FAMILIAL CEREBELLAR ATAXIA IN THE BULL MASTIFF

A THESIS SUBMITTED FOR THE DEGREE OF
MASTER OF VETERINARY MEDICINE

Stuart Carmichael, B.V.M.S., M.R.C.V.S.,
Department of Veterinary Surgery,
University of Glasgow Veterinary School,
Bearsden Road,
Glasgow, G61 1QH.

February 1987.

ACKNOWLEDGEMENTS

I should like to take this opportunity to thank Mr. and Mrs. Campbell for providing me with clinical cases and breeding records and thank Dr. M. Harvey for assistance with the genetic part of the study. I am extremely grateful to Mrs. S. Hazelton for typing the manuscript and thank Mr. A. May for providing the photographs and much helpful assistance and advice.

The Wellcome Trust provided financial support and I am thankful to them for the opportunity to undertake these studies. I would like to express my sincere gratitude to Professor D.D. Lawson for his interest, advice and encouragement in the time I have spent in the Department of Veterinary Surgery at the University of Glasgow. In particular I am indebted to Dr. I.R. Griffiths for providing me with constant inspiration and for sharing a little of his knowledge with me. Without his assistance and patience this study would not have been possible.

Lastly, I should like to take this chance to thank my parents, who over the years have unselfishly provided the opportunity for me to develop my education and always offered me support and encouragement. I dedicate this study to them.

SUMMARY

The aim of this study was to provide information about the nature and incidence of cerebellar disease in dogs with specific reference to familial conditions. Investigative studies were carried out on six closely related Bull Mastiff pups which were showing signs of cerebellar damage. The clinical details of each pup were recorded. The salient features of the condition were those of cerebellar ataxia and a proprioceptive defect often accompanied by hypermetria. The affected animals also demonstrated signs of visual impairment and bizarre behavioural changes. In each case euthanasia was carried out as a result of continued deterioration and unresponsiveness to treatment.

Pathological investigations were carried out on all six of the pups. The brain and selected samples of other tissues were obtained for histopathological examination. Two of the six cases were perfusion-fixed under general anaesthesia and positive pressure ventilation.

Pathological changes were confined to the brains of the affected animals. A mild to moderate degree of hydrocephalus was present in each case. This was demonstrated in Case 2 by ventriculography. The hydrocephalus was communicating in nature. Specific changes were detected in the deep cerebellar nuclei, the lateral vestibular nuclei and the inferior colliculi in each case. The changes were bilaterally symmetrical and consisted of areas of vacuolation and axonal degeneration. Light microscopy studies suggested that both oligodendroglia and axons were showing abnormalities. The distribution of the changes suggested that one of the main pathological features was degeneration of the distal

Purkinje cell axons. Electron microscopy confirmed that vacuolation was occuring in the distal extension of the oligodentroglia. Axonal degeneration was also studied and many axons with accumulations of organelles detected.

An attempt was made to correlate clinical findings with the pathological changes observed, by reviewing the literature concerning cerebellar pathology and its resultant clinical effects. Disease processes and experimentally induced conditions were both considered. As a result, it was ascertained that the majority of the clinical signs exhibited could be ascribed to the observed pathology. Some changes, for instance, bizarre behavioural changes could not be satisfactorily explained and likewise no clinical signs attributable to the lesions in the inferior colliculi were determined. No specific pathological changes were found as a result of hydrocephalus but it was impossible to evaluate the significance of this clinically.

Very similar pathological findings have been observed as a result of some metabolic disorders. In chronic thiamine deficiency both the nature of the pathology and the distribution were similar to that seen in the mastiffs, likewise similar changes are seen in isoniazid toxicity and in many cases of anoxic brain damage. This is taken as an indication that the affected cells in these areas have common metabolic requirements.

The pups examined were all closely related. An indication was given that many more pups had been affected. The pedigrees of all of these animals were analysed and showed that the condition has in all probability an autosomal recessive pattern of inheritance. A source dog was detected through which all of the affected animals could have inherited the trait. This dog,

however, is not likely to be the animal in which the trait has arisen.

Familial ataxia in the Bull mastiff would therefore appear to be a neuronal abiotrophy; that is, premature death of certain cell types as a result of a genetically determined metabolic abnormality and is inherited as an autosomal recessive trait.

LIST OF TABLES

LIST OF FIGURES

NOTE: All figs. after Fig. 9 depict the animals in the study.

Case 1	Figs. 13, 15 - 19
Case 2	Figs. 10, 11
Case 3	Figs. 12, 16 - 32
Case 6	Figs. 20 - 25

GENERAL INTRODUCTION

Although relatively rare, hereditary cerebellar disease is being reported with increasing frequency in animals. Despite new conditions being recognised, the aetiology is still obscure and the clinical manifestations as a result of the pathology in the cerebellum poorly understood. Six related Bull Mastiff dogs with cerebellar ataxia were investigated in detail. Information about a further eleven cases was obtained and pedigree information analysed. The purpose of this study was to investigate this hitherto undescribed problem in the Bull Mastiff, identify pathological changes and establish if a familial basis existed. In addition, it was hoped that the results of the study might improve understanding of conditions with similar pathology in the cerebellum.

SECTION 1

ANATOMY AND FUNCTION OF THE CEREBELLUM

ANATOMY OF THE CEREBELLUM

DEVELOPMENT

The cerebellum is formed from the alar plate of the metencephalon. The dorso-lateral portion of this bends medially to form the bilateral rhombic lips and with proliferation and flexure of the brain stem these meet in midline to form the primary cerebellar structure. The cerebellar plate, so formed, consists of three layers; a neuroepithelial, a mantle and a marginal layer. Two migratory paths stem from these germinal cells. Firstly there is migration of differentiating mantle cells into the substance of the rhombic lip. These cells cease division and differentiate to form the Purkinje cell layer and also the neurones of the deep cerebellar nuclei, which are formed and mature at birth (de Lahunta 1980(a)). The second route is actively dividing germinal cells from the neuroepithelial layer which migrate to the surface of the rhombic lip. These continue dividing once their location is reached, forming the external germinal layer. Differentiation occurs along the ventral border of this zone and the cells stop dividing and migrate a second time into the substance of the cerebellum immediately below the pre-formed Purkinje layer, to form the granular layer of the cerebellum. Most of the cells in the external layer develop in this manner but there are also some stellate neurones produced which persist in the external layer.

The internal germinal layer ceases activity long before birth when it is present only as the ependymal layer of the fourth ventricle but the external germinal layer (Fig. 1) is active post-gestation and can persist for 75 days in the puppy (Pheimister and

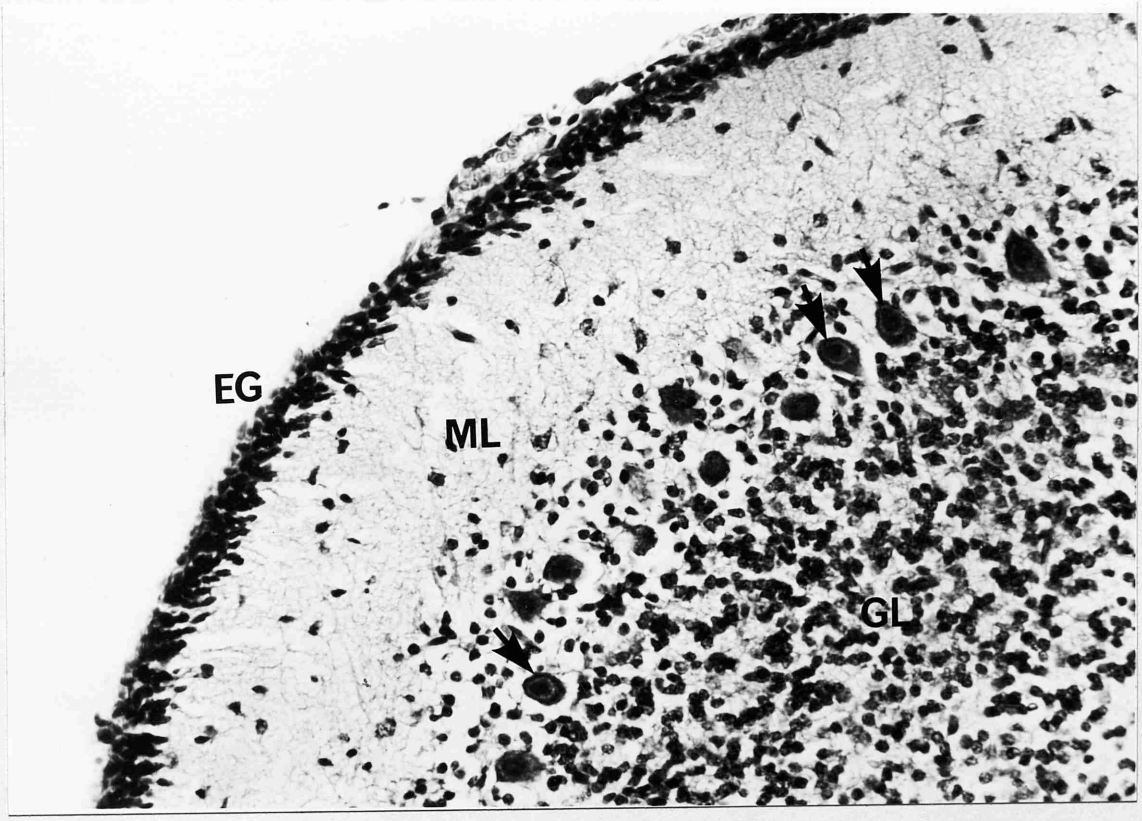


FIG. 1: IMMATURE CEREBELLAR CORTEX
THE EXTERNAL GERMINAL LAYER (E.G.) PERSISTS IN THE POST-GESTATIONAL PERIOD. CELLS MIGRATE THROUGH THE MOLECULAR LAYER (M.L.) TO THE GRANULE CELL LAYER (G.L.) PURKINJE CELLS (ARROWED) ARE FORMED BY THE TIME OF GESTATION

H AND E X 250

Young, 1968). Once differentiation is complete most of the cells from this area are present in the cellular granular layer with only a few cell bodies of the stellate neurones at the site of the external germinal layer. This latter zone is now largely accellular consisting of axonal processes from the granule layer cells and is termed the molecular layer (Fig. 2). In this manner the three layers of the definitive cerebellar cortex are formed. From external to internal they are the molecular layer, the Purkinje cell layer and the granule cell layer (Fig. 3). In addition, the neurones of the deep cerebellar nuclei are located in the cerebellar medulla and are formed from the same source as the Purkinje cell layer.

ANATOMY

The gross external appearance of the cerebellum is of a main body and a small caudal lobe, the flocculo-nodular lobe divided by the caudolateral fissure (Fig. 4). The main body has a central portion, the worm like vermis and two lateral hemispheres. This body is further sub-divided by the transverse primary fissure into rostral and caudal lobes (Fig. 5). The surface of the cerebellum is marked by many lines demarcating smaller lobules and the individual folia (Figs. 6 and 7).

Several other methods of naming the cerebellum have been employed based on its phylogenetic status or on a functional basis. Using the first system it is divided into an archicerebellum corresponding to the flocculo-nodular structure, the most primitive part of the cerebellum in evolutionary terms, the paleocerebellum corresponding to most of vermis, and the neocerebellum which is best developed in the higher mammals and

FIG. 2 MATURE CEREBELLAR CORTEX

NORMAL CORTICAL ARRANGEMENT CONSISTS OF 3 MAIN ZONES THE PURKINJE CELL LAYER (ARROWS) DIVIDES THE EXTERNAL MOLECULAR LAYER (ML) AND THE INTERNAL GRANULE CELL LAYER (GL)
THE CEREBELLAR MEDULLA CAN ALSO BE SEEN (CM)

H AND E X 100

FIG. 3 MATURE CEREBELLAR CORTEX

A HIGHER POWER VIEW OF FIG. 2 SHOWS NORMAL PURKINJE CELLS (P) THESE ARE FLASK SHAPED USUALLY. APICAL DENDRITES (ARROWED) CAN BE SEEN EXTENDING INTO THE MOLECULAR LAYER (ML)

H AND E X 400

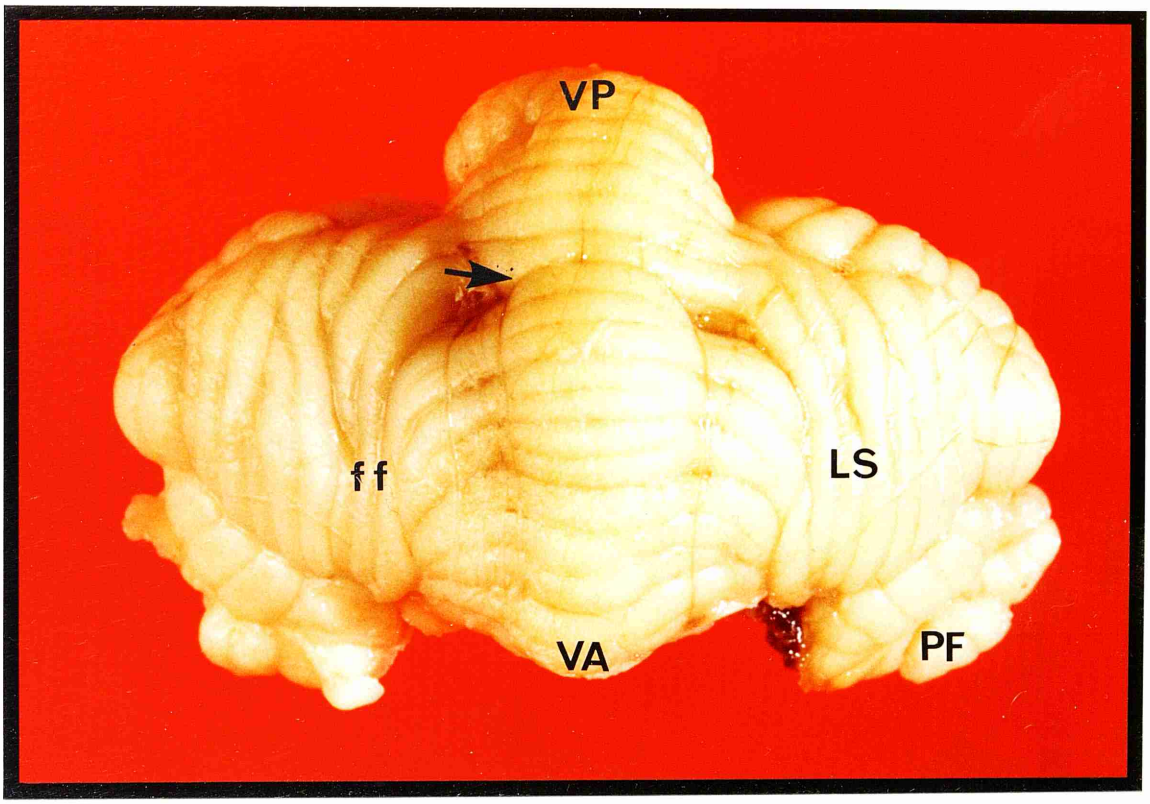
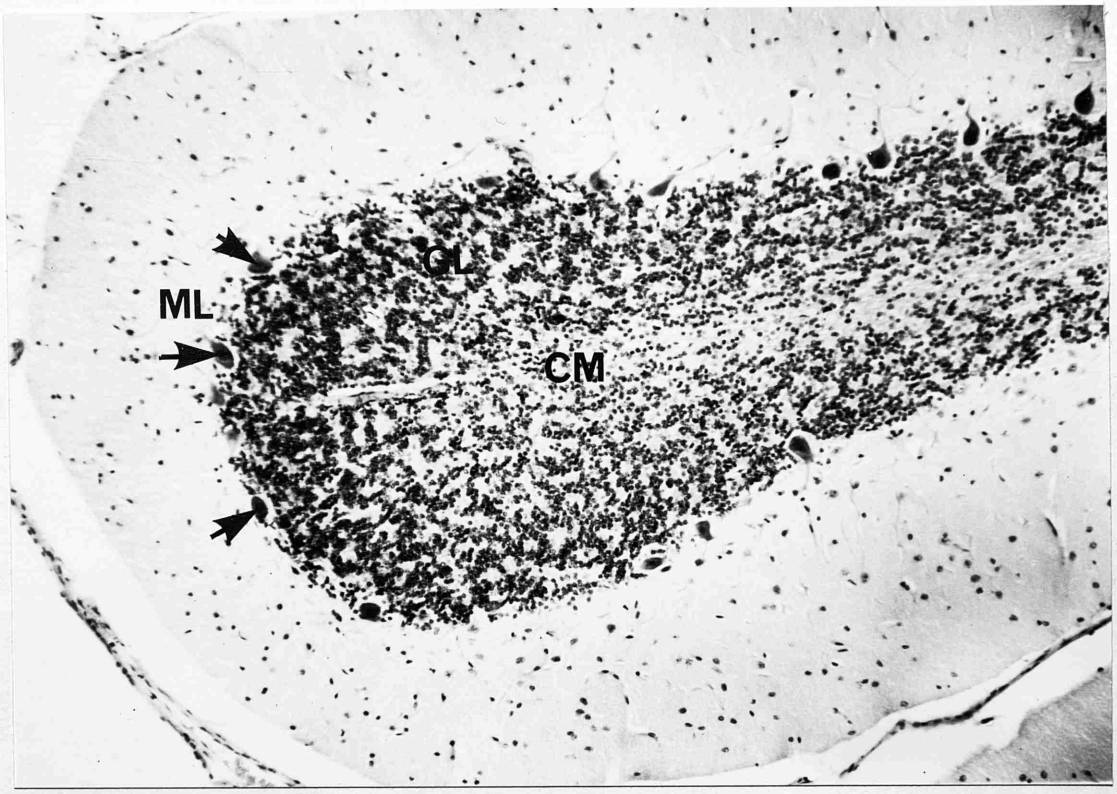


FIG. 4 CEREBELLUM - DORSAL ASPECT
VA: VERMIS, PORTION OF ANTERIOR LOBE, VP: VERMIS, PORTION POSTERIOR LOBE, PF: VENTRAL PARAFLOCCULUS, LS: LATERAL PORTION OF LOBUS SIMPLEX. ARROWED, PRIMARY FISSURE. F:FOLIA OF LATERAL HEMISPHERE


ML
GL
CM

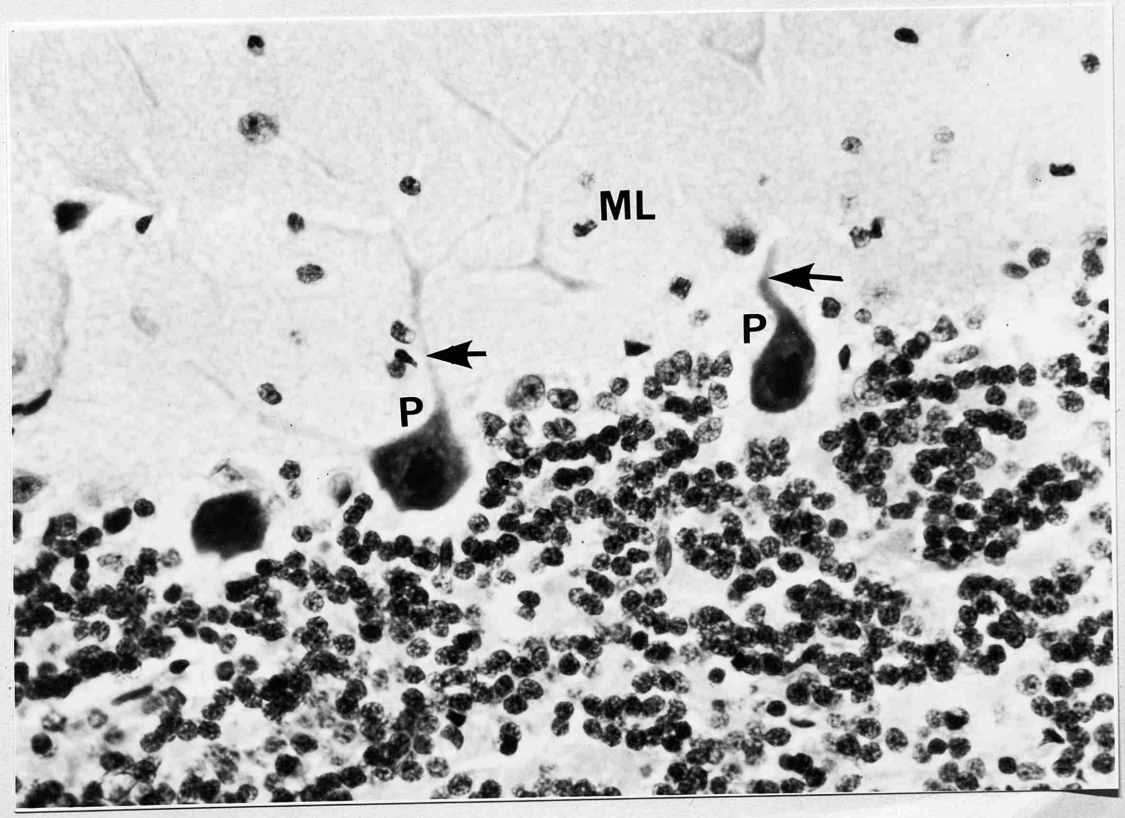

ML
P
P

FIG. 5 THE CEREBELLUM HAS BEEN SECTIONED TO SHOW THE ARRANGEMENTS OF THE CORTEX AND MEDULLA. THE PRIMARY FISSURE (ARROW) CAN ALSO BE SEEN.

