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Comparative prenatal development of the spinal cord in normal and dysraphic dogs

by

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INTRODUCTION

Spinal dysraphism is an abnormal condition in both man and animal which is characterized by a variety of structural and funtional anomalies of the spinal cord, vertebral column, muscles, and skin (McGrath, 1965; Till, 1969; James and Lassman, 1972). The disease entity in the dog affects primarily the spinal cord and, therefore, is referred to as neurospinal dysraphism (NSD). The mode of inheritance of canine NSD is a mutant gene with some degree of dominance in a one-locus, two-allele, three genotype system with some reduction of penetrance and variable expressivity (Shelton, 1977). The disease occurs naturally in the Weimaraner dog. Dogs with NSD exhibit specific locomotor and sensory deficits of the pelvic limbs, manifesting a characteristic hopping gait with synchromous movement. Frequently the legs are abducted and over-extended, and there is bilateral ataxia of the pelvic limbs. Proprioceptive and tactile reflexes are either present or absent in pelvic limbs. The histopathologic lesions of the spinal cord are varied, but include myelomeningocoele, diastematomyelia, absent or misshapen dorsal and ventral horns, hydromyelia, and syringomyelia (McGrath, 1965).

Because of the variety of lesions observed with spinal dysraphism there is discrepancy in the literature as to their critical period of development. The observation of clinical signs in newborn pups suggests a prenatal onset in the dog (Draper et al., 1975; Shelton, 1977). The question is, does the genetic expression occur during the embryonic period of organogenesis or during the fetal period as differentiation as well as organogenesis proceed? Emery and Lendon (1973) attributed the initiation

of spinal dysraphism in man to the abnormal closure of the caudal neuropore during embryogenesis. In contrast, McGrath (1965), while studying the development of the spinal cord in dysraphic Weimaraner dogs, concluded that spinal cord abnormalities were instituted during the last one-third of gestation.

The fundamental hypothesis of this research was that dysraphic changes are expressed early in the embryonic process. To confirm this hypothesis, normal and dysraphic canine embryos were surgically removed between day 24 and 28 of gestation and serially sectioned tissues of the spinal cord in the area of the caudal limb buds were compared by light microscopy. Quantification of any differences between normal and dysraphic embryos was performed by statistically comparing transverse diameter measurements of the spinal cord.

A continuation of the primary hypothesis was that established dysraphic lesions like those observed postnatally in dysraphic dogs would be present by the beginning of the last one-third of gestation. To substantiate this supposition, normal and dysraphic fetal dogs between day 41 and 44 of gestation were surgically removed and serially sectioned tissues of the spinal cord and vertebral column from the lumbosacral region were compared by light microscopy. Quantification of any differences between normal and dysraphic fetuses was performed by comparing transverse diameter measurements of the spinal cord. The dorsal root ganglia in the caudal lumbar region were compared by statistically analyzing ganglionic area differences between these two groups. It was presupposed that ganglia in the dysraphic fetuses would most likely have smaller

areas to account for the clinically observed sensory deficits in dysraphic dogs.

The premise of this study is to answer the basic question of whether NSD is a primary neural tube defect or is the consequence of secondary intervening factors. Furthermore, in course of comparison of normal embryos and fetuses with the dysraphic specimens, the normal prenatal development of the canine spinal cord was established.

Because of its natural occurrence, its genetic etiology, and its similarity to human neurospinal dysraphism, canine spinal dysraphism is an ideal model to study the development of this condition. A knowledge of the pathogenesis of NSD in dogs would aid in understanding the lesions and its clinical manifestations in man and animals.

LITERATURE REVIEW

To better understand various theories regarding the origin of dysraphic lesions, a review of the normal prenatal development of the spinal cord in vertebrates, particularly in man and dog, is necessary. This information is also required for determining the exact stages of prenatal development where similar investigations should be performed. Finally, literature pertinent to neurospinal dysraphism and its development are reviewed to determine the types of lesions likely to be encountered.

Prenatal Development of the Spinal Cord and Adnexa

The early prenatal development of the vertebrate neural tube will be reviewed with emphasis on the embryogenesis of the spinal cord in the dog and cat. The discussion of prenatal maturation of the wall of the neural tube, as its occurs in man, into a definitive spinal cord will include facts relating to the formation of the various neural plates (alar, basal, etc.), changes in the size and shape of the central canal, and myelinization. The review of prenatal development of the adnexa of the spinal cord will include information on the formation of the meninges and differentiation of the cells within the dorsal root ganglia.

The following general information relating to prenatal development of the vertebrate spinal cord is derived from research on chick, mouse, pig, and human embryos (Hamilton et al., 1962; Arey, 1965; Langman, 1975, Moore, 1977). The neural plate is the precursor of the neural tube, which in turn, evolves into the brain and spinal cord. The neural plate develops from the

neuroectoderm rostral to the level of the primitive streak. Because of ectodermal cellular proliferation at the lateral edges of the neural plate a shallow groove is formed. Further proliferation of these peripheral ectodermal cells causes the walls of the neural groove (neural folds) to become more pronounced and to approximate each other at the dorsal midline of the embryo. The folds finally fuse dorsally to form the neural tube enclosing a central canal.

Little information is available on early neural embryonic development in the dog. Houston (1968) described the prenatal development of the dog up to day 25 of gestation. He correlated the age of gestation in days and somite numeration (pairs of somites), so that comparisons between the dog and other animals could be accomplished by using either of these criteria. By embryonic day 15 the presomite embryo had differentiated into the three germ layers, with a distinct embryonic disc and primitive streak. A neural plate of three to five compact cell layers extended rostrally from the primitive streak. The presence of a primitive streak was also indicated by other investigators by embryonic day 15 in the dog (Holst and Phemister, 1971; Evans and Sack, 1973).

Houston (1968) observed the presence of four somites early on day 16 of gestation in the dog. Two distinct neural folds were present on each side of a well-defined neural plate. By the end of day 16 of gestation the embryo had reached the seven somite stage and closure of the neural tube had occurred between the second and third rhombomeres. The initial closure of the neural tube near the third rhombomere on embryonic day 16 was concordant with the findings of Evans and Sack (1973), who described the dog embryo at this stage as a neurula.

The three primary brain vesicles appeared in dog embryos during day 17 of gestation (seven to twelve somite stage) and the neural tube became completely closed even at the cranial and caudal neuropores by the beginning of day 18 (15 somites) (Houston, 1968). In contrast, Evans and Sack (1973) reported the neural tube was closed only to the level of the eighth somite during embryonic day 17.

The early neural development in the cat (Schulte and Tilney, 1915) and dog (Houston, 1968) closely resemble each other. The closure of the neural tube in the cat, however, was not a uniformly continuous process in both directions from the level of the third rhombomere. Closure was retarded at the sites of the large cranial nerve ganglia. Furthermore, Schulte and Tilney (1915) observed a delayed separation of the ectoderm from the neural tube at the sites of ganglionic enlargements.

Closure of the neural tube in man begins also at the same seven somite stage similar as the dog and cat, but initial closure occurs in the region of the fourth or fifth somite (Bartelmez and Evans, 1926) rather that at the level of the third rhombomere. The extremities of the neural tube, as in the dog, have direct connections with the amniotic cavity via cranial and caudal neuropores. These pores close in the human embryo at approximately the 20 and 25 somite stages, respectively (Moore, 1977).

During the initial closure of the neural tube, the wall is composed of a single layer of pseudostratified columnar neuroepithelial cells (Sauer, 1935). This epithelium proliferates into three relatively distinct layers, viz., an internal ependymal or matrix cell layer; an intermediate mantle cell layer; and an outer marginal layer. The neuroepithelium of the

ependymal layer serves as the origin of both primitive neurons (neuroblasts) and spongioblasts (excluding microglial primordia) of the central nervous system. When primitive neurons are produced, they migrate to the mantle layer to mature. When the production of primitive neurons ceases, the neuroepithelial cells begin to produce the spongioblasts that mature into the various macroglial cell types. The marginal layer is composed primarily of processes from the developing neurons in the mantle layer. As the spinal cord matures, some of the macroglial cells migrate to the marginal layer to support and nourish the neuron cell processes. The mantle and marginal layers eventually become the gray and white matters, respectively, of the definitive spinal cord (Langman et al., 1966).

Continued cellular proliferation by the ependymal cells produces sufficient numbers of primitive neurons resulting in ventral and dorsal thickenings of the mantle layer. The ventral enlargements later become the basal plates, which, in turn, become the ventral columns of the gray matter. The smaller dorsal thickenings are called the alar plates, which eventually become the dorsal columns of the gray matter. Expansion of the alar plates in a medial direction compresses the dorsal portion of the central canal until the two alar plates are in close apposition. The junction between these two plates becomes the dorsal median septum, which is composed of glial cell processes and pia mater. The ventral median fissure forms in the region ventral to the central canal in a similar manner to further reduce the size of the central canal, however, the ventral columns remain relatively separated as compared to the dorsal columns (Hamilton et al, 1962).

Gamble (1969) examined embryonic and fetal human spinal cords by both light and transmission electron microscopy. The cells in the mantle layer of an embryonic specimen (1.5 cm crown-rump length) defied classification as either neurons or glial cells. In the fetal material (12.0 cm crown-rump length), however, these cells could be identified as neurons by their content of granular endoplasmic reticulum. The neurons had the greater amount of ergastoplasm.

In addition to the alar and basal plates, roof and floor plates are present in the embryonic spinal cord. Gamble (1969) observed that the roof plate, dorsal to the central canal, and the floor plate, ventrally, consisted essentially of ependymal and neuroglial cells. In embryonic specimens (1.5 cm), the outer limits of the ependymal layer could be observed only in the roof and floor plate regions where this layer abutted directly upon the marginal layer. A few floor plate ependymal cells developed individual sheaths for longitudinally coursing nerve fibers. The phenomenon of axons being ensheathed by ependymal cells was a transient condition because Gambel (1969) did not observe a similar situation in human fetal material (12.0 cm).

The growth of the alar and basal plates has considerable influence on the size and shape of the central canal. The central canal immediately after fusion of the neural folds is represented only as a dorsoventrally elongated slit. With the formation of the alar and basal plates, the lumen of the central canal becomes rhomboid in shape. The dorsal and ventral corners of the rhomboid are in the area of the roof and floor plates. The lateral corners of the rhomboid form a longitudinally oriented sulcus limitans on each side. Continued growth of the alar and basal plates compresses the

central canal more so dorsally than ventrally leaving only the ventral part of the canal to persist in the adult. The most caudal part of the human central canal does not undergo as extensive reduction in size, but is represented as a small dilatation, the terminal ventricle, which extends into the initial portion of the filum terminale (Hamilton et al., 1962).

Regarding the procedure of myelinization, the oligodendrocytes form the myelin sheaths in the central nervous system (Moore, 1977). Myelinization of the fiber tracts in the spinal cord commences in the cervical part of the cord and proceeds caudally. This process begins during midfetal life and continues during the first year after birth in humans. The only signs of myelinization in the 1.5 cm human embryo involved the ependymal cells of the floor plate (Gamble, 1969). Myelinated axons, however, were observed scattered through the marginal layer in the 12.0 cm human fetal specimen. These myelinated axons were usually single, but groups of three to five fibers were sometimes found.

Concerning the meninges, Sensenig (1951) described the embryology of the spinal meninges in humans in connection with the development of the vertebral canal and its contents. The pia mater, arachnoid, and dura mater develop from undifferentiated mesenchyme, the meninx primitiva, that immediately surrounds the neural tube in early embryos. Later in gestation, as early as the 16.0 mm stage embryo, the meninx primitiva differentiates into an inner endomeninx and an outer ectomeninx. The endomeninx develops into the leptomeninges (arachnoid and pia mater). The mesenchymal cells adjacent to the spinal cord differentiate into the pia mater while the arachnoid forms from the outer portion of the endomeninx. The pia mater and arachnoid are

connected by arachnoid trabeculae that develop in the loose mesenchyme of the endomeninx. The ectomeninx proceeds to differentiate into dura mater and separates from the periosteum of the vertebral canal. The initial separation of dura mater from the rudiments of the vertebral canal was observed between day 30 and 42 of gestation in human embryos. By day 47 (30.0 mm crown-rump length) complete separation of the dura mater from the periosteum had occurred in the thoracic region. By the 90.0 mm stage, a distinct dura mater with a cell free subdural space and large venous channels in part of the epidural space was evident.

Although not classified as part of the central nervous system or spinal cord, the dorsal root ganglia are in close approximation to the spinal cord and vertebral canal. The primordial dorsal root ganglia can be discerned as early as the 4.0 or 5.0 mm stage in human embryos (Arey, 1965). The individual cells, however, are not marked as either primitive neurons or supportive cells till the human embryo reaches the 80.0 mm stage (Sensenig, 1951; Hamilton et al., 1962). Neurons can be indentified by the presence of an increased amount of granular endoplasmic reticulum, which occurs with maturation.

Spinal Dysraphism in Man

Various meanings of spinal dysraphism are present in the literature. Spinal dysraphism according to Lichtenstein (1940) relates to developmental malformations resulting from failure of fusion of dorsal midline structures. In contrast, Gardner (1961, 1966, and 1973), Padget (1968 and 1970), Rokos (1975), and Rokos and Knowles (1976) reported that dysraphic-like lesions result from the reopening of a previously closed neural tube.

Myelodysplasia was used by Fuchs (1909) and Benda (1954 and 1959) to describe the neural changes in spinal dysraphism. Malformations of the spinal cord range from hydromyelia (fluid-filled enlargement of the central canal) to diastematomyelia (doubling of the spinal cord). Schneiderling (1938) concluded that diastematomyelia occurs due to each one-half of the neural tube closing separately, instead of forming a single neural tube. Lichtenstein (1940) preferred the term diastematomyelia over hemidydemia (partial twinning) and diplomyelia (two separate cords), because the condition arises from the separation of a single spinal cord primordium.

