer surrounding the brain and spinal cord was interrupted at the attachments of the spinal nerves (Fig. 150). As the individual nerve fibres entered or left the spinal cord, they pierced the intima pia.

The intima pia was firmly anchored to the surface of the spinal cord by the external (superficial glial) limiting membrane (Fig. 58). The limiting membrane was composed of processes of fibrous astrocytes. The cell bodies of astrocytes could be observed in the limiting membrane. The glial fibres and astrocytes were particularly prominent in the regions where the dorsal and ventral spinal roots penetrated the pia mater (Fig. 77).

4.4 HISTOCHEMISTRY

4.4.1 Carbohydrates

Intense PAS activity was noticed in the luminal content and in the ependyma (Fig. 62). The neuroglia also showed a positive reaction (Fig. 62). Weak to moderate activity was seen in the ganglionic neurons and capsule (Fig. 203).

The luminal content and ependymal cells also showed positive reaction for the glycogen (Fig. 204). In addition to this, neurons, neuroglia and neuropile (Fig. 205) and spinal meninges (Fig. 206) were also positive for glycogen.

4.4.2 Proteins

The Nissl bodies appeared in the neurons of the spinal cord at fourth month, but were not demonstrable with the usual histological staining methods, such as Haematoxylin and eosin, but needed special staining methods like PTAH (Fig. 82). These bodies became better developed, were larger and had a coarse appearance at the beginning of the fifth month (Figs. 83, 84, 85, 86). Towards the end of gestation, the Nissl bodies became very clear (Fig. 86). These appeared in the ganglionic neurons also from fourth month onwards (Fig. 190). They differed form those of the multipolar neurons in that they had a finely granular appearance (Fig. 193).

4.4.3 Lipids

By the third month, white matter showed a clear presence of lipids especially in the tracts bordering the ventral median fissure (Fig. 207), indicating an early myelination at this funiculus. By fourth month, gray matter also showed diffused positive reaction, occasionally indicating the presence of myelinated nerve fibres. Blood vessels and meninges showed negative reaction (Fig. 208).

Eventhough the lipids appeared for the first time in the white matter during the third month of gestation, a well-defined myelin sheath was observed around the axons only by the fifth month. Towards the end of gestation, concentric layers of myelin sheath also could be observed around axons. White matter showed intense positive reaction for the presence of lipids in the full term foetus. The ependymal cells and meninges were negative for lipids (Fig. 209).

Lamina I and II were negative for lipids, indicating the absence of myelinated fibres even by the end of gestation. Lamina III, eventhough belonged to gray matter, presented myelinated fibres (Fig. 210).

The dorsal root ganglia presented myelinated fibres in the full term foetus. The cytoplasm of ganglionic cells was moderately positive for lipids (Fig. 211).

4.4.4 Oxidative Enzymes

Alkaline phosphatase was localized in blood vessels (Fig. 212), in the nucleoli of neurons and in the neuropile. Strong alkaline phosphatase activity was also noticed in the white matter, glial cell nuclei, pia-arachnoid, ependyma and internal and external limiting membranes (Fig. 213). Ependyma and internal limiting membrane also showed the presence of this enzyme. The activity was more in the white matter than in the gray matter towards the end of gestation (Fig. 214). The cytoplasm of neurons showed a negative reaction. Alkaline phosphatase was also seen in the nucleolus and peripheral part of the cytoplasm in ganglionic neurons (Fig. 215).

Acid phosphatase was localized in the neuroglial nuclei, which showed strong positive reaction. Moderate acid phosphatase activity was seen in the cytoplasm of ganglionic neurons (Fig. 216). When stained for acid phosphatase, the ganglionic substance showed the presence of pigments in the myelin as brown deposits along the course of the nerves in the full term foetus. These deposits were not observed in the gray matter or white matter.

Table 1. Body weight, CRL, sex and age of foetuses

Body weight (g)	CRL straight (mm)	CRL curved (mm)	Sex	Age (in days)
0.279	14.00	23.00	I	24
0.365	15.00	26.00	I	26
0.401	15.00	35.00	I	26
0.428	15.00	26.00	I	26
0.429	15.00	38.00	I	26
0.480	16.00	26.00	I	27
1.304	25.00	49.00	I	40
1.305	25.00	49.00	I	40
4.351	39.00	53.00	I	47
4.789	40.00	61.00	I	48
11.504	60.00	86.00	M	54
13.612	71.00	93.00	M	55
17.669	75.00	94.00	F	57
18.103	75.00	94.00	M	57
20.030	76.00	96.00	M	58
20.315	76.00	96.00	F	58
25.907	83.00	105.00	M	61
27.000	83.00	110.00	F	61
27.686	86.00	115.00	M	62
28.628	87.00	115.00	F	62
46.263	95.00	126.00	F	67
50.468	102.00	129.00	F	69
88.000	120.00	160.00	M	76
94.000	130.00	171.00	F	77
110.000	130.00	156.00	F	80
115.000	130.00	161.00	M	81
130.000	151.00	188.00	M	83
157.000	155.00	182.00	F	86
220.000	183.00	217.00	F	93
240.000	188.00	233.00	F	95
240.000	192.00	252.00	F	95
250.000	190.00	232.00	M	96
250.000	183.00	254.00	F	96
250.000	203.00	254.00	M	96
280.000	203.00	254.00	F	98
320.000	200.00	251.00	M	101
325.000	198.00	234.00	M	102
360.000	205.00	246.00	F	104
700.000	265.00	346.00	F	123
740.000	275.00	340.00	M	124
750.000	264.00	328.00	M	125
760.000	295.00	344.00	M	125
810.000	281.00	309.00	M	127
1040.000	295.00	332.00	F	128
1400.000	340.00	414.00	F	130
1630.000	395.00	436.00	M	140
1680.000	390.00	442.00	M	142
1800.000	405.00	447.00	F	144
2080.000	385.00	417.00	F	145
2260.000	365.00	418.00	M	146
2300.000	370.00	421.00	M	147
2880.000	415.00	452.00	M	150

I- Indifferent F- Female M- Male

Table 2. Age, body weight, CRL, sex and number of foetuses

Group	Age in days	Body weight (g)	CRL		Number of foetuses	Sex		
			Straight (mm)	Curved (mm)		Indifferent	Male	Female
I	1-30	0.279-0.480	14-16	23-38	6	6	-	-
II	31-60	1.304-20.315	25-76	49-96	10	4	4	2
III	61-90	25.907-157.000	83-155	105-188	12	-	5	7
IV	91-120	220.000-360.000	183-205	217-254	10	-	4	6
V	121-term	700.000-2880.000	265-415	309-452	14	-	9	5

Table 3. Body weight, CRL straight and CRL curved of goat foetuses at different stages of gestation

Parameters	Group I (n=6)		Group II (n=10)		Group III (n=12)		Group IV (n=10)		Group V (n=14)	
	Range	Mean± SE	Range	Mean± SE	Range	Mean± SE	Range	Mean± SE	Range	Mean± SE
Body weight (g)	0.279-0.480	0.397± 0.028	1.304-20.315	11.298± 2.450	25.907-157.000	74.996± 13.381	220.000-360.000	273.500± 14.569	700.000 - 2880.000	1487.857± 190.734
CRL straight (mm)	14.000-16.000	15.000± 0.258	25.000-76.000	56.200± 6.855	83.000-155.000	112.667± 7.674	183.000-205.000	194.500± 2.647	264.000-415.000	338.571± 15.201
CRL curved (mm)	23.000-38.000	29.000± 2.449	49.000-96.000	77.100± 6.855	105.000-188.000	143.167± 8.571	217.000-254.000	242.700± 4.074	309.000-.452.000	389.000± 13.921

Table 4. Correlation of body weight, age, CRL straight and CRL curved with body parameters

Sl.No.	Parameter	Correlation coefficient (r)			
		Body weight	Age	CRL straight	CRL curved
1	Body weight	1.000**	0.877**	0.915**	0.888**
2	Age	0.877**	1.000**	0.989**	0.991**
3	CRL straight	0.915**	0.989**	1.000**	0.994**
4	CRL curved	0.888**	0.991**	0.994**	1.000**
5	Total body length	0.940**	0.978**	0.992**	0.984**
6	Vertebral column length	0.920**	0.979**	0.995**	0.992**
7	Vertebral column tail length	0.933**	0.980**	0.994**	0.991**
8	Total bend length of foetus	0.911**	0.993**	0.995**	0.997**
9	Tail length	0.939**	0.936**	0.943**	0.940**
10	Fore limb length	0.932**	0.986**	0.991**	0.986**
11	Hind limb length	0.919**	0.989**	0.992**	0.990**
12	Liver weight	0.953**	0.946**	0.945**	0.937**

** $P<0.01$

Table 5. Regression between various parameters for total period of gestation

Parameters	Regression coefficient (b) ± S E (b)
Age and body weight (g)	21.571 ± 1.810**
Age and CRL straight (mm)	3.505 ± 0.088**
Body weight and CRL straight (mm)	0.134 ± 0.009**
Age and CRL curved (mm)	3.810 ± 0.087**
Body weight and CRL curved (mm)	0.141 ± 0.011**
CRL curved and CRL straight (mm)	0.916 ± 0.016**
Age and vertebral column length (mm)	3.755 ± 0.118**
Body weight and vertebral column length (mm)	0.130 ± 0.007**
CRL straight and vertebral column length (mm)	1.068 ± 0.024**
CRL curved and vertebral column length (mm)	0.976 ± 0.030**
Age and vertebral canal length (mm)	3.278 ± 0.101**
Body weight and vertebral canal length (mm)	0.130 ± 0.007**
CRL straight and vertebral canal length (mm)	0.931 ± 0.021**
CRL curved and vertebral canal length (mm)	0.852 ± 0.026**
Age and weight of spinal cord (g)	0.111 ± 0.009**
Body weight and spinal cord weight (g)	0.005 ± 0.000**
Spinal cord length and spinal cord weight (g)	0.042 ± 0.002**
Volume and weight of spinal cord (g)	1.039 ± 0.008**
Age and volume of spinal cord(ml)	0.105 ± 0.009**
Body weight and spinal cord volume (ml)	0.005 ± 0.000**
Spinal cord length and spinal cord volume (ml)	0.040 ± 0.002**
Age and spinal cord length (mm)	2.293 ± 0.034**
Body weight and spinal cord length (mm)	0.106 ± 0.006**
CRL straight and spinal cord length (mm)	0.768 ± 0.017**
CRL curved and spinal cord length (mm)	0.705 ± 0.019**

** $P < 0.01$

Table 6. Body parameters of foetal goats during different stages of gestation

Parameters	Group II (n=6)		Group III (n=12)		Group IV (n=10)		Group V (n=14)	
	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE
Total body length (mm)	11.000 – 45.500	31.670 ± 4.278	49.600 – 90.000	70.567 ± 5.050	113.000 – 130.000	122.800 ± 1.692	160.000 – 290.000	222.357 ± 13.288
Vertebral column length (mm)	34.000-59.000	50.200± 3.207	68.000 – 129.000	94.333± 6.418	157.000 – 178.000	167.700± 2.390	237.000 – 403.000	309.000± 15.204
Vertebral column tail length (mm)	40.000-77.000	62.500± 4.776	83.000 – 155.000	114.167± 7.933	196.000 – 221.000	211.400± 2.252	290.000 – 517.000	380.429± 20.029
Total bend length of foetus (mm)	59.000 – 123.000	98.600± 8.262	132.000 – 242.000	181.917± 11.614	286.000 – 326.000	312.100± 3.860	410.000 – 624.000	512.571± 20.154
Tail length (mm)	6.000 – 18.000	12.300± 1.732	11.000 – 26.000	19.833± 1.705	38.000 – 50.000	43.700± 1.334	32.000 – 114.000	71.643± 6.000
Fore limb length (mm)	7.400 – 46.800	27.900± 4.516	47.000 – 100.000	67.200± 5.688	126.000 – 150.000	135.200± 2.128	206.000 – 357.000	269.357± 12.792
Hind limb length (mm)	7.000 – 42.000	26.770± 4.129	44.000 – 114.000	67.417± 6.747	125.000 – 150.000	136.800± 2.205	202.000 – 320.000	264.214± 11.937
Liver weight	0.530 – 2.380	1.389± 0.206	2.160 - 13.050	6.355± 1.145	16.770 - 21.720	18.647± 0.442	36.143 – 80.000	50.067± 4.036

Table 7. Regression of body parameters with age, body weight, CRL straight and CRL curved

Body parameters	Regression coefficient (b) ± SE (b)			
	Age	Body weight	CRL straight	CRL curved
Total body length (mm)	2.407±0.083**	0.095±0.005**	0.688±0.014**	0.512±0.014**
Total bend length of foetus (mm)	5.142±0.101**	0.194±0.014**	1.452±0.024**	1.297±0.014**
Tail Length (mm)	0.793±0.049**	0.033±0.002**	0.225±0.013**	0.163±0.014**
Forelimb length (mm)	3.075±0.082**	0.119±0.007**	0.810±0.019**	0.730±0.021**
Hindlimb length (mm)	3.018±0.069**	0.115±0.008**	0.853±0.017**	0.728±0.019**
Liver weight (g)	0.661±0.035**	0.027±0.001**	0.186±0.010**	0.145±0.007**

** $P < 0.01$

Table 8. Total length and regional length of vertebral column and total length of vertebral canal and precoccygeal vertebral column in goat foetuses

	Parameter	Group II (n=6)		Group III (n=12)		Group IV (n= 10)		Group V (n=14)	
		Range	Mean ±SE	Range	Mean ±SE	Range	Mean ±SE	Range	Mean ±SE
1	Total length of vertebral column (mm)	45.000 – 73.000	62.750± 4.267	76.000 – 148.000	108.833± 7.581	178.000 – 201.000	188.200± 2.435	250.000 – 464.000	349.643± 19.067
2	Regional length of vertebral column (mm)								
	a) Cervical	14.000 – 18.000	16.667± 0.843	17.000 – 33.000	24.417± 1.721	39.000 – 45.000	42.700± 0.844	60.000 – 104.000	78.643± 3.600
	b) Thoracic	16.000 – 21.000	19.167± 0.872	22.000 – 43.000	31.917± 2.451	54.000 – 59.000	55.200± 0.554	80.000 – 138.000	104.429± 5.421
	c) Lumbar	10.000 – 12.000	11.667± 0.333	12.000 – 27.000	20.083± 1.417	34.000 – 37.000	35.000± 0.298	45.000 – 83.000	64.929± 3.688
	d) Sacral	5.000 – 6.000	5.833± 0.167	6.000 – 12.000	8.917± 0.621	13.000 – 19.000	15.700± 0.616	23.000 – 46.000	31.143± 1.950
	e) Coccygeal	13.000 – 16.000	15.333± 0.494	16.000 – 33.000	23.500± 1.569	37.000 – 42.000	39.600± 0.600	48.000 – 94.000	72.857± 4.816
3	Vertebral canal length (mm)	50.000 – 63.000	58.833± 2.358	65.000 – 125.500	92.925± 6.496	155.000 – 174.000	161.800± 1.925	230.000 – 407.000	304.429± 16.023
4	Precoccygeal vertebral column length (mm)	45.000 – 57.000	53.333± 2.076	59.000 – 115.000	85.333± 6.089	141.000 – 159.000	148.600± 1.863	210.000 – 370.000	278.786± 14.486

Table 9. Correlation of vertebral column and spinal cord parameters in goat foetuses

Parameters	Correlation coefficient (r)							
	Age	Body weight	CRL straight	CRL curved	Vertebral column length	Spinal cord weight	Spinal cord volume	Spinal cord length
Regional length of vertebral column								
a) Cervical	0.984**	0.925**	0.986**	0.982**	0.992**	0.928**	0.917**	0.995**
b) Thoracic	0.984**	0.935**	0.991**	0.986**	0.996**	0.944**	0.936**	0.996**
c) Lumbar	0.979**	0.934**	0.984**	0.977**	0.994**	0.939**	0.928**	0.994**
d) Sacral	0.968**	0.952**	0.973**	0.967**	0.990**	0.940**	0.929**	0.990**
e) Coccygeal	0.965**	0.935**	0.983**	0.977**	0.987**	0.952**	0.946**	0.984**
f) Total	0.981**	0.949**	0.990**	0.982**	1.000**	0.953**	0.944**	0.998**
Spinal cord weight	0.893**	0.979**	0.936**	0.913**	0.953**	1.000**	0.999**	0.939**
Spinal cord volume	0.879**	0.975**	0.927**	0.903**	0.944**	0.999**	1.000**	0.929**
Spinal cord length	0.988**	0.935**	0.992**	0.987**	0.998**	0.939**	0.929**	1.000**
Regional length of spinal cord								
a) Cervical	0.986**	0.904**	0.990**	0.989**	0.987**	0.929**	0.919**	0.990**
b) Thoracic	0.985**	0.932**	0.990**	0.986**	0.995**	0.942**	0.932**	0.996**
c) Lumbar	0.979**	0.937**	0.986**	0.979**	0.994**	0.937**	0.927**	0.993**
d) Sacral	0.908**	0.767**	0.882**	0.891**	0.883**	0.739**	0.728**	0.897**
e) Coccygeal	0.201^{NS}	0.117^{NS}	0.135^{NS}	0.132^{NS}	0.164^{NS}	0.014^{NS}	0.006^{NS}	0.181^{NS}
f) Cervical enlargement length	0.979**	0.926**	0.983**	0.976**	0.986**	0.934**	0.924**	0.987**
g) Lumbar enlargement length	0.971**	0.899**	0.740**	0.970**	0.978**	0.890**	0.881**	0.978**
Length of filum	0.909**	0.971**	0.940**	0.920**	0.960**	0.971**	0.967**	0.948**

** P < 0.01 NS – Non significant

Table 10. Regional length expressed as percentage of total length and precoccygeal length of vertebral column during gestation

Regions	Group II (n=6)		Group III (n=12)		Group IV (n=10)		Group V (n=14)		Total gestation (n=42)		r value*
	Range	Mean ±SE	Range	Mean ±SE	Range	Mean ±SE	Range	Mean ±SE	Range	Mean ±SE	
Percentage of total length											
Cervical	22.581 - 25.000	24.205 ± 0.411	19.101 - 24.096	22.478 ±0.434	21.891 - 23.684	22.678 ±0.257	20.531 - 25.333	22.670 ±0.370	19.101 - 25.333	22.836 ±0.208	0.992 **
Thoracic	26.667 - 28.767	27.863 ±0.331	26.829 - 31.707	29.165 ±0.429	28.272 - 30.337	29.351 ±0.274	28.571 - 32.400	29.942 ±0.325	26.667 - 32.400	29.282 ±0.204	0.996**
Lumbar	16.438 - 19.355	17.039 ±0.465	15.789 - 20.482	18.453 ±0.361	18.325 - 19.101	18.608 ±0.112	17.100 - 20.000	18.556 ±0.252	15.789 - 20.482	18.322 ±0.167	0.994**
Sacral	8.219 - 9.677	8.519 ±0.233	7.216 - 9.211	8.212 ±0.169	6.842 - 9.453	8.325 ±0.248	8.115 - 9.914	8.886 ±0.162	6.842 - 9.914	8.508 ±0.105	0.990**
Coccygeal	20.968 - 25.000	22.375 ±0.558	19.277 - 25.610	21.693 ±0.498	20.787 - 21.579	21.037 ±0.092	17.143 - 24.571	20.698 ±0.543	17.143 - 25.610	21.302 ±0.254	0.987**
Percentage of precoccygeal length											
Cervical	28.571 - 32.143	31.188 ±0.547	24.638 - 33.333	28.989 ±0.671	27.660 - 30.201	28.721 ±0.339	26.154 - 30.769	28.357 ±0.370	24.638 - 33.333	29.029 ±0.284	0.996**
Thoracic	34.694 - 36.842	35.894 ±0.338	34.328 - 39.796	37.249 ±0.521	35.762 - 38.298	37.171 ±0.345	34.783 - 38.832	37.470 ±0.337	34.328 - 39.796	37.110 ±0.218	0.998**
Lumbar	21.053 - 24.490	21.946 ±0.538	20.339 - 25.373	23.555 ±0.390	23.179 - 24.113	23.565 ±0.130	21.296 - 25.231	23.225 ±0.306	20.339 - 25.373	23.218 ±0.186	0.997**
Sacral	10.526 - 12.245	10.973 ±0.267	9.459 - 11.864	10.485 ±0.198	8.725 - 11.950	10.542 ±0.310	10.390 - 12.432	11.116 ±0.173	8.725 - 12.432	10.779 ±0.120	0.993**

** P <0.01

Table 11. Vertebral length of goat foetuses (Mean± S.E), mm

Vertebra	Group II (n=6)	Group III (n=12)	Group IV (n=10)	Group V (n=14)
C1	2.417±0.083	3.067±0.288	5.800±0.249	8.750±0.499
C2	3.417±0.083	5.058±0.421	8.450±0.411	16.929±0.905
C3	2.458±0.100	3.607±3.330	6.200±0.133	12.429±0.732
C4	2.458±0.100	3.450±0.298	5.750±0.201	11.036±0.656
C5	2.458±0.100	3.158±0.255	5.950±0.050	10.821±0.720
C6	2.458±0.100	3.133±0.266	5.450±0.157	9.750±0.412
C7	1.833±0.167	2.883±0.193	5.100±0.277	8.286±0.384
T1	1.417±0.083	2.642±0.245	3.800±0.200	8.286±0.515
T2	1.417±0.083	2.400±0.196	4.350±0.130	8.071±0.460
T3	1.417±0.083	2.400±0.202	4.250±0.112	7.607±0.492
T4	1.417±0.083	2.400±0.188	4.700±0.111	7.821±0.398
T5	1.417±0.083	2.300±0.178	3.700±0.238	7.357±0.357
T6	1.500±0.010	2.267±0.186	3.800±0.200	7.714±0.549
T7	1.500±0.010	2.425±0.266	4.250±0.112	7.571±0.465
T8	1.417±0.083	2.250±0.192	4.000±0.030	7.500±0.429
T9	1.417±0.083	2.225±0.186	4.100±0.145	8.214±0.595
T10	1.500±0.020	2.267±0.196	4.200±0.170	7.821±0.418
T11	1.583±0.017	2.608±0.185	4.500±0.149	8.321±0.418
T12	1.617±0.031	2.792±0.237	4.600±0.145	8.607±0.575
T13	1.750±0.050	2.808±0.231	4.950±0.138	9.179±0.534
L1	1.917±0.083	3.217±0.246	5.300±0.153	10.054±0.500
L2	1.958±0.042	3.283±0.245	5.300±0.153	11.554±0.927
L3	1.958±0.042	3.067±0.284	5.900±0.100	11.696±0.748
L4	1.958±0.042	3.408±0.198	6.000±0.040	10.339±0.602
L5	1.958±0.042	3.675±0.333	6.200±0.133	10.929±0.539
L6	1.917±0.083	3.433±0.301	6.300±0.213	10.286±0.722
S1	2.000±0.200	3.283±0.340	5.300±0.213	10.429±0.761
S2	1.417±0.083	2.033±0.155	3.600±0.221	7.214±0.604
S3	1.417±0.083	1.800±0.095	3.400±0.163	7.071±0.399
S4	1.000±0.020	1.717±0.114	3.100±0.100	6.357±0.414
Cy1	1.417±0.083	2.000±0.136	3.150±0.107	6.643±0.341
Cy2	1.417±0.083	2.250±0.129	3.550±0.189	6.714±0.354
Cy3	1.333±0.105	2.275±0.145	3.450±0.189	6.875±0.490
Cy4	1.333±0.105	2.517±0.211	3.050±0.157	6.714±0.507
Cy5	1.333±0.105	2.108±0.225	3.450±0.157	6.286±0.384
Cy6	1.333±0.105	2.150±0.210	3.050±0.050	7.000±0.611
Cy7	1.000±0.000	1.908±0.177	3.350±0.150	5.857±0.294
Cy8	1.000±0.000	1.825±0.134	3.050±0.050	5.714±0.425
Cy9	1.000±0.000	1.742±0.131	2.600±0.163	5.714±0.658
Cy10	1.000±0.000	1.558±0.152	3.000±0.000	5.429±0.532
Cy11	1.000±0.000	1.167±0.094	2.200±0.133	4.714±0.462
Cy12	1.000±0.000	1.000±0.163	2.100±0.100	4.286±0.412
Cy13	1.000±0.000	0.913±0.135	2.200±0.133	0.929±0.385
Cy14	0.167±0.167	0.830±0.083	1.400±0.400	0.800±0.500

Table 12. Correlaton of vertebral canal length with different parameters

Parameters	Correlation coefficient (r)
Body weight	0.939**
Age	0.983**
CRL straight	0.990**
CRL curved	0.984**
Total body length	0.990**
Vertebral column length	0.983**
Vertebral column tail length	0.989**
Total bend length of foetus	0.991**
Tail length	0.965**
Regional length of vertebral column	
a) Cervical	0.996**
b) Thoracic	0.999**
c) Lumbar	0.997**
d) Sacral	0.993**
e) Coccygeal	0.988**
f) Total	0.997**
Regional length of spinal cord	
a) Cervical	0.990**
b) Thoracic	0.998**
c) Lumbar	0.994**
d) Sacral	0.902**
e) Coccygeal	0.191^{NS}
f) Total	0.992**

** P<0.01 NS – Non significant

Table 13. Vertebral canal width at different ages (Mean±S.E), mm

Vertebra	Group II (n=6)	Group III (n=12)	Group IV (n=10)	Group V (n=14)
C1	3.167±0.021	3.933±0.170	6.700±0.597	9.571±0.429
C2	2.500±0.015	3.083±0.135	4.680±0.150	7.429±0.173
C3	2.500±0.015	3.083±0.135	4.530±0.226	7.393±0.197
C4	2.500±0.015	3.083±0.135	4.530±0.226	7.357±0.225
C5	2.400±0.063	3.183±0.199	4.980±0.224	7.429±0.173
C6	3.100±0.010	3.500±0.151	5.000±0.224	8.750±0.250
C7	3.000±0.063	3.533±0.183	5.530±0.226	9.286±0.201
T1	2.900±0.106	3.250±0.149	5.380±0.302	9.000±0.216
T2	2.900±0.106	3.267±0.143	4.780±0.187	8.721±0.254
T3	2.800±0.253	3.167±0.112	4.630±0.259	7.893±0.197
T4	2.400±0.063	3.033±0.157	3.950±0.138	7.429±0.251
T5	2.333±0.105	2.967±0.176	3.750±0.171	7.121±0.169
T6	2.333±0.105	2.967±0.176	3.750±0.171	7.121±0.169
T7	2.200±0.063	2.967±0.176	3.700±0.153	6.679±0.113
T8	2.200±0.063	2.967±0.176	3.750±0.171	6.607±0.119
T9	2.200±0.063	2.967±0.176	3.750±0.171	6.571±0.156
T10	2.200±0.063	2.967±0.176	3.750±0.171	6.536±0.185
T11	2.200±0.063	2.967±0.176	3.700±0.153	6.536±0.185
T12	2.200±0.063	2.967±0.176	3.700±0.153	6.571±0.195
T13	2.200±0.063	2.967±0.176	3.700±0.153	6.643±0.169
L1	2.367±0.042	3.200±0.187	4.020±0.117	6.893±0.223
L2	2.367±0.042	3.200±0.187	4.530±0.226	7.457±0.300
L3	2.533±0.148	3.200±0.187	4.600±0.153	7.629±0.306
L4	2.867±0.042	3.310±0.169	4.670±0.176	8.107±0.253
L5	3.000±0.025	3.310±0.169	4.660±0.207	8.179±0.238
L6	2.933±0.042	3.420±0.169	4.730±0.221	8.286±0.221
S1	2.833±0.105	3.367±0.227	3.730±0.054	7.143±0.308
S2	2.467±0.211	3.217±0.220	3.100±0.100	6.393±0.469
S3	2.033±0.169	2.683±0.142	2.990±0.064	4.679±0.456
S4	1.933±0.232	2.650±0.137	2.130±0.277	4.393±0.439
Cy1	1.733±0.169	2.333±0.241	2.130±0.277	3.357±0.243
Cy2	1.667±0.211	2.208±0.255	1.350±0.076	2.607±0.175
Cy3	1.333±0.105	0.925±0.260	0.300±0.153	2.286±0.155
Cy4	1.333±0.105	0.758±0.251	0.200±0.015	1.429±0.327

Table 14. Weight, volume and total length, regional length, length of cervical and lumbar enlargements of spinal cord and length of filum terminale

Parameter	Group II (n=6)		Group III (n=12)		Group IV (n=10)		Group V (n=14)	
	Range	Mean± S.E	Range	Mean± S.E	Range	Mean± SE	Range	Mean± S E
Weight of spinal cord (g)	0.146 – 0.166	0.160± 0.003	0.172 – 0.693	0.379± 0.052	0.904 - 1.445	1.126± 0.071	4.010 – 11.520	7.859± 0.801
Volume of spinal cord (ml)	0.200 – 0.200	0.200± 0.000	0.200 – 0.700	0.375± 0.049	0.800 – 1.000	0.940± 0.031	3.500 – 11.000	7.450± 0.809
Total length of spinal cord (mm)	38.000 – 61.000	52.625± 3.495	63.000 – 123.000	90.917± 6.290	141.000 – 157.000	150.800± 2.164	207.000 – 337.000	264.179± 12.308
Regional length of spinal cord (mm)								
a) Cervical	13.000 – 18.000	16.500± 0.957	17.000 – 30.000	23.417± 1.302	37.000 – 45.000	41.800± 1.172	60.000 – 87.000	76.893± 2.960
b) Thoracic	18.000 – 21.000	19.833± 0.477	22.000 – 44.000	31.917± 2.482	50.000 – 62.000	53.900± 1.197	79.000 – 130.000	101.821± 5.055
c) Lumbar	10.000 – 12.000	11.5± 0.837	12.000 – 26.000	19.417± 1.282	30.000 – 35.000	33.700± 0.496	45.000 – 81.000	61.786± 3.564
d) Sacral	5.000 – 6.000	5.667± 0.211	6.000 – 12.000	8.833± 0.649	11.000 – 16.000	14.300± 0.496	13.000 – 27.000	17.679± 0.943
e) Coccygeal	4.500 – 4.500	4.500± 0.000	3.500 – 12.000	7.250± 1.008	5.000 – 10.000	6.900± 0.482	5.000 – 12.000	5.607± 0.952
Length cervical enlargement (mm)	4.500 – 9.000	7.583± 0.898	9.200 – 16.000	12.400± 0.744	16.000 – 22.000	18.250± 0.629	28.000 – 50.000	36.107± 1.581
Length of lumbar enlargement (mm)	8.000 – 9.500	9.167± 0.247	9.500 – 20.000	15.225± 1.117	22.000 – 28.000	26.400± 0.718	28.500 – 56.000	39.786± 2.278
Length of filum terminale (mm)	0.000 – 2.000	1.333± 1.033	1.000 – 4.500	2.092± 0.424	6.000 – 17.000	11.000± 1.155	18.000 – 70.000	40.250± 3.988

Table 15. Correlation and regression of body parameters with spinal cord weight, volume and length

Parameter	Spinal cord weight		Spinal cord volume		Spinal cord length	
	Correlation coefficient (r)	Regression coefficient (b) ±SE (b)	Correlation coefficient (r)	Regression coefficient (b) ±SE (b)	Correlation coefficient (r)	Regression coefficient (b) ±SE (b)
Total body length	0.949**	0.026±0.003**	0.942**	0.222±0.003**	0.992**	1.203±0.025**
Total bend length of foetus	0.925**	0.011±0.001**	0.914**	0.009±0.001**	0.995**	0.486±0.008**
Tail length	0.912**	0.063±0.012**	0.904**	0.053±0.010**	0.966**	3.161±0.274**
Forelimb length	0.939**	0.020±0.002**	0.927**	0.017±0.010**	0.994**	0.835±0.027**
Hindlimb length	0.936**	0.020±0.002**	0.925**	0.017±0.002**	0.993**	0.842±0.024**
Liver weight	0.931**	0.102±0.006**	0.919**	0.087±0.006**	0.971**	4.058±0.186**

**P< 0.01

Table 16. Regional length of spinal cord expressed as percentage of total length

Regions	Group II (n=6)		Group III (n=12)		Group IV (n=10)		Group V (n=14)		Total (n=42)	
	Range	Mean± S.E	Range	Mean ± S.E	Range	Mean ± S.E	Range	Mean ± S.E	Range	Mean ± S.E
Cervical	26.000 – 30.000	28.572 ± 0.755	22.973 – 28.986	26.030 ± 0.573	26.144 – 29.221	27.677 ± 0.447	25.816 – 31.439	29.267 ± 0.430	22.973 – 31.439	27.864 ± 0.326
Thoracic	33.333 – 36.000	34.561 ± 0.475	32.353 – 37.255	34.887 ± 0.499	34.194 – 39.490	35.732 ± 0.514	36.891 – 40.909	38.474 ± 0.304	32.353 – 40.909	36.237 ± 0.334
Lumbar	19.672 – 20.755	20.016 ± 0.162	18.868 – 24.638	21.476 ± 0.563	19.608 – 23.404	22.373 ± 0.359	20.455 – 24.329	23.248 ± 0.364	18.868 – 25.329	22.072 ± 0.273
Sacral	9.434 – 10.000	9.851 ± 0.090	8.140 – 11.110	9.716 ± 0.247	7.006 – 10.638	9.518 ± 0.399	5.428 – 9.174	6.756 ± 0.298	5.428 – 11.111	8.701 ± 0.263
Coccygeal	6.557 – 7.980	6.996 ± 0.250	4.286 – 13.953	7.811 ± 0.887	3.185 – 6.536	4.570 ± 0.298	1.060 – 4.587	2.217 ± 0.308	1.060 – 13.953	5.058 ± 0.457
Cervical enlargement	9.000 – 15.000	12.990 ± 1.195	12.326 – 14.706	13.753 ± 0.215	11.348 – 14.013	12.080 ± 0.289	12.539 – 15.081	13.717 ± 0.202	9.000 – 15.081	13.234 ± 0.221
Lumbar enlargement	15.574 – 16.981	15.966 ± 0.214	13.971 – 18.137	16.700 ± 0.328	14.013 – 19.858	17.572 ± 0.645	13.258 – 16.617	14.982 ± 0.257	13.258 – 19.858	16.230 ± 0.250

Table 17. Correlation between regional length of vertebral column and corresponding length of spinal cord

Regions	Correlation coefficient (r)			
	Group II (n=6)	Group III (n=12)	Group IV (n=10)	Group V (n=14)
Cervical	0.991**	0.989**	0.971**	0.901**
Thoracic	0.974**	0.998**	0.942**	0.990**
Lumbar	0.878**	0.974**	0.560 ^{NS}	0.957**
Sacral	0.632^{NS}	0.992**	0.275 ^{NS}	0.730**
Coccygeal	0.135 ^{NS}	0.754**	0.268 ^{NS}	0.114 ^{NS}

** P<0.01 NS – Non significant

Table 18. Length of the spinal cord segments in foetal goat (Mean±S.E), mm

Segments	GroupII (n=6)	GroupIII (n=12)	GroupIV (n=10)	GroupV (n=14)
C1	2.000±0.001	2.917±0.260	5.300±0.111	9.143±0.573
C2	3.000±0.010	3.533±0.142	7.000±0.211	13.143±0.864
C3	2.333±0.105	3.308±0.339	6.900±0.208	12.429±0.669
C4	2.333±0.105	3.167±0.264	6.000±0.149	11.071±0.559
C5	1.917±0.083	3.100±0.222	5.300±0.186	9.071±0.221
C6	1.667±0.211	2.758±0.126	4.800±0.226	8.214±0.447
C7	1.667±0.211	2.550±0.141	3.800±0.226	7.357±0.372
C8	1.750±0.171	2.267±0.143	2.700±0.238	6.465±0.401
T1	1.333±0.105	2.567±0.244	3.400±0.180	6.786±0.281
T2	1.333±0.105	2.375±0.223	3.550±0.302	7.286±0.483
T3	1.333±0.105	2.292±0.189	3.800±0.200	7.071±0.425
T4	1.500±0.020	2.533±0.202	4.050±0.090	7.321±0.398
T5	1.500±0.020	2.350±0.157	3.300±0.170	7.607±0.426
T6	1.500±0.020	2.308±0.171	4.100±0.100	7.036±0.350
T7	1.500±0.020	2.483±0.215	3.700±0.213	7.393±0.487
T8	1.500±0.020	2.233±0.190	4.200±0.291	7.429±0.429
T9	1.500±0.020	2.408±0.202	4.250±0.134	8.143±0.430
T10	1.500±0.020	2.408±0.202	4.400±0.125	7.929±0.412
T11	1.567±0.021	2.667±0.218	5.050±0.05	8.286±0.518
T12	1.600±0.037	2.783±0.242	4.900±0.125	9.750±0.599
T13	1.700±0.063	2.633±0.253	5.200±0.111	10.000±0.419
L1	1.927±0.083	3.225±0.207	5.300±0.153	10.964±0.566
L2	1.958±0.042	3.258±0.179	5.500±0.167	10.929±0.559
L3	1.958±0.042	3.517±0.277	6.200±0.200	11.179±0.624
L4	1.958±0.042	3.350±0.273	5.800±0.200	11.107±0.738
L5	1.958±0.042	3.317±0.229	5.400±0.306	8.964±0.531
L6	1.917±0.083	3.150±0.273	5.200±0.200	8.643±0.651
S1	2.000±0.015	3.333±0.216	5.500±0.307	6.286±0.450
S2	1.333±0.135	2.158±0.189	4.000±0.211	4.714±0.194
S3	1.333±0.105	1.758±0.129	3.100±0.233	4.071±0.322
S4	1.000±0.030	1.583±0.203	2.300±0.153	2.821±0.384
Cy1	1.000±0.030	1.708±0.132	2.000±0.075	1.750±0.455
Cy2	1.000±0.030	1.542±0.145	1.700±0.170	1.136±0.400
Cy3	1.000±0.030	1.425±0.171	1.300±0.213	1.079±0.280
Cy4	1.000±0.030	1.233±0.196	1.000±0.149	1.007±0.156
Cy5	0.500±0.004	0.658±0.226	0.600±0.153	0.875±0.053

Table 19. Width of spinal cord segments in goat foetuses (Mean ± S.E), mm

Segments	Group II (n=6)	Group III (n=12)	Group IV (n=10)	Group V (n=14)
C1	2.002±0.189	2.567±0.265	4.150+0.378	6.310±0.735
C2	1.670±0.104	2.358±0.196	3.150±0.150	6.014±0.247
C3	1.670±0.104	2.167±0.163	3.020±0.093	5.686±0.293
C4	1.670±0.104	2.000±0.147	3.020±0.093	5.686±0.283
C5	1.703±0.125	2.033±0.148	3.070±0.073	6.050±0.249
C6	2.037±0.147	2.408±0.205	3.640±0.122	6.679±0.270
C7	2.037±0.147	2.633±0.205	3.850±0.076	7.436±0.255
C8	2.037±0.147	2.783±0.205	4.200±0.133	7.521±0.273
T1	2.037±0.147	2.650±0.166	3.930±0.052	6.879±0.309
T2	1.937±0.083	2.417±0.167	3.490±0.127	6.121±0.390
T3	1.737±0.147	2.075±0.158	3.270±0.113	5.200±0.296
T4	1.670±0.104	1.992±0.147	3.020±0.113	4.821±0.207
T5	1.637±0.083	1.992±0.147	2.820±0.073	4.779±0.205
T6	1.637±0.083	1.992±0.140	2.750±0.095	4.779±0.205
T7	1.637±0.083	1.992±0.147	2.750±0.095	4.757±0.209
T8	1.637±0.083	1.808±0.058	2.690±0.115	4.743±0.212
T9	1.637±0.083	1.975±0.148	2.660±0.127	4.743±0.212
T10	1.637±0.083	1.808±0.058	2.660±0.127	4.757±0.209
T11	1.637±0.083	2.008±0.146	2.770±0.098	4.770±0.179
T12	1.637±0.083	2.008±0.146	2.780±0.101	4.700±0.179
T13	1.637±0.083	1.842±0.062	2.780±0.101	4.750±0.185
L1	1.637±0.083	1.875±0.055	2.960±0.114	4.886±0.195
L2	1.637±0.083	1.875±0.055	3.140±0.107	4.950±0.193
L3	1.637±0.083	1.858±0.058	3.140±0.107	5.043±0.190
L4	1.970±0.104	2.208±0.115	3.610±0.142	0.457±0.214
L5	2.003±0.125	2.350±0.109	3.770±0.119	6.150±0.198
L6	2.003±0.125	2.525±0.150	4.160±0.130	6.750±0.193
S1	2.003±0.125	2.467±0.158	4.090±0.159	5.750±0.169
S2	1.810±0.004	2.175±0.116	3.430±0.116	4.579±0.142
S3	1.303±0.062	1.758±0.128	2.680±0.153	3.386±0.100
S4	1.203±0.002	1.542±0.091	1.820±0.121	2.464±0.133
Cy1	1.037±0.020	1.275±0.084	1.180±1.117	1.821±0.100
Cy2	1.003±0.002	1.008±0.062	0.900±0.125	1.457±0.139
Cy3	0.803±0.002	0.817±0.074	0.750±0.083	1.036±0.082
Cy4	0.803±0.002	0.542±0.056	0.500±0.010	0.857±0.063
Cy5	0.803±0.002	0.475±0.025	0.500±0.001	0.607±0.057

Table 20. Height of spinal cord segments (Mean ± S.E), mm

Segments	Group II (n=6)	Group III (n=12)	Group IV (n=10)	Group V (n=14)
C1	1.510±0.004	1.800±0.071	3.150±3.136	5.307±0.189
C2	1.510±0.040	1.683±0.091	2.780±0.098	5.000±0.157
C3	1.510±0.040	1.683±0.091	2.790±0.090	4.786±0.146
C4	1.510±0.040	1.700±0.095	2.790±0.094	4.821±0.145
C5	1.510±0.040	1.683±0.091	2.820±0.095	5.000±0.152
C6	1.610±0.004	1.875±0.134	3.900±0.313	5.636±0.158
C7	1.610±0.004	2.008±0.178	3.690±0.145	6.100±0.167
C8	1.610±0.004	2.058±0.186	3.720±0.123	5.829±0.178
T1	1.610±0.004	2.008±0.179	3.460±0.152	5.186±0.195
T2	1.610±0.004	1.808±0.125	3.230±0.222	4.821±0.180
T3	1.510±0.004	1.625±0.091	3.080±0.269	4.329±0.144
T4	1.510±0.004	1.625±0.091	2.820±0.202	4.186±0.168
T5	1.510±0.004	1.608±0.087	2.740±0.214	4.150±0.158
T6	1.510±0.004	1.608±0.087	2.540±0.136	4.114±0.156
T7	1.510±0.004	1.608±0.087	2.540±0.136	4.114±0.156
T8	1.510±0.004	1.608±0.087	2.540±0.136	4.114±0.156
T9	1.510±0.004	1.608±0.087	2.500±0.149	3.993±0.117
T10	1.510±0.004	1.608±0.087	3.020±0.354	3.864±0.096
T11	1.510±0.004	1.608±0.087	3.020±0.354	3.864±0.096
T12	1.510±0.004	1.608±0.087	2.500±0.149	3.850±0.094
T13	1.510±0.004	1.608±0.087	2.460±0.148	3.764±0.130
L1	1.510±0.004	1.642±0.095	2.540±0.136	3.821±0.136
L2	1.510±0.004	1.642±0.095	2.550±0.132	3.864±0.131
L3	1.510±0.004	1.725±0.133	2.710±0.114	3.879±0.133
L4	1.610±0.004	1.850±0.137	3.090±0.120	4.564±0.148
L5	1.610±1.004	1.908±0.173	3.180±0.089	4.864±0.168
L6	1.610±1.004	1.908±0.173	3.000±0.149	5.371±0.217
S1	1.610±1.004	1.892±0.173	2.860±0.194	4.700±0.271
S2	1.610±1.004	1.775±0.123	2.450±0.302	4.079±0.306
S3	1.210±0.004	1.467±0.102	1.600±0.163	3.179±0.255
S4	1.210±0.004	1.300±0.064	1.500±0.167	2.536±0.275
Cy1	0.810±0.004	0.983±0.046	0.900±0.100	1.644±0.262
Cy2	0.810±0.004	0.933±0.067	0.250±0.083	0.800±0.267
Cy3	0.810±0.004	0.725±0.094	0.250±0.083	0.686±0.215
Cy4	0.810±0.004	0.467±0.105	0.055±0.041	0.400±0.133
Cy5	0.020±0.002	0.033±0.022	0.055±0.041	0.036±0.036

Table 21. Allometric growth of spinal cord and vertebral column at different regions

Regions	Group II (n=6)		Group III (n=12)		Group IV (n=10)		Group V (n=14)		Total gestation period (n= 42)	
	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE
Cervical	0.929 – 1.000	0.988± 0.012	0.900 – 1.000	0.968± 0.014	0.930 – 1.023	0.978± 0.010	0.837 – 1.051	0.983± 0.017	0.837 – 1.051	0.978± 0.007
Thoracic	1.000 – 1.125	1.040± 0.026	0.974 – 1.023	1.000± 0.004	0.926 – 1.051	0.976± 0.013	0.920 – 1.023	0.977± 0.007	0.920 – 1.125	0.992± 0.006
Lumbar	0.917 – 1.000	0.986± 0.014	0.870 – 1.000	0.972± 0.014	0.857 – 1.000	0.963± 0.014	0.794 – 1.020	0.954± 0.018	0.794 – 1.020	0.966± 0.008
Sacral	0.857 – 1.000	0.988± 0.012	0.833 – 1.000	0.972± 0.028	0.579 – 1.071	0.925± 0.049	0.434 – 0.833	0.579± 0.027	0.434 – 1.071	0.835± 0.032
Coccygeal	0.176 – 0.522	0.298± 0.032	0.205 – 0.388	0.262± 0.009	0.119 – 0.244	0.174± 0.011	0.035 – 0.185	0.084± 0.013	0.035 – 0.522	0.192± 0.017

Table 22. Allometric growth rate of spinal cord and vertebral column at different segments

Segments	Group II (n=6)	Group III (n=12)	Group IV (n=10)	Group V (n=14)
C1	0.827	0.951	0.914	1.045
C2	0.878	0.698	0.828	0.776
C3	0.949	0.902	1.110	1.000
C4	0.949	0.918	1.040	1.003
C5	0.780	0.982	0.891	0.838
C6	0.678	0.880	0.881	0.842
C7	0.909	0.884	0.745	0.888
C8	0.955	0.858	0.711	0.780
T1	0.941	0.972	0.895	0.819
T2	0.941	0.990	0.816	0.903
T3	0.941	0.955	0.894	0.930
T4	1.059	1.055	0.862	0.936
T5	1.059	1.022	0.892	1.034
T6	1.000	1.018	0.927	0.912
T7	1.000	1.024	0.871	0.976
T8	1.000	0.992	1.050	0.990
T9	1.059	1.082	1.037	0.991
T10	1.000	1.062	1.048	1.014
T11	0.990	1.023	1.010	0.995
T12	0.989	0.997	1.065	1.133
T13	0.971	0.938	1.05	1.089
L1	1.005	1.002	1.00	1.091
L2	1.000	0.992	1.038	0.946
L3	1.000	1.147	1.051	0.956
L4	1.000	0.983	0.967	1.074
L5	1.000	0.903	0.87	0.820
L6	1.000	0.918	0.825	0.840
S1	1.000	1.015	1.038	0.603
S2	0.941	1.061	1.111	0.653
S3	0.941	0.977	0.912	0.576
S4	1.000	0.922	0.742	0.444
Cy1	0.706	0.854	0.635	0.263
Cy2	0.706	0.685	0.479	0.169
Cy3	0.750	0.626	0.377	0.156
Cy4	0.750	0.541	0.328	0.150

Table 23. Length of dorsal root ganglia of foetal goat (Mean ± S.E), mm

Segments	Group III (n=12)	Group IV (n=10)	Group V (n= 14)
C1	1.417±0.183	2.450±0.090	3.429±0.318
C2	1.450±0.194	2.270±0.065	4.671±0.365
C3	1.383±0.174	2.170±0.065	3.357±0.153
C4	1.250±0.144	1.920±0.073	3.286±0.102
C5	1.350±0.165	2.020±0.020	3.593±0.109
C6	1.500±0.213	2.120±0.066	3.886±0.195
C7	1.500±0.213	2.160±0.078	5.100±0.148
C8	1.517±0.244	2.190±0.468	5.143±0.237
T1	1.250±0.144	1.570±0.142	4.643±0.365
T2	1.033±0.107	1.280±0.098	4.143±0.353
T3	0.950±0.081	1.280±0.098	3.179±0.285
T4	0.883±0.066	1.280±0.098	2.607±0.113
T5	0.883±0.066	1.300±0.111	2.393±0.122
T6	0.883±0.066	1.100±0.100	2.200±0.147
T7	0.817±0.061	1.300±0.111	2.371±0.157
T8	0.883±0.066	1.450±0.090	2.200±0.147
T9	0.883±0.066	1.300±0.111	2.129±0.162
T10	0.883±0.066	1.320±0.125	2.129±0.162
T11	0.983±0.121	1.320±0.125	2.300±0.176
T12	0.983±0.121	1.380±0.111	2.343±0.187
T13	0.983±0.121	1.470±0.104	2.371±0.152
L1	1.117±0.116	1.570±0.221	2.771±0.135
L2	1.117±0.116	1.720±0.290	2.964±0.173
L3	1.150±0.135	1.780±0.266	3.271±0.267
L4	1.283±0.216	1.980±0.914	3.429±0.852
L5	1.317±0.216	2.020±0.063	3.621±0.764
L6	1.317±0.216	2.100±0.316	4.207±1.052
S1	1.133±0.104	1.490±0.486	3.529±0.628
S2	0.967±0.141	1.440±0.386	2.857±1.099
S3	0.933±0.132	1.280±0.253	2.107±0.764
S4	0.150±0.045	1.100±0.316	1.286±0.508
Cy1	0.150±0.045	1.100±0.316	1.286±0.508
Cy2	0.117±0.037	1.000±0.001	0.928±0.182
Cy3	0.117±0.037	1.000±0.001	0.928±0.182

Table 24. Width of dorsal root ganglia of foetal goat (Mean ± S.E), mm

Segments	Group IV (n=10)	Group V (n=14)
C1	1.140±0.099	2.415±0.213
C2	1.120±0.080	2.431±0.174
C3	1.270±0.091	2.469±0.170
C4	1.630±0.126	2.431±0.174
C5	1.650±0.130	2.731±0.184
C6	1.980±0.085	3.277±0.156
C7	2.560±0.133	3.885±0.197
C8	2.310±0.193	3.523±0.289
T1	1.620±0.099	3.408±0.296
T2	1.520±0.075	2.538±0.232
T3	1.000±0.001	2.192±0.133
T4	1.050±0.050	2.069±0.156
T5	1.100±0.100	2.069±0.156
T6	1.050±0.050	2.000±0.170
T7	1.100±0.100	2.000±0.170
T8	1.050±0.050	2.000±0.170
T9	1.050±0.050	2.000±0.170
T10	1.080±0.080	2.000±0.170
T11	1.100±0.100	2.069±0.156
T12	1.160±0.098	2.115±0.151
T13	1.340±0.140	2.131±0.147
L1	1.640±0.060	2.354±0.187
L2	1.570±0.070	2.577±0.232
L3	1.420±0.114	2.938±0.327
L4	1.750±0.112	2.808±0.308
L5	1.780±0.079	2.846±0.173
L6	1.450±0.117	2.900±0.280
S1	1.180±0.140	2.746±0.22
S2	1.160±0.119	1.962±0.192
S3	1.010±0.057	1.577±0.096
S4	1.010±0.057	1.346±0.131
Cy1	0.910±0.048	0.962±0.200
Cy2	0.360±0.147	0.308±0.133
Cy3	0.360±0.147	0.308±0.133

Table 25. Micrometrical data at the first month of gestation (Mean ± S.E), μm

Parameter	Regions						
	Cervical	Cervical enlargement	Thoracic	Lumbar	Lumbar enlargement	Sacral	Coccygeal
Neural tube height	587.500± 53.021	654.375± 54.963	551.667± 35.688	523.125± 33.713	597.500± 40.327	363.333± 11.118	210.000± 15.732
Roof plate height	22.500± 3.354	20.625± 2.745	20.000± 2.795	18.813± 2.894	15.000± 0.001	13.333± 1.667	13.500± 1.300
Floor plate height	70.000± 9.220	80.625± 4.858	61.722± 8.110	63.750± 4.701	91.667± 4.773	47.500± 10.680	25.500± 3.824
Lumen height	466.667± 33.133	486.875± 29.760	459.222± 30.022	436.875± 23.622	455.000± 25.593	317.500± 11.726	165.000± 10.062
Alar plate height	232.500± 21.823	228.750± 16.949	255.000± 17.678	243.750± 20.975	252.500± 21.360	176.667± 24.080	80. 342± 2.697
Basal plate height	280.000± 41.473	318.750± 33.391	235.000± 19.843	245.625± 16.020	290.000± 25.000	181.667± 27.588	122.367± 8.127
Neural tube width - upper	475.000± 17.607	534.375± 28.053	391.667± 21.570	468.750± 16.223	482.500± 55.644	240.000± 13.693	132.000± 9.950
Neural tube width - lower	532.500± 24.418	697.500± 61.651	453.889± 36.570	575.625± 45.966	655.000± 93.327	318.333± 10.240	87.000± 17.348
Lumen to neural tube left margin -upper	182.500± 22.389	221.250± 25.630	168.333± 12.693	185.625± 15.510	206.667± 32.395	105.833± 5.512	52.500± 4.743
Lumen to neural tube left margin - lower	237.500± 16.163	322.500± 32.445	227.556± 16.604	261.625± 20.506	312.500± 47.971	142.500± 4.330	57.000± 1.837
Lumen to mantle layer left margin - upper	102.500± 2.500	155.000± 25.884	146.667± 14.530	140.625± 14.983	155.000± 25.884	77.778± 3.447	*
Lumen to mantle layer left margin - lower	162.500± 2.500	290.625± 28.338	206.111± 11.925	220.000± 18.028	275.000± 41.833	128.333± 3.632	*
Lumen width- upper	95.000± 3.162	84.375± 2.745	66.667± 6.180	75.000± 6.708	77.500± 2.500	65.000± 3.536	52.500± 7.500
Lumen width- lower	90.000± 33.317	80.625± 12.006	22.500± 2.165	43.125± 9.997	55.000± 11.402	45.000± 6.124	15.000± 0.500

Table 25 Continued .....

