A possible mode of inheritance for spinal dysraphism in the dog with a more complete description of the clinical syndrome

by

Mary E. Shelton

A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
MASTER OF SCIENCE

Department: Veterinary Anatomy, Pharmacology and Physiology
Major: Veterinary Physiology

Iowa State University
Ames, Iowa

1977

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURE

DEFINITION OF SYMBOLS USED

A_4 = These are animals exhibiting severe signs of spinal dysraphism including bilateral hopping gait, conformational abnormalities and sensory deficits of the hind limbs. These animals are homozygotes, DyDy.

A_2 = These are animals exhibiting some sensory and reflex deficiencies and are synonymous with a heterozygote $Dy^{+}Dy$.

A_1 (Ne) = These are animals exhibiting very slight to no sensory or reflex deficiencies. These animals are extracted from dysraphic stock animals.

N = These animals are animals produced from a Weimaraner not affected with spinal dysraphism crossed to a normal outcross animal.

N_0 = These are normal outcross animals. The breed has had no previous history of spinal dysraphism.

INTRODUCTION

The malformations generally attributed to faulty neurulation are among the most severe anomalies found in man and other animals. Many of these are heredopathies which are incompatible with independent or prolonged existence outside the uterus. Neurospinal dysraphism, an abnormal fusion of dorsal midline structures in the embryo, is one such disease entity. Although different aspects of the syndrome have been long recognized and described in man and animals there are conflicts regarding the etiologic relationships in different species.

Benda (1959) characterized cleft formations as a major dysraphic congenital malformation of the nervous system which are often associated with severe osseous and cutaneous defects. He suggested a genetic and accidental cause for the neurospinal dysraphism which manifests itself in cutaneous, mesodermal and neural pathology. Subsequently numerous genealogical examinations have been conducted in an effort to describe population frequencies of certain dysraphic lesions. Because of the broad nature and the many conditions included in the defined neurospinal dysraphic syndrome, authors have studied collectively or separately many of the developmental abnormalities. Therefore, in the etiologic descriptions in man, single changes or combinations of changes are examined using mainly survey data. This has resulted in a definition of neurospinal dysraphism with as yet an undetermined genetic etiology.

Most studies in mice, cats, dogs, and other species have not provided conclusive answers as to the etiology of the condition because the disease syndrome can be so variable. One form, canine dysraphism, can be exactly defined. Because this form of dysraphism is similar to a mild form of the conditions observed in man, considerable information would be gained by determining the inheritance pattern of this syndrome. Identification and search for the related abnormalities in the canine population is important because it would add insight into etiologic relationships of lesions, allow a better description of the disease syndrome and possibly define a canine model of a human disease.

The purpose of the research was to prove and determine the mode of inheritance of spinal dysraphism in the dog. At the same time the syndrome was studied and classified in order to provide a better clinical description of the disease and to determine the relationships of neural and non-neural lesions to the neurospinal dysraphism syndrome.

LITERATURE REVIEW

Neurospinal Dysraphism in Man

Status dysraphicus is an all inclusive term which describes an improper union of midline structures in the embryo. The term implies a relationship between many different conditions which may involve mesodermal and ectodermal tissue changes or deficiencies (Stedman, 1976). Authors of recent genetic and pathologic descriptions of dysraphic states have replaced this term with neurospinal dysraphism (NSD), but have retained the broad definition of the syndrome.

An initial dysraphic state was described by Fuchs in 1909 as a myelodysplasia. Benda (1959) also limited his definition to nervous tissue changes. He attributed the myelodysplasia to arrested development of intrinsic spinal cord structures before complete differentiation had occurred.

Based on a clinical survey, Bremer (1926) suggested a close relationship between syringomyelia, a cavity within the spinal cord proper, and NSD. He established groupings of patients based on the occurrence of clinical signs characterized by varying degrees of the syringoid state. Bremer also incorporated other anomalies into the "syringomyelia syndrome". These included koilosternia, kyphoscoliosis, spina bifida, arachnodactyly, clinodactyly and hypertrichosis (colics, streams). Specific sensory deficits causing enuresis and absence of pain and temperature recognition with presence of

touch and pressure recognition were incorporated into the syringoid state.

A more complete definition of the neural tube malformations includes a variety of changes. Skeletal anomalies (James, 1967) have been grouped into neural arch defects which may occur with or without a meningocele or meningomyelocele and may be accompanied by vertebral body defects at any spinal level; diastematomyelia may also be present. Segmental defects of vertebral bodies may be present with frequent occurrence of scoliosis. Anterior vertebral body defects causing kyphoscoliosis and vertebral fusion have been defined. Lastly, a minor fusion abnormality which may affect vertebral bodies, laminae or spines may occur with other groups of abnormalities or as isolated features in an almost normal vertebral column.

Recently, evidence has been accumulating which defines a number of clinical syndromes under the general heading of neurospinal dysraphism because of etiologic relationships. This syndrome includes abnormal attachments of the conus medullaris to neighboring structures with anomalies of the spinal cord, vertebrae and skin (Till, 1969; James and Lassman, 1972). Familial surveys have shown an etiologic relationship of anencephaly, iniencephaly, encephalocoele, and spina bifida (Record and McKeown, 1950; Carter, David, Laurence, 1968; Carter and Evans, 1973a). Sever (1974) presented evidence that spondylolisthesis and meningomyelocele are related. Wynne-

Davies (1975) has illustrated that congenital scoliosis, involving multiple vertebrae with or without concomitant spina bifida may be etiologically related to other neural tube malformations.

Carter, et al. (1976) have further classified the abnormalities which are considered under the general heading of spinal dysraphism. All cases have an abnormal conus and may have abnormalities of the cord and meninges (diastematomyelia, meningocoele, lipomata), the skeleton (spina bifida, vertebral body abnormalities, vertebral arch abnormalities), the skin over the spine (hypertrichosis, dermal sinus, dimple, lipoma) or neurological deficits of the lower limbs (shortening of the feet, talipes equinovarus, anesthesia) and urinary incontinence.

Evidence exists which links other conditions or structural changes to the dysraphic state. These conditions include an Arnold-Chiari syndrome (Padget, 1972), renal dysplasia (Forbes, 1972), a Dandy-Walker syndrome (Padget, 1972; Benda, 1954), conditions secondary to any of the above malformations (Bokinsky, Hudson, Weil, 1973), and fibrolipomas (Emery and Lendon, 1969).

Pathogenesis of Neurospinal Dysraphism

In discussions of a possible pathogenesis of NSD many authors use spina bifida as an experimental model. Because the different types or the severity of spina bifida may be related to the time of lesion development, it is important to define this disease entity. The least severe form, spina bifida

occulta, refers to a defect in the closure of the posterior wall or lamina of the vertebrae without associated abnormalities of the spinal cord or meninges. The spina bifida cystica form includes meningoceles and myelomeningoceles, that is, any herniated nervous tissue containing cerebrospinal fluid (CSF) covered by a cutaneous epithelium. Spina bifida aperta, the most severe form of anomaly, represents early fetal transformations leading to an exposed or split spinal cord, myeloschisis. In later discussions of the genetics of human spinal dysraphism, spina bifida is not divided into the subgroups and is discussed as one disease entity.

Theories regarding the development of an open or rachischistic lesion of the neural tube fall into two basic categories. One postulate, deviating from the definition of NSD, is that the neural tube fuses normally and subsequently reopens or ruptures. Other authors hypothesize that the tube never undergoes normal closure at one or more sites. This has been thought to be due to either an overgrowth of neural tube tissue or an undergrowth of the tissue. Since the time in development that the two postulated events might occur is entirely different, an understanding of the pathogenesis is important.

Gardner (1961 and 1973) resurrected a former theory of rupture of the fused neural tube due to abnormal distention and pressure within the central canal. He described the hydromyelia as a change secondary to an inefficient filtration and drainage of CSF from the fourth ventricle with a flow

of CSF from the fourth ventricle down the central canal. This theory is dependent on finding abnormalities of the foramen magnum, with developing hydrocephalus and syringomyelia common to a high percentage of cases. Bonnevie (1934) advanced further evidence for this theory on the basis of a strain of mice having myelencephalic blebs that supposedly arose secondary to the escape of CSF from a closed neural tube. The theory assumes a common histopathological finding of remnants or an actual mesodermal or ectodermal covering of the open neural tube.

A more recent proposed pathogenesis is given by Padget (1968). She describes a normal closure of the neural tube which subsequently undergoes a process of neuroschisis, in which a cleft in the dorsal neuroectoderm may allow escape of CSF into subectodermal spaces or into the amniotic cavity. She suggested that since closure of the primitive skin is accomplished only after closure of neuroepithelium, the normal closure must be followed by a reopening. More exact and complete examination of the open tube revealed a walled cavity over the neural tube, a cavity wall, or remnants of a wall, composed of ectodermal and mesodermal cells. The primary defect, neuroschisis, allows fluid to escape which may then form walled cavities. These cavities may rupture with eversion of nervous tissue or the rupture may heal and appear closed. The neuroschisis, in any case, involves damage to neural cells, resulting

in loss of proper axonal growth, spinal cord differentiation, neuronal interaction or any directional mechanism from the nervous structures to developing mesodermal, ectodermal and endodermal structures.

A less popular hypothesis has been advanced by Vogel (1961). In his discussion, limited to anencephaly, Vogel stated that the cephalic changes are secondary to malformations in the pattern of cerebral vasculature. Although his studies have been limited to older embryos some experimental occlusions of the internal carotid artery have also suggested arrest of cerebral development. The evidence is not complete and it is unclear if early occlusions of that specific vascular supply can cause all lesions associated with anencephaly. Vogel did not postulate on the possible relationship of spinal cord developmental anomalies.

Somewhat supportive evidence for vasculature changes as a cause or effect of NSD were suggested in a recent diagnostic review of arteriovenous (AV) malformations of the spinal cord (Tobin and Layton, 1976). Anomalies known to be associated with the anastamoses include anomalies of the vertebrae, scoliosis, congenital heart defects and angiomas. Nitter and Tonnis (1950) suggested that the AV malformations may be one facet of a dysraphic state. They based their conclusion on a high percentage of cases (9 of 13) with AV abnormalities which had associated mesenchymal or ectodermal anomalies. Clinically,

however, the A-V syndrome is progressive and the onset is later in life.

Experimental studies in animals do not strengthen these encephaloclastic or myeloclastic theories. Murakami, et al. (1972), have produced reopening of the midbrain in rat fetuses whose mothers were injected with vincristine. However, the rest of the neural tube was closed. Fowler (1953) illustrated that fused neural tubes of a chick embryo can be reopened by external mechanical manipulation with a sharp instrument. The latter experiment does not substantiate the internal causes of neuroschisis proposed by Gardner (1961) or Padget (1968).

In contrast, many neuroembryologists support a non-fusion etiology for NSD. Recklinghausen (1886) favored a "developmental arrest" of neural tube tissue and implied that a reduced metabolic rate in these tissues may be manifest as a reduced mitotic activity and non-fusion of the neural crest. Warkany et al. (1958), experimentally produced myeloschisis, myelomeningocele, myelocele, and spina bifida in the same animal at different levels. The mechanism involved here in preventing neural tube closure is probably a direct toxic effect of the dye, trypan blue, on the cells of the mouse egg cylinder (Hamburgh, et al. 1975). Warkany et al. postulated that a lumbosacral dysraphism initiated at the time of closure of the posterior neuropore may lead to an open neural plate and degeneration of the central nervous tissues as well as the peripheral nerve

roots. In addition, they found evidence of regenerative changes. These changes were recognized in the formation of a sac which was formed by shedding of the exposed neural plate tissue, epithelization and vascularization of surrounding meninges. Regenerative changes were also evidenced by the production of hypertrophic skin patches at points where the cord attached directly to epidermis. Many changes were produced simultaneously, similar to the natural form of the disease.