Non-neural anomalies that often coexist with myelodysplasia include koilosternia, kyphoscoliosis, spinal bifida, arachnodactyly, clinodactyly, and hypertrichosis (Bremer, 1926). In a genetic study of human dysraphic patients, Carter et al. (1976) summarized a variety of lesions and anomalies that can accompany the primary spinal cord problem in dysraphism. Additional anomalies involved the skeleton (e.g. spina bifida occulta involving more than one vertebra, vertebral body malformations) and the skin over the spinal column (e.g. hypertrichosis, dimple, lipoma). Further, signs of neurological deficits in the pelvic limbs (e.g. analgesia with thermoanesthesia but with persistence of touch and pressure responses) were present. The confusing aspect of spinal dysraphism is that any or all degrees of dorsal midline malformations can occur in the same person.

Benda (1959) restricted dysraphic lesions to nervous tissue and thus evolved the term neurospinal dysraphism (NSD). Patients with NSD, generally, have lesions in, although not limited to, the area of the conus medullaris of the spinal cord (Till, 1969; James and Lassman, 1972). Although not

a lesion comprising nervous tissue, the consistent finding of a fibrous attachment of the conus medullaris to the vertebrae in cases of NSD was acknowledged by Till (1969) and James and Lassman (1972). The disruption of this attachment was considered paramount in the surgical relief of the tension on the spinal cord in dysraphic subjects.

In order to clarify the source of the sensory deficits (analgesia and thermoanesthesia) observed in dysraphic patients, Emery et al. (1973) examined the left seventh dorsal root ganglion in both normal and dysraphic children and found the number and apparent degree of maturation of the nerve cells in dysraphic ganglia within normal limits. In contrast, when left and right dorsal root ganglia from a child with a hemi-myelomeningocoele were compared with the result obtained from the affected (paralyzed) side, there was a reduction in number of nerve cells in the latter. Shorey (1909) and Hamburger (1934) noticed a reduction in the size of associated dorsal root ganglia following amputation of a chick wing bud early in development. Piatt (1948) and Hamburger and Levi-Montalcini (1949) reported the number of cells that mature in dorsal root ganglia depends on the presence of the peripheral nerves and sensory feedback conducted by these nerves. Emery et al. (1973) could not demonstrate conclusively whether the reduced number of nerve cells was due to failure of primary development of primitive neurons or the result of failure of maturation of primitive neurons due to lack of sensory stimulation.

Spinal Dysraphism in the Weimaraner Dog

The first reported incidence of dysraphism in the dog was in a two-year-old Newfoundland having paralysis of one pelvic limb (Liénaux, 1897).

The condition as it exists in the Weimaraner has been extensively described by McGrath (1956 and 1965), Confer and Ward (1972), Draper et al. (1975), and Shelton (1977). Spinal dysraphism has also been reported in the English Bulldog (Parker and Byerly, 1973; Parker et al., 1973), Samoyed (Furneaux et al., 1973), Dalmatian (Neufeld and Little, 1974), English Setter, Golden Retriever (Draper et al., 1975), and even mongrels (Geib and Bistner, 1967).

The characteristic clinical signs first appear at four to six weeks of age in dysraphic Weimaraner pups (McGrath, 1965). Draper et al. (1975) and Shelton (1977) were able to identify dysraphic pups at birth by the presence of a bilateral synchronous withdrawal reflex in the pelvic limbs instead of a normal crossed-extensor reflex. By the time it is weaned the typical dysraphic dog has postural abnormalities that include a crouched stance with abduction of one pelvic limb. Pin prick reflexes are attenuated in the abducted limb. Confer and Ward (1972) indicated absence of the tactile and/or visual placing relfexes, impaired abduction of the pelvic limbs, and increased pain threshold which suggest the presence of lesions interrupting the propriospinal tract.

McGrath (1965) listed other gross deformities that could accompany dysraphism in the Weimaraner dog, e.g., scoliosis, fusion of vertebrae, spondylosis, abnormal hair growth, koilosternia, and kinking of the tail. McGrath (1965) and Shelton (1977) also reported meningeal fibrosis and "adhesions" of the dura mater to the periosteum of the vertebral canal in the area of the conus medullaris.

Histopathologic findings of spinal dysraphism reported by McGrath (1965), Confer and Ward (1972), and Draper et al. (1975) were most consistently

confined to the lumbosacral area of the spinal cord. The lesions, however, were neither entirely limited to this segment nor did all of the changes exist in each of the affected dogs. The findings commonly observed at the lumbosacral region comprise absence of the ventral median fissure; enlargement, duplication, dorsoventral elongation, or displacement of the central canal; flattened ventral gray matter; thinness, absence, or neuronal deficits of the ventral gray columns; and a bluntness or absence of the dorsal gray columns (Shelton, 1977). Because of the firmness of the thigh musculature and the attenuated muscle mass, Draper et al. (1975) and Shelton (1977) suggested muscle myopathy to be a lesion associated with NSD in Weimaraner dogs. Muscle tissue, however, was not obtained for histologic examination.

Syringomyelia, cord cavitation not lined by ependymal cells, dorsal to the gray matter was reported by McGrath (1965) to be a rather consistent finding in mature dysraphic Weimaraner dogs. He postulated that spinal dysraphism, as it exists in both man and animals, may be related to a variety of congenital syringomyelia. Furneaux et al. (1973) observed syringomyelia in conjunction with spina bifida occulta in a Samoyed dog described as having "status dysraphicus". Lesions compatible with syringomyelia were only occasionally observed in dysraphic spinal cords examined by Draper et al. (1975) and Shelton (1977).

Shelton (1977) examined the genetic aspects of NSD in Weimaraner dogs after a pattern suggested by Draper et al. (1975). The proposed mode of inheritance was based on results of various breeding combinations between severely dysraphic and normal Weimaraners and with backcross breeding of the progeny. Also, purebred Norwegian Elkhounds, German Shepherds, German

Shorthaired Pointers, and an Irish Setter were procured for outcross mating. The method of genetic transmission of NSD is linked to a mutant gene (Dy) with some degree of dominance (co-dominance) in a one-locus, two-allele, three genotype system. Furthermore, this gene has some reduction of penetrance and variable expressivity. The phenotypic expression of dysraphic lesions would not necessarily occur in all of the progeny resulting from the mating of two severely dysraphic dogs because of the reduced penetrance and variable expressivity characteristics of this mutant gene.

McGrath (1965) attempted to determine the period during development in which the expression of dysraphic lesions occurred. He concluded that in view of the rather consistent localization of lesions in the lumbosacral region of the spinal cord and the severity of the abnormal morphology, dysraphism in the Weimaraner was due to abnormal development at a rather restricted time. From his preliminary studies involving development of the canine spinal cord, he believed these changes most likely occurred during the last one-third of gestation.

Etiology of Neurospinal Dysraphism

The basic cause of NSD in Weimaraner dogs is genetic, however, the development of this disease is still unresolved. From a survey of the literature the formation of dysraphic lesions can be categorized into one of the following five theories: cerebrospinal fluid accumulation; abnormally different growth rates; developmental arrests; ischemic factors; and abnormal caudal neuropore closure. Each of these aspects will be discussed.

Cerebrospinal fluid accumulation

Morgagni (1761, cited by Gardner, 1966) at first proposed that the accumulation of cerebrospinal fluid could lead to prenatal neurological defects. He suggested that the excess fluid would flow down the vertebral canal surrounding the neural tube rupturing the developing vertebrae and thereby exposing the neural tissue. More support for the development of dysraphic lesions, however, has been attributed to the accumulation of cerebrospinal fluid within the central canal of the spinal cord. This would account for hydromyelia and open cord lesions (Gardner, 1961, 1966, and 1973; Padget, 1968 and 1970; Rokos, 1975; Rokos and Knowles, 1976).

Gardner (1961, 1966, and 1973) identified a primary relationship between atresia of the lateral apertures of the fourth ventricle and dysraphic malformations. The cerebrospinal fluid rather than flowing into the subarachnoid space is hydrodynamically forced into the central canal resulting in dilatation and rupture of a previously closed neural tube. This rupture of the central canal could occur at various stages of embryologic development to account for a variety of accompanying conditions.

Gardner et al. (1957), Padget (1972), and Gruys and Bethlehem (1976) have indicated a similar situation coexisting with the Arnold-Chiari syndrome. The cerebellar herniation through the foramen magnum of the skull, which accompanies the hydrocephalus and syringomyelia seen in Arnold-Chiari cases, may occlude the flow of cerebrospinal fluid from the fourth ventricle into the subarachnoid space. The excess fluid would then be diverted through the central canal giving rise to spinal cord lesions. Gardner et al. (1957) speculated that the decussating fibers in the spinal cord at the level of the

obex might afford the necessary resistance to prevent total collapse or even dilatation of the central canal at this level.

By draining fluid from the metencephalon of early chick embryos Desmond and Jacobson (1977) demonstrated that cerebrospinal fluid pressure is necessary for normal development of the central nervous system. Following 24 hours of incubation, enough time for at least two or more cell cycles, the nervous tissue was extensively folded into the neural cavities of the spinal cord and brain.

Becker et al. (1972) reasoned that occlusion of the central canal alone is not sufficient to cause hydromyelia. In cats that had kaolin-induced hydrocephalus, massive dilatation of the central canal resulted when there was continuity between the fourth ventricle and the central canal. Hydromyelia and syringomyelia, however, did not develop when the central canal was blocked at the level of the obex and the filum terminale ligated in similarly prepared hydrocephalic cats. They concluded that the central canal dilatation in these cats results from increased choroid plexus activity in the ventricular system because the fluid formation by or across the ependyma in the isolated central canal was not sufficient to cause hydromyelia.

Hall et al. (1975) provided evidence indicating hydromyelia and syringomyelia produced in kaolin-induced hydrocephalus are due to distension and rupture of the central canal and not the result of other possible etiologies, such as, ischemic softening. Comparing lesions produced under both conditions in dogs, they observed a dilated central canal, thinning of the lining ependyma, and cavities communicating with

the central canal following treatment with kaolin. In contrast, extradural rhizotomies to produce ischemic myelopathy by interrupting the segmental vascular supply to the gray matter (Woodward and Freeman, 1956) resulted in destruction of the gray matter and cavitation, but the central canal remained small, with intact ependyma and surrounding glia. The cavities occurred in the necrotic tissue, but did not communicate with the central canal.

James et al. (1978) attempted to disprove that the central canal of the spinal cord is a significant alternate route for cerebrospinal fluid flow in hydrocephalus by the injection of non-reactive silicone rubber into the subarachnoid space caudal to the lateral apertures of the fourth ventricle in dogs. Even after 16 weeks there was still no enlargement of the central canal. These workers concluded that the excess fluid was not directed into the canal.

Abnormally different growth rates

A second theory on the development of NSD has been postulated by Patten (1953) and Barson (1970). They suggested that the morphologic changes observed in NSD may be attributed to abnormally different growth rates between the neuroectoderm and the mesoderm. If the neuroectoderm overgrew to cause thickening of the floor plate of the neural groove, the neural folds would be prevented from uniting dorsally and result in non-closure of the neural tube. Fowler (1953) implied that if NSD lesions were initiated early in gestation a primary neural tube non-closure would lead to other secondary abnormalities, such as, spina bifida. In contrast, spina bifida occluta must be a primary mesodermal malformation arising independantly later in development.

Another aspect of different growth rates concerns the formation of the head and tail folds (Barson, 1970). Disparities in the growth rates between neuroectoderm and mesoderm result in the formation of the head and tail folds during normal neurulation (Arey, 1965). Any disruption in the normal series of events at this time could result in anomalies at one or both extremities of the embryo.

Developmental arrests

A third concept concerning the development of NSD centers around developmental arrests. Recklinghausen (1886) stated that a decreased metabolic rate and concomitant decreased mitotic activity underlie the basic mode of embryonic arrests. He suggested that neuroectoderm was the primary tissue to undergo developmental arrest and result in non-fusion of the neural folds. In contrast, Marsh et al. (1885) proposed mesoderm as the arrested tissue and suggested that it failed to enclose the spinal cord, thereby leaving an exposed and unprotected neural tube.

Cameron (1957) believed that an arrest of Hensen's node (primitive knot), during regression of the primitive streak, would cause malformations of the neurospinal axis. The node gives origin to the notochord and, together with the primitive streak, is responsible for the creation of the paraxial and intermediate mesoderm. The medial sclerotomes, originating from the paraxial mesoderm, along with the notochord form the vertebral column and ribs, while the nephrogenic cord arises from the intermediate mesoderm. Malformations of the skeletal and urogenital systems would obviously result from disturbances involving the primitive knot. Smith and Stein (1962) using embryonic looptail mice and Bellairs (1963) using

chick embryos have demonstrated that normal regression of the primitive streak itself is necessary for somite formation. Delayed regression of the primitive streak would result in the skeletal and urogenital malformations along with shortened axial body lengths.

Herren and Edwards (1940) suggested that diastematomyelia, as observed in some cases of NSD, cannot be explained on the basis of arrested fetal development simply because there is no stage in normal prenatal development where the spinal cord is doubled. Lendon (1972) studied neural tube closure using tritiated thymidine on neural plates of normal mice and on mice with trypan blue induced spina bifida. He found no significant increase or decrease in mitotic activity between the two groups thereby refuting the developmental arrest theory.

Ischemic factors

A fourth theory of NSD development is secondary cavitation produced by spinal cord ischemia. Lichtenstein (1943) attributed cavitations in the cervical region of the spinal cord, associated with Arnold-Chiari malformations, to compression of the vertebral arteries and venous stasis. Tauber and Langworthy (1935) were able to replicate spinal cord cavitations merely by the ligation of the ventral spinal artery in cats. Woodward and Freeman (1956) produced cavitations dorsal to the central canal in dogs by ligating paired dorsal and ventral nerve roots extradurally over six adjacent segments. The cavities occurred in necrotic tissue and were not connected to the central canal. They were not lined by ependyma, indicating the lesions were consistent with syringomyelia. Woodward and Freeman (1956) concluded that the resulting pathology was due to

interference with the venous drainage rather than impairment of the arterial supply, thus bringing about cord edema and generalized reduction in microvascular circulation of the spinal cord.