Parameter	Regions						
	Cervical	Cervical enlargement	Thoracic	Lumbar	Lumbar enlargement	Sacral	Coccygeal
Ependymal thickness - alar plate	110.000± 6.325	103.125± 14.013	76.111± 7.158	95.625± 7.986	125.500± 20.736	65.000± 2.500	63.000± 7.348
Ependymal thickness - basal plate	57.500 ± 8.139	50.625± 7.986	51.667± 6.180	39.375± 8.475	55.000± 11.402	43.333± 3.909	63.000± 7.348
Mantle layer thickness –alar plate	57.500± 19.429	26.250± 3.750	38.333 ±6.180	39.375± 8.475	30.000± 5.477	15.000± 0.000	*
Mantle layer thickness –basal plate	157.500± 29.686	238.125± 34.537	153.333± 16.729	208.125± 45.747	220.000± 51.769	83.333± 7.546	*
Marginal layer thickness –alar plate	42.500± 10.368	75.000± 13.595	35.000± 3.536	46.875± 5.256	75.000± 22.249	17.500± 1.250	21.000± 6.452
Marginal layer thickness –basal plate	27.500± 2.500	30.000± 5.669	31.667± 4.640	33.750± 6.797	32.500± 9.014	16.667± 1.667	21.000± 6.452
Basal plate width	220.833± 32.975	324.375± 32.561	216.667± 17.159	259.750± 21.523	312.500± 47.971	141.667± 5.652	42.000± 2.000
Alar plate width	182.500± 22.389	219.375± 25.971	155.000± 15.612	185.625± 15.510	195.000± 38.536	104.167± 6.305	33.000± 3.000
Dorsal funiculus thickness	40.000± 3.162	54.375± 9.794	39.222± 2.425	45.000± 6.705	55.000± 11.402	22.500± 2.165	1.500± 0.500
Lateral funiculus thickness	35.000± 6.325	58.125± 13.724	37.556± 2.501	39.375± 3.946	52.500± 12.093	40.000± 2.500	*
Ventral funiculus thickness	45.000± 9.487	63.750± 7.365	53.333± 6.667	46.875± 5.256	62.500± 8.139	40.000± 6.614	3.000± 0.500
Width of dorsal root ganglia	114.167± 4.167	170.000± 33.690	113.333± 6.667	120.000± 6.944	133.125± 17.111	61.667± 7.265	4.500± 3.000
Length of dorsal root ganglia	305.000± 5.000	335.625± 26.583	235.000± 6.614	241.875± 17.802	330.000± 42.953	255.000± 20.463	21.000± 14.697

* not measurable

Table 26. Segment-wise height of precoccygeal spinal cord, gray matter, dorsal horn and ventral horn at second and third month of gestation (Mean ± S.E), μm

Segments	Group II				Group III			
	Spinal cord	Gray matter total	Dorsal horn	Ventral horn	Spinal cord	Gray matter total	Dorsal horn	Ventral horn
C1	1387.500 ±50.312	1012.500±36.895	465.000±6.708	547.500±30.187	1747.500±43.603	1211.500±17.218	600.000±11.180	611.500±28.398
C2	1200.000± 67.082	950.500±95.033	437.500±27.951	513.000±67.082	1522.500±90.561	1185.000±60.374	622.500±43.603	562.500±16.771
C4	1200.000± 33.541	907.500±50.312	442.500±16.771	465.000±33.541	1350.000±67.082	1005.000±20.125	517.500±3.354	487.500±16.771
C6	1462.500± 30.187	1210.000±29.069	637.500±16.771	572.500±1.118	1650.000±87.207	1335.000±40.249	712.500±50.312	622.500±10.062
C8	1575.000±22.361	1356.500±36.448	719.000±53.218	637.500±5.590	1822.500±10.062	1427.500±25.715	742.500±36.895	685.000±17.889
T2	1470.000±26.833	1230.000±20.125	592.500±16.771	637.500±30.187	1669.000±8.497	1380.000±20.125	712.500±50.312	667.500±36.895
T5	1477.500±36.895	1113.500±61.492	625.500±27.951	488.000±22.361	1515.000±60.374	1162.500±43.603	532.500±43.603	630.000±20.125
T9	1400.000±11.180	1069.500±36.448	575.500±50.312	494.000±25.044	1500.000±73.790	1222.500±30.187	645.000+33.541	577.500±43.603
T12	1372.500±10.062	1088.000±38.013	586.500±34.212	501.500±16.323	1372.500±10.613	1135.000±71.554	647.500±32.423	487.500±10.062
L1	1320.000±33.541	1060.000±49.193	567.500±41.367	492.500±7.826	1350.000±22.361	1095.000±13.416	600.000±20.125	495.000±6.708
L2	1416.500±9.165	1095.000±12.298	592.500±16.771	502.500±3.354	1455.000±24.597	1157.000±36.224	550.500±5.590	606.500±30.634
L3	1425.000±33.541	1137.500±32.423	592.500±16.771	545.000±15.652	1515.000±60.374	1290.000±93.915	622.500±43.603	667.500±50.312
L4	1470.000±31.305	1199.000±6.932	622.500±10.062	576.500±3.130	1552.500±23.479	1350.000±67.082	645.000±33.541	705.000±33.541
L6	1475.000±22.361	1276.000±55.902	563.000±55.902	713.000±5.813	1560.000±26.833	1290.000±6.708	675.000±2.236	615.000±6.708
S2	1432.900±14.713	1215.500±2.236	531.000±0.447	684.000±2.683	1517.500±25.715	1230.000±13.416	637.500±23.479	592.500±30.187
S4	1035.000±26.833	925.000±44.721	492.500±1.118	432.500±0.224	1245.000±8.944	990.000±3.130	540.000±1.342	450.000±1.789

Table 27. Segment-wise height of precoccygeal spinal cord, gray matter, dorsal horn and ventral horn at fourth and fifth month of gestation (Mean ± S.E), μm

Segments	Group IV				Group V			
	Spinal cord	Gray matter total	Dorsal horn	Ventral horn	Spinal cord	Gray matter total	Dorsal horn	Ventral horn
C1	2800.000± 76.026	2025.000±67.082	975.000±33.541	1050.000±100.623	3815.000±216.899	2557.500±197.892	1320.000±181.122	1237.500±16.771
C2	2625.000±67.282	1851.000±143.556	938.000±28.174	913.000±44.721	3727.500±177.767	2000.000±111.803	937.500±83.853	1062.500±27.951
C4	2475.000±33.541	1688.000±50.535	813.000±28.174	875.000±11.180	3000.000±100.623	2137.500±50.312	935.000±84.971	1202.500±34.659
C6	2895.000±46.957	2175.000±46.957	990.000±77.368	1185.000±54.560	3900.000±201.246	2475.000±100.623	1350.000±134.164	1125.000±33.541
C8	2910.000±12.522	2313.000±75.132	1188.000±11.180	1125.000±27.727	3925.000±212.426	2500.000±111.803	1337.500±128.574	1162.500±16.771
T2	2480.000±26.836	1860.000±26.833	945.000±20.125	915.000±6.708	3750.000±134.164	2175.000±100.623	1125.000±33.541	1050.000±67.082
T5	2220.000±53.665	1500.000±139.531	675.000±78.262	825.000±61.268	3337.500±352.181	1906.250±153.730	912.500±128.574	993.750±23.985
T9	1920.000 ±80.608	1290.000±26.833	630.000±8.944	660.000±17.889	3247.500±244.849	1785.000±107.331	719.000±13.864	1066.000±93.468
T12	1845.200±40.249	1125.000±33.541	550.000±22.361	575.000±55.902	3270.000±234.787	2033.750±225.284	950.000±122.984	1083.750±102.300
L1	1800.000±100.028	1245.000±93.915	495.000±19.230	750.000±20.125	3187.500±184.476	1810.000±82.735	850.000±11.180	960.000±93.915
L2	1950.000±33.741	1455.000±46.957	705.000±74.685	750.000±8.944	3210.000±174.413	1741.500±113.369	806.500±8.273	935.000±105.095
L3	1980.000±2.436	1470.000±6.708	675.000±13.416	795.000±20.125	3375.000±100.623	2231.250±92.238	1281.250±69.877	950.000±22.361
L4	2250.900±67.085	1875.000±33.541	900.000±33.541	975.000±27.727	3562.500±16.771	2587.500±36.895	1252.500±57.020	1335.000±26.833
L6	2400.000±33.541	1888.000±12.522	975.000±33.541	913.000± 5.814	3412.500±83.853	2662.500±23.479	1305.000±33.541	1357.500±16.771
S2	2040.000±61.708	1650.000±22.361	900.000±11.180	750.000±4.472	2038.500±10.062	1725.000±33.541	937.500±16.771	787.500±16.771
S4	1575.000±31.305	1245.000±17889	675.000±18.371	570.000±15.652	2017.500±7.826	1320.000±8.944	690.000±40.249	630.000±13.416

Table 28. Segment-wise width of precoccygeal spinal cord at second and third month of gestation (Mean+ S.E), μm

Segments	Group II					Group III				
	Spinal cord width	Central canal to spinal cord left margin	Gray matter total width	Doral horn width	Ventral horn width	Spinal cord width	Central canal to spinal cord left margin	Gray matter total width	Doral horn width	Ventral horn width
C1	1865.000± 71.554	872.500± 25.715	506.250± 8.385	375.500± 39.131	375.500± 60.373	1882.500± 63.728	896.25± 31.864	622.500± 10.062	716.500± 31.976	537.500± 39.131
C2	1530.000± 80.498	735.000± 36.895	467.000± 34.435	406.000± 41.814	375.500± 27.951	1575.000± 33.541	727.500± 57.020	525.000± 33.541	577.000± 30.411	470.000± 24.497
C4	1476.500± 77.512	708.25± 31.677	418.000± 8.944	387.500± 5.590	316.500± 12.746	1545.000± 114.039	727.500± 12.298	483.000± 25.491	537.500± 27.951	483.000± 25.591
C6	1605.000± 2.236	768.750± 0.559	551.500± 61.939	400.000± 24.150	570.000± 20.125	1695.000± 33.541	787.500± 16.771	600.000± 10.062	632.000± 12.522	560.000± 11.180
C8	1788.000± 128.798	860.250± 62.722	592.500± 30.187	594.000± 97.940	606.500± 47.628	1905.000± 46.957	892.500± 23.479	660.000± 13.416	712.500± 3.354	720.000± 13.146
T2	1657.000± 23.479	798.750± 1.046	569.000± 53.218	450.000± 33.541	585.000± 38.013	1957.500± 23.479	924.750± 11.739	750.000± 26.833	690.000± 6.708	712.500± 16.771
T5	1375.000± 22.361	665.000± 12.746	435.000± 2.236	456.000± 19.454	367.500± 10.062	1425.000± 33.541	660.000± 16.771	513.750± 15.093	460.000± 26.833	500.000± 44.721
T7	1462.500± 50.312	697.500± 26.777	444.000± 13.864	469.000± 13.834	438.000± 3.578	1650.000± 67.082	787.500± 33.541	521.500± 9.615	555.000± 13.416	457.500± 3.354
T12	1515.000± 6.708	716.250± 1.677	475.000± 6.708	512.500± 5.590	416.500± 1.565	1670.000± 44.721	782.500± 22.361	583.750± 10.621	630.000± 20.125	577.500± 16.771
L1	1350.000± 67.082	641.250± 28.510	485.000± 29.069	500.500± 27.951	519.000± 36.724	1665.000± 33.541	788.500± 16.771	506.500± 41.814	540.000± 13.416	525.000± 60.373
L2	1425.000± 33.541	675.000± 13.416	537.500± 5.390	519.000± 36.224	555.000± 46.957	1698.000± 32.199	792.500± 16.100	586.500± 66.411	570.000± 15.652	655.000± 69.318
L3	1430.100± 31.305	677.500± 12.298	567.500± 7.876	535.000± 40.249	592.500± 30.187	1650.000± 11.180	772.500± 5.590	630.000± 46.957	577.500± 3.354	650.000± 55.942
L4	1800.000± 13.416	855.000± 6.708	656.250± 5.031	665.000± 17.889	630.000± 20.125	1890.000± 26.832	900.000± 13.416	825.000± 46.957	727.500± 10.062	765.000± 40.249
L6	1890.000± 13.416	896.250± 8.385	681.500± 52.995	625.500± 27.951	750.000± 20.125	1963.000± 38.908	937.500± 19.454	720.000± 6.708	750.000± 20.572	769.000± 120.300
S2	1695.000± 33.541	810.000± 16.771	592.500± 3.354	600.000± 6.261	688.000± 19.677	1890.000± 40.249	901.000± 20.125	688.000± 20.572	687.500± 5.590	842.500± 7.826
S4	1260.000± 26.833	611.250± 15.093	433.500± 14.982	460.000± 26.833	407.000± 3.130	1560.000± 13.416	761.000± 6.708	536.250± 38.572	540.000± 13.416	517.500± 10.062

Table 29. Segment-wise width of precoccygeal spinal cord at fourth and fifth month of gestation (Mean± S.E), μm

Segments	Group IV					Group V				
	Spinal cord width	Central canal to spinal cord left margin	Gray matter total width	Doral horn Width	Ventral horn width	Spinal cord width	Central canal to spinal cord left margin	Gray matter total width	Doral horn width	Ventral horn width
C1	3250.000± 11.180	1580.00± 23.255	975.000± 8.944	850.000± 11.180	825.000± 11.180	5550.000± 603.738	2670.000± 295.161	1372.500± 90.561	1000.000± 111.803	937.500± 83.853
C2	2550.000± 6.261	1237.500± 3.354	720.000± 6.708	688.000± 12.522	563.000± 28.174	4875.000± 436.033	2351.250± 206.277	990.000± 60.374	985.000± 38.013	900.000± 4.472
C4	2526.000± 11.628	1218.000± 8.050	675.000± 6.708	705.000± 2.236	625.000± 11.180	4496.000± 338.093	2157.375± 162.059	1031.250± 58.697	750.000± 10.286	712.500± 39.131
C6	3510.000± 4.472	1687.500± 12.298	960.000± 11.180	838.000± 16.994	975.000± 33.541	5500.000± 111.803	2661.875± 52.827	1316.250± 0.956	850.000± 72.672	1481.250± 75.467
C8	3900.000± 18.783	1897.500± 8.944	1050.000± 12.522	875.000± 11.180	1038.000± 16.994	5925.000± 301.869	2855.000± 143.108	1335.000± 6.708	1006.000± 2.907	1725.000± 33.541
T2	2880.000± 10.733	1387.500± 16.771	825.000± 10.773	725.000± 2.236	750.000± 22.361	4700.000± 290.689	2267.500± 137.518	1112.500± 61.492	844.000± 42.038	1275.000± 100.623±
T5	2040.000± 5.814	982.500± 5.590	625.000± 3.578	463.000± 4.472	510.000± 4.472	4200.000± 402.492	2036.250± 196.215	678.000± 48.634	688.000± 27.727	594.000± 13.864
T7	2400.000± 2.236	1162.500± 10.062	720.000± 1.342	625.000± 6.708	460.000± 26.833	4063.000± 502.221	1970.875± 244.682	825.000± 67.082	856.500± 36.448	569.000± 13.864
T12	2460.000± 10.286	1185.000± 11.180	780.000± 25.491	663.000± 13.416	690.000± 40.249	4325.000± 391.312	2091.250± 187.271	912.500± 39.131	719.000± 13.864	718.500± 13.975
L1	2440.000± 4.919	1175.000± 20.125	675.000± 21.466	688.000± 8.050	563.000± 5.814	4200.000± 447.214	2021.250± 189.507	870.000± 46.957	700.000± 5.813	731.500± 75.355
L2	2460.000± 16.100	1177.500± 16.771	705.000± 7.603	688.000± 22.361	690.000± 4.472	4230.000± 389.076	2043.750± 179.444	922.500± 23.479	750.000± 55.902	762.500± 61.492
L3	2540.000± 12.969	1217.500± 2.012	750.000± 21.019	688.000± 35.777	700.000± 4.472	4350.000± 335.410	2101.875± 158.481	1180.000± 31.305	937.500± 27.951	950.000± 22.361
L4	2910.000± 10.826	1410.000± 9.839	1080.000± 29.069	938.000± 13.416	780.000± 17.889	5140.000± 205.718	2476.2500± 91.120	1500.000± 33.541	1094.000± 42.038	1300.000± 33.541
L6	2940.000± 98.387	1417.500± 12.746	900.000± 27.280	875.000± 11.180	1125.000± 11.180	5290.000± 308.577	2570.000± 157.643	1297.500± 23.479	1156.500± 41.814	1702.500± 10.062
S2	2030.000± 6.708	985.000± 4.025	765.000± 20.572	765.000± 15.652	975.000± 11.180	2100.000± 13.416	1000.000± 1.118	735.000± 0.447	938.000± 16.994	862.500± 27.951
S4	2010.000± 0.894	967.500± 6.485	674.500± 31.081	625.000± 4.472	530.000± 13.416	2085.000± 6.708	993.750± 1.677	690.000± 6.708	842.500± 41.367	785.750± 38.349

Table 30. Height and width of precoccygeal spinal cord structures at different regions during second and third month of gestation (Mean ± S. E)

Parameter	Group II						Group III					
	Cervical region	Cervical enlargement	Thoracic region	Lumbar region	Lumbar enlargement	Sacral region	Cervical region	Cervical enlargement	Thoracic region	Lumbar region	Lumbar enlargement	Sacral region
HEIGHT												
Spinal cord (μm)	1262.500± 65.431	1502.500± 32.755	1416.667± 30.405	1387.167± 35.133	1472.500± 35.148	1035.000± 26.833	1540.000± 100.822	1713.833± 61.528	1462.500± 64.791	1440.000± 50.133	1556.250± 32.620	1245.000± 8.944
Total gray matter (μm)	956.833 ± 68.406	1265.500± 41.242	1090.333± 33.758	1097.500± 37.665	1237.500± 56.140	925.000± 44.72	1133.833± 55.965	1380.833± 50.580	1173.330± 61.409	1180.666± 68.999	1320.000± 64.161	990.000± 3.130
Dorsal horn (μm)	448.333± 19.944	649.667± 40.985	595.833± 39.829	584.167± 28.030	592.750± 54.622	492.500± 1.118	580.000± 32.965	722.500± 46.704	608.333± 43.982	591.000± 30.985	660.000± 31.885	540.000± 1.342
Ventral horn (μm)	508.500± 49.068	615.833± 22.413	494.500± 21.694	513.333± 14.472	644.750± 39.72	432.500± 0.224	553.833± 31.247	658.333± 27.070	565.000± 38.665	589.667± 46.816	660.000± 40.620	450.000± 1.789
Mean gray matter %	75.789	84.226	76.965	79.118	84.041	89.372	73.626	80.570	80.228	81.991	84.819	79.518
WIDTH												
Spinal cord (μm)	1623.833± 108.559	1683.500± 83.060	1450.333± 41.138	1401.667± 49.694	1845.000± 31.225	1260.000± 26.833	1657.500± 40.204	1852.500± 62.179	1581.667± 170.777	1671.000± 29.029	1926.500± 48.016	1560.000± 13.416
Central canal to spinal cord left margin (μm)	771.917± 47.026	809.250± 40.589	692.917± 19.616	664.583± 20.881	875.625± 15.424	611.250± 15.093	783.750± 52.328	868.250± 31.783	744.333± 36.479	784.500± 14.335	918.750± 24.136	761.000± 6.708
Central canal to gray matter left margin (μm)	463.750± 26.571	571.000± 50.824	451.333± 11.809	530.000± 23.345	668.875± 49.139	433.500± 14.982	543.500± 36.170	670.000± 33.072	539.667± 18.458	574.333± 57.543	772.500± 52.856	536.250± 38.572
Dorsal horn (μm)	389.833± 33.708	481.333± 71.551	479.333± 17.772	518.167± 35.743	645.250± 32.369	460.000± 26.833	610.333± 45.702	678.167± 17.350	548.333± 37.454	562.500± 14.068	738.750± 21.891	540.000± 13.416
Ventral horn (μm)	353.667± 41.024	587.167± 37.652	407.333± 14.591	555.500± 40.697	690.000± 43.301	407.000± 3.130	496.333± 33.162	664.167± 35.810	511.667± 34.609	610.000± 67.688	767.000± 115.808	517.000± 10.062
Mean gray matter width %	57.253	67.855	62.217	75.624	72.507	68.810	65.581	72.335	68.240	68.741	80.197	68.750

Table 31. Height and width of precoccygeal spinal cord structures at different regions at fourth and fifth month of gestation (Mean ± S. E)

Parameter	Group IV						Group V					
	Cervical region	Cervical enlargement	Thoracic region	Lumbar region	Lumbar enlargement	Sacral region	Cervical region	Cervical enlargement	Thoracic region	Lumbar region	Lumbar enlargement	Sacral region
HEIGHT												
Spinal cord (μm)	2633.333± 85.612	2755.500± 98.678	1995.000± 94.393	1900.000± 68.932	2325.000± 81.009	1575.000± 31.305	3809.167± 177.696	3858.333± 189.040	3285.000± 282.134	3257.500± 162.018	3487.500± 89.268	2017.500± 7.826
Total gray matter (μm)	1854.667± 114.051	2116.000± 100.225	1305.000± 108.695	1390.000± 26.158	1881.500± 32.898	1220.000± 17.889	2231.667± 171.205	2383.333± 123.603	1908.333± 175.209	1927.583± 137.038	2625.000± 45.415	1320.000± 8.944
Dorsal horn (μm)	908.667± 43.186	1041.000± 64.815	618.833± 52.626	625.000± 61.339	937.500± 48.412	615.000± 18.371	1064.167± 149.100	1270.833± 118.395	860.500± 112.540	979.250± 104.286	1278.750± 62.262	690.000± 40.249
Ventral horn (μm)	946.000± 72.202	1075.000± 62.798	686.667± 67.484	765.000± 19.664	944.000± 31.451	610.000± 15.652	1167.500± 43.565	1112.500± 49.054	1047.833± 83.153	948.333± 82.519	1346.250± 29.607	630.000± 13.416
Mean gray matter %	70.430	76.806	65.414	73.158	80.925	77.777	58.587	61.771	58.092	59.174	75.269	65.428
WIDTH												
Spinal cord (μm)	2775.333± 150.449	3430.000± 188.368	2300.000± 83.236	2480.000± 22.888	2925.000± 15.615	2010.000± 0.894	4973.667± 510.863	5375.000± 338.071	4196.000± 437.500	4260.000± 394.293	5215.000± 341.309	2085.000 ±6.708
Central canal to spinal cord left margin (μm)	1345.167± 75.716	1657.500± 94.503	1110.000± 41.573	1190.000± 17.491	1413.750± 14.857	967.500± 6.485	2392.875± 246.801	2594.792± 395.089	2032.792± 212.047	2055.625± 176.939	2523.125± 168.407	993.750± 1.677
Central canal to gray matter left margin (μm)	790.000± 59.554	945.000± 42.922	708.333± 32.186	710.000± 22.586	990.000± 51.797	674.500± 31.081	1131.250± 104.757	1254.583± 57.505	805.417± 68.253	990.833± 70.112	1398.750± 63.383	690.000± 6.708
Dorsal horn (μm)	721.000± 43.955	812.667± 30.883	583.667± 39.823	688.000± 24.798	906.500± 24.185	625.000± 4.472	911.667± 85.468	900.333± 58.999	754.500± 42.844	795.833± 58.341	1125.250± 57.055	842.500± 41.367
Ventral horn (μm)	697.667± 51.004	921.000± 60.781	553.333± 53.324	651.000± 28.676	952.500± 101.438	530.000± 13.416	850.000± 69.270	1493.750± 111.418	627.250± 32.424	814.667± 71.996	1501.250± 120.509	785.750± 38.349
Mean gray matter width %	56.930	55.102	61.594	57.258	67.692	67.114	45.490	46.682	38.390	46.518	53.643	66.187

Table 32. Average percentage contribution of dorsal and ventral horn height to gray matter and precoccygeal spinal cord height at second and third month of gestation (μm)

Parameter	Group II						Group III					
	C*	CE*	T*	L*	LE*	S*	C*	CE*	T*	L*	LE*	S*
Dorsal horn to spinal cord %	35.511	43.239	42.057	42.112	40.255	47.585	37.664	42.157	41.5955	41.042	42.410	43.370
Dorsal horn to gray matter %	46.856	51.337	54.646	53.227	47.899	53.243	51.154	52.324	51.847	49.463	50.000	54.540
Ventral horn to spinal cord %	40.277	40.987	34.906	37.008	43.786	41.787	35.963	38.413	38.632	40.919	42.410	36.145
Ventral horn to gray matter %	53.144	48.663	45.353	46.772	52.101	46.757	48.846	46.677	48.153	49.351	50.000	45.454

C =Cervical region
CE =Cervical enlargement
T =Thoracic region
L =Lumbar region
LE =Lumbar enlargement
S =Sacral region

Table 33. Average percentage contribution of dorsal and ventral horn height to gray matter and precoccygeal spinal cord height at fourth and fifth month of gestation (μm)

Parameter	Group IV						Group V					
	C*	CE*	T*	L*	LE*	S*	C*	CE*	T*	L*	LE*	S*
Dorsal horn to spinal cord %	34.506	37.786	30.994	32.895	40.323	39.048	27.937	32.937	26.195	30.061	36.667	34.201
Dorsal horn to gray matter %	48.994	49.197	47.382	44.964	49.827	50.410	47.685	53.321	45.092	50.802	48.714	52.273
Ventral horn to spinal cord %	35.924	39.020	34.419	40.263	40.603	38.730	30.649	28.834	31.898	29.112	38.602	31.227
Ventral horn to gray matter %	51.006	50.803	52.618	55.036	50.173	50.000	52.315	46.678	54.908	49.198	51.285	47.727

C =Cervical region

CE =Cervical enlargement

T =Thoracic region

L =Lumbar region

LE =Lumbar enlargement

S =Sacral region

Table 34. Percentage increase in height of precoccygeal spinal cord between age groups (μm)

Parameters and period (month)	Regions					
	Cervical	Cervical enlargement	Thoracic	Lumbar	Lumbar enlargement	Sacral
Spinal cord height						
2 – 3	21.980	14.065	3.235	3.809	5.688	20.290
3 – 4	70.996	60.751	36.410	31.944	49.398	26.506
4 – 5	44.652	40.048	64.662	71.447	50.000	28.095
2 – 5	201.238	156.794	131.882	134.831	136.842	94.928
Gray matter height						
2 – 3	18.499	9.114	7.612	7.578	6.667	7.027
3 – 4	63.575	53.241	11.222	17.730	42.538	23.232
4 – 5	20.327	12.634	46.232	38.675	39.516	8.197
2 – 5	133.235	88.331	75.023	75.634	112.121	42.703
Dorsal horn height						
2 – 3	29.368	11.211	1.091	1.170	11.345	9.645
3 – 4	56.667	44.083	2.656	5.753	4.205	13.889
4 – 5	17.113	22.078	39.164	56.680	36.400	12.195
2 – 5	137.361	95.613	44.420	67.632	115.732	40.102
Ventral horn height						
2 – 3	8.915	6.901	14.257	14.870	2.365	4.046
3 – 4	70.810	63.291	21.534	29.734	43.030	35.556
4 – 5	23.414	3.488	52.597	23.965	42.611	3.279
2 – 5	129.597	80.650	111.897	84.740	108.802	45.665

Table 35. Percentage increase in width of precoccygeal spinal cord between age groups (μm)

Parameters and period (month)	Regions					
	Cervical	Cervical enlargement	Thoracic	Lumbar	Lumbar enlargement	Sacral
Spinal cord width						
2 – 3	2.073	10.039	9.018	19.215	4.417	23.810
3 – 4	67.441	85.155	45.416	48.414	51.830	28.846
4 – 5	79.210	56.706	82.435	71.774	78.290	3.731
2 – 5	206.292	219.275	189.213	203.924	182.656	65.476
Central canal to spinal cord left margin						
2 – 3	1.533	7.291	7.276	18.044	4.925	24.499
3 – 4	71.632	90.901	49.327	51.689	53.878	27.135
4 – 5	77.887	56.549	83.134	72.742	78.470	2.713
2 – 5	209.991	220.640	193.367	209.311	188.151	62.577
Central canal to gray matter left margin						
2 – 3	17.197	17.338	19.572	8.365	15.492	23.702
3 – 4	45.354	41.045	31.254	23.622	28.155	25.781
4 – 5	43.196	32.760	13.706	39.554	41.288	2.300
2 – 5	143.935	119.717	78.453	86.950	109.120	59.170
Dorsal horn width						
2 – 3	56.563	40.894	14.395	8.556	14.491	17.391
3 – 4	18.132	19.833	6.444	22.311	22.707	15.741
4 – 5	6.935	10.787	29.270	15.673	24.131	34.800
2 – 5	97.771	87.050	57.406	53.586	74.390	83.152
Ventral horn width						
2 – 3	40.480	13.114	25.614	9.811	11.159	27.150
3 – 4	40.423	38.670	8.143	6.721	24.185	2.416
4 – 5	21.835	62.188	13.359	25.141	57.612	48.255
2 – 5	140.339	154.400	53.089	46.655	117.573	93.059

Table 36. Average width of neurons in the different laminae of gray matter at fifth month of gestation (μm)

Lamina		124 days						142 days					
		C	CE	T	L	LE	S	C	CE	T	L	LE	S
I*		10.8x28.8-12.6x39.6	7.2x25.2-7.2x28.8	7.2 x18.0-10.8x36.0	7.2x39.6 - 10.8x43.2	10.8x18.0-10.8x36.0	7.2x18.0 - 7.2x28.8	10.8x46.8-14.4x46.8	3.6x28.8 - 3.6x46.8	10.8x43.2 - 8.0x43.2	7.2x36.0 - 10.8x21.6	9.0x38.8 - 10.8x32.4	3.6x5.4 - 3.6x36.0
II	I type	5.4 – 7.2	5.4 – 7.2	5.4 – 7.2	5.4 – 7.2	5.4 – 7.2	5.4 – 7.2	5.4 - 7.2	14.4 – 25.2	14.4 – 18.0	7.2 – 10.8	7.2 – 10.8	7.2 – 10.8
	II type	3.6 – 5.4	3.6 – 5.4	3.6 – 5.4	3.6 - 5.4	3.6 – 5.4	3.6 – 5.4	3.6 – 5.4	3.6 – 10.8	7.2 – 9.0	5.4 – 7.2	5.4 – 7.2	7.2 – 9.0
III		10.8 – 21.6	10.8 - 18.0	7.2 – 18.0	7.2 – 14.4	10.8 – 14.4	14.4 – 18.0	43.2 – 46.8	14.4 - 25.2	10.8 – 28.8	14.4 – 36.0	18.0- 28.8	7.2 – 10.2
IV		14.4 – 18.0	18.0 - 28.8	10.8 – 18.0	10.8 – 18.0	10.8 – 32.4	10.8- 14.4	10.8- 21.6	14.4- 36.0	10.8- 36.0	10.8- 43.2	14.4- 43.2	10.8- 14.4
V		14.4 – 32.4	14.4 - 25.2	14.4- 28.8	14.4 – 25.2	14.4- 21.6	10.8 – 14.4	14.4 – 39.6	14.4 – 36.0	14.4 – 25.2	10.8 – 32.4	14.4 - 36.0	14.4 – 18.0
VI		14.4 – 18.0	10.8 - 28.8	21.6 – 36.0	10.8 – 18.0	10.8 – 18.0	10.8- 14.4	14.4 – 36.0	14.4 – 36.0	14.4 – 21.6	14.4 – 21.6	14.4 - 18.0	14.4 – 18.0
VII		18.0 – 21.6	14.4 - 36.0	18.0 – 21.6	18.0 – 28.8	18.0 – 21.6	18.0 – 25.2	18.0- 46.8	43.2 – 54.0	21.6- 32.4	14.4 – 36.0	21.6- 50.4	18.0- 36.0
VIII		10.8 – 14.0	10.8- 18.0	10.8- 14.4	10.8 – 14.4	14.4 – 18.0	10.8- 14.4	10.8 – 14.4	10.8 – 18.0	14.4 – 18.0	10.8 – 14.4	14.4 - 18.0	10.8 – 14.4
X		10.8 – 18.0	10.8 - 14.4	10.8 – 14.4	10.8 – 14.4	10.8 – 14.4	10.8- 14.4	14.4- 18.0	14.4- 18.0	14.4- 18.0	19.8 – 25.2	25.2- 36.0	9.0- 14.4

* width x length

Table 37. Segment-wise width and height of substantia gelatinosa at different ages (Mean ± S.E), μm

Segments	Group II		Group III		Group IV		GroupV	
	Width	Height	Width	Height	Width	Height	Width	Height
C1	500.000± 55.902	300.000± 0.000	671.500± 21.690	312.500± 27.951	1000.000± 18.257	750.000± 17.892	1287.500± 16.771	881.500± 41.814
C2	469.000 ±42.038	250.000± 0.000	512.500± 5.590	294.000± 36.224	813.000± 4.747	663.000± 22.639	1138.000± 22.361	775.5000± 27.951
C4	468.500± 14.087	231.500± 8.273	481.000± 19.677	237.500± 5.590	688.000± 16.067	563.000± 8.398	950.000± 33.541	750.5000± 27.951
C6	606.500± 36.448	243.000± 2.907	613.000± 33.541	252.500± 1.118	1000.000± 10.954	775.000± 27.386	1137.500± 5.590	937.500± 83.853
C8	650.000± 11.180	262.500± 5.590	681.500± 2.907	281.500± 14.087	1063.000± 23.004	750.000± 11.180	1159.000± 12.969	750.000± 55.902
T2	596.500± 14.982	269.000± 8.497	669.000± 47.405	300.000± 22.361	938.000± 13.876	588.000± 16.797	1156.500± 14.087	738.000± 33.541
T5	550.500± 5.590	281.500± 14.087	594.000± 13.864	344.000± 13.864	813.000± 4.747	563.000± 2.921	938.000± 0.000	719.000± 97.940
T9	475.000± 11.180	250.000± 0.000	563.000± 0.000	281.500± 14.087	838.000± 13.876	688.000± 23.735	937.500± 27.951	562.500± 55.902
T12	443.500± 2.907	231.500± 8.273	594.000± 13.864	300.500± 5.590	563.000± 23.004	500.000± 9.859	844.000± 42.038	625.000± 55.902
L1	512.500± 5.590	281.500± 14.087	531.500± 14.087	294.000± 19.677	938.000± 10.954	750.000± 13.510	1000.000± 55.902	562.500± 27.951
L2	519.000± 8.497	250.000± 0.000	544.000± 8.497	325.000± 22.361	1000.000± 9.129	437.000± 12.780	1125.000± 55.902	625.000± 27.951
L3	562.500± 27.951	250.000± 0.000	563.000± 0.000	269.000± 19.677	938.000± 2.921	625.000± 4.382	1212.500± 16.771	594.000± 13. 864
L4	631.500± 25.268	291.500± 9.615	712.500± 16.771	294.000± 19.677	1125.000± 45.643	600.000± 20.448	1487.500± 5.590	706.500± 64.175
L6	719.000± 13.866	281.500± 14.087	812.500± 27.951	344.000± 13.864	1250.000± 18.257	625.000± 6.208	1562.500± 27.951	813.000± 0.000
S2	412.500± 16.771	200.000± 22.361	531.000± 42.038	281.500± 14.087	750.000± 14.606	500.000± 5.477	1331.500± 14.087	531.000± 13.864
S4	294.000± 8.497	137.500± 5.590	331.500± 8.273	218.500± 14.087	750.000± 8.764	437.000± 12.050	750.000± 3.651	500.000± 3.651

Table 38. Region-wise average of width and height of substantia gelatinosa at different ages (Mean ± S.E), μm

Regions	Group II		Group III		Group IV		Group V	
	Width	Height	Width	Height	Width	Height	Width	Height
Cervical	479.167± 41.650	260.500± 4.515	555.000± 41.068	281.333± 30.196	833.667± 57.337	658.667± 34.169	1125.167± 66.699	802.500± 40.848
Cervical enlargement	617.677± 25.830	262.667± 6.520	654.500± 36.115	278.000± 17.597	1000.333± 22.8222	704.333± 37.070	1151.000± 12.291	808.500± 73.684
Thoracic	489.667± 21.402	254.333± 13.200	583.667± 13.071	308.667± 16.659	738.000± 55.528	583.667± 34.941	906.500± 35.215	635.500± 72.986
Lumbar	531.333± 19.835	260.500± 10.500	546.167± 11.125	296.000± 23.016	958.667± 13.070	604.000± 57.530	1112.500± 60.810	593.833± 21.324
Lumbar enlargement	675.25± 36.472	286.500± 15.835	762.500± 41.458	319.000± 26.290	1187.500± 29.463	612.500± 7.219	1525.000± 33.850	759.750± 66.161
Sacral	353.250± 38.272	168.750± 22.631	431.250± 69.616	250.000± 25.720	750.000± 8.764	468.500± 18.187	1040.75± 168.356	515.500± 15.500

Table 39. Average size of ventral horn nuclei and neurons at fourth and fifth month of gestation (μm)

	Nucleus	102 days						124 days						142 days					
		C	CE	T	L	LE	S	C	CE	T	L	LE	S	C	CE	T	L	LE	S
Total size (length x width)																			
1	Dorsomedial	112.5 x 112.5	250.0 x 250.0	105.0 x 75.0	100.0 x 87.5	125.0 x 125.0	87.5 x 100.0	187.0 x 187.0	350.0 x 350.0	150.0 x 150.0	175.0 x 175.0	187.0 x 275.0	212.5 x 212.5	287.5 x 287.5	375.0 x 375.0	162.0 x 162.0	275.0 x 275.0	337.5 x 337.5	250.0 x 250.0
2	Dorsolateral	-	187.0 x 312.5	-	-	100.0 x 250.0	-	-	187.0 x 350.0	-	-	312.5 x 312.5	-	-	375.0 x 375.0	-	-	375.0 x 375.0	-
3	Ventromedial	137.5 x 137.5	312.5 x 312.5	162.5 x 150.0	125.0 x 250.0	162.0 x 167.0	75.0 x 120.0	187.5 x 187.5	250.0 x 375.0	100.0 x 100.0	150.0 x 162.5	250.0 x 300.0	187.0 x 187.0	312.5 x 312.5	500.0 x 500.0	120.0 x 120.0	200.0 x 200.0	462.0 x 500.0	137.5 x 137.5
4	Ventrolateral	-	162.0 x 250.0	-	212.5 x 312.5	137.5 x 287.5	-	-	250.0 x 250.0	-	150.0 x 150.0	250.0 x 437.0	-	-	500.0 x 620.0	-	175.0 x 187.0	625.0 x 537.5	-
5	Central	-	215.5 x 215.5	-	-	187.0 x 187.0	-	-	250.0 x 250.0	-	-	250.0 x 250.0	-	-	312.0 x 100.0	-	-	262.5 x 262.5	-
6	Retrodorsolateral	-	162.5 x 162.5	-	-	187.5 x 187.5	-	-	187.0 x 187.0	-	-	175.0 x 187.0	-	-	400.0 x 400.0	-	-	312.5 x 312.5	-
Neuron size (width)																			
1	Dorsomedial	14.0 – 25.2	21.6 - 28.8	18.0– 21.4	18.0- 21.6	18.0– 21.6	10.8– 18.0	18.0- 25.2	18.0– 43.2	18.0- 21.6	18.0- 25.2	18.0– 21.6	14.4- 18.0	28.8– 36.0	37.5– 75.0	18.0- 25.2	10.8– 28.8	21.6– 25.2	18.0- 25.2
2	Dorsolateral	-	14.4 – 36.0	-	-	28.8 – 36.0	-	-	36- 43.2	-	-	43.2 - 46.8	-	-	43.2– 50.0	-	-	57.6– 75	-
3	Ventromedial	18.0 – 25.2	21.6 – 28.8	22.8- 26.6	21.6- 25.6	18.0 – 32.4	18.0- 28.8	25.5– 32.4	21.6- 36.0	18.0- 21.6	21.6 - 37.5	21.6– 37.5	14.4- 21.6	28.8– 39.6	28.8– 37.5	14.0 - 32.4	32.4- 39.6	28.8– 46.8	7.2 – 21.6
4	Ventrolateral	-	21.6 32.4	-	21.6- 25.6	18.0 – 36.0	-	-	10.8 - 43.2	-	25.0– 36.0	18.0– 43.2	-	-	43.2- 50.4	-	32.4- 39.6	43.2- 62.5	-
5	Central	-	14.4 – 36.0	-	-	28.8- 32.4	-	-	28.8- 36.0	-	-	21.6- 36.0	-	-	26.0 –50.0	-	-	43.2- 54.0	-
6	Retrodorsolateral	-	28.8- 32.4	-	-	21.6- 32.4	-	-	25.2- 43.2	-	-	21.6- 39.6	-	-	14.4 54.0	-	-	32.4- 72.0	-

Table 40. Thickness of funiculi of white matter in the precoccygeal spinal cord (Mean± S.E), μm

	Group II			Group III					Group IV					Group V				
	DF*	VF*	LF*	DF*		VF*		LF*	DF*		VF*		LF*	DF*		VF*		LF*
	V*	V	T*	T	V	T	V	T	T	V	T	V	T	T	V	T	V	T
C*	60.0 ± 5.4	165.0 ± 16.43	255.5 ± 5.4	99.5 ± 3.8	81.8 ± 2.3	96.5 ± 5.0	215.5 ± 4.2	277.5± 9.8	133.1 ± 11.4	84.3 ± 2.9	187.5 ± 20.9	530.0 ± 19.2	645.0 ± 16.4	285.8 ± 23.2	747.0 ± 25.7	389.4 ± 34.2	986.2 ± 41.9	1281.7 ± 64.4
CE*	83.1 ± 0.5	160.0 ± 8.3	230.0 ± 6.8	78.0 ± 1.1	142.5 ± 7.2	100.0 ± 9.9	179.1 ± 2.3	274.1 ± 10.6	204.1 ± 9.5	232.6 ± 26.1	241.5 ± 20.6	534.3 ± 13.7	693.3 ± 27.1	250.0 ± 21.7	428.7 ± 50.6	317.0 ± 5.7	1014.5 ± 17.1	1289.2 ± 29.2
T*	103.5 ± 3.1	155.8 ± 4.3	202.6 ± 4.9	85.4 ± 10.7	147.5 ± 8.8	108.3 ± 4.7	214.3 ± 15.7	224.6 ± 1.6	39.1 ± 7.4	177.6 ± 4.9	182.5 ± 17.4	448.3 ± 24.5	526.3 ± 28.4	56.2 ± 11.8	356.3 ± 22.4	247.9 ± 10.5	927.1 ± 17.3	1170.8 ± 17.3
L*	70.5 ± 4.1	127.6 ± 1.0	195.0 ± 2.2	89.5 ± 5.7	125.0 ± 4.1	125.5 ± 5.7	155.5 ± 2.1	277.6 ± 17.0	95.8 ± 2.6	126.0 ± 2.3	162.3 ± 13.6	405.6 ± 2.2	702.6 ± 16.9	252.0 ± 33.6	401.3 ± 13.5	304.1 ± 32.3	952.1 ± 19.9	996.7 ± 6.5
LE*	56.2 ± 0.1	165.0 ± 17.3	232.5 ± 12.9	131.2 ± 7.2	118.8 ± 0.6	162.5 ± 7.2	180.7 ± 0.4	297.0± 8.9	193.5 ± 3.7	132.5 ± 18.7	168.5 ± 10.6	375.0 ± 43.3	575.0 ± 28.8	306.2 ± 18.0	341.2 ± 32.4	381.2 ± 3.6	720.0 ± 38.9	900.0 ± 43.3
S*	37.7 ± 0.1	127.5 ± 12.9	147.5 ± 7.2	-	82.5 ± 4.3	-	102.7 ± 22.9	168.7 ± 10.8	-	94.0 ± 10.9	-	137.5 ± 7.2	218.7 ± 32.4	-	75.0 ± 21.6	-	171.2 ± 26.7	285.0 ± 26.0

C - cervical region
CE - cervical enlargement
T - thoracic region
L - lumbar region
LE - lumbar enlargement
S - sacral region

DF – dorsal funiculus
VF – ventral funiculus
LF – lateral funiculus
V – vertical thickness
T – transverse thickness

Table 41. Height and width of fasciculus gracilis and fasciculus cuneatus during different stages of gestation (Mean ± S.E), μm

Regions	Group II				Group III				Group IV				Group V			
	FG*		FC*		FG*		FC*		FG*		FC*		FG*		FC*	
	Height	Width	Height	Width	Height	Width	Height	Width	Height	Width	Height	Width	Height	Width	Height	Width
Cervical	121.500 ± 20.791	42.000 ± 3.742	121.500 ± 20.791	355.500 ± 23.740	292.500 ± 17.642	37.500 ± 2.500	60.000 ± 7.416	511.500 ± 24.744	810.000 ± 8.216	114.000 ± 22.045	475.000 ± 10.173	630.000 ± 56.125	1269.000 ± 104.168	275.750 ± 39.813	969.000 ± 141.860	1245.000 ± 93.005
Cervical enlargement	142.500 ± 10.308	44.250 ± 6.562	142.500 ± 10.308	379.000 ± 23.068	414.000 ± 29.597	21.750 ± 1.750	82.500 ± 2.500	658.500 ± 50.022	739.200 ± 25.200	30.200 ± 0.200	225.200 ± 0.200	810.200 ± 0.200	1519.500 ± 99.701	249.000 ± 16.763	497.500 ± 33.510	1605.000 ± 57.228
Thoracic	146.250 ± 9.437	32.500 ± 1.685	146.250 ± 9.437	352.500 ± 0.754	330.083 ± 22.136	43.208 ± 5.176	251.333 ± 22.899	712.583 ± 19.569	447.500 ± 34.659	45.167 ± 0.167	97.500 ± 3.354	750.000 ± 67.082	990.00 ± 27.136	236.250 ± 22.243	401.250 ± 16.732	1010.000 ± 77.04

FG* - Fasciculus gracilis
FC* - Fasciculus cuneatus

Table 42. Segment-wise height and width of central canal and ependymal layer thickness in the precoccygeal spinal cord (Mean ± S.E), μm

Segments	Group II			Group III			Group IV			Group V		
	Central canal		Ependymal thickness	Central canal		Ependymal thickness	Central canal		Ependymal thickness	Central canal		Ependymal thickness
	Height	Width		Height	Width		Height	Width		Height	Width	
C1	152.500± 4.330	120.000± 25.981	30.012± 6.078	262.500± 16.870	90.000± 1.826	37.500± 3.354	160.000± 2.887	90.000± 1.826	30.000± 2.236	165.000± 7.678	210.000± 17.321	26.250± 1.677
C2	127.500± 3.433	60.000± 8.660	30.000± 0.365	247.500± 10.227	90.000± 3.651	30.000± 0.913	130.000± 5.774	75.000± 4.017	30.000± 0.365	127.500± 4.952	172.500± 21.651	26.250± 1.677
C4	127.500± 3.433	60.000± 0.015	30.000± 6.078	187.500± 3.819	90.000± 1.461	30.000± 2.236	135.000± 0.577	90.000± 5.447	30.000± 0.146	141.250± 9.612	181.250± 13.975	22.050± 0.201
C6	180.000± 0.012	67.500± 4.330	22.500± 3.354	217.500± 16.869	120.000± 5.774	25.000± 2.236	195.000± 2.309	135.000± 5.447	30.000± 0.365	180.000± 6.952	176.250± 6.149	22.500± 0.020
C8	187.500± 3.819	60.000± 0.577	35.000± 4.472	231.500± 19.503	120.000± 2.887	37.500± 2.737	180.000± 1.155	105.000± 1.095	30.000± 0.183	141.250± 9.529	215.000± 15.652	28.800± 0.438
T2	197.500± 3.096	67.500± 3.819	35.000± 8.944	231.500± 19.503	105.000± 2.887	37.500± 2.737	150.000± 4.041	105.000± 7.303	30.000± 0.730	212.500± 5.881	165.000± 15.652	28.800± 0.358
T5	232.500± 4.425	82.500± 1.443	33.000± 5.367	221.500± 15.046	105.000± 02.039	33.750± 0.456	165.000± 3.464	90.000± 16.432	32.400± 0.803	197.500± 2.141	142.500± 16.771	22.500± 3.354
T7	227.500± 12.633	67.500± 4.330	15.000± 0.210	232.500± 4.425	75.000± 3.464	31.000± 0.913	135.000± 1.555	75.000± 5.477	30.000± 1.461	197.500± 2.141	121.250± 12.857	18.300± 1.476
T12	225.500± 0.100	45.000± 3.354	22.500± 3.354	225.000± 0.200	105.000± 0.577	22.000± 0.730	135.000± 5.774	90.000± 2.191	30.000± 1.095	187.500± 3.819	127.500± 10.062	20.100± 2.281
L1	225.000± 20.207	67.500± 10.062	22.500 ± 1.118	240.000± 13.904	88.000± 6.351	30.000± 0.671	165.000± 2.887	105.000± 1.461	30.000± 3.286	180.000± 2.887	157.500± 23.479	26.000± 1.789
L2	262.500± 3.819	75.000± 6.708	30.000± 0.010	240.000± 13.904	113.000± 4.619	30.000± 0.010	135.000± 1.732	105.000± 0.730	22.500± 0.913	187.500± 3.819	142.500± 30.187	26.000± 2.191
L3	262.500± 23.550	75.000± 6.708	30.000± 0.365	212.500± 3.819	105.000± 1.155	30.000± 0.365	135.000± 5.771	90..000± 3.651	22.500± 3.651	187.500± 3.774	146.250± 18.447	29.250± 3.019
L4	192.500± 5.881	90.000± 0.000	30.000± 2.191	225.000± 20.453	90.000± 4.041	30.000± 2.191	180.000± 5.196	105.000± 3.651	30.000± 0.548	187.500± 3.430	187.500± 23.478	30.000± 1.555
L6	200.000± 22.435	97.500± 3.354	18.000± 2.012	257.500± 4.958	88.000± 2.887	30.000± 1.876	150.000± 2.309	105.000± 0.730	30.000± 2.191	195.000± 20.125	150.000± 6.708	30.000± 0.913
S2	285.000± 2.887	75.000± 0.025	18.000± 0.730	250.000± 3.651	88.000± 5.774	30.000± 0.025	210.000± 5.774	60.000± 5.447	30.000± 0.730	200.000± 1.826	100.000± 1.826	30.000± 0.913
S4	277.500± 4.333	37.500± 1.118	15.000± 1.826	250.000± 3.651	38.000± 1.732	30.000± 0.730	300.000± 11.547	75.000± 5.447	30.000± 0.671	337.500± 0.913	97.500± 0.913	26.000± 0.730

Table 43. Region-wise height and width of central canal and ependymal layer thickness in the precoccygeal spinal cord (Mean±S.E), μm

Regions	Group II			Group III			Group IV			Group V		
	Height	Width	Ependymal thickness	Height	Width	Ependymal thickness	Height	Width	Ependymal thickness	Height	Width	Ependymal thickness
Cervical	135.833± 8.333	80.000± 20.000	30.000± 4.174	232.500± 22.913	90.000± 0.000	32.500± 2.500	141.667± 9.280	85.000± 5.000	30.133± 0.133	144.583± 10.953	187.917± 11.327	24.850± 1.400
Cervical enlargement	188.333± 5.069	65.000± 2.500	30.800± 4.167	226.833± 4.667	115.000± 5.000	33.333± 4.167	175.000± 13.229	115.000± 10.000	30.000± 0.426	177.917± 20.594	185.417± 15.144	26.700± 2.100
Thoracic	228.333± 2.205	65.000± 10.897	23.500± 5.220	226.333± 3.245	95.000± 10.000	28.917± 3.548	145.000± 10.000	85.000± 5.000	30.800± 0.800	194.167± 3.333	130.417± 6.305	20.300± 1.217
Lumbar	250.000± 12.500	72.500± 2.500	27.500± 2.500	230.833± 9.167	102.000± 7.371	30.000± 0.349	145.000± 10.000	100.000± 5.000	27.083± 1.083	185.000± 2.500	148.750± 4.507	25.000± 2.500
Lumbar enlargement	196.250± 3.750	93.750± 3.750	24.000± 3.464	241.250± 16.250	89.000± 1.000	30.000± 2.034	165.000± 15.000	105.000± 0.000	30.000± 1.370	191.250± 3.750	168.750± 18.750	30.000± 1.234
Sacral	281.250± 3.750	56.250± 18.750	16.500± 1.500	250.000± 0.000	63.000± 25.000	30.000± 0.378	255.000± 45.000	67.500± 7.500	30.000± 0.701	268.750± 68.750	98.750± 0.722	28.000± 2.000

Table 44. Region-wise dorsal surface to central canal height and ventral surface to central canal height (Mean ± S.E), μm

Regions	Group II		Group III		Group IV		Group V	
	DMS – CC*	VMF – CC*	DMS - CC	VMF - CC	DMS - CC	VMF - CC	DMS - CC	VMF - CC
Cervical	615.833 ±25.672	500.833 ±27.338	750.833 ±46.965	541.667 ±41.062	1162.500 ±27.951	1357.500 ±25.715	1658.083 ±178.209	1935.333 ±163.386
Cervical enlargement	800.833 ±46.033	465.000 ±31.937	840.000 ±64.807	635.000 ±56.745	1050.000 ±22.361	1200.000 ±89.443	1630.833 ±135.810	2032.500 ±137.147
Thoracic	725.833 ±22.227	458.333 ±26.257	738.333 ±26.667	478.333 ±50.525	862.500 ±22.822	1087.500 ±39.131	1269.167 ±34.854	1819.333 ±185.318
Lumbar	728.333 ±18.514	400.000 ±25.000	766.667 ±34.512	426.667 ±23.898	705.000 ±1.826	945.000 ±20.125	1264.167 ±69.467	1800.833 ±102.522
Lumbar enlargement	722.500 ±60.467	553.750 ±70.100	830.000 ±26.693	468.750 ±34.301	1155.000 ±69.318	1015.000 ±5.477	1549.750 ±67.387	1745.000 ±95.960
Sacral	527.500 ±12.298	220.000 ±9.168	675.000 ±33.541	285.000 ±38.013	1004.167 ±8.944	385.833 ±38.274	1125.000 ±11.180	450.000 ±22.361