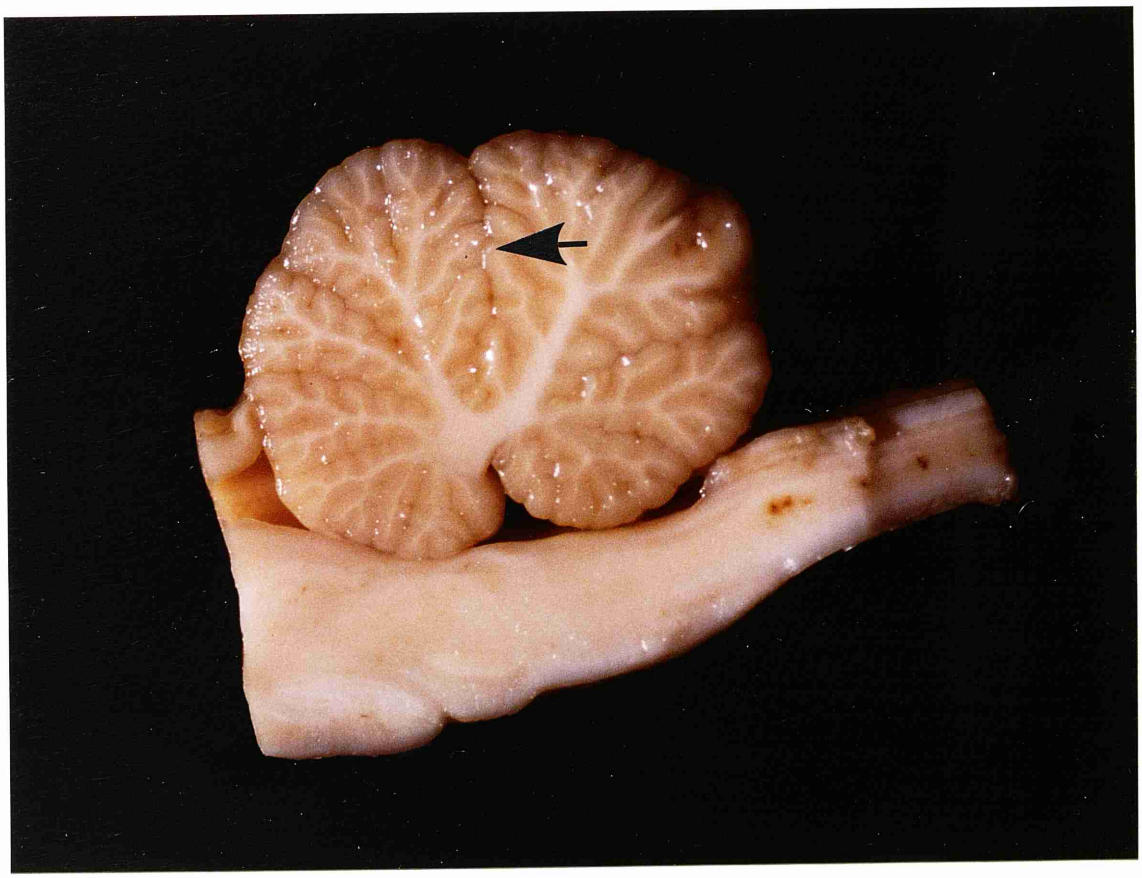
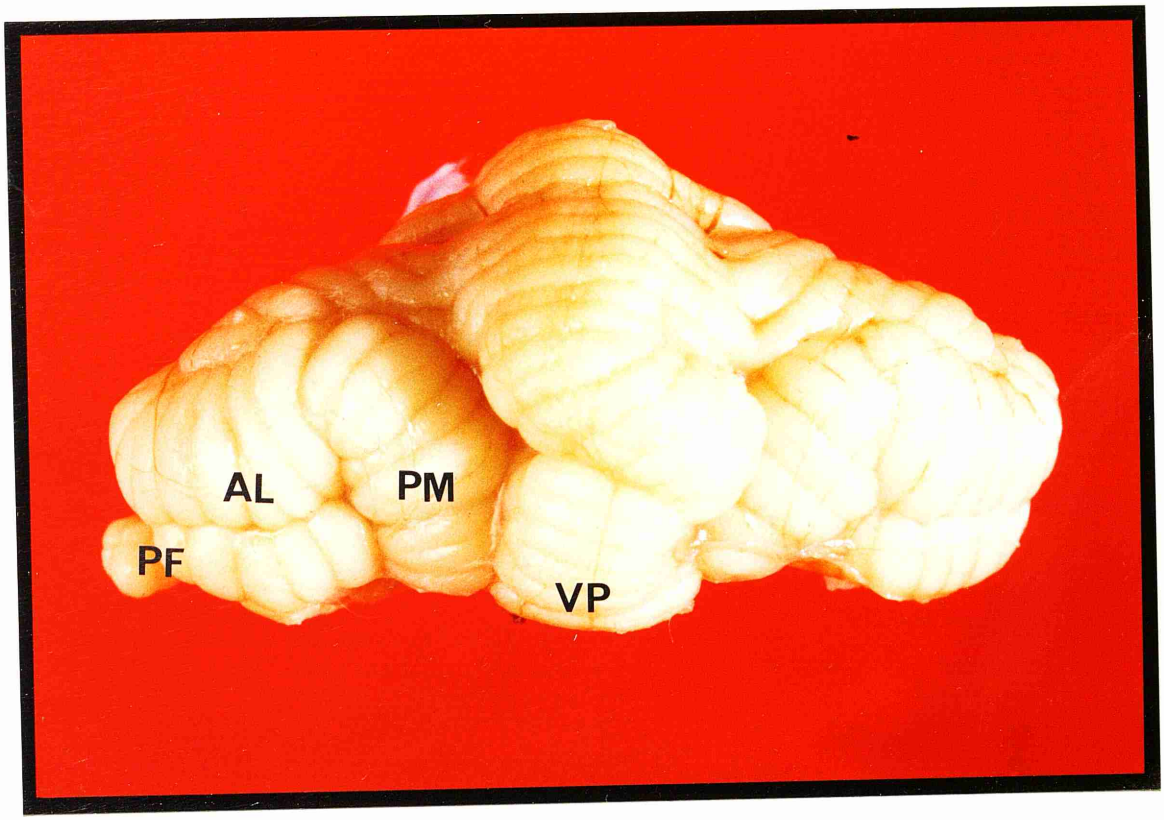
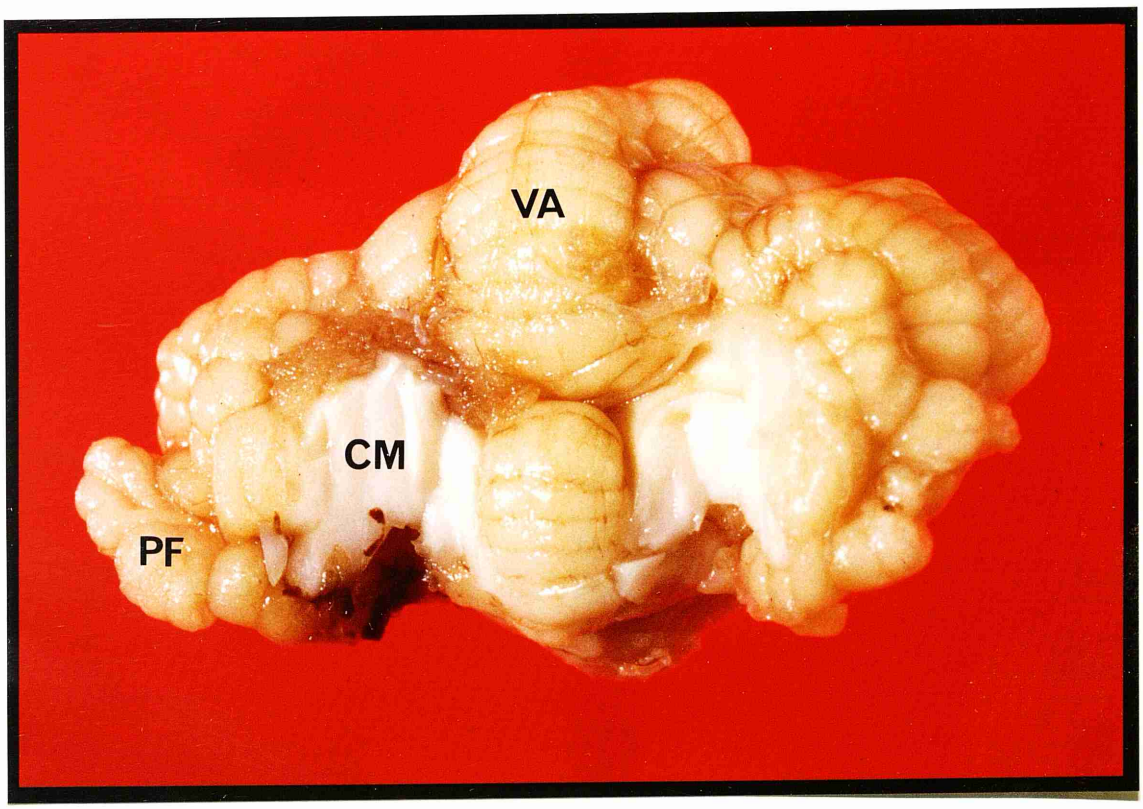


FIG 6 CEREBELLUM CAUDAL ASPECT
VP: VERMIS, PORTION POSTERIOR LOBE PF: PARAFLOCCULUS
PM: PARAMEDIAN LOBE AL: ANSIFORM LOBE

FIG. 7 CEREBELLUM CRANIAL ASPECT
VA: VERMIS, PORTION OF ANTERIOR LOBE,
PF: PARAFLOCCULUS, CM: WHITE MATTER COMPOSING CORPUS MEDULLARE


AL
PM
PF
VP


VA
CM
PF

especially the primates. This last area represents the most recent evolutionary advance comprising the cerebellar hemispheres and the middle portion of the vermis, the pars intermedia.

The first attempts at functional classification of the cerebellum described three main divisions, the vestibulo-cerebellum, being the flocculo-nodular formation, the spinocerebellum which is the vermis and the pontocerebellum comprising the lateral hemispheres. This classification was based on the afferent projections to the cerebellum. Although in general, most of the cerebral projections terminate in the lateral hemispheres, the spinal in the vermis and the vestibular in the flocculo-nodular area, there are very considerable areas of overlap and this terminology was discontinued.

The further method of functional division of the cerebellum is into saggital strips on the basis of the cortical projections to the deep cerebellar nuclei. Jansen and Brodal (1940) described the main areas of conticonuclear projections with the most median strips projecting to the fastigial and vestibular nuclei, an intermediate zone projecting to the interpositus and a lateral area projecting to the dentate. The shape of these zones has been further modified following experiments by Voogd (1969) showing an alteration to the longitudinal pattern proposed by Jansen and Brodal.

The cerebellum is attached to the brain stem by means of three peduncles on each side of midline. The rostral joins the cerebellum to the mesencephelon and contains mainly efferent fibres and some afferent. The middle peduncle attaches to the region of the pons and has afferent fibres only. The caudal cerebellar peduncle attaches in the medulla and contains mainly

afferent fibres.

The substance of the cerebellum comprises a central medulla, the cerebellar white matter and an outer cortex (Fig. 5). The medulla dorsal to the 4th ventricle contains the deep cerebellar nuclei, three paired structures on each side of mid-line which are named from medial to lateral, the fastigial, the interpositus and the dentate nuclei. The remainder of the medulla consists of axonal processes projecting to and from the cortex. The cerebellar cortex is a three layered structure. The outer layer is largely acellular consisting mainly of axonal processes originating from the granule cell layer, or climbing fibres from the inferior olivary nuclei. A few cell bodies of stellate, or on the inner surface basket cells, are found in this area. These are interneurones inhibitory to the Purkinje cells which comprise the middle layer of the cortex. The Purkinje cell layer consists of a single line of Purkinje cell bodies which are comparatively large and triangular in shape. Dendrites from these cells form elaborate projections into the molecular layer synapsing with interneurones and mainly with the parallel fibres from granule cells (Fig. 3). Granule cell activity is facilitatory to Purkinje cells. The axons from the Purkinje cells traverse the lower granule cell layer passing into the cerebellar medulla where most terminate in the deep cerebellar nuclei although direct projections from the cerebellar cortex to lateral vestibular nucleus have been demonstrated (Dow, 1936; 1938; Angaut and Brodal, 1967). The granule cell layer contains the cell bodies of many small, dark- staining granule cells. Also present are some larger Golgi cells. Afferent fibres to the cerebellum mostly synapse in the granule cell layer. Granule cell axons

ascend to the molecular layer where they bifurcate to give two 'parallel fibres' running in opposite directions. These synapse with and are facilitatory to Purkinje dendrites. Other synapses are made with Golgi, basket and stellate interneurones which are also facilitatory. These cells in turn synapse with the Purkinje cells (basket, stellate) and granule cells. The cortex is represented diagramatically in Fig 8.

There is evidence that information is carried into the cerebellum by three types of fibre. The first of these is called a 'mossy' fibre, the name being due to the appearance of the telodendrons. This fibre has a widespread origin carrying information from the spinal cord and from the pontine, vestibular, reticular and trigeminal nuclei and can be found ascending in all the cerebellar peduncles. After giving off a collateral synaptic branch to the deep cerebellar nuclei it ascends to the granule cell layer, synapsing with granule cell bodies, which in turn contact Purkinje cells. Each mossy fibre interacts with many granule cells.

The second type of afferent is the climbing fibre. This has a single source in the inferior olivary nuclei and entering the cerebellum via the caudal peduncle ascends through the granule cell layer to the molecular layer where it winds round a single Purkinje cell dendritic system. Each climbing fibre although contacting only one Purkinje cell makes multiple synapses with this.

There is evidence for a third fibre called an aminergic fibre (Bloedel and Courville,1981) with terminations which differ from both mossy and climbing fibres. These project both to the granule cell layer and the molecular layer of the cortex. A sub-

Fig 8

CEREBELLAR CORTEX - Diagramatic Representation

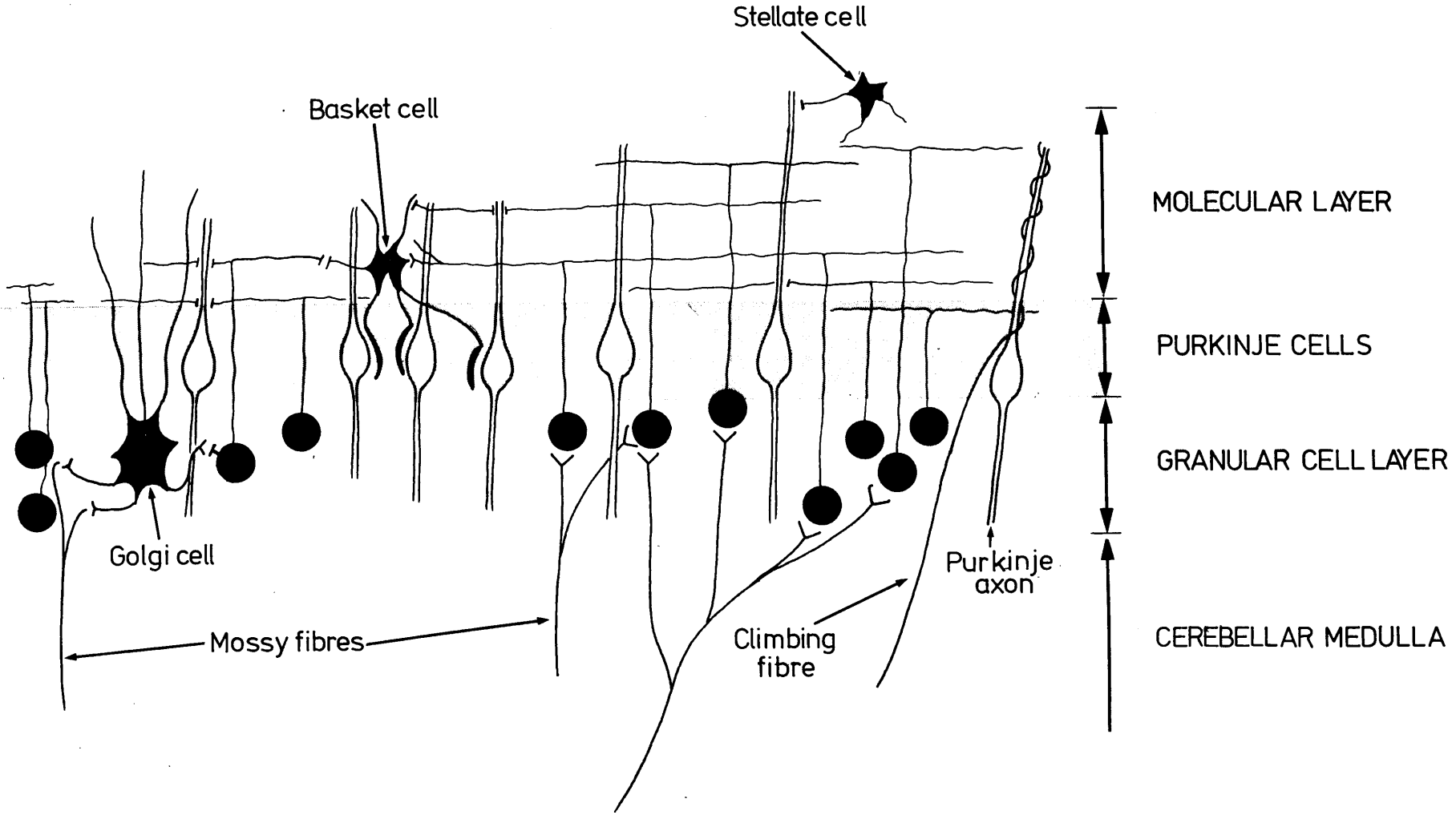


FIG. 8 A DIAGRAMATIC REPRESENTATION OF CEREBELLAR CORTEX

division can be applied to this group according to the transmitter substances produced, one being serotonin and the other noradrenalin.

Purkinje cells are facilitated directly by the climbing fibres and indirectly by the mossy fibres via the granule cells (Fig. 9). Excitatory impulses are also provided to the basket, stellate and Golgi cells by the parallel granule cell axons. All of these interneurones produce an inhibitory effect on the Purkinje cell, stellate and basket cells by direct synapse and Golgi cell by inhibition of the granule cell (Eccles, 1966). This means that the Purkinje cell is initially facilitated by incoming stimuli via the granule cell and the climbing fibre then inhibited immediately after, through the action of the interneurones. This will limit the area of cortex reacting to a particular incoming impulse (Brodal, 1969).

In summary, (Table 1) information is carried to the cerebellum from the spinal cord in the dorsal and ventral spinocerebellar tracts, and in the cuneocerebellar tract from the cervical region. These projections (with the exception of the ventral spinocerebellar fibres) ascend in the caudal peduncle and are carried by mossy-type fibres.

Information from the cerebrum is principally carried from the pontine nuclei via the middle peduncle, again mostly by mossy fibres.