By means of injection preparations in rabbits and a monkey (Macacus rhesus), Krough (1945) observed that there is no difference in the density of the capillary network between the center of the spinal cord and the periphery. Furthermore, the arteries branch into capillaries primarily near the circumference of the cord, while the veins are formed at the center. Following the results of cord ischemia produced by obstructing the abdominal aorta, he concluded that the tissue most resistant to occlusion was positioned close to the arterial ends of the capillaries. Woodward and Freeman (1956) utilized this information to theorize that the consistent location of cavitation dorsal to the central canal was the result of not having well-defined venous drainage of this area. Venous blood must drain through the entire substance of the spinal cord dorsally to reach the large surface veins. The region ventral to the central canal is relatively well-drained.

In a study of spinal cord defects in Manx cats, Martin (1971) observed a similar type of cavity formation dorsal to the central canal, communicating with the latter. He studied isolated spinal cord segments from cats which were transected at L4, crushed at S3, and which had the dorsal roots transected. A cavity appeared in the dorsal portion of the spinal cord. Upon further examination, it was revealed that the vessels overlying the lesion were obliterated, while the vessels ventral to the cord remained patent.

James and Lassman (1972) noticed that in some cases of human spina bifida occulta, the lumbosacral spinal cord was fixed caudally by fibrous bands coursing from the spinous processes of the vertebrae to the dura mater. They suggested that the fixation of the spinal cord would prevent the cord from changing its position within the vertebral canal due to the differential growth rate of vertebral elements. The traction force applied to the tethered spinal cord could then result in ischemia created either by deficient arterial supply or venous drainage. In addition, the pressure created by the traction could cause a failure of neuronal conduction.

Abnormal caudal neuropore closure

Warkany et al. (1958) experimentally produced myeloschisis, myelomeningocoele, and spina bifida at various levels of the spinal cord in the same rat by the use of trypan blue. This teratogenic agent was administered subcutaneously on the eighth day after conception. Because this time coincides with closure of the caudal neuropore in rats, they concluded that lumbosacral dysraphism would result if the caudal neuropore was prevented from having normal closure.

Lendon and Emery (1970) noticed a high occurrence of forking of the central canal in the conus medullaris of apparently normal infant spinal cords (29 of 100 cords from children under one year of age). A similar situation was observed in human embryos by Ikeda (1930, cited by Emery and Lendon, 1973). It was further suggested by Emery and Lendon (1973 that a proportion of the human population may be prone to partial spinal cord duplication, at least in the terminal part of the cord. They resolved that the most common factor in partial cord duplication and

meningomyelocoele formation centered around abnormal neurulation at the level of the caudal neuropore. These authors indicated that the lumbar and sacrocaudal parts of the spinal cord in humans canalize from a solid rod of tail-bud tissue. This would result in irregular growth of the neural tube by the addition of small cavities which may lead to central canal duplication. Consequently, a meningomyelocoele may arise from faulty canalization of tail-bud tissue.

Lendon (1968) observed that central canal duplication in the terminal cord region was a rare phenomenon in rats. This finding was later confirmed by Hughes and Freeman (1974), who found an open neuropore at the tip of the tail in approximately 10-day-old rat and mouse embryos. They suggested that closure of the caudal neuropore in rodents occurs in a even manner during growth of the tail. On the other hand, they reported that closure of the caudal neuropore occurs prior to tail-bud formation in the pig. The central canal in pig embryos (5.0 mm stage) was observed ending two sections (14 μm) cranial to the tip of the tail. By the 7.5 mm stage, the tip of the tail extended even further beyond the end of the central canal. In summary, Hughes and Freeman (1974) suggested that closure of the caudal neuropore occurs before formation of the tail-bud in man and pig and after tail-bud formation in rodents. Furthermore, growth of the caudal neural tube in man occurs by the process of canalization, while in pig and rodents caudal development of the neural tube results by intrinsic growth into the tail-bud.

MATERIALS AND METHODS

Subjects

Embryos and fetuses were harvested from four normal mongrel bitches and five dysraphic Weimaraner bitches. The four normal mongrel bitches were mated to normal male dogs and yielded a total of 28 progeny. The five dysraphic bitches were mated with dysraphic males. These matings yielded 36 offspring.

Animals used for normal matings were obtained from Laboratory Animal Resources, Iowa State University. The dogs used to produce the dysraphic litters belonged to a genetic colony of dysraphic Weimaraner dogs maintained at the Veterinary Medical Research Institute, Iowa State University.

Harvest Times

Embryos were harvested between 24 and 28 days of gestation. This time was chosen to allow for any dysraphic changes to become evident following closure of the neural tube on the 17th day (Houston, 1968). Fetuses were obtained between 41 and 44 days of the same gestation period. This time corresponded to the beginning of the last trimester. Thus each normal and dysraphic bitch experienced two cesarean operations.

In order to obtain embryos and fetuses of known gestational ages it was necessary to determine day one of gestation. This was accomplished by using the procedures of Holst and Phemister (1971). Weekly vaginal smears were obtained from all bitches and examined to determine the end of anestrus and the beginning of proestrus. Once a bitch was in proestrus, smears were made on alternate days to identify the onset of estrus. Bitches

were bred either by artificial insemination or by natural service. Breeding dates were recorded for all bitches. Each bitch was bred at least twice. Daily vaginal smears were utilized during the estrus period to determine the commencement of metestrus. Day one of gestation was then approximated by retrospectively counting two days from the onset of metestrus as suggested by Holst and Phemister (1971).

Surgical Procedures

Embryos were surgically removed according to procedures developed by Evans and Sack (1973) and improved upon by Lowrey (1975). Prior to induction of anesthesia, atropine sulfate (0.01 mg/Kg body wt) was administered subcutaneously. Anesthesia was induced by administering five percent thiamyl sodium intravenously. Anesthesia was maintained by using a mixture of methoxyflurane and oxygen in five bitches. In the remaining four animals anesthesia was maintained with a halothane-oxygen mixture.

Embryos were harvested by aseptic surgical techniques and immediately transferred to a buffered neutral formalin (BNF) solution. These procedures were repeated on succeeding uterine enlargements until all embryos, except the most caudal one, had been removed from the left horn. The most caudal enlargement in the left horn was left intact to serve as a barrier against possible disruption of the contents in the right horn.

After removal of the embryos from the left horn, the abdominal wall was closed with two layers of simple interrupted stainless steel sutures (20 gauge). The skin sutures were removed five to seven days post-operatively.

Following surgery, progesterone (25 mg) was administered intramuscularly to inhibit spontaneous abortion. This procedure was found to be

unnecessary and was subsequently dropped from the regime.

Between 41 and 44 days of gestation, the uterus was re-entered to remove the littermates of the 25 day embryos. Using one incision near the body of the uterus, it was possible to evert the uterus to retrieve all fetuses. Closure of the uterus was achieved with a single layer of Cushing sutures utilizing 4-0 chromic gut with a swedged-on needle. The abdominal wall was closed as in the first operation.

After removing a fetus, the fetal membranes were immediately incised and separated leaving a 3 to 5 cm umbilical stalk with the specimen. The fetus was then immersed in a ten percent BNF solution. Within 30 minutes following surgery, the umbilical vein of each fetus was infused with 15 to 30 ml of a ten percent BNF solution. The fetuses were kept immersed in formalin.

Age and Size of Embryos and Fetuses

The number and gestational age of the embryos removed from each normal and dysraphic bitch are presented in Table A1. Two specimens from each of the litters were randomly chosen for histological studies, resulting in a total of eight normal and ten dysraphic embryos. The mean gestational ages were 26.25 and 26.00 days for the normal and dysraphic specimens, respectively. A summary of gestational age data for the two groups of embryos is in Table A2.

Crown-rump measurements of the normal and dysraphic 24 to 28 day embryos are listed in Table A3. The measurements for the embryos utilized for histologic studies had a mean of 14.69 mm for the eight normal and 14.20 mm for the ten dysraphic specimens (Table A4).

Listed in Table A5 are the number of normal and dysraphic fetuses obtained and their gestational age. Contained in Table A6 are the data relevant to the ages of the eight normal and ten dysraphic fetuses randomly selected for histologic examination. The mean gestational ages were 42.75 and 42.10 days for normal and dysraphic fetuses, respectively.

Measurements obtained from the crown of the head to the caudal contour of the body (croup) of the fetuses were recorded (Table A7). Table A8 contains the information regarding the size of the fetuses utilized for histologic investigation. The mean body lengths of these specimens were 92.00 mm for the normal fetuses and 96.5 mm for the dysraphic fetuses.

Table 1 represents a summary of the data pertaining to the gestational ages and physical body measurements of the embryos and fetuses selected for histologic examination. There were no significant differences in the mean gestational ages or in the mean body length measurements between the normal and dysraphic groups as determined by one-way analysis of variance procedures.

Table 1. Summary of data on embryos and fetuses selected for histologic examination

	Number examined	Mean gestational age	Mean length in mm
Embryos			
Normal	8	26.25 days	14.69
Dysraphic	10	26.00 days	14.20
Fetuses			
Normal	8	42.75 days	92.00
Dysraphic	10	42.10 days	96.50

Histologic Procedures

Following fixation, the embryos were passed through a series of graded alcohol and xylene, and finally embedded in paraffin. The embryos were positioned so that cross-sections from the caudal end would be obtained first. The entire embryo was serially sectioned at 7 μm intervals, mounted on glass slides, and stained with Harris hematoxylin-eosin.

For fetal materials, a wedge-shaped portion of the back, caudal to the throacolumbar junction, including the spinal cord was removed. Beginning from the caudal end, three 1 cm segments were obtained from the wedge-shaped back, ensuring that the lumbosacral region of the spinal cord would be included for histologic study. Prior to serial passage of tissues in the Autotechnicon (The Technicon Company, Chauncey, New York), the cranial end of each segment was identified with a drop of Mercurochrome as a reference point. Each segment was embedded in paraffin, so that the unmarked caudal portion would be sectioned first. The caudal one-half of each segment was serially sectioned at 7 μm intervals. The fetal serial sections were also stained with Harris hematoxylin-eosin.

The initial centimeter of the portion of the tail that extended beyond the croup in the fetal specimens was also sectioned and stained. These sections were used to compare the morphology of the terminal spinal cord in both normal and dysraphic fetuses.

The sections of both embryonic and fetal tissues underwent microscopic examination. In the embryonic sections only the caudal one-half of each embryo was studied because dysraphic lesions in the Weimaraner dog commonly occur in the lumbosacral region of the spinal cord (McGrath, 1965); Draper

et al., 1975; Shelton, 1977). All slides obtained from the fetal tissues were examined and any microscopic abnormalities were recorded.

Data Analysis

In the region of the caudal limb buds, the transverse diameters of the entire spinal cord and of the gray matter, including the central canal, were measured in both normal and dysraphic embryos. An ocular grid was utilized for making these histological measurements. Normal versus dysraphic values were compared by a one-way analysis of variance procedure to determine if there were any differences in the transverse diameters of the spinal cord and of the gray matter. Ratios of gray matter to spinal cord transverse diameter values were similarly analyzed to determine any differences in the amount of marginal layer (future white matter) present.

Quantification of any differences between normal and dysraphic spinal cords at the fetal stage was accomplished by analysis of transverse diameter measurements. These measurements were recorded from the caudal lumbar region. As with the embryonic specimens, the values obtained for the spinal cord and gray matter transverse diameters as well as the ratios of spinal cord to gray matter diameters were compared by one-way analysis of variance procedures.

The cross-sectional areas of the dorsal root ganglia in the caudal lumbar region of normal and dysraphic fetuses were compared by one-way analysis of variance procedures. After identifying the tissue section which contained the mid-portion of a ganglion, the slide was projected

onto a screen. The outline of the ganglion was traced onto graph paper so that an area measurement could be ascertained. In addition, dorsal root ganglia areas were examined for the presence of any right-left differences within a given normal or dysraphic fetus.

RESULTS

Histologic Findings

Embryonic stage

In both normal and dysraphic embryos, mitotic figures were observed in the ependymal layer frequently at right angles, occasionally parallel, to the lumen of the central canal of the spinal cord.

The central canal was elongated dorsoventrally across almost the entire diameter of the spinal cord. The sulcus limitans, at a transverse plane with the canal, was present on its lateral wall (Figure 1). In both embryonic groups the lateral walls at times approximated to each other, and this was considered to be an artifact.