DMS – CC: dorsal median septum to central canal distance
VMF – CC: ventral median fissure to central canal distance

Table 45. Region-wise average of percentage of dorsal median septum to central canal distance and ventral median fissure to central canal distance to spinal cord height

Regions	Group II		Group III		Group IV		GroupV	
	DMS – CC*	VMF – CC*	DMS - CC	VMF – CC	DMS - CC	VMF – CC	DMS - CC	VMF - CC
Cervical	48.779	39.670	48.755	35.173	44.146	52.234	43.529	50.807
Cervical enlargement	53.300	30.948	49.013	37.051	38.113	43.557	42.268	52.678
Thoracic	51.235	32.353	50.484	32.707	43.233	54.511	38.635	55.383
Lumbar	52.505	28.836	53.241	29.630	37.105	49.737	38.808	55.283
Lumbar enlargement	49.066	37.606	53.333	30.120	49.677	43.656	44.437	50.036
Sacral	33.493	14.000	33.457	14.126	63.757	24.497	55.762	22.305

DMS – CC: dorsal median septum to central canal distance
VMF – CC: ventral median fissure to central canal distance

Table 46. Micrometrical data of the coccygeal region (Mean ±S.E), μm

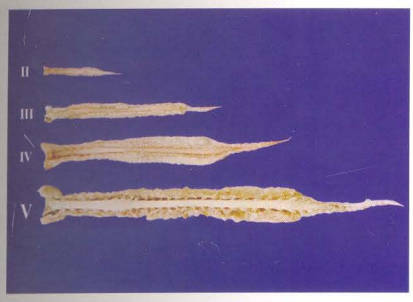
Parameter	Group II	Group III	Group IV	Group V
Total spinal cord width	551.250±124.800	646.667±162.700	1555.000±2.887	1654.000±9.452
Total gray matter width	368.250±85.776	508.750±111.060	1075.000±2.887	1280.000±8.944
Dorsal horn width	172.125±39.027	254.250±55.540	293.000±8.050	612.500±7.217
Ventral horn width	180.500±40.845	250.000±56.236	281.250±8.385	492.500±1.443
Spinal cord height	491.250±45.569	573.750±74.593	1002.500±1.443	1462.500±12.500
Gray matter height	385.225±33.452	444.750±60.030	832.000±1.155	1211.250±11.250
Dorsal horn height	185.000±14.142	207.500±18.136	451.000±0.577	656.250±6.250
Ventral horn height	198.000±15.406	249.750±70.870	380.500±0.300	555.000±9.000
Dorsal funiculus	28.500±8.660	35.250±7.361	61.875±0.125	97.000±0.224
Ventral funiculus	75.000±0.000	89.250±7.073	110.000±0.000	154.000±0.000
Lateral funiculus	71.000±0.020	88.500±7.500	109.250±0.250	150.750±19.566
Central canal height	251.250±25.000	281.250±31.250	297.500±25.000	372.500±1.118
Central canal width	41.625±1.546	50.000±0.000	62.250±0.112	73.750±1.250
Ependymal layer thickness	29.000±0.573	23.300±0.981	22.500±1.118	27.500±2.500
Substantia gelatinosa width	213.000±57.735	272.667±27.534	313.250±0.250	314.000±0.447
Substantia gelatinosa height	143.500±10.681	147.667±2.539	162.250±0.250	162.500±0.224

Table 47. Average size of the cells and thickness of capsule in dorsal root ganglia at different ages (μm)

Foetal groups	Neurons (with nuclear size)	Regions						Satellite cells	Capsule thickness
		Cervical	Cervical enlargement	Thoracic	Lumbar	Lumbar enlargement	Sacral		
Group III	Large	14.4 (7.2) - 14.4 (9.0)	14.4 (7.2) - 18.0 (7.2)	14.4 (10.8) - 18.0 (7.2)	18.0 (7.2) - 21.6(10.8)	18.0 (10.8) - 21.6 (10.8)	21.6 (14.4) - 18.0 (7.2)	3.6	3.6 - 5.4
	Small	7.2 (5.4) - 10.8 (9.0)	10.8 (7.2) - 7.2 (5.4)	7.2 (5.4) - 12.6 (7.2)	7.2 (3.6) - 9.0 (7.2)	12.6 (7.2) - 9.0 (7.2)	10.8 (7.2) - 7.2 (5.4)		
Group IV	Large	36.0 (14.4) – 21.6 (10.8)	46.8 (14.4) - 54.0 (14.4)	28.8 (12.6) - 21.6 (14.4)	21.6 (7.2) - 32.4 (10.8)	28.8 (10.8) - 25.2 (12.6)	25.2 (12.6) 10.8 (7.2)	3.6 - 5.4	3.6 - 5.4
	Small	10.8 (5.4) - 10.8 (7.2)	18.0 (5.4) - 28.8 (5.4)	18.0 (7.2) - 18.0 (10.8)	14.4 (10.8) - 18.0 (10.8)	16.2 (10.8) - 18.0 (10.8)	10.8 (7.2) - 14.4 (10.8)		
Group V	Large	28.8 (7.2) - 32.4 (7.2)	39.6 (14.4) - 46.8 (10.8)	28.8 (10.8) - 36.0 (7.2)	32.4 (7.2) - 36.0 (7.2)	36.0 (10.8) - 50.4 (10.8)	36.0 (10.8) - 43.2 (10.8)	5.4 - 7.2	9.0 -10.8
	Small	10.8 (5.4) - 18.0 (10.8)	14.4 (5.4) - 28.8 (7.2)	14.4(7.2) - 18.0 (7.2)	18 (7.2) - 21.6 (7.2)	18.0 (7.2) - 28.8 (7.2)	14.4 (10.8) - 25.2 (10.8)		

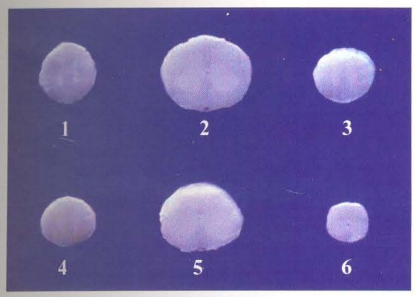
Table 48. Nucleus-cytoplasmic ratio of ganglionic neurons at different ages

Foetal groups	Neurons	Regions					
		Cervical	Cervical enlargement	Thoracic	Lumbar	Lumbar enlargement	Sacral
Group III	Large	1:1.60 - 1:2.00	1: 2.00 - 1: 2.50	1: 1.33- 1: 2.50	1: 2.00 - 1: 2.50	1: 1.67 - 1: 2.00	1: 1.50 - 1: 2.50
	Small	1: 1.33 - 1: 1.20	1: 1.33 - 1: 1.50	1: 1.33 - 1: 1.75	1: 2.00 - 1: 1.25	1: 1.25 - 1: 1.75	1: 1.50 - 1: 2.50
Group IV	Large	1: 2.00 - 1: 2.50	1: 3.25 - 1: 3.75	1: 1.50 - 1: 2.29	1: 3.00	1: 2.00 - 1: 2.67	1: 2.00
	Small	1: 1.50 - 1: 2.00	1: 2.50 - 1:3.00	1: 1.67 - 1: 2.50	1:1.33 - 1: 1.67	1:1.50 - 1: 1.67 -	1: 1.50 - 1:1.33
Group V	Large	1: 4.00 - 1: 4.50	1: 2.75 - 1: 4.33	1: 2.67 - 1: 5.00	1: 4.50 - 1: 5.00	1: 3.33 - 1: 4.67	1: 3.33 - 1: 4.00
	Small	1: 1.67 - 1: 2.00	1: 2.67 - 1: 4.00	1: 2.00 - 1: 2.50	1: 2.50 - 1: 3.00	1: 2.50 - 1: 4.00	1: 1.33 - 1: 2.33



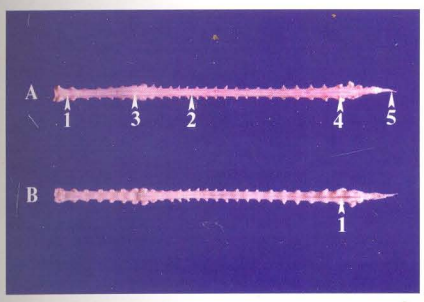
II. At second month (55 days)
III. At third month (80 days)
IV. At fourth month (101 days)
V. At fifth month (127 days)

Fig. 1 Spinal cord within vertebral canal at different stages of gestation



1. Cervical region (C2)
2. Cervical enlargement (C8)
3. Thoracic region (T4)
4. Lumbar region (L4)
5. Lumbar enlargement (L6)
6. Sacral region (S3)

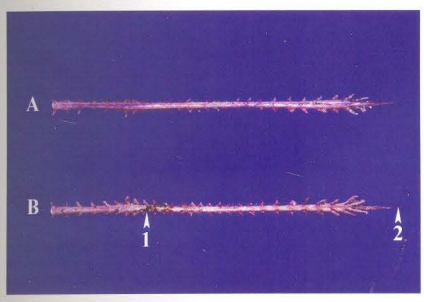
Fig. 2 Spinal cord segments of a full term foetus showing inner gray matter and outer white matter



A. Dorsal surface
1. Dorsal median sulcus
2. Dorsolateral groove
3. Cervical enlargement
4. Lumbar enlargement
5. Conus medullaris

B. Ventral surface
1. Ventral median fissure

Fig. 3 Spinal cord at 125 days of gestation



A. Dorsal surface

B. Ventral surface
1. Epidural fat
2. Filum terminale

Fig. 4 Spinal cord of a full term foetus showing increased vascularity

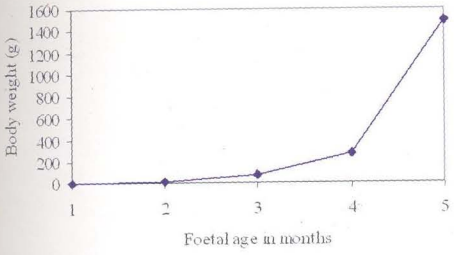


Fig.5 Relation between age and body weight

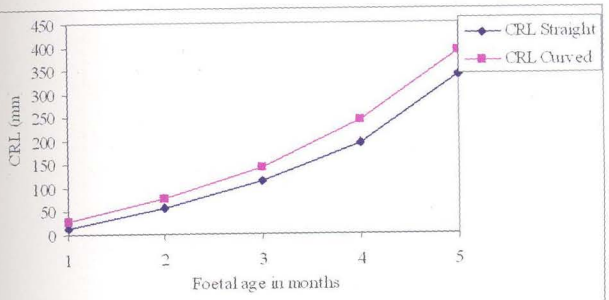


Fig.6 Relation between age and CRL

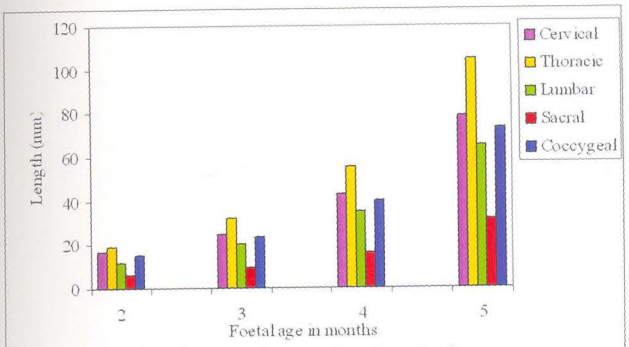


Fig.7 Regional length of vertebral column

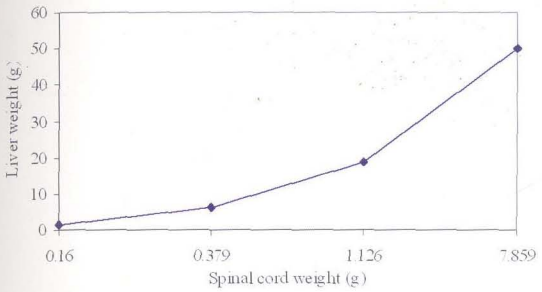


Fig. 8 Relation between spinal cord weight and liver weight

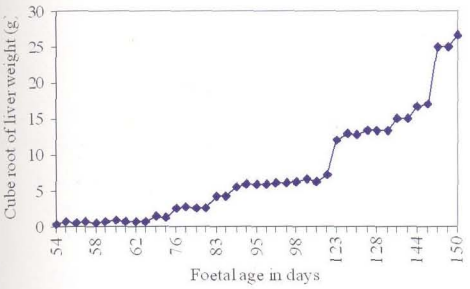


Fig. 9 Relation between age and cube root of liver weight

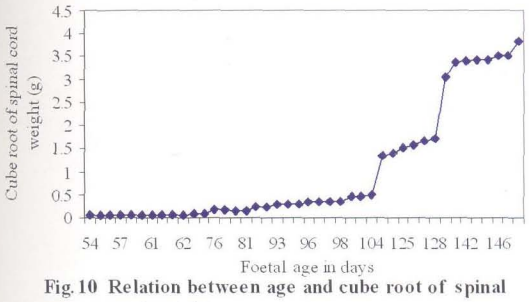


Fig. 10 Relation between age and cube root of spinal cord weight

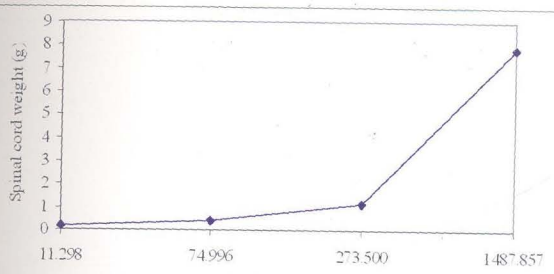


Fig. 11 Relation between spinal cord weight and body weight

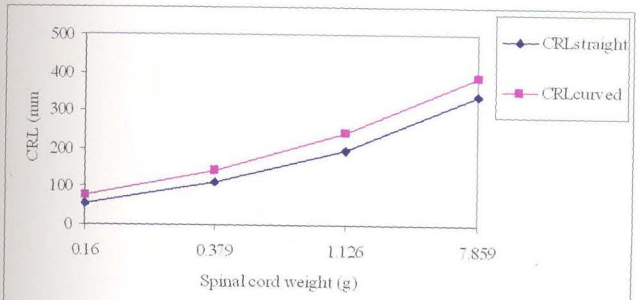


Fig. 12 Relation between spinal cord weight and CRL

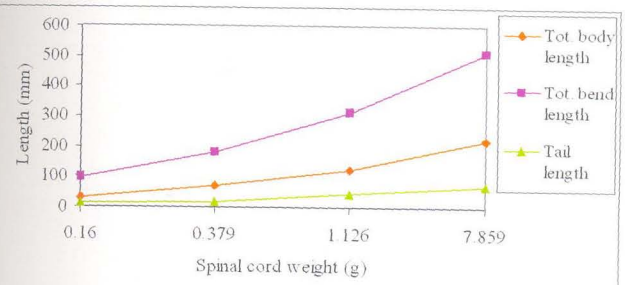


Fig. 13 Relation between spinal cord weight and total body length, total bend length and tail length

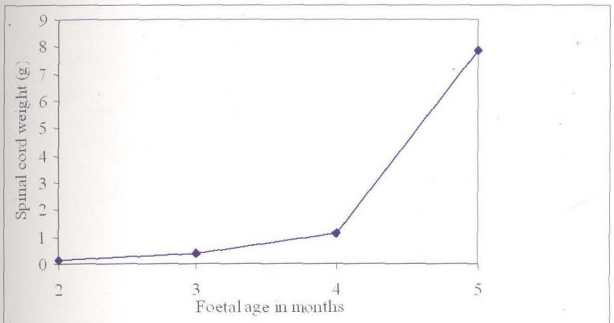


Fig.14 Relation between age and spinal cord weight

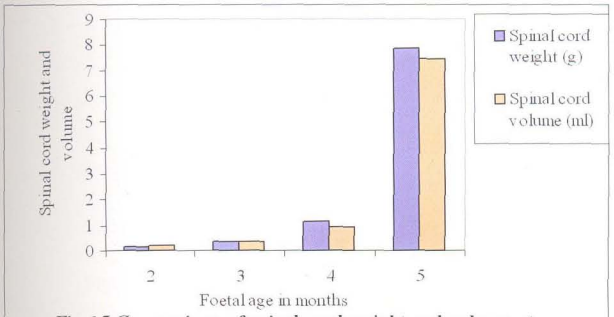


Fig. 15 Comparison of spinal cord weight and volume at different ages

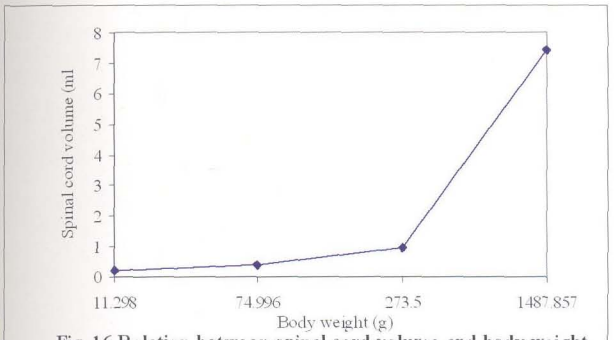


Fig. 16 Relation between spinal cord volume and body weight

Fig. 17 Relation between age and spinal cord length

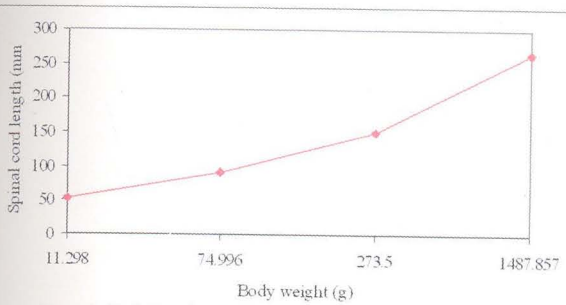


Fig. 18 Relation between body weight and spinal cord length

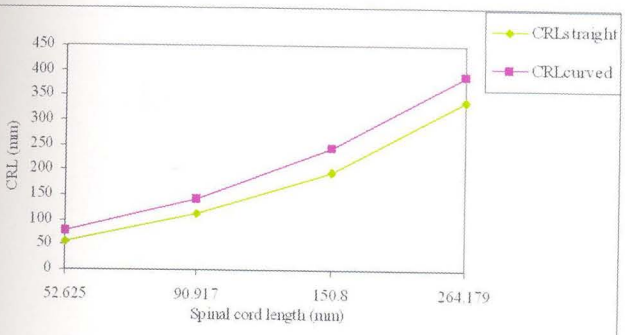


Fig. 19 Relation between spinal cord length and CRL

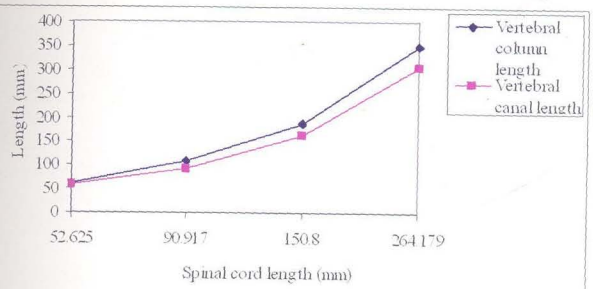


Fig. 20 Relation of spinal cord length with length of vertebral column and vertebral canal

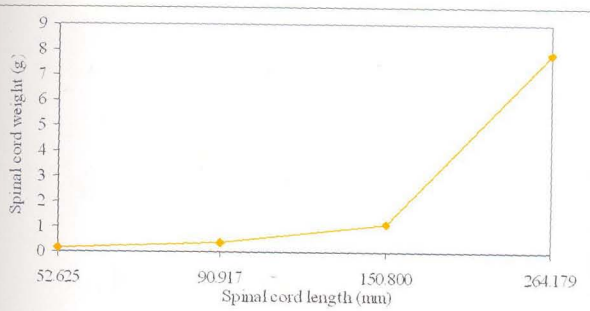


Fig. 21 Relation between spinal cord length and weight

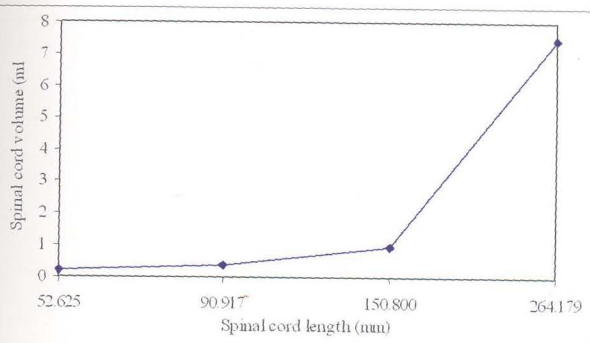


Fig. 22 Relation between spinal cord length and volume

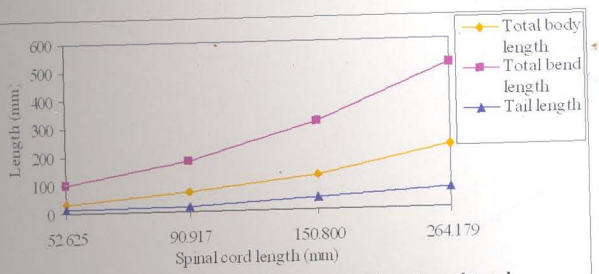


Fig. 23 Relation between spinal cord length and total body length, total bend length and tail length

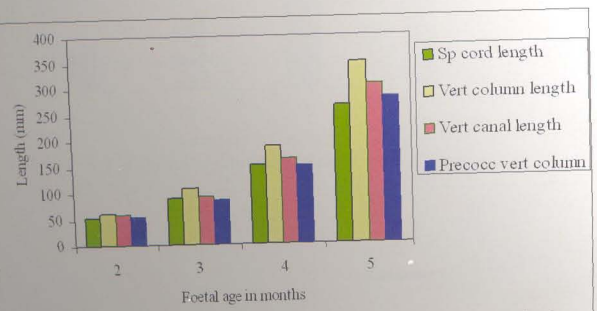


Fig. 24 Length of spinal cord, vertebral column, vertebral canal and precoccygeal vertebral column

Fig. 25 Regional length of spinal cord in goat foetuses

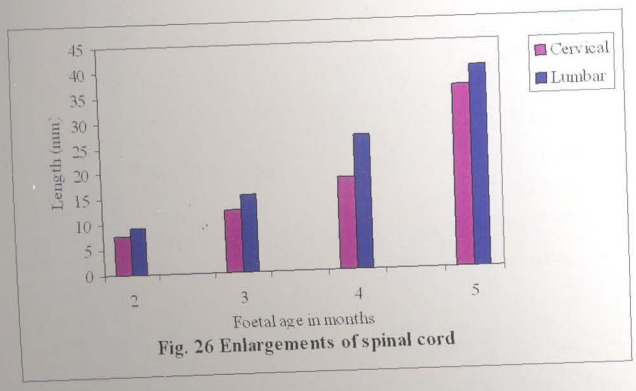


Fig. 26 Enlargements of spinal cord

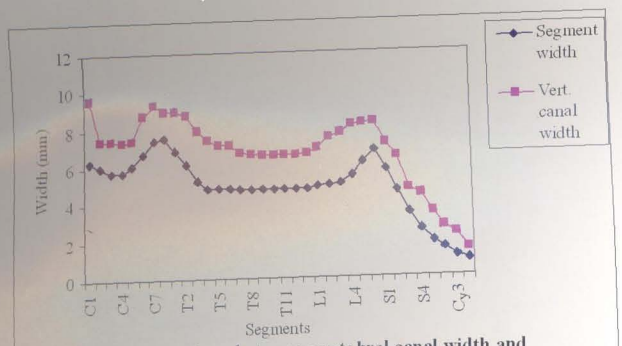


Fig. 27 Comparison between vertebral canal width and cord segment width in the last month of gestation

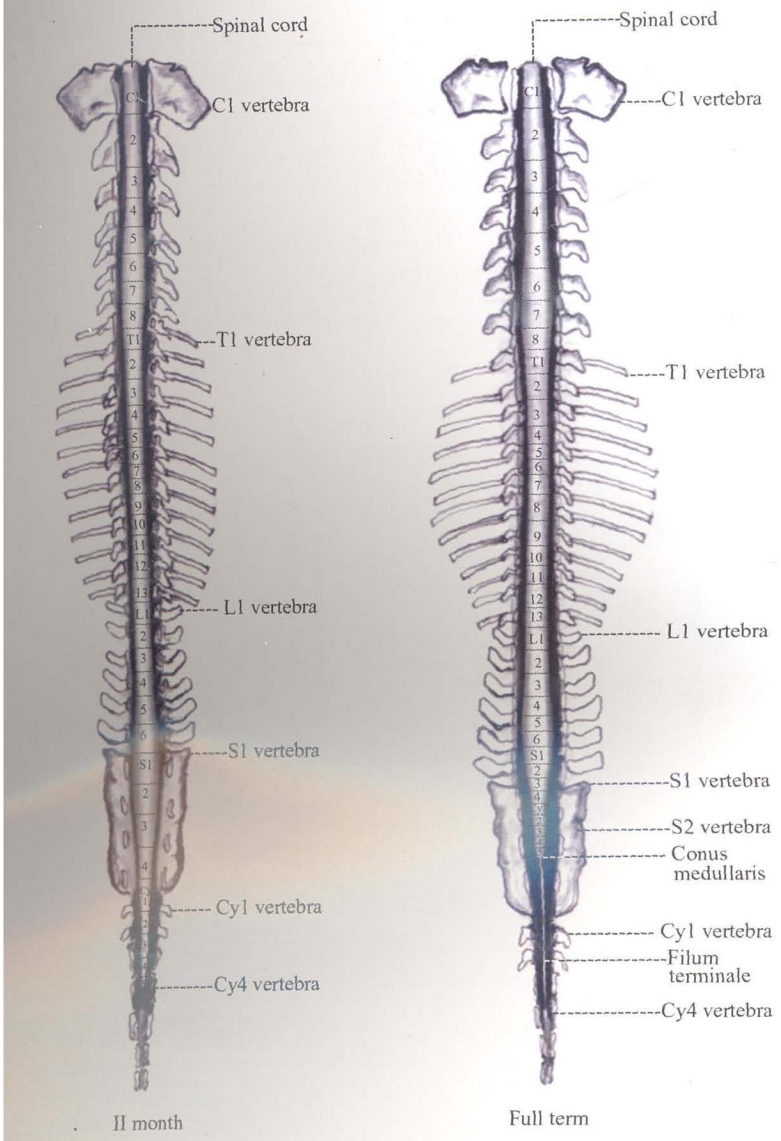


Fig. 28 Correspondence between spinal cord segments and vertebrae

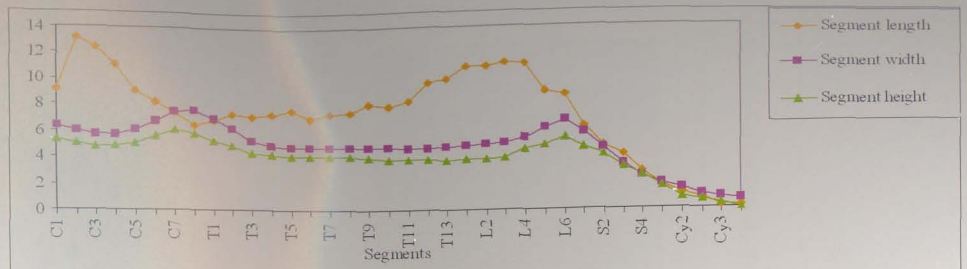


Fig. 29 Comparison between length, width and height of spinal cord segments at the last month of gestation

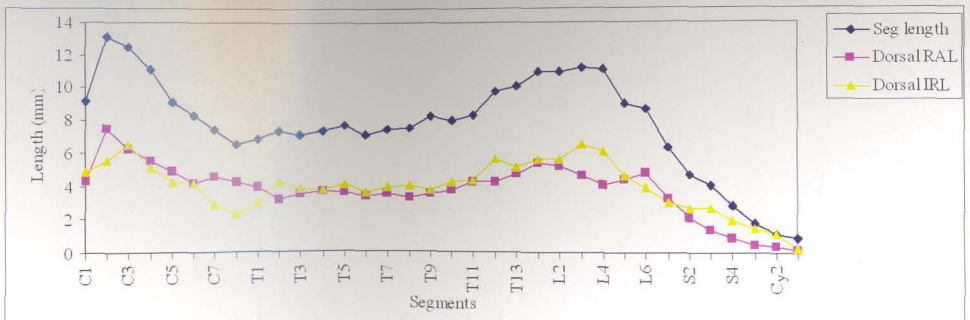
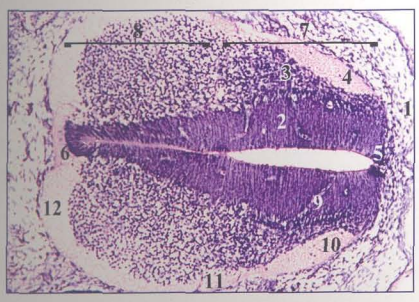
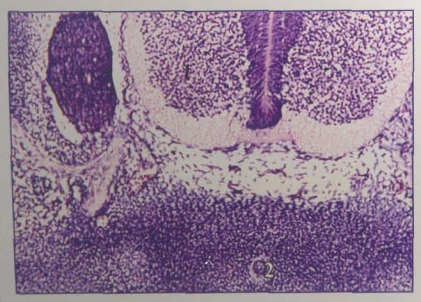


Fig. 30 Segment length, dorsal root attachment length and inter root length of spinal cord at last month of gestation



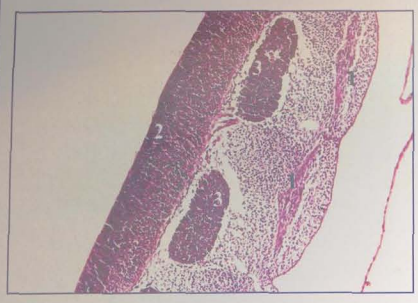
1. Dorsal body wall
2. Ependymal layer
3. Mantle layer
4. Marginal layer
5. Roof plate
6. Floor plate
7. Alar Plate
8. Basal plate
9. Channel-like blood vessel
10. Dorsal funiculus
11. Lateral funiculus
12. Ventral funiculus

Fig. 31 C.S. of neural tube at thoracic region (26 days). H & E x 100



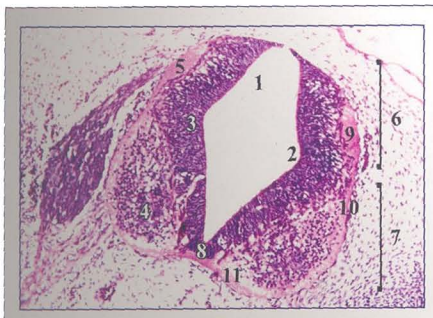
1. Neural tube
2. Notochord

Fig. 32 C.S. of neural tube at the thoracic region (26 days). H & E x 100



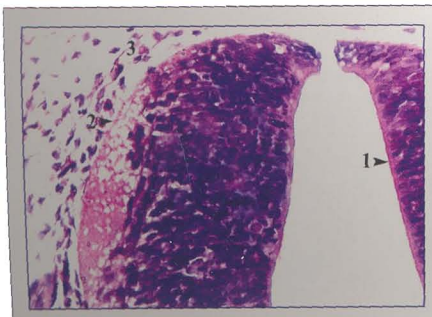
1. Somites
2. Neural tube wall
3. Ganglion

Fig. 33 L.S. of neural tube at the curved back showing somites and ganglia (24 days). H & E x 100



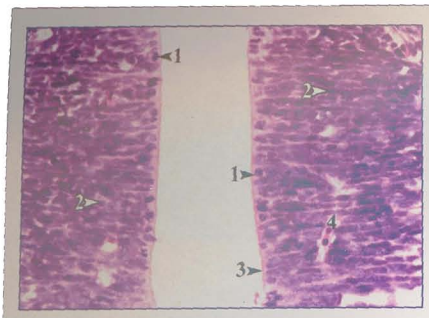
1. Diamond-shaped lumen
2. Sulcus limitans
3. Ependymal layer
4. Mantle layer
5. Marginal layer
6. Alar plate
7. Basal plate
8. Floor plate
9. Dorsal funiculus
10. Lateral funiculus
11. Ventral funiculus

Fig. 34 C.S. of neural tube at thoracic region (24 days). H & E x 100



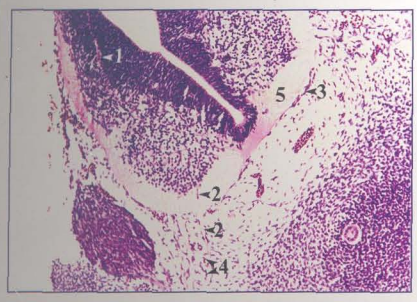
1. Internal limiting membrane
2. External limiting membrane
3. Primitive meninx

Fig. 35 C.S. of neural tube at lumbar region (24 days). H & E x 400



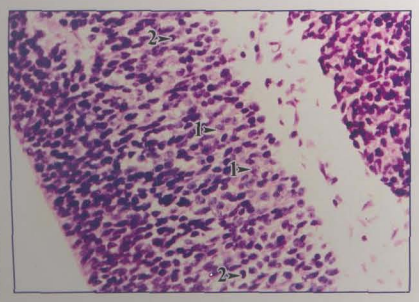
1. Germinal cells
2. Neuroepithelial cells
3. Internal limiting membrane
4. Blood vessel

Fig. 36 C.S. of neural tube wall (ependymal layer) at cervical enlargement (26 days). H & E x 400



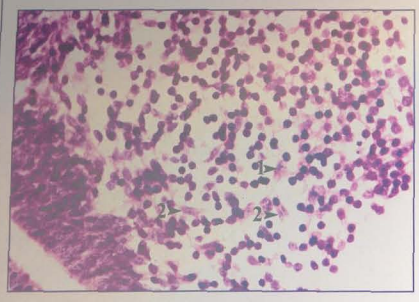
1. Blood channels
2. Ventral root fibres
3. Primitive meninx
4. Connective tissue cells and erythrocytes between ventral root fibres
5. *Marginal layer*

Fig. 37 C.S. of neural tube at lumbar region (27 days). H & E x 100



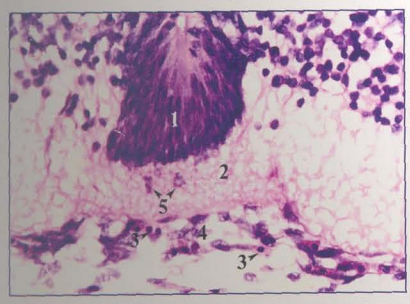
1. Neuroblasts
2. Spongioblasts

Fig. 38 L.S. of neural tube wall at curved back (24 days). H & E x 400



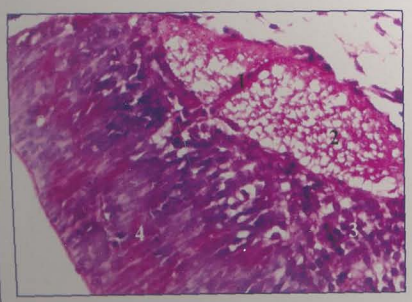
1. Multipolar neuroblast
2. Bipolar neuroblasts with elongated cell body

Fig. 39 Basal plate at lumbar region (27 days). H& E x 400



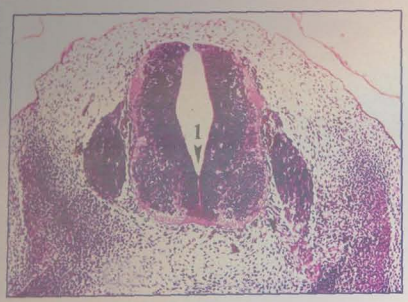
1. Ependymal layer
2. Marginal layer
3. Nucleated erythrocytes
4. Mesenchymal cells
5. Cells in the marginal layer

Fig. 40 Floor plate at 26 days. H & E x 400



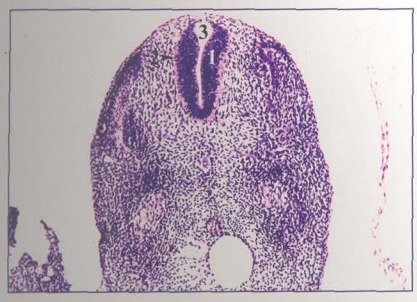
1. Blood vessel
2. Dorsal funiculus
3. Mantle layer
4. Ependymal layer

Fig. 41 C.S. of neural wall at dorsal aspect (26 days). H & E x 400



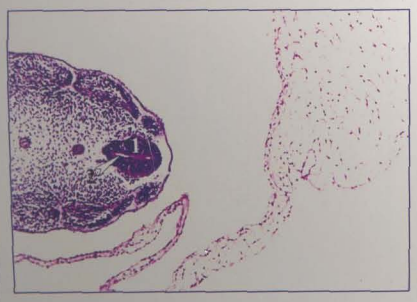
1. Lumen with ventral one–fourth fused

Fig. 42 C.S. of neural tube at lumbar region (24 days). H & E x 100



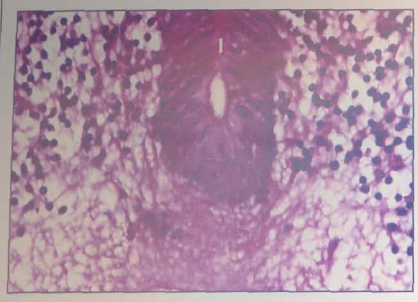
1. Ependymal layer
2. Marginal layer
3. Lumen

Fig. 43 C.S. of caudal sacral region (26 days). H & E x 100



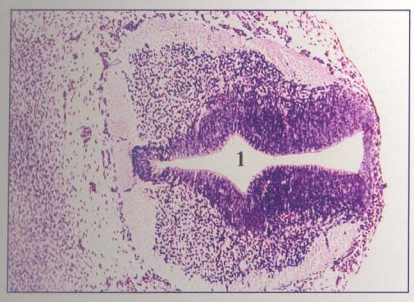
1. Ependymal layer
2. Lumen

Fig. 44 C.S. of neural tube at coccygeal region (24 days). H & E x 100



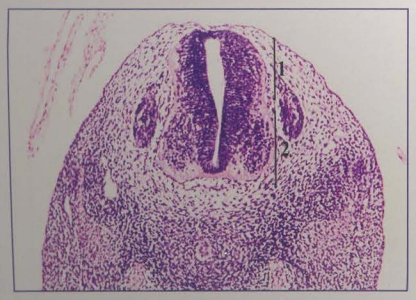
1. Fused ependymal layer

Fig. 45 Ventral aspect of neural tube at cervical enlargement (26 days). H & E x 400



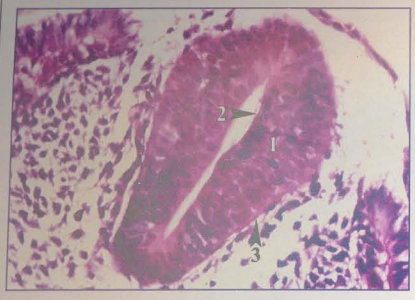
1. Lumen

Fig. 46 C.S. of neural tube at cervical region (27 days). H & E x 100



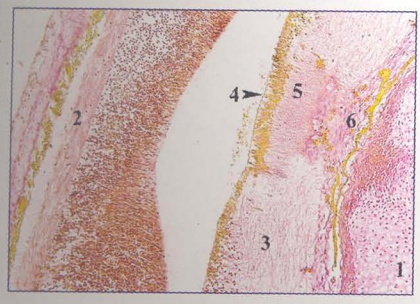
1. Alar plate
2. Basal Plate

Fig. 47 C.S. of neural tube at sacral region (26 days). H & E x 100



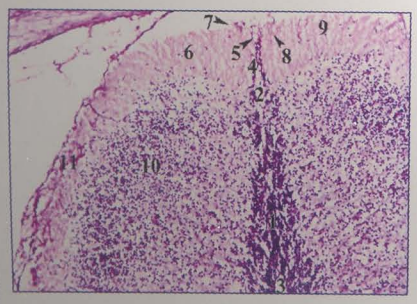
1. Ependymal layer
2. Internal limiting membrane
3. External limiting membrane

Fig. 48 C.S. of neural tube at coccygeal region (24 days). H & E x 400



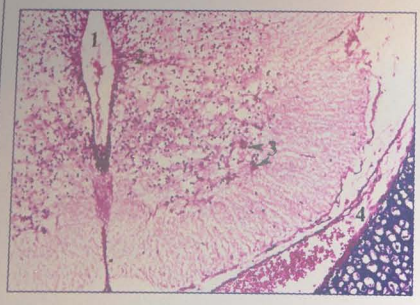
1. Vertebral body
2. Dorsal funiculus
3. Ventral funiculus
4. Internal limiting membrane
5. Ependymal processes extending towards periphery
6. Pia mater extending into ventral median fissure

Fig. 49 L.S. of spinal cord (48 days). van Gieson's x 100



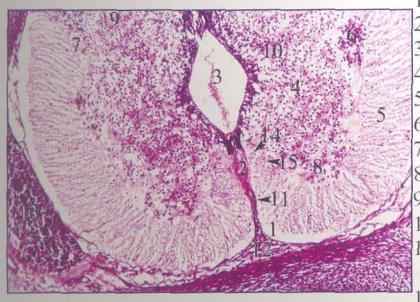
1. Fused area
2. Unfused area
3. Central canal
4. Dorsal median septum
5. Dorsal medium sulcus
6. Dorsal funiculus
7. Dorsal intermediate groove
8. Fasciculus gracilus
9. Fasciculus cuneatus
10. Substantia gelatinosa
11. Dorsolateral fasciculus

Fig. 50 C.S. of cervical region (48 days). H & E x 100



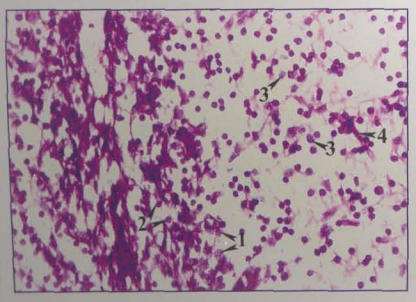
1. Central canal
2. Processes of ependymal cells extending outwards
3. Neurons of ventral horn forming nuclear aggregations
4. Epidural space

Fig. 51 C.S. of cervical region (48 days). H & E x 100



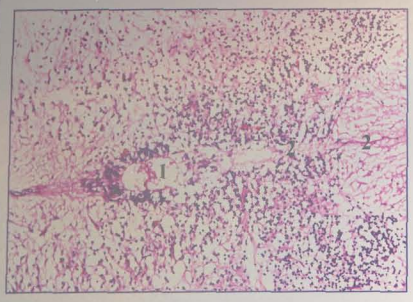
1. Ventral median fissure
2. Ventral commissure
3. Central canal
4. Gray matter
5. White matter
6. Lateral horn
7. Reticular formation
8. Narrow ventral horn
9. Nucleus proprius
10. Clark's column
11. Pia mater extending into ventralmedian fissure
12. Linea splendens
13. Ganglion
14. Medial longitudinal fasciculus
15. Vestibulospinal tract

Fig. 52 C.S. of thoracic region (48 days). H & E x 100



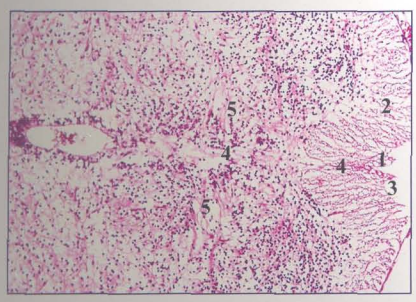
1. Astrocytes
2. Oligodendrocytes
3. Neuroblasts
4. Blood vessel

Fig. 53 C.S. of cervical region showing cell types near dorsal median septum (48 days). H & E x 400



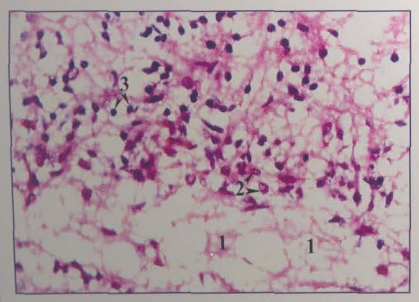
1. Central canal
2. Dorsal median septum

Fig. 54 C.S. of cervical region (54 days). H & E x 100



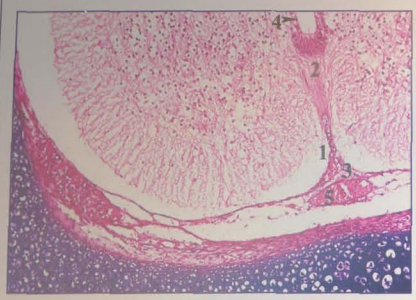
1. Fasciculus gracilis
2. Fasciculus cuneatus
3. Dorsal intermediate groove
4. Dorsal median septum
5. Fibres of astrocytes extending laterally

Fig. 55 C.S. of thoracic region (54 days). H & E x 100



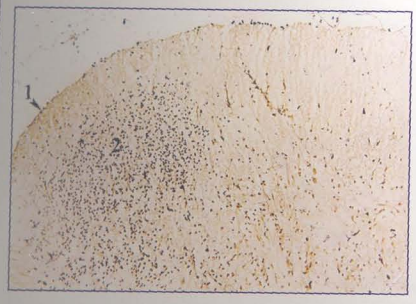
1. Dorsal median septum
2. Astrocytes
3. Oligodendrocytes

Fig. 56 Cells surrounding dorsal median septum (58 days). H & E x 400



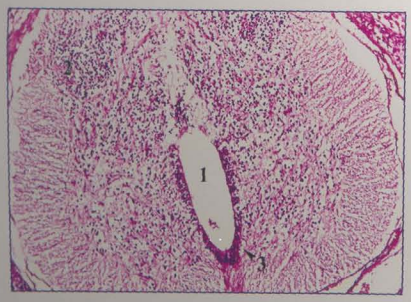
1. Ventral median fissure
2. Ventral commissure
3. Linea splendens
4. Internal limiting membrane
5. Ventral spinal artery

Fig. 57 C.S. of sacral region (54 days). H & E x 100



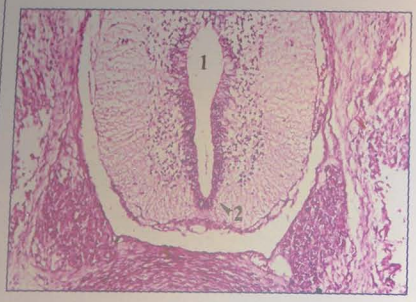
1. External limiting membrane
2. Substantia gelatinosa

Fig. 58 C.S. of cervical region (58 days). Sevier- Munger method x 100



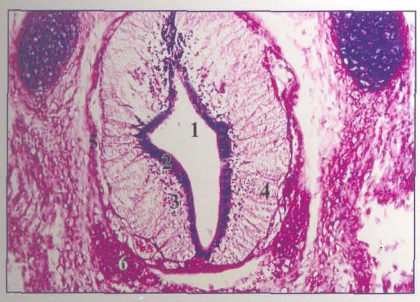
1. Central canal
2. Substantia gelatinosa
3. Medial longitudinal fasciculus

Fig. 59 C.S. of caudal sacral region (58 days). H & E x 100



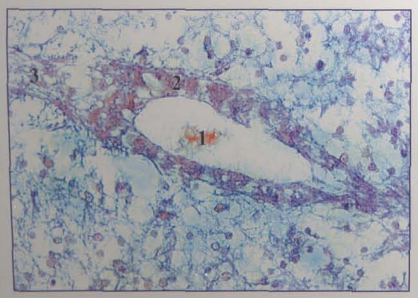
1. Terminal ventricle (lumen)
2. Medial longitudinal fasciculus

Fig. 60 C.S. of anterior coccygeal region (58 days). H & E x 100



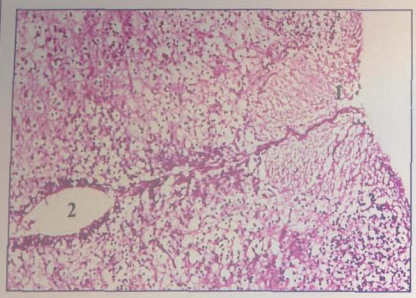
1. Terminal ventricle (lumen)
2. Ependymal layer
3. Mantle layer (gray matter)
4. Marginal layer (white matter)
5. Meninges
6. Ganglion

Fig. 61 C.S. *of conus medullaris* (58 days). H & E x 100



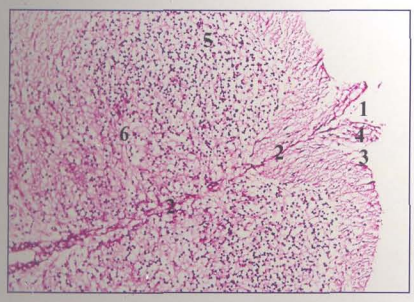
1. PAS positive luminal content
2. Ependymal cells
3. Ventral commissure

Fig. 62 Central canal at the cervical spinal cord (62 days). Aldehyde Thionine PAS method x 400



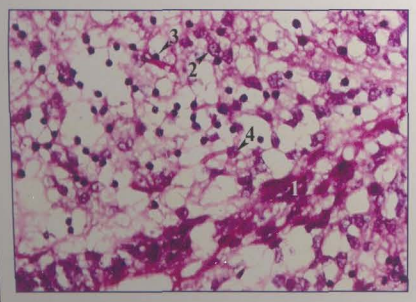
1. Undivided dorsal funiculus
2. Central canal

Fig. 63 C.S. of lumbar enlargement (62 days). H & E x 100



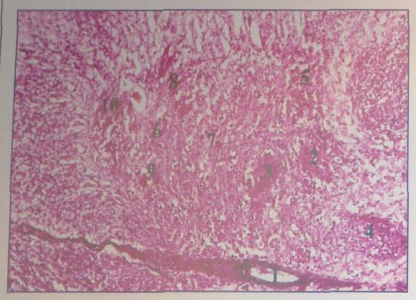
1. Dorsal median sulcus
2. Dorsal median septum
3. Dorasal intermediate groove
4. Fasciculus gracilis
5. Substantia gelatinosa
6. Lamina III

Fig. 64 C.S. of middle thoracic region (62 days). H & E x 100



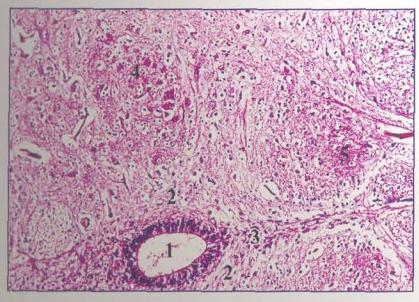
1. Dorsal median septum with cells and fibres
2. Developing neuron
3. Oligodendrocyte as Juxtavascular cell
4. Astrocyte as perivascular satellite

Fig. 65 C.S. of middle lumbar region (62 days). H & E x 400



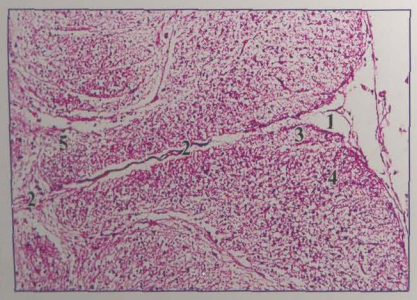
1. Central canal
2. Nucleus proprius
3. Cervical nucleus of Stilling
4. Cuneate nucleus of medulla
5. Reticular formation
6. Spinal accessory nucleus
7. Dorsomedial nucleus
8. Dorsolateral nucleus
9. Ventromedial nucleus
10. Ventrolateral nucleus

Fig. 66 C.S. of cervical region (81 days). H & E x 100



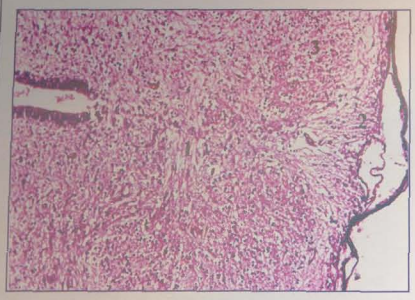
1. Central canal
2. Substantia gliosa
3. Wedge shaped area above central canal
4. Cervical nucleus of Stilling
5. Cuneate nucleus of medulla

Fig. 67 C.S. of first cervical segment (102 days). H & E x 100



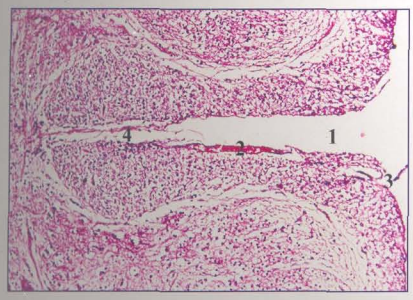
1. Dorsal median sulcus
2. Dorsal median septum
3. Fasciculus gracilis
4. *Fasciculus cuneatus*
5. Dorsal fasciculus proprius

Fig. 68 C.S. of first cervical segment (102 days). H & E x 100



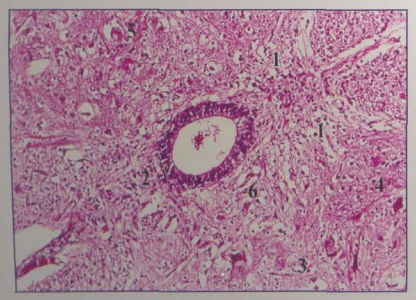
1. Dorsal commissural fibres
2. Undivided dorsal funiculus
3. Substantia gelatinosa

Fig. 69 C.S. of sacral region showing absence of dorsal median septum (102 days). H & E x 100



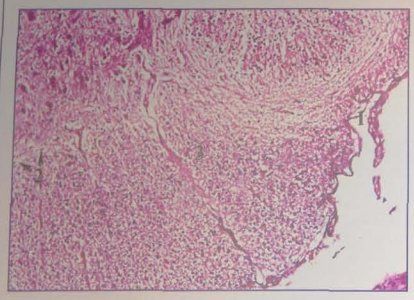
1. Dorsal median sulcus
2. Blood vessel
3. Dorsal intermediate groove
4. Pia mater extending into the sulcus