Other tracts projecting to the cerebellum are the tectocerebellar, carrying visual and auditory information, the rubrocerebellar from the red nucleus, and the reticulocerebellar. The projection from the inferior olives entering via the caudal peduncle is made up entirely of climbing fibres and although there

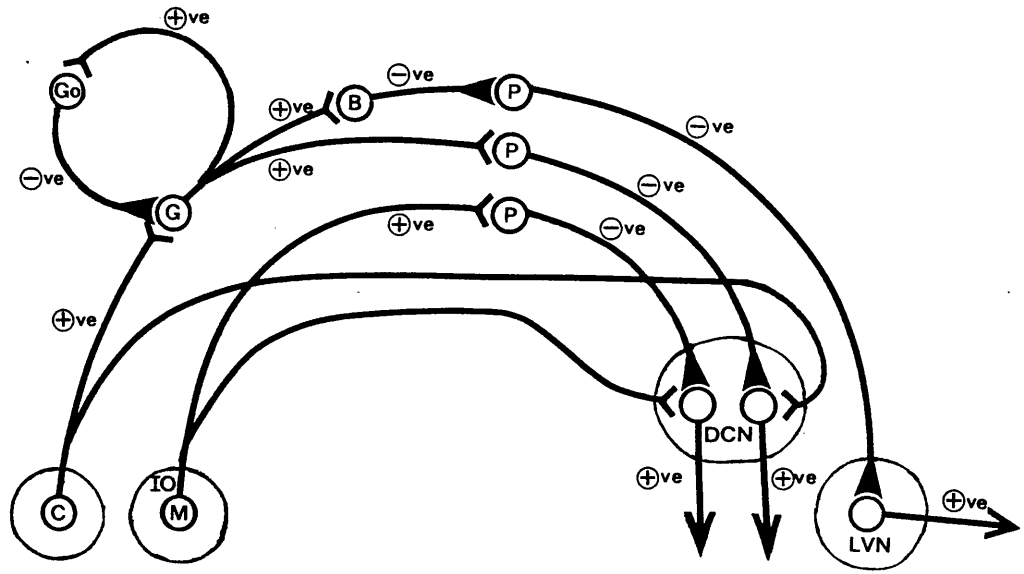


FIG. 9 PRINCIPLES OF CEREBELLAR CELLULAR ORGANISATION.
C: CLIMBING FIBRE; M: MOSSY FIBRE; G: GRANULE CELL; GO: GOLGI CELL; B: BASKET AND STELLATE CELLS.
P: PURKINJE CELL; D.C.N.: DEEP CEREBELLAR NUCLEI; L.V.N.: LATERAL VESTIBULAR NUCLEI: I.O.: INFERIOR OLIVE.

is evidence that there are some sources of extraolivary climbing fibres, these represent the largest supply. The olives receive projections from spinal and higher centre sources and so process a wide variety of information.

The projections of the cerebellar nuclei are shown in Table 2. The fastigial nuclei project mainly to the lower centres and spinal cord, the dentate to the higher centres via the contralateral ventral lateral nucleus of the thalamus while the interpositus sends projections to both.

Inhibitory Purkinje activity acts mainly to dampen the effect of discharges from the cerebellar nuclei and loss of function will result in increased facilitation of motor axons giving jerky uncontrolled movements and delay in cessation of movement.

SECTION 2

A REVIEW OF CONDITIONS SPECIFICALLY AFFECTING THE CEREBELLUM IN THE DOG

A REVIEW OF CONDITIONS SPECIFICALLY AFFECTING THE CEREBELLUM IN THE DOG

A number of conditions are described where specific abnormalities are located principally within the cerebellum rather than the variable involvement of the organ seen with an infective agent as in distemper encephalitis, Toxoplasma encephalitis or a mycotic encephalitis. A parvovirus-induced hypoplasia similar to that occurring in the feline has not been described in the dog. It would seem that viral damage other than distemper is of very rare occurrence in the cerebellum although young pups infected with herpesvirus have focal areas of cerebellar degeneration and cortical dysplasia (Percy and others, 1971), in conjunction with widespread organ involvement including focal cerebral lesions, retinal dysplasia and lesions of the viscera, lungs, liver, heart and kidney. Two pups have also been described by Harari and others (1983) with complete cerebellar agenesis. It was thought that this was due to an in-utero herpesvirus infection

Some toxic agents have also been incriminated as a cause of cerebellar lesions. Purkinje cell degeneration with absence of ascending fibres in the granule cell layer and basket cell fibres in the molecular layer has been described in dogs fed on a diet containing flour bleached by agene (NCl_3) (Silver 1949; Lewey, 1950). There were also minor changes in the deep cerebellar nuclei and diffuse involvement of the cerebrum with patchy liquefaction and necrosis. The animals developed bouts of hysteria, with convulsions, and death occurred rapidly if the diet was not discontinued. Agene was popular as a processing agent at the time of these reports but has not been used since due to its

neurotoxicity.

D.D.T. toxicity has also been shown to result in a reduction of cells in the dentate nucleus of affected dogs (Haymaker and others, 1946).

There are many reports of cerebellar conditions occurring in young pups and affecting several members of single litters. Certain of these have a suspected or proven familial basis (Tables 3 & 4). The conditions can be divided into 2 main groups: group 1, where the signs are present seemingly from birth becoming obvious when the pup attempts to ambulate and group 2 in which signs of locomotor disturbance develop only after a period of apparent normality (Table 4).

Group 1

In the first group affected breeds have included the Beagle, Berne running dog, Boston Bull Terrier, Chow, Irish Setter, Toy Poodle, Samoyed and Wire Haired Fox Terrier. Modes of inheritance have only been confirmed in the Chow and the Irish Setter, but several of the conditions in the other breeds may be genetically based.

Beagle

de Lahunta (1980(a)) described severe cerebellar ataxia, truncal ataxia pronounced hypermetria and head tremor present from birth in two Beagles from a litter of six. The condition was not progressive. Pathological changes were confined to the cerebellum with a diffuse absence of Purkinje neurones, some loss of granule cells and the presence of swollen axons in the granule cell layer. Wallerian degeneration, presumed to result from degeneration of Purkinje neurones, was found in the laminae of folia.

Boston Bull Terrier

Two litter mates from a litter of four were described by Dow (1940). These dogs were affected from birth, the most prominent clinical feature being a marked overextension of the hind legs, which although not continuous could be easily elicited by activity or pressure on the pads. The pups also exhibited a continuous head tremor of varying regularity and a markedly dysmetric gait with hypermetria present in all four legs. Frequently the hind limbs went into extensor spasms and were dragged behind the animal. Proprioceptive functions were reduced, muscle tone slightly increased and tendon reflexes hyperactive. One pup also showed a tendency to circle to the left. The condition was non-progressive over a period of 20 months in one case. Macroscopically a slight internal hydrocephalus and slight atrophy of the right side of cerebellum were noted. Histologically, areas of total atrophy of the cerebellar cortex were found mainly in the vermis, with sparing of the lateral lobules. There was a sharp demarcation between areas of normal and abnormal cerebellar cortex. There was gross atrophy of the fastigial nuclei, moderate abnormality in the interpositus and only slight changes in the dentate nuclei reflecting the cortical distribution of the lesion. The superior cerebellar peduncle was atrophic. Severe cell loss was also evident in the inferior olivary nuclei, probably secondary to the cerebellar agenesis.

Chow

Knecht and others (1979) examined twelve animals from three different but related litters showing signs of cerebellar dysfunction. All affected animals showed ataxia and hypermetria when walking but no abnormalities at rest. Proprioceptive

responses were slow but no tremor was reported. The signs were present when the pups first attempted to ambulate and no progression was observed. Six animals were available for necropsy and changes were confined to the cerebellar cortex. There was a reduced number of Purkinje and granule cells but no degenerative changes or reactive gliosis was observed. The remainder of the cerebellum and cerebellar projections showed no abnormalities. Thus it was considered to be a hypoplasia of the cerebellar cortex. An autosomal recessive mode of inheritance is proposed for this condition.

Irish Setter

There are accounts of two different types of cerebellar disturbance in this breed. de Lahunta (1980(a)) described three puppies in a litter of ten showing severe non-progressive cerebellar ataxia from the moment walking was attempted. At necropsy all three had identical, severely hypoplastic cerebelli with lissencephaly and pachygyria of the cerebrum.

Palmer and others (1973) also described a condition, present from birth, in this breed but in these cases the pathological lesions were confined to the cerebellum. Eighteen animals were examined. All of these dogs were unable to stand but propelled themselves on their bellies, so-called 'swimmers'. Several also had convulsions. Other manifestations evident were a constant head tremor, jerky inco-ordinated movements and occasional opisthotonos. The pups were to all intents blind but may have been able to differentiate between areas of light and shade. Nystagmus was also observed but light reflexes and examination of the retina showed no abnormalities.

There was a severe loss of Purkinje cells and many

degenerating Purkinje cells with vacuolation and ischaemic-type necrosis were observed. In some of the older dogs there was also a depletion of granule cells. No other lesions could be found in the central or peripheral nervous system and it is difficult to match the pathological findings with the severe clinical manifestations. An autosomal recessive mode of inheritance is proposed.

Toy Poodle

Kay and Budzilovich (1970) described cerebellar changes in two toy poodles. One of these was unable to stand from birth and at necropsy severe atrophy of the cerebellum was found, with preservation of the flocculo-nodular formation. Secondary changes were seen in the brain stem including atrophy and gliosis of the inferior olives and fibre loss in the spinocerebellar tracts.

The second case was able to walk but exhibited a dysmetric gait. Diffuse episodic myoclonic seizures were observed during which the animal was conscious but unable to stand. Necropsy at 9 weeks of age revealed agenesis of the cerebellum and diffuse atrophy of the brain stem with involvement of the inferior olives.

Samoyed

A pelvic limb ataxia in three separate litters of Samoyeds, was described by de Lahunta (1980(a), 1983). Three out of six pups were affected in one litter. The pelvic limbs were spastic, severely hypermetric and held forward under the body. The thoracic limbs, however, were only mildly affected with slight spasticity and hypermetria. The condition was considered to be non-progressive. Pathologically the most obvious findings were swollen axons in the granule cell layer and evidence of occasional

degeneration of Purkinje axons in the laminae of folia. There appeared to be no loss of Purkinje neurones.

Wire Haired Fox Terrier

de Lahunta (1980(b)) described cerebellar ataxia in two pups from a litter of three. The condition was non-progressive from birth but at the age of 1 year generalised seizures occurred. The pathological findings were those of cerebellar dysgenesis and cerebral lissencephaly, very similar to the changes found in the Irish Setter (de Lahunta 1980(a)).

Berne Running Dog

Cerebellar ataxia associated with a loss of Purkinje cells and a symmetrical olivary degeneration was reported in this breed (Good 1962). A familial basis is strongly suspected.

Two further reports describe changes analagous to the Dandy-Walker syndrome in humans (Urich, 1976) in a variety of canine breeds. Pass and others (1981) examined two animals, a Beagle and an Australian Silky terrier which exhibited severe signs of cerebellar ataxia from birth. Absence of portions of the cerebellum in both animals accompanied by mild hydrocephalus in the beagle was found on necropsy. Kornegay (1986) described 6 animals with similar signs of cerebellar disease and some evidence of vestibular involvement. The breeds represented in this study were two Bull terriers, a Labrador, a Weimeraner, a Dachshund and a dog of mixed breed. In all of the animals cerebellar dysplasia with hypoplasia of the vermis was noted pathologically. In some of the animals there was also involvement of the cerebellar hemispheres and the flocculus and one animal (Labrador Retriever) showed a mild hydrocephalus.

Group 2

In the second arbitrary group of cerebellar disorders, the animals appear normal when they are first able to walk and signs of cerebellar disturbance develop some time after this period of apparent normality. This suggests that initially these animals have either no pathological changes or insufficient disturbance to interfere with normal function but deterioration occurs due to a later-developing pathology. The breeds of dog in which such a clinical situation has been reported are Airedale, Cairn, Cocker Spaniel, Gordon Setter, Rottweiler, Kerry Blue, Great Dane, Golden Retriever and Labrador.

It seems a condition exists in the Cairn, Cocker Spaniel, Great Dane, Golden Retriever and Labrador producing very similar signs in these breeds. de Lahunta (1983) described a cerebellar ataxia which becomes apparent after 8-12 weeks of age. The condition is generally progressive but static periods have been observed. In these animals there is loss and obvious degeneration of Purkinje cells. Although not proven, a genetic basis is very strongly suspected in each breed.

Airedale

A report of cerebellar ataxia in a family of this breed was made by Cordy and Snelbaker (1952). They described at least 6 cases occurring in 3 separate litters when a grand-daughter - grandfather mating occurred using two bitches to produce the litters. Two of the cases were investigated fully. Signs became obvious at 12 weeks of age, with ataxia and hypermetria particularly of the forelegs. Eventually the dogs had great difficulty standing and remained seated with marked swaying of the head and trunk. The cerebellum of both animals appeared

smaller than normal. The main change was a diffuse loss and degeneration of Purkinje cells associated with a moderate gliosis. The granule cell layer was undisturbed and the white matter of the folia had evidence of occasional axonal loss. There was increased cellularity in the molecular layer due to cells similar in appearance to neuroblasts which, it is suggested, may have been Purkinje precursors (Innes and others, 1940). With the breeding history, although not proven, a familial condition must be suspected.

Gordon Setter

A slowly-developing cerebellar ataxia has been described in this breed (de Lahunta and others 1980; Steinberg and others, 1981). de Lahunta and others (1980) described 19 cases, 10 of which were available for necropsy. The signs became apparent in a much older age range from 6 months to 24 months. A stiff, dysmetric gait was observed with hypermetria in the thoracic limbs. Although there was some delay in performing postural tests proprioception was almost normal and no tremor was apparent. Once noticed, the condition was insidiously progressive.

The cerebellum was slightly reduced in size and pathological changes were mainly confined to the cerebellar cortex. There was a profound loss of Purkinje cells with obvious degeneration of many remining neurones. The granule cell layer showed variable cellular depletion and contained swollen Purkinje cell axons. Astrocytic proliferation occurred where Purkinje depletion was greatest. The deep cerebellar nuclei were only mildly affected with gliosis and occasional axonal swellings. An autosomal recessive mode of inheritance was proposed.

Kerry Blue Terrier

de Lahunta and Averill (1976) gave descriptions of 13 Kerry

Blue Terriers with progressive cerebellar signs which became apparent between 8 and 16 weeks of age. Initially, stiffness of the pelvic limbs and a mild head tremor were seen which progressed to a severe dysmetria with obvious hypermetria affecting all limbs. Walking became increasingly more difficult and the animals were usually unable to stand 5 months after the initial signs. There was no paresis and tendon reflexes were normal.

Early pathological changes were most profound in the cerebellar cortex with degeneration and subsequent loss of Purkinje cells and an accompanying astrocytic response. Almost the whole complement of Purkinje cells was lost when degeneration was apparent in the granule cell layer. Some swollen axons were evident in the cerebellar white matter and the deep cerebellar nuclei. Late in the disease there was degeneration in the olivary nuclei, the substantia nigra and the caudate nuclei. Changes were also observed in the medial thalamic nucleus, the optic tract and the lateral geniculate bodies. Pedigree data analyses indicate this condition is hereditary probably due to an autosomal recessive gene.

Rough Collie

Hartley and others (1978) examined 39 animals showing signs of progressive cerebellar ataxia. All but one of the animals had a sable coat. Signs were first apparent at 4-12 weeks of age, the majority showing signs by the age of 6 weeks. Initially, there was ataxia present in the hindlimbs which rapidly progressed to involve the thoracic limbs with severe dysmetria, pronounced hypermetria, swaying and falling. Some pups developed tremor of the head. All the signs were exacerbated by excitement. The condition was progressive over a period of 4 weeks in all animals

bar one and all were severely affected at the end of this period. The exception was the non-sable animal in which the signs remained static over a period of 1 year.

Grossly, there was atrophy of the vermis anteriorly and indeed this reflected the microscopial findings where the central vermis was most severely affected with some involvement of the lateral hemispheres. The initial lesion was a rapid degeneration and subsequent loss of granule cells. Purkinje cells were preserved but when the total granule cell layer was lost then they too were seen to degenerate. The result was a complete atrophy of cerebellar cortex. Wallerian degeneration was present in the white matter of the folia and peduncles and demyelination was observed at all levels in the spinal cord with the exception of the dorsal columns. There was neuronal loss in the cerebellar nuclei, the lateral vestibular nuclei, the olivary nuclei and also involvement of the reticular formation and medial longitudinal fascicle. The ventral horns of the spinal cord also showed neuronal loss. An autosomal recessive mode of inheritance has been demonstrated for this condition.

Rottweiler

An insideously developing condition has been described in the Rottweiler breed by Chrisman and others (1984) and Cork and others (1983). Five cases are described. These animals developed a mild gait abnormality before 1 year of age. The signs progressed gradually till the dog showed ataxia of all four limbs with hypermetria of the pectoral and occasionally the pelvic limbs. Most of the animals exhibited these signs between two and three years of age. Further development occurred till the animals were grossly ataxic over a period of several years. In

the later stages reduced menace response, head tremor and nystagmus became apparent.

Pathological changes were not confined to the cerebellum but were widespread throughout the central nervous system. There was, however, mild atrophy of the cerebellum and some loss of both Purkinje and granule cells. The disease was characterised by the presence of large axonal spheroids particularly in the vestibular nucleus, the lateral and medial geniculate bodies, the cuneate and gracillei nuclei, the sensory nucleus of the trigeminal nerve and the dorsal roots of the spinal cord. The spheroids were also found occasionally in the cerebellum in the granule cell layer, the medulla and deep cerebellar nuclei. They occurred most commonly in the vermis and pars intermedia. The pathological features resembled neuroaxonal dystrophy of humans An autosomal recessive mode of inheritance is suggested for this disease (Cork and others 1983).

In conclusion, specific cerebellar degeneration can occur in dogs as a result of certain viral infections or damage by toxic agents. There are also a large group of conditions which are suspected to have a genetic aetiology. These conditions may be apparent from birth or may develop sometime after birth. The cerebellar cortex and in particular the Purkinje cell system is very often affected.

SECTION 3

INTRODUCTION

Six Bull Mastiff pups showing ataxia were investigated. These animals all had very similar histories and clinical signs when examined. They also were closely related to one another. Each animal was euthanased as a result of its clinical problem and submitted for detailed pathological investigations. Further information about related animals having a similar condition was received and detailed pedigree information analysed. The nature and results of the investigation are presented in this section. The results will be considered under four main headings.

a) Clinical Details

b) Ancillary Findings

c) Pathological Findings

d) Genetic Aspects

The results are then discussed under the same headings.

MATERIALS AND METHODS

Six affected pups, three males and three females were available for investigation. A single older animal of the same breeding which died as a result of myocarditis at the age of 18 months was used as a control for pathological studies. All animals received a general clinical and a detailed neurological examination. Proprioceptive functions were examined by utilising three tests, paw position sense, reflex stepping and hip sway test in the pelvic limbs. For the paw position test, with the animal standing the leg is lifted and replaced so that the dorsum of the paw is contacting the ground with the pads uppermost as occurs when a dog knuckles on a limb. In the normal animal this abnormal positioning is corrected immediately and any delay in doing so indicates some dysfunction. This test is repeated for all feet. In the reflex stepping test the animal is again examined standing, the paw lifted and a sheet of paper or plastic placed under it and the paw replaced on the sheet. The sheet is then gradually slid behind the animal drawing the paw with it. A normal animal will only allow a minimal displacement before returning the limb to the correct position. Again any delay in this response indicates an abnormality. This is repeated in all legs. For the hip sway test the animal is once more standing. The examiner positions himself behind the dog and by applying manual pressure to each gluteal mass, deviates the hindquarters to that side. This effectively changes the centre of gravity of the animal to that side and a normal response is a rapid short step of the ipsilateral hind-limb to the side being tested to correct the balance. An abnormal response is seen when on altering the centre

of gravity the hind-legs remain positioned as they were on commencement of the test resulting in a pronounced leaning of the hindquarters.

Both tactile and ocular placing reflexes were examined. In each case the animal is lifted and carried towards a surface such as a table where it could be set down. For a positive response to the ocular test the animal should extend the leg being examined in anticipation of bearing weight immediately before being placed on the table. In the tactile test the eyes are covered and the animal is moved so that the dorsum of the paw of the leg being tested contacts the edge of the table. A positive response is a lifting clear of the paw on contact and extension in preparation for weight bearing. A negative response is observed when on contact the carpus or tarsus is passively flexed with no attempt to clear the obstacle.

The pedal, patellar, panniculus and anal reflexes were examined as described by Palmer (1976).

Pain sensation was assessed by pricking the skin with a pin, a positive response shown by turning of the head, vocalisation or aggression towards the examiner. Deep pain sensation was tested by pinching the webs of the toes with artery forceps and looking for the same response.

An attempt to estimate visual ability was made by constructing obstacle courses using boxes and chairs through which the animals were made to pass. The obstacles were arranged in such a fashion that frequent changes in direction were necessary to negotiate them.

Narrow spaces were also employed to assess fine judgement of distance and co-ordination. Objects were also rolled across in

front of the animals to estimate their ability to follow these. Stationary objects (such as a bowl of food) were also placed at various distances from the animal and the time taken to locate these objects was noted. Lengthy periods of observation of the pups' behaviour in the kennel and outside were carried out and any abnormalities also noted. The pups' responses to various stimuli such as loud noise or excitement were also recorded.

Haematology

Jugular venous samples were obtained from four of the animals (cases 1, 2, 3 and 4) and red cell count, white cell count, haemaglobin concentration and Mean Cell Volume (M.C.V.) determined.

Biochemistry

Jugular venous samples were obtained from cases 1, 2, 3 and 4 and the following parameters were examined, urea, sodium, potassium, chloride, calcium, magnesium, phosphate, glucose, bilirubin, alkaline phosphatase, ALT and AST activity, creatinine, albumin, globulin and total protein levels. A urine sample from case 2 was analysed and pH, specific gravity, protein and urea levels determined.