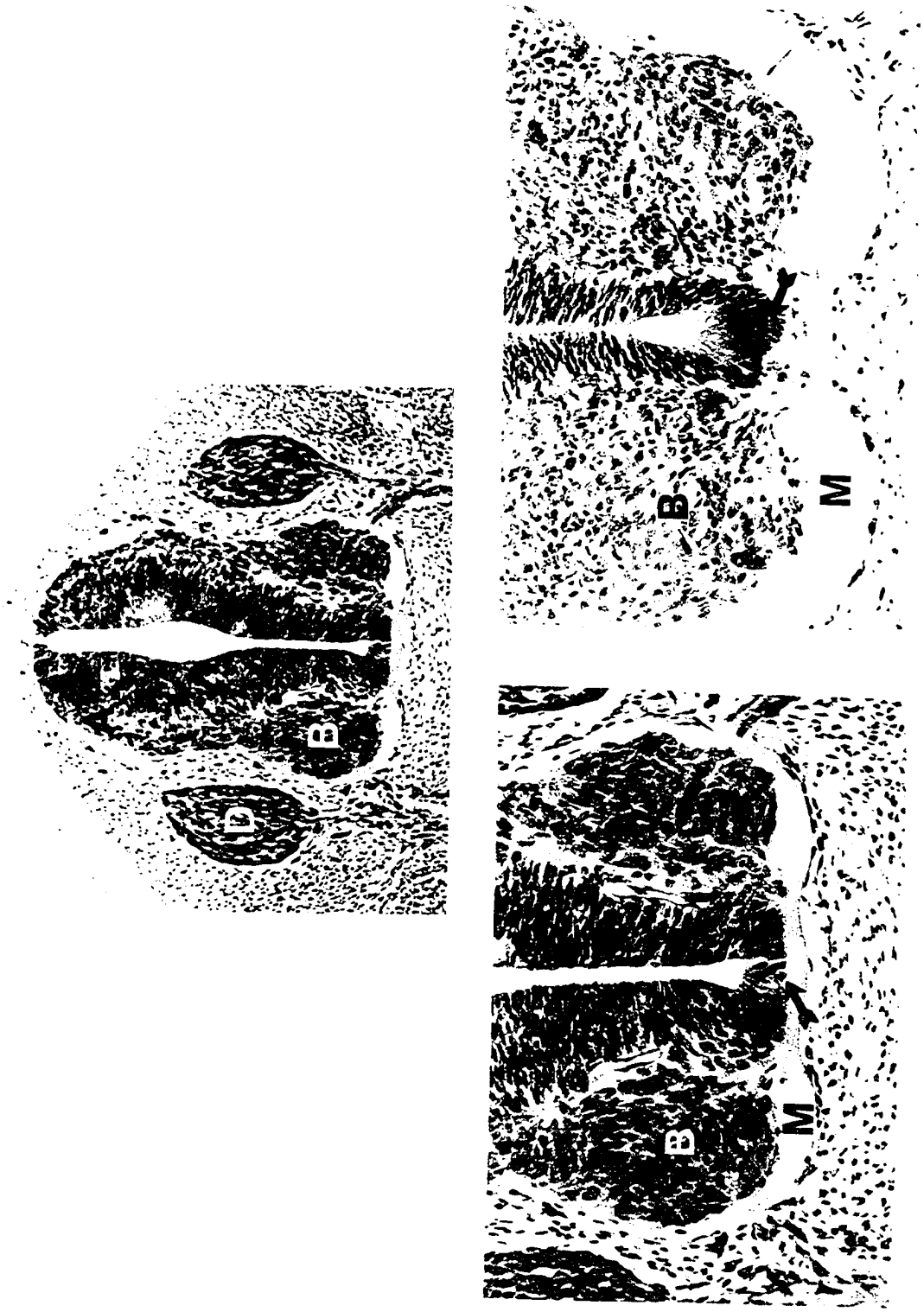
Cells from the ependymal layer had migrated by 24 to 28 days to form the mantle layer, and individual cells were difficult to distinguish from primitive neurons and spongioblasts (Figures 2 and 3). Mantle cells tended to accumulate in the ventrolateral regions forming the basal plates (primordial ventral horns). During this period, the dorsolaterally positioned alar plates (primordial dorsal horns) were not as distinct as the basal plates (Figure 1).

The thin marginal layer was differentiated by 24 days of gestation (Figure 2) consisting primarily of processes from the mantle layer cells and a few spongioblasts. By 28 days of gestation, the marginal layer was thicker, but spongioblasts were still scarce (Figure 3). The marginal layer and ependymal layer were in direct apposition dorsal to the roof plate and ventral to the floor plate in normal embryos.

Figure 1. Spinal cord, normal canine embryo, 49B, 24 days gestation. Note the presence of alar plates (A), basal plates (B), dorsal root ganglia (D), and the abrupt junction between ependymal and marginal layers. H & E. x80

Figure 2. Spinal cord, normal canine embryo, 49B, 24 days gestation. Note the presence of basal plates (B), thinness of marginal layer (M), and the abrupt junction of ependymal and marginal layers (arrow). H & E. x200

Figure 3. Spinal cord, normal canine embryo, 47B, 28 days gestation. Note the presence of basal plates (B), increased thickness of marginal layer (M), and juxtaposition of ependymal and marginal layers (arrow). H & E. x200


B
B
B
B
M
B
M

The dorsal root ganglia were well-defined by the 24th day of gestation (Figure 1). The individual cells, however, could not be identified as being either ganglion cells or spongioblasts.

Between day 24 and 28 of gestation, the meninx primitiva had differentiated into an endomeninx and an extomeninx. The endomeninx consisted of widely spaced mesenchymal cells adjacent to the spinal cord. The vascular pia mater and the avascular arachnoid that develop from the endomeninx could not be distinguished at this stage. A condensed layer of mesenchymal cells external to the endomeninx constituted the ectomeninx lying next to the developing vertebral arch.

Major morphologic discrepancies between normal and dysraphic embryos were revealed by histologic comparisons. In seven of the ten dysraphic embryos, mantle cells were dispersed between the ependymal and marginal layers ventral to the central canal (Figures 4 through 10). The aberrant position of mantle cells was generally confined to the spinal cord segments at the level of the caudal limb buds. There was a conspicuous absence of distinct basal plates in conjunction with the mantle cells being in the floor plate area.

Differences between normal and dysraphic mantle layer cells also were evident. In dysraphic specimens, the mantle cells (presumed to be primitive neurons) were spaced closer together and stained more basophilic with hematoxylin-eosin stain than their normal counterparts.

Another major finding was the abnormal termination of the neural tube. The neural tube ended at the tip of the tail bud in both normal and dysraphic embryos (Figure 11), consisting only of the ependymal layer. In three of the ten dysraphic embryos, a transversely oriented doubling of the terminal part

Figure 4. Spinal cord of dysraphic canine embryo, 51B, 28 days gestation. Arrow indicates abnormal position of mantle cells. Similar finding in Figures 5 through 10. H & E. x40

Figure 5. Spinal cord of dysraphic canine embryo, 51A, 28 days gestation. H & E. x40

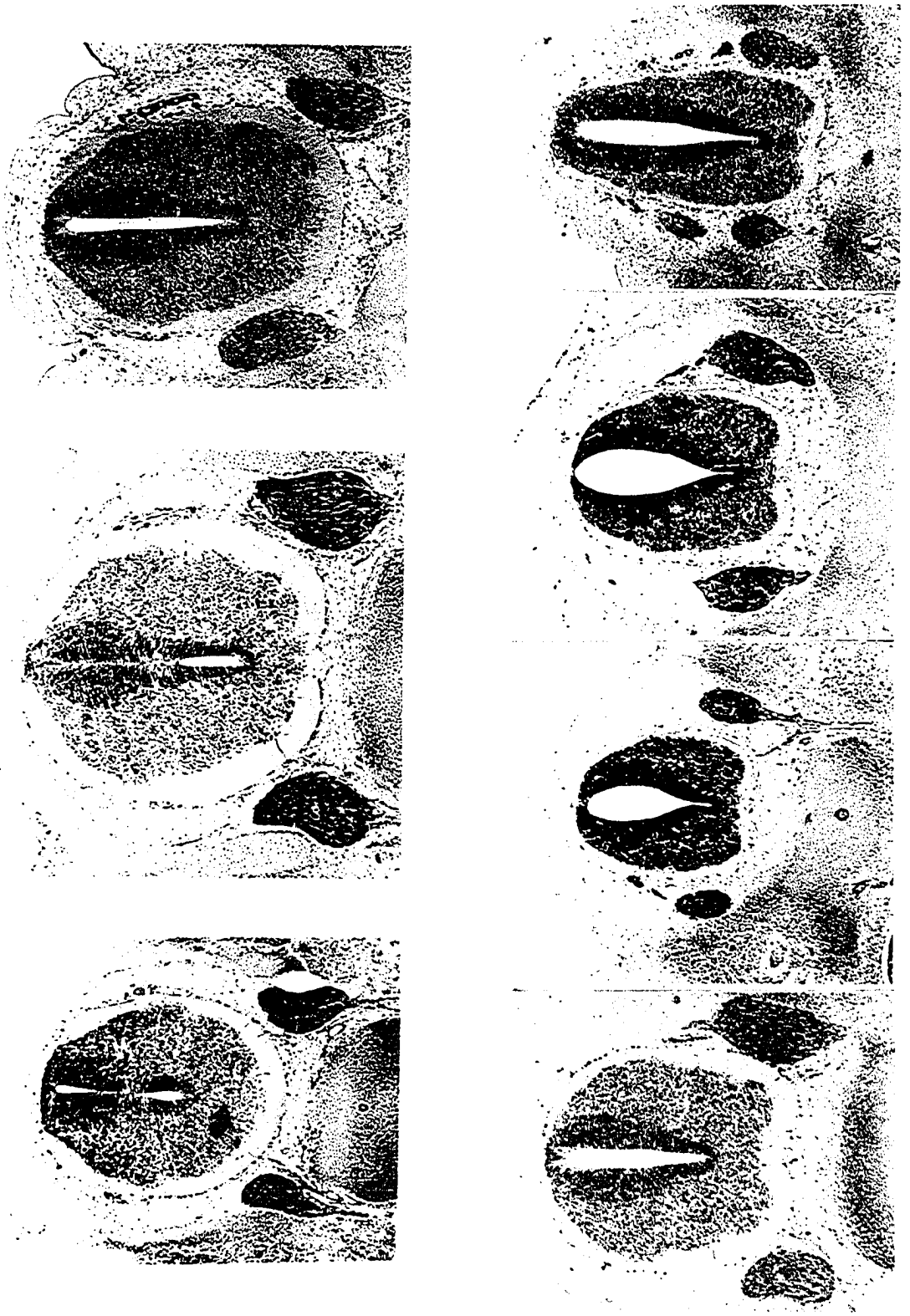
Figure 6. Spinal cord of dysraphic canine embryo, 222A, 28 days gestation. H & E. x40

Figure 7. Spinal cord of dysraphic canine embryo, 222B, 28 days gestation. H & E. x40

Figure 8. Spinal cord of dysraphic canine embryo, 70A, 25 days gestation. H & E. x40

Figure 9. Spinal cord of dysraphic canine embryo, 70B, 25 days gestation. H & E. x40

Figure 10. Spinal cord of dysraphic canine embryo, 221A, 24 days gestation. H & E. x40



(caudal 150-200 μm) of the central canal was seen. In serial sections, a progressive dorsal invagination of the ependymal layer into the central canal was observed, which continued until duplication of the neural tube was completed (Figures 12 and 13). The abnormal findings observed in each dysraphic embryo are tabulated in Table 2.

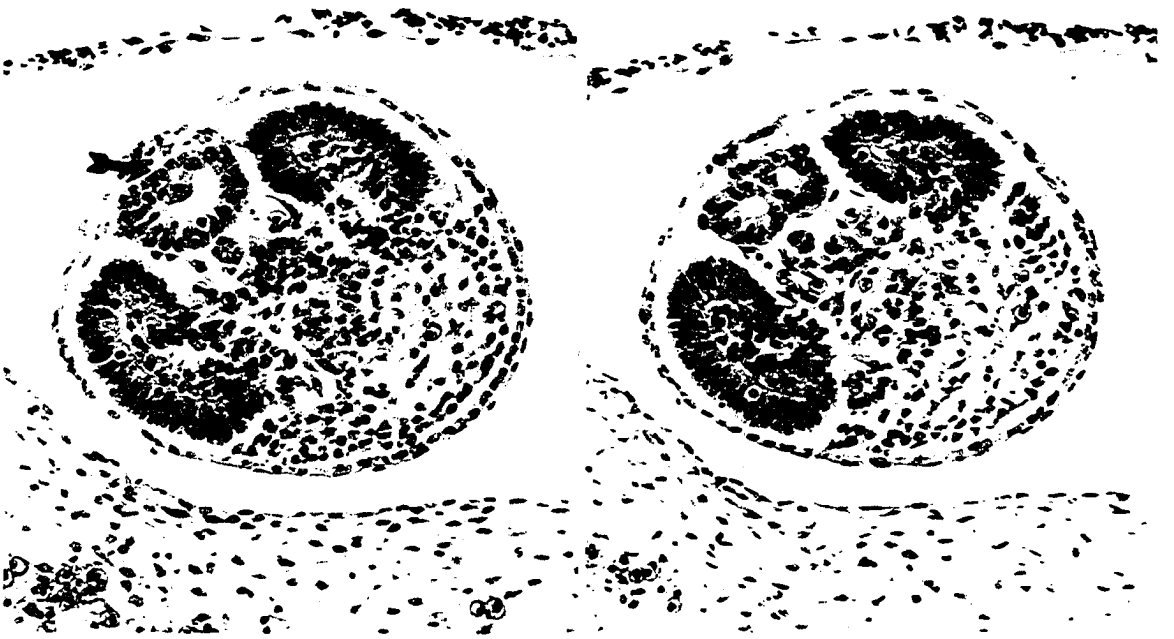
Table 2. List of abnormal findings observed in each dysraphic embryo

Embryo	Abnormal position of mantle cells	Abnormal terminal central canal
220 A	-	-
220 B	-	-
221 A	+	-
221 B	-	+
222 A	+	-
222 B	+	-
51 A	+	+
51 B	+	+
70 A	+	-
70 B	+	-

Figure 11. Terminal neural tube, normal canine embryo, 49A, 24 days gestation. Note the circular neural tube (arrow) composed of only ependymal cells. H & E. x200

Figure 12. Terminal neural tube, dysraphic canine embryo, 221B, 24 days gestation. The ependymal cells dorsally (arrow) are causing a thickening and invagination of the wall of the neural tube. H & E. x200

Figure 13. Terminal neural tube, dysraphic canine embryo, 221B, 24 days gestation. Serial section 21 µm caudal to the section in Figure 12. The ependymal cells have completely divided the neural tube to cause duplication of the central canal. H & E. x200



Fetal stage

Very few mitoses were observed in the ependymal layer of either normal or dysraphic fetuses by day 41 to 44 of gestation. The central canals in the normal fetuses were considerably reduced in size in comparison to the diameters of the spinal cords (Figure 14). The outline of the central canal was oval; the sulcus limitans was not present.