Fig. 70 C.S. of T_2 segment (102 days). H & E x 100



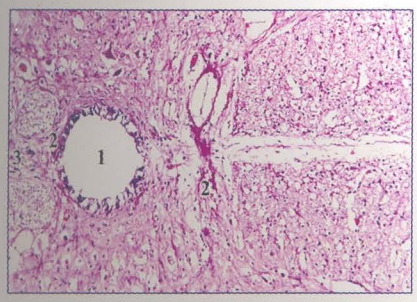
1. Dorsal gray commissure
2. Ventral gray commissure
3. Lamina VI
4. Nucleus proprius
5. Clark's column
6. Lamina X

Fig. 71 C.S. of T_2 segment (102 days). H & E x 100



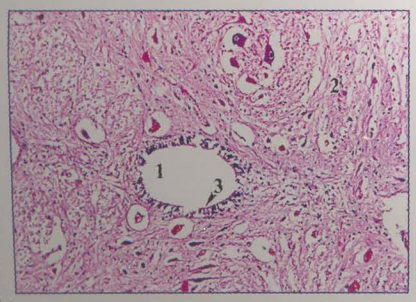
1. Dorsolateral groove
2. Lateral fasciculus proprius
3. Rubrospinal tract

Fig. 72 C.S. of T_5 segment (102 days). H & E x 100



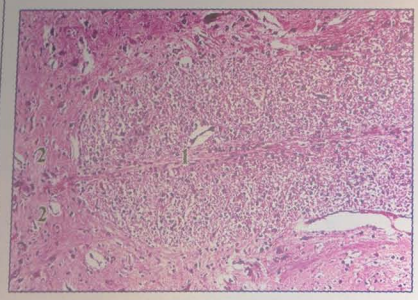
1. Central canal
2. Gray commissure
3. White commissure

Fig. 73 C.S. of L_6 segment (142 days). H & E x 100



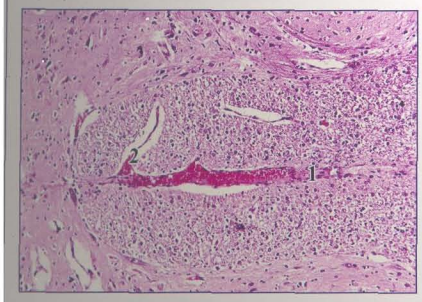
1. Central canal
2. Medial zone of lamina VI lateral to central canal
3. Internal limiting membrane

Fig. 74 C.S. of T_{10} segment (142 days). H & E x 100



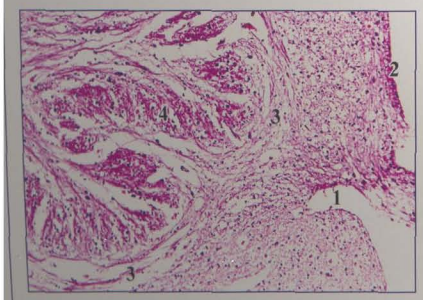
1. Dorsal median septum
2. Gray commissure

Fig. 75 C.S. of C_1 segment (124 days). H & E x 100



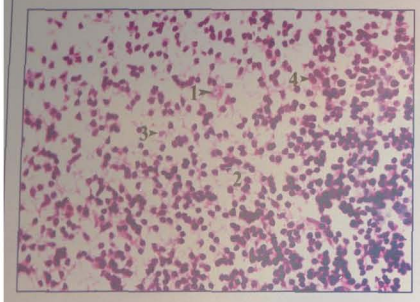
1. Dorsal median septum
2. Blood vessel and piamater extending into white matter

Fig. 76 C.S. of C_1 segment showing dorsal median septum carrying blood vessel (124 days). H & E x 100



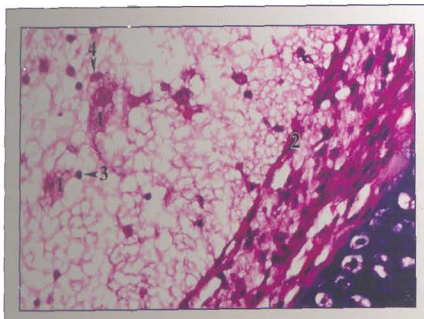
1. Dorsolateral groove
2. External limiting membrane
3. Lamina I
4. Convoluted substantia gelatinosa

Fig. 77 C.S. of C_1 segment showing well developed dorsolateral groove (142 days). H & E x 100



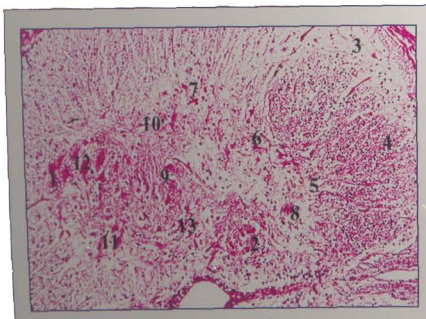
1. Developing multipolar neuroblast with vesicular nucleus and nucleolus
2. Neuropile
3. Developing protoplasmic astrocyte
4. Developing fibrous astrocyte

Fig. 78 L.S. of neural tube mantle layer (40 days). H & E x 400



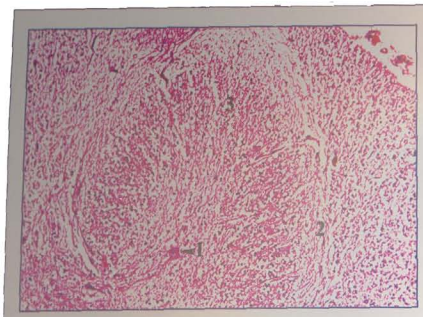
1. Neurons in ventral horn
2. Piamater
3. Oligodendrocyte as perineuronal satellite
4. Astrocyte as perineuronal satellite

Fig. 79 C.S. of middle lumbar region (62 days). H & E x 400



1. Neuron in ventral horn
2. Clark's column
3. Lamina I
4. Lamina II
5. Lamina III
6. Reticular formation
7. Lateral horn
8. Nucleus proprius
9. Dorsomedial nucleus
10. Dorsolateral nucleus
11. Ventromedial nucleus
12. Ventrolateral nucleus
13. Intermediomedial nucleus

Fig. 80 C.S. of L_1 Segment (81 days). H & E x 100



1. Neuron in lamina III
2. Lamina I
3. Conical substantia gelatinosa

Fig. 81 C.S. of C_4 segment (102 days). H & E x 100

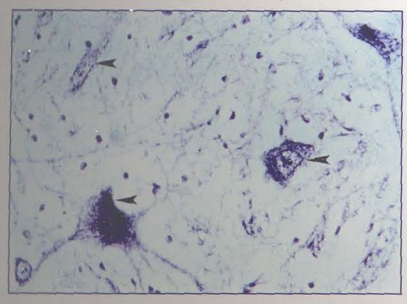


Fig. 82 Ventral horn neurons showing Nissl bodies in the cytoplasm at C_6 segment (102 days). PTAH x 400

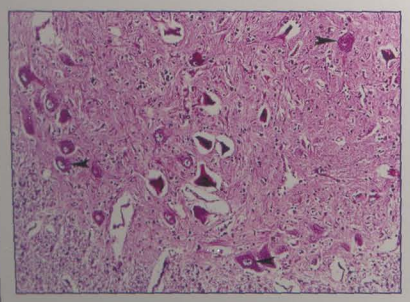
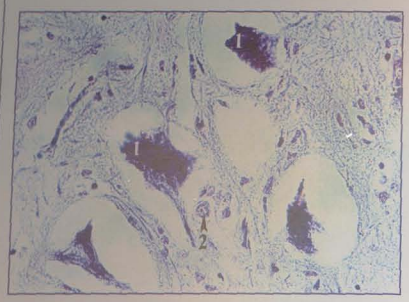
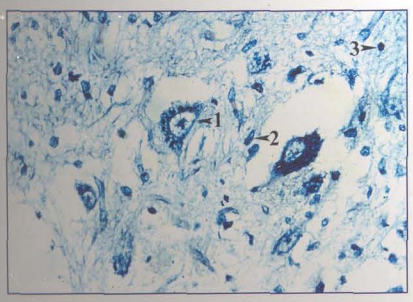


Fig. 83 Ventral horn at C_1 segment showing alpha motor neurons with Nissl bodies (124 days). H & E x 100



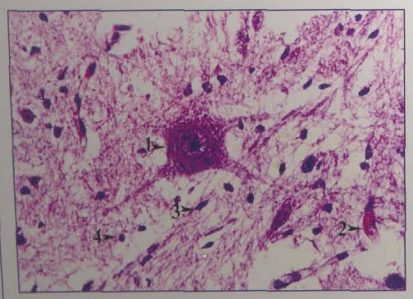
1. Alpha neuron
2. Gamma neuron

Fig. 84 Ventral horn neurons (alpha and gamma) at cervical enlargement (C8 segment) at 124 days. PTAH x 400



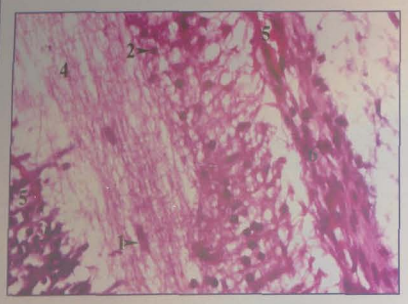
1. Neuron with Nissl bodies and eccentric nucleus
2. Astrocyte
3. Oligodendrocyte

Fig. 85 Clark's column neurons with Nissl bodies at cervical enlargement (C7 segment) at 124 days. Aldehyde Thionine PAS method x 400



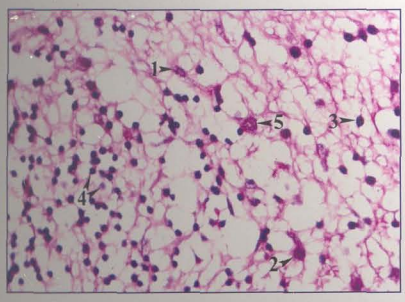
1. Neuron with Nissl bodies
2. Blood vessel
3. Astrocyte
4. Oligodendrocyte

Fig. 86 Gray matter at S_2 segment (142 days). H & E x 400



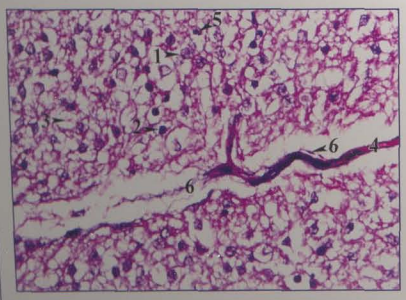
1. Astrocyte
2. Oligodendrocyte
3. Gray matter
4. White matter (Dorsal funiculus)
5. Blood vessel
6. Meninges

Fig. 87 L.S. of spinal cord (48 days). H & E x 400



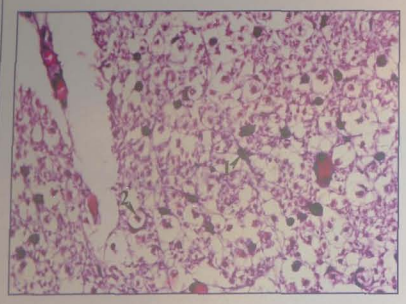
1. Fibrous astrocyte
2. Protoplasmic astrocyte
3. Oligodendrocyte
4. Cells of substantia gelatinosa
5. Cell in lamina I

Fig. 88 White matter of dorsal funiculus at lumbar region (62 days). H & E x 400



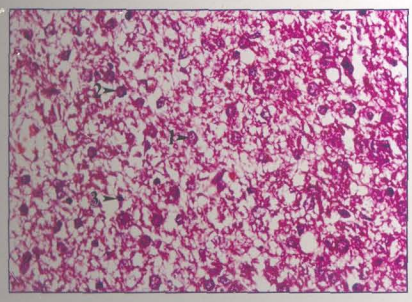
1. Astrocyte
2. Oligodendrocyte
3. Axons with surrounding vacant space
4. Blood vessel
5. Microglia
6. Pia mater

Fig. 89 Increased number of cells in the white matter at C_1 segment (102 days). H & E x 400



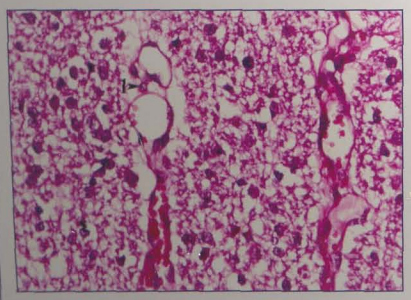
1. Fibrous astrocyte with processes
2. Axon with myelin sheath

Fig. 90 White matter at T_{10} segment (142 days). H& E x 400



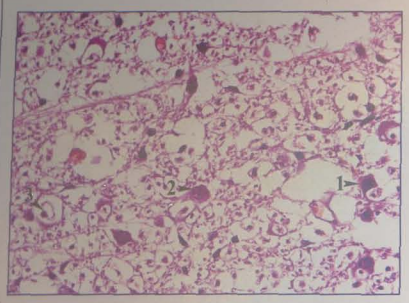
1. Astrocyte
2. Oligodendrocyte
3. Microglia

Fig. 91 White matter at C_1 segment (124 days). H & E x 400



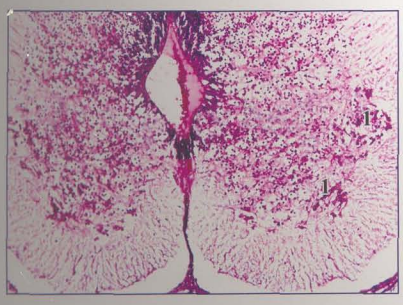
1. Perivascular satellite

Fig. 92 White matter at T_5 segment (124 days). H & E x 400



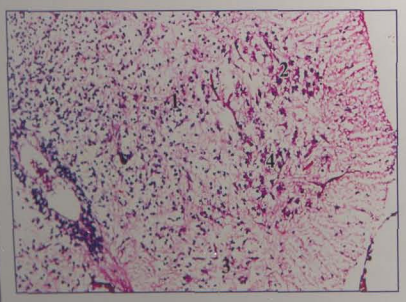
1. Oligodendrocyte
2. Neuron
3. Axon with myelin sheath

Fig. 93 White matter at L_3 segment (142 days). H & E x 400



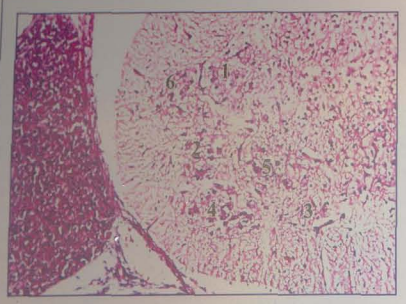
1. Nuclear aggregations

Fig. 94 C.S. of lumbar enlargement (48 days). H & E x 100



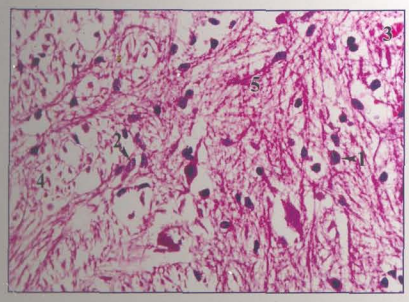
1. Dorsomedial nucleus
2. Dorsolateral nucleus
3. Ventromedial nucleus
4. Ventrolateral nucleus

Fig. 95 C.S. of cervical enlargement showing nuclear aggregations (54 days). H & E x100



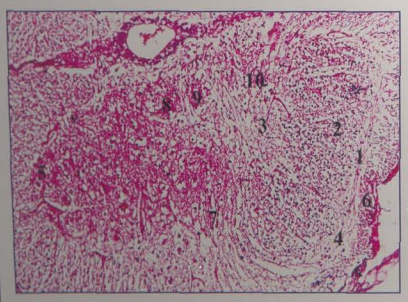
1. Dorsomedial nucleus
2. Dorsolateral nucleus
3. Ventromedial nucleus
4. Ventrolateral nucleus
5. Central nucleus
6. Retrodorsolateral nucleus

Fig. 96 C.S. of lumbar enlargement (58 days). H & E x 100



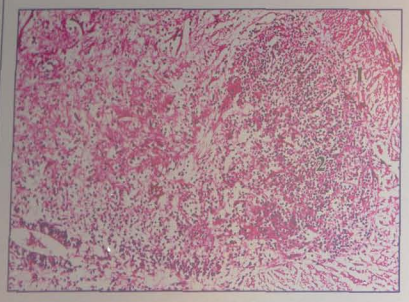
1. Oligodendrocyte
2. Astrocyte
3. Blood vessel
4. White matter
5. Gray matter

Fig. 97 Neuropile (142 days). H& E x 400



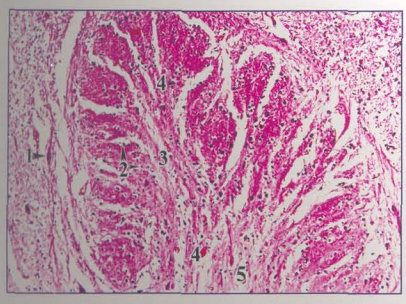
1. Apex of dorsal horn
2. Head of dorsal horn
3. Cervix of dorsal horn
4. Lamina I
5. Narrow ventral horn
6. Dorsolateral fasciculus
7. Intermediolateral nucleus
8. Intermediomedial nucleus
9. Clark's column
10. Nucleus proprius

Fig. 98 C.S. of T_8 segment (81 days). H & E x 100



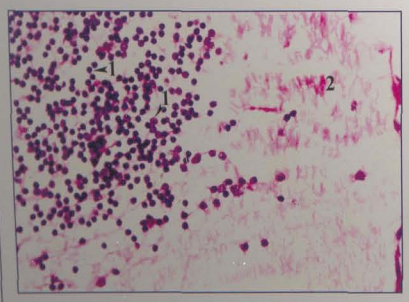
1. Developing marginal zone
2. Substantia gelatinosa

Fig. 99 C.S. of thoracic region (58 days). H & E x100



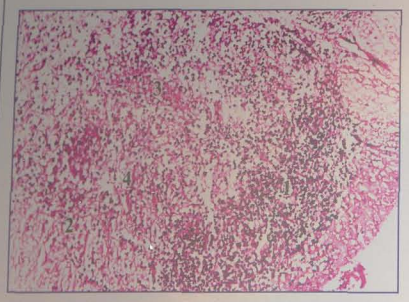
1. Cell in lamina I
2. Lamina II cells
3. Fibres in lamina II
4. Blood vessels
5. Lamina III

Fig. 100 C.S. of T_{10} segment (142 days). H & E x100



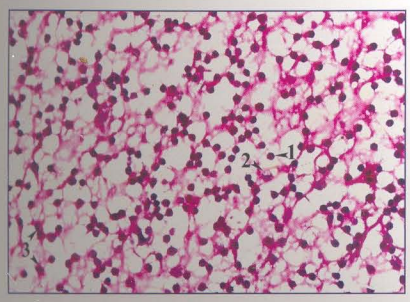
1. Cells in substantia gelatinosa
2. Dorsolateral fasciculus

Fig. 101 C.S. of cervical region (48 days). H & E x 400



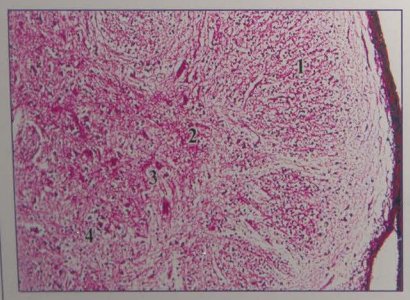
1. Substantia gelatinosa
2. Reticular nucleus
3. Nucleus proprius
4. Blood vessel

Fig. 102 C.S. of cervical region (54 days). H & E x100



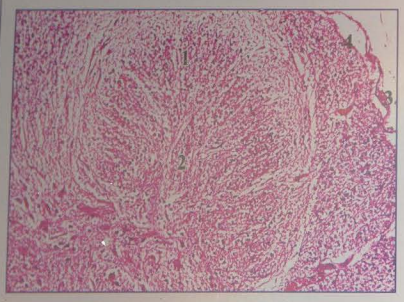
1. Cells with dark stained nuclei of lamina II
2. Pale staining cells of lamina II
3. Neuroglia

Fig. 103 Lamina II at lumbar region (81 days). H & E x 400



1. Lamina II
2. Lamina III
3. Lamina IV(nucleus proprius)
4. Clark's column

Fig. 104 C.S. of S_1 segment showing dorsal horn (102 days). H & E x100



1. Lamina II
2. Lamina III
3. Epipial layer of pia mater
4. Glial limiting membrane

Fig. 105 Dorsal horn at T_9 segment (102 days). H & E x 100

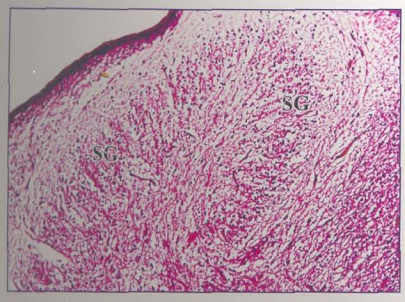
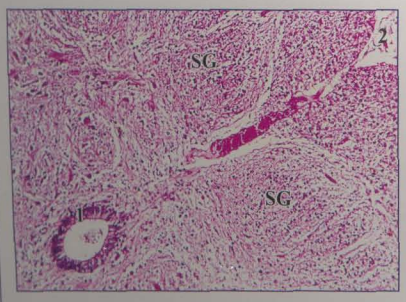
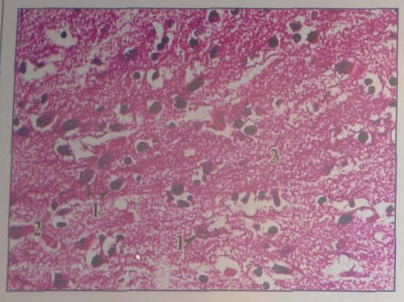


Fig. 106 C.S. of L_1 segment showing flattened substantia gelatinosa (SG) at 102 days. H & E x 100



1. Ependymal layer lining the central canal
2. Pia mater

Fig. 107 C.S. of T_9 segment showing substantia gelatinosa (SG) of either side closely approaching towards the midline (102 days). H & E x 100



1. Cells
2. Blood vessel
3. Fibres

Fig. 108 Substantia gelatinosa at L_1 segment (124 days). H & E x 400

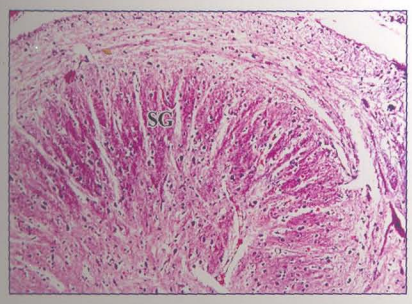
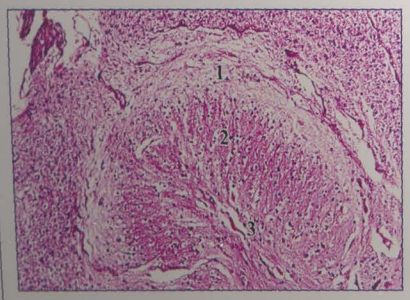
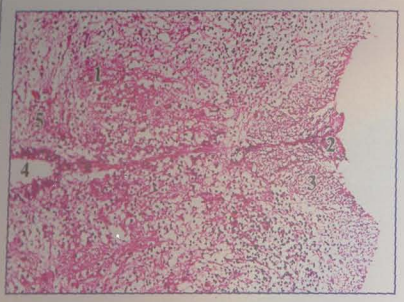


Fig. 109 Substantia gelatinosa (SG) at sacral region showing rounded periphery (142 days). H & E x 100



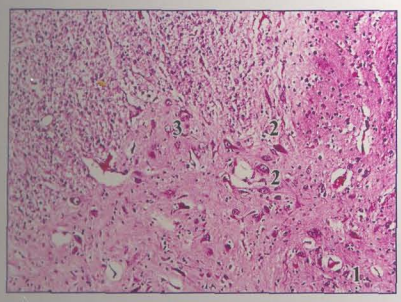
1. Lamina I
2. Lamina II
3. Lamina III

Fig. 110 Dorsal horn at T_3 segment (124 days). H & E x 100



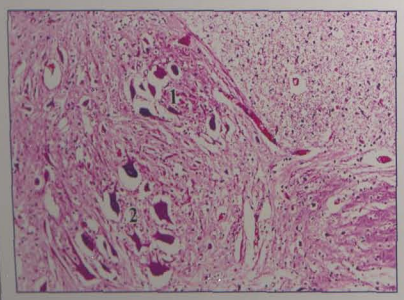
1. Nucleus proprius
2. Fasciculus gracilis
3. Fasciculus cuneatus
4. Central canal
5. Clark's column

Fig. 111 Dorsal half of the spinal cord at caudal thoracic region (62 days). H & E x 100



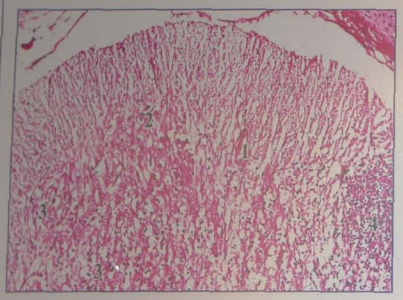
1. Lateral part of lamina IV showing cells
2. Reticular formation
3. Intermediolateral nucleus

Fig. 112 Middle lateral part of spinal cord at T_5 segment (124 days). H & E x 100



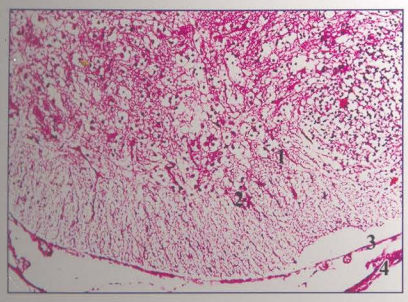
1. Nucleus proprius
2. Clark's column

Fig. 113 C.S. of lumbar region showing well developed nucleus proprius (142 days). H & E x 100



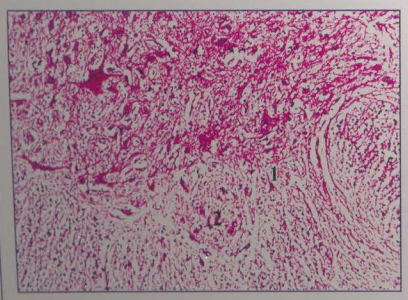
1. Reticular formation
2. Intermediolateral nucleus
3. Nuclear aggregations in ventral horn
4. Substantia gelatinosa

Fig. 114 C.S. of thoracic region (58 days). H & E x100



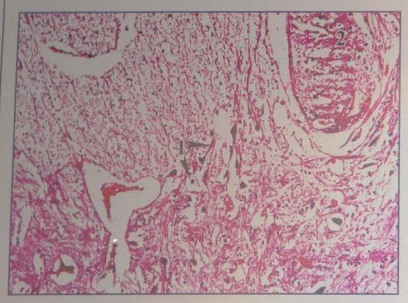
1. Reticular formation
2. Intermediolateral nucleus
3. Pia mater
4. Dura mater

Fig. 115 Lateral aspect of spinal cord at anterior lumbar region (62 days). H & E x 100



1. Reticular formation
2. Lateral horn with intermediolateral nucleus

Fig. 116 C.S. of L_4 segment at lateral aspect (102 days). H & E x 100



1. Axons in reticular formation
2. Substantia gelatinosa

Fig. 117 Middle lateral aspect of C_1 segment (142 days). H & E x 100

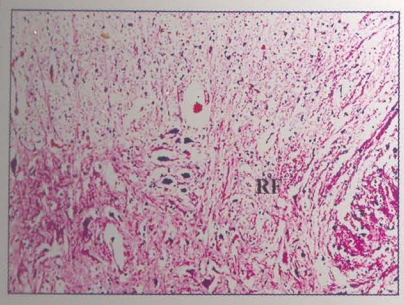
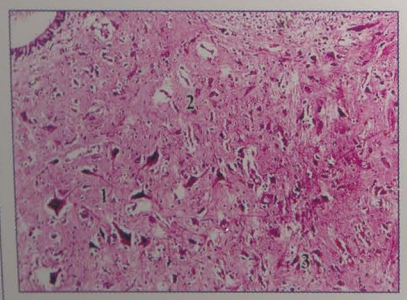
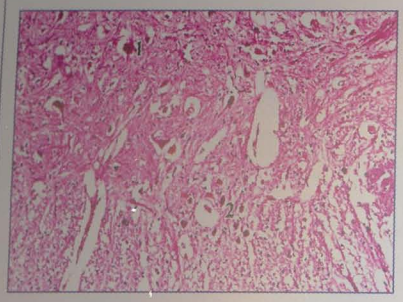


Fig. 118 Lateral aspect of L_3 segment showing less developed reticular formation (RF) at 142 days. H & E x 100



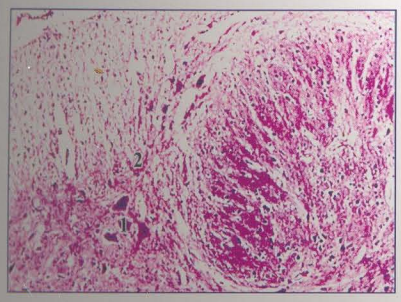
1. Cervical nucleus of Stilling
2. Medial zone of lamina VI
3. Lateral zone of lamina VI

Fig. 119 C.S. of C_1 segment showing gray matter ventral to substantia gelatinosa (124 days). H & E x 100



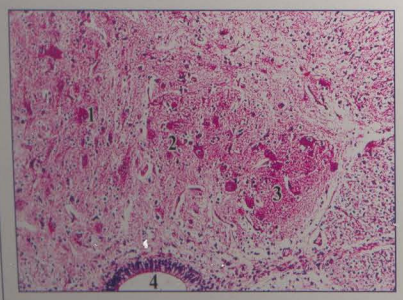
1. Lateral zone of lamina VI
2. Intermediolateral nucleus

Fig. 120 Lateral zone of lamina VI (medial to intermediolateral nucleus) at T_5 segment (142 days). H & E x 100



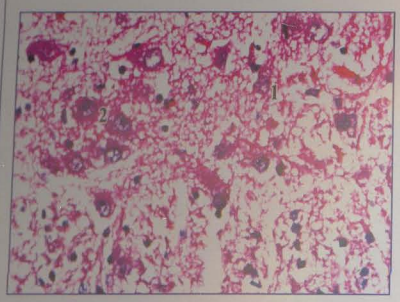
1. Intermediolateral nucleus
2. Reticular formation

Fig. 121 C.S. of sacral region (142 days). H & E x 100



1. Intermediomedial nucleus
2. Clark's column
3. Medioposterior column
4. Central canal

Fig. 122 Intermediate gray matter at L_4 segment (102 days). H & E x 100



1. Intermediolateral nucleus
2. Intermediomedial nucleus

Fig. 123 Intermediate zone of gray matter at T_{10} segment (102 days). H & E x 400

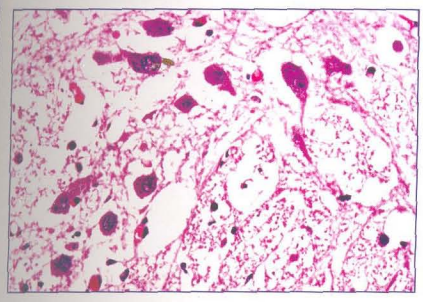
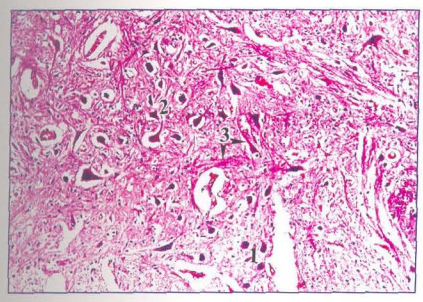
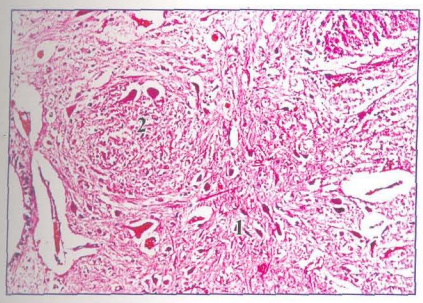


Fig.124 Cells of intermediolateral nucleus at L_2 segment (142 days). H & E x 400



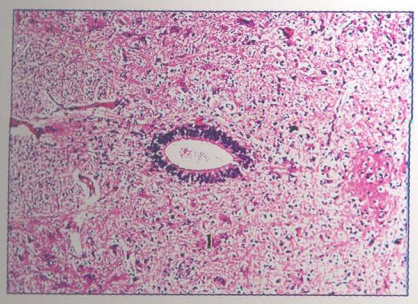
1. Intermediolateral nucleus
2. Intermediomedial nucleus
3. Processes extending between the two nuclei

Fig.125 Intermediate zone of gray matter at T_{10} segment (142 days). H & E x 100



1. Intermediomedial nucleus
2. Clark's column

Fig.126 Intermediate gray matter at T_{12} segment (142 days). H & E x 100



1. Sacral nucleus of Stilling

Fig. 127 Region surrounding the central canal of S_1 segment (102 days). H & E x 100

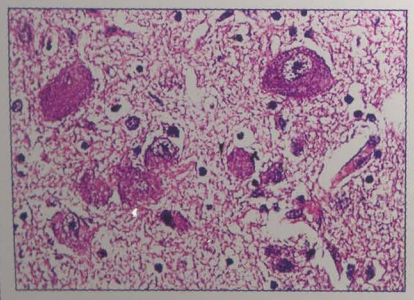
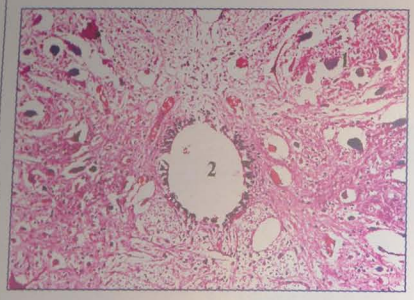


Fig. 128 Cells of Clark's column at L_4 segment (102 days). H & E x 400



1. Clark's column
2. Central canal

Fig. 129 Intermediate gray matter surrounding the central canal at L_2 segment (142 days). H & E x 100

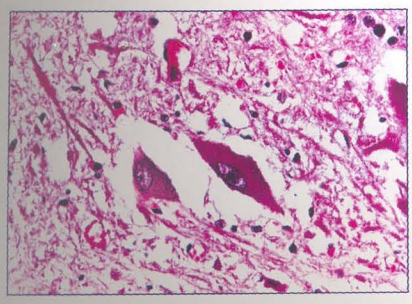


Fig. 130 Cells of Clark's column at L_3 segment (142 days). H & E x 400

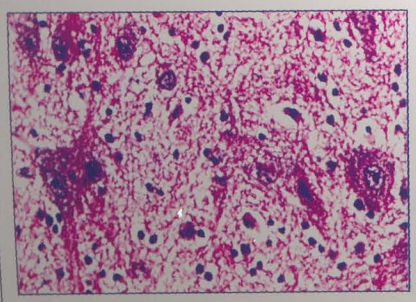
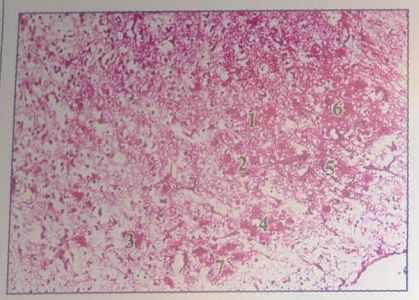
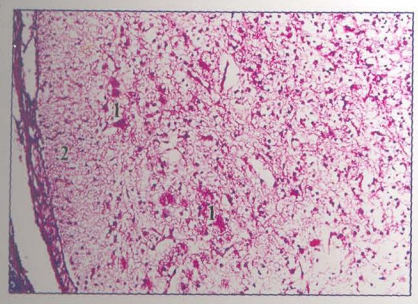


Fig. 131 Cells of laminae VIII and IX at L_4 segment (102 days). H & E x 400



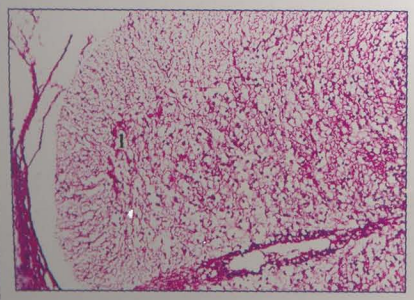
1. Dorsomedial nucleus
2. Central nucleus
3. Ventromedial nucleus
4. Ventrolateral nucleus
5. Dorsolateral nucleus
6. Retrodorsolateral nucleus
7. Phrenic nerve nucleus

Fig. 132 C.S. of ventral horn at cervical enlargement (62 days). H & E x 100



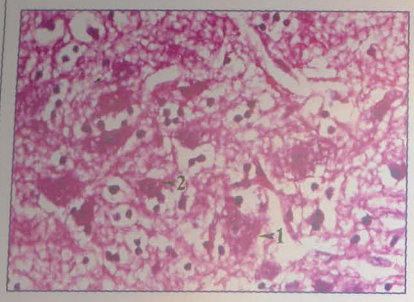
1. Nuclear aggregations
2. White matter with more cells

Fig. 133 C.S. of ventral horn at lumbar enlargement (62 days). H & E x 100



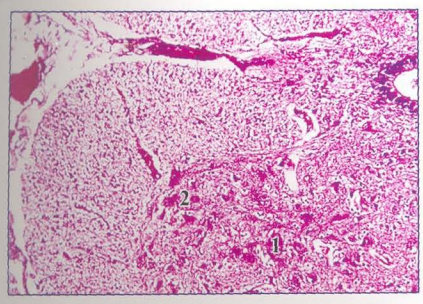
1. Nuclear aggregations

Fig. 134 Ventral horn at thoracic region (62 days). H & E x 100



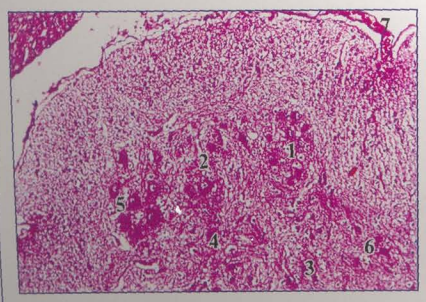
1. Alpha neuron
2. Gamma neuron

Fig. 135 Cells of lamina IX at S_1 segment (102 days). H & E x 400



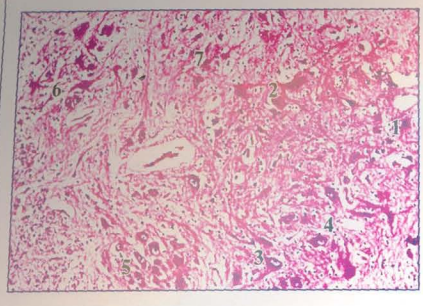
1. Dorsomedial nucleus
2. Ventromedial nucleus

Fig. 136 Ventromedial aspect of S_1 segment (102 days). H & E x 100



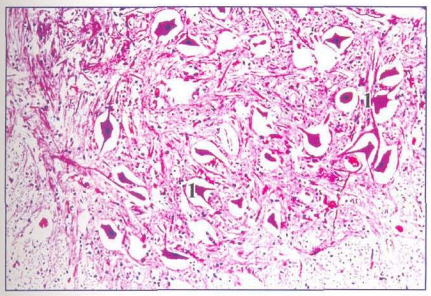
1. Retrodorsolateral nucleus
2. Dorsolateral nucleus
3. Dorsomedial nucleus
4. Central nucleus
5. Ventrolateral nucleus
6. Intermediolateral nucleus
7. Blood vessel

Fig. 137 Ventrolateral aspect of S_1 segment (102 days). H & E x 100



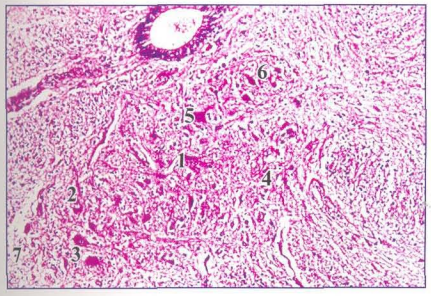
1. Ventromedial nucleus
2. Central nucleus
3. Ventrolateral nucleus
4. Phrenic nerve nucleus
5. Dorsolateral nucleus
6. Retrodorsolateral nucleus
7. Dorsomedial nucleus

Fig. 138 Nuclear aggregations at C_6 segment (124 days). H & E x 100



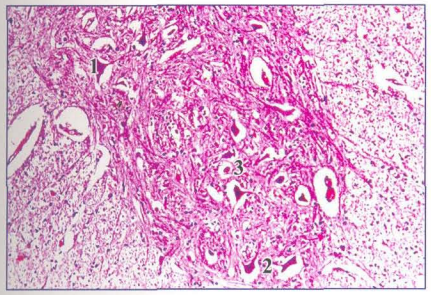
1. Nuclear aggregations

Fig. 139 Nuclear aggregations at cervical enlargement (142 days). H & E x 100



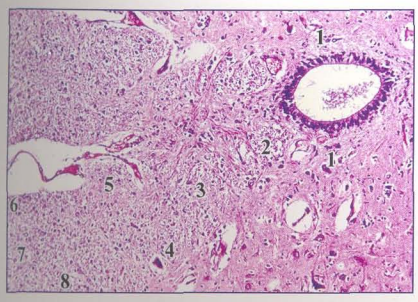
1. Dorsomedial nucleus
2. Ventromedial nucleus
3. Ventrolateral nucleus
4. Lateral horn
5. Intermediomedial nucleus
6. Clark's column
7. Ventral fasciculus proprius

Fig. 140 Nuclear aggregations at T_9 segment (102 days). H & E x 100



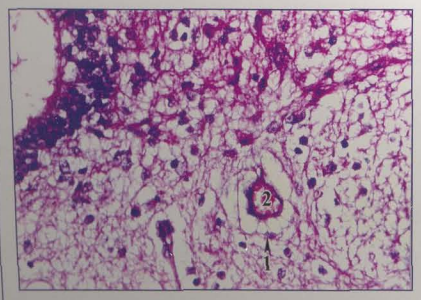
1. Dorsomedial nucleus
2. Ventromedial nucleus
3. Spinal accessory nucleus

Fig. 141 Nuclear aggregations at C_3 segment region (142 days). H & E x100



1. Lamina X
2. Medial longitudinal fasciculus
3. Medial vestibulospinal tract
4. Ventral fasciculus proprius
5. Corticospinal tract
6. Tectospinal tract
7. Lateral vestibulospinal tract
8. Reticulospinal tract

Fig. 142 Lamina X surrounding central canal at C_1 segment (124 days). H & E x 100



1. Astrocyte
2. Blood vessel

Fig. 143 Lateral funiculus at C_1 segment showing astrocytes extending processes towards blood vessel (102 days). H & E x 400

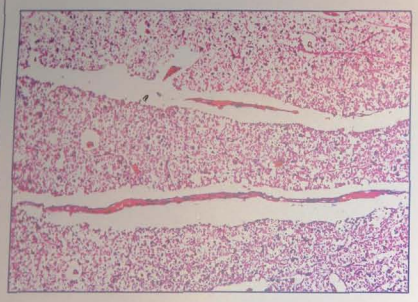


Fig. 144 Increased number of cells and blood supply at white matter of T_8 segment (142 days). H & E x 100

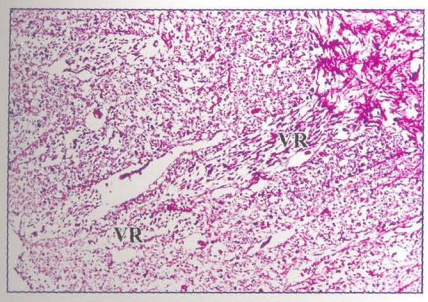


Fig. 145 C.S. of L_6 segment showing emergence of ventral root fibres (VR) in the ventral funiculus at 142 days. H & E x 100

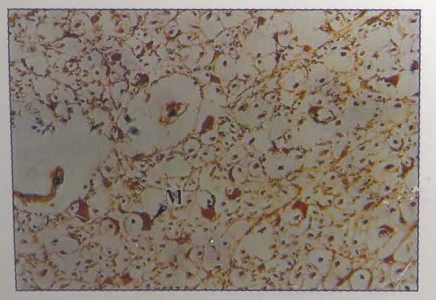
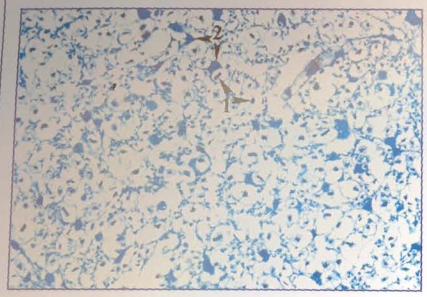
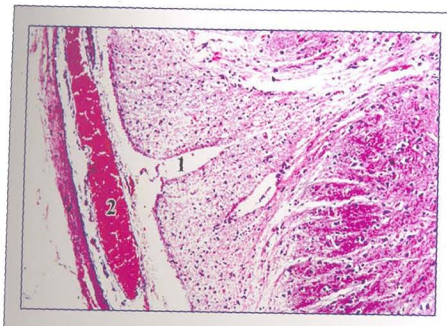


Fig. 146 C.S. of T_1 segment showing myelin sheath (M) in white matter at 142 days. Sevier-Munger method x 400



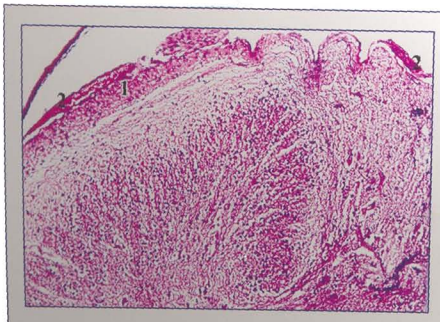
1. Axon
2. Myelin sheath

Fig. 147 White matter at ventral funiculus at S_1 segment (142 days). Luxol Fast Blue-Holmes silver nitrate method x 400



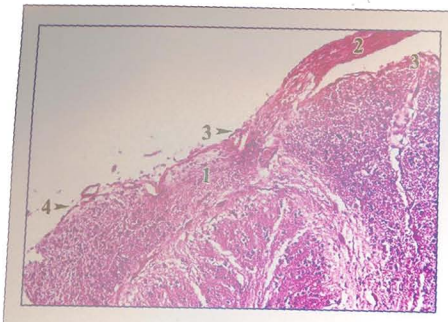
1. Dorsal median sulcus
2. Blood vessel

Fig. 148 C.S. of S_1 segment showing undivided dorsal funiculus (142 days). H & E x 100



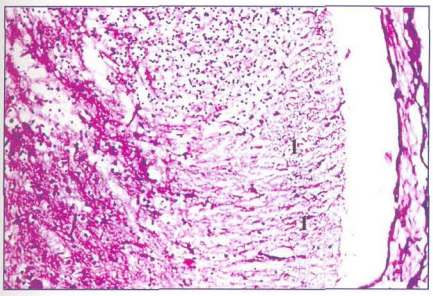
1. Dorsolateral fasciculus
2. Blood vessel

Fig. 149 C.S. of S_1 segment at the dorsolateral aspect (102 days). H & E x 100



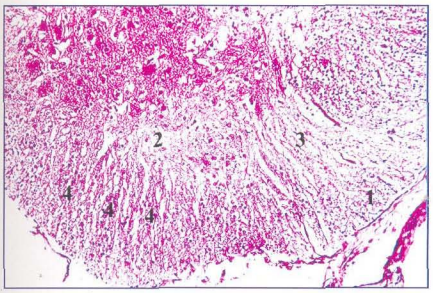
1. Dorsolateral fasciculus
2. Dorsal root of spinal nerve
3. Pia mater
4. Glial limiting membrane

Fig. 150 C.S. of T_5 segment at dorsolateral aspect (124 days). H& E x 100



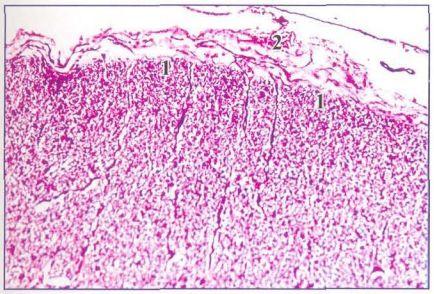
1. Spinocerebellar tracts

Fig. 151 C.S. of anterior thoracic region at the lateral funiculus (62 days). H & E x 400



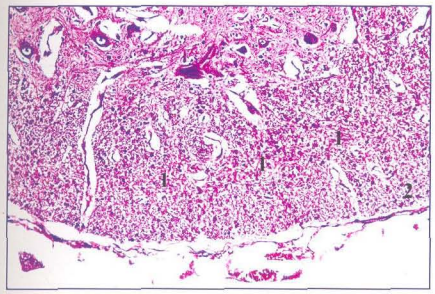
1. Spinocerebellar tracts
2. Lateral fasciculus proprius
3. Lateral corticospinal tract
4. Lateral reticulospinal tract

Fig. 152 C.S. of T_3 segment at the lateral aspect (81 days). H & E x 100



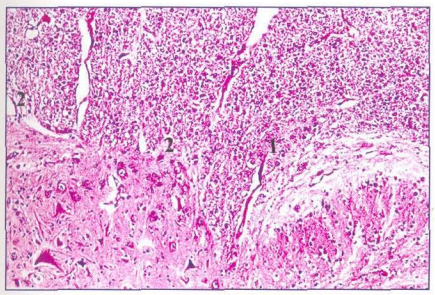
1. Spinocerebellar tracts
2. Dentate ligament

Fig. 153 C.S. of C_8 segment at lateral funiculus (124 days). H & E x 100



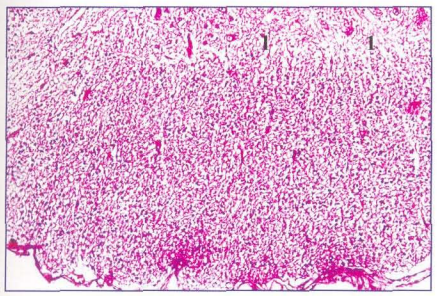
1. Lateral reticulospinal tract
2. Ventral spinocerebellar tract

Fig. 154 C.S. of C_1 segment at lateral funiculus (124 days). H & E x 100



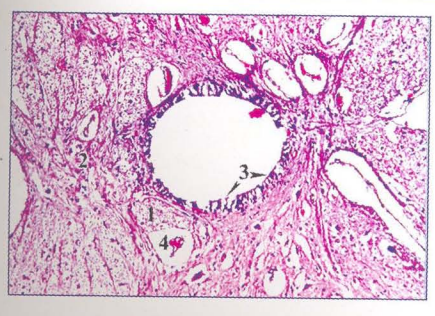
1. Rubrospinal tract
2. Lateral fasciculus proprius

Fig. 155 C.S. of T_3 segment at lateral funiculus (124 days). H & E x 100



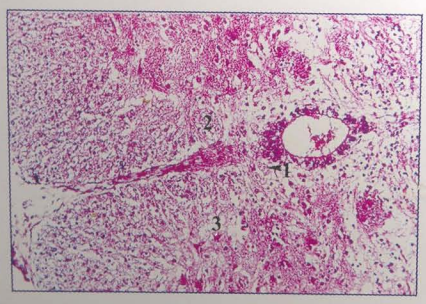
1. Lateral fasciculus proprius

Fig. 156 C.S. of T_5 segment at lateral funiculus (102 days). H & E x 100



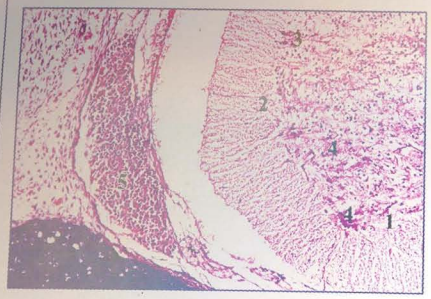
1. Medial longitudinal fasciculus
2. Ventral median raphae
3. Ephendymal cells
4. Blood vessels

Fig. 157 C.S. of central aspect of L_3 segment (142 days). H & E x 100



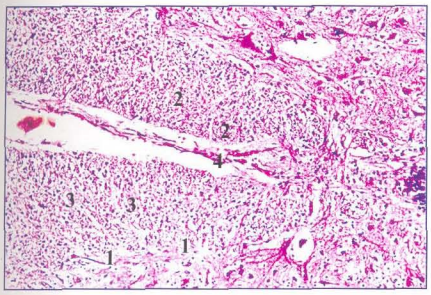
1. Medial longitudinal fasciculus
2. Vestibulospinal tract
3. Ventral fasciculus proprius

Fig. 158 Ventral part of T_3 segment (81 days). H & E x 100



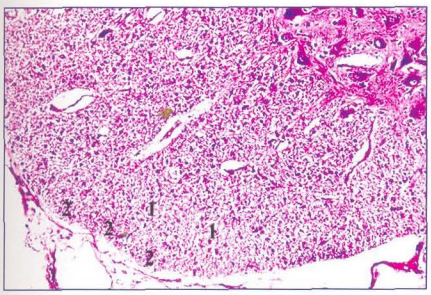
1. Ventral fasciculus proprius
2. Lateral fasciculus proprius
3. Lateral horn
4. Nuclear aggregations
5. Ganglion

Fig. 159 C.S. of lumbar region (54 days). H & E x 100



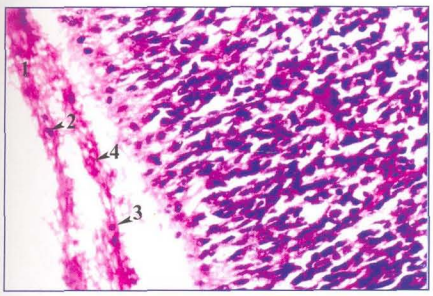
1. Ventral fasciculus proprius
2. Ventral corticospinal tract
3. Reticulospinal tract
4. Pia mater

Fig. 160 C.S. of C_6 segment at ventral funiculus (102 days). H & E x 100



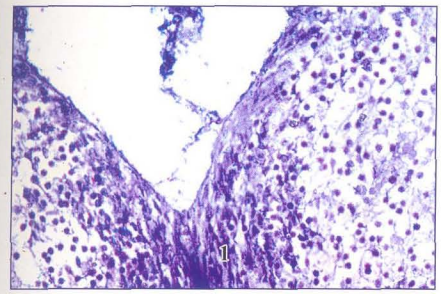
1. Vestibulospinal tract
2. Tectospinal tract