Many of the morphological characteristics of neurospinal dysraphism can be accounted for by neuroectodermal overgrowth or inhibition of mesodermal growth. It is thought that differential growth of neuroectoderm and mesoderm is necessary to bring about formation of head and tail folds during neurulation. Abnormalities at this time might explain the common involvement at one or both extreme sites (Barson, 1970). Patten's (1953) observation that an early overgrowth of nervous tissue at the point of myeloschisis is found in some cases of NSD and substantiates Barson's explanation of how neural folds may be prohibited from fusing properly. Patten again points out that the sequence of events is very important in determining the time of the maldevelopment. A primary neural tube nonclosure would secondarily lead to bony and other soft tissue abnormalities. That is, a spina bifida which coexists with a myeloschisis should be regarded as a secondary change due to

an abnormal pattern established by a change in the area into which it was to grow. Patten also strengthened the observations of neural degenerative processes by noting involvement of ventral horn motor neurons in the area of the myeloschisis.

In a study describing the occurrence of NSD in chicks exposed to specific antibody to duck brain tissue during early embryonation, Barson (1972) noted the predominance of open neural tubes which spread laterally over the dorsal surface of the embryo. He questioned whether the apparent overgrowth or misdirected growth is a cause or result of a dysraphism. That such a picture might be due to loss of a localized growth inhibition of neural crest cells after abnormal non-fusion is possible. Then any blockage of normal fusion would block the normal growth inhibition and growth of the neural tube may subsequently be excessive at this point. In contrast, the mesodermal tissues, having lost proper neural control or relationships in a non-fusion area, manifest a growth retardation. This is manifested in failure of somite segmentation and stunting in axial elongation.

In an important autoradiographic study of experimentally produced (trypan blue) spina bifida in mice, Lendon (1972) suggests that irregularities in cell divisions may not be the most important cause of NSD. Using tritiated thymidine he did not find any indication of an increased or decreased mitotic activity in early states of NSD. This suggests that

neural overgrowth in trypan blue-induced myelomeningocele is a secondary phenomenon. Because the radiolabel entry into the embryo is mechanically retarded there may be some temporal developmental relationships which are not clear. It appears that the neural overgrowth is probably secondary to a primary non-fusion. Hsu and van Dyke (1948) substantiate this fact when they noted a retardation of the mitotic rate prior to a subsequent neural overgrowth in a mutant mouse strain. The problem still exists whether the faulty closure of the neural plate is due to an arrest of its development and functions or whether overgrowth factors may be involved (Emery and Lendon, 1972). It is still unclear whether the primary defects are in the ectoderm or mesoderm and when in developmental time they occur.

In general then, the malformations may be characterized by the time of development. Malformations in early gestation (less than 25 days) are those in which nervous tissue is found externally and not covered by intact ectoderm or skin. After the posterior neuropore closes (between 25 and 45 days) any malformation is covered by intact skin. Later changes are also covered with intact skin and involve structures developing at this time. Vertebral malformation found in conjunction with neural defects arise during the last two time periods. These changes may then involve many structures forming complex anomalies (Lemire et al., 1975).

Description of Human Neurospinal Dysraphism

Clinical description of NSD includes a whole range of deformities including the skeletal and neural malformations. Individuals with one or more structural abnormalities may exhibit a combination of sensory and reflex abnormalities. Paresis and paralysis may be seen unilaterally or bilaterally. Dissociation of temperature and pain sensation may be observed over very specific dermatomes (syringomyelia) of the trunk or limbs (foot). Enuresis with secondary renal malfunction may be seen. Patellar tendon and gastrocnemius tendon reflexes may be poor or absent. Secondary to development of other neural changes, individuals may exhibit ataxia, convulsions or other signs of hydrocephalus. Because development of the caudal digestive tube is synonymous in time with that of the neural tube, dorsal intestinal fistulas may be present, the anal tone and reflex may be poor with depressed peri-anal sensation. The level and the type of deformity will determine the clinical picture of each case. This may include a complex, multiple site lesion involvement with similar complex clinical signs.

Histopathological studies on spinal dysraphic individuals have revealed various degrees of involvement at different cord levels. The primary morphological changes associated with the dysraphic state are in the spinal cord and include clefts or absences of the posterior septum, asymmetry or absence of the anterior horns, absence or thinness of the posterior horns,

anomalies of the central gray matter and absence, duplication, occlusion, enlargement or displacement of the central canal (Benda, 1959). Curtis (1934 and 1939) and Benda (1959) have associated syringomyelia with the dysraphic state. Absence of hair follicles and sweat glands and degeneration of anterior and posterior nerve roots have been described by Lichtenstein (1940).

Recently a relationship has been observed between the clinical picture and the histopathological changes of the spinal cord. Emery and Lendon (1973) have described the spinal cord changes at the level of a gross deformity (e.g., meningomyelocele or open neural tube) as well as above and below this level. At the level of the deformity a wide variety of cord lesions were found, but four groups of changes were reported. The two most common categories consisted of a spinal cord with some degree of duplication or with a flattened appearance. Dorsoventral elongation of the central canal which may or may not open to the subarachnoid space was found less commonly. A less homogeneous description defines the least common group which includes syringomyelia, arachnoid cysts, exposure to the surface or fibrolipoma. Changes seen cranial to the deformity included hydromyelia and partial diplomyelia. Normal cords, complete diplomyelic cords and double or multiple central canals were seen caudal to the gross deformity. Emery, et al. (1973) found a diminution of dorsal root ganglion neurons in

children exhibiting paralysis associated with neurospinal dysraphism. Rálĭs and Rálĭs (1972) described a decrease in the diameter and an associated loss of function of the sciatic nerve in paralyzed individuals with spina bifida. Corresponding changes, secondary to the neuronal changes, were described in the muscles of the hind limb.

Even though neurospinal dysraphism has been long recognized as a medically important condition, an etiological and syndrome definition has yet to be evolved. Genealogy studies and histopathologic changes correlated with clinical changes suggest a relationship, etiologically, between many clinical syndromes and deformities.

Spinal Dysraphism in Other Species

Exencephalic forms of spinal dysraphism have been reported in swine (Nordby, 1929), cattle (Shaw, 1938), and mice (Garber, 1952, Bennett, 1959, and Bonnevie, 1934). Spinal dysraphic changes characterized primarily by spina bifida with or without myeloschisis have been reported in the cat (James, Lassman and Tomlinson, 1969 and Kitchen, Murray and Cockrell, 1972 and Martin, 1971), rabbits (Grüneberg, 1947) and mice (Reed, 1937, Kumminek, 1959, Strong and Hollander, 1949 and Hollander, 1976). Syringomyelia has been reported in rabbits (Nachtsheim, 1931) and cats (James, Lassman, Tomlinson, 1969). The reports describe characteristic skeletal changes and histopathologic lesions characteristic of other spinal dysraphic syndromes (Patten, 1953;

Ostertag, 1930).

Spinal Dysraphism in the Dog

The first report of this condition in dogs was given by Lienaux (1897) when he observed the condition in a two year old Newfoundland. The animal exhibited slight paralysis of one pelvic limb, Harbitz (1942). More recent occurrence of spinal dysraphism in the dog has been exhibited primarily in the Weimaraner (McGrath, 1965), but has also been observed in the English Setter, Golden Retriever, (Draper, et al., 1975) English Bulldog, Huskies, (Parker and Byerly, 1973) Samoyed (Furneaux, Doige, Kaye, 1973) and mongrels (Geib and Bistner, 1967).

As previously defined, status dysraphicus is a broad term. Although each type of ectodermal or mesodermal heredopathy in man associated with neurospinal dysraphism has been described in the dog, only infrequent and sketchy pedigrees have been available and experimental breeding has been limited so that etiological relationships are uncertain. A review of hereditary malformations of the nervous system in dogs reveals that exencephaly, (Little, 1948; McGrath, 1956; Fox, 1963; Fuller, 1956; Parker, et al., 1973), spina bifida, (McGrath, 1956; Akker, 1962; Furneaux, Doige, Kaye, 1973; Parker and Byerly, 1973 and Parker et al., 1973), syringomyelia, (McGrath, 1965; Geib and Bistner, 1967; Furneaux, Doige, Kaye, 1973), multiple vertebral deficiencies, (Curtis, English, Kim, 1964) and hydromyelia (McGrath, 1965; Geib and Bistner, 1967) may be incorpor-

ated as single or multiple gene defects. Results obtained from other experimental breeding colonies have suggested a genetic pattern for certain of these conditions but no common etiology has yet been determined (Fuller, 1956; Curtis, English, Kim, 1964; McGrath, 1965).

A classical syndrome of spinal dysraphism in the Weimaraner has been described by McGrath (1965). According to McGrath the chacteristic clinical signs first appear at four weeks of age and consist of symmetrical synchronous hind limb movement at a walk or slightly faster pace. Postural abnormalities such as a crouched posture with unilateral abduction of a hind limb with or without an over-extension of one or both hind limbs in a wide based stance are often observed.

Additional but less consistent anatomical deformities include scoliosis, fusion of vertebrae, spondylosis, abnormal hair growth, koilosternia, and kinking of the tail (McGrath, 1965). Less frequently, head tilt is observed in young animals. McGrath (1965) also suggested the occurrence of a malabsorption syndrome associated with the spinal dysraphic state (Thompson, 1958; Thompson and Murchison, 1958).

Characteristic morphological changes of the spinal cord in the dog have been described. Not all of the histopathologic changes are observed in all affected dogs. Any or some of the lesions may be present at different levels of the spinal cord. The findings most common at the lumbosacral levels in the dog

are similar to those in man and include a cleft or absence of the dorsal septum; thinness, absence or neuronal deficits of the ventral gray horn; a blunted, absent or cavitated dorsal gray horn; flattened or rotated central gray matter; absence of the ventral median fissure; and enlargement, duplication, dorso-ventral elongation or displacement of the central canal. McGrath (1965) also listed spinal vascular anomalies exemplified by ectopic glial cells in the area of the ventral spinal artery and meningeal fibrosis, calcification and ossification as commonly seen changes. McGrath suggested that because of a high incidence of spinal cord cavitation or syringomyelia in the dogs that it be considered as a part of the dysraphic syndrome.

No attempt has yet been made to correlate developmental changes at the different cord levels to the clinical signs exhibited.

Genetics of Neurospinal Dysraphism

Genetics of human spinal dysraphism

Through an examination of clinical genealogies and the study of twins, the effects of noxious genes or mutant genes or environmental factors on different individuals may be ascertained. Environmental factors have been shown to cause spinal dysraphic defects. Chemical toxicity (Warkany, Wilson, Geiger, 1958), irradiation (Rugh and Grupp, 1959; Moriarty and Klingman, 1965) and plant toxins (Poswillo, et al., 1972; Renwick, 1972, and

Sever, 1973) can cause similar neural tube abnormalities. However, certain neural developmental defects have genetic etiologies.

Early descriptions by Bremer (1926) initiated the description of a complex syndrome, NSD. In this he alluded to hereditary etiologic relationships of many conditions. Definition of status dysraphicus which included syringomyelia with NSD was strengthened by this complex (Curtis 1934 and 1939), based on familial studies. He demonstrated a hereditary etiologic relationship of some syringomyelic signs to a hereditary status dysraphicus. From his survey Curtis recognized the variability in signs and suggested a genetic pattern based on a pleotropic gene with some degree of dominance. He suggested that status dysraphicus may dispose an animal to other heredopathies of the nervous system.

The familial occurrence of syringomyelia has been described repeatedly. Van Epps and Kerr (1940) recorded 26 cases of lumbosacral syringomyelia in three successive generations of four families. Mueller and Sugar (1943) have described a number of cases with familial occurrence, (although a genological search was not done). Clinical involvement in 10 unrelated families suggested a genetic etiology to Benda (1959).