The mantle layer cells had undergone extensive differentiation so that the definitive nuclei of the spinal cord could be recognized (Figure 14). The presumptive alpha motor neurons were easily discerned in the ventral horns (Figure 15).

The marginal layer was thicker than in the embryonic stage with a mean increment of 0.106 mm. In addition to an apparent increase in the number of fibers, there were glial cell nuclei visible indicating the beginning of myelinization (Figure 15).

The spinal cords in normal fetuses had a distinct ventral median fissure which almost reached the central canal. A less developed dorsal median septum was also present (Figure 14).

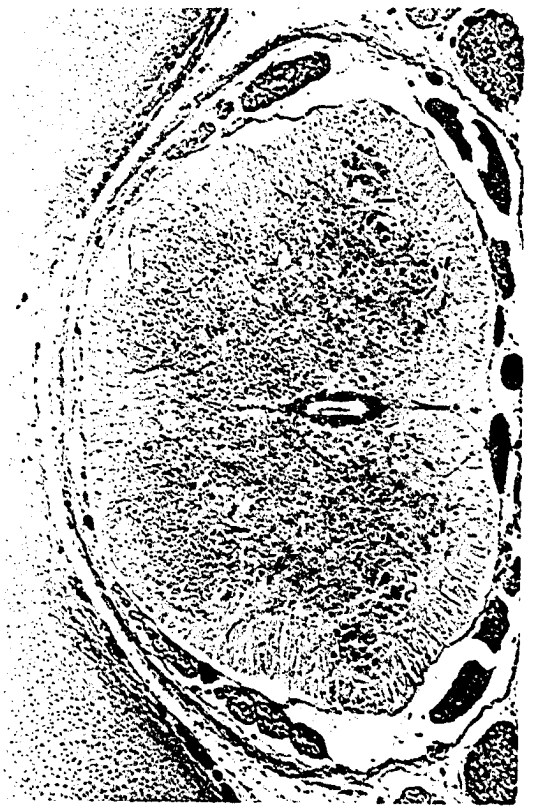
The cells within the dorsal root ganglia had differentiated so that individual neurons and supportive cells could be recognized (Figure 16).

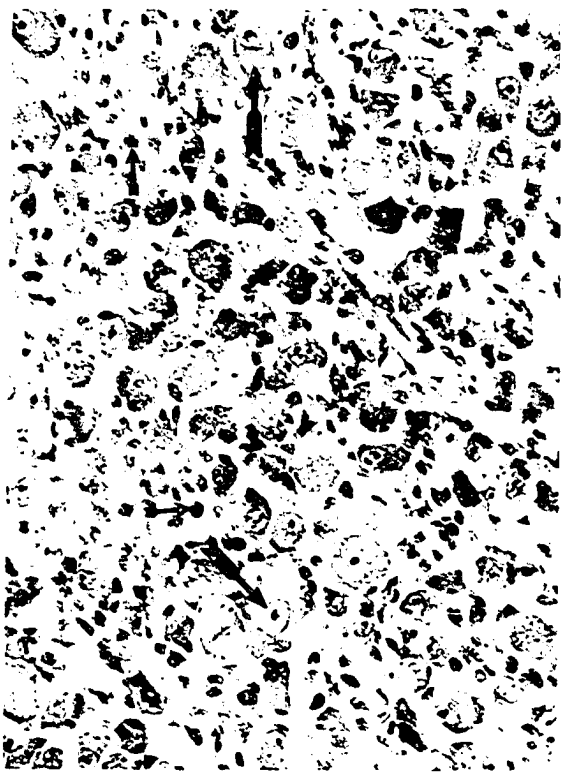
In the normal specimens, the ectomeninx had separated from the mesenchyme that forms the periosteum of the vertebral arch to become the dura mater. The outer fibrous layer of the periosteum was easily identified with its blood vessels. The dura mater consisted of an outer layer of squamous cells and a layer of underlying collagen fibers. The endomeninx had also differentiated into arachnoid and pia mater and a subarachnoid space, containing numerous

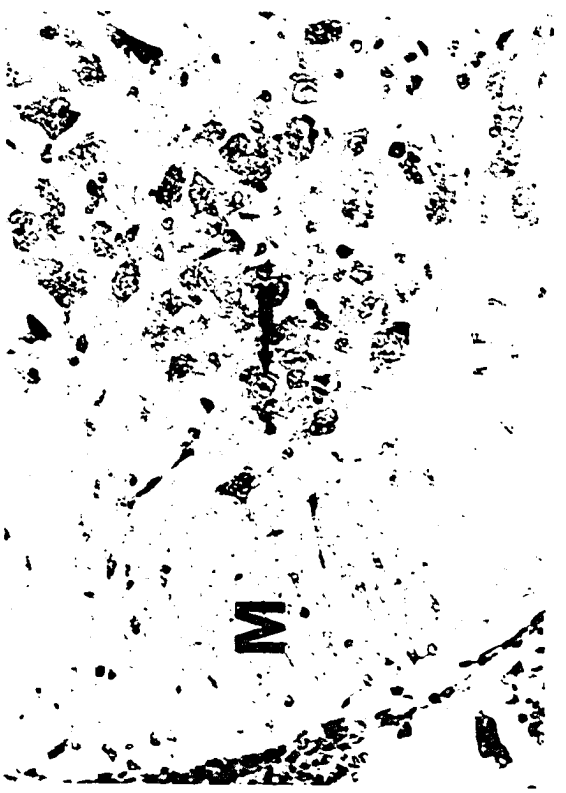
Figure 14. Lumbar spinal cord area, normal canine fetus, 58, 44 days gestation. Note the presence of dorsal and ventral horns, ventral median fissure, normal central canal, and separation of dura mater and periosteum dorsally. H & E. x40.

Figure 15. Lumbar spinal cord area, normal canine fetus, 58, 44 days gestation. Closer view of the ventral horn area from the fetus in Figure 14. Note motor nuclei (arrow) and marginal layer (M) with supportive cell nuclei. H & E. x200

Figure 16. Lumbar spinal cord area, normal canine fetus, 58, 44 days gestation. Even higher magnification of the dorsal root ganglion from the fetus in Figures 14 and 15. Note the differentiation of ganglion cells (large arrows) and supportive cells (small arrows). H & E. x 250






M

arachnoid trabeculae, had increased the gap between these layers. Vessels were present in the pia mater lying on the surface of the spinal cord (Figure 17).

In the conus medullaris region the terminal central canal was usually single in both normal and dysraphic fetuses (Figure 18). In one normal fetus and in one dysraphic fetus, however, multiple central canals were noted (Figure 19). The secondary canals converged with the lumen of the main central canal cranially. They were attenuated with an average length of 300 μm. The ependymal layer of the canals was irregular and was devoid of the mantle cell layer. This entire arrangement has the resemblance of a forking of the terminal central canal.

Histomorphologic differences between normal and dysraphic fetuses were primarily confined to the lumbosacral region of the cord. The dissimilarities noted were central canal abnormalities, failure of the ectomeninx to differentiate dorsolaterally into the dura mater by separating from the periosteum of the vertebral arch, and absence of a ventral median fissure with concomitant lack of separation between the ventral horns.

Central canal abnormalities were observed in seven of the ten dysraphic fetuses. The central canal underwent dorsoventral enlargement and narrowing at several levels of the lumbosacral spinal cord. This was the most common canal abnormality (present in five of the ten dysraphic fetuses). The enlargements were actually ventral diverticuli from the central canal (Figures 20 through 23). A diagrammatic representation of this phenomenon is presented in Figure 23.

Concerning the central canal, another malformation observed in two dysraphic fetuses was extreme reduction in size. The presence of misplaced

Figure 17. Dorsolateral margin of the spinal cord of normal canine fetus, 58, 44 days gestation. Closer view of the separation between the dura mater (D) and the periosteum (P). Note the presence of blood vessels in the pia mater and subperiosteally (arrows). H & E. x150

Figure 18. Terminal spinal cord area of normal canine fetus, 59, 44 days gestation. Note the normal central canal. H & E. x200.

Figure 19. Terminal spinal cord area of dysraphic fetus, 63, 42 days gestation. Note the multiple central canals. H & E. x200


P
D

Figure 20. Lumbosacral spinal cord area, dysraphic canine fetus, 69, 41 days gestation. Note the dorsoventral elongation of the central canal. H & E. x40

Figure 21. Lumbosacral spinal cord area, dysraphic canine fetus, 69, 41 days gestation. Serial section 14 µm caudal to the section in Figure 20. Note the lateral walls of the central canal touch to create an hourglass shaped lumen. H & E. x40

Figure 22. Lumbosacral spinal cord area, dysraphic canine fetus, 69, 41 days gestation. Serial section 7 µm caudal to the section in Figure 21. Note the presence of the diverticulum ventral to the main central canal falsely creating the appearance of two canals. H & E. x40

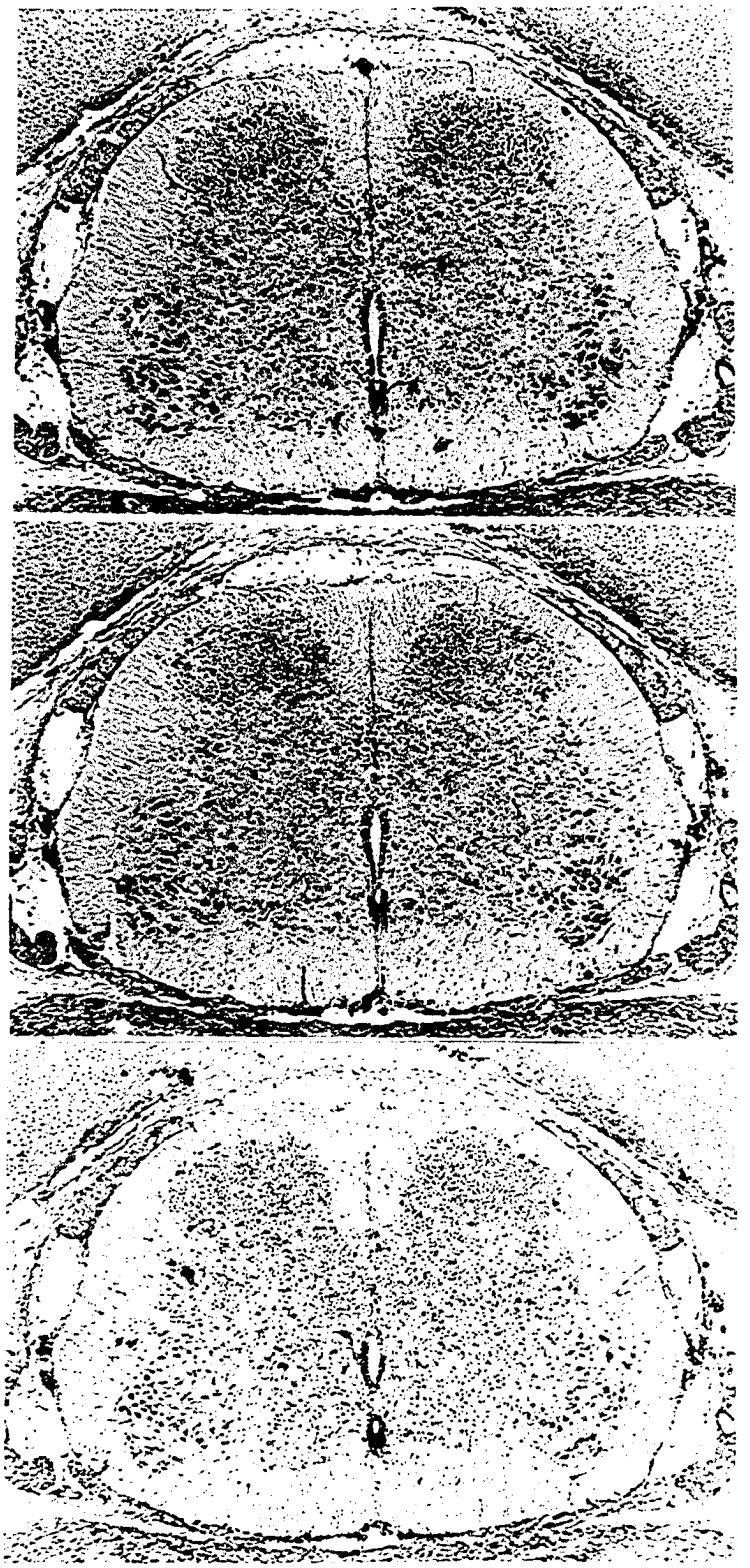
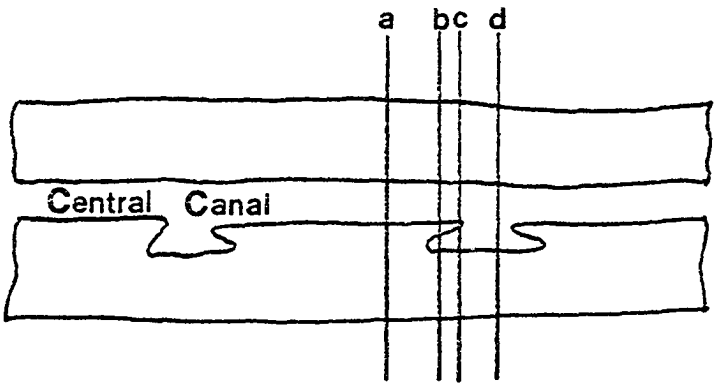
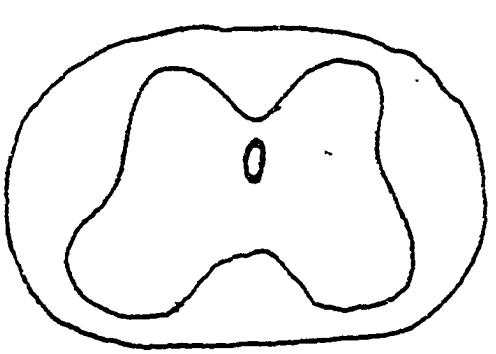


Figure 23. The top illustration represents a sagittal section of a dysraphic fetal spinal cord in a region having dorsoventral enlargement and narrowing of the central canal. The four illustrations at the bottom represent transverse sections through the spinal cord at levels a, b, c, and d. These levels portray a narrowed central canal (a), a false dorsoventral doubling (b), a dorsoventral enlargement (c), and completion of the dorsoventral enlargement (d).