Fig. 161 C.S. of C_1 segment at ventral funiculus (124 days). H & E x 100



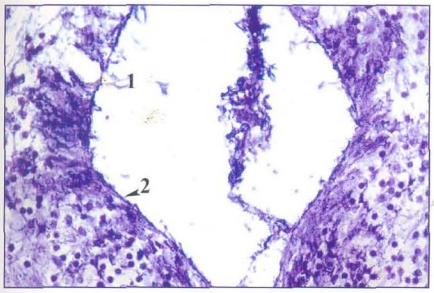
1. Secretion in the lumen
2. Denuded cell
3. Nucleated erythrocyte
4. Non- nucleated erythrocyte

Fig. 162 L.S. of spinal cord (48 days). H & E x 400



1. Ependymal cell processes clustered at the ventral aspect

Fig. 163 C.S. of lumbar region (48 days). Holzer's method x 400



1. Cilia of ependymal cells
2. Internal limiting membrane

Fig. 164 C.S. of lumbar region (48 days). Holzer's method x 400

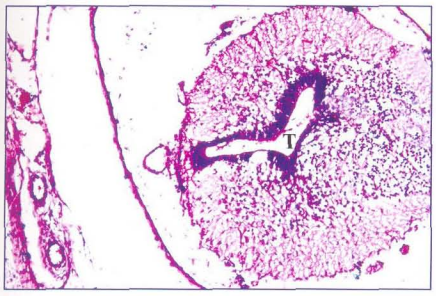


Fig. 165 C.S. of coccygeal region showing terminal ventricle (T) at 81 days. H & E x 100

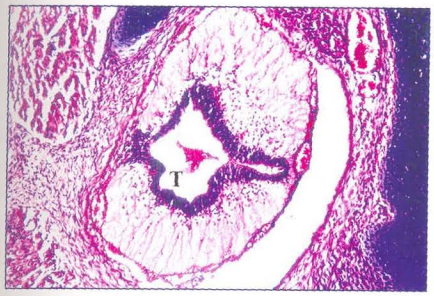
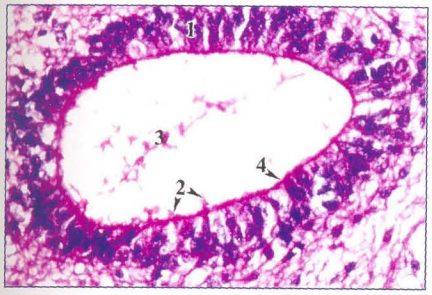


Fig. 166 Conus medullaris region with terminal ventricle (T) at 62 days. H & E x 100



1. Pseudostratified columnar cells
2. Cilia
3. Luminal content
4. Internal limiting membrane

Fig. 167 Central canal at C_1 segment (102 days). H & E x 400

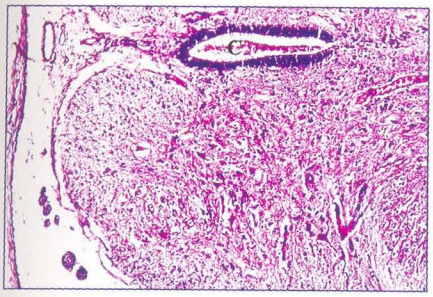
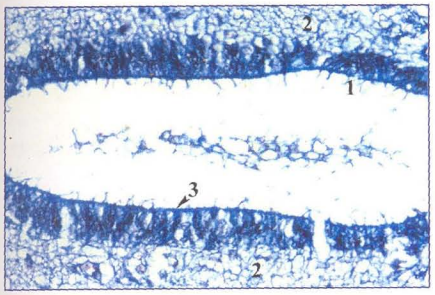
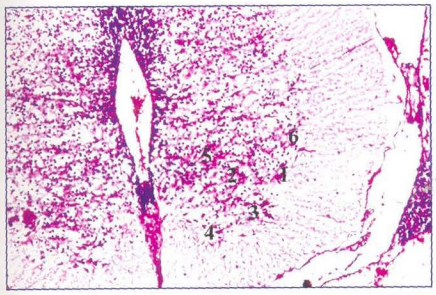


Fig. 168 C.S. of S_4 segment with elongated central canal (C) at 102 days. H & E x 100



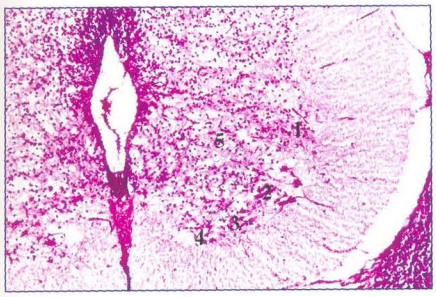
1. Cilia
2. Area gliosa
3. Internal limiting membrane

Fig. 169 Central canal at caudal sacral region (142 days). Luxol Fast Blue-Holmes silver nitrate method x 400



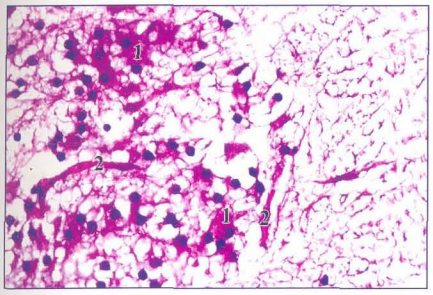
1. Ventrolateral nucleus
2. Spinal accessory nucleus
3. Phrenic nerve nucleus
4. Ventromedial nucleus
5. Dorsomedial nucleus
6. Dorsolateral nucleus

Fig. 170 C.S. of C_5 segment showing nuclei at ventral horn (48 days). H & E x 100



1. Dorsolateral nucleus
2. Ventrolateral nucleus
3. Phrenic nerve nucleus
4. Ventromedial nucleus
5. Dorsomedial nucleus

Fig. 171 C.S. of cervical enlargement showing increased vascularity and fibre content (48 days). H & E x 100



1. Neurons
2. Blood vessel

Fig. 172 Ventral horn at cervical enlargement (48 days). H & E x 400

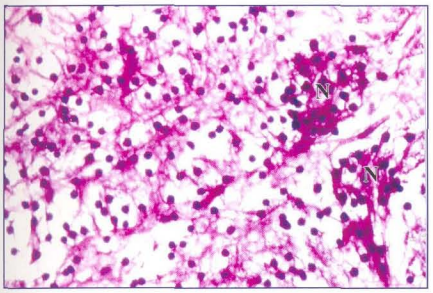


Fig. 173 Ventral horn cells forming nuclear aggregations (N) in the cervical enlargement at 48 days. H & E x 400

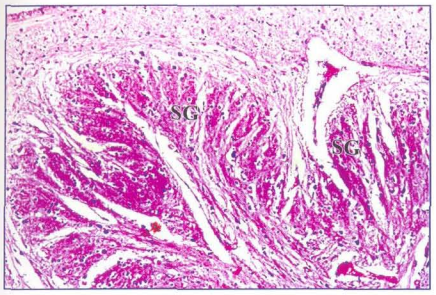
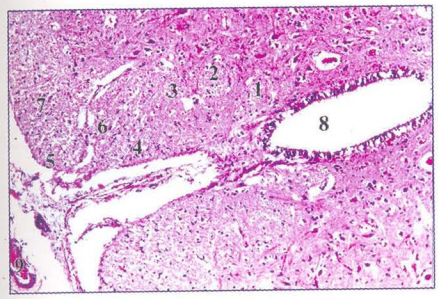
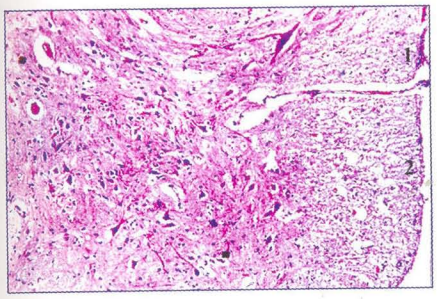


Fig. 174 Convoluted substantia gelatinosa (SG) in the L_5 segment at 142 days. H & E x 100



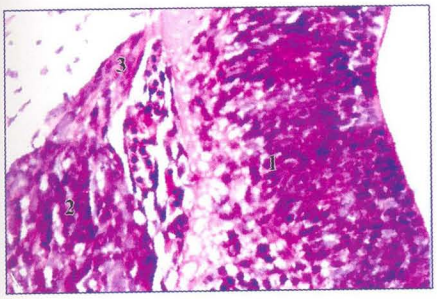
1. Medial longitudinal fasciculus
2. Medial vestibulospinal tract
3. Fasciculus proprius
4. Corticospinal tract
5. Tectospinal tract
6. Lateral vestibulospinal tract
7. Reticulospinal tract
8. Central canal
9. Ventral spinal artery

Fig. 175 Tracts at the ventral funiculus at S_2 segment (142 days). H & E x 100



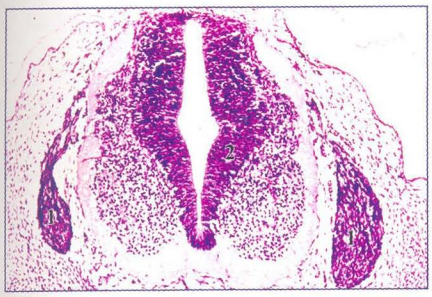
1. Dorsal spinocerebellar tract
2. Ventral spinocerebellar tract

Fig. 176 C.S. of S_1 segment at the lateral aspect (142 days). H & E x 100



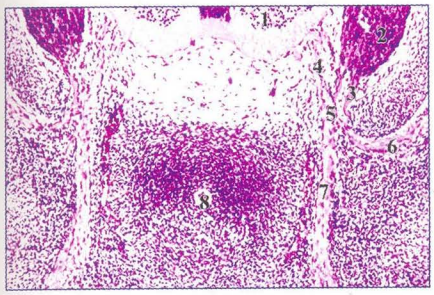
1. Neural tube wall
2. Dorsal root ganglion
3. Dorsal root fibres with connective tissue sheath

Fig. 177 C.S. of neural tube wall (24 days). H & E x 400



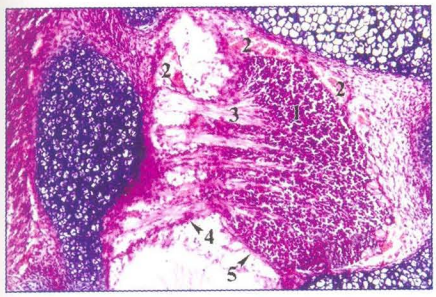
1. Dorsal root ganglion
2. Neural tube

Fig. 178 C.S. of neural tube (26 days). H & E x 100



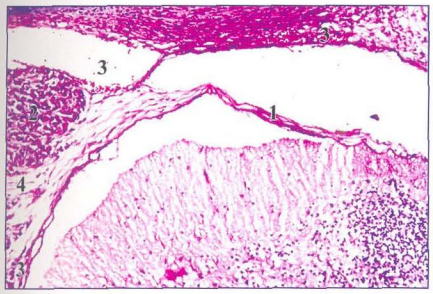
1. Neural tube
2. Dorsal root ganglion
3. Dorsal root fibres
4. Ventral root fibres
5. Spinal nerve
6. Dorsal branch
7. Ventral branch
8. Notochord

Fig. 179 C.S. of embryo showing formation and division of a spinal nerve (26 days). H & E x 100



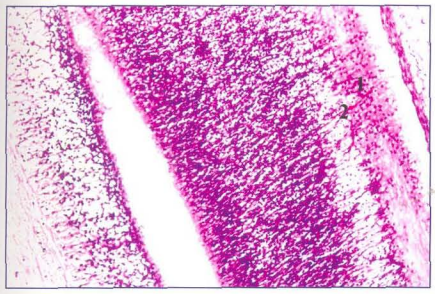
1. Dorsal root ganglion
2. Blood spaces
3. Dorsal root fibres attaching to ganglion
4. Perineurium
5. Capsule

Fig. 180 L.S. of dorsal root ganglion (48 days). H & E x 100



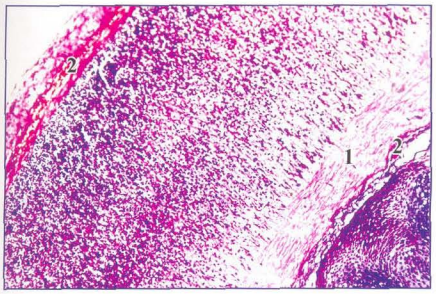
1. Dorsal root of spinal nerve
2. Ganglion
3. Blood vessels
4. Septum in the ganglion

Fig. 181 C.S. of cervical enlargement region of spinal cord (54 days). H & E x 100



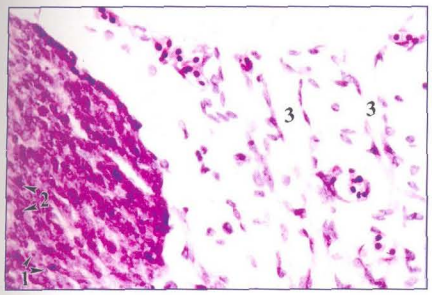
1. Dorsal root fibres
2. Blood vessels accompanying nerve fibres

Fig. 182 L.S. of spinal cord (48 days). H & E x 100



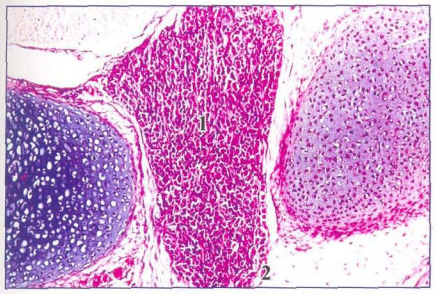
1. Ventral root fibres emerging
2. Blood vessels

Fig. 183 L.S. of spinal cord (48 days). H & E x 100



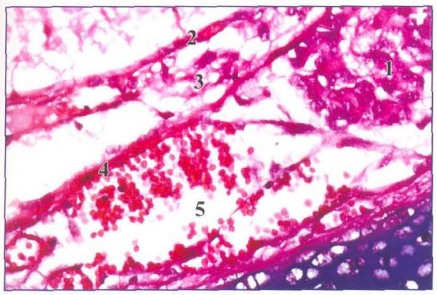
1. Ganglionic neuroblast
2. Supporting cell
3. Ventral root fibres

Fig. 184 Dorsal root ganglion (27 days). H & E x 400



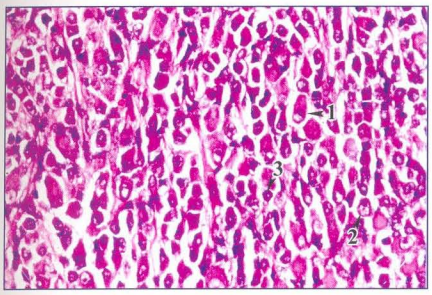
1. Ganglion
2. Capsule

Fig. 185 Dorsal root ganglion at intervertebral foramen (58 days). H & E x 100



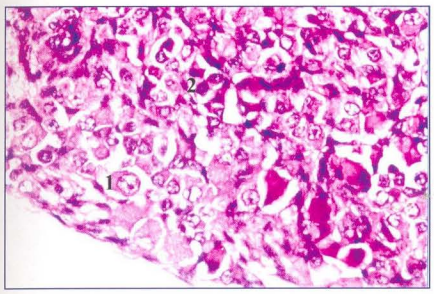
1. Ganglion
2. Pia mater
3. Arachnoid
4. Dura mater
5. Epidural space

Fig. 186 Meninges and dorsal root ganglion (54 days). H & E x 400



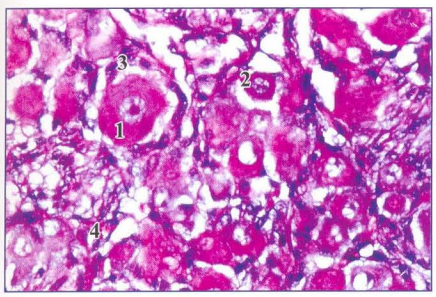
1. Large neuroblast
2. Small neuroblast
3. Satellite cell

Fig. 187 Dorsal root ganglion (58 days). H & E x 400



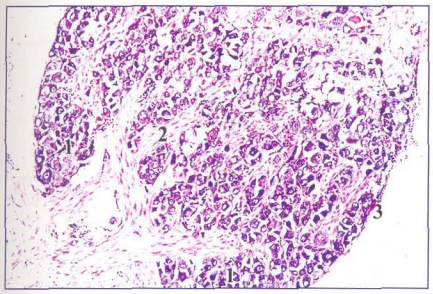
1. Neuroblast
2. Satellite cell

Fig. 188 Cells of dorsal root ganglion at anterior lumbar region (62 days). H & E x 400



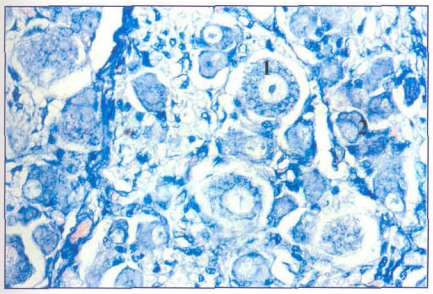
1. Large neuron
2. Small neuron
3. Satellite cell
4. Blood vessel

Fig. 189 Cells of ganglion at S_1 segment (102 days). H & E x 400



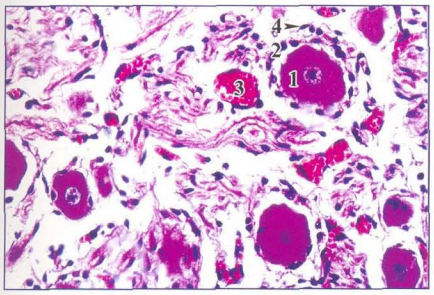
1. Neurons with Nissl bodies
2. Fibre bundle
3. Blood vessel in the capsule

Fig. 190 Dorsal root ganglion at T_5 segment (102 days). PTAH X 100



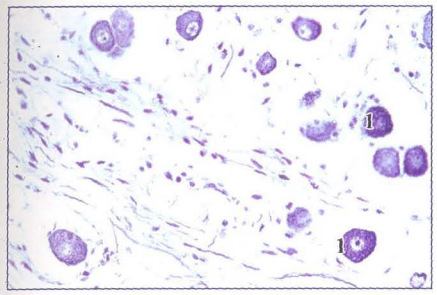
1. Neuron with granular cytoplasm and central nucleus
2. Neuron with eccentric nucleus

Fig. 191 Cells of dorsal root ganglion at L_5 segment (124 days). Aldehyde Thionine PAS method x 400



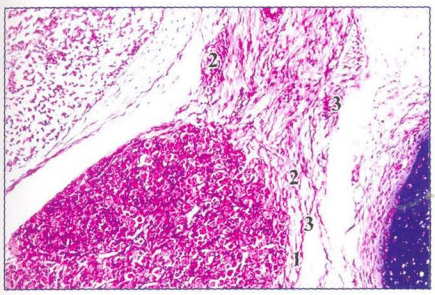
1. Neuron
2. Satellite cells
3. Blood vessel
4. Capsule cell

Fig. 192 Cells of dorsal root ganglion at C_5 segment (142 days). H & E x 400



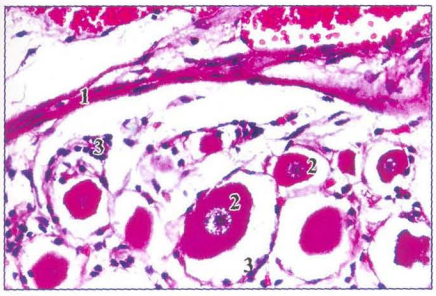
1. Ganglionic neurons with Nissl bodies

Fig. 193 Cells of dorsal root ganglion at L_5 segment (124 days). PTAH x 400



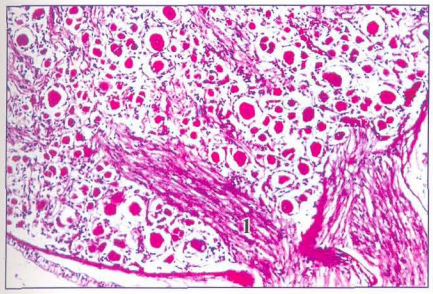
1. Capsule
2. Nerve bundles
3. Blood vessels

Fig. 194 Dorsal root ganglion at anterior lumbar region (62 days). H & E x 100



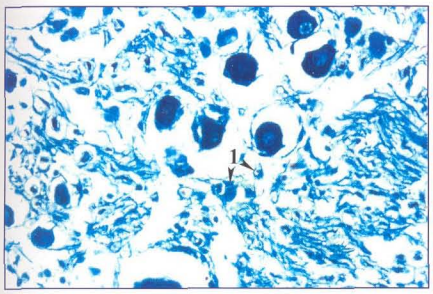
1. Ganglionic capsule
2. Neurons
3. Satellite cells

Fig. 195 Dorsal root ganglion at C_8 segment (142 days). H & E x 400



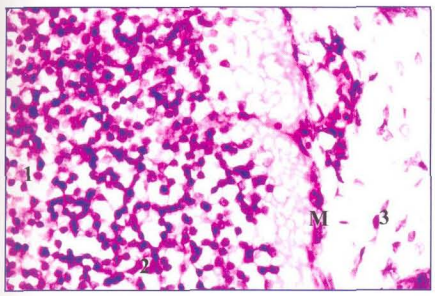
1. Nerve fibres entering the ganglion

Fig. 196 Dorsl root ganglion at C_8 segment (142 days). H & E x 100



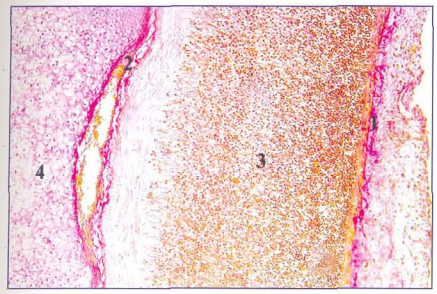
1. Myelinated axons

Fig. 197 Ganglion at S_1 segment (142 days). Luxol Fast Blue-Holmes silver nitrate method x 400



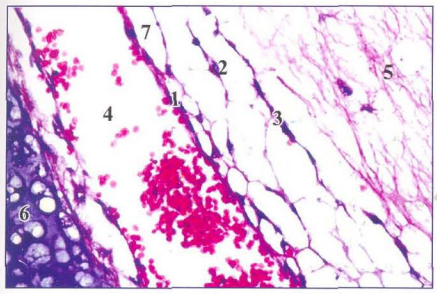
1. Neuroblast
2. Spongioblast
3. Mesenchymal cells

Fig. 198 Primitive meninx (M) in the alar plate region at 26 days. H & E x 400



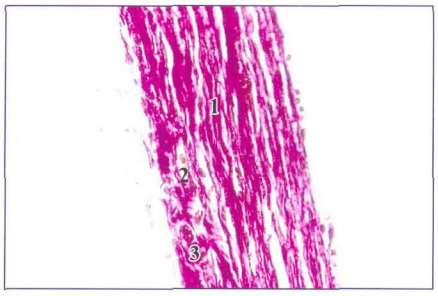
1. Meninges at the dorsal aspect
2. Meninges at the ventral aspect
3. Spinal cord
4. Vertebral body

Fig. 199 L.S. of spinal cord (48 days). Van Gieson's x 100



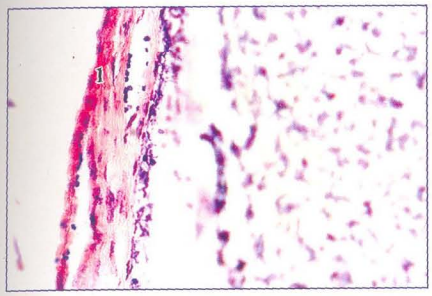
1. Dura mater
2. Arachnoid
3. Pia mater
4. Epidural space
5. White matter
6. Vertebral body
7. Subdural space

Fig. 200 L.S. of spinal cord at the ventral aspect (48 days). H & E x 400



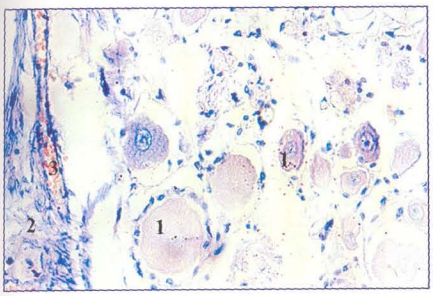
1. Collagen fibres
2. Blood vessels
3. Nerve fibre

Fig. 201 Dura mater at C_7 segment (124 days). Van Gieson's x 400



1. Elastic fibres

Fig. 202 Duramater at C_6 segment (102 days). PTAH x 400



1. Ganglionic neuron
2. Ganglionic capsule
3. Blood vessel

Fig. 203 C.S. of ganglia at C_7 segment (142 days). Aldehyde Thionine PAS method x 400

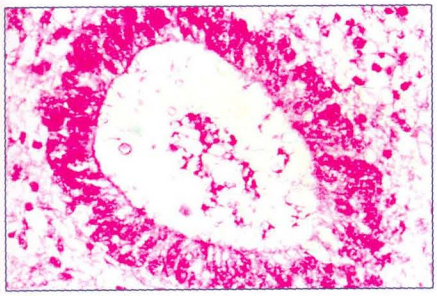
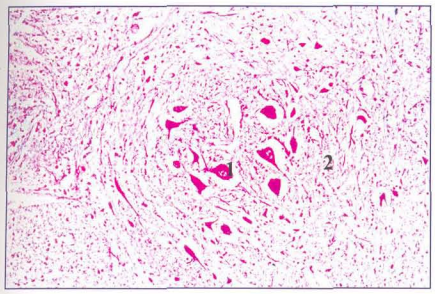
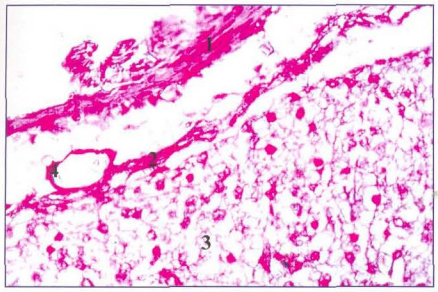


Fig. 204 Central canal at lumbar enlargement (102 days). Best's Carmine method x 400



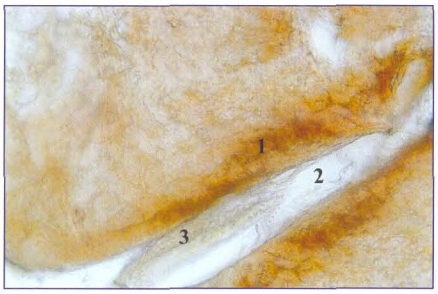
1. Neuron
2. Neuropile

Fig. 205 C.S. of L_3 segment (142 days). Best's Carmine method x 100



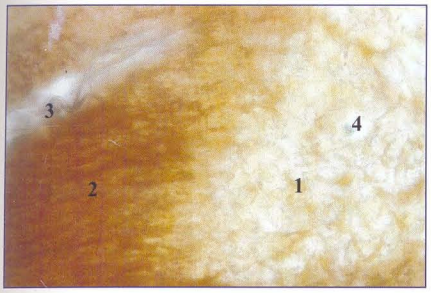
1. Dura mater
2. Pia-arachnoid
3. White matter
4. Blood vessels

Fig. 206 C.S. of L_4 segment (102 days). Best's Carmine method x 400



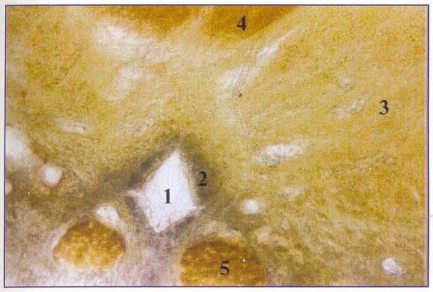
1. Tracts near the ventral median fissure
2. Ventral median fissure
3. Pia mater

Fig. 207 C.S. of C_1 segment (81 days). Oil red 'O' x 100



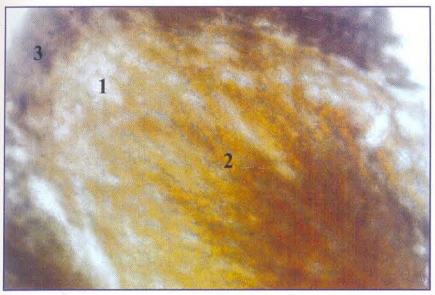
1. Gray matter
2. White matter
3. Pia mater
4. Blood vessel

Fig. 208 C.S. of C_1 segment (102 days). Oil red 'O' x 100



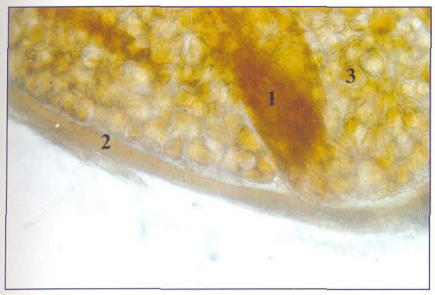
1. Central canal
2. Ependymal cells
3. Gray matter
4. White matter
5. Medial longitudinal fasciculus

Fig. 209 Area surrounding central canal at C_1 segment (150 days). Oil red 'O' x 100



1. Lamina I
2. Lamina III fibres
3. Dorsolateral fasciculus of Lissauer

Fig. 210 Dorsal horn at C_1 segment (150 days). Oil red 'O' x 100

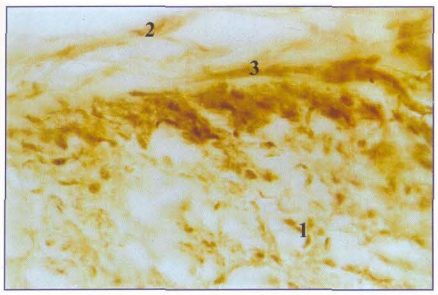


1. Myelinated fibres
2. Capsule
3. Ganglionic neurons

Fig. 211 Dorsal root ganglion at C_1 segment (150 days). Oil red 'O' x 100

1. Blood vessel
2. Glial cell nuclei

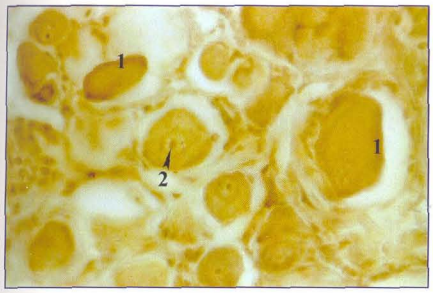
Fig. 212 Gray matter at C_1 segment showing alkaline phosphatase activity (102 days). Gomori's method x 400



1. Glial cell nuclei
2. Pia-arachnoid
3. External limiting membrane

Fig. 213 White matter at ventral funiculus showing alkaline phosphatase activity (102 days). Gomori's method x 400

Fig. 214 White matter (W) bordering ventral median fissure showing alkaline phosphatase activity (150 days). Gomori's method x 100



1. Ganglionic neurons
2. Nucleolus

Fig. 215 Dorsal root ganglion at C_1 segment showing alkaline phosphatase activity (150 days). Gomori's method x 400

1. Ganglionic neurons
2. Pigments

Fig. 216 Dorsal root ganglion at C_1 segment showing acid phosphatase activity (150 days). Gomori's method x 100

Discussion

5. DISCUSSION

The cellular thickening in the dorsal surface ectoderm, neural plate, concomitant with primitive streak regression marked the start of neurogenesis, which happened in the early embryonic life. The neural plate all along developed a median neural groove flanked by neural folds and subsequently formed the neural tube, the primordium of brain and spinal cord. The neuroepithelial cells of the neural tube wall were mitotically very active. Hence, the size of the neural tube increased rapidly especially at the cephalic end to form brain vesicles. The rest of the neural tube lying in the vertebral canal formed the spinal cord.

5.1 MORPHOLOGY

Like other ungulates, in goat also the spinal cord was an elongated structure, with the cervical and lumbar enlargements. In the present study, in goat, this morphology of the spinal cord was attained even by 24 days of gestation. Correspondingly, this was by 19 days in pig (Rugh, 1964), 21 days in cattle (Reddy, 1972) and by 25 days in man (Langman, 1981). Considering the total length of gestation, the present study showed that, this happened in foetal goat almost at the same time as in the pig, but at a later stage than in man and cattle.

The embryonic spinal cord all along its length was enveloped by meninges by 48 days, whereas it was during the 14^{th} week of gestation in man (Arey, 1957) and by 41 to 44 days in the dog (Engel and Draper, 1982b). So, the differentiation of meninges happened at an early stage in the goat foetus, than the other two.

Cross-sections through different levels of the cord revealed that the spinal cord had a greater transverse diameter than dorsoventral diameter in most of its segments, which is similar in the case of other domestic animals also. As in all other vertebrates, in goat also, the spinal cord presented the internally placed gray matter and external white matter. This description of the spinal cord is found true with descriptions given by the earlier workers in vertebrates (Papez, 1967) and in mammals (Clark, 1984).

The spinal cord exhibited variations in the gray-white matter relationships at different regions in the present study. The enlargements had characteristic laterally bayed out horns, but a narrow horn was seen in cervical, thoracic and lumbar levels. The amount of white matter was found to increase from caudal segments towards the

cervical level. The sacral region was characterized by its quadrangular shape. Caudally, the cord tapered abruptly to become conus medullaris, from which extended the filum terminale in the caudal part of vertebral canal. Kappers *et al.* (1967) opined that it is due to the increased demand of nerve fibres for nerve supply towards limbs, the two enlargements appeared in the spinal cord at corresponding levels. Jenkins (1978) also observed that these variations in the gray-white matter relationships are because of the variation in the size and number of fibres composing the spinal nerves, which attach to the cord at different levels in mammals.

Eventhough its formation started at the second month by 48 days as revealed by microscopic examination, a grossly distinguishable dorsal median sulcus and ventral median fissure could be seen all along the length of spinal cord only by fourth month of gestation. In the fifth month of gestation, the dorsolateral groove also could be distinguished. No similar groove was visible on the ventral aspect. The dorsal intermediate groove also could not be distinguished grossly even towards the end of gestation except in the cervical region. This morphology of the spinal cord concurs with the observations given by earlier workers in vertebrates (Papez, 1967) and in mammals (Kappers *et al.*, 1967; Jenkins, 1978; Clark, 1984).

Spinal cord exhibited well developed plexus of blood vessels on its surface by fifth month. The pia mater extended into all sulci, starting from second month onwards. The spinal cord was supplied by the ventral spinal artery and by the paired dorsolateral spinal arteries on the respective aspects. These vessels extended along the entire length of the cord. These findings are in accordance with the findings of Dyce *et al.* (1996), who described the spinal cord as being supplied by these three arteries that run its length. According to them, all three are periodically reinforced by branches from regional arteries, viz. vertebral in the neck and intercostals, lumbar and sacral arteries in the trunk. These entered the intervertebral foramina, accompanied the roots of the spinal nerves and formed plexuses on the surface of the cord with which the major longitudinal arteries connected.

From 48 days of gestation, the tooth-like lateral projections of pia mater, the dentate ligament, anchored the spinal cord in the dural tube. This projection extended from the level of foramen magnum to S4 segment of the cord in goat in the present

study, whereas it was only upto the L7 segment of the spinal cord with the cord terminating at the level of L7 vertebra in cat (Mc Clure *et al.*, 1973).

5.2 MORPHOMETRY

5.2. 1 Body Parameters

In the present study, the mean body weight showed a progressive increase during the gestation with a greater growth rate during fifth month and showed a positive and highly significant correlation with age, CRL straight and curved. A conspicuous increase of weight in comparison to other body parametres appears logical as it represents a sum of increase of all parts of the body including the viscera. So it is concluded that the maximum rate of growth in the present study was noticed in the fifth month. This observation is in accordance with observations of Majumder *et al.* (1986) in black Bengal goat. Mc Donald *et al.* (1988) also reported that the growth in foetal body weight exceeded the relative growth of all other characters during the last trimester of gestation in Australian foetal goat. But Ramkumar *et al.* (1988) reported the period of maximum growth during 60 to 120 days in foetal goat. This change may be attributed to the regional variation in the accessibility of maternal nutrition.

The present study showed that the rate of increase in body weight was very rapid during last three months of foetal age, indicating greater rate of growth during the later stages of gestation. This agreed with the findings of Roberts (1976) in ox, Mc Donald *et al.* (1988) in Australian foetal goat, and Jeetandra *et al.* (1995) in buffaloes.

CRL straight and curved and other body parameters also showed a progressive increase during the period of study and exhibited positive and highly significant correlation with body weight and age. Majumder *et al.* (1986) also observed that the CRL showed a positive correlation with increase of foetal age in black Bengal goat.

In the present study, CRL straight and curved showed little difference in their rate of increase. MC Donald *et al.* (1988) also found that there was little difference between the relative growth of CRL straight and curved, vertebral column length and thoracic circumference over trimesters of gestation.

5.2. 2 Vertebral Column

The vertebral column consisted of 7 cervical, 13 thoracic, 6 lumbar, 4 sacral and 12 to 15 coccygeal vertebrae. Chandna and Tyagi (1981) reported the vertebral formula of goat as C7 T13 L6 S4 and Cy 16-20. The minor change in the number of coccygeal vertebrae may be attributed to a regional variation among local breeds of goats.

5.2. 2. 1 Total Length of Vertebral Column

The total length of vertebral column showed a progressive increase during gestation and exhibited a positive and highly significant correlation with body weight, age and CRL straight and curved. The length of vertebral column was highly dependant on these parameters. It indicated a simultaneous growth of the axial skeleton relative to the foetal development. It also indicated that the vertebral column length can be a more satisfactory basis than CRL for studying foetal growth. Kadu and Kaikini (1987) reported similar observations in caprine foetuses.

5.2. 2. 2 Regional Length of Vertebral Column

In the present study, the thoracic region formed the longest region of the vertebral column, which substantiate the findings in domestic animals by Sisson and Grossman (1953). In the present study, the cervical region contributed more significantly to the length of precoccygeal vertebral column and total length of vertebral column in comparison with lumbar region. Rao and Tewari (1974) reported that the same was true in horse, cow, bull, ox, and buffalo, but the reverse was true in sow, dog and man.

5.2. 2. 3 Vertebral Length

The longest vertebra was C2 at all ages, in the present study. The minimum length in the cervical region was measured for C7. Similar observations are reported by Taluja and Shrivastava (1989) in goat foetuses.

Longest thoracic vertebra was T13, closely followed by T12 and T11 in all the four age groups. The lumbar region presented little variation between length of its component vertebrae. In the sacral region, S1 was the longest and S4 was the shortest. Taluja and Shrivastava (1989) also reported that these three thoracic vertebrae were the longest in the thoracic region.

5.2. 2. 4 Vertebral Canal Length

The vertebral canal extended from first cervical to fourth coccygeal vertebra. The vertebral canal length increased progressively during gestation and showed highly significant positive correlation with body weight, age, CRL straight, vertebral column length and spinal cord length during all stages of gestation, but it did not show any correlation with the coccygeal part of spinal cord. The relative contributions of the caudal vertebrae were ignored because after attaining the maximum proportional growth, the wave of growth alterered back to the loin region, which continued to grow later than any other part of the body.

In the present study, the rate of growth of vertebral canal length exceeded that of the spinal cord during all stages of growth, contributing to the cranial ascend of the spinal cord in the vertebral canal as age advanced. No data are available for comparison in goat foetuses.

5.2. 2. 5 Vertebral Canal Width

In the present study, in all age groups, the maximum width of vertebral canal was found at the level of atlas. Apart from atlas, the maximum width corresponded to the segments of vertebrae, which accommodated the enlarged segments of spinal cord. It was at C6 and L5 vertebrae in the second month and at C7 and L6 vertebrae in the following months. These observations confirmed the findings observed by earlier workers. Sisson and Grossman (1953) also reported that the greatest cross-sectional area was at the level of atlas. According to Rao and Tewari (1974) in ox and buffalo, apart from the atlas, the vertebral canal had the greatest cross-sectional area at the junction of C7 and T1 and at L6 vertebrae. They opined that the cross-sectional area correspondingly increased to accommodate the enlarged segments. No comparable data are available in foetal goat.

5.2. 3 Morphometry of Spinal Cord

5.2. 3. 1 Spinal Cord Weight

The mean spinal cord weight showed a progressive increase during gestation. The correlation between spinal cord weight with body weight of foetus, age, CRL straight and curved, vertebral column length and spinal cord length and volume were positive and highly significant. Spinal cord weight was highly dependant on age, body

weight and spinal cord length. It was more dependant on age, followed by spinal cord length and then by body weight. Hence it is indirectly interpreted that the spinal cord functions are more dependant on age in prenatal and probably in the postnatal life.

The percentage contribution of spinal cord to the body weight was more during early gestation. This parameter showed a decreasing trend upto fourth month, followed by an increase in the fifth month. According to Arey (1957), the spinal cord over grew brain during the postnatal years, increasing from 0.9 to 2 percent of the brain weight in man.

The present study showed a direct relationship between spinal cord weight and body weight. The spinal cord weight also had a positive and highly significant correlation with all body parameters measured. These nonsignificant correlations probably indicate that while one of the associated body measurements is increasing the other is relatively constant (viz. practically constant). This confirmed the finding of Latimer (1938) in cat and Taluja *et al.* (1989b) in goat foetuses, who also noticed a direct relationship between the spinal cord weight and body weight. But Sharma and Rao (1971b) could not report any relationship between the cord weight and other body parameters in buffalo.

Richardson and Hebert (1978) observed a linear relation between the cube root of organ weight and gestational age in ovine foetuses between 50 and 140 days of age and this linear relationship became sigmoid for the weight of the brain, cerebellum and spinal cord. In the present study, eventhough the relative growth of spinal cord weight and liver weight was linear, the cube root of weight of spinal cord and liver confirmed the findings of Richardson and Hebert (1978). This showed that, from the fifth month of gestation onwards, the spinal cord differed from other visceral organs in its rate of growth, showing a greater variability towards the end of gestation and probably during the postnatal growth also. The rate of increase in spinal cord weight according to age was more during fifth month.

5.2. 3. 2 Volume of Spinal Cord

The volume of the spinal cord showed a highly significant positive correlation with spinal cord weight, spinal cord length, body weight, age, CRL straight and vertebral column length. The spinal cord volume changed significantly with age, body weight and spinal cord length. The rate of increase was found to be more towards the

fifth month of gestation. Taluja *et al.* (1989b) also observed a progressive increase of the volume in goat foetuses according to age, confirming the observations of the present study.

5.2. 3. 3 Total Length of Spinal Cord

The spinal cord length was highly dependant on age, CRL straight and curved and body weight, with more dependence on age and CRL than body weight. The spinal cord length showed a highly significant and positive correlation with spinal cord weight and volume, vertebral canal length, vertebral column length, body weight, age, CRL straight, and curved and other body parameters measured in the present study. A direct relationship between the cord length and body length was reported in cat (Latimer, 1938; Kreig and Groat, 1944), in buffalo (Sharma and Rao, 1971b) and in foetal goat (Taluja and Shrivastava, 1982). But Greenaway *et al.* (2001), could not find any correlation between length of the spinal cord and age, weight and sex.

Sharma and Rao (1971b) also reported a linear correlation between spinal cord length and cranio-caudal axis length, body length, height and weight of the animal and cord weight. They also observed that the spinal cord length was highly dependant on these parameters. In the present study also, the spinal cord length showed significant change depending on a unit change in age, CRL straight and curved, body weight and other body parameters. This may be because of the increased number of spinal nerve fibres needed for supply towards the musculoskeletal system as the body grows.

Regression analysis showed that the rate of increase of spinal cord length in relation to age was exceeded by those of vertebral canal and vertebral column. This contributed to the cranial ascend of the caudal end of spinal cord within the vertrbral canal.

5.2. 3. 4 Regional Length of Spinal Cord

The maximum regional cord length was observed in the thoracic region in all groups followed by cervical, lumbar, sacral and coccygeal regions. This was in accordance with earlier reports in man (Lassek and Rasmussen, 1938), and in sheep (Ghazi and Gholami, 1993). In the central European hedgehog, Malinska *et al.* (1972)

reported that the coccygeal region was longer than the sacral region. Present study substantiated the findings of previous workers with regard to the maximum regional cord length in cow, horse, dog (Sisson and Grossman, 1953) and foetal goat (Taluja and Shrivastava, 1982). The regional thoracic cord length expressed as percentage of total length of spinal cord increased from second to fifth month of gestation.

Minimum mean regional cord length was recorded in the coccygeal region during all months of gestation. The regional coccygeal cord length expressed as percentage of total cord length increased upto third month. Thereafter, it decreased towards the end of gestation. This observation confirms the findings of Fletcher and Kitchell (1966), who stated that the general phenomenon of the vertebral column outgrowing the spinal cord in length is not a passive relative cranial migration of the cord in domestic animals, but is a non-uniform differential growth of specific spinal cord areas.

A highly significant positive correlation was observed between total cord length and length of different cord regions except coccygeal region. The cervical, thoracic and lumbar regions of spinal cord exhibited significant positive correlation and changed significantly with corresponding regional length of vertebral column.

5.2. 3. 4. 1 Cervical and Lumbar Enlargements of Spinal Cord

The last three cervical and first two thoracic segments formed the cervical enlargement where as the lumbar enlargement was formed by last three lumbar and first two sacral spinal cord segments. The length of cervical enlargement showed a lesser contribution to total length of spinal cord. Sharma and Rao (1971a) observed the changes in relative length of the spinal cord and the extent of enlargements in a number of domestic and laboratory animals and opined that the dynamics of the spinal cord is an active process connected with the functions of the limbs, viz. those animals using the forelimbs more effectively, have better developed cervical enlargement. They also stated that goat has a shorter cervical enlargement, which fits well into their feeding habits. The goats put their whole weight on their hind limbs, resting their forelimbs on the shrubs while they nibble the leaves. This is in contrast to the nature of buffaloes where it was proposed that there is even distribution of work load between the two pairs of limbs.

The cervical enlargement extended from C6 to T3 vetrebrae during the second month and from C6 to T2 vetrebrae towards the end of gestation. The lumbar enlargement extended from L4 to S2 vertebrae at second month. It was from L4 vertebra to the caudal end of L6 vertebra towards the end of gestation. This change in location was attributed to the slower growth rate of spinal cord when compared to that of vertebral column and vertebral canal as age advanced.

The length of these enlargements exhibited positive and highly significant correlation with body weight, age, CRL straight, vertebral column length and weight, volume and length of spinal cord, which suggested the relative development of the peripheral nervous system corresponding to the growing musculoskeletal system of the limbs.

5.2. 3. 5 Spinal Cord Segments

The foetuses under study had 36 pairs of spinal nerves: 8 cervical, 13 thoracic, 6 lumbar, 4 sacral and 5 coccygeal. Ghoshal (1975) reported that the number of spinal nerves varied depending on the number of the thoracic, lumbar and sacral vertebrae present in sheep and goat. According to Ulinski (1997), the distinct differences in the numbers of spinal cord segments between species, reflected individual morphology and suited the functional requirements of the animal. The size and internal structure of the spinal cord segments were related to the specialization of the post cranial body.

5.2. 3. 5. 1 Segment Length

In the present study, the longest cervical segment was C2 in all the age groups studied. It was closely followed by C3 in last three months of gestation. The serial number of longest segment in the present study was identical to that in buffalo (Sharma and Rao, 1971b) and goat foetuses (Taluja, *et al.,* 1983). According to Sharma *et al.* (1973) in adult goat and Hussain *et al.* (1990) in calves, the longest spinal cord segment was C3. Ghazi and Gholami (1993) opined that in sheep C1 was the longest in all ages except in new born lambs where the segment C3 was longest.

The shortest segment in cervical region fell among the segments of enlargements with the C8 forming the shortest in last three months whereas in 2nd month it was C6 and C7 in the present study. In calves Hussain *et al.* (1990) and in

sheep Rao (1990a) and Ghazi and Gholami (1993) reported C8 as the shortest cervical segment.

The segments of enlargements were among the shorter cord segments in the present study. This observation confirms the findings of Fletcher and Kitchell (1966) in dog.

The longest thoracic segment was T13 in the period studied except in the third month, where the segment T12 was the longest. T1, T2 and T3 were among the shortest segments in the thoracic region with T1 being the shortest in second, fourth and fifth month. In the lumbar region, L3 formed the longest except in the second month when L2 to L5 segments exhibited the greatest length. The shortest lumber segment was L6 in all these four groups. In the sacral region, S1 segment was the longest and S4 the shortest in all age groups. Rao (1990a) reported that in sheep, the longest cord segments were at C2, T13, L3 and S1 levels and the shortest were at C8, T1, L6 and S4 levels.

In the present study, the segment length gradually decreased after the level of S1 segment towards the last coccygeal segment in all age groups.

5.2. 3. 5. 2 Segment Width

Maximum width belonged to segments of cervical and lumbar enlargements during the period under study, with the C8 and L6 being the widest at cervical and lumbar enlargements respectively. The width of spinal cord segments decreased gradually after S1 segment to the coccygeal region. Dellmann and Mc Clure (1975) opined that the increased number of nerve cells and fibres meant for supply towards the limbs contributed to the formation of these enlargements.

5.2. 3. 5. 3 Segment Height

The maximum segment height also belonged to the segments of cervical and lumbar enlargements. It varied between different segments of these regions during gestation period with C8 being highest in third, C6 in fourth and C7 in fifth month. At lumbar enlargement, L5 and L6 formed the segments with maximum height at third month, and later L5 at fourth and L6 at fifth month. The segment height decreased from sacral region to coccygeal region.

The present study confirmed the findings of earlier workers who reported the location of greatest diameters of spinal cord at cervical and lumbar enlargement levels in horse (Dellmann and Mc Clure, 1975), in calves (Hussain *et al.*, 1990) and in sheep (Rao, 1990a). Parmar *et al.* (2000) reported the occurrence of minimum segment width and height in coccygeal part in calves.

5.2. 4 Allometric Growth of Spinal Cord and Vertebral Column

In the present study, the thoracic region exhibited an allometric growth rate above one in second and third month of gestation, indicating an increased growth rate of spinal cord over that of vertebral column, but in fourth and fifth month cervical region showed greater growth rate than thoracic region. In sheep, Ghazi and Gholami (1994) also showed that although vertebral column grew faster than the spinal cord, this was not true in all regions. They also found that the index of allometric growth was not constant in all regions of the spinal cord during prenatal and postnatal lives. According to them in sheep of all age groups, both cervical and thoracic regions exhibited growth rate over one, indicating a similar growth rate. In the postnatal life of camel Ghazi *et al.* (1998a) found that the allometric growth rate was highest in the thoracic region followed by cervical, lumbar, sacral and coccygeal regions.

Cervical and lumbar regions showed isometric growth rate during all stages of gestation in the present study. The sacral region was also isometric upto fourth month but there was a marked decline in growth rate of this region during the fifth month of gestation. In albino mouse, Sakla (1969) also found that the cervical region was the most rapidly lengthening region of the cord followed by sacral and lumbar regions.

A drastic change in the growth rate of the spinal cord segments in relation to vertebral segments occurred in the caudal part of spinal cord and the growth index decreased cranio-caudally in this study. A similar pattern was also reported in sheep (Ghazi and Gholami, 1994) and in camel (Ghazi *et al.*, 1998a).

The similar observations in dog by Fletcher and Kitchell (1966) and in buffalo by Sharma and Rao (1971b), the differential growth of spinal cord segments and vertebral bodies and the resultant differences in the root emergence pattern at various segments, indicated that the growth was an active process in determining the segment-vertebra relationship.

According to Ghazi *et al.* (1998 a), during a short period of early embryonic life the spinal cord grew at almost the same rate as the vertebral column thus displaying an isometric growth. This phase was of short duration since the adjacent vertebral segments increased in length more rapidly than the segments of spinal cord. The growth of the spinal cord segments in relation to respective vertebrae thus exhibited negative allometry, which continued even into adult life. This was found true in the present study also.

5.2. 5 Correspondence between Spinal Cord Segments and Vertebrae

All cord segments did not correspond to the transverse and vertical planes of the corresponding vertebrae in the present study. This substantiates the observations in cat (Krieg and Groat, 1944), dog (Fletcher and Kitchell, 1966) and adult buffalo (Sharma and Rao, 1971b).

In the present study, upto second month, the spinal cord extended the entire length of vertebral canal. In the third and fourth month, the cranial ascent was initiated and the lumbar region reached only upto middle of L6 vertebra. In the fifth month, lumbar region reached only upto caudal end of L5 vertebra. So a corresponding cranial ascent was noticed in sacral and coccygeal regions. This agrees with the findings of Kappers *et al.* (1967) who found that unlike the spinal cord of birds and reptiles, the mammalian spinal cord seldom extends through the entire length of the vertebral canal. But they opined that in early embryonic life, the cord occupies the whole extent of the canal. Later, due to unequal growth of the skeleton and the spinal cord there is a lack of correspondence between the length of vertebral canal and of the spinal cord so that in carnivores, the conus reached upto the end of lumbar column, while in ungulates it extended even to middle of sacral region. This feature well explains the recapitulation theory because in man it terminates at a much cranial level in the lumbar region.

At all stages of growth, the rate of growth of vertebral column exceeded that of the spinal cord in the present study. Barry (1956) also stated that eventhough in the early embryonic life the spinal cord extended upto the end of the vertebral canal, in subsequent prenatal and postnatal development, the growth of vertebral column was faster than that of the spinal cord, producing a relative shortening of the latter and an apparent cranial displacement of its segments. He also felt that during this

differentiation process the spinal cord played a relatively passive role and the vertebral column simply overgrew the cord.

In all age groups, C1 to C7 spinal cord segments lay within their corresponding vertebra. Due to the presence of C8 segment, cranial and middle thoracic segments upto T6 or T10 lay within the next vertebra. The caudal thoracic from T8 or T10 and cranial lumbar segments upto L3 or L4 again lay within their corresponding vertebra. This was in accordance with the observation of Hopkins (1935). Barry (1956) also divided the spinal cord segments in man into four regions based on changes in angulation of spinal nerves, but these regions differed from those described for domestic animals.

Hopkins (1935) identified four regions in terms of segments-vertebrae relationships in domestic animals. Cranial cervical region, caudal cervical to cranial thoracic regions, caudal thoracic to cranial lumbar region and remainder of the cord. In the first and third regions the segments lay within the corresponding vertebrae and in second and fourth regions segments lay cranial to the corresponding vertebrae. The same finding was reported in cat (Thomas and Combs, 1962), in monkey (Thomas and Combs, 1965) and in horse (Dellmann and Mc Clure, 1975). These observations concur with the present study also.