An examination of monozygotic twins points to the possibility that heredity may not be the complete etiology. Discordant involvement in twins indicates environmental factors may be involved. Compilation of several genealogies indicate dominance or incomplete dominance while some indicate recessiveness

(Moriarty and Klingman, 1965).

Because there is more than one gene that controls the development of all mesodermal and ectodermal tissues associated with the nervous system (Verschuer by Moriarty and Klingman, 1965), a genetic description of status dysraphicus is not available, however, modes of inheritance for specific clinical syndromes within the broad disease description have been proposed.

In an epidemiological study, Sever (1974) revealed a hereditary etiologic relationship between spina bifida with meningomyelocele and spondylolisthesis. Sever (1974) suggested an autosomal dominant inheritance pattern with variable expressivity. Wynne-Davies (1975) stated more emphatically that multiple vertebral abnormalities, spina bifida and anencephaly share a common genetic etiology.

The low frequency found in a compilation of cases of spina bifida, encephalocele, anencephaly, iniencephalus and microcephalus (Carter, David and Laurence, 1968) are compatible with the hypothesis that there may be a common etiology consisting of polygenic inheritance and intrauterine factors (Carter, 1969). The low incidence rates of spina bifida and anencephaly among twins and a higher incidence in siblings led Yen and MacMahon (1968) to postulate low penetrance rates of one major gene with some degree of dominance. Carter and Evans (1973a) resurrected a multifactorial etiology for general neural tube malformations

with the variability also determined by environmental factors. They did state that there are no indications for any single gene-determined component among this group of malformations. In a later paper, Carter and Evans (1973b) prove that there is no sex prediliction for spina bifida. Further Carter and Evan's compilation of previous studies indicate that the risk to children of affected males is at least as high as the risk to children of female patients, the risk being approximately 3%.

In genealogic studies of families with major neural groove closure anomalies, Polman (1950) and McKusick (1971) suggested an autosomal recessive inheritance. Penrose (1957) found a lower incidence of such major defects suggesting a multiple gene inheritance with environmental factors and chromosomal translocation determining the phenotype. Then later in a single family survey, Wright et al. (1974) disclosed the possibility that chromosomal translocations may be the primary factor causing craniorachischisis (anencephaly plus spina bifida). Other isolated reports of cytogenetic studies suggest chromosomal abnormalities but reveal a lack of information relating the cause and effect of the chromosomal abnormalities (Spellman, 1966).

In 1962 Wiltse defined the etiology of spondylolisthesis as due to a hereditary defect characterized by dysplasia of the vertebral arches. The dysplasia causes strain upon the

interarticularis region of the affected vertebrae. Further compilation of his data suggested a lesion transmitted as a single recessive gene with incomplete penetrance and without sex linkage. Amuso and Mankin (1967) presented a contrasting genetic pattern in a family with spondylolisthesis and simultaneous spina bifida. Unlike previous published reports, the transmittance in this family suggested to the authors that the trait was passed through three generations as an autosomal dominant. In contrast, Lorber (1965) has suggested that spina bifida is transmitted via an autosomal recessive gene. Although he did review incomplete genealogies, he found a 6.8% incidence of gross neural tube malformations and a higher incidence in families with more than one offspring.

In a necropsy study of children who died of meningomyelocoele, Emery and Lendon (1973) supported an etiologic relationship between spinal dyschesia, lipoma and vertebral body abnormalities. Carter, Evans, and Till (1976) found a 4% incidence of anencephaly and spina bifida in sibs of patients with spinal dysraphism leaving little doubt that neural tube malformations (anencephaly and myelomeningocele) are etiologically related to spinal dysraphism. The proportion of siblings with neural tube malformations is similar when patients have spinal dysraphism or other neural tube malformations. It therefore appears that the classical, broadly defined neural tube malformations (spinal dysraphism) including severe and mild spina

bifida, congenital scoliosis and spondylolisthesis are genetically determined and etiologically related. Unfortunately, concurring data has not yet allowed a firm definition of the mode of inheritance of the broad condition or of any one specific syndrome.

Genetics of spinal dysraphism in other species

Genetic descriptions of neural heredopathies in mice are most common. Although many genes determine the development of the nervous system, primarily single gene changes have been produced in mice. An autosomal recessive gene (Bonnevie, 1934) has been shown to produce myelencephalic blebs while an autosomal dominant (Russell, 1947) has been found to produce early myeloschisis in the lumbosacral region. Spina bifida with myeloschisis in the lumbosacral region has been produced consistently with the curly tail trait (Grüneberg, 1954). Semidominant lethal patterns have been used to explain a short tail strain of mice with spina bifida. Another semidominant explanation is proposed for the fused vertebrae strain with high incidences of spina bifida. Strong and Hollander (1949) suggested a semidominant lethal inheritance for complete craniorachisis in loop tail mice. Most recently the recessive mutant "snubnose" has been shown to exhibit spina bifida as one of its major effects (Hollander, 1976).

An autosomal recessive gene was proposed by Grüneberg (1947) to produce a myeloschisis syndrome in rabbits. Crary,

Fox and Swain (1966) discovered a form of spina bifida which carried an achondroplastic gene. The bifidic defect, affecting the entire spine, was transmitted as a lethal autosomal recessive. Syringomyelia in rabbits has been defined as a main gene effect which was in general recessive (Nachtsheim, 1931). He suggested that interaction of the gene with modifiers explained the variable expression of the syndrome.

Hughes and Hart (1934) attributed exencephaly in swine to a "recessive factor", but more accurate descriptions were not available.

Although the tailless feature of the Manx cat is thought to be an autosomal dominant gene with partial lethality (Todd, 1961), descriptions of the inheritance pattern of the spina bifida and syringomyelic lesions associated with this breed characteristic have not been possible.

Genetics of canine spinal dysraphism

Although spinal dysraphism has been recognized in dogs, there have been few suggestions of a genetic etiology. Fuller (1956) found that stillborn pups from inbred beagles had malformations of the skull which included overshot jaws, twisted ears and iniencephaly. Analysis of this syndrome indicated an inheritance pattern based on a recessive gene. A paradoxically lethal hereditary open fontanelle syndrome without a central nervous abnormality of cocker spaniels (Pullig, 1952) has been described. In the same breed, Little (1948) reported

a single instance of a "skull not grown together". In an investigation of a "stub" tailed condition of beagles, Curtis, English and Kim (1964) experimentally reduced the number of caudal vertebrae and produced a case of bifid centrum of the sacral vertebrae and two cases of spina bifida of the lumbar vertebrae. The "stub" tail condition appeared to be inherited as an autosomal dominant factor with reduced penetrance and variable expressivity. They suggested a multifactorial cause for the variable expressivity of the skeletal changes. They further suggested that the skeletal abnormalities were due to notochordal defects which produced changes in the sclerotome. Other reports of dogs with possible hereditary deformities of the spine (Suu by Kalter, 1968; Burns and Fraser, 1966) described a shortened spine with vertebral bifidity. However, the spinal cord was not investigated and the genetic pattern was not ascertained.

McGrath (1965) has presented the only discussion of the genetic etiology of spinal dysraphism in dogs but has not elucidated the pattern of inheritance. Geographic origin and litter incidence allowed him to suggest a primary genetic etiology rather than an environmental influence. He did not find a predominance in either sex. In his paper McGrath has alluded to an etiologic relationship between syringomyelia and spinal dysraphism in the Weimaraner. Although he has presented numerous abbreviated pedigrees, the results, based

on his definition of the clinical syndrome in offspring and parents, do not lend definitive data relative to the recessiveness, dominance or penetrance pattern of the gene or genes involved. He did suggest a variable expressivity as evidenced by gradations and variability of clinical signs and histopathologic lesions.

It is apparent from the literature that 1) the disease syndrome, neurospinal dysraphism, includes a vast number and type of developmental anomalies, 2) some of these abnormalities are prevalent within family lines, 3) many vertebrate species have similar spontaneous or induced involvements, and 4) the etiologic interrelationships of the complex syndrome are far from being understood.

Studies of specific forms of NSD have allowed some exact genetic information in the murine species (Hollander, 1976) and in man (Sever, 1974). Although the canine model of the syndrome is well-known, a complete description of the disease syndrome along with a definition of the inheritance pattern is lacking. Published pedigrees (McGrath, 1965) and isolated reports (Draper, et al., 1975), although contradictory have allowed an insight into a possible mode of inheritance of NSD in the canine. Based on descriptions of canine neurospinal dysraphism as one genetic entity or disease syndrome, the gene or genes involved seem to have some degree of dominance. From analysis of published pedigrees (McGrath, 1965) it

was observed that crosses between affected animals yielded some normal progeny. The affected animals thus appeared to be heterozygotes. The homozygote affected animals, not recognized in these crosses, were thought to have died in utero or before the condition could be evaluated. These findings suggested a codominant lethal gene as the mode of inheritance. Because "normal" Weimaraner crosses produced some affected offspring, reduced penetrance would have to be involved. Through examination of pedigrees and some previous work (Draper, et al., 1975) a codominant lethal inheritance pattern with reduced penetrance was suggested as the genetic etiology of canine NSD. The purpose of this research was to test this working hypothesis.

In order to prove a codominant hypothesis, it was necessary to find crosses with wild type (a breed not affected with the trait in question) yielding non-wild type (affected) F_1. If the F_1 animals looked like the affected parent, the parent would be homozygous for at least one dominant mutant. If, on the other hand, the F_1 animals are defined as intermediate, the affected parent type would be homozygotes for a partial-dominant, intermediate or codominant mutant(s). The F_2, backcross and testcross data would then be used to determine a frequency of affected types and as such would indicate how many mutants were involved (e.g., the irreducible ratio or group frequency indicates the minimum number of mutants involved. A ratio of fourths indicates one mutant in an F_2).

MATERIALS AND METHODS

Animals

Breeding stock

Throughout a three year period 12 purebred Weimaraners with a characteristic "bunny hop gait" and abducted hind limb(s) were obtained from private homes from various regions of the United States. All animals were at least six weeks of age when obtained. Nine adult purebred Norwegian Elkhounds, two adult purebred German Shepherds, one Irish Setter and three German Shorthaired Pointers were also procured for outcross mating. In addition, normal Weimaraners were kept at three private homes and also used in outcross breedings.

Management

Animals were kept in a closed canine research colony in semi-open 12' x 50' kennels. All dogs received canine distemper, hepatitis, leptospirosis and Bordetella bronchiseptica vaccinations regularly and routinely (or as needed) and received pyrantel tartrate or piperazine as an anthelminthic. Other prophylactic and emergency treatments were administered as needed. Water and feed were available ad libitum from the age of 4 weeks throughout the time spent in the colony.

Procedures

Experimental matings

Reciprocal mating of affected animals and the normal

animals were performed. Females in estrus were observed for natural mating. If this was not recorded then the appropriate male was ejaculated and the female was artificially inseminated on the first and fourth day of estrus. F_2 and F_3 animals were obtained from reciprocal matings. Backcross breeding to normal (testcross) and affected stock was also performed. Again, reciprocal crosses were recorded. All mating pairs were recorded, offspring counted and sex of offspring defined for each mating. Table 1 outlines all types of experimental matings. A sample mating scheme and the actual results are given in Figure 1.