Cerebrospinal fluid

Samples were obtained from cases 3, 4 and 5 and analysed for total protein and glucose concentrations. The animals were first sedated using an acetylpromazine - pentazocine mixture intravenously or general anaesthesia was used with thiopentone induction and halothane maintenance. These measures were

necessary to achieve relaxation for positioning and to prevent problems caused by movement during the procedure. The animal was placed on a table in right lateral recumbency. An assistant supported the head ensuring that the long axis was at right angles to a line projected from the cervical spinal canal and that the head was not tilted up or down. The landmarks for the site of cisternal puncture are; caudally, a line dropped perpendicularly between the wings of the atlas and anteriorily the prominent occipital tubercule which also marks mid-line. The site of entry is at a point mid-way between these landmarks and exactly in mid-line which is usually marked by a palpable furrow representing the midline fibrous raphe of the semispinalis muscles. A 1 1/2 inch 21 g hypodermic needle was inserted at this spot after cleansing the skin and advanced at right angles into the sub-arachnoid space of the cisterna magna. On penetrating the meninges a flow of cerebrospinal fluid was obtained and no further advancement attempted. The sample was collected in a sterile bijoux bottle.

Bromosulphthalein Dye Test (BSP)

Liver clearance of BSP was determined on 2 occasions in case 2. After obtaining a control blood sample, a dose of 5mg/kg was injected intravenously and a second blood sample taken exactly 30 minutes later. The concentration of the dye remaining in the blood was measured and expressed as a percentage of the initial amount injected.

Radiography

Plain films of the skull were taken in 2 animals. Positive contrast radiography was utilised on 2 occasions. In case 4,

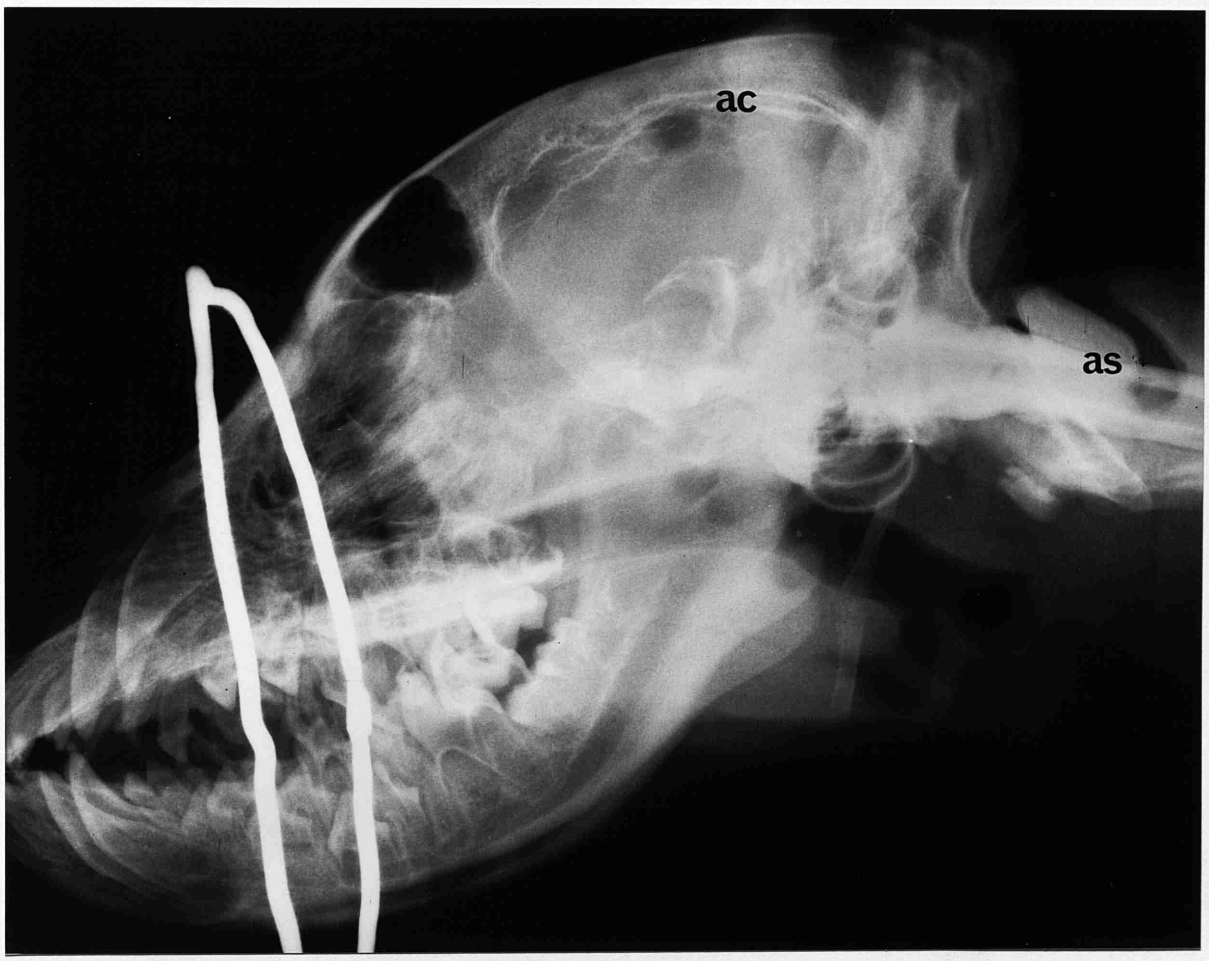


FIG. 10 LATERAL RADIOGRAPH OF HEAD TAKEN AFTER INJECTION OF POSITIVE CONTRAST INTO LATERAL VENTRICLES. CONTRAST HAS PASSED INTO THE SUB-ARACHNOID SPACE OF THE CEREBRUM A.C. AND THE SPINAL CORD A.S.

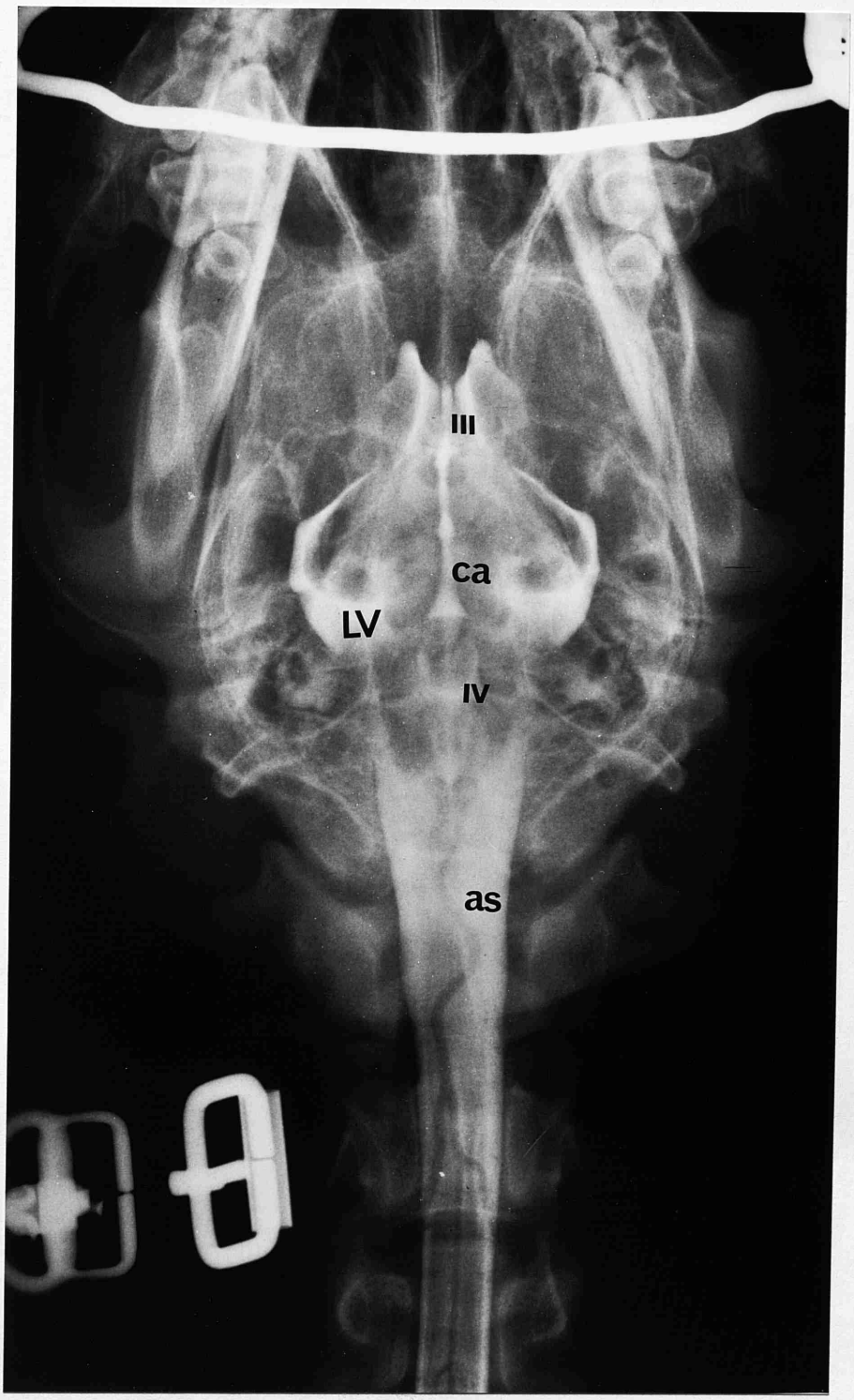


FIG. 11 DORSO-VENTRAL RADIOGRAPH TAKEN AFTER VENTRICULOGRAM SHOWS VENTRICULAR SYSTEM. L.V. DILATED LATERAL VENTRICLE; III THIRD VENTRICLE; C.A. CEREBRAL AQUEDUCT; IV FOURTH VENTRICLE; A.S. SPINAL SUB-ARACHNOID SPACE.

oesophageal function was monitored on an image intensifier following the administration of oral barium.

Ventriculography was attempted in case 2 using Meglumine Iothalomate 60% solution. After inducing general anaesthesia and surgical preparation of the dorsum of the head, a two inch skin incision was made in midline along the saggital crest. The fascia overlying the right temporalis muscle was incised and the muscle bluntly reflected from the skull a distance of 1/2 - 3/4 inch from mid line. The point of insertion of the needle is midway along a line projected from the lateral canthus of the eye to the external occipital tubercule. A cranial tunnel is created using a Steinman pin and a Jacob's hand chuck and a 21g 1 1/2 inch needle is advanced through this keeping the point parallel to mid-line until ventricular fluid is aspirated. In this case 5cc Meglumine Iothalomate (Conray) was injected into the right ventricle. Radiographs were taken immediately and after a short time delay (Fig. 10, 11).

Electrocardiography

This was performed on one case (case 2) by routine methods.

Necropsy and Pathological Sampling

All six cases were available for necropsy. In addition, the brain was obtained from one normal animal which was closely related to the others. The brains from cases 2,4,5 and 6 in addition to the control dog were removed immediately after euthanasia and immersion-fixed in 10% formal saline. Cases 1 and 3 were perfusion-fixed under general anaesthesia and positive pressure ventilation. Following heparinisation a left lateral

thoracotomy was performed to give access to the heart and descending aorta. A cannula was inserted retrogradely into the descending aorta and the common aorta was clamped above the heart. A tube was placed in the right ventricle to allow drainage of blood and fixative into a bucket. The perfusate was pumped through the cannula so that the brain and cervical cord were fixed. In case 1, 10% formol saline was used after a preliminary perfusion with saline while in case 3 a paraformaldehyde-g glutaraldehyde fixative similar to that described by Karnovsky (1965) was used. In this case the initial perfusate of 2 litres contained 2% paraformaldehyde and 2% glutaraldehyde in 0-08M cacodylate buffer (pH7.2) with added calcium and was at a temperature of 37°C. Four litres of a stronger fixative with 4% paraformaldyhyde and 5% glutaraldehyde in the same buffer at 4°C were then infused. The brain and cervical cord were left in situ for 3 hours, removed and further fixed for 2 days either in formalin or in 2.5% glutaraldehyde. Plastic or paraffin sections were prepared from the material.

Plastic sections were prepared by post-fixation in 1% osmium for 1 1/2 hours, dehydrated through alcohol at increasing concentrations from 50% to 100% for 85 minutes with two final 15 minute periods through propylene oxide and embedded in Araldite. 1 um thick sections were cut from the blocks and stained with Toluidine Blue.

Paraffin sections from the brain were processed on an automatic Histokinette. The procedure involves 12 individual steps as detailed in table 5. The tissues were then blocked out in fresh wax and sections cut at 8 um. Stains utilised in paraffin-embedded material were haematoxylin and eosin, luxol

fast blue - cresyl violet, cresyl violet, Holzer and Holmes silver impregnation. In addition to brain, spinal cord in case 1 and spinal cord and lumbar ganglia in case 3 were also examined. Samples of viscera from thorax and abdomen in cases 1, 3 and 4 were taken, immersion-fixed in 10% formalin and prepared as paraffin sections stained with haematoxylin and eosin (see Table 6).

RESULTS

a) CLINICAL DETAILS

Individual case descriptions of six Bull Mastiff dogs are given below. In all animals bar one, the clinical signs developed between the ages of four to eight weeks. The exception was one dog (case 3) in which the signs first became apparent at seven months of age. The condition appeared to be progressive and all of the animals were destroyed within four months of showing clinical signs. There was no sex bias, three males and three females being affected. Details of each case are shown in Table 7.

Case 1: This male pup was presented at seven weeks of age. It was dull and generally disinterested and did not play as much as normal pups. In addition, it was reported to be bumping into objects and had difficulty following articles moved across its visual field although the owner was certain that the pup had reasonable vision. Attempts to follow objects were usually accompanied by irregular jerking movements of the head. It had exhibited ataxia of the hindquarters which was variable from day to day, and on occasions was hypermetric. Several unusual signs were reported which were consistently repeated, such as alternately lifting the right or left foreleg whilst eating, starting to move backwards rather than forwards when called and having great difficulty feeding from a bowl without compressing the food hard against the base. All of these reported signs were observed during its stay in hospital. The dog was also said to have been having fits and showing hysterical behaviour. Although

demonstrations of the latter were observed no convulsions occurred during hospitalisation.

On examination the animal was hypermetric with a variable ataxia of the hind-limbs. Proprioception was slightly reduced as indicated by reflex stepping, hip-sway and paw position tests and placing reflexes were also slightly slow. Local limb reflexes appeared normal as did muscle tone. No paresis was observed. No abnormalities could be detected on examination of the eyes, the animal was able to see, had normal photomotor reflexes and menace responses and no retinal changes were observed.

The dog's condition showed gradual deterioration and eventually euthanasia was performed at seventeen weeks of age, that is ten weeks after the apparent onset of the condition.

Case 2: This male pup was presented for examination at nine weeks of age. Since the owners had obtained the pup three weeks previously it had shown variable ataxia. The animal had been generally dull and tired very easily, although still willing to play. It had proved difficult to train, especially toilet training, and was reported to be bumping into objects. Other signs included wide circling and occasional falling to the right, knuckling of the forelimbs and compulsive forward movements.

On clinical assessment, proprioceptive functions were slow although local reflexes and muscle tone were present and normal. The animal was ataxic but no hypermetria was observed. Vision seemed to be impaired to a degree although sight was present and photomotor reflexes were normal. Menace response was present.

The animal deteriorated rapidly and was finally destroyed after showing a reaction to the contrast material used in the

ventriculogram.

Case 3: This male pup was much older than the others being presented at 10 months of age. Clinical signs had been manifest for the previous three months, the dog showing progressive ataxia and bouts of hysterical behaviour which were described as 'fits' but were certainly not generalised convulsions. The animal was also reported to walk into objects although apparently able to see.

On examination there was obvious ataxia of the hind-limbs and a short-stepping forelimb gait. The hind-limbs also appeared to be paretic with a noticeable sagging of the stifle and hock as weight was borne by the limb. There was also a tendency for the animal to sway more to the right than the left. A marked tremor of the head and neck was present which became pronounced on feeding. Slowed proprioceptive responses with delayed replacement and reaction to paw position, reflex stepping tests and a pronounced uncorrected sway in the hip-sway test were detected in the hind-limbs. The forelimbs were affected to a much lesser degree. Local reflexes were all intact and muscle tone appeared to be normal. Although vision was present and menace response and photomotor reflex responses apparently normal, the animal seemed unable to follow a moving object. No bumping into objects was observed despite attempting to induce this by placing obstacles in the animal's path. There was an intermittent and variable nystagmus to the right.

Euthanasia was carried out 10 days after admission at the owners request.

Case 4: This young female pup was initially presented at 12 weeks of age because of vomiting. It had also been unsteady on its feet from four weeks of age. The pup, the smallest of nine, had not received any colostrum. In addition to the vomiting and ataxia it also had a chronic pneumonia with a persistent nasal discharge. No further neurological defects were noticed on initial examination. It was re-examined two weeks later. At this time the owners reported that the pup was bumping into objects, seemed unwilling to step over objects and had an unsteady gait. The pup had also been observed standing with either a forelimb or a hind-limb raised whilst eating.

There was a marked ataxia of the fore and hind-limbs and also slight hypermetria. Responses to proprioceptive tests were all reduced but local reflexes and muscle tone appeared normal. There was no obvious weakness. Vision seemed abnormal in the same manner as described in the previous cases although the dog was able to see and menace and photomotor responses were normal. All the behavioural abnormalities reported by the owners were observed during hospital stay. The pup had great difficulty eating, pressing food into the base of the bowl and if the food was moved it had a problem relocating the bowl. It was also observed raising a leg on occasions when eating. This was not a constant feature at every meal nor was there any consistency in which leg was raised. The animal had great difficulty stepping over objects in its path or descending from its kennel, repeatedly hesitating before emerging.

Euthanasia was carried out at 16 weeks of age due to the failure of the pup to improve though no marked deterioration was observed.

Case 5: This was an 18 week old female pup which had been showing gait abnormalities from six weeks of age. The owners reported that the pup had a pronounced sway whilst walking and tended to fall over to either side. There also seemed to be exaggerated limb movement. Although some initial deterioration had been observed the condition now appeared to be static.

The animal showed marked ataxia of fore and hind-limbs falling to either left or right. There was also pronounced hypermetria most obvious in the forelegs but also present in the hind-limbs. Proprioceptive reflexes were reduced in the forelegs but apparently normal in the hind-limbs and local limb reflexes and muscle tone were normal. There was again a doubt whether vision was normal. Palpebral and photomotor reflexes were normal although menace response was slow, but this is not an uncommon finding in young pups. Euthanasia was performed at 23 weeks of age.

Case 6: This pup, an 11 week old female, was presented with a visual problem. The animal had been bumping into objects and having difficulty finding objects or food placed at some distance although it seemed that a degree of vision was present. There was also some weakness of the hind-limbs which, according to the owner, had shown improvement over the previous week.

The most obvious clinical feature was a slight degree of hypermetria. Placing reflexes were very slow in the hind-limbs but the other proprioceptive tests, paw position, hip-sway and reflex stepping gave normal responses. The dog's body twisted to the right when the animal was held up and on occasions the animal

would exhibit a crab-like gait walking towards the right. When objects were placed in its path the animal would walk into them and experienced difficulty finding objects placed well within its visual field. Ocular reflexes were normal and there were no signs of cranial nerve dysfunction. Menace response was present. This pup was destroyed at 12 weeks of age.

The clinical signs of all 6 cases are summarised in Tables 8 and 9. Each pup was reported to have a visual problem which was recognised by the pups frequently bumping into objects. Several (cases, 1, 4 and 6) were reported and observed to have difficulty finding objects. The nature of the defect was more complex than simple visual loss as all the pups appeared able to see objects and no abnormalities were detected on examination of the eyes except slowed menace responses. The defect was most obvious if the animals attempted to follow a moving object visually, which most were unable to do. Several also exhibited a very characteristic hesitancy in stepping over objects or descending a low step.

All of the animals showed ataxia which was variable from day to day. This was most obvious in the hind-limbs although the pectoral limbs were obviously involved in several pups. Hypermetria was demonstrated by four of the six pups and was most pronounced in the hind-limbs. Most of the pups also showed an obvious proprioceptive deficit.

Five of the six pups showed signs at an early age, before 9 weeks. The exception was case 3 which was reported to have initially demonstrated signs at 6 to 7 months of age.

Another characteristic feature was the manifestation of bizarre clinical signs by many of the pups as described in the

individual case reports. The condition was insidiously progressive in each case and all the animals were euthanased.