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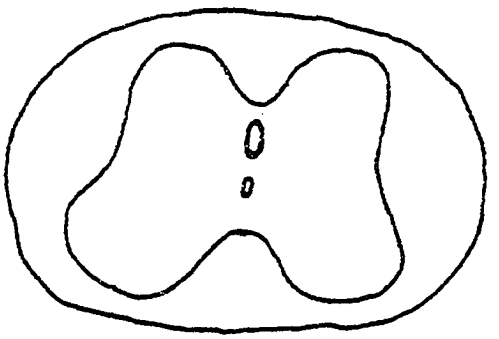
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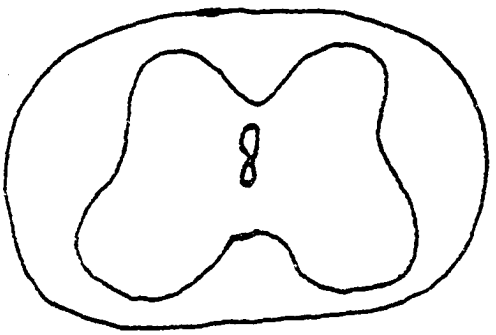
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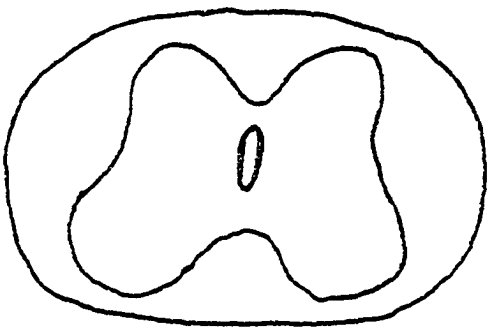
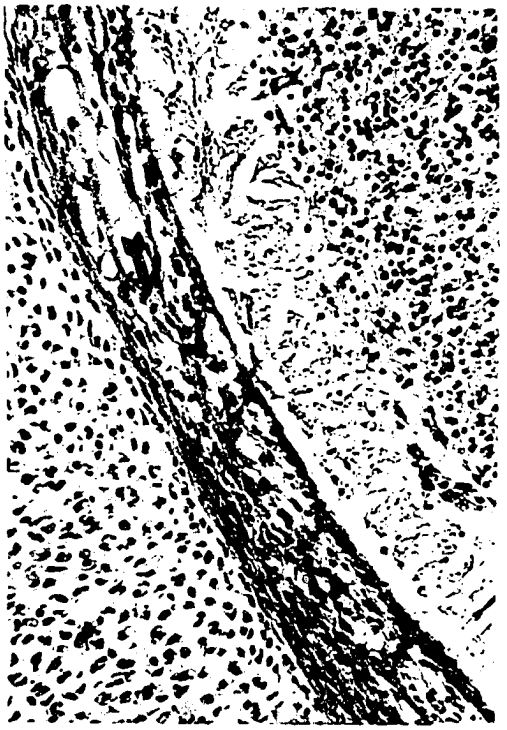
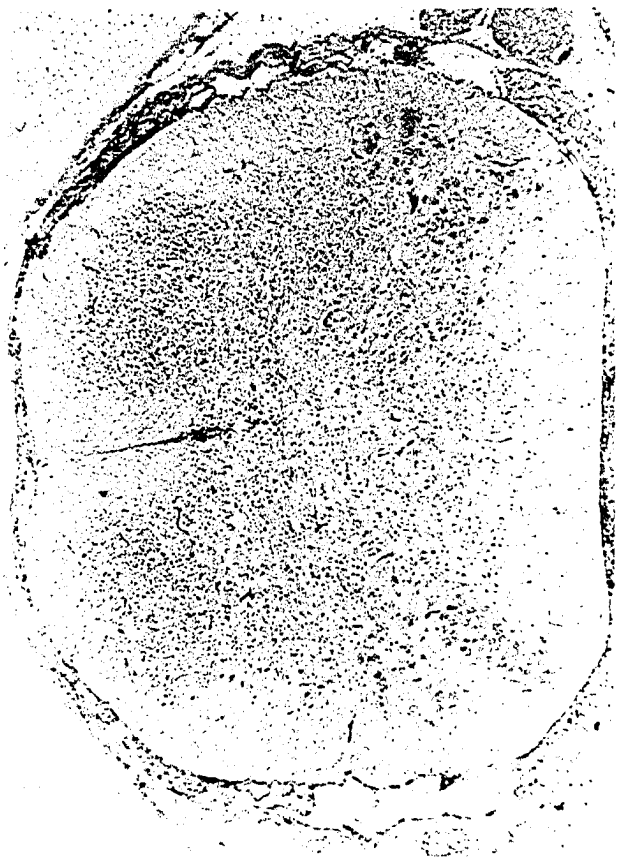

d

Figure 24. Lumbosacral spinal cord area, dysraphic canine fetus, 253, 41 days gestation. Note the small central canal, failure of separation of dura mater and periosteum, fusion of ventral horns, and absence of a ventral median fissure. H & E. x40

Figure 25. Dorsolateral margin of the spinal cord of dysraphic canine fetus, 253, 41 days gestation. Closer view of the lack of differentiation and separation between dura mater and periosteum (arrow) of the fetus in Figure 24. Note the absence of blood vessels in this undifferentiated area. H & E. x150

Figure 26. Lumbosacral spinal cord area, dysraphic canine fetus, 249, 44 days gestation. Another example of reduction in size of central canal, fusion of ventral horns, and absence of a ventral median fissure. H & E. x40





gray matter ventral to the central canal (Figures 24 and 26) seemingly compressed the canal to account for the reduction in size.

Failure of differentiation of the ectomeninx dorsolaterally into dura mater was observed in eight of the ten dysraphic fetuses. This was depicted by the lack of separation between the ectomeninx and periosteum of the vertebral arch in the lumbosacral region (Figure 25). Blood vessels were not seen in the undifferentiated layers.

Another conspicuous morphologic difference was related to the absence of the ventral median fissure of the spinal cord in dysraphic specimens. Seven of the ten dysraphic fetuses either had a less developed or completely absent ventral median fissure in the lumbosacral region. Its absence was accompanied by a loss of distinct separation between both ventral horns and some flattening of this portion of the cord ventrally (Figures 24 and 26). A tabulation of the abnormal findings observed in each dysraphic fetus is presented in Table 3.

Table 3. Tabulation of abnormal findings observed in each dysraphic fetus

Fetus	Abnormal central canal	Failure of dura mater to differentiate	Absence of ventral median fissure
249	+	+	+
250	+	+	+
251	+	+	-
252	-	+	-
253	+	+	+
254	+	+	+
62	-	-	+
63	+	-	+
68	-	+	-
69	+	+	+

Quantitative Analysis

Embryonic stage

Diameter of the gray matter The transverse diameters of the gray matters in the region of the caudal limb buds were recorded. This dimension ranged from 0.525 to 0.844 mm in the normal embryos with a mean of 0.689 mm. On the other hand, the dysraphic embryos had a mean diameter of 0.588 mm with a range from 0.388 to 0.750 mm. These data are summarized in Table 4. Individual specimen data are presented in Tables A9 and A10. A one-way analysis of variance procedure was used to determine that the dysraphic embyros had significantly smaller transverse diameters of the gray matter ($F=5.50$, $P<0.05$) than the normal embryos.

Diameter of the spinal cord The spinal cords of normal embryos had a mean transverse diameter of 0.826 mm with a range from 0.562 to 1.125 mm, while the transverse diameters of dysraphic spinal cords ranged from 0.375 to 0.900 mm with a mean of 0.634 mm. These data are summarized in Table 4 with individual measurements presented in Tables A9 and A10. The transverse diameters of the dysraphic spinal cords were significantly smaller ($F=5.89$, $P<0.05$) than the normal spinal cord diameters. This was ascertained by a one-way analysis of variance procedure.

Ratios of the gray matter vs. the spinal cord diameter The ratios of gray matter diameter to spinal cord diameter in normal and dysraphic embryos are listed in Tables A9 and A10, respectively, and summarized in Table 4. The mean ratio for the normal embryos was 0.843, while that for the dysraphic specimens was 0.887. Although there was no significant difference ($F=3.10$, $P<0.10$, there was a clear tendency for the dysraphic

specimens to have proportionally less marginal layer thickness than in the normal embryos.

Table 4. Summary of the transverse diameter measurements of the gray matter (GM) and of the entire spinal cord (SC) with their ratios in normal and dysraphic embryos

	Mean diameter of gray matter in mm	Mean diameter of spinal cord in mm	Mean ratio of GM/SC
Normal embryos	0.689	0.826	0.843
Dysraphic embryos	0.558*	0.634*	0.887

*$P<0.05$

Fetal stage

Diameter of the gray matter The transverse diameters of the gray matter in the caudal lumbar region were recorded. Mean dimensions of 1.052 mm and 1.288 mm were recorded from normal and dysraphic fetuses, respectively. These data are summarized in Table 5. Individual specimen recordings are presented in Tables A11 and A12. No significant difference between these diameters was evident by a one-way analysis of variance procedure ($F=2.99$, $P<0.25$).

Diameter of the spinal cord The spinal cords of normal fetuses had a mean transverse diameter of 1.400 mm while the dysraphic fetuses had a mean spinal cord diameter of 1.608 mm (Table 5). Individual measurements are presented in Tables A11 and A12 for the normal and dysraphic fetuses, respectively. A one-way analysis of variance procedure was use to indicate no significant difference ($F=1.77$, $P<0.25$) between these measurements.

Ratios of the gray matter vs. the spinal cord diameter The ratios of gray matter to the spinal cord diameter in normal and dysraphic fetuses are presented in Table A11 and A12, respectively, and summarized in Table 5. The ratios of the normal fetuses had a range from 0.714 to 0.788 with a mean of 0.750. The values in the dysraphic fetuses ranged from 0.714 to 0.848 with a mean of 0.796. A significant difference ($F=7.20$, $P<0.05$) between the ratios in the normal and dysraphic groups was determined by a one-way analysis of variance procedure.

Table 5. Summary of the transverse diameter measurements of the gray matter (GM) and of the entire spinal cord (SC) with their ratios in normal and dysraphic fetuses

	Mean diameter of gray matter in mm	Mean diameter of spinal cord in mm	Mean ratio of GM/SC
Normal fetuses	1.052	1.400	0.750
Dysraphic fetuses	1.288	1.608	0.796*

*$P<0.05$

Area of dorsal root ganglia Area measurements of both left and right, either the sixth or seventh lumbar, dorsal root ganglia of the fetuses were made. The dorsal root ganglia data from both groups are listed in Table A13. The overall mean area of left and right ganglia in the normal fetuses was 0.444 mm^2, while that obtained from the dys-

raphic fetuses was 0.352 mm^2. No significant difference between these mean areas ($F=3.93$, $P<0.10$) was evident.

Comparison of left and right dorsal root ganglia The ratio of the area of the smaller dorsal root ganglion to the larger dorsal root ganglion in each fetus was recorded in both groups (Table A14). The mean ratio in the normal group of fetuses was 0.937, while 0.865 was the mean ratio obtained from the dysraphic fetuses. There was no significant difference ($F=4.23$, $P<0.10$) between the two groups of ratios suggesting no measurable difference between the size of left and right dorsal root ganglia in each fetus.

DISCUSSION

The fundamental hypothesis of the present research was that dysraphic changes are expressed during the period of embryogenesis in Weimaraner dogs with hereditary neurospinal dysraphism. To test this hypothesis the histologic findings observed in the normal canine embryos in this study will be compared first with previous reports concerning similar sized canine and human embryos. Next, perceived differences between the normal canine embryos and canine embryos obtained from dysraphic matings will be presented. The importance of these differences in the development of abnormal findings present in postnatal dysraphic dogs will be indicated. A discussion of the statistical results relating to diameter measurements of the spinal cord will conclude the section on embryonic findings.

A similar approach will be used to substantiate that established dysraphic lesions, like those observed postnatally in dysraphic dogs, are present at the beginning of the last one-third of gestation. The histologic structure of the spinal cord and adnexa in the normal canine fetuses will be compared with other reported findings and then related to the abnormal observations found in the dysraphic canine fetal spinal cords. This will be followed by observations relating to the statistical measurements of the spinal cords and dorsal root ganglia in the normal and dysraphic fetuses. The relationship between the various proposed theories on the development of neurospinal dysraphism and the embryonic and fetal findings in the present study will ensue.

In the embryonic specimens, the mantle and marginal layers were abruptly adjacent to each other in the normal canine embryos (14.69 mm average crown-rump length) in the roof as well as the floor plate areas. Anderson (1970) in a 10.5 mm dog embryo and Gamble (1969) in a 15.0 mm human embryo observed similar histomorphology. The degree of development of the alar plates, basal plates, and overall thickness of the marginal layer as observed in the normal canine embryo was similar to the extent of development of these same structures seen in the human embryo by Hamilton et al. (1962). Additionally, the endomeninx and ectomeninx had differentiated by 24 to 28 days of gestation in the normal canine embryos. Similar findings were reported in human embryos by Sensenig (1951).