Neurological and physical examination

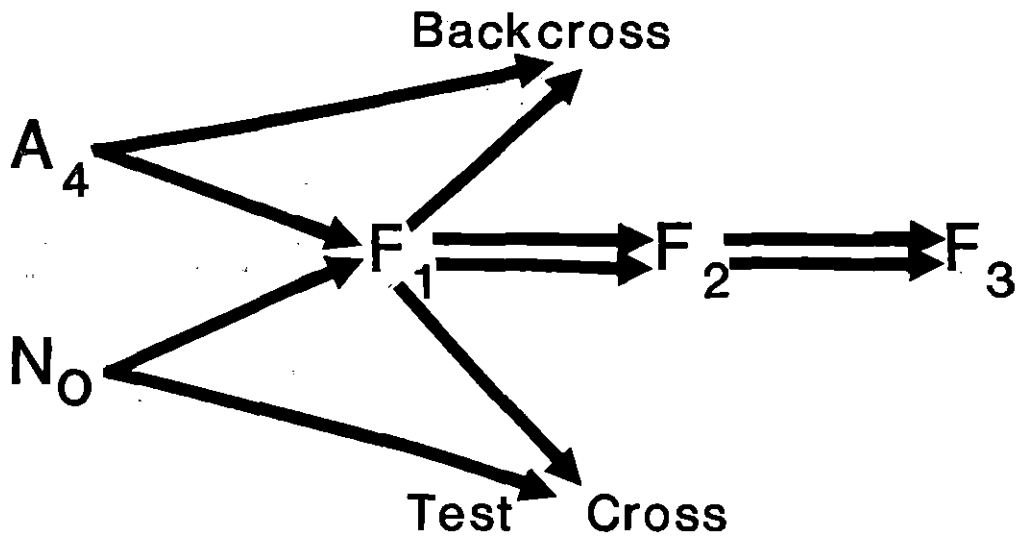
At least two completely independent neurological examinations were performed on all stock animals and their offspring. Two experienced neurologists worked together in each examination to ensure proper positioning of the dog for each test to allow better cooperation by the animal. To prevent bias on the part of the examiners, each person performed different tests during the examinations. The first examination was done at three months of age and the second one was done at least one month later. To insure an unbiased interpretation of the examination one of the two examiners was ignorant of the parentage of the animals and, therefore, was ignorant of any expected results.

Hopping, proprioception, visual and tactile placing

Table 1. Experimental Mating Scheme of Spinal Dysraphic Weimaraners and Normal Breeds

A_4 SEVERELY AFFECTED WEIMARANER

N_O NORWEGIAN ELKHOUND
GERMAN SHEPHERD
GERMAN SHORTHAIR POINTER (FEMALE)
IRISH SETTER (FEMALE)



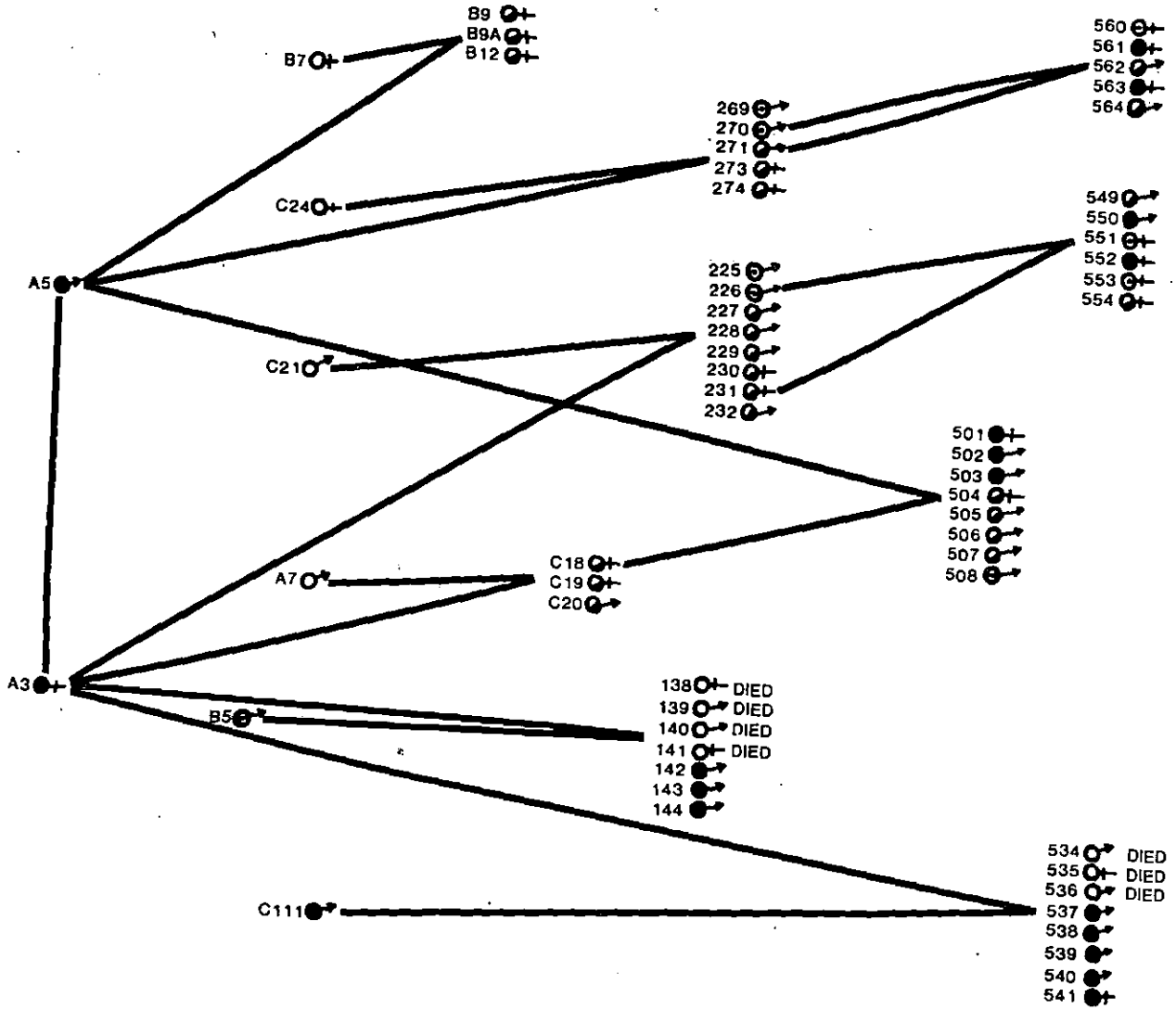


Figure 1. Sample Mating Results. Phenotype Given as a Category of Spinal Dysraphism, A_4=●, A_2=◎, A_1=⊙, N_0=○.

responses to specific stimuli were recorded as normal, hyper-responsive, hypo-responsive or absent. Paramedian and perineal sensations were checked and recorded as normal, depressed or absent. Entire pelvic limb sensory efficiency was evaluated by response to a light pin prick. Responses were recorded as normal, depressed or absent. Specific anatomical areas exhibiting deficit were recorded. During the sensory testing, apprehensive behavior was avoided as much as possible. Any such behavior which could not be overcome was noted and the animal was rechecked carefully later or at the second examination. The withdrawal reflex was tested for the presence of a bilateral response. At this time some reference was made if the examiner thought an increased threshold was needed to cause the reflex withdrawal to pain. Locomotor patterns were observed at various speeds to determine the presence or absence of the characteristic bilaterally synchronous "hopping" gait of the pelvic limbs.

Body conformation was evaluated and any recognized abnormality limb abduction, limb over-extension, over-rotation of pes, arachnodactyly, weakness, head tilt, scoliosis, tail kink, or dwarfism was recorded and described according to the anatomical region involved (Table 2).

Post Mortem Analysis

Upon the death of any colony animal, it was perfused with 10% buffered neutral formalin (BNF) and a complete necropsy was

Table 2. Clinical Findings Associated with Spinal Dysraphism in the Dog. (354 dogs examined)

CLINICAL FINDING	CATEGORY	NUMBER OF DOGS WITH THE VARIOUS SYNDROMES			
		A_4	A_2	A_1	NOT CLASSIFIED
TALIPES EQUINOVARUS		9	0	0	0
OCCIPITAL DYSPLASIA		2	0	1	0
OPEN FONTANELLES		1	0	1	1
CEREBELLAR HERNIATION		2	0	0	0
VESTIBULAR SYNDROME		2	0	0	0
DWARF		0	3	0	4
SCOLIOSIS KINKY TAIL		4	0	0	1
RENAL APLASIA		1	0	0	6

then performed. Abnormalities of the skeletal system (open fontanelles, occipital dysplasia, spondylosis) or other systems (renal aplasia, cardiac abnormalities or other gross changes) were recorded and appropriate tissue samples were taken for histology. Spinal cords of animals less than 7 weeks of age were removed in situ.

Histological Preparation

Only the spinal cord caudal to the T_{12} level was prepared. Each spinal cord level was cut in approximately 1 cm lengths so that equal divisions were obtained. The cut tissues were identified as a thoracic, lumbar or sacral level and the position of the block within each level, designated A, B, C or D. The sections were embedded in paraffin so that sections would be taken from the cranial side of each tissue block. Three serial 10 μ sections were cut from each block and placed on a microscopic slide. The tissues were stained with cresylecht violet (Nissl stain) for identification and cytoarchitectonic studies and counting of neurons within both dorsal and ventral horns (specific laminae).

The procedure for young animals, less than 8 weeks of age, was different. The spinal cord was left within the vertebral column. The vertebral column was bisected through the twelfth intervertebral disc. Each vertebra caudal to this level was separated through the intervertebral disc spaces. Similar to the larger cords, the level of the tissue block was identified

and embedded so that 10 μ sections would be taken from the cranial side of each block. The individual vertebrae with the spinal cord in situ was placed in saturated (10-15%) disodium ethylene diamine tetracetic acid (EDTA). The decalcification was complete after two months at which time the tissue was also embedded in paraffin for sectioning. The same Nissl stain was used in the puppy cord sections.

Cursory examination of the sections was done upon recognition of a gross pathological lesion, serial sections of the cord above and below the level of the lesion was done to determine the extent of the lesion and possible relationship to other structures within the cord.

Data Analysis

A Chi square analysis was used to give the probability of the expected and measured type of animals in the progeny.

RESULTS

Neurological Examination Results

Because quantitative genetics depends on a definition of affectedness and normalcy, a method of differentiating, identifying and measuring the types of spinal dysraphism was necessary. Clinical evaluations with emphasis on neurological examinations were used to classify all stock and progeny animals into grades of dysraphism or normality.

An animal's behavior and experience can modify its response to either painful or non-painful stimuli; two neurologic examinations were done and the results of both compared. The resultant response was obtained by adding the tests so that a negative response one time and a positive the next would nullify any depression of the reflex tested. The light tactile stimulation (placing or pin prick), inhibited in apprehensive animals, was considered a very minimal neural deficit.

The most severely affected animals, designated A_4, show the characteristic hopping gait, usually have an abnormal conformation with one or both hind limbs abducted and over extended. These animals show an inability or decreased ability to perform the hopping test. Characteristically these animals have a loss or depression of proprioceptive reflexes and an absence of touch and/or visual placing responses in both hind limbs. Sensation was diminished over the dorsal, lateral and medial surfaces of the crus, tarsal and metatarsal regions. A

bilateral synchronous withdrawal was consistently found. An increased threshold to the withdrawal response to painful stimuli was suspected in many cases but was not measured. In addition to the above findings, approximately 95% of young, severely affected animals showed a unilateral inward rotation of the pes, a condition resembling talipes equinovarus. When young A_4 animals (greater than six weeks and less than four months of age) were tested for placing responses a pronounced inhibition of the proper lift and place response was produced if one limb was flexed and the opposite limb was stimulated. Other sensory input would not produce the inhibition. (It appears as if the bilateral flexor movement of the pelvic limbs is a necessary reflex or at least a low threshold reflex.)