According to Dellmann and Mc Clure (1975) in cattle, the cervical segments were displaced cranially and all thoracic and first two lumbar segments were displaced caudally. The segment L3 was located at the corresponding vertebral level but there was cranial displacement for the rest of the segments. According to them, in sheep and goat, the condition was similar but the cranial displacement was not so marked as in cattle.

5.2. 6 Level of Termination of Spinal Cord

The spinal cord extended the entire length of the vertebral canal upto fourth coccygeal vertebra till 54 days of gestation. In the latter half of second month, it was at third coccygeal vertebra. By third month, the level was between first and second coccygeal vertebra. By fourth month, it varied from first coccygeal to fourth sacral. During fifth month, the level of termination varied from rostral end of S4 through middle of S3 to caudal end of S2. This rostral ascend may be due to the faster rate of the growth of the vertrebral column than the spinal cord at these respective stages of

prenatal growth. This partially agrees with the findings of Taluja and Shrivastava (1984) in foetal goat. They reported that the level of termination of spinal cord varied from caudal end of S4 to rostral end of Cy1 vertebra in the first trimester, caudal end of S3 to rostral end of S4 in second trimester and caudal end of S2 to rostral end of S3 in third trimester of gestation.

Sharma and Rao (1971b) observed that in buffalo the cord ended at third sacral and the level of termination did not vary from day-old to 16 years of age suggesting that the spinal cord attained its definite level of termination even at birth. As per earlier reports the level of termination in adult goat was at S3 (Sharma *et al.,* 1973) at S2 levels (Dellmann and Mc Clure, 1975 and Chandana and Tyagi, 1981).

In the present study, the level of termination of spinal cord in the goat foetus almost came close to that reported in adult goat, confirming the reports of Sharma and Rao (1971 b). The knowledge about the level of termination is important during the administration of epidural anaethesia to avoid injury to the spinal cord.

From the caudal end of the spinal cord a thin strand of pia mater extended caudally in the dural sac from 54 days of gestation. According to Bowsher (1967), the filum terminale was a strand of primitive neuroectoderm by which, the lower end of the spinal cord in man (opposite the second lumbar vertebra) was tethered to the back of the coccyx. The filum terminale extended caudal to the base of the tail in rabbit (Kozma *et al.,* 1974). According to Chandna and Tyagi (1981) in goat, the filum terminale extended upto second coccygeal vertebra.

5.2. 7 Spinal Nerves

There were 36 pairs of spinal nerves in the foetuses under study: 8 cervical, 13 thoracic, 6 lumbar, 4 sacral and 5 coccygeal. According to Ghoshal (1975), the number of spinal nerves varied depending on the thoracic, lumbar and sacral vertebrae present in sheep and goat.

5.2. 7. 1 Spinal Nerve Roots

Each spinal nerve was formed by two roots. The dorsal roots had a small number of large coarse, rootlets whereas the ventral roots consisted of many small rootlets with less tensile strength. This was in accordance with findings of Thomas and Combs (1962) in cat.

5.2. 7. 1. 1 Root Emergence Length

In the present study, the dorsal roots emerged after piercing the dura mater over a greater area than those of ventral roots. Histological observations of Sanomiya (1927) indicated that there were more of glial tissues and a greater number of nerve fibres in the dorsal roots, which might account for this gross difference. Root emergence length was slightly greater in cervical, rostral thoracic and lumbar region of the cord. It decreased through sacral region. Ventral root emergence length remained less variable in the thoracic region. This is in accordance with the findings of Sharma and Rao (1971a) in buffaloes and Sharma *et al.* (1973) in goats.

The greatest root emergence length was noticed dorsally at C8 in last two months of gestation. But ventrally it was at C1, C2, C3 and C8 at fourth month and C3 at fifth month. Greatest root emergence length were recorded at C3, T1, L4 and S1 segments in sheep (Rao, 1990a). This observation is identical with those recorded in thoracic and sacral regions in the present study.

5.2. 7. 1. 2 Root Attachment Length

In general, ventral roots originated over greater area when compared to corresponding dorsal roots. This is in accordance with the study by Thomas and Combs in cat and monkey (1962 and 1965 respectively) and Sharma and Rao (1971a) in buffalo. Sharma *et al.* (1973) reported that this observation was true in goats in prelumbar segments. In the present study, the lumbar and sacral segments presented ventral root attachment length either greater than or equal to those at the dorsal aspect.

The maximum root attachment length was associated with the longest spinal cord segment in the present study, C2 dorsally in fourth and fifth month. On ventral aspect it was at C3 in fourth and C2 at fifth month of gestation. The root attachment length decreased after L6 towards the coccygeal region. So there stands a relation between the root attachment length and segment length. Being greater in the longer ones and decreased in the shorter ones. So as stated by Sharma *et al.* (1973), the root attachment length contributes to the segment length.

The greatest root attachment length was recorded at C3 in dogs (Fletcher and Kitchell, 1966), at C2 through C3 in buffalo (Sharma and Rao, 1971a) and at C3 in goats (Sharma *et al.*, 1973) and sheep (Rao, 1990a).

Taluja *et al.* (1983) reported maximum RAL at C7 in all age groups in goat foetuses. Taluja *et al.* (1999) reported maximum RAL at C5, C7 and C8 in calves also. RAL increased from T11 to L1 in fourth month and from T8 to L1 in fifth month of gestation in the present study. Sharma *et al.* (1973) reported that this second increment of RAL lay at T11 to L1 dorsally at goats.

5.2. 7. 1. 3 Inter Root Length

Maximum inter root length was at C2 in fourth month and C3 in fifth month. Maximum IRL was recorded at C2 in goat foetuses (Taluja *et al.,* 1983), C3 in sheep (Rao, 1990a) and C2 in calves (Taluja *et al.,* 1999; Parmar *et al.,* 2000).

Cervical enlargement region showed a decrease in inter root length in both fourth and fifth month rarely with, no measurable distance at C8 segment. In the fifth month, lumbar enlargement region also showed a decrease in inter root length after L6. Longest inter root length was at T11 at fourth month and at T12 in fifth month. Minimum inter root length was at T1 segment. Similar pattern occurred in camel (Gholami *et al.,* 1998).

The segments of cervical and lumbar enlargements had shorter length for root attachment and shorter interval between adjacent roots than the segments in other regions of cord in the present study. This is found true with the findings of Fletcher and Kitchell (1966) in dog.

5. 2. 7. 2 Dorsal Root Ganglia

The dorsal root of each spinal nerve presented a swelling made up of sensory unipolar nerve cells and fibres as the dorsal root ganglion from 24 days of gestation onwards in the present study. Grossly, the shape of dorsal root ganglion was ovoid, or spindle shaped, with a size of the mustard or wheat seed. It was elongated distal to the caudal lumbar region upto second or third coccygeal segment. The size varied among the groups and regions of the spinal cord. These ganglia were situated at proximity to the dorsolateral aspect of the cord at the intervertebral foramina. The description is in accordance with that given by Taluja and Shrivastava (1988) in goat foetuses.

The ganglia did not present any grossly distinguishable difference in size and shape between regions upto second month of gestation. After the level of S3 segment, the length of ganglia decreased during second month of gestation. Later, the

maximum length of dorsal root ganglia was recorded at C8 segment. Sisson and Grossman (1953) also reported the presence of the largest one at the region of cervical enlargement in domestic animals. This may be attributed to the large amount of sensory fibres being carried by the enlarged segments of the spinal cord.

5.3 HISTOLOGY

During the first month, the primordium of spinal cord had the shape of a closed neural tube by 24 days, which was located between the body wall dorsally and notochord ventrally. The neural tube began to close by 19 days in pig (Rugh, 1964), 21 days in cattle (Reddy, 1972) and by 25 days in man (Langman, 1981). The present study showed that the neural tube closure happened in foetal goat more or less at the same time as in the pig, but at a later stage than in man and cattle.

Somites could be identified in the 14 mm embryo by 24 days, which became less distinct as age advanced. No comparable observation in goat foetus could be reviewed but this agrees with the findings of Gasser (1979) who found that during the period of somite break up in rat, the neural tube grew dorsally, away from the notochord, which lay adjacent to the ventral surface of the neural tube and the somite remnant moved laterally and dorsally.

Patten (1968) stated that all mammals showed the same developmental pattern and therefore a 5 mm pig embryo was an exact replica of a four week-old human embryo; and a 10 mm pig embryo was an equivalent of a human embryo of the sixth week.

5.3.1 Morphogenesis and Histogenesis of Spinal Cord in the First Month of Gestation

By 24 days of gestation, the 14 mm embryo presented three layers in the neural tube wall: an inner ependymal layer, a middle mantle layer and an outer marginal layer. So it made an early appearance when compared with the occurrence in dog but later than the occurrence in man and pig. The differentiation of these three layers occurred in the wall of neural tube in 9 mm embryos by 19 days in pig (Patten, 1948), by fourth week of gestation in man (Arey, 1957) and by 24 days of gestation in dog (Engel and Draper, 1982a).

Larsell (1951) observed that the arrangement of nuclei in the primitive epithelium made it possible to differentiate three zones or layers. Bohme (1988) opined that the three layers were formed by mitosis in the periluminal area, by migration of cells and by outgrowth of axons.

The lumen of the neural tube presented a lateral sulcus, the sulcus limitans, which divided the lateral walls or lateral plate into a dorsal alar plate and a ventral basal plate. Arey (1957) opined that the sulcus limitans appeared as a result of the proliferation of cells in the wall of the neural tube. So the presence of sulcus limitans at the anterior part of the spinal portion of the neural tube indicated a greater degree of proliferation and differentiation here.

The dorsal and ventral margins were thin and were formed by the roof plate and floor plate respectively. Towards the lumen, the neural tube was bounded by an internal limiting membrane and peripherally its extend was marked by an external limiting membrane. This agrees with the findings of Keith (1947) by the beginning of fifth week in man. Similar findings in pig embryos by 26 days of gestation were reported by Rugh (1964) and Huettner (1967).

Arey (1957) also opined that the internal and external limiting membranes marked the extent of spinal cord. Bruny and Reddy (1987) found that the radially directed processes formed from the base of many of the cells of the neuroepithelium and contributed to the formation of the external glial limiting membrane. Bohme (1988) also observed that while both the alar and basal plate were composed of multiple cell layers representing the developing gray matter of the spinal cord, the roof plate contained matrix (ependymal) cells only initially, the peripheral surface of which formed the glia limitans.

5.3.1.1 Ependymal Layer

The ependymal layer was a stratified layer composed of neuroepithelial cells by 24 days in the present study. The ependymal cells, began to undergo mitosis in pig embryos by 18 days and a middle mantle layer and an outer marginal layer got differentiated in 9 mm embryos by 19 days. So in the present study, the time of the differentiation of the three layers from the ependymal layer, corresponded to the same stage of gestation in pig.

In the present study, the individual cells were without any clear-cut outlines, at this stage. As reported by Patten (1948) in pig, the original cells proliferated very rapidly during the neural groove stage and immediately after closure of the tube. These cells lost their original clear-cut outlines later and merged into a syncytium. He also opined that eventhough earlier studies stated that the neuroepithelial cells merged into a syncytium, more recent studies suggested that in such material although the membranes became delicate and inconspicuous, if a well-preserved material was carefully studied, the cells could be seen to retain their membranes intact.

Among the ependymal cells, towards the internal limiting membrane, some spherical, pale staining cells with round nuclei were also seen, which represented the germinal or mitotic cells in the present study. This is in accordance with the reports of Patten (1948), who found that these cells were believed to be dividing at the time when the material was collected and fixed. Larsell (1951) reported that the rounded cells in mitotic division, the germinal cells, produced daughter cells, which crowded outward in the wall of the tube so that the wall soon showed many layers of nuclei. Thus, the ependymal zone included germinal cells and epitheloid cells whose nuclei lay near the lumen. In vertebrates, Shumway and Adamstone (1954) found that the neural tube originated from the neural plate, which arose from cells, which could be identified even in the blastula stage. De Lahunta (1983) also found that the cell membrane of each cell connected to both sides of the neural tube but nuclei were at different levels depending on the stage of mitosis. At interphase, the nuclei were located on the external surface of the tube and during mitosis it migrated to the luminal surface.

In the present study, the thickness of the wall of the neural tube increased as the age advanced. According to Jacobson and Tam (1982) in mouse embryos also, there was considerable increase in the volume of the neural plate during closure, and cell proliferation and enlargement of daughter cells seemed to account for this growth.

The ependymal layer increased in depth as it was followed from the floor plate to roof plate, whereas the mantle layer diminished in thickness as it passed into the alar plate. The ependymal layer was the prominent component of the roof plate and floor plate. The roof plate contained only of the ependymal layer, whereas the floor plate was thicker than the roof plate and consisted of ependymal and marginal

layers but no mantle layer. A similar observation was made by Sturrock (1982b) in foetal rabbit. As per the reports of Sadler (2004) the roof and floor plates did not contain neuroblasts and served as pathways for nerve fibres crossing from one side to the other.

5.3.1.2 Mantle Layer

In the present study, the mantle layer was formed by 24 days. So, the differentiation of the mantle layer happened at an earlier stage than that of dog, in which, the cells from the ependymal layer migrated to form the mantle layer by 24 to 28 days (Engel and Draper, 1982a).

The mantle layer presented densely packed nuclei and showed two types of cells: the neuroblasts and spongioblasts. The neuroblasts had a large, round or ovoid vesicular nucleus with homogenous chromatin and faint nucleolus and had a paler cytoplasm than the spongioblasts. In the mantle layer, the smaller cells with round nuclei, which represented spongioblasts were more. Similar observations were made by Rugh (1964) in pig, Hannah and Nathaneil (1975) in rat and Langman (1981) in man.

Larsell (1951) also reported that the primitive epithelium gradually differentiated into spongioblasts and neuroblasts. According to Arey (1957) the neuroblasts are the precursors of neurons and the spongioblasts give rise to the neuroglia. According to Ulinski (1997) as neuronal precursors were generated during the early life of neural tube, they migrated radially into the walls of the neural tube, leading to an increase in its width.

Jenkins (1978) noticed that by proliferation and differentiation the ependymal cells gave rise to a thick middle mantle layer, which later developed nerve cells and neuroglia and formed the future gray matter.

During the first month, the maximum alar plate height was at thoracic region but the basal plate height was maximum at the cervical enlargement followed by cervical region. The mantle layer was thin in the alar plate and extended dorsally to the site of entry of the dorsal root. The basal plate was always thicker than the alar plate and bulged laterally to mark the beginning of the ventral horn. These changes

happened at a later stage than in man, as Arey (1957) reported these changes in embryos of 10 to 15 mm in man by fourth week.

By 27 days, the basal plate showed beginning of differentiation of neurons. So the basal plate differentiated earlier than the alar plate. Since, the basal plate is motor in function and the alar plate sensory, this explains the early development of motor activity in this species, which in turn is classified as early maturing animals having mature young ones. This agrees with the opinion of Harrison (1978) that the region of formation of neuroblasts was mainly in the ventral aspect of the neural tube, which began to bulge on either side so that the shape of the tube changed and the central canal became ventrally compressed.

Two types of neuroblasts could be identified by 27 days: a multipolar neuroblast with polygonal cell body and a bipolar neuroblast with elongated cell body. Ghosh (2002) also demonstrated that in animals at the beginning of development, the embryonic nerve cell or the neuroblast passed through a bipolar stage before reaching the multipolar stage. So the bipolar neuroblast represented an intermediate stage in the differentiation of multipolar neuroblast.

In the present study, the multipolar neuroblasts appeared for the first time by 27 days. So, the cellular differentiation started by the first month itself in the goat embryo. In pigs, Rugh (1964) noticed that the neurons appeared first at about 14 days from the neural ectoderm as neuroblasts that later formed 'proneurons' and finally permanent neurons. The differentiation of neurons started at a later stage in goat than that in pig.

The proneurons, formed a second layer beneath the spongioblasts in the present study and showed eosinophilic, pale cytoplasm and vesicular nucleus with faint nucleolus. The shape of the cell body varied from polygonal to elongated-shape. Hannah and Nathaniel (1975) reported that in rat, the neuroblasts at birth could be distinguished from other cellular elements by a round or ovoid nucleus with the homogenous nuclear chromatin and by paler cytoplasm which confirms the observations in this study.

5.3.1.3 Marginal Layer

The marginal layer formed the outermost zone and contained the processes or fibres arising from the neuroblasts in the mantle layer. This peripheral region generally lacked neuroblasts or cells in this first month, but occasionally spongioblasts were observed in the marginal layer especially at floor plate and at the entry and exit of nerve roots, representing the cells, which accompany their processes. Arey (1957) also opined that the marginal layer was a fibrous mesh and lacked cells in early months of gestation at fourth week.

The marginal layer started differentiation into dorsal, lateral and ventral funiculi in the present study, at an earlier stage when compared with that in dog as reported by Engel and Draper (1982a). They observed that the layer was differentiated in dog, only by 24 days, and by 28 days it became thicker.

In the first month, the marginal layer was absent at the dorsal aspect of the neural tube. Blood vessels entered from the periphery through marginal layer to reach the mantle layer and ependymal layer. Towards the end of first month, a fibrillar meshwork, the myelospongium was laid down by the better-developed cell processes. This concurs with the finding of Larsell (1951) who also found that the cell membranes separated the individual cells, many of which extended through the thickness of the tube wall. He observed that as the wall of the tube thickened, the outer portions of some of the cells became fibrous and a loose myelospongium resulted in the outer portions of the wall.

According to Kent (1969), many of the nerve fibres that grew into the marginal layer turned upward or downward in the cord or brain for long distances and synapsed with neurons elsewhere in the CNS. Jenkins (1978) also noticed that the axonal processes of the nerve cells in the mantle layer extended outward and formed the outermost, marginal layer, which formed the future white matter.

5.3.1.4 Central Canal

Gilbert (1997) opined that the neurulation process occurred by two ways: in primary neurulation, the chordamesoderm directed the ectoderm overlying it to form a hollow tube. In secondary neurulation, the neural tube arose from a solid cord of cells that sank into the embryo and subsequently cavitated to form a neural tube. In

mammals (e.g. mice and man) the head and trunk underwent primary neurulation and the region posterior to hind limb i.e.tail (at 35 somite level) followed secondary neurulation.

In the present study, the lumen of the neural tube was diamond-shaped in cross-section by 24 days in the anterior half of the spinal portion of the neural tube. Thereafter, the ventral one-fourth of the neural canal was in close apposition or even fused except in the lumbar enlargement region where the neural canal again became wider without any ventral fusion. In the caudal sacral region, the neural canal showed a wider upper (one-third) and narrow middle and lower portions. Harrison (1978) stated that as the ventral aspect of the neural tube began to bulge on either side, the shape of the tube changed and the lumen became compressed ventrally to become diamond-shaped in cross-section.

In coccygeal region, the canal was narrow and extended dorsoventrally. Even the caudal most portion of the tail showed an oval neural tube with a rounded lumen. This indicated that a patent and intact neural tube existed at the tail at this age.

5.3.1.5 Regional Differences

Ulinski (1997) reported that the spinal cord had an explicit segmental organization that was related to segmentation of the postcranial body. According to him there were distinct differences in the number of spinal cord segments between species that reflected individual morphology. Long animals had many spinal cord segments, while short animals have only a few. He also opined that the number of cord and body segments was directly related to the locomotor specializations of the animal, and closely related animals with different locomotor strategies had greater or smaller numbers of cord segments. The size and internal structure of the spinal cord segments were clearly related to specializations in the postcranial body. The cervical and lumbar regions of the cord showed distinct enlargements that reflected the accumulation of a greater number of neurons within the spinal cord at these levels. The number of neurons within a cord segment varied according to the size and character of the part of the body it served.

In 16mm embryos aged 27 days, the cranial one-fourth of the spinal portion of the neural tube showed the luminal surfaces of the lateral walls approaching towards each other both at dorsal and ventral aspects of the lumen, probably to start fusion to

effect the reduction in size of the lumen. The caudal three-fourth of neural tube showed the lumen with fusion only at the ventral part. It is according to the earlier reports by Jenkins (1978) that the differentiation of structures in the spinal cord proceeds from before backwards.

In the coccygeal region, cross-section of the neural tube was simpler in structure, and the neural tube wall consisted only of external and internal limiting membranes and the ependymal layer. Here, the thickness of the wall was essentially due to the ependymal zone. Roof plate and floor plate were thicker. Dorsal and ventral funiculi could be seen but were less distinguishable. Lateral funiculi were not seen. No distinguishable alar or basal plates and mantle layer could be identified. According to Arey (1957), the laggard differentiation of the tail in comparison to higher levels, was reflected in the less specialized spinal cord and somites. According to Hughes and Freeman (1974) the closure of the caudal end of the neural tube occurred after tail bud formation, in animals with normal length tails (rat and sheep). This observation is confirmed in the present study.

The enlargements of the spinal cord could be identified at regions corresponding to limb buds in 14 mm embryo by 24 days in the present study. Arey (1957) reported the presence of brachial plexus at a comparatively early stage, even in 10 mm pig embryo and the enlargements were well developed in 18 mm pig embryos by 26 days of gestation.

5.3.2 Morphogenesis and Histogenesis during Second to Fifth Month of Gestation

5.3.2.1 Morphogenesis

5.3.2.1.1 Morphogenesis in the Second Month

By the beginning of second month, the primordium of spinal cord presented the form of a neural tube. By the middle of second month, the changes were in progress and the dorsal and ventral parts of the lumen of the neural tube were obliterated by the apposition of the lateral plates. The basal plates had grown ventrolaterally and a ventral median fissure was formed leading to the development of the ventral funiculi in the ventral part of the marginal zone. Arey (1957) found that as the proliferative activity in the spinal cord reached its height, the rapidly thickening walls of the basal plates overlapped the laggard floor plate and lead to the formation of the ventral median fissure. According to the observations made in cat, Bohme

(1988) opined that the ventral median fissure was developed because the floor plate of the spinal cord hardly changed its thickness during development but ventral horns of gray matter extended ventrolaterally and defined the ventral fissure.

The spinal cord varied in size at different levels being enlarged at the cervical and lumbar enlargements and reduced at the conus, but its pattern of structure was uniform throughout its length. By the middle of the second month, the outer white matter and inner gray matter could be distinguished. Jenkins (1978) opined that the spinal cord retained the original neural tube relationship of mantle (gray matter) and marginal (white matter) layers during later stages of development also.

By 48 days, the developing spinal cord consisted of fibres, constituting the white matter and longitudinal column of cells forming the gray matter. The gray matter included numerous nerve fibres also, originating from its cells or terminating in relation to them. The white matter occurred in three longitudinal columns, viz. dorsal, ventral and lateral funiculi of nerve fibres, on each side, extending the length of the cord. In each side of the cord, the gray matter was divided into dorsal and ventral horns. In the thoracic, anterior lumbar and middle sacral levels, in addtion there was a lateral projection of gray matter on each side, the lateral horn. In the groove between the dorsal and ventral or lateral horns there were extensions of gray matter into the adjacent white matter, the two being intermingled as the reticular formation. Therefore, in the present study, the differentiation of gray matter happened by 48 days, evidently at a later stage than in man, where it was during the 11^{th} week of development, the mantle zone differentiated into dorsal and ventral horns (Keith, 1947).

According to Arey (1957) it was in embryos of 10 to 15 mm in man, that the mantle layer thickened on each side ventrolaterally to form the ventral horn, which in later stages supplied migrant cells that organized into a lateral horn and only the foetuses in the fourth month of gestation had their gray matter arranged in the permanent form. Buchanan (1957) opined that the relationship between the gray and white matter in the adult spinal cord corresponded to that of the mantle and marginal layers in the developing neural tube. De Lahunta (1983) described the spinal cord development as the best example of the symmetric development of the neural tube by layers.

As the stage of the neural tube terminated between 40 and 48 days in the present study, the dorsal funiculi were being formed in the dorsal marginal zone by the middle of second month. The dorsal halves of either side started to fuse. The dorsal median sulcus and dorsal median septum started to extend between the right and left dorsal funiculi developing on each side of the original roof plate, but still there was a gap between the two dorsal halves of the spinal cord indicating that the fusion between the dorsal halves of spinal cord was not complete. Aggregations of the neuroepithelial cells in the ependymal zone of approaching walls of the lumen were also seen on either side of dorsal midline above the central canal. The septum was formed of astrocytes and a few oligodendrocytes. Larsell (1951) and Arey (1957) found that as alar plates thickened, the roof plate was obliterated and facing ependymal layers united into the dorsal median septum.

The ventral commissure, connected the two halves of the spinal cord ventrally. The pia mater, which descended into ventral median fissure presented well developed blood vessels including the ventral spinal artery by 54 days. Keith (1947) reported that the dorsal median sulcus and ventral median fissure developed at an earlier stage, viz. in the seventh week in man. Engel and Draper (1982b) found that it was by 41 to 44 days, after the middle of gestation in dogs, the ventral median fissure was distinct and reached the oval central canal and a less developed dorsal median septum was also formed. Dyce *et al.* (1996) reported that the branches of the ventral spinal artery supplied the 'core' of the spinal cord, the gray matter and the adjacent layer of white matter by its approach through the ventral median fissure.

In the present study by the middle of second month, the walls of the neural tube became progressively thicker. This is in accordance with the reports of Larsell (1951) and Arey (1957), who found that during later stages of development, thickening of the wall of the spinal portion of the neural tube happened by migration of spongioblasts and neuroblasts proliferating in the ependymal zone and by mitosis in the mantle zone. Coincidental with this growth came a relative narrowing and reduced extent of the central canal.

By the end of the second month, there was a reduction in the size of the central canal. This was not caused by a simple fusion of opposite walls but by displacement of cells of the ependymal layer from the lumen to the dorsal gray

commissure and by replacement of the migrated cells by adjoining cells of the ependymal layer. Keith (1947) reported that it was in the in the ninth week in man, the dorsal part of the central canal was obliterated by the apposition of the lateral plates, the ependymal zone reduced, the dorsal funiculi and dorsal median septum were formed. Bohme (1988) opined that the proliferation in the wall of the neural tube, obliteration of the lumen and formation of the dorsal septum signalled the beginning of development of the spinal cord, which occurred in cat during 24 to 58 days of gestation.

5.3.2.1.2 Morphogenesis in the Third Month

By the third month, the dorsal median sulcus was shallow at the caudal lumbar and sacral region and was absent at the coccygeal region. The septum exhibited thin fibres also and at the caudal sacral region, the septum was absent. The absence of these structures from the sacral region onwards may be attributed to the lesser differentiation towards the caudal end of the spinal cord.

5.3.2.1.3 Morphogenesis in the Fourth Month

From fourth month onwards, by 102 days, the basic structure of spinal cord in the present study corresponded to the adult structure described in animals by several workers (Kappers *et al.*, 1967; Papez, 1967; Dellmann and Mc Clure, 1975; Jenkins, 1978; Clark, 1984; King, 1987). From this age onwards, the central canal presented an oval shape. Immediately surrounding the central canal was a light granular area composed mainly of neuroglia, the central gelatinous substance (substantia gliosa).

According to Truex and Carpenter (1969), the ependymal cells lining the central canal and the subjacent glial tissues constitute a potential barrier between the cerebrospinal fluid (CSF) and the interstitial fluid of the brain, forming the brain – CSF barrier. They opined that these structural elements pose little obstruction to the passage of fluids between the CSF and extracellular compartments. Therefore, substances, injected into the CSF, can gain access to the interstitial fluid and then to the neuroglia and neurons of the brain. The presence of these structures in the spinal cord indicate that probably, a similar structure may be present in the cord also during the developmental stages, when there was a patent central canal.

A dorsolateral groove and gray and white commissures were identified and the adult form was reached in the fourth month, obviously, at a later stage than that in man. Keith (1948) observed that the adult form was reached in the 13th week in man. But here after, the differentiation was at a faster rate that the multipolar neurons took their stations and attained Nissl bodies, which was indicative of an early onset of activity making the kid a completely developed animal at birth.

Ghosh (2002) found that gradually the diamond-shaped canal became triangular in outline during development in animals and the differentiation of structures in the spinal cord proceeded from before backwards and this sequence of events were almost the same in all domestic animals. He also opined that the transformation of the embryonic organization into that of the mature spinal cord resulted from massive proliferation, asymmetric movement of immature neurons and development of neuronal processes.

5.3.2.1.4 Morphogenesis in the Fifth Month

By the fifth month, the central canal varied in shape from round to ovoid. The dorsal median septum was well organized and extended into the clearly distinguishable gray commissure by the fifth month. Blood vessels from pia mater descended into the dorsal median septum and sulcus. This description of the spinal cord is in accordance with the observations made by Kelly *et al.* (1984) in man.

5.3.2.1.5 General Micrometrical Observations

The cervical enlargement had the maximum height during the entire gestation followed by the lumbar enlargement for the major part of gestation. The gray matter height and dorsal horn height was also maximum at cervical enlargement during greater part of gestation, but by the fifth month, it was at lumbar enlargement. The maximum ventral horn height was also seen at the enlargements with the lumbar enlargement predominating during the major part of the gestation. All these values decreased towards the coccygeal region. Taluja *et al.* (1990) also found that the height of spinal cord was maximum at cervical enlargement in goat foetuses. This increased size was attributed to the increased number of nerve fibres originating for supply towards the limbs.

In dogs, the mean ratio of gray matter diameter to spinal cord diameter showed a decreasing trend as the age advanced from 24 days (Engel and Draper, 1982 a) to 44 days of gestation (Engel and Draper, 1982b). In the present study also, sparing a few exceptions, the percentage contribution of gray matter height, dorsal horn height and ventral horn height to the total spinal cord height showed a gradual decrease indicating a corresponding increase in the white matter towards the end of gestation due to the development of fibre tracts. The percentage of gray matter width decreased after third month of gestation at all regions indicating a comparative increase in the growth of lateral funiculus at later stages of gestation. Vertical gray matter percentage started to decrease at an earlier stage indicating early growth of ventral funiculus.

The gray matter height expressed as percentage of spinal cord height was maximum at lumbar enlargement from third to fifth month indicating a dominance of gray matter at the lumbar enlargement, which in turn relates to the increased need for use of the hindlimbs by goat. The ventral horn height as percentage of gray matter total height was more than that of dorsal horn at lumbar enlargement, indicating the dominance of the motor component. Instead, at the cervical enlargement, the dorsal horn contribution was more indicating a comparative increase in the sensory component.

The greatest values for total width of spinal cord and distance from central canal to spinal cord left margin varied between the enlargements at different stages of gestation but at fourth and fifth month, the values at cervical enlargement exceeded that of lumbar enlargement, indicating the dominance of the total width at the cervical enlargement during later stages of gestation. The distance from central canal to gray matter left margin was also more at the enlargements. The spinal cord width was maximum at L6 segment during second and third month but it was at C8 during fourth and fifth month. In goat foetuses, Taluja *et al.* (1990) also found that the width was greatest at the enlargements, which might be due to the difference in size of the nerve roots and the relative quantum of gray matter.

The total width of spinal cord was greater than the height at all levels studied. This partially concurs with the observation in goat foetuses by Taluja *et al.* (1990) who also found that the transverse distance was greater than the vertical distance at all the levels except in the cervical region in the first trimester of gestation.

A greater rate of growth in spinal cord height was seen during the latter half of gestation in all regions. This partially agrees with the observation in goat foetuses by Taluja *et al.* (1990) who found a greater rate of increase for the height and other vertical distances in later half of gestation in all regions except at L5 segment. In the present study, the spinal cord width at sacral region showed a decreasing growth rate towards the end of gestation indicating the dedifferentiation towards the caudal end.

5.3.2.2 Histogenesis

5.3.2.2.1 Neurons

By the beginning of the second month, the multipolar neurons first appeared in the ventral horn. This may account for the fact that the motor activity develops earlier than the sensory perceptions and confirms the opinion of Jenkins (1978) that in mammals the ventral horn differentiated before dorsal horn. He opined that in each lateral plate the neuroblasts became grouped from ventral to dorsal horn into: somatic motor, splanchnic motor (both in the basal plate), splanchnic sensory and somatic sensory (both in the alar plate). Sims and Vaughn (1979) found that in cervical spinal cord of mouse also, the ventral motor neurons were the first neurons generated, which developed by the embryonic day 8.8. Interneurons and dorsal root ganglionic neurons developed by 9.5 days. According to Bohme (1988) also, initially proliferations in the basal plate prevailed over those of the alar plate in cat.

Larsell (1951) reported that a young neuron could be recognized with the formation of processes and neurofibrillae. According to Sturrock (1983) it was very difficult to differentiate between astrocytes and differentiating neurons in the (dorsal horn) gray matter. In the present study also, the smaller neurons were very difficult to be differentiated from astrocytes confirming the earlier reports.

Ulinski (1997) found that the shape of the adult brain and spinal cord was determined by the spatial and temporal pattern of cell divisions in the neural tube. The migration of the neuronal precursors or neuroblasts resulted in diffuse distribution of neurons in the wall of the neural tube or in definable aggregations as nuclei or as sheets or layers of cells. In the present study, by the middle of second month, as the dorsal horn presented neurons with indistinct cell boundaries, the ventral horn neurons formed aggregations and were better differentiated.

Eventhough the neurons of both dorsal and ventral horns were better differentiated by the third month of gestation, the nerve cell boundaries became clear only by fourth month. All the neurons of spinal cord including the large alpha and small gamma neurons of ventral horn exhibited increased cytoplasmic content by the beginning of fifth month. By the fifth month, typical multipolar neurons with well developed Nissl bodies were seen. Taluja and Shrivastava (1988b) also stated that in goat foetuses, the cytoarchitecture of neurons was indistinct upto fourth month of gestation.

The Nissl bodies became better developed in all the neurons of the spinal cord by the beginning of the fifth month. According to Jenkins (1978), the Nissl bodies are concerned with protein synthesis and the replacement of neuroplasm and there is decrease in number of Nissl bodies as a result of physiological changes. De Lahunta (1983) found that the neurons stop dividing as they acquire functional maturity. It is called the birthdate of neuron. According to him, the neurogenic cells in the ventral part of the spinal cord and hind brain are the first to stop dividing, with the birth dates of dorsal and lateral horns following. In dog, cortical neurons stop proliferation by third to fourth month of postnatal life and by three years in man. In precocial animals such as foal and calf, most cortical neurons are formed by birth. Taluja and Shrivastava (1988b) stated that deep purplish Nissl substance was characteristic of neurons in all regions of the cord in last trimester of gestation of goat foetuses. As per these earlier reports, it is probable that there is a relation between the functional maturity of neurons and the presence of Nissl bodies. If so, the birth date of neurons in the present study was attained by fifth month of gestation, evidently at an earlier stage than dog and man and this also may account for the presence of better developed reflex activities in the new born kid.

In the present study, the shape of the neurons varied according to their specific location. Most of the neurons were multipolar, but some were spindle-shaped or spherical in shape especially in the lateral horn. According to Jenkins (1978) also, the multipolar neuron is the most common type of neuron found within the central nervous system of higher mammals. He also opined that the polarity of the neuron is designated according to the number of its dendrites, and the most outstanding physiological advantage of the multipolar neuron is that the many dendrites permit

more axodendritic synapses. This may account for the well developed ability of the spinal cord to mediate reflex actions.

Buchanan (1957) also stated that most of the neurons in the CNS were multipolar possessing an axon and two or more dendrites. Those with long axons were designated as Golgi type I and those with short and freely branching axons were classified as Golgi type II. Banks (1981) stated that in domestic animals, neurons varied in size, shape and number and length of the processes. Spindle-shaped ones were bipolar neurons and were seen in areas of special visceral and somatic sensation (Banks, 1981; King, 1987). This confirms the occurrence of spindle-shaped neurons at the lateral horn in the present study, which is thought to be the location of autonomic motor neurons.

Eventhough the nucleus was centrally positioned in most of the neurons, a characteristic eccentric nucleus was found in the Clark's column by the fifth month. Kelly *et al.* (1984) also reported the presence of this feature in the cells of Clark's column. Banks (1981) also stated that the neurons were with a centrally placed, round large vesicular nucleus (3 to18 μm in diameter) and a prominent nucleolus.

The neurons in the present study had a size of 3.8 μm to 5.6 μm in the neuroblastic stage by the second month and attained a maximum width of 75 μm at the cervical and lumbar enlargements during the fifth month. According to Jenkins (1978), neurons vary in size probably more than any other type of cell in the body. Banks (1981) stated that the neuron cell body varied from four to135 μm in diameter in domestic animals.

The number of neuroblastic cells per unit area was more during the first month ranging between 93 and 105, which decreased to 5 to 6 alpha neurons per unit area towards the end of gestation. Eventhough the number of neurons per unit area decreased, the cells were increased in size and were much more differentiated. The total size of the cord also increased towards the end of gestation. So, the growth of the spinal cord in the present study, meant the better differentiation of individual cells and the increase in the total size of the cord. Montagna (1960) opined that the number of neurons in an individual species is relatively constant and does not increase after late embryonic life. Dropp (1972) could not detect any mast cell in the spinal cord. This observation was confirmed in the present study.

The number and size of the neurons were more at the enlargements. This concurs with the reports of Fletcher (1993), who opined that the neurons assumed a variety of shapes and sizes related to their function, whereas the size of the cell body was related to that of its processes. According to Ulinski (1997), the number of neurons in a cord segment varied as per the size and character of the part of the body it supplied. Peterson (1966) confirmed that there is an inverse relation between neuronal size and firing rates and between size and the rate of protein synthesis per square micron of cytoplasm with a direct relation between the rate of firing and that of protein synthesis. There was also an inverse relation between neuronal size and the ratio of the neuronal surface area to volume.

According Larsell (1951), the nerve cells of the gray matter were multipolar and ranged in size from neurons of small size to the large motor neurons of the ventral column, some of which were among the largest cells in the body. Many of these cells of the dorsal column also attained a considerable size. The cells of the ventral and lateral horns gave rise to the motor root fibres of the spinal nerves and to the preganglionic sympathetic fibres respectively and were frequently called the ventral horn cells. The remaining nerve cells sent their fibres to various parts of the central nervous system either on the same or the opposite side. Most of these fibres passed into the white matter, where they formed fibre tracts to the brain or connected different levels of the cord. There were also cells of Golgi type II, which gave rise to axons that terminated in the gray matter of the same or the opposite side, within the same segment of cord.

5.3.2.2.2 Neuroglia

In addition to the various types of neurons, the spinal cord contained the interstitial cells called as the neuroglia. Four types of neuroglia could be identified towards the end of gestation as astrocytes, oligodendrocytes, microglia and ependyma. According to Truex and Carpenter (1969) neuroglia were classified as macroglia (ie., astrocytes and oligodendrocytes), ependyma and microglia.

Similar supportive cells were present in all vertebrates and some of the invertebrates (Coggeshall and Fawcett, 1964). Jenkins (1978), stated that it is erroneous to consider them as true connective tissue cells because all neuroglia, which

with the exception of one type were formed from ectoderm, whereas the connective tissue cells were derived from mesoderm. He also opined that the neuroglia are not merely the supportive cells of the central nervous system, but, they also act as regulators of the chemical milieu of the nerve cells and therefore as important controllers of neuronal metabolism.

Fletcher (1993) stated that neuroglial cells were relatively small; and with routine stains only their nuclei and perikarya were evident. This finding is confirmed in the present study.

According to Arey (1957), the astrocytes first appeared in the third month in man. The oligodendrocytes developed at a later period than astrocytes. In man, Rugh (1964) also observed that the neuroglia were abundant by ten weeks. As per the reports of Sturrock (1982a), spongioblasts were seen as cells with dark nucleus and scanty cytoplasm in the developing gray matter from 12 days of gestation in rabbit. In the present study, differentiating astrocytes and oligodendrocytes could be identified by 40 days. So the differentiation occurred almost at the same time as that in man.

Processes of all the developing cells made up the neuropile from second month onwards. As the vascularity increased according to advancement of age, more neuroglia were seen as perivascular satellites. By third month, the neuroglia became more distinguishable and the density of the cells increased as the age advanced. The astrocytes had a size of 1.8 μm in white matter and 3.6 μm in gray matter. Sturrock (1982a) opined that the differences in orientation were probably responsible for the differences in glial nuclear diameter between the gray and white matter. From fourth month onwards, the cell density was more.

In the present study at all ages, the number of neuroglia were more than that of the developing neurons. Bowsher (1967) stated that within the CNS, the neuroglia outnumbered neurons in the ratio of about 10:1 in vertebrates. According toTruex and Carpenter (1969) the fact that the neuroglial elements may comprise almost half the total volume of the human brain indicated their important and specialized role.

By the beginning of second month, among neuroglia the astrocytes had larger and paler nucleus than oligodendrocytes. Protoplasmic astrocytes were predominantly seen in gray matter. Fibrous astrocytes were predominant in white matter but were also seen towards the periphery of the gray matter. From the middle of the second

month onwards, astrocytic nuclei could be seen as pale stained and ovoid. The astrocytes were the largest among neuroglia upto the end of the gestation. Jenkins (1978) found that these were the largest and most elaborate of all the glial elements forming most of the 'packing tissue' of the neuropile of the nervous system and formed the blood-brain barrier in mammals. It was also reported that as a result of an injury to brain or spinal cord, astrocytes tend to multiply and enlarge. The result of this proliferation is called the 'gliosis' and the culmination of the process is the 'glial scar', which close any gaps caused by loss of cells or myelin. Banks (1981) noticed that the astrocytes are responsible for the repair of the nervous system defects and these cells can become hypertrophic, hyperplastic and phagocytic. They also serve to isolate neuronal receptor surfaces by serving an insulating function.

It was noticed that in routine staining, astrocytes were recognized by a large pale round nucleus, which was the largest among glial nuclei and with silver stains, astrocytes were seen to possess numerous processes. These findings concur with the observations of Fletcher (1993) in domestic animals.

Cox (1977) reported that the nuclei of astrocytes had a diameter of 8 to 10 μm in adult nervous system. In the present study, the developing nervous system presented a nuclear size of 5.4 to 7.2 μm for the astrocytes. The smaller size may be attributed to a change in the developing stage.

The astrocytes had perivascular feet and some of their processes formed a superficial glial limiting membrane beneath the pia mater of the cord. This concurs with the reports of Fielder and Drommer (1976) in pig who stated that the membrane consisted mainly of fibrous astrocytes and was with a continuous basement membrane on its surface, which also was found true in the present study. According to King (1987), astrocytes formed a continuous single cell layer immediately beneath the pia mater, which covered nearly all (about 85 per cent) of the basal plate of all blood vessels in the CNS by means of their perivascular feet and got fused with the ependymal cells. In cat spinal cord, Uehara and Ueshima (1988) described that this membrane consisted of fibrous astocytes and their processes were less complex than those in the parietal cortex and contained foot processes, consisting of end-feet, ascending processes and cylindrical processes. They also reported that the end–feet had numerous fine processes and some long, unbranched processes.

The dorsal median septum was also formed mostly of astrocytes and a few oligodendrocytes. This is in accordance with the findings of Fletcher (1993) who opined that in addition to forming the glial limiting membrane, the end feet of astrocytes formed the septa in spinal cord. According to Peplow (2004), the astrocytes if bound with glutamate were capable of affecting the communication between neurons.

In the mantle layer, oligodendrocytes were seen by the beginning of the second month. Among neuroglia, oligodendrocytes predominated and resembled the developing spongioblasts by the middle of the second month. Oligodendrocytes occurred as three types, viz. perineuronal satellites in gray matter, interfascicular cells in white matter and juxtavascular cells near blood vessels. Similar reports are made by Banks (1981), who opined that the perineuronal oligodendrocytes may perform some type of nutritive function.

Truex and Carpenter (1969) described the oligodendrocytes as the smallest among macroglia cells, numerous at birth and becoming less conspicuous in the adult brain and spinal cord. In contrary, Dekaban (1956) described the oligodendrocytes as the most numerous cells in the nervous system of adult mammals.

In the present study, other oligodendrocytes with definite cell boundaries without forming the myelin sheath were also seen associated with nerve tracts as interfascicular cells and as free cells in white matter. Jenkins (1978) opined that the location of oligodendrocytes associated with nerve tracts gives a picture analogous to that of the neurilemmal cells of the peripheral nervous system.

In the marginal layer cell nuclei were also seen in the second month. According to Sturrock (1982a), cells with dark nucleus and scanty cytoplasm which represented neuroglia were present in the developing white matter from 14 days in rabbit. From third month onwards, in the present study, the glial cells in the white matter became better differentiated with a clear distinction between fibrous and protoplasmic astrocytes. Oligodendrocytes started to curve around axons to form myelin sheath towards the end of gestation. These observations concur with the earlier reports by Jenkins (1978). He also stated that oligodendrocytes gave rise to myelin in the CNS forming myelin sheath around more than one axon at a time. This was not observed in the present study owing to a change during the developing stages.

According to Fletcher (1993) also, a single oligodendrocyte was known to contribute myelin sheaths to about 50 myelinated axons.

Oligodendrocytes were with round nucleus and scanty cytoplasm. This is in accordance with the reports of Cox (1977) and Banks (1981). Cox (1977) also reported that the nucleus of these cells was smaller than that of the astrocyte with a diameter of 7 μm in the adult nervous system. In the present study, the nuclear size reached only upto 3.6 to 5.4 μm owing to the occurrence of a smaller sized nucleus in the developing nervous system.

The microglia were the last type of neuroglial cells to develop in the present study and were detected only from fourth month onwards. Harrison (1978) also reported that these were the final type of neuroglial cell to develop in the mammalian neural tube as the blood vessels grew into the neural tube. They opined that these cells developed from the adventitia of the blood vessels and were therefore, mesodermal in origin in contrary to other neuroglia, which were ectodermal in origin.

Penfield (1932) described their process of development as "fountains" of microglia budding off from blood vessels. These act as scavengers of the central nervous system being able to phagocytose products of the disintegration of neurons following injury. Jenkins (1978) also opined that since microglia act as a counterpart of the macrophages of other parts of the body, they may be considered as true members of the reticuloendothelial system. Sturrock (1982a) demonstrated microglial cells at 14 days in rabbit.

The microglia were smaller and similar to oligodendrocytes and occurred both in white and gray matter being more in the latter in the present study. This was in accordance with the findings of Jenkins (1978) and Banks (1981). Fletcher (1993) noticed that the microglial cells had antigen-presenting and phagocytic capabilities. They are sparce and difficult to be found in the normal tissue.

The ependymal cells around the central canal formed a stratified layer by the middle of second month. In adult vertebrates, the ependymal cells represented some of the germinal cells, which were left behind in the germinal layer and formed the non-nervous lining of the lumen (Huettner, 1967; Banks 1981). Kent (1969) noticed that in cyclostomes, the ependyma was the only type of neuroglia found, but in higher vertebrates, the neuroglial cells became increasingly abundant and diversified.

Ependymal cells developed cilia from the middle of second month onwards. Banks (1981) reported the embryonic ependyma as ciliated low cuboidal or low columnar cells. The latter form was retained in the adult. In the adult, the basal borders resided on a basement membrane and were separated from the nervous tissue. He also found that the ciliated ependymal cells were responsible for the movement of cerebrospinal fluid within the ventricular system. According to him, the cerebrospinal fluid and thus the ependymal cells, which help to form this modified transudate may be involved in the transport of hormones.

Processes arose from the basal ends of ependymal cells and extended through the gray matter in the present study, in accordance with the reports of Kingsley (1962) and Banks (1981) who found that the basal modifications and cellular processes extended through the mantle layer of the developing nervous tissue in younger animals.

From fourth month onwards, the ependymal cells formed a ciliated pseudostratified layer. The height of the cilia varied greatly. The cilia were very short or almost absent upto lumbar region by the end of gestation, but were present at sacral and caudal levels. Fletcher (1993) found that zonulae adherentes near the luminal border joined the ependymal cells and the luminal surface of each cell had microvilli and numerous motile cilia. Large molecules from the extracellular space of the central nervous system passed between the ependymal cells to reach cerebrospinal fluid (CSF). At certain sites neuron processes extended between the ependymal cells to contact CSF serving a receptive function. Rajtova and Kokardova (1998) found that the external morphology of ependymal cells was independent of their developmental stage, resembling that of postnatal ependymocytes in sheep and goat foetuses. The surfaces of proximal apical membranes of ependymal cells were covered with cilia, microvilli and tiny spherical protrusions of secretion.

Kelly *et al.* (1984) observed that the ependyma consisted of cells lining the cavities of the spinal cord and brain. He opined that their long axes were perpendicular to the cavity and although they appeared as columnar or cuboidal epithelium, this was a false impression. Ependymal cells are derived as the most apical cells of the neural tube epithelium. In the adult therefore, they were closely adherent cell bodies lining the apical (ventricular) surface of the CNS epithelium. The

basal surface was the outer perimeter of the brain and spinal cord. These cells had inner processes ramifying deeply into the epithelial walls of the CNS and sometimes providing end feet to the outer glia limitans or to perivascular areas. Ependymal processes were not unlike those of fibrous astrocytes in their fine structure. They may have, in certain forms and in certain places cilia, which protrude into the lumen.

5.3.2.2.3 Gray Matter

The gray matter extended the length of the cord, as a mass of nerve cells and fibres with neuroglial elements and blood vessels. By the fifth month, the neuropile network, consisted of nonmyelinated axons, oligodendrocytes, astrocytes and the neuroglial processes.

In the present study, the tubular form of the primordium (neural tube) extended upto the middle of the second month. The gray matter became arranged as horns from this age onwards. With the formation of dorsal funiculi, the gray matter of dorsal alar plate, at first united by the roof plate, became widely separated to form the dorsal horns. The dorsal horn could be divided into a pointed apex, an expanded head, and a constricted cervix. The ventral horn of gray matter contained the motor cells, which gave rise to the fibres of the ventral root of the spinal nerves. It varied greatly in size and form at different levels of cord. This basic structure corresponds to that described in man by Larsell (1951) and in animals by many earlier workers (Kappers *et al.*, 1967; Papez, 1967; Dellmann and Mc Clure, 1975; Jenkins, 1978; Clark, 1984; King, 1987).

According to Papez (1967) the dorsal horn consisted of receptive cells, which serve as a nucleus of termination of the afferent fibres of the spinal nerves. He also found that the fibres of the dorsal afferent nerve roots and collaterals from the fibres of the fasciculus gracilis and cuneatus terminated on the dorsal horn.

The gray matter presented dorsal and ventral horns by the middle of the second month. The dorsal and ventral gray commissures joined the gray matter of the two halves of the cord from fourth month onwards. The lateral horns projected laterally from the lateral intermediate substance from middle of the second month onwards and was prominent at thoracic, anterior lumbar and middle sacral levels. Keith (1947) found that it was during the 11^{th} week of development in man, the mantle zone differentiated into dorsal and ventral horns and it was in the 13^{th} week,

that the adult form was reached. According to Sturrock (1982b), the dorsal horns were present from 20 days of gestation onwards in rabbit. Therefore in the present study, it happened at the corresponding time as that occurred in man.

In the present study, lamination of gray matter started from second month onwards but the lamination process was complete only during the fourth and fifth month of gestation. All the ten laminae could not be differentiated by the second month, but in the ventral horn the beginning of formation of nuclear aggregations in the lamina IX were seen. These aggregations became better defined towards the end of second month at the enlargements. By the beginning of the third month, the lamination of gray matter was not clear except at lamina II (substantia gelatinosa), VII (intermediolateral nucleus) and IX (nuclear aggregations in the ventral horn), but towards the end of this month other laminae started to differentiate and became better differentiated by the fourth month. The gray matter presented ten laminae towards the end of gestation. The cell size and thickness of different laminae varied between regions of the cord, which confirmed the findings of Jenkins (1978). The density of nerve fibres of the gray matter became more at enlargements from the middle of second month onwards in the present study. It appeared that the development of the nuclear aggregations in the present study occurred corresponding to the progression of development of the muscles and skeleton of the foetus.

Rexed (1952) described the architectural organization of neurons in the cat spinal cord. He believed that a similar lamination or zoning of gray matter existed in all higher mammals and this lamination of cell groups in the spinal cord resolved much of the confusion in terminology, and has became a widely used method for localizing axonal degeneration in the mammalian spinal cord. According to Truex and Carpenter (1969) adult human spinal cord from different segment levels showed ten laminae, which constituted regions with characteristic properties, but their boundaries were zones of transition. Rexed's cytoarchitectonic organization of gray matter was composed of nine cell layers and the region ten surrounded the central canal. Some of the layers corresponded to recognized cell columns and nuclei and others were a regional admixture of cells and fibres.

Jenkins (1978) also opined that the organization and relative positions of the laminae were not uniform throughout the cord. This observation was confirmed in the present study.

The cells of the gray matter were arranged in distinct groups, the nuclei or cell columns. The neurons started to form nuclear aggregations from the middle of second month onwards in the present study. Keith (1947) reported that in man, by third month the nerve cells took up their permanent stations in the gray horns as nuclear aggregations. Larsell (1951) found that the adult pattern of nuclear arrangement was laid down in human embryos by fourteen weeks.

Larsell (1951) described that in transverse sections of the cord these nuclei were apparent as cell groups distinguishable by their location, the size and form of cells and their internal structure. According to him, the cell columns may also be recognized in longitudinal sections of cord, but the knowledge as to their topography and functional significance, is still incomplete, while terminology varied greatly with different investigators.

5.3.2.2.3.1 Dorsal Horn

Eventhough the dorsal horns started to show better differentiation towards the latter half of third month, their structure became similar to that of the adults only during fourth and fifth month of gestation. Taluja *et al.* (1991) also found that the nuclear pattern of gray matter of the spinal cord in goat foetuses during last trimester of gestation was similar to that in buffalo calf (Rao, 1970) and albino rat (Zargar *et al.*, 1975).

In the present study, the adult structure of dorsal horn was attained between fourth and fifth month of gestation, when, it presented a marginal zone, the substantia gelatinosa, nucleus proprius and Clark's column. In the groove between the dorsal and ventral or lateral horns the reticular nucleus was also seen. This agrees with findings of Kappers *et al.* (1967) who also reported the presence of three divisions in the dorsal horns of mammals: a marginal zone, the substantia gelatinosa and the body of the dorsal horn. It appeared that the development of the dorsal horn eventhough happened at a later stage than that of the ventral horn, makes the new born kid capable of sensory perceptions even at birth.