In 70 percent of the dysraphic canine embryos in this study the mantle layer cells had migrated ventral to the central canal between the ependymal and marginal layers and interfered with the normal developing basal plates. This deviation from normal morphology was considered a dysraphic lesion and could easily account for some of the pathologic findings encountered in postnatal dysraphic dogs as reported by McGrath (1965), Confer and Ward (1972), Draper et al. (1975), and Shelton (1977). Mantle cells in the floor plate area would allow abnormal fusion of the ventral horns, which develop from the basal plates. Furthermore, the physical presence of mantle cells in the floor plate would reduce the depth of the ventral median fissure or cause it not to develop at all. The development of the ventral commissure as well as the propriospinal tract and other tracts would be severely hampered by an abnormal floor plate. Reduced maturation of the propriospinal

tract was indicated by Confer and Ward (1972) to account for the absence of some reflexes and an increased pain threshold. More likely, these deficits are related to the disruption of the normal architecture of the gray matter in Rexed's lamina VIII which is concerned in part with proprioception. Additionally, the altered shape of the gray matter could apply pressure on those tracts positioned laterally and dorsally which are concerned with proprioception (e.g. spincerebellar and fasciculus gracilis) and pain (e.g. spinothalamic).

By light microscopic observations, it was ascertained that the neural tube terminates at the tip of the tail-bud in both normal and dysraphic embryos. This would be in agreement with the findings of Hughes and Freeman (1974). They concluded that closure of the caudal neuropore occurs after tail-bud formation in animals with normal length tails (rat and sheep) and before tail-bud formation in animals with tails that undergo any reduction in length (chick, pig, and man). Three of the ten dysraphic embryos had a transversely oriented doubling of the terminal 150 to 200 μm of the neural tube. The ependymal layer invaginated dorsally into the central canal until duplication was complete. Even with this low incidence, it would seem that lateral doubling of the terminal neural tube could be an expression of neurospinal dysraphism. The reduced penetrance and variable expressivity traits of the neurospinal dysraphism gene in Weimaraner dogs (Shelton, 1977) would account for the low incidence of this finding. Information is still lacking concerning normal embryology of the tail-bud and thus prevents any conclusion concerning the development of this lesion. One can only speculate, as did Emery and Lendon (1973), that this lesion could be associated with abnormal closure of the terminal neural tube.

The transverse diameters of the mantle layer and the entire spinal cord in dysraphic embryos, at the level of the caudal limb-buds, were significantly smaller than in normal specimens. This confirms that individual cells within the mantle layer of dysraphic embryos were less differentiated being more compact and basophilic with hematoxylin-eosin stains. Mantle cells, as they mature, develop projections, which cause the cells to be further apart (Hamilton et al., 1962). In agreement with present findings, Emery et al. (1973) reported an overall diminution in the numbers of large and middle-sized neurons in the spinal cords of children with neurospinal dysraphism. The delayed maturation as evidenced in the embryonic stage in the present study could be the first indication that some of these cells would fail to mature.

Because the neurons in the mantle layer produce axons that reach into the marginal layer to form spinal tracts (Hamilton et al., 1962), a thin marginal layer would likewise occur if these neurons failed to mature. The ratios of the gray matter to spinal cord diameters were statistically compared between the two embryonic groups and were not found to be significantly different. By having no meaningful difference between these ratios but significantly smaller gray matter and spinal cord transverse diameters in the dysraphic embryos would indicate the marginal layer (white matter) in the dysraphic group was also significantly smaller. The variance in the ratios was significant at $P<0.10$ with the dysraphic embryos having the larger ratio, a finding which would further suggest less development of the marginal layer in the dysraphic specimens.

Concerning the developmental changes that occurred in the canine fetal spinal cords by day 44 of gestation, the definitive position of spinal cord nuclei was already evident in the normal canine fetuses (92.0 mm crown-rump length). A distinct ventral median fissure and less developed dorsal median septum approached the oval-shaped central canal. Supportive cells were present in the marginal layer of normal fetuses indicating the beginning of the myelinization process. In general the morphology of the normal canine fetal spinal cords (41 to 44 days of gestation) mirrored the anatomy of spinal cords from normal human fetuses between 80.0 and 120.0 mm crown-rump length as reported by Sensenig (1951), Hamilton et al. (1962), and Gamble (1969).

The dorsal root ganglia in normal canine fetuses (92.0 mm crown-rump length) were located at the intervertebral foramina. The individual cells within the ganglia could easily be identified as supportive cells or neurons. A similar status was observed in normal human fetuses (90.0 mm crown-rump length) (Hamilton et al., 1962).

By 44 days of gestation the spinal cord dura mater in the dog had separated from the periosteum of the vertebral arch in the lumbosacral region which is compatible with the finding in normal human fetuses during the fourteenth week of gestation (Sensenig, 1951; Hamilton et al., 1962). Venous channels were evident in the epidural space of normal canine fetuses (92.0 mm crown-rump length) in the present study. Likewise, Sensenig (1951) indicated the presence of large venous channels in the epidural space in normal human fetsues (80.0 mm crown-rump length). Pia mater and arachnoid had also differentiated from the endomeninx in the normal canine fetuses

(92.0 mm crown-rump length) and in normal human fetuses of corresponding crown-rump length (Sensenig, 1951; Hamilton et al., 1962).

Fetuses (41 to 44 days of gestation) from the dysraphic Weimaraner dogs had abnormal findings that could easily represent an exacerbation of the anomalies observed in the dysraphic embryos (24 to 28 days of gestation). Again, the absence of the ventral median fissure and concomitant fusion of the ventral horns would likely result if mantle cells persisted ventral to the central canal as they were in dysraphic embryonic spinal cords. This aberrant positioning of the mantle cells would also account for the flattening of the ventral horns and disruption of the spinal nuclei that is found in dysraphic fetal specimens.

Diverticuli from the central canal were observed to form ventrally causing the canal to elongate dorsoventrally. Cross-sections at different levels of the diverticuli falsely resembled dorsoventral doubling of the central canal. The canal abnormality in the present study was considered to be identical to the forked central canal observed by Lendon and Emery (1970) in children with meningomyelocoele. A reasonable explanation on the occurrence of the "doubling" could rest in the distribution of gray matter. Enlargement of the alar plates normally causes compression on the dorsal part of the central canal to reduce the size of the lumen (Hamilton et al., 1962). With the more ventral position of the mantle cells in both dysraphic embryos and fetuses, the canal would be compressed from the sides to at first result in an hourglassed-shaped canal on cross-section and subsequently by the formation of diverticuli. Complete obliteration of the central canal occurred in two dysraphic canine fetuses.

Occlusion of the central canal was reported by McGrath (1965), Confer and Ward (1972), Draper et al. (1975), and Shelton (1977) in postnatal dysraphic Weimaraner dogs. Obliteration of the central canal could result in a rise in the cerebrospinal fluid pressure cranial to the lesion. The increased pressure would easily lead to the formation of hydromyelia according to Gardner (1961, 1966, and 1973) and Padget (1968 and 1970). Hydromyelia occurs in dysraphic Weimaraner dogs (McGrath, 1965; Draper et al., 1975; Shelton, 1977).

In one normal and one dysraphic canine fetus multiple central canals occurred in the terminal part of the spinal cord. Multiple central canals in the terminal cord of normal children were proposed by Hughes and Freeman (1974) to be the consequence of canalization of tissue in the tail-bud region. The terminal central canal in the dog, however, should develop by intrinsic growth in the tail-bud rather than by canalization according to Hughes and Freeman (1974). This terminal area could be more prone to primary embryonic maldevelopment simply because of the normal absence of notochordal tissue ventral to the neural tube to direct the construction of the terminal spinal cord.

The lack of separation of the dura mater from the periosteum of the vertebral arch has been described as a lesion present in both the dysraphic Weimaraner dog (McGrath, 1965; Shelton, 1977) and man (Till, 1969; James and Lassman, 1972; Carter et al., 1976). Sensenig (1951) observed complete detachment between dura mater and periosteum in human fetuses of 80.0 mm crown-rump length. Therefore, the failure of separation of these layers as identified in eight of the dysraphic canine fetuses (96.5 mm crown-rump length) was considered pathologic. The lesions were generally

confined to the dorsolateral surfaces of the spinal cord in the lumbosacral region.

Blood vessels were absent in those areas where failure of the dura mater to differentiate from the periosteum occurred in the dysraphic canine fetuses. Woodward and Freeman (1956) concluded that the absence of venous drainage from the spinal cord would create cavitations in the spinal cord similar to clinical cases of syringomyelia. They experimentally produced cavitations dorsal to the central canal by ligating spinal vessels coursing with the nerve roots to produce venous stasis, ischemia, and cord edema. They postulated that the area dorsal to the central canal was subject to cavitations because the venous blood must drain through the entire substance of the spinal cord dorsally to reach the large surface veins. The area ventral to the central canal was free of cavitations due to the presence of a well-developed venous drainage. Syringomyelia in Weimaraner dogs has only been reported as a postnatal finding and this was in mature dogs by McGrath (1965). Draper et al. (1975) and Shelton (1977) occasionally encountered cases of syringomyelia in dysraphic Weimaraner dogs.

James and Lassman (1972) suggested that the failure of the separation of the dura mater in the caudal portion of the spinal cord would tether the cord and prevent it from "migrating" cranially. Spinal cord development does not maintain pace with vertebral development, thus resulting in the spinal cord terminating further cranially at maturity than at birth. The tension created on a tethered spinal cord might further lead to occlusion of vessels and produce cord cavitations (James and Lassman, 1972).

The reduced incidence of syringomyelia reported in dysraphic Weimaraner dogs as compared to man could be due to the fact that the canine spinal cord does not "migrate" as far cranially as does the human cord (L7 in the dog and L1 in man). The tension on the cord would not be as severe in the Weimaraner dog as it is in man.

There were no significant differences between the transverse diameter measurements of both the gray matter and the entire spinal cord in both the normal and dysraphic fetuses. One would expect smaller diameter measurements in the dysraphic subjects to coincide with the neural deficits reported by Emery et al. (1973) and with the smaller cord measurements obtained from the dysraphic canine embryos. A possible explanation for the lack of significantly smaller measurements in the dysraphic fetuses may be attributed to breed size. Although the normal fetuses did not differ significantly in size ($P<0.05$) from the dysraphic fetuses, their mean body length was 92.0 mm as compared to 96.5 mm for the dysraphic fetuses. The Weimaraner dog is a relatively large breed with an average adult weight of 60 to 90 pounds, while beagle crosses were included among four of the eight normal fetuses (average adult weight, 20 to 35 pounds).

There was significance, however, between the ratios of gray matter to entire diameter of the spinal cord in the fetal specimens. The dysraphic fetuses had a larger ratio of gray matter to spinal cord diameter indicating either less white matter development or greater diameter of the gray matter. Because it has already been shown that no significant difference in gray matter exists, the significance must lie with the less amount of white matter in the dysraphic fetuses. A reduced thickness of white matter would occur developmentally if sufficient neurons in either

the gray matter or dorsal root ganglia failed to mature and produce processes. Again, Emery et al. (1973) did report deficiency in the number of certain types of neurons in the spinal cords of children with neurospinal dysraphism. The differences obtained in the present study might have been even greater if fetuses from similar size dogs were used thoughout all comparisons.

Normal neuronal development in the dorsal root ganglia requires the presence of peripheral nerves and sensory feedback conducted by these nerves (Piatt, 1948; Hamburger and Levi-Montalcini, 1949; Carr and Simpson, 1978). Emery et al. (1973) observed considerably reduced number of cells in a dorsal root ganglion on the paralyzed side of a human infant with dysraphism. Because a sensory deficit is commonly observed with neurospinal dysraphism in Weimaraner dogs (McGrath, 1965; Confer and Ward, 1972; Draper et al., 1975; Shelton, 1977), mid cross-sectional areas of the dorsal root ganglia in the caudal lumbar region were expected to be smaller in dysraphic than normal fetuses. Although no significant differences were identified between the two groups, there was a clear tendency for the dysraphic fetuses (0.3515 mm^2) to have smaller dorsal root ganglion areas than the normal (0.444 mm^2) dorsal root ganglion areas. These findings would no doubt reach significance if breed size was a contributing variable.

There was no significant difference between the ratios of the areas of the smaller to the larger dorsal root ganglia in normal and dysraphic fetuses. This statistical measurement was used to determine if a tendency existed for the dorsal root ganglia on one side to be smaller

in the dysraphic fetuses.

The hereditary aspect of dysraphism has been studied in Weimaraner dogs and found to be transmitted by a co-dominant gene with reduced penetrance and variable expressivity (Shelton, 1977). The crossing of severely dysraphic male and female Weimaraner dogs should, therefore, yield litters with a high percentage of individuals expressing some phenotypic signs of dysraphism. Indeed, histologic abnormal findings were evident in 80 percent of the embryos and 100 percent of the fetuses obtained from these dysraphic matings.

Considering the various theories proposed for the development of dysraphic lesions, no one theory accounts for all of the lesions reported. The migration of the mantle cells to an aberrant position in the floor plate, as observed in the present study, is the first lesion expressed in the development of NSD in Weimaraner dogs. The accumulation of excess cerebrospinal fluid creating hydromyelia, as proposed by Gardner (1961, 1966, and 1973), Padget (1968 and 1970), Rokos (1975), and Rokos and Knowles (1976), is not the primary initiating factor causing these cells to migrate abnormally. Also, the Arnold-Chiari syndrome, which causes hydromyelia by occluding the lateral apertures of the fourth ventricle (Gardner et al., 1957; Padget, 1972; Gruys and Bethlehem, 1976), has not been reported as a dysraphic lesion in dogs. Cerebrospinal fluid pressure is necessary for normal development of the central nervous system (Desmond and Jacobson, 1977), but the ependyma lining the central canal does not produce sufficient fluid to create hydromyelia in the isolated spinal cord (Becker et al., 1972). Therefore, the excess fluid and increased

pressure result from ventricular choroid plexus activity. An occlusion of the central canal, as observed in the present study, by the abnormal position of the mantle cells ventral and lateral to the canal might lead to hydromyelia cranial to the blockage as a secondary lesion.

Syringomyelia produced by ischemia is another secondary lesion occurring in NSD. The failure of separation of the dura mater from the periosteum of the vertebral canal dorsally can produce syringomyelia as a consequence of two factors. First, the absence of vessels in the area that fails to differentiate causes an interference with the venous drainage with cord edema as the result (Woodward and Freeman, 1956). The second factor, the tethering of the spinal cord by its dural attachment, will cause tension on the cord surface which can occlude vessels and may impede nervous conduction (James and Lassman, 1972). Again, syringomyelia is a lesion that appears only in adult Weimaraner dogs (McGrath, 1965) and is not as severe as the condtition in humans possibly because the normal site of spinal cord termination in the adult dog is further caudal than in man.