A category, A_3, has been reserved for those animals which show reflex and locomotion abnormalities similar to A_4 group but which show little, if any, sensory deficits. The hopping gait is observed at a faster gait with alternate stepping observed at a walk. The significance of these animals in the scheme of the disease syndrome is still unclear, however, neurophysiological and histopathological studies have yet to be completed. These animals are considered in the A_4 category here.

A third well-delineated group designated A_2, showed minimal but consistent, neurological deficits. The animals did have a normal gait and conformation. Sensory abnormalities

were limited to the pelvic limbs, and included depression of the proper response to abnormal proprioceptive stimuli. This was evident when the pes was placed on a surface in plantar flexion and the animal did not return the paw to its proper standing position for a few seconds. Diminished uni-ped hopping responses were recognized when an animal knuckled or dragged the pes before trying to hop. The length of the hopping step was noted as normal or long. The proprioception and hopping was recorded as absent or more often as hyporesponsive or a normal response. Tactile and visual placing responses were depressed in some cases. Tactile deficits of the perineum and of the crus, tarsal and metatarsal regions were also exhibited. The sensation, tested by a light pin prick, was characteristically absent on the dorsal and lateral surfaces. The pes was sensitive on all surfaces.

Only those animals exhibiting a combination of tactile or proprioceptive deficiencies of the pelvic limbs with other sensory or reflex deficiency affecting the same or other areas were considered part of the A_2 group. In other words, those animals with two or more of the above deficiencies are considered within this grouping.

The last animal grouping combines the normal animals and those animals which may have an abnormal genotype but do not have an abnormal phenotype (reduced penetrance). The animals which show only a very minimal tactile deficit or sensory

deficit to a pin prick are considered here. Again because of behavioral modification of the responses, a minimal deficit is questionable. Animals in this category are designated A_1 or N_e if they are from stock animals and N_0 if they are normal outcross animals (different breed).

Experimental Matings and Progeny

After categorizing all stock and progeny animals on the basis of a neurological examination the different affected groups were mated or backcrossed to normal or affected animals. The results appear in Table 3. Purebred A_4 stock animals were produced from apparently normal Weimaraner parents. Other A_4 individuals have been obtained from $A_4 \times A_4$ matings, $A_2 \times A_2$ mating, $A_3 \times A_4$ matings and $A_1 \times A_2$ matings. Animals of the A_2 group were produced in $A_4 \times N_0$ and $A_2 \times N_0$ outcross matings and in $A_2 \times A_4$, $A_2 \times A_2$ and $A_2 \times A_1$ matings. $A_4 \times N_0$ and $A_2 \times N_0$ outcross matings as well as $A_2 \times A_2$ and $A_4 \times A_2$ matings have produced the last category of individuals, A_1 or N_e.

Three hundred and fifty-four animals were produced from 50 matings. Of these, 236 animals were examined and classified into a group (A_4, A_2 or A_1). Animals which were not classified died before a neurological examination could be performed. All 20 offspring produced from purebred A_4 Weimaraners were classified A_4. Seven or 47% of the progeny of the $A_2 \times A_4$ matings were classified A_4. $A_2 \times A_2$ matings produced seven A_4 animals or 25% of the total progeny. Three A_4 animals were produced in

Table 3. Results of Experimental Matings: Phenotype Classification of Progeny Based on a Clinical Neurological Examination.

CROSS	LITTERS ♂x♀/♀x♂	PROGENY						
		A_4	A_2	A_1 (N_e)	SUBTOTAL	NOT CLASSIFIED	NEONATAL DEATHS	TOTAL
$A_4 \times A_4$	5	20	0	0	20	1	8	29
$A_4 \times A_2$	3/0	7	6	2	15	2	2	19
$A_2 \times A_2$	7	7	19	2	28	21	8	57
Total	15	34	25	4	63	24	18	105
$A_4 \times N_o$	9/4	0	41	17	58	14	21	93
$A_4 \times A_1$	/1	3	0	0	3	1	3	7
$A_2 \times N_o$	3/6	0	28	27	55	1	9	65
$A_2 \times A_1$	1/4	6	13	8	27	2	2	31
Total	28	9	82	52	143	18	35	196
$N_o \times N$	5	0	1	9	10	7	14	31
$N_o \times A_1$	1/2	0	12	8	20	1	2	23
Total	7	0	13	17	30	8	16	54
TOTAL	50							354

the only litter of an A_4 x A_1 mating. The balance of the A_4 animals were produced in five A_2 x A_1 matings in which six or 22% of the classified animals were in the A_4 group.

A_2 animals were produced in all but two mating categories, the A_4 x A_4 and the A_4 x A_1. The A_4 x A_2 mating resulted in six or 40% A_2 progeny. Nineteen or 68% of the progeny from A_2 x A_2 matings were described as A_2. In the three A_4 x N_0 matings 71% or 41 animals were classified in the A_2 group. Fifty percent or 28 animals in the nine A_3 x N_0 matings were A_2 and similarly, 48% or 13 animals from the five A_2 x A_1 matings were A_2. One animal (10%) from the five N_0 x N_0 matings was an A_2 phenotype while 60% or 12 animals of the three N_0 x A_1 matings were of the A_2 phenotype.

A_1 animals were produced in all except the A_4 x A_4 and the A_4 x A_1 crosses. Two animals classified A_1 were produced from three A_1 x A_3 and the seven A_3 x A_3 matings. These comprised 13% and 7% of the progeny, respectively. Seventeen or 29% of all offspring of the 13 A_4 x N_0 matings were of the A_1 phenotype. Forty-nine percent or 27 offspring from the A_3 x N_0 matings were classified in the A_1 group. The A_2 x A_1 matings yielded 8 or 30% of the A_1 phenotype offspring. A_1 animals comprised 90% and 40% of the N_0 x N progeny and the N_0 x A_1 progeny, respectively.

DISCUSSION

Definition of the Neurospinal Dysraphic Genetic Character

Results of the categorization of NSD animals, based on the phenotype defined using a clinical neurological examination, are shown in Table 3. These have provided some indication of the mode of inheritance of NSD in the Weimaraner. Analysis of the data has strongly supported some hypotheses and negated others. The most plausable genetic pattern involves a gene with some degree of dominance since outcrosses to unrelated normals of other breeds yield affected animals.

The proposed inheritance pattern may be based on intermediate dominance, that is, a co-dominant mutant. The A_4 and A_3 grades represent the homozygous mutant, $\underline{Dy}\underline{Dy}$; and the A_2 grade represents the heterozygote mutant $\underline{Dy}^{+}\underline{Dy}$. Again, grade 1 and normal animals are considered together. Review of the results shown in Table 4 support this hypothesis with some reservation or modification. The $A_4 \times A_4$ mating results fit the hypothesis perfectly and the $A_4 \times A_2$ matings fit the hypothesis with the exception of the two A_1 animals. No animals in this category are expected. The $A_2 \times A_2$ mating results yield all of the expected classes and are within acceptable limits using the Chi square test for probability ($p=.07$). There are more A_2 animals than expected and fewer A_1 animals than expected. In the affected group matings then, there must be some

Table 4 Results of Experimental Matings. Comparison of Numbers of Progeny Animals in Actual and Expected Phenotype Category Based on a Clinical Neurological Examination.

	Progeny					
CROSS	A_4	A_2	$A_1(N_e)$	SUBTOTAL	X^2	P
$A_4 \times A_4$						
ACTUAL	20	0	0	20		
EXPECTED	20	0	0			
$A_4 \times A_2$						
ACTUAL	7	6	2	15	*	
EXPECTED	7.5	7.5	0			
$A_2 \times A_2$						
ACTUAL	7	19	2	28	5.36	.07
EXPECTED	7	14	7			
$A_4 \times N_o$						
ACTUAL	0	41	17	58	*	
EXPECTED	0	58	0			
$A_4 \times A_1$						
ACTUAL	3	0	0	3	* *	
EXPECTED	0	3	0			
$A_2 \times N_o$						
ACTUAL	0	28	27	55	.018	.95
EXPECTED	0	27.5	27.5			
$A_2 \times A_1$						
ACTUAL	6	13	8	27	* *	
EXPECTED	0	13.5	13.5			
$N_o \times N$						
ACTUAL	0	1	9	10	* * *	
EXPECTED	0	0	10			
$N_o \times A_1$						
ACTUAL	0	12	8	20	* *	
EXPECTED	0	0	20			

* MUST ASSUME REDUCED PENETRANCE FOR FIT.

* * $A_1(N_e)$ SOMETIMES MAY BE VARIABLE EXPRESSIONS OF THE AFFECTED TRAIT AND A REDUCED PENETRANCE CLASSIFICATION.

* * * ANOTHER NEURAL CONDITION WHICH MAY MODIFY THE NEURAL DEFICITS.

modifications to allow a fit. In the A_4 x A_2 matings some A_1 animals were produced. This could be accounted for by reduced penetrance in these two animals. The paucity of A_1 animals in the A_2 x A_2 matings, in contrast to the A_1 x A_2 group, may suggest that the A_2 category is defined by clinical signs which can be mimicked in behaviorally modified animals or signs which may be widespread in the canine population. The numbers are small, however.

In the affected x normal matings the hypothesis does not fit. The A_4 x N_0 mating results should yield all A_2 animals, however, some A_1 animals have been defined. The A_4 x A_1 (N_e) mating should produce progeny similar to the A_4 x N_0 matings. Surprisingly, A_4 progeny were produced without the appearance of A_2 or A_1 animals. These completely unexpected results are based on only one litter and then on only three animals from that litter. They may be accounted for by assuming that the N_e present was actually a failure of penetrance for an A_4 or A_2 animal. The A_2 x N_0 matings fit the co-dominant hypothesis closely ($p=.95$) while the A_2 x N_e (A_1) does not. The appearance of A_4 from the latter mating is again not expected and the ratio of A_2 to A_1 animals should be a 1:1. The 13:8 ratio of A_3 to A_4 animals approximates this ratio. The appearance of the A_1 animals in the A_4 outcrosses suggests, as in the affected matings, a reduced penetrance. The $A_1(N_e)$ matings suggest that some of the N_e animals are genetically A_2 animals.

There is some evidence again for reduced penetrance or variable expressibility in these animals. In fact, it appears that all $A_1(N_e)$ animals which have been experimentally mated are A_2 animals. $A_1(N_e)$ animals have yet to be defined and experimentally mated and proven as normal animals.

In the normal x normal matings there were unexpected A_2 animals produced in both matings, the N x N and the N_0 x A_1 (N_e). The appearance of the one A_2 animal in the N x N mating suggests that the clinical signs used in defining this condition may be present in a normal population or that behavioral modification or other factors may be more involved as in a phenocopy. The one A_2 animal was produced from an F_1 hybrid. The parent of this hybrid was thought to be free of the dysraphism as defined, but the Weimaraner parent in question may have had another as yet undefined syndrome. Mating this animal to proven A_2 animals has yet to be accomplished. The $A_1(N_e)$ animals are genetically A_2 animals. Further mating of the $A_1(N_e)$ matings are planned to better define an A_1 or N_e animal.

The fit of the data to the co-dominant hypothesis is not perfect, but the levels of probability are better than for any other hypothesis. Even similar assumptions of reduced penetrance and variable expressivity did not allow development of any other acceptable hypothesis. Variable expressivity is suggested by the variable clinical signs seen in the A_2 and A_1

(N_e) animals. Previous work has been suggestive of reduced penetrance and variable expressivity as part of the genetic pattern (McGrath, 1965).

Other hypotheses which were considered included a co-dominant lethal gene. This hypothesis was negated because the number of dead pups in affected parent litters was the same or smaller than in outcross litters. A second hypothesis based on a recessive gene was discounted because many F_1 breed outcrosses progeny exhibit some abnormal clinical signs. A multiple genetic etiology (or genes) of equal importance collaborating to produce the abnormality, NSD in the Weimaraner, was nullified primarily because of the deficiency of normal progeny in the breeding population.