The dorsal horn was narrow in the thoracic segments and was wider at the enlargements. This observation confirmed the findings of Papez (1967) who observed that the dorsal horn size corresponded to the sensory field of distribution of dorsal roots and inversely to the length of cord segment. He opined that it was because of this reason that the long thoracic segments had a very narrow or short dorsal horn in man. It is also reported that most of the neurons in the dorsal horn are relay neurons receiving primary peripheral afferent axons and sending their own axons to other spinal cells or to the higher centres in the brain stem or cerebellum. Most of them are also internuncial in function having connections with both primary relay and motor cells in the spinal cord itself.

5.3.2.2.3.1.1 Lamina I

By the end of second month, cells which represented the future marginal zone appeared as a single layer towards upper margin of dorsal horn and covered the apex of the dorsal horn as a cap with cells of varying sizes and shape. These cells were scattered, arranged tangential to the surface of the dorsal horn and were situated in the lamina I. These findings are in accordance with the findings of Clark (1984) who found that these cells were with flattened disc-like dendritic domains. The largest of these elements, the marginal cells of Waldeyer, are now known to be responsive to nociceptive information involving tissue distruction, whether of mechanical or thermal nature. Their input appears to arise primarily from fine myelinated (and perhaps some unmyelinated) fibres of primary afferent source, and their axons project to the sagittal plane. Spines cover the dendrite branches of these propriospinal pathways.

The lamina I was found in all the segments studied and so extended throughout the length of the cord. Larsell (1951) also reported that these cells were association neurons of the dorsal gray column.

From fourth month onwards, lamina I became measurable as a layer. The cell size and number varied between regions. By the fifth month, the cells were better differenciated and the segments of lumbar enlargement presented more number of cells per unit area. This agrees with the findings of Truex and Carpenter (1969) who reported that in man the cells were most numerous in the lumbosacral segments, less so in the cervical and least in the thoracic segments.

In the present study, lumbar enlargement presented the maximum size for substantia gelatinosa and more number of cells in lamina I. This observation was in accordance with the findings of Papez (1967), who found that the Waldeyer's nucleus is always related both in size and disposition to the substantia gelatinosa. The cells of substantia gelatinosa and those in the Waldeyer's nucleus send their axons into the fasciculus proprius that surrounds the dorsal horn. They conduct thermal (pressor and depressor) and painful sensibilities.

5.2.2.2.3.1.2 Lamina II

Lamina II corresponded to the substantia gelatinosa and extended the entire length of the cord. This formed a cap over the dorsal horn. It was located beneath marginal zone and presented two types of cells, which were very small with only a rim of perinuclear cytoplasm. Hannah and Nathaneil (1975) also found that the cells of this layer were small with large oval nuclei and scanty cytoplasm at birth. Todd and Lewis (1986) also recognized two main cell types in lamina II of the rat.

This layer presented convolutions towards the end of third month in some segments in the present study. By this age, the lamina II was organized into form and presented prominent fibres. The frequency of occurrence of convolutions increased as age advanced. Kappers *et al.* (1967) also found that the substantia gelatinosa was well developed in most mammals especially in ungulates where it formed convolutions. They identified the substantia gelatinosa as a portion of the pathway for pain and temperature. They also opined that the convolutions were surface extensions of this gray matter and such surface extensions are typical of highly organized sensory nuclei with pronounced local functions. According to them, the substantia gelatonosa varies in its degree of development in different mammals and there is reason to suppose that this variation is influenced to some extend by the development of the peripheral sensibility of the organs particularly those associated with the hairs.

Larsell (1951) regarded this nucleus as the chief sensory nucleus of the spinal cord for afferent impulses. Clark (1984) opined that the lamina II corresponded to the substantia gelatinosa. He described the neurons as frequently bipolar in structure. Spines covered the dendritic branches of these cells, thereby marking them, with one exception, as the only spine-bearing neurons in the adult mammalian spinal cord.

The neurons were tightly packed in this layer upto the end of third month and by this age the layer was penetrated by fibre bundles. As a result, the cells became loosely packed later and were arranged in vertical or radial orientation in clumps or miniature palisades. The lamina II had different shapes at different segments.

In the present study at caudal thoracic region the substantia gelatinosa was flattened and approached the same of the other side so that the dorsal horns were at close proximity. Kappers *et al.* (1967) reported a similar condition in carnivores. No information is available for comparison in other species.

Jenkins (1978) observed that this nucleus extended the whole length of the cord and was the largest in the lumbosacral and first cervical segments in mammals. In the present study, the maximum width was exhibited by L6 segment at all ages. The maximum regional average value for width for the entire gestation was at lumbar enlargement. Since the largest substantia gelatinosa was seen among the largest segments of the spinal cord there existed a relationship between the size of substantia gelatinosa and the size of the spinal cord segments. Papez (1967) also opined that it is a long column that extends the entire length of the cord and receives an abundance of collaterals from the entering dorsal roots of the spinal nerves. Hence it is regarded as the proper sensory nucleus of the cord. The cutaneous sensibility in particular is discharged into the cells of substantia gelatinosa.

5.3.2.2.3.1.3 Lamina III

In the present study, this layer was represented by a less packed area. Larger nerve cells appeared by the end of third month. This zone entered into the substantia gelatinosa towards the end of gestation. The cell size in the present study ranged from 10.8μm by fourth month and 46.8μm towards the end of gestation. Maxwell (1985) reported that lamina III consisted of large projection neurons and small interneurons.

5.3.2.2.3.1.4 Lamina IV

By the beginning of the third month lamina IV was not clear but by the end of the third month, it contained nucleus proprius, which occupied the central part of the head and cervix of dorsal horn as an indistinct cell column, corresponding to the lamina III and IV. Even by the fifth month, it was a poorly defined cell column with diffuse cell borders consisting of spindle-shaped and polygonal cells. According to

Papez (1967), nucleus proprius corresponded in size to the substantia gelatinosa. Truex and Carpenter (1969) also found that the cell column contained small to medium sized cells and was largest in the lumbosacral region and smallest in the thoracic region. This observation is confirmed in the present study.

As per the reports of Larsell (1951), the cells had either polygonal or spindle-shape and gave origin for spinothalamic and spinotectal tracts. These also gave rise to fibres, which entered the white matter on the same side. He also reported that the lateral base group of the posterior cell column consisted of Golgi type II cells. They were interneurons in the gray matter. According to Papez (1967), the large cells that form nucleus proprius sent their fibres into the lateral columns of the same and of the other side. He also opined that they are concerned with the conduction of tactile sensibilities upwards to the brain-stem and thalamus (spino-thalamic) tract. So it is confirmed in the present study as the larger segments of lumbar enlargement had a large sized nucleus proprius and the smaller sized thoracic segments had small sized nucleus proprius.

5.3.2.2.3.1.5 Lamina V

In the present study, the cells of lamina V, which were of medium to small size were most numerous in the zone between the ventral part of the dorsal horn and the lateral funiculus of white matter. Larsell (1951) reported that Bok designated this region as the spinal reticular nucleus. The axons of the cells appeared to pass in part to the lateral and ventral funiculi of the opposite side by way of the ventral white commissure and in part to the lateral funiculus of the same side.

Lamina V was described by Truex and Carpenter (1969) as a broad zone extending across the cervix of the dorsal horn, having medial and lateral divisions except in the thoracic region. In the present study, the lamina V attained this form only from fourth month onwards. It started to appear by the middle of the second month. The layer presented nucleus reticularis in the groove between the dorsal and ventral or lateral horns, which presented extensions of gray matter as reticular processes into the adjacent white matter. The gray and white matter became intermingled as the reticular formation.

The reticular formation was seen along the entire length of the spinal cord being well developed in the anterior cervical segments in the present study. As per the

earlier reports of Taluja *et al.* (1991) the reticular formation existed in the lumbar enlargement region alone. The reticular formation is considered as the seat of consciousness making the animal aware about the changes in its surroundings (Dyce *et al.*, 1996).

5.3.2.2.3.1.6 Lamina VI

This layer became identifiable with indistinguishable boundaries by fourth month and was located lateral to the central canal. It was typical only at enlargements confirming the reports of Larsell (1951). It consisted of a compact medial zone and a less compact lateral zone in this study. This was in accordance with the reports of Truex and Carpenter (1969) in man. They found that many dorsal root group I muscle afferents terminate in the medial zone but descending pathways project to cells in the lateral zone. Some axons leave the gray matter to enter fasciculus proprius system and lateral white matter.

5.3.2.2.3.2 Lateral Horn

5.3.2.2.3.2.1 Lamina VII

This layer was represented by most of the intermediate zone of gray matter, which presented intermediolateral nucleus, intermediomedial nucleus, cervical nucleus of Stilling and Clark's column. The size of the neurons in this zone was highly variable. Grottel (1979) found that the neurons of various centers of intermediate gray matter differed more with the range of their dendrites than with the size of their perikaryons.

5.3.2.2.3.2.1.1 Intermediolateral nucleus

By the middle of second month, the intermediolateral nucleus was seen at thoracic, anterior lumbar and middle sacral segments. The neurons in this nucleus were not as differentiated as those of the ventral horn. By the end of third month, the nucleus was better differentiated with spindle-shaped cells. Truex and Carpenter (1969) described these visceral efferent neurons as smaller than the somatic motor cells, which was found true with the present study also. Jenkins (1978) also identified the lateral horn as the nucleus of origin for the general visceral efferent neurons of the autonomic nervous system.

According to Papez (1967), the cells of the lateral horn contribute (preganglionic) fibres that pass through the white rami communicans and terminate on cells of sympathetic (visceral) ganglia. This sympathetic column of cells is absent in the cervical region. It is prominent in the thoracic region where it gives rise to the cervical sympathetic chain, the cardiac plexus, white rami communicans of the intercostal nerves and the splanchnic nerves. It appears again in the lower sacral segments that supply the hypogastric plexus.

By fourth month, the intermediate gray included two dispersed nuclear groups. The medial part was medioposterior column and the lateral part was intercornual column. According to Papez (1967) the medioposterior column receive a large number of collaterals from the dorsal root zone that forms the lateral half of the fasciculus gracilis. The cells of this nucleus give off axons that form the large fasciculi proprii (reflex tracts) that surround the ventral horns of both sides. From this nucleus a large number of these fibres cross and form a prominent ventral commissure and ventral fasciculus proprius of the cord. For this reason, the medioposterior column is also called the commissural nucleus. The intercornual column gives rise to fibres that surround the lateral and ventral sides of the ventral horn and are known as the lateral faciculus proprius. He also believed that these columns and fasciculi proprii are an essential part of the spinal mechanism for integration of spinal reflexes. It is known that the fibres of the fasciculi proprii terminate on the motor cells of the ventral horn and there is some evidence that the cerebrospinal tracts end on the cells of the medioposterior column.

Slawomirski *et al.* (1973) reported that the intermediolateral nucleus in dog extended from C8 to L3 segment. It was located C8 to L4 level in cats (Okamoto, 1977) and at T1 to L3 in horses both in adults (Welento *et al*, 1979) and in prenatal period (Arciszewski *et al.*, 1999b).

In the present study, the lateral horn extended from C8 or T1 to anterior lumbar segments, similar to the reports by Jenkins (1978) in mammals. It was also seen at the sacral region. In the present study, the intermediolateral nucleus appeared by the middle of second month of gestation at the thoracic segments. The lateral horn with intermediolateral nucleus was well developed by the the beginning of fifth month. It became surrounded by fibres in the fourth month. Rethelyi (1976) also

revealed a regular orientation of dendrites in the intermediate region forming a circular or elliptical gray matter in cats.

Rao (1970) in buffalo calf, Zargar *et al.* (1975) in albino rat and Taluja *et al.* (1991) in foetal goat in the fifth month of gestation reported the presence of substantia intermedia lateralis and centralis in the lateral and medial aspects (respectively) of lateral horn.

5.3.2.2.3.2.1.2 Intermediomedial Nucleus

The intermediomedial nucleus appeared for the first time in the present study by the end of third month in the thoracic and lumbar regions ventral to Clark's column. By the end of fifth month, the nucleus became better developed but became small at L1 and T12 segments. The Nissl bodies were finer and uniformly distributed in the intermediomedial and intermediolateral nuclei.

Welento *et al.* (1977) observed the presence of the nucleus intermediomedialis of the pig from C1 to Cy2 or Cy3, whereas in horses Arciszewski *et al.* (1999a) found that this nucleus in prenatal period extended from C1 to S5 as an interrupted band of cells.

In the present study, the processes extended from the neurons at lateral horn towards the neurons of intermediomedial nuclei. Grottel and Teresa (1979) also noticed that the dorsal dendrites of neurons of lateral horns in the thoracic segments reached lamina II and the ventral ones reached the lateral group of motor nuclei. The dendrites directed medially run to the medial area of intermediate gray while those directed laterally enter the lateral funiculus to run a short distance.

5.3.2.2.3.2.1.3 Cervical Nucleus of Stilling

Cervical nucleus of Stilling was noticed by the end of third month at the cervical region in the medial part of base of dorsal column. It was ill-developed in regions other than the cervical region. During fourth month also, the cervical nucleus was seen in the anterior cervical segments, and at the same position in the sacral region, cells representing sacral nucleus of Stilling was seen. By fifth month it was noticed with elongated cells. Its position corresponded to that of the Clark's column in

thoracic region. This finding is in accordance with those of Larsell (1951) who also observed that this nucleus become continuous with the lateral cuneate nucleus of the medulla.

5.3.2.2.3.2.1.4 Clark's Column

Clark's column appeared by middle of second month on the lateral aspect of the central canal. It became more clear by the end of second month. By fourth month, it presented neurons with eccentrally placed nucleus. The column was seen from T1 level extending backwards, well developed from T9 to L2 level but was not prominent behind this level. It partially agreed with the observations of Grant and Rexed (1958) in cat, who reported that the column extended from T1 to L3 segment. According to Truex and Carpenter (1969) also, this nucleus was particularly prominent in segments T10 to T12 in man. It was also in accordance with the findings of Dellmann and Mc Clure (1975), who opined that it was well defined in C8 segment and extended through the thoracic and anterior lumbar regions being most prominent in T10, T12 and L1 segments in domestic animals.

Papez (1967) found that the Clark's column stands in intimate relation to the fasciculus gracilis, which sends numerous collaterals into it. The cells of Clark's column give origin to dorsal spinocerebellar tract of the same side and also to the crossed ventral spinocerebellar tract of the other side. It is probable that proprioceptive impulses are sent by these tracts into the cerebellar cortex (vermis). These tracts are also believed to conduct exclusively deep or muscular sensibility to the cerebellar cortex.

5.3.2.2.3.3 Ventral Horn

The ventral horn contained lamina VIII and IX. The present study revealed that the shape and size of the horns varied from one region to the other and the ventral horns were wider in the enlargements and narrower in the anterior cervical and thoracic levels. Similar observations were made by Clark (1984) in man.

5.3.2.2.3.3.1 Lamina VIII

From fourth month onwards, lamina VIII contained small and medium sized cells and was not sharply differentiated from lamina VII. This description is in accordance with the findings of Clark (1984).

Clark (1984) opined that by definition the lamina VIII excluded motor neuron pools and consisted entirely of interneurons. Although the axons of all these cells projected via propriospinal tracts to a number of different spinal levels, serving as receptor sites for fibres of suprasegmental origin, whose excitation patterns were then synaptically transferred to the nearby motor neurons. The vestibulospinal, pontine reticulospinal and tectospinal tracts and the medial longitudinal fasciculus end as synapse on lamina VIII interneurons in order to affect, ultimately, the final common pathway in man. In cat, the lateral corticospinal tract synapsed on such cells, eventhough in primates and man the internuncial cell had been largely eliminated from the descending loop.

5.3.2.2.3.3.2 Lamina IX

By the middle of second month towards the ventrolateral aspect, the formation of nuclear aggregations were seen. These aggregations became better defined by the end of second month. Lateral and medial nuclear masses were seen and the former always had sharply limiting boundaries at enlargements whereas the latter were less defined and had a diffused border with lamina VIII.

The medial nuclear group was smaller and most distinct in cervical and lumbar enlargements, whereas the lateral nuclear group extended throughout the whole cord. This was in accordance with the findings of earlier workers who also found that the nuclear pattern of spinal cord gray matter in buffalo calf (Rao, 1970) and albino rat (Zargar *et al.*, 1975, 1979) was similar to that in goat foetuses at fifth month of gestation (Taluja *et al.*, 1991).

Truex and Carpenter (1969) reported that the medial group innervated the short and long muscles attached to the axial skeleton. The lateral nuclear group became considerably enlarged with a number of subgroups in cervical and lumbar enlargements. This group innervated the most distal portions of the extremities.

In the present study, the enlargements presented central and retrodorsolateral nuclei also, in addition to those described by these earlier workers (Rao, 1970, Zargar *et al.*, 1975, 1979, Taluja *et al.*, 1991). According to Larsell (1951), the central nucleus innervates the muscles attached to the shoulder and pelvic girdle. He also found that the retrodorsolateral nucleus gives rise to motor fibres to the distal muscles of the digits.

Papez (1967) found that the number of cell groups in the spinal cord is much smaller than the number of muscles. It appears that these cell groups supply groups of muscle which have a unity of action or whose actions are integrated to perform a common movement.

By the third month, the ventral horn presented multipolar neurons with large vesicular and eccentrically placed nucleus and eosinophilic cytoplasm. Lamina IX was distinguishable even by the middle of second month and was composed of the largest cells of the spinal cord namely, the alpha motor neurons. This is in accordance with the findings of Clark (1984) who found that lamina IX consisted of a number of groups of somatic motor neurons, segregated by function. The larger alpha motor neurons innervated striated muscle fibres (extrafusals) and the smaller gamma motor neurons innervated the intrafusal fibres of muscle spindles.

The large motor neurons of ventral horn were the largest in the enlargements in the present study. These neurons were smaller in other segments. Scattered among the large ventral horn cells were smaller cellbodies of gamma neurons. These findings agree with the reports of Truex and Carpenter (1969) who described these neurons as contributing axons to the ventral roots of the spinal nerves and had a transverse diameter of 30-70 μm and the cell body was 100 μm in length. Such elongated multipolar neurons had 3-20 dendrites and axons with diameter of 10-13 μm. Rafalowska (1977) found that in cat, the ventral horn neurons smaller than 30 μm were connected with oxygen metabolism, while large cells were connected with glycogen metabolism. According to Fletcher (1993), volume of the cellbody of the ventral horn neurons was proportional to the volume of its axon and the size of the motor unit being innervated.

Rao (1970) grouped the neurons of spinal cord into three categories based upon their sizes: small (15 to 25 μm), medium (25 to 35 μm) and large (over 35 μm) while studying the nuclear pattern of spinal gray matter in buffalo. In the present study neurons were still smaller owing to a change in the developing stage.

5.3.2.2.3.3.3 Lamina X

This layer surrounded the central canal. By the fifth month, the neurons in lamina X could be clearly identified without much regional variation in size.

According to Papez (1967), functionally these cells are homologous with the cells of the reticular formation in the brain stem.

5.3.2.2.4 White Matter

In domestic animals, the white matter of spinal cord could be divided into three white columns or funiculi by the entering dorsal and emerging ventral rootlets of the spinal nerves on each side as per earlier reports (Dellmann and Mc Clure, 1975). The present study confirmed this information and the funiculi were dorsal, ventral and lateral. These funiculi took their form by middle of second month.

All the funiculi showed a progressive increase in the thickness during the gestation period and a tremendous increase was noticed towards the latter half of gestation especially between fourth and fifth month of gestation. This may account for the well developed reflexes possible in full term foetuses and new born kids.

A remarkable increase was also noticed in the amount of white matter from the sacral region towards the cervical region in the fifth month of gestation. This observation is found to agree with the observations of Truex and Carpenter (1969). He found that since all levels of the spinal cord were connected with the brain by long ascending and descending fibres, the white matter increased from lower to higher levels of the cord and Jenkins (1978), who opined that in mammals the cervical segments contained the largest number of fibres.

According to Nyberg (1966) in adult animals, the ventral funiculus was larger than the lateral funiculus. In contrary to this, in the present study, the lateral funiculus exceeded other funiculi in thickness in all regions at all ages. This difference may be attributed to a change during the developing stages.

According to Dellmann and Mc Clure (1975), the funiculi in the white matter of the spinal cord in animals were formed of numerous fibre tracts or fasciculi which were of three types: ascending, descending and intersegmental. They also opined that these tracts were developed to different degrees in different species of domestic animals.

In the present study, fibre tracts like fasciculus gracilis, fasciculus cuneatus, dorsolateral fasciculus of Lissaeur, medial vestibulospinal tract and medial longitudinal fasciculus could be identified by the middle of second month at 48 days.

This age of onset corresponded to that in man, as Keith (1947) reported that most of the ascending and descending tracts in the ventrolateral marginal zone were formed in the third month of gestation.

Except for the above mentioned tracts, other tracts were very difficult to be identified. But at their respective area of location clustering of fibres or cells could be seen. The definite levels of origin and termination were also very difficult to be identified. This agrees with the reports of Reith and Ross (1977) who observed that although the fibres which had common origin and destinations in the physiological sense were arranged in tracts, these tracts could not be distiguished unless they have been marked by special techniques such as causing injury to the cellbodies from which they arise. King (1987) also reported that almost all tracts were mixed with other tracts in the spinal cord and were not separated into well defined bundles. The only general exception was the gracile and cuneate fascicles, but in several domestic species for example, in sheep, even these tracts were blended with other tracts.

From the middle of the second month onwards, occasionally blood vessels could be seen in the white matter in the present study. It was according to the observations made by Dyce *et al.* (1996), who found that the greater part of the white matter is supplied by radial twigs from the dorsolateral arteries and surface plexus.

By fourth month, blood vessels presented perivascular satellites. These cells may contribute to the formation of blood-brain barrier.

The vascularity and the cell density in the white matter increased as age advanced upto the end of gestation in the present study, indicating the onset of myelination. So it is assumed that the onset of myelination was in the third month of gestation in the present study, hence the motor and sensory impulses through the cord became specific and it is ready for reflex activity.

A quantitative study of vascularization of the prenatal rabbit spinal cord by Sturrock (1982b) revealed that the onset of myelination coincided with the beginning of a rapid increase in vascularity in both gray and white matter. But in the mouse, the percentage vascularity in both gray and white matter remained fairly constant from onset of myelination by 18 days of gestation to 5 days postpartum, when myelination was fairly well established. Logan *et al.* (1983) found that myelination in the CNS commenced by 8 to 9 days post partum in the rat.

The processes of multipolar neurons extending through the white matter could be well demonstrated towards the end of gestation as both bundles and tracts. Klemm (1996) opined that eventhough the neurons stopped to divide shortly after birth, their axons and dendrites grew extensively during the preadult period.

Oligodendrocytes formed myelin sheaths around axons by the fifth month, hence deriving the name for the white matter. Other oligodendrocytes without forming the myelin sheath were also associated with nerve tracts and also free in white matter. Eventhough a typical myelin sheath was observable only towards the end of gestation, clear and vacant spaces occurred around axons from fourth month itself representing sites of myelination. Histochemical studies revealed the presence of lipids in the white matter even in the third month of gestation. Towards the end of gestation, the white matter became a mixture of myelinated and nonmyelinated axons, blood vessels and neuroglia. Collagen was absent in the spinal cord tissue except in the blood vessels, which agrees with the reports of Clark (1984).

Different levels of the cord revealed variations in the gray and white matter relationship in the present study. The gray matter percentage showed a decreasing trend during the entire gestation period as the age advanced, indicating a gradual increase in the white matter percentage. Sparing a few exceptions, the percentage contribution of gray matter height showed a gradual decrease as the age advanced, indicating the development of the ventral funiculus indirectly, since the contribution from the dorsal funiculus at the dorsal aspect was always less. The transverse gray matter percentage showed a decreasing trend only after the third month. It indicated an increase in the growth of the lateral funiculus at a later stage of gestation when compared with that of the ventral funiculus. Jenkins (1978) opined that variations in the gray and white matter relationship at different levels of the cord occurred because of the variation in the size and number of fibres forming the spinal nerves, which attached to the cord at different levels. He also found that the ventral funiculus primarily contained descending phylogenetically older tracts in mammals. So naturally, its differentiation happened earlier than other funiculi in the present study.

5.3.2.2.4.3 Dorsal Funiculus

The dorsal funiculus presented fasciculus gracilis and fasciculus cuneatus from the middle of the second month onwards from the C1 segment upto caudal

thoracic region. The dorsal funiculus was undivided after this level. These observations agree with those of Jenkins (1978) who reported that in mammals some tracts like fasciculus cuneatus were not present in caudal part of the cord. He also stated that the fasciculus gracilis is composed of axons from the cell bodies in the dorsal root ganglia of the nerves innervating the caudal trunk and hindlimbs, whereas the fasciculus cuneatus is composed of similar axons from the forelimb, cranial trunk and neck. These tracts convey impulses of conscious proprioception for the sensations of position and movement.

The fasciculus gracilis was ill-defined by the middle of the second month, very narrow towards the end of the second month but became better developed by third month. By fourth and fifth month from S1 segment and caudally, no dorsal median septum was present to separate the dorsal funiculi of either side. At sacral region, dorsal commissure could be seen as fibres crossing from one side to the other and connecting the dorsal part of two halves of the spinal cord.

By fourth month, the dorsal fasciculus proprius was seen as bundles of axons at the dorsal aspect of gray commisure bordering the dorsal funiculus. As reported by Sisson and Grossman (1953) and Buchanan (1957), the fasciculus proprius was the intersegmental tract of each funiculus and acquired the name designating its location viz. dorsal, lateral and ventral fasciculus proprius. They also opined that it was composed of ascending and descending fibres, which bordered the gray matter and originated and terminated within the spinal cord to connect the spinal neurons of intrasegmental and intersegmental levels. Papez (1967) opined that these connections through fasciculus proprii form one of the chief integrating mechanisms, which connects the dorsal (sensory) and the ventral (motor) roots. It is also reported that the dorsal fasciculus proprius gets its name from the fact that its fibres are derived from the cells of the dorsal horn and are limited to the cord. They mediate some of the intersegmental reflexes of sensibility.

5.3.2.2.4.4 Lateral Funiculus

Lateral white column contained both ascending and descending fasciculi and lateral fasciculus proprius. The ascending tracts were: the dorsolateral fasciculus of Lissauer and the dorsal and ventral spinocerebellar tracts. The descending tracts in the

lateral funiculus were the lateral corticospinal, rubrospinal and lateral reticulospinal tracts as reported in mammals by Jenkins (1978).

At the dorsolateral aspect of the dorsal funiculus, from middle of second month onwards, the formation of dorsolateral fasciculus of Lissauer was seen. It became better developed by fourth month and later. Larsell (1951) opined that this zone contained few myelinated fibres. It received the lateral, largely unmyelinated divisions of the posterior roots. Jenkins (1978) reported that this tract convey impulses of pain and temperature sense.

By the beginning of third month at the periphery of the lateral aspect of the lateral funiculus, the dorsal and ventral spinocerebellar tracts were seen with indistinguishable boundaries. As reported by Jenkins (1978) in mammals, these tracts were located at the periphery of the lateral funiculus in the present study. He also stated that these tracts convey propioceptive impulses from muscle and tendon receptors to the cerebellum, which exerts its tonic and synergizing influence on the motor pathways for regulation of voluntary motor activity.

The extent of these tracts could not be understood in the present study. Grant and Rexed (1958) reported that the dorsal spinocerebellar tract (Flechsig's fasciculus) was composed of myelinated fibres, which had their cellbodies within the Clark's column ascending from L3 or L4 segments in cat to enter cerebellum via restiform body. According to Hubbard and Oscarsson (1962) in the cat, the ventral spinocerebellar tract originated from the base and neck of the dorsal horn in lumbar segments and ascended through the brain stem and rostral cerebellar peduncle into the cerebellum. Dellmann and Mc Clure (1975) described the presence of the spinocerebellar tracts in sheep, goat and ox.

Towards the end of third month, lateral corticospinal tract was seen lateral to reticular formation. The location of the tract agrees with the reports of Jenkins (1978) in mammals as the lateral corticospinal (crossed pyramidal) tract occupied the central area of the upper quadrant of the lateral funiculus, medial to the dorsal spinocerebellar tract dorsal to the rubrospinal tract, lateral to the lateral fasciculus proprius and ventral to the dorsolateral fasciculus of Lissauer. According to him, the corticospinal and rubrospinal tracts excite the flexor mechanisms. So, this may account for the movability of the kid at the day-old stage itself.

The respective area of location of the lateral reticulospinal tract appeared towards the end of third month lateral to the ventral horn and lateral fasciculus proprius at the medial aspect of lateral funiculus. This location agrees with the description given by Jenkins (1978) in mammals for the tract.

By the fourth month, in addition to these tracts, the lateral funiculus presented rubrospinal tract near the corticospinal tract, which became better developed later. In animals, the rubrospinal tract appeared to take the place of crossed pyramidal tract in man (Sisson and Grossman, 1953) which was inconspicuous in the latter (Austin, 1961). In cat, it was traced upto the sacral segments (Nyberg, 1966) and was much larger in most mammals than in birds and reptiles and extended farther caudally (Kappers *et al.*, 1967). The tract was demonstrated in goat and ox (Dellmann and Mc Clure, 1975) and arose from red nucleus and was located ventrolateral to and partially intermingled with fibres of the lateral corticospinal tract, being medial to the dorsal spinocerebellar tract in mammals (Jenkins, 1978). Dyce *et al.* (1996) described that the tract reached upto the most caudal part of the cord and it was an important tract in carnivores and was the best developed of all motor pathways in ungulates.

Lateral fasciculus proprius was seen bordering the gray matter by the end of third month and became well distinguishable by fourth month. According to Clark (1965) in man, the earliest tracts in the marginal zone differentiated as intersegmental tracts in the second month. But in the present study, it became demonstrable only by the third month. This change may be attributed to a species variation.

Papez (1967) opined that the lateral fasciculus proprius is derived chiefly from the intercornual column and is a reflex bundle.

5.3.2.2.4.5 Ventral Funiculus

Jenkins (1978) found that the ventral funiculus primarily contained descending phylogenetically older tracts in mammals.

All tracts in the ventral funiculus except medial longitudinal fasciculus and medial vestibulospinal tract, were mixed with other tracts and were not seperated into well-defined bundles in the present study. Medial longitudinal fasciculus appeared by the middle of the second month and and became well defined from the end of second month onwards. Verhaart and Beusekom (1958) described it as a very old tract that

extended from the rostral midbrain upto lumbar levels in dog and second thoracic segment in cat. According to Jenkins (1978), this tract serves as a connection between vestibular and motor nuclei of cranial nerves, III, IV and VI to account for vestibular reflexes involving eye movements.

The medial vestibulospinal tract was seen at the base of ventral median fissure ventral to medial longitudinal fasciculus from middle of the second month onwards, which became well developed by the end of third month. Dellmann and Mc Clure (1975) reported that the vestibulospinal tracts were demonstrated in goat and ox. Jenkins (1978) opined that this tract started from the medial vestibular nucleus, descended within the medial part of the ventral funiculus in the area of the medial longitudinal fasciculus and extended upto the mid-thoracic segments of the cord.

The ventral fasciculus proprius, which was seen bordering the gray matter, was ill developed by the end of second month, and became better developed from the third month onwards in the present study.

As to the other tracts in the ventral funiculus, the ventral corticospinal tract and ventral reticulospinal tract appeared by fourth month near the ventral median fissure and other tracts like, tectospinal and lateral vestibulospinal tracts were also seen. None of these tracts could be identified as well defined bundles.

According to Nyberg (1966), reticulospinal tract was the only pathway by which spinal cord received impulses from higher centers in cyclostomes and were the first to possess the phylogenetic signs of cephalization. In addition to this, in higher animals, there were the vestibulospinal, tectospinal and the corticospinal system. Jenkins (1978) repoted that the ventral reticulospinal fibres extended from the caudal pontine reticular area upto the lumbosacral segments but the extent of this tract could not be detected in the present study.

Aggregations of axons and cells at the reported location of the ventral cotricospinal tract might represent the same but their extent could not be detected clearly as it was mixed with the other tracts of the ventral funiculus in this study. According to Kappers *et al.* (1967), the ventral corticospinal tract was smaller than the lateral corticospinal tract and was traced upto lumbar levels in higher mammals but was rare in the cat spinal cord. Jenkins (1978) found that the ventral corticospinal tract bordered the ventral median fissure and consisted of those corticospinal fibres,

which did not cross at the medullary level in mammals. King (1987) found that in ungulates, the entire pyramidal system was small and ended at the cervical region. The fibres decussated in the pyramids and mainly descended as the ventral tract alongside the ventral median fissure.

According to De Lahunta (1983), tectospinal tract arose from the rostral colliculus, descended in the periphery of the ventral funiculus bordering the ventral median fissure and terminated in the upper four cervical segments and it served as an afferent pathway for visuospinal and auditospinal reflexes. In the present study also, aggregations of axons and cells at the reported location of this tract was seen from fourth month onwards.

An ill-defined lateral vestibulospinal tract was also seen at the ventral funiculus. Nyberg and Mascitti (1964) detected that this tract contained descending fibres from the lateral vestibular nucleus (of Dieter) in the medulla and extended the whole length of the cord in the cat. Jenkins (1978) found that the reticulospinal tract and vestibulospinal tracts, which descend in the ventral funiculus, excite the extensor mechanisms and contribute to the maintenance of postural tone. This may account for the well developed ability for maintenance of posture in the new born kid. In short, all these tracts were present by the fifth month of gestation, indicating that the kid can be considered as a mature young one at the day of birth itself.

5.3.2.2.5 Central Canal

The central canal extended through the entire length of the cord in the present study. It continued cranially through the lower part of the medulla oblongata, into the fourth ventricle of brain as reported by earlier workers (Larsell, 1951; Leach, 1952; Jenkins).

The central canal was positioned eccentrically in the gray commissure as soon as the latter was formed in the fourth month. Larsell (1951) also reported the eccentric position of central canal in the gray commissure.

The development of the neural tube resulted in a relative reduction of its lumen accompanied by increased thickness of its wall. The central canal, which was diamond-shaped in cross-section upto 40 days of foetal age (by the beginning of second month) changed its shape into round or oval in cross-section as age advanced.

This change was gradual and occurred due to the union of the dorsal walls resulting in obliteration of dorsal part of the neural cavity and rounding off of the persisiting ventral portion into the definitive central canal. So by the fourth month, the height of the central canal in cross-section was reduced to attain an adult size and shape. These changes happened between nine weeks and three months of gestational age in man (Arey, 1957) and between 28 and 34 foetal days in cat (Bohme, 1988). According to Bohme (1988), it was characterised by an elongation and shifting of the dorsal ependymal matrix cells and by the apposition of lateral walls in the same region. He found that it was the increase in the size of the dorsal funiculi which caused the elongation of the ependymal cells, the basal processes of which remained to form the dorsal glial septum. The proliferation of neurons and the resultant growth of the dorsal gray horn was also believed to be responsible for the narrowing of the lumen.

In the present study, the evidence for the obliteration of the lumen was indicated by the displaced lumen-lining cells being in contact with the undifferentiated ependymal cells. This was according to the findings of Bohme (1988), who also found that in the zone of contact microvilli and cilia interdigitated and the typical protrusions of embryonic ependymal cells were reduced.

In the present study, these dual events of wall apposition and the shifting of matrix cells were conspicuous until 58 days of gestation. As a result, the lumen of the developing spinal cord was gradually reduced. By 54 days of gestation, the lumen was reduced and had become the central canal of the spinal cord. It attained the adult shape by fourth month only. Bohme (1988) also opined that a wedge-shaped area of matrix cells connected with the dorsal glial septum remained until two or three months after birth in cat. After that time the central canal was lined by a single layer of mature ependymal cells and the matrix cells disappeared two or three months after birth.

The position of the central canal is believed to be central in the spinal cord as per its nomenclature used in various textbooks (Trautmann and Fiebiger, 1957; Dellmann and Mc Clure, 1975; Dellmann and Brown, 1981).

In the present study during fourth and fifth month of gestation, the percentage ratio between different distances revealed that the position of the central canal a little dorsal in the cervical, cervical enlargement, thoracic and lumbar regions of the spinal

cord but ventral or central in lumbar enlargement and ventral in sacral regions. This partially agrees with the observations of Taluja *et al.* (1990) in goat foetuses as they observed the location of the central canal a little dorsal, ventral and approximately central in cervical, thoracic and lumbar regions respectively. They opined that this feature probably helped to maintain the caudal flow of CSF. However, the upper end of the central canal started approximately from the central level at the second and third month of gestation in most of the regions but at the sacral region the central canal was always located at ventral position.

By third month of gestation, the central canal was elongated and narrow at cervical and thoracic segments but at the segments of enlargement, the canal was wider. The shape was oval from fourth month onwards. At sacral and coccygeal regions, the central canal was dorsoventrally elongated and presented the terminal ventricle towards the caudal end. These findings are in accordance with those of Larsell (1951) in man, Kappers *et at.* (1967) in mammals, Pearson and Sauter (1971) in human, rabbit and monkey foetuses, Sturrock (1981) in mouse and Bruni and Reddy (1987) in rat. Pearson and Sauter (1971) described the terminal ventricle in the human foetuses between 100 and 200 mm CRL as a translucent pyriform area with small outpouchings and duplications of the central canal at the lower end of the conus medullaris.

In the present study, the central canal at the conus was lined by a single flattened layer of epithelium. Bruny and Reddy (1987) also found that in the rat, the central canal of the conus differed from more rostral segments of the cord and it consisted of a large tube comparable in size to that at thoracic and cervical levels lined by a single flattened layer of ependymal cells. They also reported that proliferation of ependymal cells was also observed in the mammalian central canal in response to a variety of insults. Channels formed between adjacent ependymal cell processes like germinal epithelium provide the pathway for guidance and direction of regenerating axons towards their destination.

The shape of the central canal varied from round to oval upto the sacral region during the last two months of gestation. Goller (1962) opined that the shape of the cross-section and position of the central canal showed only very slight variations among cattle, but Sturrock (1982a) reported that the central canal varied in shape from

circular to slit-like in different regions in rabbit by 20 days of gestation. Bjugn *et al.* (1988) found that in animals showing inadequate tissue preservation the central canal was partially collapsed and in animals showing good tissue preservation, the central canal was always round to ovoid in cross-section at all levels examined.

However, according to Truex and Carpenter (1969) in man, a sharply defined central canal was seen only in foetal and newborn spinal cords whereas in the adult, the ependymal lining was discontinuous and the canal disappeared after 40 years.

The lumen was lined by stratified columnar cells during early stages. Upto fourth month, till the adult shape was attained, the ependymal layer was thicker at the ventral aspect of the lumen and thinner at enlargements. From fourth month onwards, the layer formed a pseudostratified ciliated columnar layer. According to Bjugn *et al.* (1988), the ependyma had the features of a simple, cuboidal epithelium and had no regional variations in structure along the length or circumference of the central canal.

The cells presented cilia from middle of second month onwards, upto the beginning of the fifth month. The cilia were very short or almost absent upto lumbar region towards the end of gestation, but were present at sacral and caudal levels. Larsell (1951) also found that the central canal was lined with columnar ependymal epithelial cells, ciliated in the embryo and to some extend in the adult, and is filled with cerebrospinal fluid. Kohno (1969) also found that in rat, the cervical and upper thoracic cord ependymal cells were unique with respect to the sparse distribution of cilia at their surface.

While studying the cerebrospinal fluid contacting surface of the ependyma in mouse, Bjugn *et al.* (1988) noticed that the individual cells were seen to have highly variable number of kinocilia. Of the kinociliary-poor cells, a few were, in addition, almost free of microvilli.

The luminal content started to appear in the central canal of goat embryo by 40 days in the present study. It may indicate the onset of secretory activity of the ependymal cells. It may also indicate the onset of activity of the choroid plexus of the ventricles of the brain and the presence of cerebrospinal fluid in the lumen. Banks (1981) opined that the ependymal cells contribute significantly to the formation of cerebrospinal fluid. This formative process is not confined to areas in which the

ependymal cells are juxtaposed to the choroid plexuses but occurs at scattered sites throughout the ventricular system of the central nervous system.

The lumen presented cell debris along with clusters of denuded cells and erythrocytes in the present study. According to Truex and Carpenter (1969) also, the lumen contained a variety of debris including round cells, macrophages and neuroglial processes. Bjugn *et al.* (1988) during scanning electron microscopic studies found that in mouse, within the central canal and along its entire length, Reissner's fibre was present but no cells were observed in the lumen. They also reported that in all segments examined the central canal was found to be free of amorphous material apart from the luminal vesicles or apical protrusions, which were interpreted as apocrine secretion observed on the spinal cord ependyma. According to Sturrock (1981) the material, which filled the lumen below the cervical cord was caused by inadequate fixation.

5.3.2.2.6 Regional Differences

5.3.2.2.6.1 Cervical Region

The cervical region presented the maximum thickness of dorsal funiculus (vertical thickness) and second highest values for ventral and lateral funiculi at the fifth month indicating an increased amount of white matter at the cervical region. According to Truex and Carpenter (1969) also, the cervical segments are characterized by the large amounts of white matter.

Gray matter was characterized by the presence of well developed reticular formation, cervical nucleus of Stilling and spinal accessory nucleus. By the latter half of third month, the cervical nucleus of Stilling appeared at the medial part of the dorsal horn. The nucleus proprius was also seen as ill-defined column of cells. Truex and Carpenter (1969) also observed that the reticular nucleus was prominent in cervical region.

The spinal accessory nucleus was seen from C1 level upto C5 segment at the ventrolateral aspect of ventral horn by the end of second month onwards. By the fifth month, the nucleus was seen in the middle of ventral horn. Fleiger (1970) found that in pig also, the nucleus of the accessory nerve extended from the posterior end of the olivary nucleus to the posterior sections of the first and fifth cervical segments of the

cord respectively. The present observations confirm the findings of Dellmann and Mc Clure (1975) in domestic animals that this nucleus was situated in the cervical part except in caudal two segments. Ullah and Salman (1986) also observed in rabbit, that the spinal nucleus of accessory nerve extended from the caudal part of the medulla oblongata to the cranial part of sixth cervical segment of spinal cord.

A phrenic nerve nucleus was also seen at caudal part of cervical region at C4 to C6 segments from the latter half of second month onwards. It was located between ventromedial and ventrolateral nuclei at the ventral margin of the ventral horn. Keswani and Hollinshed (1956) reported that the phrenic nucleus in man occupied the most medial portion of the ventromedial group of cells in the ventral gray horn of the third, fourth and fifth cervical segments. However, the phrenic nucleus in the rabbit (Ullah, 1978) and cat (Takahashi and Ninomiya, 1985) extended from the 4th to 6th cervical segment as a longitudinal column of cells lying between the ventromedial and ventrolateral columns at ventral margin of the ventral gray horn of the spinal cord confirming the observations in the present study.

5.3.2.2.6.2 Cervical Enlargement

The cervical enlargement had the maximum height of the spinal cord during the entire gestation period. The gray matter height and dorsal horn height was maximum at cervical enlargement at all age groups except during the fifth month of gestation. As to the total width of spinal cord and distance from central canal to spinal cord left margin, the cervical enlargement dominated during later stages of gestation, viz. at fourth and fifth month. During second and third month, this region occupied the second position behind lumbar enlargement. Eventhough cervical enlargement was having greater width than lumbar enlargement at fourth and fifth month of gestation, its increased width was mostly due to increased amount of white matter at cranial levels of the spinal cord. This region presented the maximum values for the vertical thickness of ventral and lateral funiculi at the fifth month. The thickness of all funiculi except transverse thickness of dorsal funiculus at the fifth month, were more at the cervical enlargement than the lumbar enlargement during the last two months. These findings agree with the reports of Truex and Carpenter (1969) who opined that the segments of cervical enlargement had increased size, large amounts of white matter and the prominent gray matter.

Maximum spinal cord height and gray matter total height were at C8 at all ages. The maximum value for dorsal horn height varied between C8 and C6. The cervical enlargement had prominent appearance of gray matter histologically. From second month onwards at C6, the ventral horn bulged laterally with increased fibre content and vascularity. This increased size is attributed to the greater number of nerve fibres, which originate from this area.

From the level of C8 onwards, intermediolateral nucleus, reticular formation, intermediomedial nucleus and Clark's column were also visible from middle of second month onwards. In the ventral horn by the second month, the nuclear aggregations were seen, which were dorsolateral, dorsomedial, ventrolateral and ventromedial nuclei. From the beginning of third month onwards the ventral horns became still wider and the lateral nuclei were more organized than the medial nuclei. Dorsomedial nucleus was better organized all along the cervical enlargement at this age. By this age, at the cervical enlargement, the lateral nuclear group presented central, ventrolateral, dorsolateral and retrodorsolateral nuclei. From the end of third month onwards, the ventral horns bulged laterally with nuclear groups well developed but at T1 the ventral horn again became narrow at the ventral side. The cell size of multipolar neurons was more at the enlargements especially towards the end of gestation. Kappers *et al.* (1967) opined that the increased size of the ventral horns is attributed towards the increased need for the origin of nerve fibres for supply towards the limbs in vertebrates. This is found true in the present study also.

5.3.2.26.3 Thoracic Region

This region presented the second lowest value for the total width behind sacral region in the precoccygeal region during last three months of gestation. This agrees with the findings of Truex and Carpenter (1969) who opined that the thoracic segments had smaller size and thinner gray columns. The smaller diameter of slender thoracic segments was primarily due to marked decrease in gray matter. Jenkins (1978) also found that thoracic segments were characterized by small amount of gray matter and comparatively large amount of white matter.

The maximum values for gray matter total height and dorsal and ventral horn heights varied between the segments. All width were more at T12 segment at all ages. During the fifth month at the T12 segment the dorsal and ventral horn width were less

compared to that of lumbar cord. But it was more prominent than ventral horn observed in T5 and T8 segments. This is in accordance with the reports of Larsell (1951) who opined that thoracic segments display slightly different appearance at higher and lower levels. The transition occurred because the cranial thoracic nerves supply motor fibres only to axial musculature, viz. back and intercostals, whereas the lower thoracic nerves supply same muscles, as well as motor fibres to abdominal musculature and therefore had more pronounced ventral horn.

The thoracic region was characterized by a narrow ventral horn, Clark's column and prominent lateral horn with intermediolateral nucleus from second month onwards. In addition to all these, reticular formation and nucleus proprius were also seen in all thoracic segments. This confirms the description of thoracic segments given by Truex and Carpenter (1969) in man and Jenkins (1978) in mammals.

5.3.2.2.6.4 Lumbar Region

The spinal cord height and gray matter total height increased from L1 towards L3 segment at all ages. The appearance at L1 was similar to caudal thoracic segments. A rounded Clark's column was present in this region. This prominent group of neurons formed a continuous column of cells from lumbar region cranially upto C8 segment. The description corresponds to that given by Larsell (1951) in man.

The lateral horn disappeared at the level of L2 and reappeared again at L4 segment. This horn represented the location of autonomic motor neurons, which are confined to the thoracic and anterior lumbar and middle sacral levels.

The lumbar region presented ventromedial and dorsomedial nuclei in the ventral horn. L1 segment also presented a well developed ventrolateral nucleus for supplying the abdominal musculature, along with caudal thoracic segments.

The dorsal funiculus was undivided in this region. This observation agrees with those of Jenkins (1978) who reported that in mammals some tracts like fasciculus cuneatus were not present in caudal part of the cord.

5.3.2.2.6.5 Lumbar Enlargement

This region presented massive gray horns with laterally bayed out ventral horns. The spinal cord height was more at L6 when compared to L4 and S2 segments at all ages, whereas the maximum value for height varied between L4 and L6

segments eventhough L6 had the maximum values for most of the age groups. The segments of this region were enlarged in area and had a greater width. The total width of spinal cord, distance from central canal to spinal cord left margin and ventral horn width were more at L6 segment at all ages, but the maximum values for distance from central canal to gray matter left margin and dorsal horn width varied between L4 and L6 at different ages. Truex and Carpenter (1969) observed that these changes were the result of the large number of incoming sensory fibres and outgoing motor fibres of the lumbar and sacral plexuses.

The gray matter height expressed as percentage of spinal cord height was maximum at lumbar enlargement in all age groups from third to fifth month. The maximum mean value for the distance from central canal to left gray matter margin was recorded at lumbar enlargement during the period from second to fifth month of gestation followed by cervical enlargement. Dorsal and ventral horns were widest at lumbar enlargement during this period indicating that eventhough cervical enlargement was having greater width than lumbar enlargement at fourth and fifth month of gestation, its increased width was mostly due to increased amount of white matter at cranial levels of the spinal cord. The well developed lumbar enlargement region is a special feature associated with goats, as they use the hindlimbs more, due to their peculiar grazing habits.

The ventral horn height expressed as percentage of gray matter total height exceeded that of dorsal horn for major part of gestation at lumbar enlargement. The ventral horn bayed out laterally and presented prominent nuclear aggregations. By the middle of second month, the ventral horn became wider and exhibited ventromedial, ventrolateral, dorsomedial and dorsolateral nuclei but the dorsomedial nucleus was dispersed in form. Towards the end of second month, the nuclear groups were larger indicating that the ventral horn became functional by this age.

5.3.2.2.6.6 Sacral Region

The height and width were less at the sacral region when compared with other regions located at its cranial level. The percentage contribution of the dorsal horn to spinal cord height was the greatest at the sacral region upto the third month, but for last two months, this region acquired the second position, where the maximum value was at the lumbar enlargement. This indicated the presence of a proportionately larger

dorsal horn in this region. As to the percentage increase of spinal cord width, central canal to spinal cord left margin and central canal to gray matter left margin, the sacral region showed a decreasing trend towards the end of gestation. Pearson and Sauter (1971) also noticed that the gray matter became reduced in size and spread dorsally in the caudal sacral and coccygeal segments of the spinal cord in man, rabbit, monkey embryos and foetuses.

From second month onwards, at segment S1, the spinal cord was smaller when compared with lumbar segments and at S2 and S4 it was still smaller. This may be due to the lesser differentiation of spinal cord towards its caudal region.

Central canal became narrower at S1, was elongated and located more ventrally at S2 and was wider at S4 segment. This contributes for the formation of the terminal ventricle to enhance the flow of the CSF towards the caudal part of the spinal cord.

Eventhough dispersed substantia gelatinosa and undivided dorsal funiculus were seen at S1 segment during second month, from fourth month onwards S1 showed a large substantia gelatinosa with rounded periphery. S2 was with a fused dorsal horn and substantia gelatinosa at all ages but between the dorsal funiculus still a gap existed at the end of third month, which represented a dispersed dorsal median septum. These findings are in accordance with the reports of Truex and Carpenter (1969) that the sacral segments had small diameter, thick quadrangular gray matter with pronounced substantia gelatinosa, short gray commissure and small amount of white matter in man.

Nucleus proprius and reticular formation were seen at S4 level. So the reticular formation extends all along the length of the spinal cord.

The lateral horn and intermediolateral nucleus was seen upto S2 segment. It was dispersed thereafter. It indicates the location of autonomic visceral neurons in the sacral region.

The ventral part of the spinal cord and the vental horn were wider upto S2 segment. The ventral horn projected laterally and had well developed nuclear aggregations at S1, which formed a part of the lumbar enlargement. At S4 segment, the nuclear aggregations were dispersed, but the ventromedial and dorsomedial nuclei

were seen. Jenkins (1978) also opined that the cord was with ventral aspect larger than the dorsal at the sacral region.

From S4 segment onwards, the white matter was decreased in amount. By the fifth month, at S1 segment the dorsal white commissure could be seen as white fibres crossing from one side to the other and connecting the dorsal part of the two halves of the spinal cord. At S4 segment, the gray commissure was shorter and the distance between the two substantia gelatinosa and the dorsal horn was reduced. These changes indicated a lesser differentiation of the spinal cord towards its caudal end, confirming the fact that the differentiation starts from the cranial level.

Sacral region exhibited an undivided dorsal funiculus and a ventral funiculus with medial longitudinal fasciculus and other tracts from second month onwards. Other fibre tracts bordering the ventral median fissure like corticospinal, tectospinal, lateral vestibulospinal and reticulospinal tracts were also seen in the ventral funiculus from fourth month onwards. In addition, the dorsal and ventral spinocerebellar tracts were seen in the lateral funiculus at S1 segment. Jenkins (1978) also observed that the dorsal funiculus of the sacral cord consisted only of fasciculus gracilis.

5.3.2.2.6.7 Coccygeal Region

The anterior segments of the coccygeal region were similar to the caudal sacral segments. The medial longitudinal fasciculus was still seen. The dorsal median septum was dispersed. The substantia gelatinosa of either side communicated. The dorsal horn was large but ventral horn was smaller. The central canal started from about the middle of the spinal cord and extended upto the ventral side. The dorsal and ventral funiculi were smaller. The lateral funiculus was wider. Jenkins (1978) also described the coccygeal segments as characterized by their small overall size, reduced amount of white matter and many nerve roots were descending inside the dura, entering into the formation of the cauda equina.

Still caudally, the structure was similar but spinal cord became smaller in size, the gray matter was reduced and the region presented the terminal ventricle. At the conus medullaris the structure was simple and was similar to the neural tube. The ependymal layer was extensive and was composed of neuroepithelial cells. The mantle layer or the gray matter was thin and was composed of neuroblastic and spongioblastic nuclei. Only very few cells were seen next to the ependymal zone in

the gray matter. The white matter was more extensive. The central canal extended dorsoventrally and formed the terminal ventricle. Vessels were seen on the ventral and dorsolateral aspects. At caudal coccygeal region, nerve bundles were seen inside the dura.

The findings in the present study, concur with those of Pearson and Sauter (1971) who reported that with the disappearance of the tail, there was a dedifferentiation of the caudal end of the spinal cord in man. The lower end of the spinal cord developed upto a point and then there was reverse differentiation of the tissues in the cord to a more primitive state. The lower end of the cord continued to develop but in a reverse order. For this reason, the coccygeal segments of the cord were better developed in embryos than in older foetuses. In younger embryos the coccygeal cord had developed ependymal, mantle and marginal layers. However, in older foetuses the mantle layer gradually disappeared in the lower coccygeal segments leaving an ependymal layer covered by a marginal or fibre layer. The precoccygeal segments of the cord were affected very little by the process of dedifferentiation and continued in a progressive developmental pattern. The transition from the well-developed sacral cord of an older foetus into the atrophic coccygeal cord was abrupt, involving only one or two segments.