(b) ANCILLARY INVESTIGATIONS

Haematology and Biochemistry

Results of the haematological and blood biochemical investigations are shown in Table 10. No haematological abnormalities were found. Biochemical values were, in general, also normal. All of the pups sampled showed high alkaline phosphatase levels, a finding which is normal for the young growing pup. Three of the four cases (1, 3, 4) also showed high values for blood glucose determination and two (cases 1 and 3) showed elevated blood calcium levels. Case 4 had a persistently basal blood magnesium. The result of a bromosulphthalein dye test, performed twice in case 2, showed normal liver clearance. Cerebrospinal fluid analysis in 3 cases showed no abnormalities. A urine sample from case 2 was normal apart from a slight proteinuria.

Radiography

No abnormalities were detected in plain film radiography. Positive contrast radiography was utilised in cases 2 and 4. In case 4 a barium swallow revealed no evidence of megaoesophagus and oesophageal motility was normal as shown by flouroscopy. In case 2 ventriculography demonstrated enlarged lateral, third and fourth ventricles, indicative of hydrocephalus (Figs. 10, 11). The contrast passed freely through the third ventricle, the cerebral aqueduct and the fourth ventricle into the spinal subarachnoid space. This would suggest that there was no blockage

and that this was a communicating form of hydrocephalus.

Electro cardiography

No abnormalities were detected.

In conclusion the only significant abnormality detected by the ancillary tests was a communicating hydrocephalus.

(c) PATHOLOGICAL FINDINGS

Gross inspection of the brain indicated that the cerebrum and cerebellum were of normal size and appearance. There was no evidence of cerebellar atrophy nor alteration of the surface anatomy of either the cerebellum or cerebrum. On sectioning the brain a moderate to severe internal hydrocephalus was observed (Fig. 12) with dilatation of all the ventricles and the cerebral aqueduct. In the majority of brains a brownish discoloured area was visible in the region of the deep cerebellar nuclei in slices of formalin-fixed material.

Under low power microscopy the degree of hydrocephalus could readily be ascertained with dilatation of the lateral ventricles (Figs. 13, 15) and third ventricle (Fig. 13) when compared to the control (Fig. 14). Dilatation of the cerebral aqueduct and fourth ventricle could also be seen (Figs. 13, 15, 17). Bilaterally symmetrical areas with a spongy, vacuolated appearance were observed in the region of the deep cerebellar nuclei (Fig. 16) and in a well demarcated zone at the lateral aspect of the base of the inferior colliculi (Fig. 17).

The vacuoles in these areas were non-staining (Fig. 18, 19) and were accompanied by a reactive gliosis with an increase in both microglia and astrocytes. The neurones observed in the

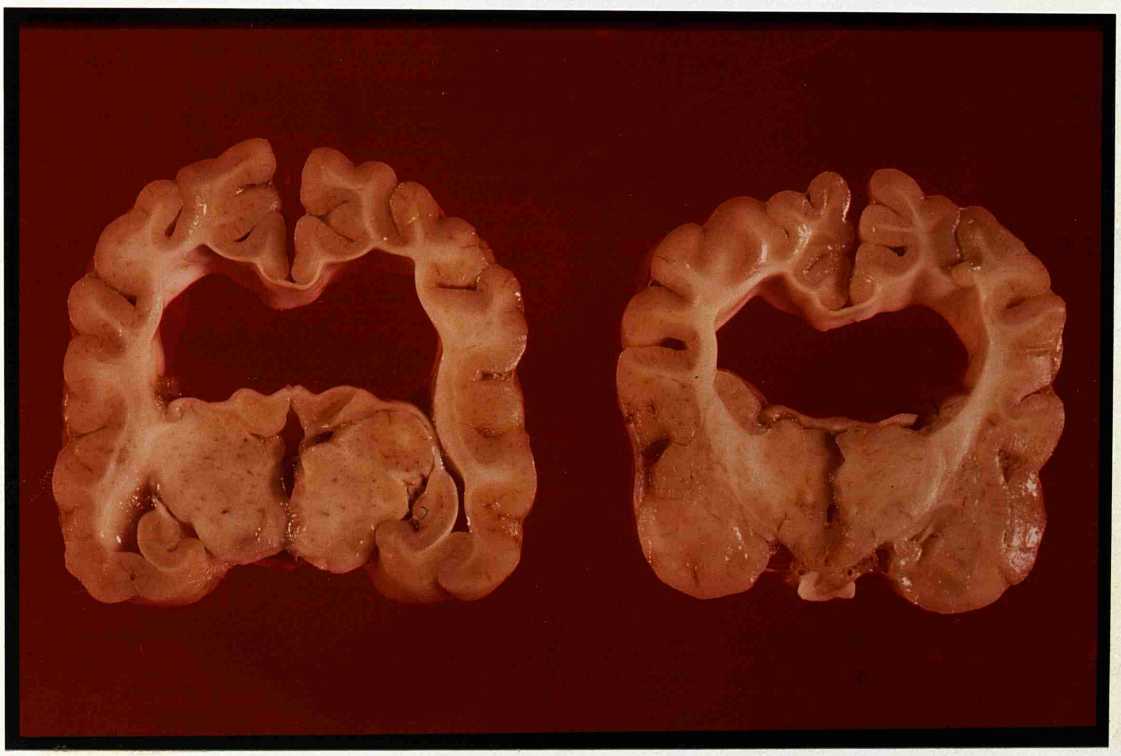


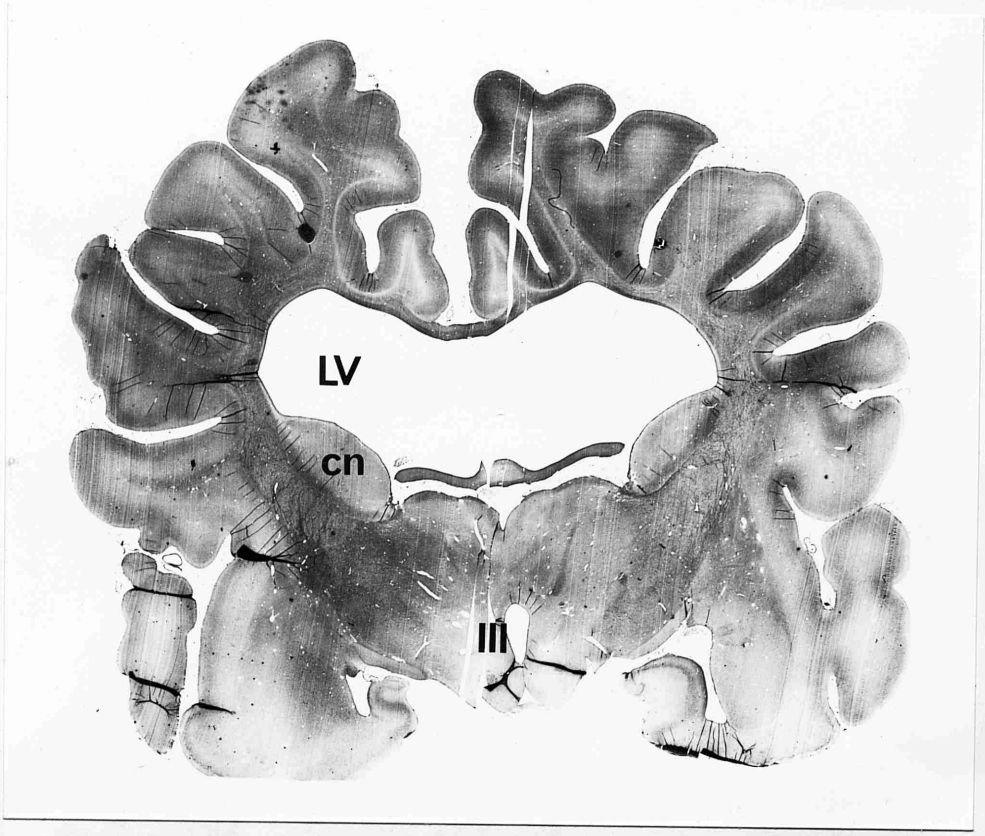
FIG. 12 THE BRAIN OF AN AFFECTED PUP HAS BEEN SECTIONED TO SHOW THE DEGREE OF HYDROCEPHALUS PRESENT.

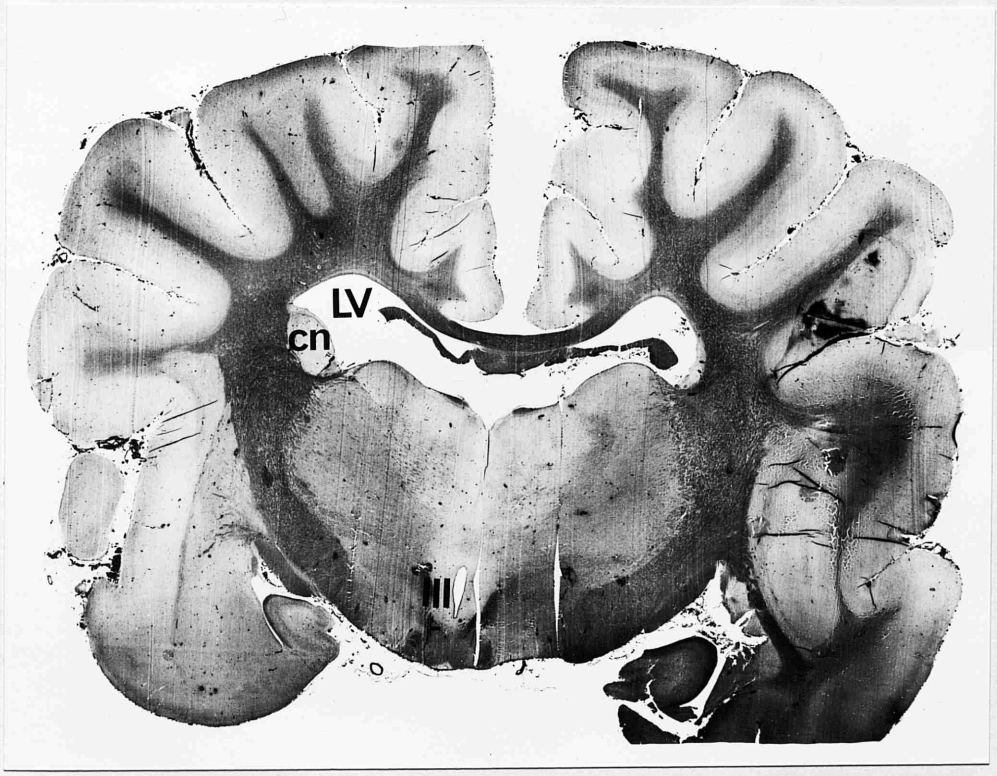
FIG. 13 LOW POWER SECTION OF THE BRAIN AT THE LEVEL OF THE CAUDATE NUCLEUS (C.N.) SHOWING DISTENSION OF BOTH THE LATERAL VENTRICLES (L.V.) AND THE THIRD VENTRICLE (III).

H AND E X 10

FIG. 14 LOW POWER SECTION OF THE BRAIN OF AN AGE-MATCHED CONTROL AT THE SAME LEVEL AS FIG 11 SHOWING NORMAL APPEARANCE OF THE STRUCTURES.
L.V.: LATERAL VENTRICLES; III: THIRD VENTRICLE;
C.N.: CAUDATE NUCLEUS

H AND E X 10


LV
cn
III


LV
cn
III

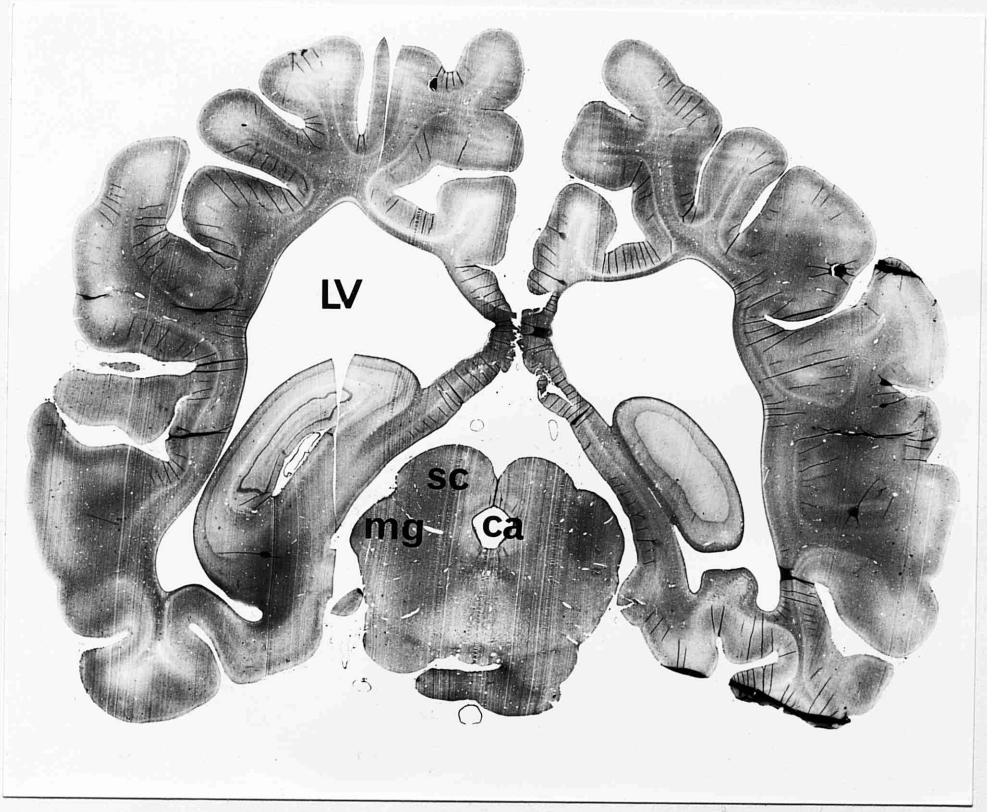


FIG. 15 LOW POWER SECTION AT THE LEVEL OF THE SUPERIOR COLLICULUS (S.C.). GROSS DISTENSION OF THE LATERAL VENTRICLES (L.V.) AND CEREBRAL AQUEDUCT (C.A.) CAN BE SEEN.
M.G: MEDIAL GENICULATE.

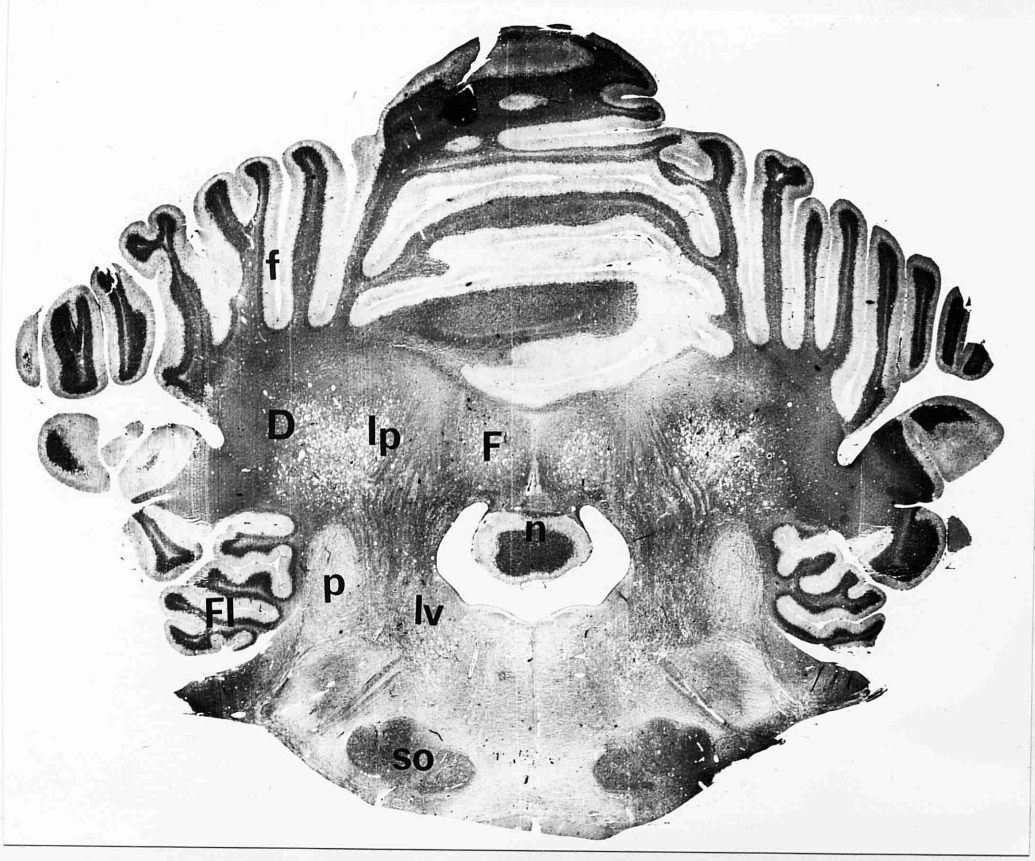
H AND E X 10

FIG. 16 LOW POWER SECTION AT LEVEL OF CEREBELLUM. NORMAL CEREBELLAR CORTICAL ORGANISATION CAN BE OBSERVED IN THE FOLIA (F). AREAS OF VACUOLATION ARE OBVIOUS IN THE DEEP CEREBELLAR NUCLEI (F, IP, D).
F: FASTIGIAL NUCLEI; IP,D MERGED INTERPOSITUS AND DENTATE NUCLEI;
S.O. SUPERIOR OLIVE; P: INFERIOR CEREBELLAR PEDUNCLE:
L.V: LATERAL VESTIBULAR NUCLEUS; FI: FLOCCULUS;
N: NODULUS.

H AND E X 10

FIG. 17 SECTION THROUGH MID-BRAIN AT THE LEVEL OF THE INFERIOR COLLICULUS. DISTINCT FOCUS OF VACUOLATION (ARROWS) CAN BE SEEN CONFINED TO A LOCALISED AREA IN THE LATERAL ASPECT OF THE INFERIOR COLLICULUS (IC). THE LESIONS ARE BILATERALLY SYMMETRICAL.