Therefore, based on the goodness of fit, a co-dominant or incompletely dominant gene is hypothesized as the major genetic control of the defined clinical syndrome of NSD in the dog. The main mutant gene may be symbolized by Dy. The A_4 animals would be symbolized by $\underline{DyDy}$ while the heterozygote, A_2, would be symbolized by $\underline{DyDy}^+$.

Breeding Population and Progeny

The number of reciprocal crosses for each breeding class could justifiably be increased. However, other investigations on sex predilection of NSD have shown that the occurrence role in males and females (Carter and Evans, 1973b) and bitches and dogs (McGrath, 1965) are similar.

The use of purebred normal Weimaraners (not extracted from our breeding population) in the crosses with the affected animals (A_1 and A_3) and with normal extract animals would be highly desirable. Although it was important to define the genetic mode by matings with a canine breed without a history of NSD syndrome (outcrosses), it is also important to use normal Weimaraners. Two reasons predominate: (1) Gene modifiers in the Weimaraner breed may affect the phenotype such that a classification scheme as defined and used here may not fit all families. (2) Similarly because of these possible gene pool differences or a differing genetic penetrance (Sang, 1963) the recognition of the A_2 or "carrier" group may not be possible in as high a percentage of cases, or the A_2 groups may show additional clinical signs and be more obvious.

Understanding any breed differentiation is of extreme importance in the genetic counseling of Weimaraner breeders. It is also of interest in that gene pools or derivatives of these pools in human populations (town, cities, marriage of cousins, etc.) affected with NSD may affect the frequency and phenotypic expression of a specific lesion. If such a phenomenon existed in geographic areas irrespective of gene pools, the occurrences could suggest a toxic or major environmental factor causing the NSD syndrome.

Concerning the above points, the wide geographic distribution of affected stock animals in the canine colony suggests a

genetic etiology. Pedigrees available for all stock animals demonstrate the absence of close inbreeding. The reproduction of the syndrome in expected frequencies also substantiates this. Supportive or detracting evidence that a different genetic background of a specific breed may affect the phenotype is relatively lacking. Within a pure breed, matings have been scarce. Only one purebred Weimaraner, produced from an affected by normal mating, has been examined. He is normal. Mating of this animal with A_2 animals produces the type and frequency expected for a heterozygote cross (1:2:1). Because experimental matings with known heterozygotes and this male produce some A_4 and A_2 animals, there appears to be reduced penetrance in the Weimaraner in question. An accurate interpretation and comparison of the pure breed is not possible until more affected Weimaraners (A_4 and A_2) are examined and categorized, and then subsequently mated to substantiate their classification.

Other aspects which point to a genetic determination of the canine NSD is the small number of affected animals obtained from a specific source or litter. Rebreeding of the normal source animals to different, usually unrelated, normal animals at the same locality does not usually result in any affected (A_4) progeny. Also, preliminary histopathology and clinical pathology studies have not been suggestive of any toxic, bacterial or viral disease agent.

The number of neonatal (2 weeks of age) deaths was in

certain cases considerable. Every puppy was necropsied and any abnormalities were recorded. Tissue sample from liver, kidney, lung, spleen and abnormal tissues were taken. Examination of these tissues did not, in many cases, reveal a cause of death. Common causes for the neonatal losses included primarily behavioral and environmental effects which could not be amended easily. The conditions for housing were only satisfactory. Overly excited first litter bitches combined with adverse (cold to freezing) weather conditions were the cause of many deaths. A very small bitch with many small pups, trauma due to over-protection by the female and poor maternal behavior were causes which constituted the balance of lost pups. Mortality rates for the affected crosses and the affected normal outcrosses were similar, 17% and 19%, respectively. More dilution of the condition and a similar death rate exhibited by the outcross groups, suggests that a concentration of the mutant effects does not have a lethal phenotypic effect. The possibility of intrauterine death or reabsorption has been considered but the question of early death has not been answered.

The techniques, procedures and individual tests used in the examination are well-known. However, observing and correct recording of results depends on an immediate interpretation of the response. Such an interpretation is not difficult for an experienced diagnostician if the deficiencies are motor or

reflex abnormalities. The technique is even more illustrative if the proper response is either absent or present, or more correctly absent, in degrees or grades. That is, the animal is able to respond even though the response may be delayed, slowed, dysmetric, or totally different than expected.

An interpretation problem and recognition of even greater magnitude involves the evaluation of certain sensory systems. Here again the tests are common, but even expert interpretation is not a good quantitative measurement. Due to this inability to accurately measure a response (degree of absence of a response), the sensory testing results were recorded as present or absent. To add some reliability to this then, more than one sensory test involving one area was performed. The exact location of abnormalities in the combination of tests allowed some measurable comparison.

Difficulties in the classification scheme are inherent in that the intermediate group is defined primarily by these ambiguous sensory deficits alluded to above. As was mentioned earlier, some of these same deficits can be observed in a behaviorally abnormal animal (frightened, apprehensive, excited, etc.). Conversely, some of the sensory and reflex tests may be exaggerated in an abnormal animal so that a slow response may appear normal. The difference then between an A_2 group animal and a normal animal may be slight. There are two reasons why the effects of the tests upon the classification scheme may be

diminished. First, two neurological examinations were done to ensure that the response abnormalities were similar. If they were not, it was assumed that a behavioral factor may have been involved at the time of either testing procedure. If the abnormalities were similar in degree and involving the same limb or area, the specific deficit was assumed to be present. Second, only those apparently normal animals with more than one sensory deficit were classed with the A_2 animals. The animals with only slight deficits were grouped with the normal class (A_1 and N_e).

As was pointed out earlier in the description of the genetic character, the co-dominant hypothesis of NSD in the dog is not entirely consistent with the expected, however, no other mode could be found to better fit the data. Additional data, of course may resolve the fit. The data and classification scheme thus defined seems adequate. However, consistent recognition of the carrier or heterozygous animals has still not been attainable. Expert performance and interpretation of the neurological examination seems plausible as a defining character. This is not always possible. To an untrained observer a heterozygote is a normal animal and only the right chance mating to another heterozygote will result in any progeny so seriously affected (A_4) that they can be readily recognized. Until the time that the experimental mating producing A_4 animals is procured, the bitch or dog may be perpetuating the heterozygote condition in one-half of all their progeny. The trait

to these breeders appears as a recessive. That is, the homozygote is the only one exhibiting obvious signs. The F_1 progeny of a recessive and outcross mating appear outwardly normal while the F_2 offspring will segregate into categories, the number of which will suggest the number of gene pairs involved.

In effect we have established a threshold scale based on a clinical evaluation of the phenotype. Threshold is determined by recognition of certain sensory and motor abnormalities in the A_2 group. We would expect this scale to be the same for different treatments (breeds of animals) unless the distribution of affected animals is not normal. It is convenient to grade the affected animals (mutants) into large categories, without accurately dividing intermediate types because the number of animals incorporated into each group can therefore be large and may overcome any loss of grading accuracy, It is important, however, to insure that the chosen categories do not eliminate information and that precautions are taken to minimize personal errors in classification. The two neurological examinations with interpretation by more than one observer and a combination of tests hopefully decreased any such bias.

Examination of the data suggested a co-dominant hypothesis and allowed the classification of the three different groups. Matings, with subsequent classification of progeny, revealed certain inadequacies of the data, or of the classification

scheme threshold. In the affected normal matings the deficiency of phenotypes vary. In the outcross mating of A_4 animals, 30% of the animals are classified as A_1 or N_e or an estimated reduced penetrance of 30%. None of these types were expected. Mating of the A_1 animals to other affected animals prove most of them to be A_2 genetically. In the A_2 outcross matings, however, the proportion of A_2 and N_e animals is as expected (1:1). There seems to be a difference in penetrance and/or expressivity affecting the same, A_2, class.

Penetrance and expressivity are properties of phenotypic expression of populations homozygous for the gene concerned but not necessarily homozygous for other genes (Falconer, 1965). There is a physiological developmental threshold which determines that phenotype. We might expect a similar penetrance for affected x normal outcross matings (A_4 x N_0 and A_3 x N_0) as we have mixed the population (A_2) by extensive breeding. However, the threshold of the defined phenotype appears different in the F_1 backcross progeny. By definition, the populations may differ in gene penetrance because they have different genetic backgrounds. The A_2 population may be enough different to affect the expression of the gene(s). The problem is, therefore, to determine the threshold of the affected animal in a given population (breed).

A backcross to normal Weimaraners, A_2 x N_w may give a different threshold of recognition and therefore a different

penetrance with a different ratio of A_4 animals. This might fit better with the similar population in the A_4 and N_0 matings, as all the A_4 are Weimaraners. The A_2 and N_0 matings involve non-Weimaraner breeds to a greater extent and the recognition threshold may be different. Comparison of results obtained from non-Weimaraner A_4 individuals crossed with normal outcross and normal Weimaraners may also lead to a determination of these two thresholds.

This again only strengthens the argument that an in breed (within the Weimaraner) study for comparison to the breed mating data is of great importance to determine the tests needed and an interpretation of those tests which can be used to suggest further definitive testing. The terms, penetrance and expressivity, are used to hide our ignorance of underlying reactions, however, expressivity, often used with the term penetrance, can be measured if the penetrance classes can be divided and classified unequivocally. Since the same genes affect both penetrance and expressivity, a measure of these would be ideal. Then a measure of the gene action can be clarified and the carrier state adequately defined..

Quantitative Measurements

The shortcomings of the present classification scheme involve the breed of dog used for defining the groups, the ambiguity of the neurological examination and the actual modification of results and grouping due to behavioral changes.

There are means whereby the individuals could be more closely evaluated and categorized. Quantitative measurements of the reflex and sensory tests are possible. This would entail some psychophysiological testing procedures and would allow measurement of the time delay, extent or absence of a response to a measured adequate stimulus. At the same time, the individual responding muscles could be evaluated as to resting activity, the response time and the activity during and after response. The sensory nerves could also be directly stimulated and the response measured (H-wave). This in effect would give a measure of the sensory and reflex deficits.

The conformational changes might be similarly measured so that different grades of abnormalities may be recognized. The measurement might consist of joint angle measurements of a standing animal. Simultaneous monitoring of muscle activity would be helpful. The recording scheme here could then be used to monitor the changes during locomotion. The time sequence of the muscle and joint activity might help to determine where the peripheral deficiencies, if any, will be found (i.e., in joint angle activity or tactile receptors).

Although the results have not been included here, the information gained by a quantitative histological examination of sequential spinal cord levels would be invaluable. Initial studies of the spinal cord sections in A_4 animals give an indication that there may be a deficiency of neurons in certain

areas of the spinal cord with an increase of neurons in other areas. If the levels of the central nervous system abnormalities can be identified and evaluated quantitatively, then another measured means of classification would be available. Comparison and correlation of the sensory and reflex changes with the histological changes might better identify or confirm the genetic etiologic diagnosis.

Further Analysis of Neurospinal Dysraphism in the Canine

Clinical description

Although the canine model of NSD is well-known, a complete and accurate description of the disease syndrome is lacking. In McGrath's monograph on the histology, genetics and clinical syndrome of NSD, the discussion and conclusions are based on very few pedigrees, the relationships of which are not clear. Many of the animals which were examined and subsequently classified were not evaluated at all quantitatively. Animals with an "abnormal posture or crouchy gait" were grouped differently than "affected" animals. The difference between these animals is not elucidated. Progeny of matings of the above groupings were classified differently. Those from an "affected litter with subtle clinical signs or subtle dysraphic lesions" and clinically affected and clinically normal were the groupings used. The subtle signs are not listed nor is there any indication that these signs are the same or vary with individual

animals.