Because the notochord is a primary inducer of axial growth in the embryo (Arey, 1965), it would appear that this structure may cause the mantle cells to gravitate towards the midline as soon as they are released from the ependymal layer. The onset of this taxis would be important. If it occurs prior to closure of the neural tube, open cord lesions would result. This would appear as an abnormally different growth rate with the neuroectoderm causing a thickening of the floor plate of the neural groove as reported by Patten (1953) and Barson (1970) when, instead, neuroepithelial

cells are being drawn towards the midline with no increase in numbers.

After closure of the neural tube, a taxis by the notochord on the mantle cells would lead to lesions observed in a milder form of NSD as occurs in Weimaraner dogs. A focal activation of induction of the notochord at its termination would account for the high incidence of lesions in the lumbosacral region. Cameron (1957) proposed an arrest in the development of the primitive knot during regression of the primitive streak as causing malformations of the notochord and neurospinal axis. Smith and Stein (1962) and Bellairs (1963) have also indicated that shortened body lengths, skeletal, and urogenital malformations can result from delayed regression of the primitive streak. These lesions have been reported in cases of NSD in Weimaraner dogs (Draper et al., 1975; Shelton, 1977) thus adding support to this theory. Lendon (1972), however, found no significant changes in mitotic activity in the neural plates of normal mice and on mice with induced spina bifida.

Warkany et al. (1958) and Emery and Lendon (1973) have suggested that abnormal caudal neuropore closure would initiate several of the dysraphic findings. The support behind this theory lies with the observation that dysraphic lesions can be produced by subjecting embryos to a teratogenic compound (trypan blue) at the time of closure of the caudal neuropore. Research needs to be performed to better understand what biochemical inducers are being released at the time the caudal neuropore closes resulting in the abnormal migration of mantle cells. In the Weimaraner dog this induction is under genetic influence.

SUMMARY

1. The normal prenatal development of the canine spinal cord was examined by light microscopy in embryos and fetuses of average gestational ages of 26.25 and 42.75 days and crown-rump lengths of 14.69 and 92.00 mm, respectively. These findings were compared with the development of the spinal cord in embryos and fetuses obtained by mating severely dysraphic Weimaraner dogs. These embryos and fetuses had average gestational ages of 26.00 and 42.10 days and crown-rump lengths of 14.20 and 96.50 mm, respectively.

2. The histology of the spinal cord in the canine embryos was comparable to the development in human embryos between six and seven weeks of gestation (10.0 to 17.0 mm crown-rump length). Likewise, normal canine fetal spinal cords had anatomical similarities with spinal cords in human fetuses of 14 weeks gestation (90.0 mm crown-rump length).

3. Dysraphic lesions were, indeed, expressed early in gestation during the embryonic stage in 70 percent of the dysraphic specimens. The primary lesion consisted of mantle cells aberrantly positioned ventral to the central canal in the floor plate area. The importance of this finding in the development of several dysraphic lesions observed postnatally was presented. A plausible explanation for the aberrant migration of mantle cells towards the midline in the dysraphic embryos is abnormal notochordal induction.

4. The dysraphic embryos, in general, had significantly smaller gray matter and spinal cord diameters. This confirmed the histologic observation that the mantle cells in the dysraphic embryos were less differentiated being

more compact and basophilic with hematoxylin-eosin stains.

5. In 30 percent of the dysraphic embryos there was a lateral doubling of the terminal neural tube.

6. There was a failure of the dura mater to differentiate and separate from the periosteum of the vertebral canal in 80 percent of the dysraphic fetuses. This lesion was considered important in the production of syringomyelia postnatally by preventing the normal development of vessels extradurally and by secondary compression of existing vessels as a result of the spinal cord being tethered caudally.

7. The ventral median fissure was absent in 70 percent of the dysraphic fetuses along with concomitant fusion of the ventral columns. This disruption of the normal architecture of the gray matter, also observed postnatally, was believed to account for some of the sensory and motor deficits reported in clinical cases.

8. Central canal abnormalities were evident in 70 percent of the dysraphic fetuses. These findings included the presence of diverticuli and reduction in the size of the central canal. The aberrant mantle cells were thought to have created these lesions. It was further proposed that occlusion of the central canal would lead to secondary hydromyelia cranial to the blockage.

9. The dysraphic fetuses had significantly larger ratios of gray matter to spinal cord diameters. This indicated less white matter development.

10. There were no significant differences in the transverse diameter measurements of the gray matter and spinal cord in the dysraphic fetuses.

The lack of expected significance was attributed to differences in breed size.

11. Although not significant, there was a tendency for the dysraphic fetuses to have smaller cross-sectional areas of the dorsal root ganglia in the caudal lumbar region. This would account for some of the sensory deficits observed postnatally.

12. There was no significant difference between the areas of the left and right dorsal root ganglia in the dysraphic fetuses, thus suggesting no prevalence for dysraphism to affect one side over the other.

13. Multiple central canals were present in one normal and one dysraphic fetus in the terminal portion of the spinal cord.

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APPENDIX

Table A1. Number of normal and dysraphic embryos obtained and their gestational age

Normal dogs	Embryos removed	Age of embryos	Dysraphic dogs	Embryos removed	Age of embryos
Dog 4	4	24 days	Dog A3F	4	28 days
Dog 975	4	26 days	Dog C51F1	5	24 days
Dog 258	3	27 days	Dog C51F2	4	25 days
Dog 930	5	28 days	Dog A6F	4	25 days
Means	4	26.3 days	Dog 646	3	28 days
			Means	4	25.8 days

Table A2. Number of normal and dysraphic embryos utilized for histologic examination from each dog and their gestational age

Normal dogs	Embryos utilized		Age of embryos	Dysraphic dogs	Embryos utilized		Age of embryos
Dog 4	2		24 days	Dog A3F	2		28 days
Dog 975	2		26 days	Dog C51F1	2		24 days
Dog 258	2		27 days	Dog C51F2	2		25 days
Dog 930	2		28 days	Dog A6F	2		25 days
Total	8	Mean	26.25 days[a]	Dog 646	2		28 days
				Total	10	Mean	26.0 days[a]

[a]No significant difference in mean gestational ages even at $P<0.25$, $F=0.0986$.

Table A3. Crown-rump measurements of normal and dysraphic embryos

Normal dogs	Number examined	Average C-R length[a]	Dysraphic dogs	Number examined	Average C-R length[a]
Dog 4	4	11.63	Dog A3F	4	16.75
Dog 975	4	14.38	Dog C51F1	5	11.50
Dog 258	3	14.83	Dog C51F2	4	13.25
Dog 930	5	17.80	Dog A6F	4	13.00
Means	4	14.84	Dog 646	3	16.00
			Means	4	13.88

[a]Average C-R length = measured in mm.

Table A4. Crown-rump measurements of normal and dysraphic embryos utilized for histologic examination

Normal dogs	Number utilized	Average C-R length[a]	Dysraphic dogs	Number utilized	Average C-R length[a]
Dog 4	2	11.50	Dog A3F	2	16.75
Dog 975	2	14.50	Dog C51F1	2	11.75
Dog 258	2	14.75	Dog C51F2	2	13.25
Dog 930	2	18.00	Dog A6F	2	13.12
Total	8	Mean 14.69[b]	Dog 646	2	16.12
			Total	10	Mean 14.20[b]

[a]Average C-R length = measured in mm.

[b]No significant difference in mean crown-rump measurements even at $P<0.25$, $F=0.213$.

Table A5. Number of normal and dysraphic fetuses obtained and their gestational age

Normal dogs	Fetuses removed	Age of fetuses	Dysraphic dogs	Fetuses removed	Age of fetuses
Dog 4	3	42 days	Dog A3F	3	44 days
Dog 975	4	43 days	Dog C51F1	6	41 days
Dog 258	3	42 days	Dog C51F2	4	41 days
Dog 930	2	44 days	Dog A6F	0	
Means	3	42.7 days	Dog 646	3	42 days
			Means	4	41.8 days

Table A6. Number of normal and dysraphic fetuses utilized for histologic examination from each dog and their gestational age

Normal dogs	Fetuses utilized		Age of fetuses	Dysraphic dogs	Fetuses utilized		Age of fetuses
Dog 4	2		42 days	Dog A3F	3		44 days
Dog 975	2		43 days	Dog C51F1	3		41 days
Dog 258	2		42 days	Dog C51F2	2		41 days
Dog 930	2		44 days	Dog A6F	0		
Total	8	Mean	42.75 days[a]	Dog 646	2		42 days
				Total	10	Mean	42.10 days[a]

[a]No significant difference in mean gestational age, $P<0.25$ and $F=1.34$.

Table A7. Body length measurements of normal and dysraphic fetuses

Normal dogs	Number examined	Average length[a]	Dysraphic dogs	Number examined	Average length[a]
Dog 4	3	91.67	Dog A3F	3	100.67
Dog 975	4	88.50	Dog C51F1	6	92.50
Dog 258	3	94.00	Dog C51F2	4	94.25
Dog 930	2	103.00	Dog 646	3	99.33
Means	3	93.08	Means	4	95.75

[a]Average length = measured in mm.

Table A8. Body length measurements of normal and dysraphic fetuses utilized for histologic examination

Normal dogs	Number utilized	Average length[a]	Dysraphic dogs	Number utilized	Average length[a]
Dog 4	2	90.00	Dog A3F	3	100.67
Dog 975	2	86.00	Dog C51F1	3	91.67
Dog 258	2	89.00	Dog C51F2	2	94.00
Dog 930	2	103.00	Dog 646	2	100.00
Total	8	Mean 92.00[b]	Total	10	Mean 96.50[b]

[a]Average length = measured in mm.

[b]No significant difference in mean body lengths, $P<0.25$ and $F=2.40$.

Table A9. Transverse diameter measurements of the gray matter (GM) and of the spinal cord (SC) in normal embryos and the ratios of these measurements (GM/SC)

Embryos	Diameter of gray matter in mm	Diameter of gray matter in mm	Ratio of GM/SC
46A	0.750	0.844	0.889
46B	0.609	0.750	0.812
47A	0.703	0.938	0.750
47B	0.844	1.125	0.750
49A	0.525	0.562	0.933
49B	0.656	0.750	0.875
50A	0.675	0.797	0.847
50B	0.750	0.844	0.889
	Mean 0.689	Mean 0.826	Mean 0.843

Table A10. Transverse diameter measurements of the gray matter (GM) and of the spinal cord (SC) in dysraphic embryos and the ratios of these measurements (GM/SC)

Embryos	Diameter of gray matter in mm	Diameter of gray matter in mm	Ratio of GM/SC
220A	0.375	0.412	0.910
220B	0.338	0.375	0.901
221A	0.544	0.600	0.907
221B	0.506	0.562	0.900
222A	0.656	0.750	0.875
222B	0.750	0.862	0.870
51A	0.712	0.900	0.791
51B	0.562	0.656	0.857
70A	0.525	0.562	0.934
70B	0.609	0.656	0.928
	Mean 0.558	Mean 0.634	Mean 0.887

Table A11. Transverse diameter measurements of the gray matter (GM) and of the spinal cord (SC) obtained from the level of the caudal lumbar region in normal fetuses and the ratios of these measurements (GM/SC)

Fetuses	Diameter of gray matter in mm	Diameter of spinal cord in mm	Ratio of GM/SC
52	1.125	1.500	0.750
53	0.938	1.312	0.714
54	0.844	1.125	0.750
55	1.219	1.594	0.765
56	0.844	1.125	0.750
57	1.031	1.359	0.759
58	1.106	1.500	0.738
59	1.312	1.688	0.778
	Mean 1.052	Mean 1.400	Mean 0.750

Table A12. Transverse diameter measurements of the gray matter (GM) and of the spinal cord (SC) obtained from the level of the caudal lumbar region in dysraphic fetuses and the ratios of these measurements (GM/SC)

Fetuses	Diameter of gray matter in mm	Diameter of spinal cord in mm	Ratio of GM/SC
249	1.725	2.062	0.836
250	1.406	1.688	0.833
251	1.500	2.025	0.741
252	1.031	1.350	0.764
253	1.669	1.969	0.848
254	1.406	1.688	0.833
62	0.938	1.172	0.800
63	0.656	0.919	0.714
68	1.031	1.312	0.786
69	1.519	1.894	0.802
	Mean 1.288	Mean 1.608	Mean 0.796

Table A13. Areas of dorsal root ganglia (DRG) in normal and dysraphic fetuses in the lumbosacral region

Normal fetuses	Area of lf. DRG in mm^2	Area of rt. DRG in mm^2	Dysraphic fetuses	Area of lf. DRG in mm^2	Area of rt. DRG in mm^2
52	0.392	0.397	249	0.510	0.691
53	0.485	0.461	250	0.416	0.436
54	0.382	0.333	251	0.318	0.279
55	0.652	0.613	252	0.416	0.480
56	0.377	0.387	253	0.284	0.338
57	0.319	0.279	254	0.407	0.392
58	0.451	0.451	62	0.137	0.157
59	0.534	0.593	63	0.475	0.539
			68	0.059	0.083
Overall mean	0.444		69	0.314	0.299
			Overall mean	0.352	

Table A14. Ratios of areas of dorsal root ganglia (smaller area to the larger area) in normal and dysraphic fetuses in the lumbosacral region

Normal fetuses	Ratio	Dysraphic fetuses	Ratio
52	0.987	249	0.738
53	0.951	250	0.954
54	0.872	251	0.877
55	0.940	252	0.867
56	0.974	253	0.840
57	0.875	254	0.963
58	1.000	62	0.873
59	0.900	63	0.881
		68	0.706
Mean	0.937	69	0.952
		Mean	0.865