H AND E X 25


f
D
lp
F
n
p
Fl
lv
so

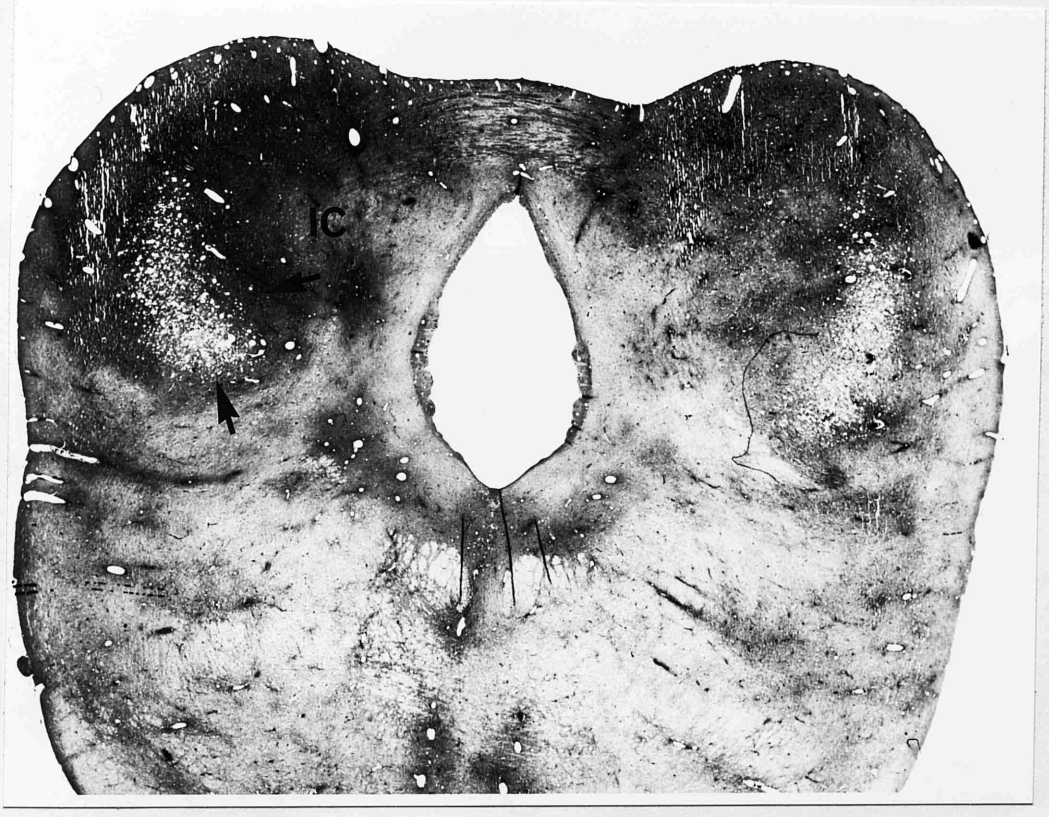
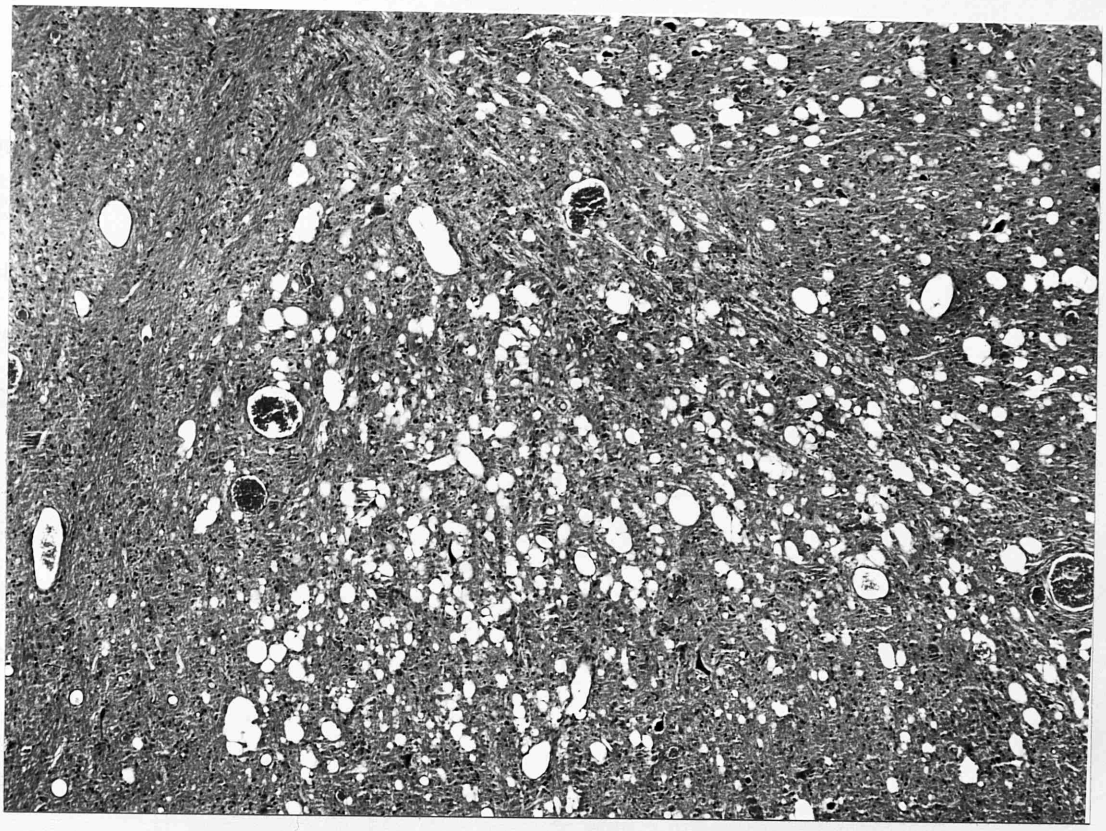

IC

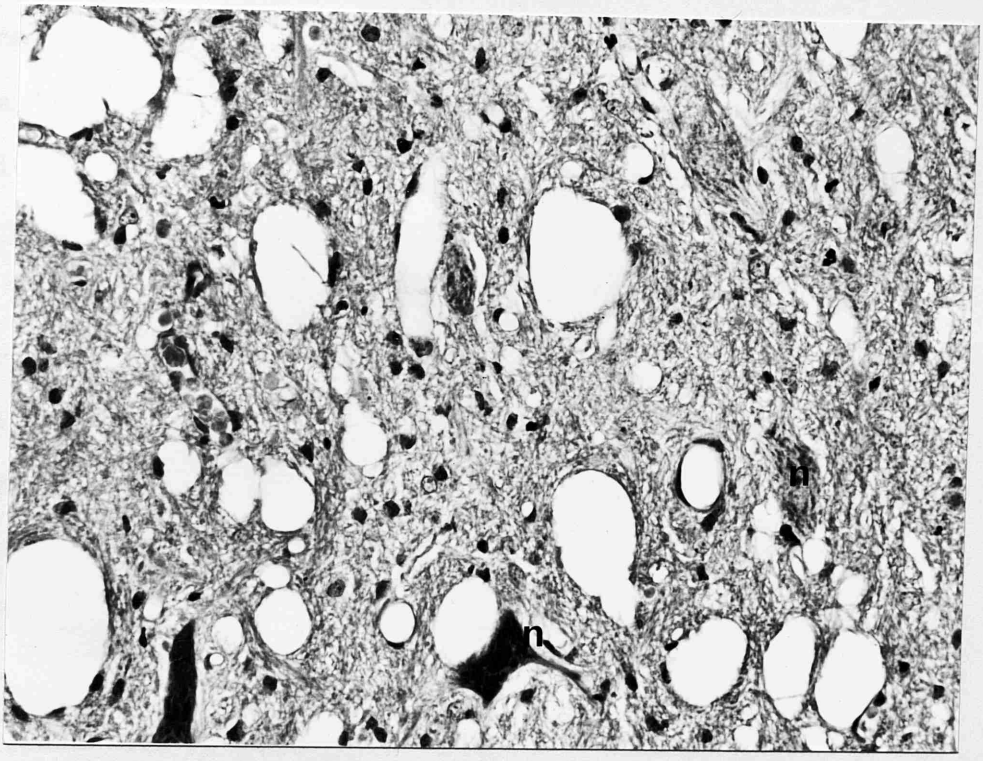
FIG. 18 FASTIGIAL NUCLEUS SHOWING WIDESPREAD VACUOLATION GIVING A 'LACE-LIKE' APPEARANCE.

H AND E X 100

FIG. 19 FASTIGIAL NUCLEUS. THIS SECTION SHOWS THE VACUOLES IN MUCH MORE DETAIL. SEVERAL NORMAL NEURONES (N) CAN BE SEEN ONE OF WHICH IS CLOSELY ASSOCIATED WITH A VACUOLE.

H AND E X 250





sections appeared normal although in close proximity to and sometimes distorted by the vacuoles (Fig. 19). There was also evidence of axonal degeneration and small numbers of axonal spheroids were noted in all of the deep cerebellar nuclei (Fastigial, Interpositus and Dentate) and in the inferior colliculus.

Similar changes were also noted in the region of the lateral vestibular nuclei. Here, however, the degree of vacuolation was minimal but there was a very marked glosis (Fig. 20) and many axonal spheroids were observed (Fig. 21). This last feature was apparent as small, densely-stained round structures on haematoxylin and eosin sections consisting of an intensely pink centre surrounded by a pale halo (Fig. 21). They were much more numerous in the lateral vestibular nuclei than in the deep cerebellar nuclei or the inferior colliculus. Axonal spheroids represent some form of axonal pathology (Lampert, 1967) and their increased density is a result of increased numbers of axoplasmic organelles. The neurones visible in the lateral vestibular nucleus appeared to be of normal appearance (Fig. 21) and there was no obvious neuronal loss.

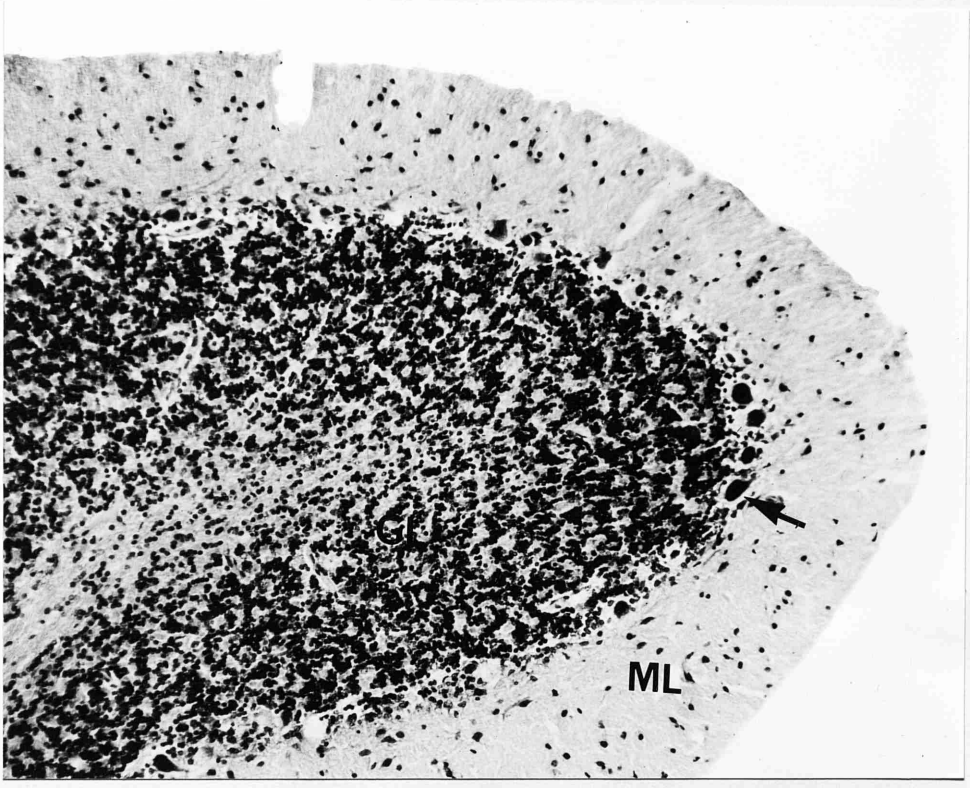
The remainder of the cerebellum was examined for changes. There was no obvious loss of either granule cells or Purkinje cells in the cerebellar cortex which appeared morphologically normal with a mature molecular layer and no evidence of the external germinal layer (Fig. 22). There was a normal complement of Purkinje cells (Fig. 23) and the perikarya of individual neurones were unremarkable. A normal arrangement of basket cells was visualised on silver impregnated preparations (Fig. 24, 25) with their descending collateral axonal branches surrounding the

FIG. 22 THE CEREBELLAR CORTEX IS SHOWN WITH NORMAL CORTICAL ORGANISATION AND NO OBVIOUS CELL LOSS.
ML: MOLECULAR LAYER; G.L.: GRANULE CELL LAYER:
PURKINJE CELLS ARE ARROWED

H AND E X 100

FIG. 23 NO PURKINJE CELL LOSS WAS APPRECIATED IN ANY OF THE CASES. THIS SECTION SHOWS PURKINJE CELLS (ARROWED) OF NORMAL APPEARANCE AND COMPLEMENT IN THE CORTEX.

H AND E X 250


ML

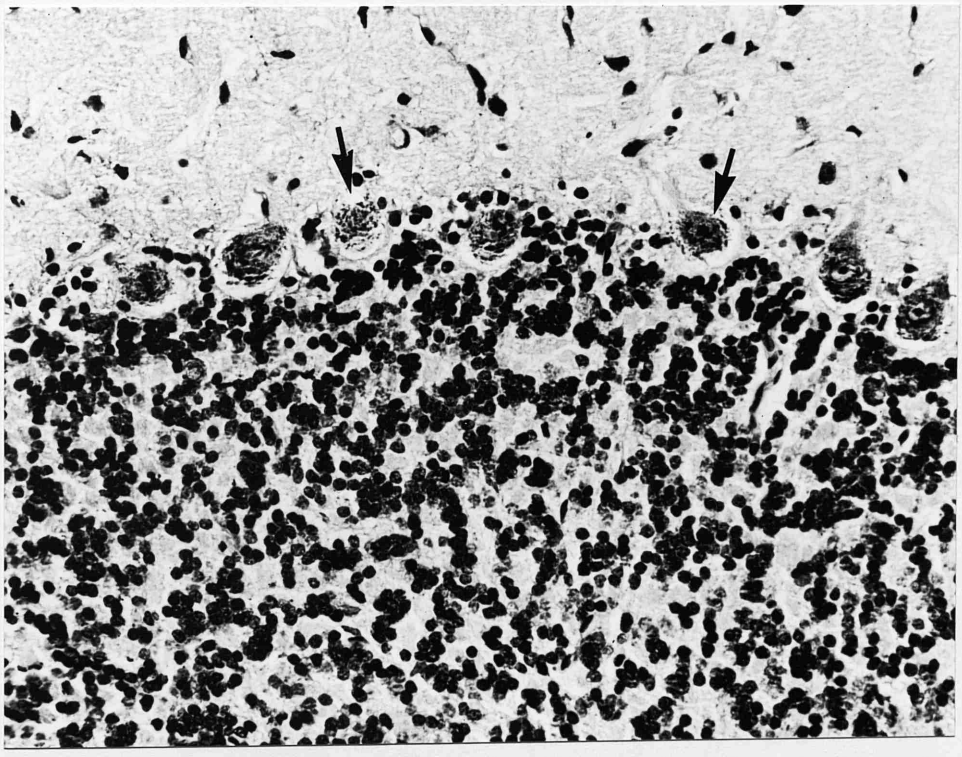
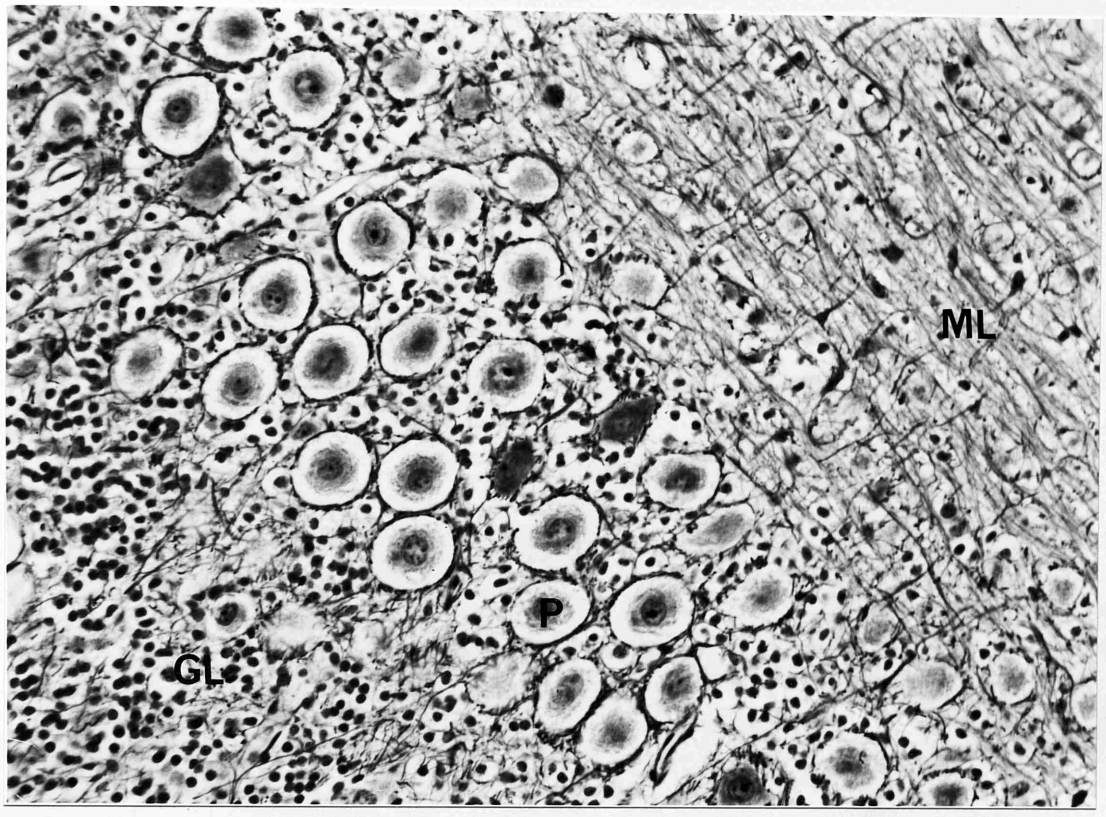


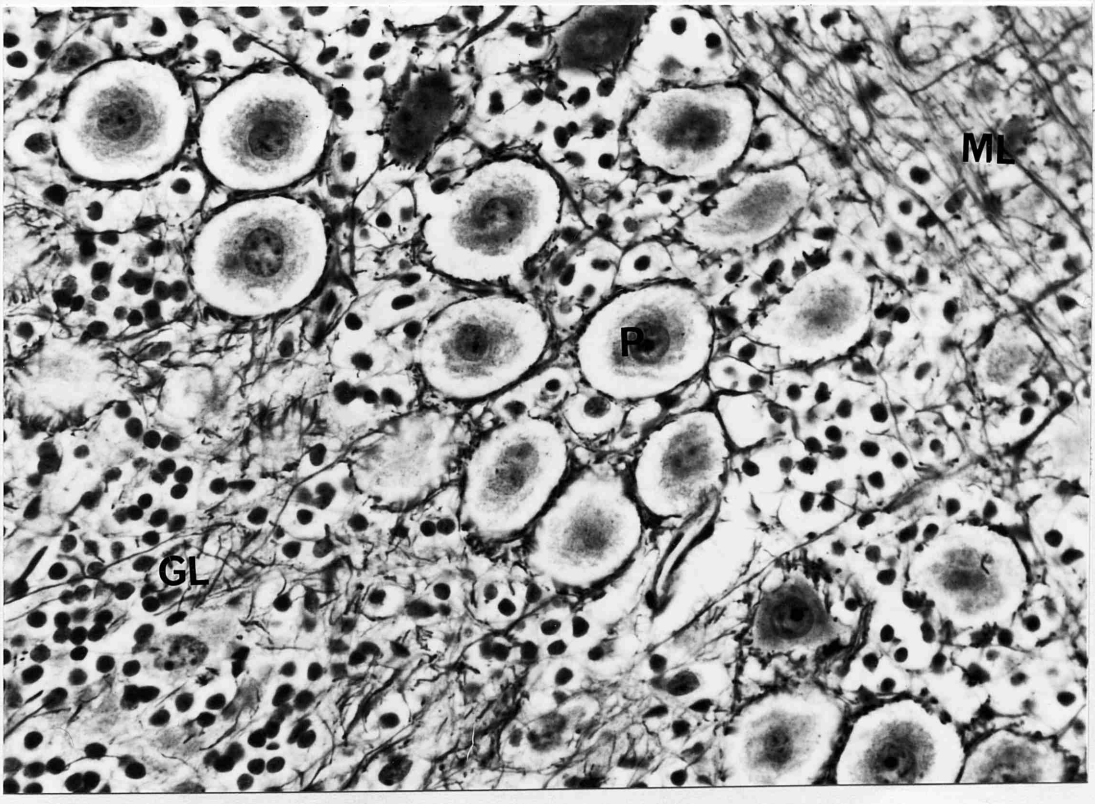
FIG. 24 CEREBELLAR CORTEX STAINED WITH HOLMES STAIN SHOWS THE NORMAL ARRANGEMENT OF BASKET CELL AXONS SURROUNDING THE PURKINJE CELLS. THE PROJECTIONS FROM THE GRANULE CELLS CAN BE DISTINGUISHED RUNNING IN THE MOLECULAR LAYER PARALLEL TO THE PURKINJE CELL LAYER. EACH OF THESE PARALLEL FIBRES CONTACTS MANY PURKINJE DENDRITES.
P: PURKINJE CELL, ML; MOLECULAR LAYER,
GL: GRANULE CELL LAYER

HOLMES X 250

FIG. 25 A HIGHER MAGNIFICATION OF FIG. 24 SHOWS PURKINJE CELLS SURROUNDED BY BASKET CELL FIBRES.
P: PURKINJE CELL, GL.: GRANULE CELL LAYER,
ML: MOLECULAR LAYER

HOLMES X 400


ML
P
GL


ML
P
GL

cell bodies of the Purkinje cells. On the same section the arrangement of parallel fibres projecting from the granule cells can be seen in the molecular cell layer (Fig. 24). Occasional axonal torpedoes observed in the granule cell layer (Fig. 26) were present in small numbers throughout the layer and in the cerebellar medulla. Axonal torpedoes which represent degenerating axons (Blumcke and others, 1966; Chou and Hartmann, 1965) were the only pathological change present in the cerebellar cortex and medulla.

Toluidine Blue stained sections showed that the large vacuoles were partially or completely surrounded by myelin (Fig. 27, 28). Often the outer and inner lamellae had separated to the vacuole leaving the inner lamellae surrounding the axon. In many areas multiple vacuoles separated by small myelin strands were seen surrounding a single axon (Fig. 28). Most of the axons associated with the vacuolated myelin were in stages of degeneration (Fig. 27, 28) although some normal myelinated axons were observed in the sections. In other areas, swollen axons with densely staining axoplasm were associated with attenuated myelin sheaths and represented the spheroids seen in the paraffin sections. Microglial cells and hypertrophic astrocytes (Fig. 27) were present in increased numbers and in close proximity to the vacuoles.