5.3.3 Spinal Nerves

In the first month of gestation by 24 days, the dorsal and ventral roots of spinal nerves were formed. The dorsal roots formed a compact bundle where as the ventral root consisted of separate fibre bundles. As per the reports of Keith (1947) it was in the early second month of gestation in man, the fibres of the dorsal root entered the marginal zone of the dorsal side of the cord and formed the rudiment of dorsal funiculi.

Papez (1967) reported that the fibres of the dorsal root conduct impulses from the periphery towards their afferent cell bodies in the dorsal root ganglia. He also reported that their axons, which enter the tip of the dorsal horn form medial and lateral divisions. The medial division carries impulses of touch, pressure or kinaesthesia (vibration sense) and terminates in the gracile and cuneate nuclei of medulla. Some medial fibres from muscle stretch receptors, penetrate into the ventral horn, synapse and form the basis of the monosynaptic segmental reflex arc, which is

responsible for various 'jerk' reflexes. Some other medial fibres carry proprioceptive sensations and terminate in the Clark's column, which in turn gives rise to the dorsal spinocerebellar tract. All these impulses are confined to the side of the cord on which they entered. The fibres of the lateral division of the dorsal root end in synaptic contact with the cells of the dorsal horn, so that two kinds of secondary fibres arise. The long ascending axons cross over to the ventral and lateral funiculi of the opposite side of the cord and extend upto thalamus, tectum, medulla or to the reticular formation. The longest fibres carry impulses of pain, temperature and touch. Short (spino-spinal or spinal reticular) axons run up or down the cord, crossed or uncrossed, to make contact with other cells in the spinal cord. The axons of the ventral horn cells (lower motor neurons) emerge out as the ventral root and forms the final common pathway whereby motor impulses are transmitted to effector organs.

Ulinski (1997) also stated that each segment of the cord contained motor neurons, whose axons exit the cord via a single ventral root, and each segment was related to a dorsal root ganglion that sends sensory fibres into the cord.

5.3.3.1 Dorsal Root Ganglia

In the first month by 24 days, the longitudinal section of curved back exhibited that, the dorsal root ganglia were lying in a series like bead-like enlargements, one behind the other at regular intervals, agreeing with the position of somites. This indicated that the segmentation of the neural crest had taken place by 24 days in the present study. This was noticed by Rugh (1964) in pigs before 10-somite stage (before 15 days), Reddy (1972) in bovine embryos at 26 days and by Engel and Draper (1982a) in dogs at 24 days of gestation. Rugh (1964) also reported that in 10 mm pig embryos by 22 days, these ganglia began to send peripheral and central outgrowths. So the formation of ganglia occurred at the same stage corresponding to that in pigs, earlier than that in dogs and at a later period than that in the bovine.

Arey (1957) also found that the proliferating crest cells gave rise to bead-like enlargements, the dorsal root ganglia, interconnected for a short time by parts of the crest substance which disappeared later.

At all levels the dorsal roots joining the ganglia could be seen, which indicated that the angulation of spinal nerves was yet to start by 24 days. The supporting elements of ganglia were without any clear-cut cell boundaries, in the meshes of

which the neuroblasts were found. These findings were in accordance with those of Arey (1957) in man. As per earlier reports (Ulinski, 1997) the dorsal root ganglia were aggregations of neural crest cells found in each segment of the body. The neurons in the ganglia were sensory cells and bring information from the body surface into the spinal cord. The ganglia were also clusters of nerve cells involved in carrying information to or from the brain.

In the present study, the cell boundaries could not be differentiated upto 48 days. Thereafter, the ganglionic neurons and satellite cells became distinguishable. The satellite cells had rounded nuclei and appeared identical with oligodendrocytes. In the fifth month, some satellite cells were found to coil around axons forming myelin sheath. These were according to the reports of Harrison (1978) who also opined that some of these cells developed into neurilemmal cells. According to Jenkins (1978), the satellite cells were of ectodermal origin and homologous to the Schwann cells of the peripheral nervous system. Banks (1981) described that these cells formed a cellular capsule around the cell bodies of ganglionic neurons and this capsule is probably continuous with the sheath of Schwann. He also opined that these cells are probably closely related to oligodendrocytes.

In addition to satellite cells, which presented round nuclei, a second type of supporting cell, the capsule cell, could be identified from 124 days onwards. The capsule cells had fusiform, darkly stained nuclei and formed an outer layer covering the inner layer of satellite cells. Jenkins (1978) stated that these cells are of mesodermal (connective tissue) origin and extend to become continuous with the endoneurium of the peripheral process of the neuron. He also opined that this layer of capsule cells should not be confused with the capsule of the entire ganglion.

The nucleus was round, vesicular and eccentrically placed upto fifth month of gestation, representing the intermediate neuroblast but towards the end of gestation, the nucleus became centrally positioned. So in the present study, a definite neuroblast occurred only towards the end of gestation. Pannese (1974) also observed that in chick embryos, the intermediate neuroblast presented eccentric nucleus. He opined that the nuclear eccentricity would be indicative of a marked synthetic activity. He reported that the increase in the volume of the dorsal root ganglia occurred at the beginning due to the recruitment of additional migrating cells moving away from the

neural crest and to the proliferation of the cells, which have reached their final position in the ganglia. Later the increase in volume of the dorsal root ganglia depended on the gradual and considerable growth of the cell body of the individual elements, which differentiated into neuroblasts and then developed into mature nerve cells. Finally, the increase in number of the satellite cells and development of the interstitial spaces and blood vessels contributed to the further increase in volume of the ganglia. These observations were found true with the present study also.

Two types of nerve cell bodies were seen, large and small. Taluja and Shrivastava (1988) also reported the presence of these two types of cells in goat foetuses. Jenkins (1978) opined that the processes of the large cell bodies are heavily myelinated whereas those of the small cell bodies are very thinly myelinated or nonmyelinated. In the presence study also, some nerve fibres possessed thicker myelin sheath than others and some nonmyelinated fibres were also seen.

Clark (1984) opined that the neuronal cell bodies for all somatic and visceral sensory input from body to the cord was located in the dorsal root ganglia. Orendacova and Anna (1979) found that the axons of the nerve cells were equally distributed all along the circumference of the ganglion in the axis of the cervical sensory ganglia of dog.

The cytoplasm of the ganglionic neurons were granular and presented vesicles by fifth month. Manocha and Shantha (1969) opined that the cytoplasm of the ganglionic cells in rabbit was neither fluid nor semifluid but was of a soft gelatinous nature and the vacuoles in these neurons varied in size from submicroscopic to the size of lipid spheroids and were expected to provide a suitable substrate for metabolic reactions.

Nissl bodies could be identified in the cytoplasm of ganglion cells by fourth month of gestation onwards, but these finely granular Nissl bodies differed from the coarse ones, which could be observed in the multipolar neurons of the cord. Manocha and Shantha (1969) found that the amount and distribution of Nissl bodies vary with the activity of neurons and in these ganglia, the form varied from granular to large masses. Kelly *et al.* (1984) found that the smaller neurons, which gave rise to unmyelinated nerve fibres, contained closely packed Nissl substance, giving these cells a dark appearance in conventional staining. The large neurons, had Nissl

substance separated into groups by microtubules and neurofilaments, which course through the cell body and extended into axonal processes. This gave these cells a lighter appearance.

Pannese (1974) noticed that in chick embryos, the nucleus-cytoplasmic ratio was very high in undifferentiated cells and the ratio decreased later with cell differentiation. In the present study also, the nucleus-cytoplasmic ratio decreased as the age advanced. According to Adam *et al.* (1987) and Rao (1990b) the nucleus-cytoplasmic ratio was a good index of the functional activity of cells. So it indicated that towards the end of gestation the functional activity of cells increased.

The ganglia had a rich blood supply, contained nerve fibre bundles and were located within the intervertebral foramina by second month, but the presence of cortical and central regions as reported by Rao (1990b) in the ganglia in sheep, was not seen in the present study.

Ganglionic capsule was not developed in the first month of gestation. The capsule appeared by 40 days and thereafter, the ganglia presented a distinct connective tissue capsule. No comparable data is available.

5.3.4 Meninges

According to Hyman (1942), in vertebrates, the central nervous system did not fill the bony canal in which it lay, but was separated from it and protected, supported and nourished by fluid and by connective tissue membranes, the meninges. In cyclostomes and fishes there was only one layer, the primitive meninx. Amphibians, reptiles and birds had two, an outer dura and an inner secondary meninx. In mammals, there were three meninges, which consisted of from without to within: the tough dura mater and the delicate arachnoid and pia mater, both differentiations of the secondary meninx.

Only a vascular layer, which was composed of mesenchymal cells and blood cells represented the primitive pia mater of the meninges by 24 days. By 26 days, it was organized into a single celled layer of flat cells. By 27 days, the meninx presented more than one layer of flattened cells, which did not form a continuous layer especially at the dorsal aspect. This is in accordance with the reports of Keith (1947) who opined that in man, the neural tube was enclosed by the upgrowth of mesoderm

during the fourth week of gestation forming the primary sheath of the neural tube. By the middle of the second month, this sheath divided into an inner or pial layer and an outer or arachno-dural layer by a cleft, which formed the subarachnoid space. Arey (1957) also reported that all the three layers were of mesenchymal origin in man, which also was found true in the present study. In lower vertebrates, the dura developed from the surrounding mesenchyme and the arachnoid and pia were derived from the neural crest (Clark, 1984; Ghosh, 2002).

Meninges showed differentiation of an outer fibrous layer, which represented dura mater by 40 days, in addition to the existing vascular layer of the first month. But the arachnoid was formed only by 48 days. Thus, the three layers of meninges could be separately differentiated as distinct membranes by 48 days in the present study, where the total gestational length was 150 days.

In dog, it was between 24 and 28 days of gestation that the primitive meninx differentiated into an endomeninx and an ectomeninx (Engel and Draper, 1982a). By 41 to 44 days of gestation, in dog the ectomeninx (the future dura) was separated from the mesenchyme that formed the periosteum of the vertebral arch and the endomeninx was also differentiated into the arachnoid and pia mater with a subarachnoid space containing numerous arachnoid trabeculae (Engel and Draper, 1982b).

As per these reports, in dog, which is having a gestational length of 60 to 70 days, the differentiation of meninges happened only by 41 to 44 days. In human foetus with a gestational length of 40 weeks, it happened during the 14^{th} week of gestation (Arey, 1957). So, the differentiation of meninges happened at an early stage in the goat foetus, which is considered as an early maturing young one than the other two. This also accounts for the functional competancy of goat at birth. No other data are available in goat foetuses for comparison.

Meninges were attached to dorsal wall of the vertebral column by 48 days of gestation. Vertebral arches were not united. Keith (1947) found that the arches do not unite upto third month of gestation in man.

5.3.4.1 Dura Mater

Dura mater differentiated by 40 days consisted of a thin connective tissue layer. Later, it became thicker and contained collagen and elastic fibres and blood

vessels by third and fourth month. This description is in accordance with the findings of Fletcher (1993).

By 48 days, the dura was closely adherent to the internal surface of vertebral canal dorsally. No space was seen between the meninges dorsally, but ventrally the vertebral body and laterally vertebral laminae were seen. On the ventrolateral aspect the meninges were separated from the vertebral canal by an epidural space. At this ventrolateral aspect, the three layers of meninges could be identified.

Between dura and the arachnoid was the subdural space. According to Truex and Carpenter (1969), the spinal dura contains lymphatics, which open on both of its surfaces. The subdural space was moistened by fluid and communicated by clefts with the tissue spaces in the sheaths of nerves and through them with the deep vessels of the neck and groin. The subdural space has no direct communication with the subarachnoid space.

The midline fold of dura as reported to be present in the lumbar region by Parkin and Harrison (1985) was not observed in the present study.

After the level of conus medullaris, the dura formed the coccygeal ligament, which anchored the spinal cord in the vertebral canal (Jenkins, 1978). The ligament extended caudally to attach to the fourth coccygeal vertebra, so that the caudal part of the dural sac was occupied by the filum terminale and cauda equina. Since the spinal cord terminated at the caudal part of second sacral vertebra towards the end of gestation, this region is most suitable for tapping the cerebrospinal fluid, since there is less chance for injury to the spinal cord.

An epidural space containing blood vessels and connective tissue was seen between vertebral canal and dura mater. Epidural fat could be demonstrated around the nerve roots as brown deposits towards the end of fifth month of gestation. Ramsey (1959a) opined that, in cat since it was almost without fibrous elements, it was well adapted for lubrication of the moving structures about it. Other fat deposits were different from the epidural fat in that these were modified with increased amounts of connective tissue to withstand pressure. It also gave insulation to the dural contents, viz. nerve roots and blood vessels, against shock and mechanical injuries from surrounding bony parts. Ramsey (1959b) also reported that the distribution of epidural fat was characteristic for each species with more fat being found dorsally

than ventrally and more in the caudal half of the epidural space than in the dorsal half in general, being disposed metamerically at the intervertebral junctions. The difference in the distribution in the present study may be attributed to changes during the developing stages. Banks (1981) opined that the brown fat represents a stage in the development of the white fat. The brown colour of this tissue results from numerous mitochondria and the corresponding high quantities of the cytochrome oxidase system.

5.3.4.2 Arachnoid

Arachnoid consisted of several thin connective tissue trabeculae extending between dura mater and pia mater by 48 days. A subarachnoid space could be seen between arachnoid and pia mater and contained arachnoid trabeculae, blood vessels and nerves. These findings agree with those of Fletcher (1993) who also reported the presence of macrophages sporadically in the arachnoid of domestic animals. Macrophages were not observed in the present study.

Bowsher (1967) reported that it was through the caudal end that, the neuraxis was invaginated into its membranes, which were called the leptomeninges. The parietal layer was called the arachnoid mater and visceral layer formed the pia mater. Truex and Carpenter (1969) opined that the subarachnoid space may be regarded as dilations of pial spaces by which the embryologically single leptomeninx is transformed into the double, incompletely separated pia-arachnoid of the adult. They also observed that the subarachnoid space in life is filled with the cerebrospinal fluid, which acts as a protective layer that cushions and buffers the delicate neural tissues against trauma. The fluid also provides a pathway for chemical substances to reach the intercellular spaces of the brain, as well as pathways for neural metabolites.

The arachnoid, trabeculae and epipial surface all were covered with flattened cells in the present study. Fielder and Drommer (1976) stated that in pig, the subarachnoid space was lined with a layer of mesothelial cells, which was confirmed in the present study. Truex and Carpenter (1969) found that when certain substances are injected in the subarachnoid space, these cells may swell and participate in phagocytic activity by ingesting particles of the foreign material. They may even become detached and form free macrophages.

5.3.4.3 Pia Mater

Pia mater was adherent to the spinal cord, covering all sulci and fissures and was a thin membrane composed of flattened cells and intermittent blood vessels by 48 days. This finding is in accordance with those of Bowsher (1967) who also reported that, of the two layers of the leptomeninges, the visceral layer was closely adherant to the nervous substance and formed the adventitial coat of its blood vessels.

The pia was incorporated into the dorsal median septum and dipped into the ventral median fissure and presented blood vessels. The description concurs with that made by Sisson and Grossman (1953) and Jenkins (1978).

The pia mater consisted of two layers, viz. the inner intima pia and the outer epipial tissue. According to Truex and Carpenter (1969), like arachnoid the intima pia is avascular and derives its nutrition by diffusion from the cerebrospinal fluid and underlying nervous tissue. They also reported that in the interstices of the epipial layer are irregular spaces filled with cerebrospinal fluid, which communicate superficially with the subarachnoid space and more deeply, with the perivascular spaces of the nervous tissue.

The intima pia was firmly anchored to the surface of the spinal cord by the external (superficial glial) limiting membrane. At points where blood vessels entered the spinal cord, the intima pia invaginated to form the outer wall of the perivascular space. The blood vessels, including the ventral spinal artery, lay within the epipial layer. These observations are in accordance with those made by Truex and Carpenter (1969), who observed that the arteries of the brain and spinal cord are invested with connective tissue of the pia-arachnoid as they lie in the subarachnoid space. They observed that the pia and subjacent glial membrane blend with the vessel wall before it penetrates into the substance of the brain or spinal cord. The smaller branches of the arterial tree, within the nervous tissue, have only thin neuroglial membrane investments, which persist down to the capillary level. The capillary endothelium, its continuous and homogenous basement membrane, and the numerous processes of the astrocytes are all that separate the plasma in the vessel from the intercellular spaces within the central nervous system. These are the structures that constituted the blood-brain barrier, which functions as a filter that permits the exchange of many substances

from blood to the extracellular compartment, viz. intercellular fluid or interstitial fluid. It appears to be impermeable to other substances like vital dyes.

Lateral condensation of epipial layer was seen on the sides of spinal cord forming the dentate ligament, which anchored the spinal cord within the dura mater. This was in accordance with the findings of Fletcher (1993).

The epipial layer surrounding the brain and spinal cord was interrupted at the attachments of the spinal nerves. The glial fibres and astrocytes were prominent in the regions where the dorsal and ventral spinal roots penetrated the pia mater. Truex and Carpenter (1969) reported that in the region of the dorsolateral sulcus, such glial elements constitute a barrier to the regenerating nerve fibres of injured dorsal doots.

Morse and Low (1972a) described the pia as a complete cell layer, but Fielder and Drommer (1976) found that in pig the spinal pia mater was fibrous, composed mainly of collagenous fibres. The prolongations of the pia-arachnoid as reported to be present in relation to the dorsal extremity of the dorsal median septum in mice, by Nawar (1980), were not seen in the foetal goat in the present study.

5.4 HISTOCHEMISTRY

5.4.1 Carbohydrates

The luminal content and ependymal cells stained positive with PAS and Best's Carmine. This is in accordance with the reports of Sarnat *et al.* (1975) in cats, dogs and monkeys and Taluja and Shrivastava (1988b) in goat foetuses.

In addition to this, neurons of the spinal cord, neuroglia and neuropile and spinal meninges were also positive for Best's Carmine. These observations concur with the findings of Sahu and Rao (1975), who found the presence of glycogen in all components of the spinal cord and spinal meninges except the neuronal nucleus in buffalo. They observed that it was depleted from all the sites following injury due to trauma. Sarnat *et al.* (1975) also reported that the neuropile of gray matter, composed mostly of neuroglia, was rich in glycogen in various vertebrates including cat, dog and monkey. Taluja and Shrivastava (1988b) also found a similar reaction in ependyma and nucleus and cytoplasm of multipolar neurons of spinal cord in goat foetuses.

Kappers *et al.* (1967) pointed out that glycogen is known to be an oxygen storer and its presence points to a role in the metabolism of the nervous system. Truex and Carpenter (1969) opined that glycogen disappeared after birth or at the beginning of aerobic respiration, suggesting that the developing nervous tissue uses the energy released by the anerobic metabolism of glycogen. Jenkins (1978) reported that glycogen was not found in the nervous system of the adult, but within the embryo, it was found in the ependymal cells, choroid plexus and neurons. Sadler (1978) reported that the extracellular macromolecules like carbohydrates increased immediately prior to the neural tube closure in mice but decreased after the contact between opposing neural folds was initiated.

5.4.2 Proteins

The Nissl bodies appeared in the neurons of the spinal cord by fourth month, but were not demonstrable with the usual histological staining methods as Haematoxylin and Eosin, but needed special staining methods like PTAH and Aldehyde Thionine methods. Taluja *et al.* (1991) also could demonstrate the Nissl bodies at the last trimester of gestation.

The Nissl bodies became better developed, were larger and had a coarse appearance from the beginning of the fifth month. Nissl bodies appeared in the ganglionic neurons also from fourth month onwards. They differed from those of the multipolar neurons in that they had a finely granular appearance. Manocha and Shantha (1969) also found that in large motor neurons of spinal cord in rabbit, these bodies formed large, block-like masses ('Nissl- Schollen') in the neuroplasm of the cell body and proximal part of dendrites.

Bowsher (1967) stated that the amount of Nissl substance in a cell diminished after prolonged activity. According to Jenkins (1978), the Nissl bodies are the counterparts of the granular endoplasmic reticulum in other cells and decrease in number as a result of physiological changes, viz. in old age, overexertion, disease and metabolic disturbances. It is also thought that the Nissl bodies are concerned with protein synthesis and the replacement of neuroplasm. The injuries to any part of the neuron may be reflected by a change in the distribution and number of the Nissl bodies within the cell body. Klemm (1996) opined that most of the protein synthesis

in the nervous system occurred in Nissl granules and these proteins in general occurred in complex with lipids.

5.4.3 Lipids

The progressive myelination during foetal life was subjected to variation between regions and groups. In the present study, the lipids appeared for the first time in the white matter during the third month of gestation, eventhough a well defined myelin sheath was observed around the axons only towards the end of gestation. Clark (1965) also reported that before any fibres at all had acquired a medullary sheath, the myelin substance was already present in nervous tissues. According to Banks (1981), the myelinated fibres are the largest and most rapidly conducting fibres in the body. The myelin functions in a manner similar to the insulator on an electric conductor.

The onset of myelination in the present study corresponded to the reports in the other species. The myelin first appeared in the spinal cord by the middle of the foetal life in man (Arey, 1957), in the 17^{th} week of pregnancy in cattle (Kostyra, 1958) and even in the premyelination period (36^{th} to 60^{th} day of foetal life) in the pigs (Majstruk, 1967). According to Majstruk (1967), in pigs (active myelination) the lipid content, mainly of cerebrosides, increased in the thickening myelin sheaths from the 66^{th} day of foetal life. Berthold *et al.* (1983) and Nilsson and Berthold (1988) reported that in cat, the myelination started between 40^{th} and 45^{th} days after mating.

By the third month of gestation, the tracts bordering the ventral median fissure showed clear presence of lipids. This was in confirmation to the finding that the tracts in the ventral funiculus undergo myelination first showing that these tracts are phylogenetically older than those at the other funiculi. According to Sturrock (1982b) in rabbit also, the myelinated axons first appeared in the ventral white matter at 24 days and in the rest of the white matter at 26 days.

An early myelination in the ventral funiculus, which contains the reticulospinal tract and vestibulospinal tracts concerned with the extensor mechanisms and the maintenance of postural tone, accounts for the well established ability of the newborn kid to maintain posture even at the day of birth.

Kappers *et al.* (1967) suggested that a nerve fibre becomes functionally active when it becomes myelinated and it is essential for a nerve fibre to become myelinated

before it can participate in reflex acticvity. Langman (1981) opined that the tracts in the nervous system became myelinated at about the time they started their function.

According to Patterson *et al.* (1971) in sheep, the chemical growth (accumulation of DNA, protein and lipids) proceeded linearly in the spinal cord and the cord differed from the remainder of the central nervous system in growing more after the period studied (i.e. 50-day-old-foetus to five-week-old lambs) than before it. This was attributed to lipid accumulation. The present study confirms this observation as the intensity of reaction, which indicated the presence of lipids, increased as the age advanced. Jenkins (1978) stated that the rate of nerve impulse conduction tends to be directly proportional to the thickness of myelin sheath.

Towards the end of gestation, concentric layers of myelin also could be observed around axons. Jenkins (1978) also described that chemically myelin is a lipoprotein consisting of thin concentric lamellae of protein alternating with layers of lipids in mammals. Banks (1981) opined that the formation of myelin sheath influences the speed of conduction along nerve cell processes. So an early myelination in the white matter in the present study, may contribute to the faster rate of sensory perception and well developed motor activities of the new born kid.

Gray matter also was diffusely positive for fat. It may also be due to the presence of myelinated nerve fibres. Majno and Karnovsky (1958) also demonstrated that the phospholipids were scattered over the white matter and partly over the gray matter.

According to Clark (1984) in mammals, the input towards the lamina I appears to arise primarily from fine myelinated (and perhaps some nonmyelinated) fibres of primary afferent source. In the present study the lamina I did not show any myelinated fibres even in a full term foetus.

Lamina II was negative for myelinated fibres and it is in accordance with the findings of Kappers *et al.* (1967), who reported that substantia gelatinosa contained only nonmyelinated fibres.

5.4.4 Oxidative Enzymes

Alkaline phosphatase activity was noticed in blood vessels, nucleoli of neurons and neuropile. Strong alkaline phosphatase activity was also noticed in the

white matter, glial cell nuclei, ependyma and internal and external limiting membranes. These observations are in accordance with those made by Manocha and Shantha (1969) in rabbit spinal cord. Sahu and Rao (1975) noticed that in buffalo, alkaline phosphatase was present in the nucleus and on the neuronal surface. In goat foetuses, Taluja and Shrivastava (1988b) observed a weak alkaline phosphatase activity in multipolar neurons of the spinal cord. This reaction was not found in the present study and the cytoplasm of neurons showed a negative reaction. The enzyme plays an important role in the movement of ions or organic molecules across the cell membranes.

The alkaline phosphatase activity was more in the white matter, where differentiation was going on, than in the gray matter, which exhibited a better differentiation in the form of neurons towards the later stages. The gray matter including the cell bodies of neurons showed only a diffuse positive reaction. This concurs with the observations of Najpande and Srivastava (1974) who reported that the enzyme is localized in the areas where active differentiation is taking place, while it disappears from other parts where differentiation phase has already been completed.

Acid phosphatase was localized in the neuroglial nuclei, which showed strong positive reaction. Truex and Carpenter (1969) reported that the ependymal epithelium exhibited high oxidative activity as reflected by its high enzyme content, viz. acid and alkaline phosphatases and adenosine triphosphatase. Cox (1977) opined that the normal astrocytes had a low activity of oxidative enzymes but a vigorous activity of glycogen-synthesizing enzymes, whereas reactive astrocytes showed considerable activity of oxidative metabolism.

Moderate acid phosphatase activity was seen in the cytoplasm of ganglionic neurons. Manocha and Shantha (1969) also noticed the acid phosphatase in these locations, in rabbit. Sahu and Rao (1975) found that the acid phosphatase activity increased in injured neurons in the spinal cord of buffalo. Acid phosphatase activity was indicative of the lysosomal activity. So the activity was reduced in the foetal stage in the present study.

Ganglionic substance exhibited the presence of pigment granules in the myelin as brown deposits along the course of the nerves in the ganglia in a full term foetus when stained for the enzymes. These deposits were not observed in the gray matter or

white matter. Manocha and Shantha (1969) reported that porphyrin was present in the myelin and this accounts for the presence of brown pigments along the course of the nerves in the ganglia. Jenkins (1978) stated that the number of iron containing granules increases with age, but their true significance is not known. According to Banks (1981), oligodendrocytes and Schwann cells are responsible for myelin formation in the central and peripheral nervous systems respectively and their methods and end products are slightly different. This difference may probably account for the absence of pigment granules in the gray and white matter.

Summary

6. SUMMARY

Prenatal development of the spinal cord in goat was studied using 52 foetuses of various ages, to trace its morphogenesis and histogenesis.

As in other mammals, the spinal cord formed a long, roughly cylindrical structure with cervical and lumbar enlargements. It was anchored in the vertebral canal, enclosed by meninges from second month of gestation onwards. The cord was protected by the epidural fat towards the end of gestation. It terminated as the conus medullaris, which varied from the level of fourth coccygeal vertebra by 54 days of gestation to caudal end of S2 vertebra towards the end of gestation.

The weight, volume and length of the cord had significant correlation in between them and with age, body weight, CRL and vertebral column length and other body parameters. The rate of increase in cord weight was more during fifth month. The spinal cord weight contributed 0.980 per cent to the body weight during the second month, which decreased to 0.552 per cent by fifth month. The cord weight was dependant on its volume. The weight and volume of the spinal cord were highly dependant on age, body weight and spinal cord length, with more dependency on age, followed by cord length and body weight. The cord length had significant correlation with vertebral canal length. At all stages, the rate of growth of vertebral column and vertebral canal exceeded that of spinal cord leading to a cranial ascend of the cord in the vertebral canal after 54 days of gestation.

The maximum and minimum regional cord length was measured in the thoracic and coccygeal regions, respectively. A highly significant positive correlation was observed between total cord length and length of different cord regions except coccygeal region. Regional length of the vertebral column was dependant on the regional length of the spinal cord except at the coccygeal region and changed accordingly.

The last three cervical and first two thoracic segments formed the cervical enlargement where as the lumbar enlargement was formed by last three lumbar and first two sacral spinal cord segments. The percentage contribution to the total length of spinal cord by lumbar enlargement exceeded that of cervical enlargement at all ages, which has been attributed to the peculiar feeding habit of goats.

There were 8 cervical, 13 thoracic, 6 lumbar, 4 sacral and 5 coccygeal spinal cord segments. The longest cervical segment was C2, thoracic T13 and T12, lumbar L3 and sacral S1. Maximum width and height belonged to segments of enlargements. The size of spinal cord segments decreased after S1 segment towards the coccygeal region.

The thoracic region exhibited an increased growth rate over that of vertebral column during second and third month. Cervical and lumbar regions showed isometric growth rate during all stages. The sacral region also was isometric upto fourth month, which declined in the fifth month. The growth rate of the spinal cord segments in relation to the vertebral segments decreased in the coccygeal part of the cord.

All cord segments did not correspond to the corresponding vertebrae. Upto 54 days of gestation, the spinal cord extended the entire length of vertebral canal. In the latter half of second month, the cord terminated at third coccygeal vertebra, at third month between first and second coccygeal, at the fourth month varied from fourth sacral to first coccygeal and during fifth month from cranial end of S4 through middle of S3 to caudal end of S2 vertebra.

Each spinal nerve arose by dorsal and ventral roots. Root emergence length was slightly greater in cervical, rostral thoracic and lumbar region of the cord. It decreased through sacral region. Ventral roots attached and originated over greater area than corresponding dorsal roots. The segments of enlargements had shorter lengths for root attachment and shorter interval between adjacent roots than other segments.

The meninges became evident as distinct membranes from 48 days. The dura was a thick membrane whereas the arachnoid presented connective tissue trabeculae extending between it and pia mater. The pia mater presented blood vessels, which in the fifth month exhibited plexuses, which were more distinct at enlargements. A dentate ligament was seen as projections of pia mater upto S4 level. Epidural fat was seen around the nerve roots at the end of gestation.

Histological studies revealed that during first month of gestation, in an embryo having CRL 14 mm by the age of 24 days of gestation, the primordium of the spinal cord had the shape of a closed neural tube, with ependymal, mantle and marginal layers. The neural tube lay between body wall and notochord. The lumen was

diamond-shaped in cross-section with a sulcus limitans, which divided the sidewalls into alar and basal plates. At the dorsal and ventral walls were the roof plate and floor plate respectively. The tube was bounded by internal and external limiting membranes.

During first month, the ependymal layer was a stratified layer of neuroepithelial cells and germinal cells. The mantle layer presented neuroblasts and spongioblasts. By 27 days, the basal plate region showed beginning of differentiation of neurons. The marginal layer consisted of the processes of the neuroblasts. The dorsal, lateral and ventral funiculi started to form by 24 days. The mantle layer became reduced in the caudal sacral region and was absent in the coccygeal region. In the coccygeal region, the neural tube was simpler in structure with only the limiting membranes, thin marginal layer and a thick ependymal layer.

By 24 days, the lumen showed fusion at the ventral one-fourth in the caudal half of the spinal portion of the neural tube. The coccygeal region showed complete fusion and caudal most portion was with an oval neural tube and a rounded lumen. As the fusion progressed, by 27 days, a diamond-shaped canal was seen only in the anterior half of the cervical region.

The stage of the neural tube terminated towards middle of the second month, when the lumen was reduced by the apposition of lateral plates. By 48 days, the dorsal median sulcus, dorsal median septum and ventral median fissure were formed. The outer white matter and inner gray matter became distinguishable. The white matter presented dorsal, ventral and lateral funiculi. The gray matter exhibited dorsal, ventral and lateral horns. A dorsal intermediate groove appeared on the dorsal funiculus. By the end of second month, a ventral commissure connected the two halves of the spinal cord. The pia meter descended into the sulci and fissures and presented the linea splendens.

By third month, the dorsal median septum exhibited fibres, in addition to cells. The dorsal median sulcus became shallow at the caudal lumbar and sacral regions and was absent at the coccygeal region. At the caudal sacral region, the septum was absent.

From fourth month onwards, the central canal presented an oval shape. A dorsolateral groove was identified and the gray and white commissures connected the

two halves of the spinal cord. At this age, the basic structure of spinal cord corresponded to the adult structure. By the fifth month, the lumen varied in shape from round to ovoid, dorsal median sulcus and the dorsal median septum became well developed.

The cervical enlargement had the maximum height at all ages, with maximum gray matter and dorsal horn height except at fifth month, where it was at lumbar enlargement. The maximum ventral horn height was also seen at the enlargements. Sparing a few exceptions, the percentage contribution of gray matter height, dorsal horn height and ventral horn height to the total spinal cord height showed a gradual decrease as age advanced, owing to an increase in ventral funiculus. The gray matter height expressed as percentage of spinal cord height, was maximum at lumbar enlargement. The greatest values for total width of spinal cord and distance from central canal to spinal cord left margin varied between cervical and lumbar enlargements at different stages of gestation. The total width of spinal cord was greater than the height all levels studied during second to fifth of gestation except at thoracic region in the second month. Dorsal and ventral horns were widest at enlargements. The percentage of gray matter width decreased after third month of gestation indicating a comparative increase in the growth of lateral funiculus at later stages of gestation. Vertical gray matter percentage decreased after second month especially at cervical enlargement and cervical regions indicating an early growth of ventral funiculus.

The multipolar neurons first appeared in the ventral horn, by the beginning of second month, indicating an earlier development of motor activities than sensory functions. The cell boundaries of neurons became clear and the cells presented Nissl bodies by fourth month. The cytoplasmic content increased and the Nissl bodies became well differentiated by the fifth month, which was indicative of progression towards the functional maturity. The shape of the neurons varied according to their location. The nucleus was centrally positioned in all neurons by the fifth month, but an eccentric nucleus was found in the Clark's column.

Of the four types of neuroglia, the ependyma presented a stratified layer around the central canal from the beginning of gestation and developed cilia from the middle of second month. From fourth month onwards, they formed a pseudostratified

layer. The cilia were short or absent upto lumbar region towards the end of gestation, but were still present at sacral and caudal levels. The astrocytes appeared by the beginning of the second month. At all ages, the density of neuroglia were more than that of the developing neurons. The protoplasmic astrocytes had a star-shape, oval nucleus and wavy and branching cytoplasmic processes. Fibrous astrocytes had elongated thin nuclei and longer processes. From the middle of the second month onwards, astrocytic nuclei were pale. Astrocytes were the largest among neuroglia. They had perivascular feet and formed glial limiting membrane and the septa. Oligodendrocytes appeared by the beginning of the second month and had smaller, dark and rounded nuclei, fewer and smaller processes than those of astrocytes, and scanty cytoplasm. They occurred as perineuronal satellites, interfascicular cells and juxtavascular cells and formed myelin sheath towards the end of gestation. Microglia appeared by fourth month and occurred both in white and gray matter, being more in the latter.

The gray matter was a mass of nerve cells, neuroglia and fibres. The processes of all developing cells made up the background meshwork, the neuropile. The dorsal horn consisted of an apex, head and cervix. The dorsal horns presented a structure similar to that of the adults only by fourth and fifth month, when it presented a marginal zone, the substantia gelatinosa, nucleus proprius, nucleus dorsalis and reticular nucleus. The dorsal horn was narrow in the thoracic segments and wider at enlargements. The ventral horn contained the motor cells, giving rise to the fibres of the ventral root of the spinal nerves. The lateral horns projected laterally from the intermediate gray matter from the middle of second month onwards and was prominent at thoracic, anterior lumbar and middle sacral levels.

The lamination of gray matter started towards the end of second month, as the ventral horn presented the nuclear aggregations in the lamina IX especially at enlargements. By the beginning of the third month, the lamina II and VII also appeared and towards the end of third month, other laminae started to differentiate and became better differentiated by the fourth month.

The lamina I appeared from third month onwards and covered the surface of the dorsal column as a cap with a thin layer of scattered cells of varying size and shape. The cells were arranged tangential to the surface of the dorsal horn and

extended throughout the length of the cord. From fourth month onwards, this lamina became measurable as a layer. By the fifth month, the lumbar enlargement presented more number of cells.

Lamina II corresponded to the substantia gelatinosa and extended the entire length of the cord. This formed a cap over the dorsal horn and was located beneath marginal zone. This layer was organized into form, presented prominent fibres and convolutions towards the end of third month. It presented different shapes and two types of cells, which were tightly packed upto fourth month. Later, the layer was penetrated by fibre bundles and the cells became loosely packed. The size was more at enlargements.

Lamina III was represented by a less packed area with larger cells. It appeared by the end of third month. By the fifth month this zone entered into the substantia gelatinosa.

By the end of the third month, lamina IV appeared as an indistinct cell column and even by the fifth month, it was poorly defined and presented nucleus proprius, which was larger in the lumbosacral region.

Lamina V started to appear by the middle of the second month and presented nucleus reticularis and reticular processes with small to medium-sized cells. The lamina had medial and lateral divisions from fourth month onwards and extended the entire length of the spinal cord.

Lamina VI became identifiable as a broad layer with indistinguishable boundaries by fourth month, lateral to the central canal and consisted of a compact medial zone and a lateral less compact zone.

Lamina VII represented the intermediate zone of gray matter, which presented intermediolateral nucleus, intermediomedial nucleus, cervical nucleus of Stilling and Clark's column.

Cervical nucleus of Stilling was noticed by the end of third month in the cervical region at the base of dorsal column. At the same position in sacral region, sacral nucleus of Stilling was seen.

The lateral horn with intermediolateral nucleus extended from C8 or T1 to anterior lumbar segments and was seen at sacral region. The neurons were not as

differentiated as that of the ventral horn. The nucleus was better differenciated with spindle-shaped cells by the end of third month, was seen as a clear cell column in lateral horn and included two dispersed nuclear groups by fourth month, viz. the medioposterior and intercornual columns.

The intermediomedial nucleus appeared for the first time in the present study by 81 days in the thoracic and lumbar regions ventral to Clark's column. By 142 days, the nucleus became better developed but was smaller at L1 and T12. The Nissl bodies were finer in the intermediomedial and intermediolateral nuclei.

Clark's column was an indistinct column by 62 days, seen at the lateral aspect of the central canal from T1 level extending backwards. It became clear by 81 days at the anterior lumbar region. By fourth month, it had neurons with eccentrally placed nucleus. By fifth month, it was well developed from T9 to L2 level.

The shape and size of the horns varied from one region to the other so that the ventral horns were wider in the cervical and lumbar enlargements but were narrower in the anterior cervical and thoracic levels.

The lamina VIII and IX together formed the ventral horn, where a clustering of nerve cells were seen by the middle of second month at the ventrolateral aspect as nuclear aggregations. By fourth month, the nuclear aggregations were well defined especially at the enlargements. Lateral and medial nuclear masses were seen. By third month, the ventral horn presented multipolar neurous with vesicular nucleus and eosinophilic cytoplasm. From fourth month onwards lamina VIII appeared as a mixture of small and medium sized cells but was not differentiated from lamina VII. Lamina IX was composed of the alpha and gamma cells. The former were larger at the enlargements.

An increase in the white matter was noticed between fourth and fifth month and also from sacral region towards cervical region in the fifth month. The lateral funiculus exceeded all other funiculi in thickness.

The fibre tracts like fasciculus gracilis, fasciculus cuneatus, dorsolateral fasciculus of Lissaeur, medial vestibulospinal tract and medial longitudinal fasciculus could be identified by the middle of second month. For other tracts, definite levels of origin and termination were very difficult to identify except for clustering of fibres or

cells at their respective area of location. From the middle of the second month onwards, the vascularity and the cell density were increased as age advanced. Towards the end of gestation, the white matter was a mixture of myelinated and nonmyelinated axons, blood vessels and neuroglia and rare neurons.

The dorsal funiculus presented fasciculus gracilis and fasciculus cuneatus separated by the dorsal intermediate groove from middle of the second month onwards extending from the first cervical segment upto caudal thoracic region. The dorsal funiculus was undivided after this level. By fourth month, the dorsal fasciculus proprius also was seen.

Lateral funiculus presented dorsolateral fasciculus of Lissauer by the middle of second month, the spinocerebellar tracts, lateral corticospinal tract, lateral reticulospinal tract and lateral fasciculus proprius at the medial aspect of lateral funiculus by the third month and rubrospinal tract by fourth month.

All tracts of ventral funiculus except medial longitudinal fasciculus and medial vestibulospinal tract were mixed with other tracts and were not seperated into well-defined bundles. Medial longitudinal fasciculus, located on either side of the base of ventral median raphae and medial vestibulospinal tract, ventral to medial longitudinal fasciculus were seen from middle of the second month onwards. Ventral fasciculus proprius was seen bordering the gray matter by third month. Other tracts in the ventral funiculus appeared towards the end of third month, but were not well-defined bundles.

The central canal extended the entire length of the cord. The central canal was diamond-shaped upto 40 days and was reduced later, due to the union of the dorsal walls resulting in obliteration of lumen between 81 and 102 days. It was positioned eccentrically in the gray commissure by fourth month. During last two months, the lumen was a little dorsal upto lumbar region but ventral or central later to maintain the caudal flow of CSF. At sacral and coccygeal regions, the canal presented the terminal ventricle. The lumen contained a granular eosinophilic material from 40 days onwards.

The cervical region presented the maximum vertical thickness of dorsal funiculus and second highest values for ventral and lateral funiculi during the fifth month. The region presented well developed reticular formation, cervical nucleus of Stilling and spinal accessory nucleus. The nucleus proprius was ill-defined. The

dorsal horn was wider than the ventral horn. The ventral horn was with pointed ventral ends. From second month onwards, the ventral horn presented spinal accessory nucleus and phrenic nerve nucleus in addition to ventromedial, ventrolateral and in distinct dorsomedial nuclei in the ventral horn.

The cervical enlargement had the maximum height of the spinal cord during the entire gestation period. The gray matter height and dorsal horn height were maximum at cervical enlargement at all age groups except in the fifth month. It had maximum values for total width of spinal cord and distance from central canal to spinal cord left margin at fourth and fifth month. The thickness of all funiculi except transverse thickness of dorsal funiculus at the fifth month were more at the cervical enlargement than the lumbar enlargement at the last two months. The cervical enlargement had prominent appearance of graymatter. The ventral horn bulged out and from C8 onwards, intermediolateral nucleus, reticular formation, Clark's column were visible from middle of second month. From C6 onwards, the nuclear aggregations as dorsolateral, dorsomedial, ventrolateral and ventromedial nuclei were well developed in the ventral horn by the second month. From the beginning of third month onwards, the lateral nuclei were more organized and presented central, ventrolateral, dorsolateral and retrodorsolateral nuclei. The cell size of multipolar neurons was more at the end of gestation.

Thoracic region had the second lowest value for the total width behind sacral region in the precoccygeal region for last three months. During the fourth and fifth month, the minimum values for dorsal and ventral horn width were at this region. The region presented narrow ventral horn, Clark's column, prominent lateral horn, reticular formation, nucleus proprius and conical substantia gelatinosa. The ventral horn presented the ventromedial and dorsomedial nuclei.

Lumbar region presented an undivided dorsal funiculus, a rounded Clark's column, laterally elongated substantia gelatinosa, nucleus proprius and reticular formation. By the fifth month, the Clark's column and nucleus proprius were large. Lateral horn disappeared at the level of L2 segment. The ventral horn presented ventromedial and dorsomedial nuclei. L1 segment presented a ventrolateral nucleus also.

Lumbar enlargement segments had greater transverse diameter, presented massive gray columns with bayed out ventral horns and well developed nuclear aggregations. The maximum values for gray matter height expressed as percentage of spinal cord height, dorsal and ventral horn width and the distance from central canal to left gray matter margin were recorded at lumbar enlargement from second to fifth month. The ventral horn height expressed as percentage of gray matter total height exceeded that of dorsal horn. More cells per unit area were seen in lamina I. Substantia gelatinosa was laterally elongated by fourth month and was convoluted and prominent by fifth month. From the latter half of third month onwards, nucleus proprius was larger. Reticular formation, intermediomedial nucleus and well developed Clark's column were also seen. The lateral horn was seen at L4, but became dispersed at L5. By the middle of second month, the ventral horn had ventromedial, ventrolateral, dorsomedial and dorsolateral nuclei and by the end of second month, central and retrodorsolateral nuclei. Dorsal funiculus was undivided.

All height and width were less at the sacral region, but the region was with a larger dorsal horn. The percentage contribution of dorsal horn to spinal cord height was greatest at the sacral region upto three months, but during last two months, this region occupied a second position. The percentage of transverse measurements, showed a decreasing trend towards the end of gestation at this region. From second month onwards, lumen became narrow at S1, was elongated and located more ventrally at S2 and was wider at S4 segment. From fourth month onwards, S1 showed a large substantia gelatinosa with rounded periphery. S2 was with dispersed dorsal median septum, dorsal white commissure, fused dorsal horns and substantia gelatinosa, nucleus proprius and reticular formation. The sacral nucleus of Stilling was seen at S1 segment. Upto S2, intermediate horn was seen and ventral part of the spinal cord and the ventral horn were wider. From S4 onwards, the nuclear aggregations were dispersed and the white matter was decreased. This region exhibited an undivided dorsal funiculus, medial longitudinal fasciculus and other tracts from second month onwards. Tracts bordering the ventral median fissure and spinocerebellar tracts were also seen from fourth month onwards.

The anterior coccygeal segments were similar to the caudal sacral segments, with a medial longitudinal fasciculus, dispersed dorsal median septum, communicating substantia gelatinosa, large dorsal horn and smaller ventral horn. The

central canal extended from the middle of the cord towards the ventral side. Dorsal and ventral funiculi were smaller but the lateral one was wider. Still caudally, the spinal cord was similar but smaller, with reduced gray matter. It presented terminal ventricle. At the conus, the structure was similar to that of neural tube.

In the first month, the dorsal roots formed a compact bundle with spindle-shaped ganglia but the ventral root consisted of separate fibre bundles by 24 days. The angulation of spinal nerves was yet to start by first month. The ganglia were seen as aggregations of unipolar nerve cells with indistinct cell boundaries upto 48 days. Thereafter, the ganglionic neurons and satellite cells became distinguishable. The former were intermediate neuroblasts with round, vesicular and eccentric nucleus upto fifth month of gestation, but by 142 days the nucleus became centrally positioned. The nucleo-cytoplasmic ratio decreased as the age advanced. The ganglia had a rich blood supply, contained nerve fibre bundles and were located within the intervertebral foramina from second month onwards. Ganglionic capsule appeared from 40 days. The capsule also presented blood vessels.

Pia mater, dura mater and arachnoid were differentiated by 24, 40 and 48 days of gestation respectively. In the first month, a layer of mesenchymal cells and blood cells represented the pia mater by 24 days, which was organised into a single layer of flat cells by 26 days and presented more than one layers by 27 days. An outer fibrous layer representing dura mater was differentiated by 40 days, in addition to the existing vascular layer of pia mater. Meninges were attached to dorsal wall of the vertebral column by 48 days of gestation, when the arachnoid also became identifiable. Dura mater was thin at this stage, with an epidural space at ventral and lateral aspects containing blood vessels and connective tissue. Later, dura became thicker and contained collagen fibres, elastic fibres, nerves and blood vessels from third month onwards. Arachnoid consisted of thin trabeculae and polygonal cells extending between dura mater and pia mater by 48 days with a subarachnoid space containing arachnoid trabeculae, blood vessels and nerves. Pia mater consisted of collagen fibres, flattened nuclei of fibroblasts. It was adherent to the spinal cord, covered all sulci and fissures and was a thin and vascular by 48 days. A lateral condensation of pia mater, the dentate ligament, attached the pia-arachnoid to the dura. A fold of pia mater, linea splendens, extended into the ventral median fissure.

The luminal content and ependymal cells stained positive with PAS and Best's Carmine. In addition to this, neurons, neuroglia and neuropile and meninges were also positive for Best's Carmine. Proteins appeared as Nissl bodies in the neurons of the spinal cord and ganglia by 102 days. Those in the former had a coarse appearance by 124 days, but the latter was finely granular. The lipids first appeared by the third month in the tracts bordering the ventral median fissure, but a definite myelin sheath was observed by 142 days. Gray matter also showed the presence of very few myelinated fibres. Alkaline phosphatase was noticed in blood vessels, nucleoli of neurons and neuropile, white matter, glial cell nuclei, ependyma and internal and external limiting membranes. Acid phosphatase was localized in the neuroglial nuclei and cytoplasm of ganglionic neurons. Pigment granules were observed in the ganglion in a full term foetus.

This study revealed that growth of the spinal cord in goat was more dependant on age than on body weight. It had a greater growth rate during the fifth month, attained its adult structure with the neurons showing evidences of functional maturity by fourth month of gestation. Its tracts became myelinated towards the end of gestation, to make the new-born kid well suited to be classified as a mature young one.

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PRENATAL DEVELOPMENT OF SPINAL CORD IN GOATS (*Capra hircus*)

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ABSTRACT

Prenatal development of the spinal cord in goat was studied using 52 foetuses of various ages, to trace the morphogenesis and histogenesis of spinal cord and its relation with developing vertebral column, foetal age and body weight.

The weight, volume and length of the cord had significant correlation in between them and also with age, body weight, crown rump length, vertebral column length and other body parameters and also were more dependant on age, than on others. The maximum rate of growth was during the fifth month. The rate of growth of vertebral column and vertebral canal exceeded that of spinal cord.

The maximum and minimum regional cord length were measured in the thoracic and coccygeal regions, respectively. A significant correlation existed between total cord length and length of different cord regions except coccygeal region. The lumbar enlargement contributed more to the total length than cervical enlargement at all ages. The size of spinal cord segments decreased after S1 towards the coccygeal region.

The growth rate of cord regions declined in the fifth month caudal to sacral region. Up 54 days, the spinal cord extended the entire length of vertebral canal but at the fifth month, the termination was at caudal end of S2 vertebra.

By 24 days, the neural tube was with ependymal, mantle and marginal layers, diamond-shaped lumen with a sulcus limitans, alar, basal, roof and floor plates and limiting membranes.

The stage of the neural tube terminated by 48 days. The dorsal median sulcus and septum, ventral median fissure, dorsal intermediate groove, white matter with dorsal, ventral and lateral funiculi and gray matter with dorsal, ventral and lateral horns and a ventral commissure were seen. By fourth month, the basic structure corresponded to the adult structure.

The maximum values for width and height were at the enlargements. The total width of cord was greater than the height at all levels studied. The percentage of gray matter width decreased after third month indicating a comparative increase in the growth of lateral funiculus. Vertical gray matter percentage decreased earlier indicating early growth of ventral funiculus.

The multipolar neurons first appeared in the ventral horn, by 40 days. The cells had clear boundaries by fourth month. Among neuroglia, the ependyma presented a stratified layer around the central canal from the beginning of gestation. The astrocytes and oligodendrocytes appeared by 40 days. Oligodendrocytes formed myelin sheath towards the end of gestation. Microglia appeared by fourth month.

In the gray matter, the ventral horns were wider in the enlargements. The lateral horn appeared by middle of the second month at thoracic, anterior lumbar and middle sacral levels. The lamina I appeared by third month. Lamina II was organised into form towards the end of third month. Lamina III appeared by the end of third month and by fifth month entered into substantia gelatinosa. Lamina IV was poorly defined even by the fifth month, and presented nucleus proprius. Lamina V appeared by 48 days with nucleus reticularis and extended the entire length of the spinal cord. Lamina VI was a broad layer by fourth month. Lamina VII presented intermediolateral and intermediomedial nuclei, cervical nucleus of Stilling and Clark's column. The lamina VIII and IX formed the ventral horn. By 48 days, nuclear aggregations appeared and were well defined by fourth month. Medial nuclear groups were seen in all regions and lateral groups only at enlargements.

In the white matter fibre tracts developed from 48 days. The white matter increased towards the end of gestation.

The central canal varied in cross-section from diamond-shape to oval by 102 days. The sacral and coccygeal regions presented a terminal ventricle. The lumen contained a granular material from 40 days. Cilia appeared by 48 days and were absent towards the end of gestation, except at sacral and caudal levels.

The cervical region had well developed reticular formation, cervical nucleus of Stilling, spinal accessory nucleus and phrenic nerve nucleus.

The cervical enlargement had the maximum segment height. From C8 onwards, intermediolateral nucleus, reticular formation, Clark's column were seen by middle of second month.

Thoracic region presented Clark's column, lateral horn, reticular formation, nucleus proprius and conical substantia gelatinosa.

Lumbar region presented a rounded Clark's column, elongated substantia gelatinosa, larger neurons in lamina I, nucleus proprius and reticular formation.

Lateral horn disappeared at L2 and reappeared at L4 segment. The dorsal funiculus was undivided from this level onwards.

Lumbar enlargement had maximum gray matter height percentage, dorsal and ventral horn width and distance from central canal to left gray matter margin. The ventral horn height percentage exceeded that of dorsal horn. More cells were seen in lamina I. Larger substantia gelatinosa, nucleus proprius, reticular formation, intermediomedial nucleus, well developed Clark's column and nuclear aggregations were seen.

The sacral region had larger dorsal horn and substantia gelatinosa, ventrally located lumen, sacral nucleus of Stilling by fourth month at S1 segment. S2 segment was with nucleus proprius, lateral horn, reticular formation and fused dorsal horns. From S4 segment, nuclear aggregations dispersed and white matter was decreased.

The anterior coccygeal segments were similar to the caudal sacral segments. Conus was similar to the neural tube.

The ganglia were with indistinct cell boundaries upto 48 days, later the unipolar neurons and satellite cells became distinguishable. Capsule appeared from 40 days.

Pia mater, dura mater and arachnoid were differentiated by 24, 40 and 48 days of gestation respectively. A dentate ligament was seen as projections of pia mater upto S4 segment. Epidural fat appeared by the end of gestation.

The luminal content and ependymal cells stained positive with PAS and Best's Carmine. Neurons, neuroglia, neuropile and meninges were also positive for Best's Carmine. Proteins appeared as Nissl bodies by 102 days. The lipids appeared by third month. Alkaline phosphatase was noticed in blood vessels, nucleoli of neurons and glia, neuropile, white matter, ependyma and limiting membranes. Acid phosphatase was localized in the neuroglial nuclei and ganglionic neurons.