Many of the published pedigrees (McGrath, 1965) were incomplete as the progeny were often deceased. Histological interpretations were then used to classify these animals. However, the cause of death of these animals was often due to viral diseases affecting the central nervous system or systemic diseases. Other histological (pathological) records indicate only the non-specific changes present in the central nervous system of these animals. There is no defined mode of classification, let alone a quantitative one.

According to McGrath (1965) the clinical syndrome of canine NSD is recognized only after an animal gains a standing posture, or at about 3 weeks of age. The other deficits have been outlined earlier. Based on more controlled and extensive neurological examinations additional clinical signs have been defined in obviously affected (A_4) animals and in the A_2 or carrier animals. As was defined in the categorization, the signs are common for a group. Proprioception tactile deficits are common in the A_4 group in addition to the signs noted by McGrath (hopping and sensation deficiencies).

The time of recognition of the syndrome is significantly sooner than the 3 weeks noted above. The characteristic bilateral withdrawal response is present (at birth) in 1/3 - 1/2 the number expected. The remainder of the A_4 puppies can be identified at 2 days of age. These puppies can be differen-

tiated from the normal or carrier animals because a bilateral synchronous withdrawal reflex is present instead of the normal crossed-extensor reflex.

A very interesting finding, usually at the time of the first neurological examination, was the apparent necessity for both hind limbs to withdraw together. If when evaluating the withdrawal response and the placing responses one limb was flexed (to check the contralateral limb) the other (tested) limb would not respond. When the flexed limb was extended both limbs would flex upon stimulation. It therefore appears that the flexed position of the limb inhibits any similar movement (flexion) by the opposite limb. The neural mechanisms, probably at a local cord level, may then contain an inhibitory pathway ensuring the characteristic bilateral synchronous movement of the hind limbs.

As was mentioned earlier the occurrence of other conformational abnormalities is common (Table 2). In the progeny or produced A_4 animals, approximately 90% have an unilateral inward rotation of the pes. The rotation is commonly seen if the animals are suspended from the axilla. In the last year the talipes equinovarus has been noted on the right hind foot in all cases. There does not appear to be any difference in the reflex and sensory losses, over each paw, however.

Examination of some dogs at necropsy revealed a thin fibrous connection of the dura mater to the dorsal periosteum

of the vertebral canal. The connection was commonly seen at lumbar levels 5 or 6. The exact number of these connections is not known nor is there yet enough information to correlate this with category A_4 or A_2. One A_2 animal had a fibrous dorsal covering (spina bifida) in the mid-sacral region. This animal, produced from a German Shorthaired Pointer and an A_4 Weimaraner, may be indicative of another breed predilection.

Similar problems are encountered when trying to correlate other conditions associated with NSD with the classification of the animal. Many of the conditions, such as Arnold-Chiari syndrome or open fontenelles, seem to be fatal early in life. Before the pups attain 4 weeks of age, they usually die. Careful and satisfactory examinations of these animals has only recently been done. Therefore, only sketchy data is presented. Education of the investigator on what conditions to look for early in life have helped in the collection of the data. More complete data is forthcoming. Documentation of these conditions will be done using radiographic, photographic and some histopathologic procedures.

Syndromes related to NSD

The "malabsorption syndrome" synonymized by McGrath (1965) with leiomyometaplasia was seen only rarely in the present colony situation. McGrath did not describe any clinical signs associated with the syndrome, the age of appearance of the syndrome or the persistence or loss of clinical signs. All

animals in his study were 8 months of age or greater. No mention of histological interpretation is given. A syndrome characterized by almost a cessation of growth, loss of tissue mass, 4-6% dehydration with a normal appetite was observed in a small percentage (1.4%) of all animals. The clinical syndrome was usually recognizable at seven weeks of age. Based on weekly weights from two litters, there is a significant difference ($p < .05$) in the normal and affected animals' growth rates. The average weekly weight of the diseased animals was significantly ($p < .05$) lower than normal animals in that litter. Female animals comprised 80% (4 of 5) of such affected animals. The final adult weight is less than normal animals in that litter. The animals do reach puberty, will breed, will support gestation and will produce adequate quantities of milk. Other tests were done in an effort to further describe and clarify the condition. The absorptive tests and radiology which were carried out do not suggest a malabsorption syndrome as the cause of the poor development. Because of these results, we have reserved the term "dwarfism" for the NSD associated condition. Current work is being done to 1) reproduce the condition by specific breedings, 2) establish hormonal causes for the initiation of the syndrome, 3) define any anatomical variation in the anterior pituitary, gastrointestinal tract, thyroid, adrenals and pancreas.

SUMMARY

A study was done to define a possible mode of inheritance of neurospinal dysraphism in the dog. Intimately involved and imperative to the study was a more complete definition of the disease entity. A categorization of the different forms of the disease syndrome was established and a genetic symbol was suggested.

Purebred male and female Weimaraners exhibiting signs of neurospinal dysraphism were mated to breeds known to be unaffected with the syndrome. These included Norwegian Elkhounds, German Shepherds and an Irish Setter. One mating category included the German Shorthaired Pointer, a breed developed from the same lines as the Weimaraner and reported to have some dysraphic individuals. Progeny from the above matings were backcrossed to normal parents or parent-type, to affected parent-type or to other F_1 progeny. F_2 and F_3 progeny were produced and mated in backcross matings to normal and affected and to other like progeny.

The over 300 animals produced from reciprocal matings listed above were classified on the basis of deficits or deficiencies outlined during a clinical neurological examination. Three categories were defined, A_4 or severely affected animals, A_2 or moderately affected animals and $N_e(A_1)$ or apparently normal animals. The progeny of the matings of the affected and normal were similarly classified. Ratios of the

A_4, A_2 and A_1(Ne) progeny were compared to a possible genetic inheritance pattern.

A classification scheme of the spinal dysraphic syndrome was proposed and used successfully in this study. The classification of progeny produced from experimental breedings and subsequent check matings of these animals have allowed an intermediate or co-dominant genetic definition of NSD in the dog. The gene symbol, Dy, was postulated.

The clinical neurological syndrome and associated syndromes were defined.

REFERENCES

Akker, S. van den. 1962. Arnold-Chiari malformations in animals. Acta Neuropath., Suppl. 1:39-44.

Amuso, S. J., and H. J. Mankin. 1967. Hereditary spondylolisthesis and spina bifida. J. Bone and Joint Surger. 49A: 507-513.

Barson, A. J. 1970. Spina bifida: The significance of the level and extent of the defect to the morphogenesis. Dev. Med. Child Neurol. 12:129-144.

Barson, A. J. 1972. Craniospinal dysraphie in the chick embryo produced by tissue-specific antibodies. Dev. Med. Child Neurol. 14(Suppl. 27):17-24.

Benda, C. E. 1954. The Dandy-Walker Syndrome or the so-called atresia of the foramen of Magendie. J. Neuropath. and Exper. Neurol. 13:14-29.

Benda, C. E. 1959. Dysraphic states. J. Neuropath. and Exper. Neurol. 18:56-74.

Bennett, D. 1959. Brain hernia, a new recessive mutation in the house mouse. J. Hered. 50:265-268.

Bokinsky, G. E., L. D. Hudson, and J. V. Weil. 1973. Impaired peripheral chemosensitivity and acute respiratory failure in Arnold-Chiari malformation and syringomyelia. New England J. of Med. 18:947-948.

Bonnevie, K. 1934. Embryological analysis of gene manifestation in Little and Bagg's abnormal mouse tribe. J. Exp. Zool. 67:443-520.

Bremer, F. W. 1926. Klinische Untersuchungen zur Atiologie der Syringomyelie, der "Status Dysraphicus." Dtsch. Z. Nervenheilk. 95:1-103.

Burns, M., and M. N. Fraser. 1966. Genetics of the dog. The basis of successful breeding. Philadelphia: Lippincott.

Carter, C. O. 1969. Spina bifida and anencephaly: A problem in genetic-environmental interaction. J. Biosocial Sci. 1:71-83.

Carter, C. O., P. A. David, and K. M. Laurence. 1968. A family study of major central nervous system malformations in South Wales. J. Med. Genet. 5:81-106.

Carter, C. O., and K. Evans. 1973 (a). Spina bifida and anencephalus in greater London. J. Med. Genet. 10:207-234.

Carter, C. O., and K. Evans. 1973 (b). Children of adult survivors with spina bifida cystica. Lancet. 2:924-926.

Carter, C. O., K. A. Evans, and K. Till. 1976. Spinal dysraphism: Genetic relation to neural tube malformations. J. Med. Genet. 13:343-350.

Crary, D. D., R. R. Fox, and P. B. Swain. 1966. Spina bifida in the rabbit. J. Hered. 57:236-243.

Curtis, F. 1934. Uber Degenerationszeichen Eugenik, A. 2:3.

Curtis, F. 1939. Status dysraphicus und Myelodysplasie Fortschr. Erbpath. Rassenhyg. 3:199-258.

Curtis, R. L., D. English, and Y. S. Kim. 1964. Spina bifida in a "stub" dog stock, selectively bred for short tails. Anat. Rec. 148:365. (Abstr.)

Draper, D. D., J. P. Kluge, and W. J. Miller. 1975. Neurologic, pathologic, and genetic aspects of spinal dysraphism in dogs. Zbl. Vet. Med., Reihe C, Bd. 4, Hoft 4. (Abstr.)

Emery, J. L., and R. G. Lendon. 1969. Lipomas of the cauda equina and other fatty tumors related to neurospinal dysraphism. Dev. Med. Child Neurol. 20(Suppl.):62.

Emery, J. L., and R. G. Lendon. 1972. Clinical implication of cord lesions in neurospinal dysraphism. Dev. Med. Child Neurol. 14(Suppl. 27):45-51.

Emery, J. L., and R. G. Lendon. 1973. The local cord lesion in neurospinal dysraphism (meningomyelocele). J. Path. 110:83-96.

Emery, J. L., H. Nunn, and R. Singhal. 1973. The cell population of dorsal root ganglia in children with neurospinal dysraphism. Dev. Med. Child Neurol. 15:467-473.

Falconer, D. S. 1965. The inheritance of liability to certain diseases estimated from the incidence among relatives. Annals of Human Genetics. 29:51-76.

Forbes, M. 1972. Renal dysplasia in infants with neurospinal dysraphism. J. Path. 107:13-19.

Fowler, I. 1953. Responses of the chick neural tube in mechanically produced spina bifida. J. Exp. Zool. 123: 115-151.

Fox, M. W. 1963. Developmental abnormalities of the canine skull. Can. J. Comp. Med. 26:219-222.

Fuchs, A. 1909. Uber den Klinischen Nachweis Kongenitaler Defektbildungen in den unteren Rukenmark sab schnitten (Myelodysplasie). Wien. Med. Wschr. 59:2142-2147.

Fuller, J. L. 1956. The inheritance of structural defects in dogs. Mich. St. Univ. Vet. 16:103-108.

Furneaux, R. W., C. E. Doige, and M. M. Kaye. 1973. Syringomyelia and spina bifida occulta in a Samoyed dog. Can. Vet. J. 14:317-321.

Garber, E. D. 1952. A dominant, sex-linked mutation in the house mouse. Science. 116:89.

Gardner, W. J. 1961. Rupture of neural tube: Cause of myelomeningocele. Arch. Neurol. 4:1-7.

Gardner, W. J. 1973. The dysraphic states: From syringomyelia to anencephaly. Excerpta Medica. Amsterdam, Netherlands.

Geib, L. W., and S. I. Bistner. 1967. Spinal cord dysraphism in a dog. J. Am. Vet. Med. Ass. 150:618-620.

Grüneberg,, H. 1947. Animal genetics and medicine. New York, Hoeber.