These findings were re-inforced by the results of electron microscopy studies on affected tissue by Dr. I.R. Griffiths. The vacuoles were identified between the myelin lamellae pushing these apart (Fig. 29, 30) and contained varying amounts of myelin debris or in some cases surrounded a degenerating axon (Fig. 29). Many normal axons were present and axons associated with vacuoles were

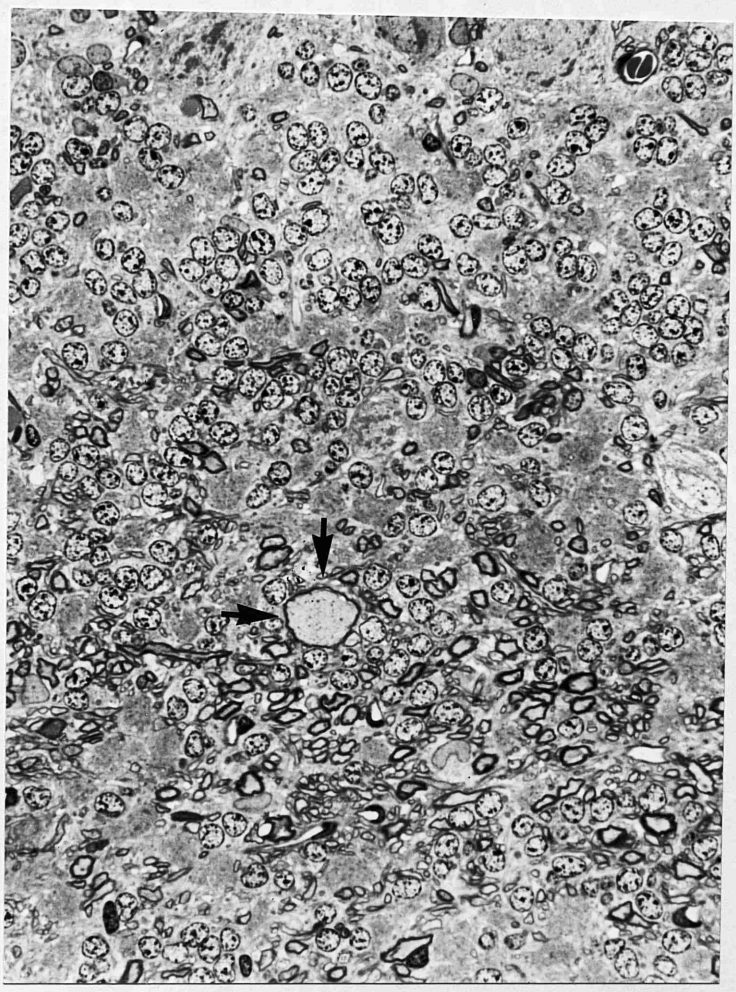


FIG. 26 CEREBELLAR CORTEX; OCCASIONAL DISTENDED AXONS (ARROWED) WERE OBSERVED IN THE GRANULE CELL LAYER. THE AXON IS ENLARGED, INCREASED IN DENSITY AND SURROUNDED BY ATTENUATED MYELIN. MANY OTHER NORMAL AXONS CAN BE SEEN.

TOLUIDINE BLUE X 400

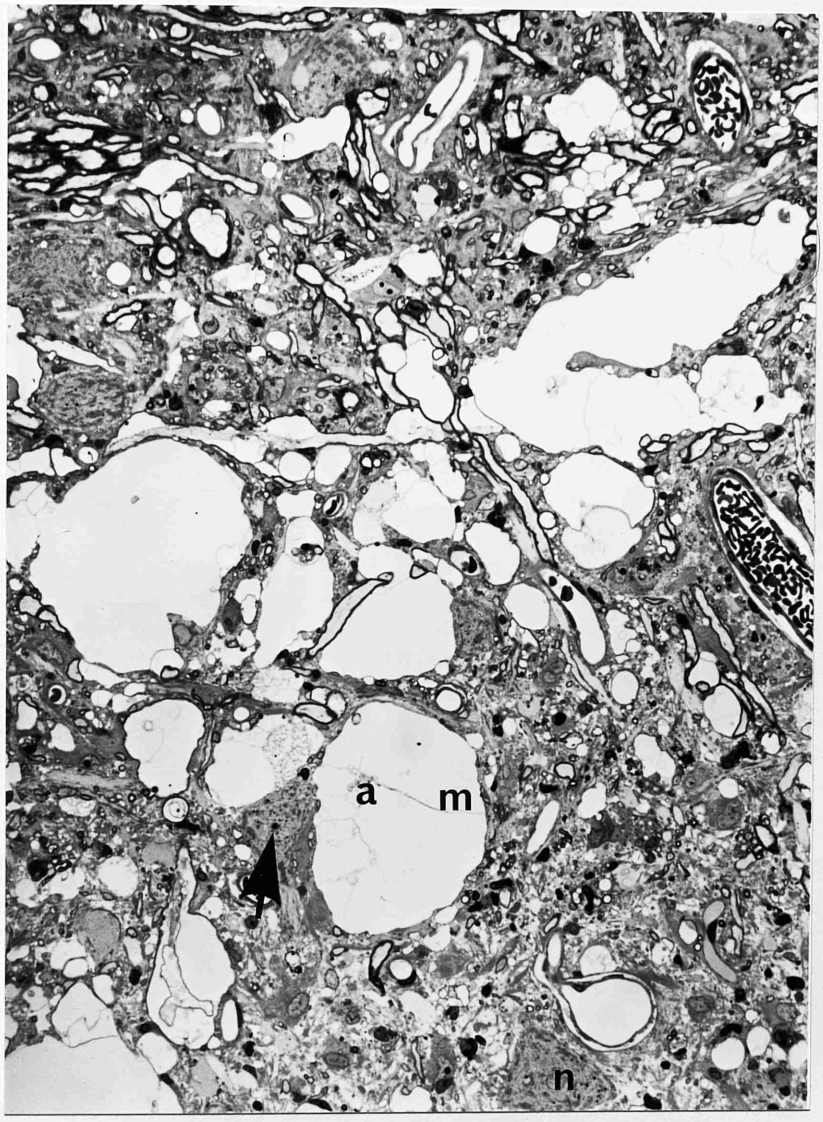


FIG. 27 SECTION OF THE FASTIGIAL NUCLEUS SHOWING MULTIPLE VACUOLES SEVERAL OF WHICH ARE SURROUNDED BY ATTENUATED MYELIN. AXONS (A) AND STRANDS OF MYELIN (M) CAN BE SEEN WITHIN SOME VACUOLES. HYPERTROPHIC ASTROCYTES ARE ALSO PRESENT (ARROWED) IN ADDITION TO NORMAL NEURONES (N).

TOLUIDINE BLUE X 250

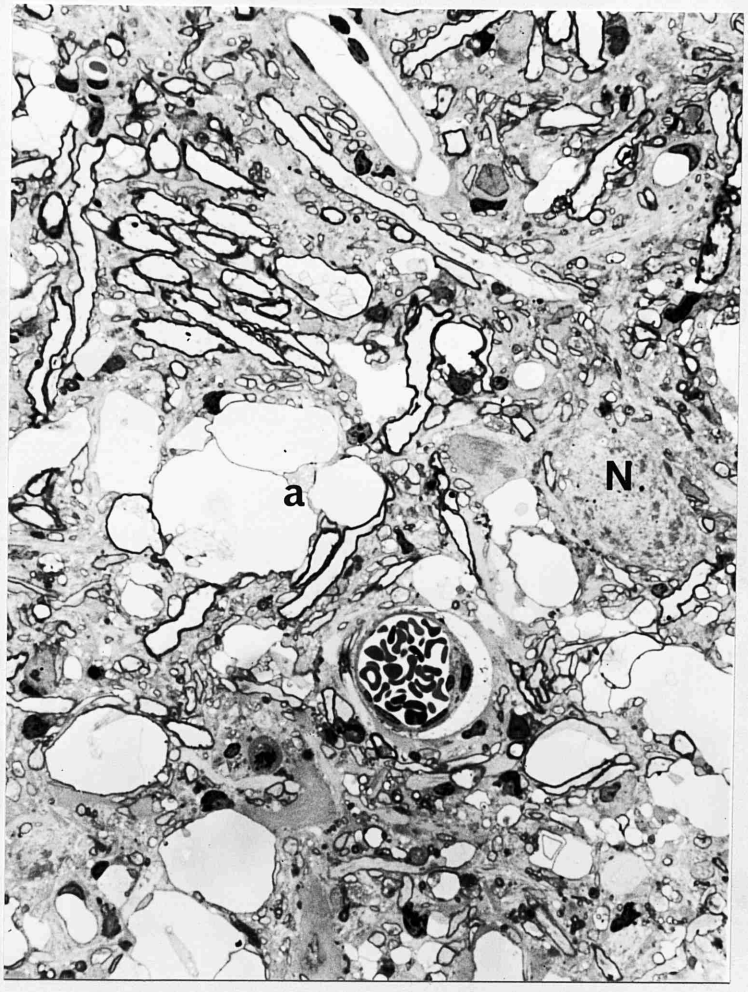


FIG. 28 FASTIGIAL NUCLEUS. THE LARGE VACUOLE IN THE CENTRE OF THE SECTION CONTAINS AN AXONAL REMNANT (A) AND MYELIN STRANDS. NORMAL NEURONES (N) ARE FOUND IN CLOSE PROXIMITY TO THE DEGENERATING AXONS AND MYELIN.

TOLUIDINE BLUE X 250

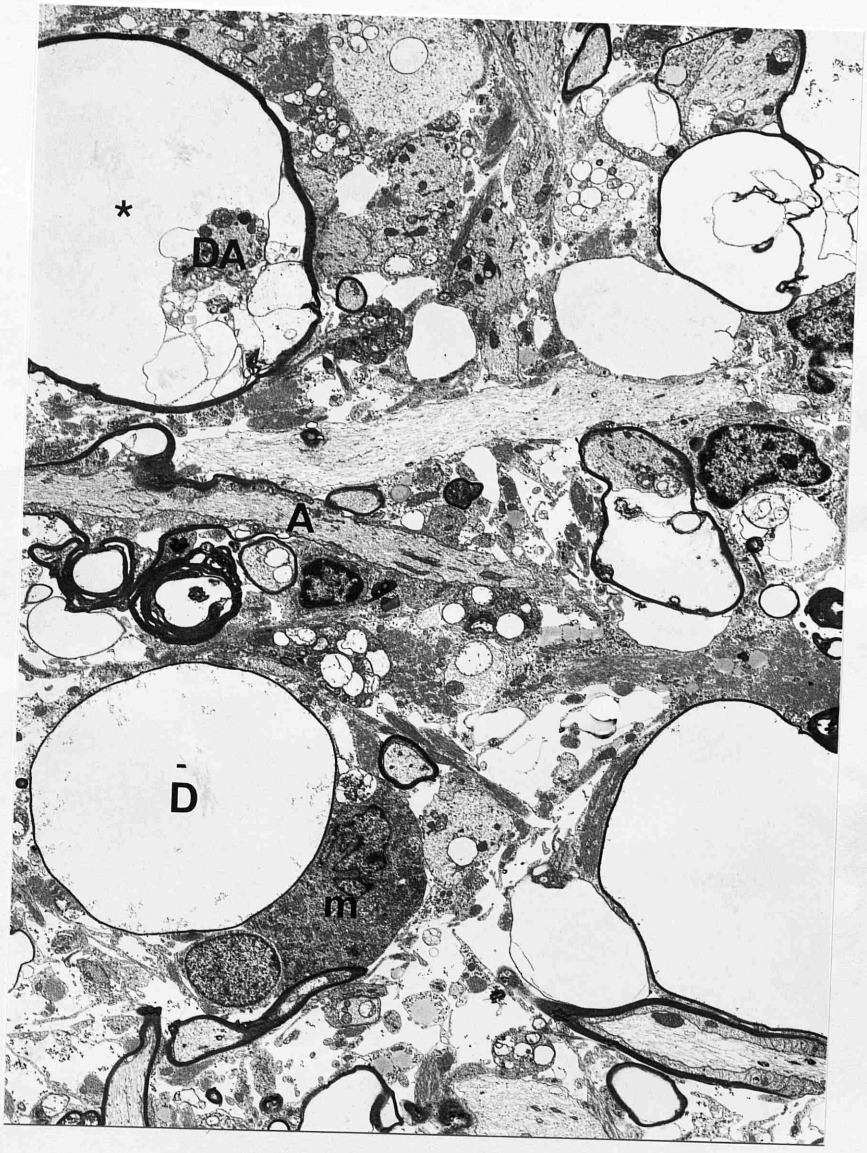


FIG. 29 FASTIGIAL NUCLEUS: MANY CLEAR VACUOLES CAN BE SEEN IN THIS SECTION ONE OF WHICH (ASTERIX) CONTAINS THE REMNANTS OF A DEGENERATING AXON (D.A.) MYELIN STRANDS CAN BE SEEN WITHIN THE VACUOLE SURROUNDING THE AXON. ANOTHER AXON (A) TRAVERSES THE SECTION. OTHER VACUOLES CONTAIN MYELIN DEBRIS (D) AND A MICROGLIAL CELL (M) IS SEEN IN CLOSE PROXIMITY TO A VACUOLE.

X3000

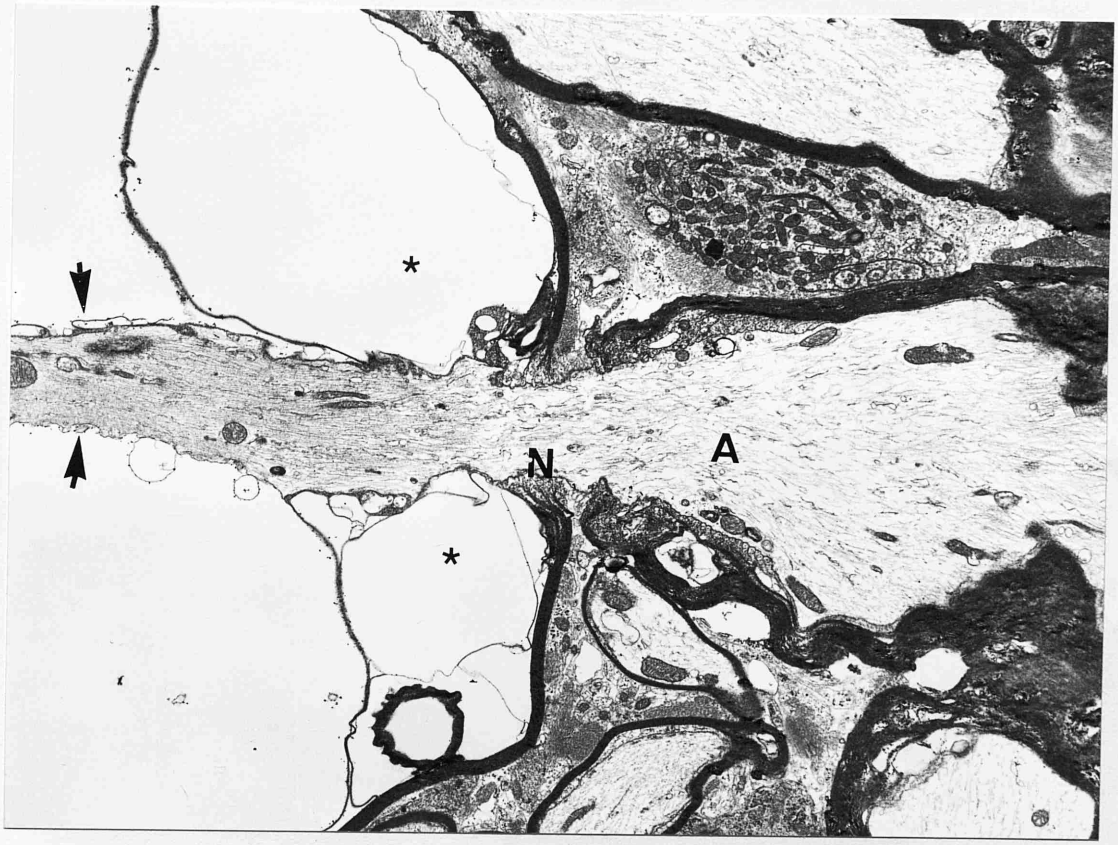


FIG. 30 FASTIGIAL NUCLEUS: THE AXON (A) TRAVERSING THE SECTION APPEARS NORMAL ON THE RIGHT HAND SIDE OF THE PLATE WITH ITS ASSOCIATED MYELIN SHEATH. THE SHEATH IS VACUOLATED TO THE LEFT OF THE NODE (N). THE VACUOLES (ASTERIX) ARE SEPARATED BY STRANDS OF MYELIN AND A THIN MYELIN SHEATH (ARROW) STILL INVESTS THE AXON.

X 6000

surrounded by the inner myelin laminae (Fig. 30). Microglia were again evident and closely associated with the vacuoles. Axons revealed various degrees of degeneration. The darkly staining axons contained accumulations of organelles (Fig. 31) accounting for the staining characteristics. Mitochondria were increased in number and many bizarre forms were present (Fig. 32). Autophagic vacuoles present contained accumulations of breakdown products.

All of the pathological changes were confined to the areas detailed. No other changes were detected in either the central or peripheral nervous systems other than the degree of hydrocephalus.

(d) GENETIC STUDIES

Information about the pedigrees of each of the 6 confirmed clinical cases was requested and obtained from the owners. Since 3 of the pups came from the same litter this gave information from only 4 matings. Further information was obtained by examination of the entire breeding records of a small breeder covering the period when the problem had been noticed. This information included 17 separate matings (involving 5 sires and 9 bitches) with the production of 104 individual pups, 49 dogs and 55 bitches (Table 12). Further pedigree information from these sires and bitches was also supplied. This breeder had been responsible for bringing 2 of the affected pups to the hospital, case 2 and case no. 3 and also gave details of a further 11 pups which she identified as having the same clinical signs as the affected animals and had been euthanased as pups. These details were combined with the information obtained earlier and a

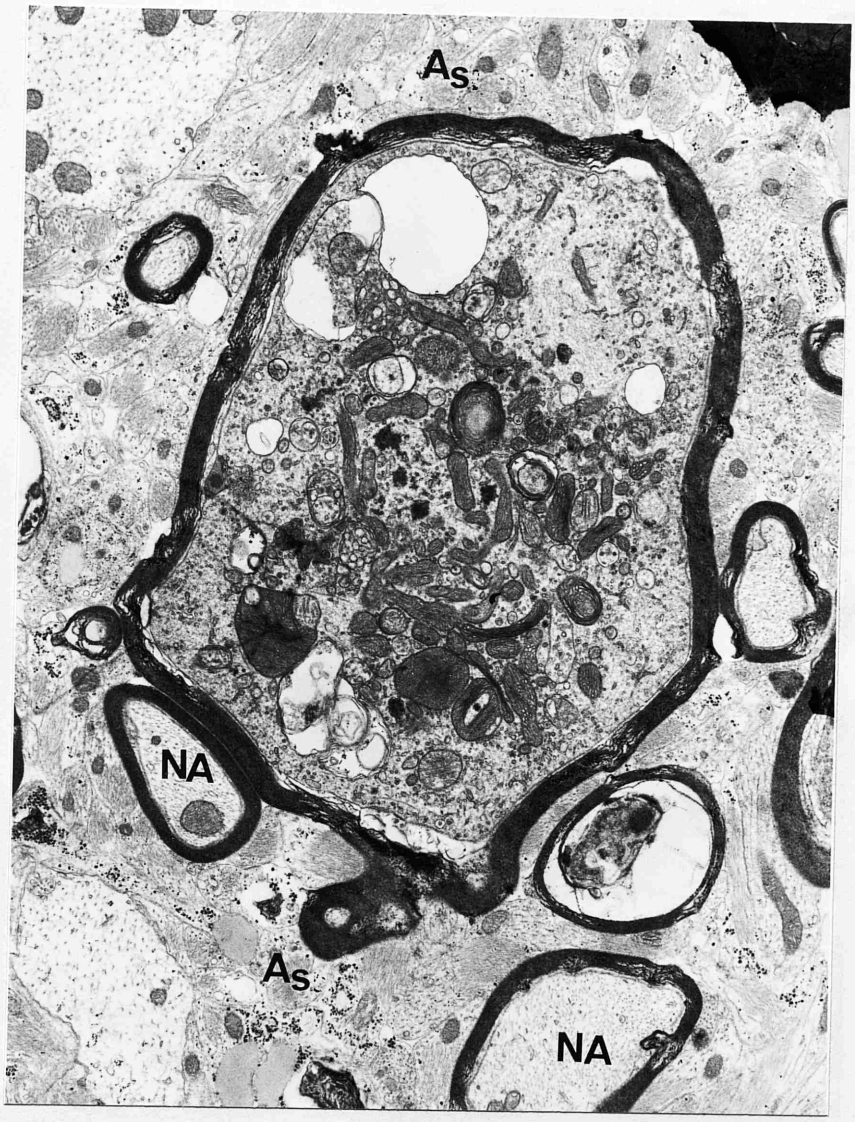


FIG 31 AN AXON IN THE REGION OF THE INFERIOR COLLICULUS IS SHOWN. AN ACCUMULATION OF ORGANELLES CAN BE SEEN WITHIN THE AXOPLASM AND MANY ASTROCYTIC PROCESSES CAN BE DETECTED (A_s) SURROUNDING THE AXON. NORMAL AXONS CAN ALSO BE SEEN (N.A.)