Grüneberg, H. 1954. Genetic studies on the skeleton of the mouse. VIII. Curly tail. J. Genet. 52:52-67.

Hamburgh, J., M. Erlich, G. Nathanson, and I. Pesetsky. 1975. Some additional observations relating to the mechanism of trypan blue induced teratogenesis. J. Exp. Zool. 192:1-12.

Harbitz, F. 1942. Stredte bemerkninger om svulster hos dyr. Norsk. vet. tskr. 54:193-224; 227-308.

Hollander, W. F. 1976. Genetic spina bifida occulta in the mouse. Am. J. Anat. 146:173-180.

Hsu, C. Y., and J. A. van Dyke. 1948. An analysis of growth rates in neural epithelium of normal and spina bifidous (myeloschisis) mouse embryos. Anat. Rec. 100:745. (Abstr.)

Hughes, E. Y., and H. Hart. 1934. Defective skulls inherited in swine. J. Hered. 25:111-115.

James, C., and L. P. Lassman. 1972. Spinal dysraphism. Butterworths, London.

James, C. C. M., L. P. Lassman, and B. E. Tomlinson. 1969. Congenital anomalies of the lower spine and spinal cord in Manx cats. J. Path. 97:269-276.

James, J. I. P. 1967. Scoliosis, Chapter 8, pp. 96-136. Livingstone, London.

Kalter, H. 1968. Teratology of the Central Nervous System. University of Chicago Press, Chicago.

Kitchen, H., R. E. Murray, and B. Cockrell. 1972. Animal model for human disease: Spina bifida, sacral dysgenesis and myelocele. Am. J. Path. 60:203-206.

Kumminek, K. 1959. Die Morphologie der unteren Wirbelsaule bei einer Spontanen Kurzschwanzmutation der Hawmaus. Biol. Zbl. 78:719-758.

Lemire, R. J., J. E. Loeser, R. W. Leech, and E. C. Alvord, Jr. 1975. Normal and abnormal development of the human nervous system. Harper and Row, Publishers, Inc., Hagerstown, MD.

Lendon, R. G. 1972. An autoradiographic study of induced myelomeningocele. Dev. Med. Child Neurol. 14(Suppl. 27):80-86.

Lichtenstein, B. W. 1940. "Spinal dysraphism," spina bifida and myelodysplasia. Arch. Neurol. Psychiat. 44:792-810.

Lienaux, E. 1897. Un cas de syringomyelia Chez le Chien. Ann. Med. Vet. 46:486-495.

Little, C. C. 1948. Genetics in cocker spaniels. Observations on hereditary and on physiology of reproduction in American Cocker Spaniels. J. Hered. 39:181-185.

Lorber, J. 1965. The family history of spina bifida cystica. Pediatrics. 35:589-595.

Martin, A. H. 1971. A congenital defect in the spinal cord of the Manx cat. Vet. Path. 8:232-238.

McGrath, J. T. 1956. Neurological examination of the dog with clinicopathological observations. Lea and Febiger, Philadelphia.

McGrath, J. T. 1965. Spinal dysraphism in the dog. With comments on syringomyelia. Path. Vet. 2(Suppl.):1-36.

McKusick, V. A. 1971. Mendelian inheritance in Man, 3rd ed. Johns Hopkins Press, Baltimore.

Moriarty, J. A., and W. O. Klingman. 1965. Congenital and prenatal diseases. in Clinical Neurology, P. B. Baker, ed., pp. 1923. Harper and Row, New York.

Mueller, C. R., and S. J. Sugar. 1943. Familial syringomyelia. J.A.V.M.A. 122:743-744.

Murakami, U., K. Hoshino, and M. Inoye. 1972. An experimental observation on the morphogenesis of reopening of the cranium. Proc. Congen. Anom. Res. Assoc. Japan. 12:157-171.

Nachtsheim, H. 1931. Uber eine erbliche Neurenkrankheit (Syringomyelie) beim Kaninchen. Z. Pelztier Rauchwarenk. 3:254-249.

Nitter, K., and W. Tonnis. 1950. Symptomatologie, Diagnostik und Behandlungser gebnisse der Rukonmarks und Wirbelangiome. Zenthabl Neurochir. 10:317-333.

Nordby, J. E. 1929. An inherited skull defect in swine. A preliminary report on the inheritance of a frontoparietal skeletal defect involving the meningocoele and proencephalus types of cranioschisis in swine. J. Hered. 20: 229-232.

Ostertag, B. 1930. Weitere Undersuchungen uber vererbbare Syringomyelie des Kaninchens. Dtsch. Z. Neurenheilk. 116:147-154.

Padget, D. H. 1968. Spina bifida and embryonic neuroschisis: A causal relationship. Johns Hopkins Med. J. 123:233-252.

Padget, D. H. 1970. Neuroschisis and human embryonic maldevelopment. J. Neuropathol. Exp. Neurol. 29:192-216.

Padget, D. H. 1972. Development of so-called dysraphism: With embryonic evidence of clinical Arnold-Chiari and Dandy-Walker malformations. Johns Hopkins Med. J. 130: 127-165.

Parker, A. J., and C. S. Byerly. 1973. Meningomyelocoele in a dog. Vet. Path. 10:266-273.

Parker, A. J., R. D. Park, C. S. Byerly, and J. L. Stowater. 1973. Spina bifida with protrusion of spinal cord tissue in a dog. J. Am. Vet. Med. Ass. 163:158-160.

Patten, B. M. 1953. Embryological stages in the establishing of myeloschisis with spina bifida. Am. J. Anat. 93:365-395.

Penrose, L. S. 1957. Genetics of anencephaly. J. Mental Deficiency Research. 1:4-15.

Polman, A. 1950. Anencephaly, spina bifida, and hydrocephaly: A contribution to our knowledge of the causal genesis of congenital malformations. Genetica. 25:29-78.

Poswillo, D. E., D. Sopher, and S. Mitchell. 1972. Experimental induction of foetal malformation with "blighted" potato; a preliminary report. Nature. 239:462-464.

Pullig, T. 1952. Inheritance of a skull defect in Cocker Spaniels. J. Hered. 43:97-99,

Rálĭs, Z., and H. M. Rálĭs. 1972. Morphology of peripheral nerves in children with spina bifida. Dev. Med. Child Neurol. 14(Suppl. 27):109-116.

Recklinghausen, F. von. 1886. Untersuchungen uber die Spina bifida. Berlin, Georg Reimer.

Record, R. G., and T. McKeown. 1950. Congenital malformations of the central nervous system. III. Risks of malformation in sibs of malformed individuals. Br. J. Prev. Soc. Med. 4:217-220.

Reed, S. C. 1937. The inheritance and expression of used, a new mutation in the house mouse. Genetics. 22:1-13.

Renwick, J. H. 1972. Hypothesis: Anencephaly and spina bifida are usually preventable by avoidance of a specific but unidentified substance present in certain potato tubers. Br. J. of Prev. and Soc. Med. 26:67-88.

Rugh, R., and E. Grupp. 1959. Exencephalia following x-irradiation of the preimplantation mammalian embryo. J. Neuropath. Exp. Neurol. 18:468-481.

Russell, W. L. 1947. Splotch, a new mutation in the house mouse, Mus musculies. Genetics. 32:102. (Abstr.)

Sang, J. H. 1963. Penetrance, expressivity and thresholds. J. Hered. 54:143-157.

Sever, J. L. 1973. Potatoes and birth defects: Summary. Teratology. 8(3):319-320.

Sever, L. E. 1974. A case of meningomyelocele in a kindred with multiple cases of spondylolisthesis and spina bifida occulta. J. Med. Gen. 11:94-96.

Shaw, A. O. 1938. A skull defect in cattle. J. Hered. 29: 319-320.

Spellman, M. P. 1966. Chromosome investigation of anencephaly and other congenital C.N.S. abnormalities in pork. Irish J. Med. Sci. 6:393-398.

Stedman's Medical Dictionary. 1976. 23rd Edition. Williams and Wilkins Company. Baltimore, Md.

Strong, L. C., and W. F. Hollander. 1949. Hereditary loop-tail in the house mouse accompanied by imperforate vagina and with lethal craniorachischisis when homozygous. J. Hered. 40:329-334.

Suu, S. 1956. Studies on the short-spine dogs. I. Their origin and occurrence. Res. Bull. Fac. Agr. Gifu Univ. 7:127-134.

Thompson, S. W. 1958. Leiomyometaplasts, particles of unknown significance in smooth muscle cells. Amer. J. Path. 35:273-283.

Thompson, S. W. and T. E. Murchison. 1958. A histochemical study of five cases of leiomyometaplasts in dogs. Cornell Vet. 48:448-457.

Till, K. 1969. Spinal dysraphism: A study of congenital malformations of the back. Dev. Med. Child Neurol. 10: 471-478.

Tobin, W. D., and D. D. Layton. 1976. The diagnosis and natural history of spinal cord anteriovenous malformations. Mayo Clin. Proc. 51:637-646.

Todd, N. B. 1961. The inheritance of taillessness in Manx cats. J. Hered. 52:228-232.

Van Epps, C., and H. D. Kerr. 1940. Familial lumbosacral syringomyelia. Radiology. 35:160-173.

Verschuer, O. von. 1959. Genetik des menschen. Urban and Schwarzenberg. Munchen, Berlin.

Vogel, F. S. 1961. The anatomic character of the vascular anomalies associated with anencephaly. Am. J. Pathol. 39:163-174.

Warkany, J., J. G. Wilson, J. F. Geiger. 1958. Myeloschisis and myelomeningocele produced experimentally in the rat. J. Comp. Neurol. 109:35-64.

Wiltse, L. L. 1962. The etiology of spondylolisthesis. J. of Bone and Joint Surgery. 44:539-569.

Wright, Y. M., and W. E. Clark, and W. R. Bregg. 1974. Craniorachischisis in a partially trisomic II Fetus in a family with reproductive failure and a reciprocal translocation t(6pt;11q-). J. Med. Genet. 11:69-75.

Wynne-Davies, R. 1975. Congenital vertebral anomalies aetiology and relationship to spina bifida cystica. J. Med. Gen. 12:280-288.

Yen, S., and B. MacMahon. 1968. Genetics of anencephaly and spina bifida. Lancet. 2:623-626.

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to Dr. Donald Draper for serving as my major professor, research associate and research advisor. He has given much time and assistance throughout the study. His expertise in clinical neurology as well as neuroanatomy and neurophysiology and many other areas of internal medicine have been informative and invaluable in the performance of the research. His patience and assistance in preparation of this thesis were helpful. I feel he gave me an insight into research writing and has done much for my career in veterinary medicine.

I would like to express my extreme thanks to Dr. Wilmer Miller for his time and experience in planning the proper matings and for his invaluable assistance in interpretation and analysis of the resultant data. He has served as a willing associate throughout my research.

I would also like to thank Dr. Robert Carithers for agreeing to serve on my committee and for his interest, suggestions and assistance in my research and importantly for the assistance in treating animals in emergency situations and for supplying the necessary vaccinations for all colony animals.

I would like to thank Dr. Charles Drewes and Dr. Neal Cholvin for accepting positions on my committee.

The various caretakers for the colony animals have offered me valuable assistance. I would like to list them all as the

quality of work and their concern for the animals has been consistently excellent.

I would like to express my appreciation to the Department of Veterinary Anatomy, Pharmacology and Physiology for the monetary support rendered for histological supplies and preparations. In particular, Rose Aspengren and Grace Faber have been especially helpful. The departmental support for the gastrointestinal part of the study is extremely appreciated.

Finally, the financial support from the various Weimaraner clubs, Dr. Draper and the College of Veterinary Medicine in the maintenance and support of the Canine Genetic Research Colony is greatly appreciated.