X 11,000

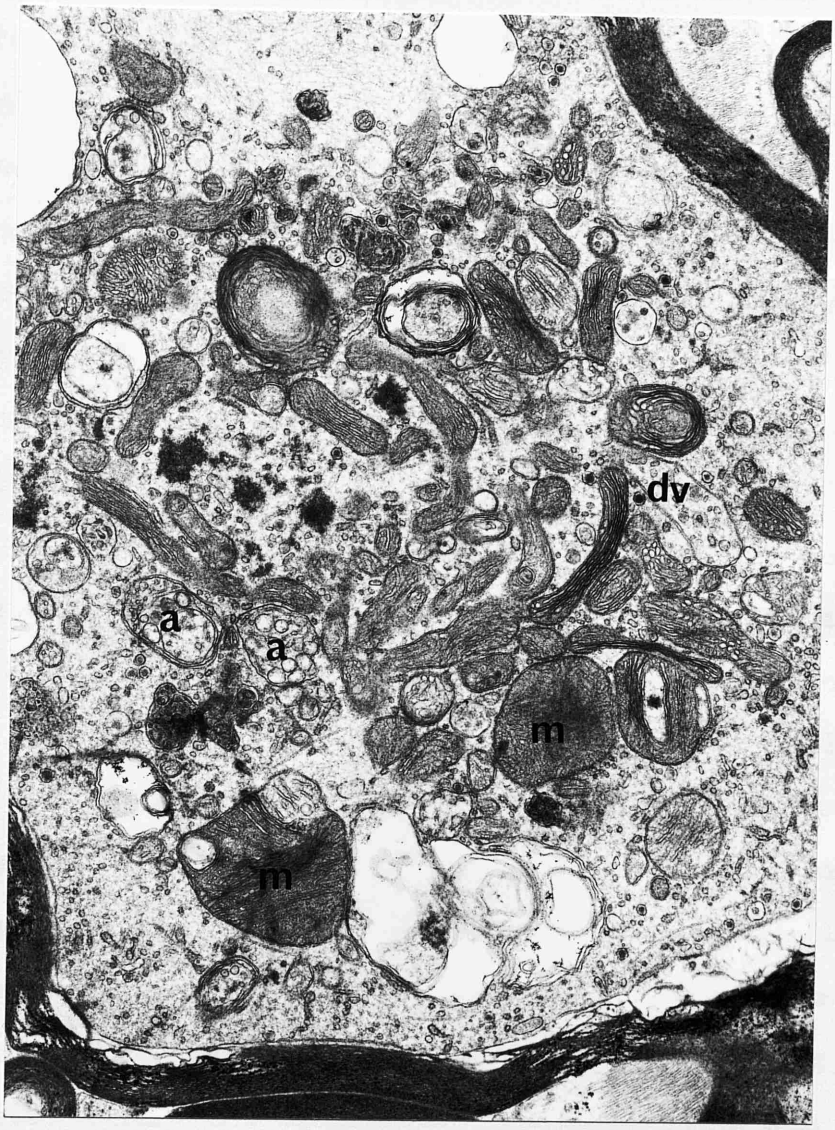


FIG 32 THIS SHOWS THE SAME AXON AS DEPICTED IN FIG 31. HIGHER MAGNIFICATION ALLOWS A MORE DETAILED OBSERVATION OF THE ORGANELLES BIZARRE MITOCHONDRIA (M), AUTOPHAGIC VESICLES (A) AND MANY EMPTY VESICLES, SOME DENSE CORED (DV) CAN BE SEEN. THERE ARE ALSO MANY MICROTUBULES PRESENT.

X 18,000

geneological table was constructed (Fig. 33) which included all of the affected animals both confirmed and suspected cases.

From the supplied data it was noted that certain males and females featured prominently in the pedigrees. Further investigation of the pedigrees of the confirmed cases showed a very obvious close relationship between these (Fig. 34 and 35) cases 1, 2, 3 & 4 having the same sire I and cases 5 and 6 being products of separate litters from a repeated mating (A-B). Furthermore the dog I and dam K were closely related to A & B (Figs. 36 and 37). These facts suported the hypothesis of the condition being hereditary. From the supplied information 87 normal and 17 abnormal pups were produced and the parents of all litters were normal. These findings eliminate both a sex-linked and a dominant mode of inheritance. The most likely mode of inheritance is a single autosomal recessive gene as both parents in every case were normal. It is possible to trace the pedigrees of both the confirmed cases (Fig. 38) and both confirmed and suspected cases (Fig. 39) to the same source dog (S). Calculation of the segregation ratio of abnormals to normals gives a ratio of 17:87 which is significantly different from the 1:3 ratio expected with an autosomal recessive inheritance ($x^2 = 4.13$, $P < 0.05$). However close scrutiny of the pedigrees reveal two annomalies which may be biasing the results. Firstly, Dog D (Fig. 33) produced 3 litters (4, 10 and 13) out of 2 bitches (N and K) both of which had produced abnormal pups by other sires (litters 5, 11 and 13) and therefore, following the hypothesis, were presumed to be carriers. Twenty four pups were produced in litters 4, 10 and 13 but not a single pup was found to be abnormal. This information would indicate that dog D is not a

Fig 33

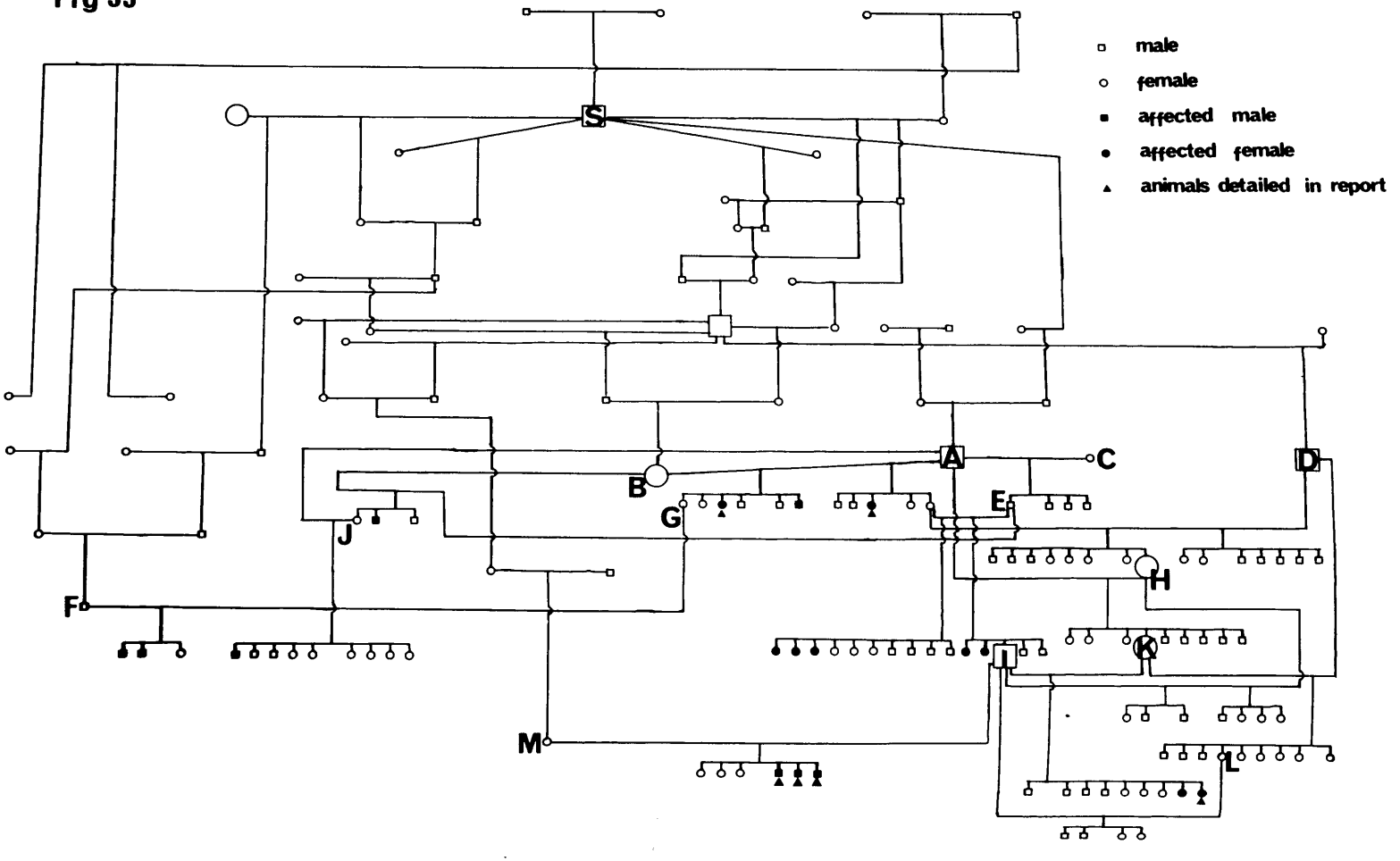

male
female
affected male
affected female
animals detailed in report
S
A
B
C
D
E
F
G
H
I
J
K
L
M

Fig 34

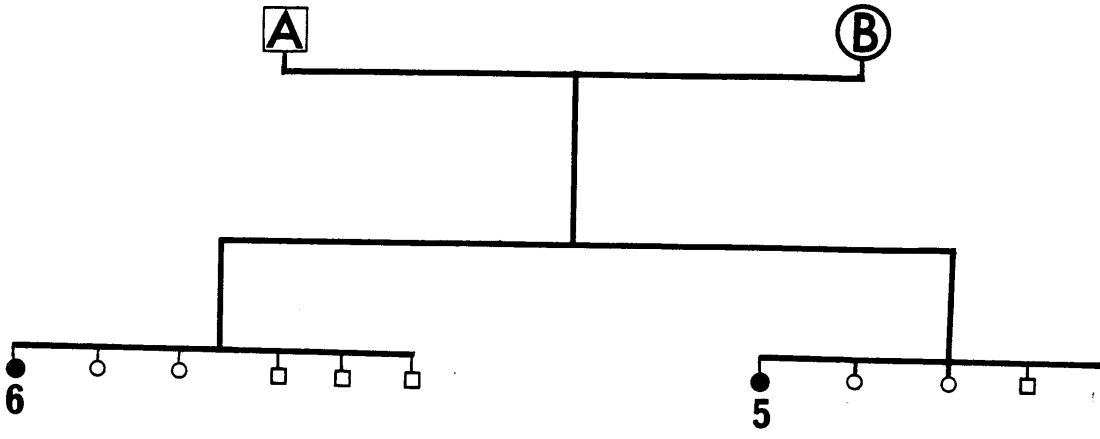


FIG. 34 RELATIONSHIP BETWEEN AFFECTED PUPS. 5 AND 6 HAVE COMMON PARENTS ALTHOUGH THEY ARISE IN DIFFERENT LITTERS.

Fig 35

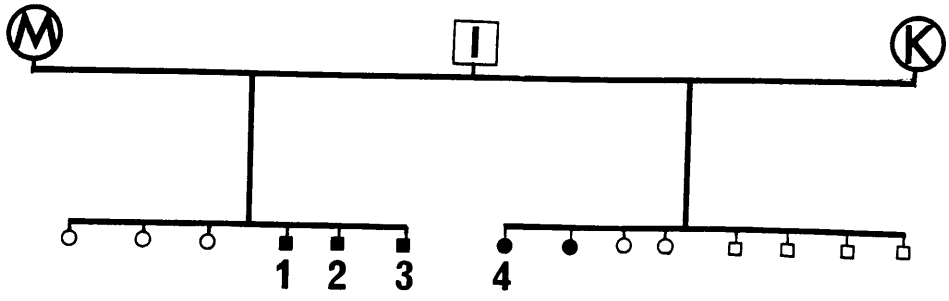


FIG. 35 RELATIONSHIP BETWEEN AFFECTED PUPS. CASES 1, 2, 3 AND 4 HAVE A COMMON SIRE (I).

Fig 36

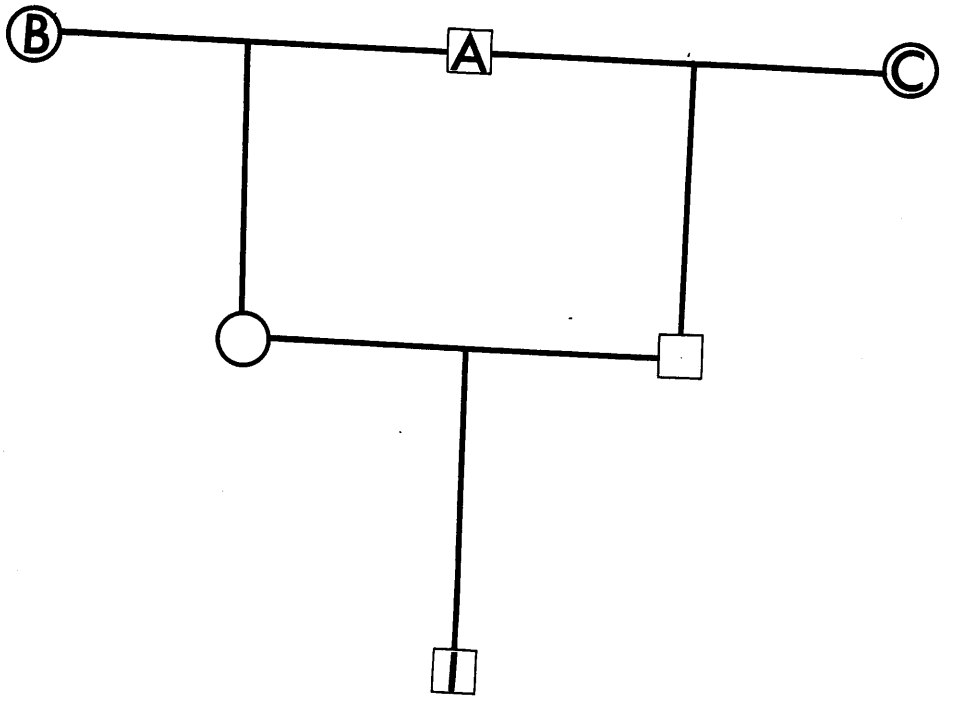


FIG. 36 SHOWING THE RELATIONSHIP BETWEEN I AND A AND B. IT CAN BE SEEN THAT DOG I IS VERY CLOSELY RELATED TO A AND B, A IS THE SIRE OF BOTH I's PARENTS.

Fig 37

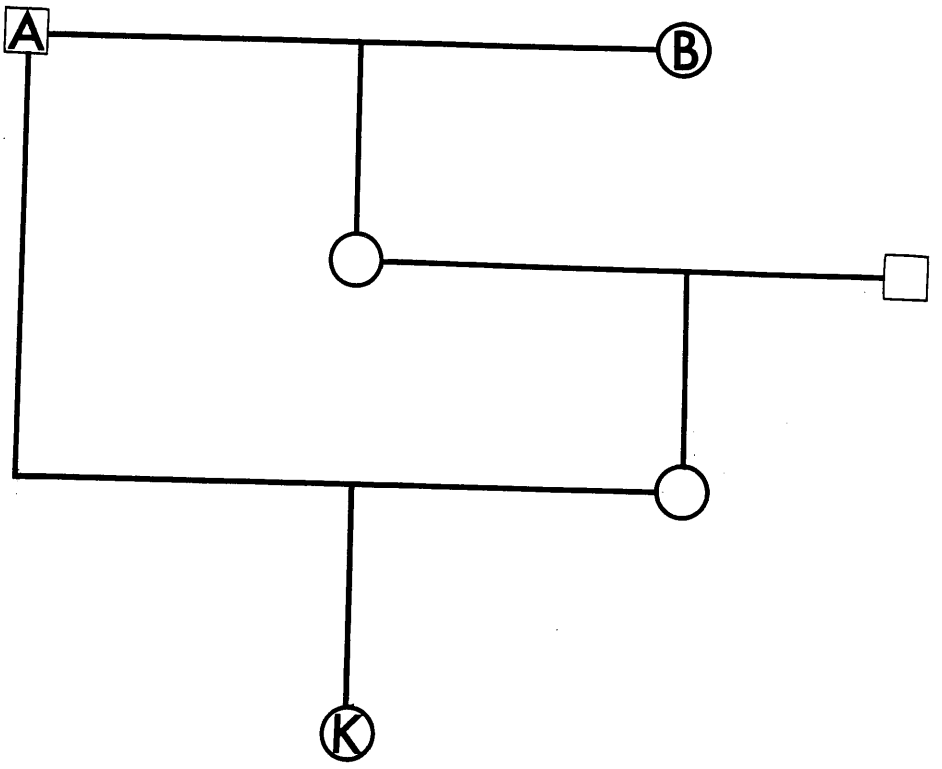
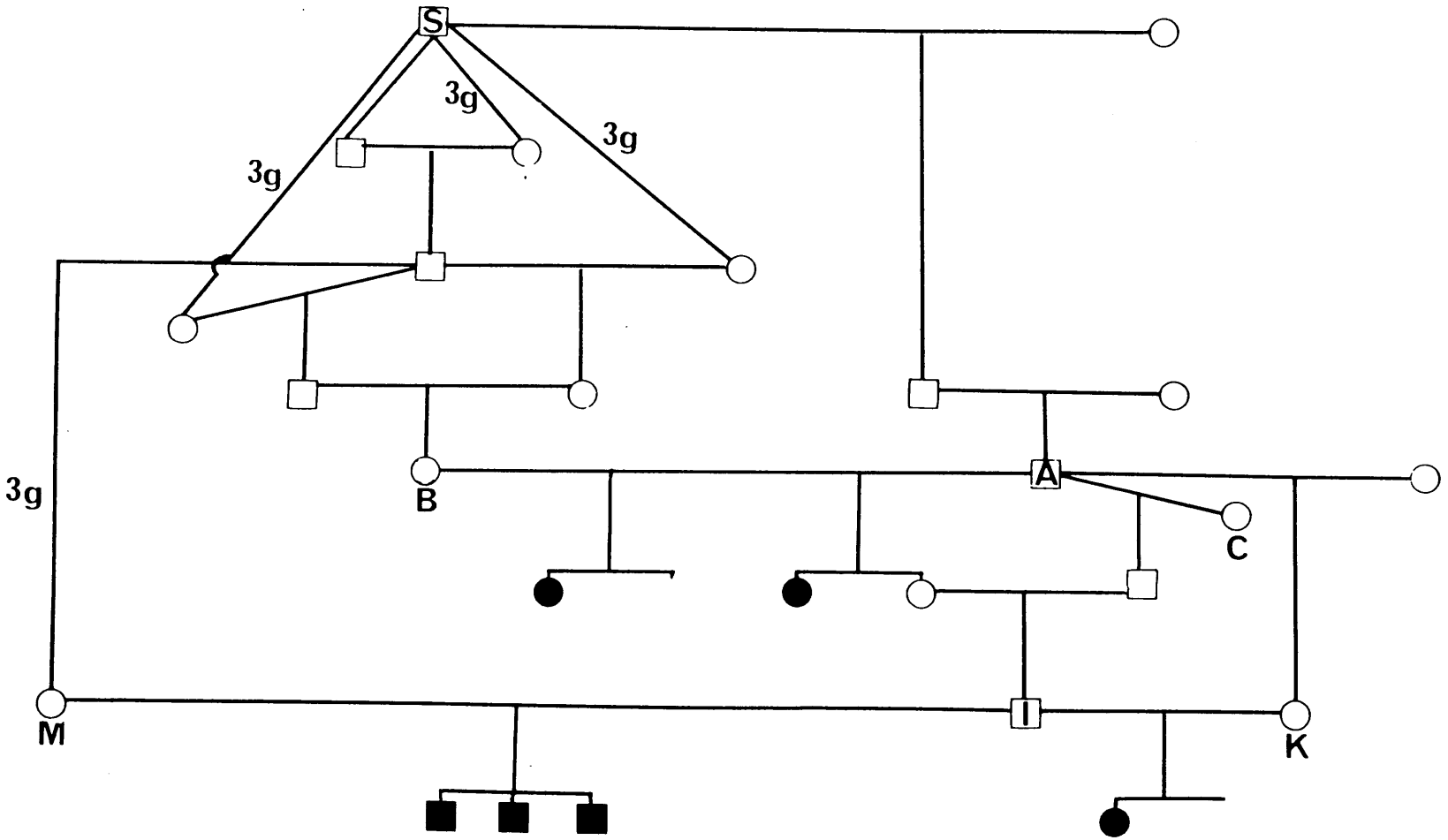


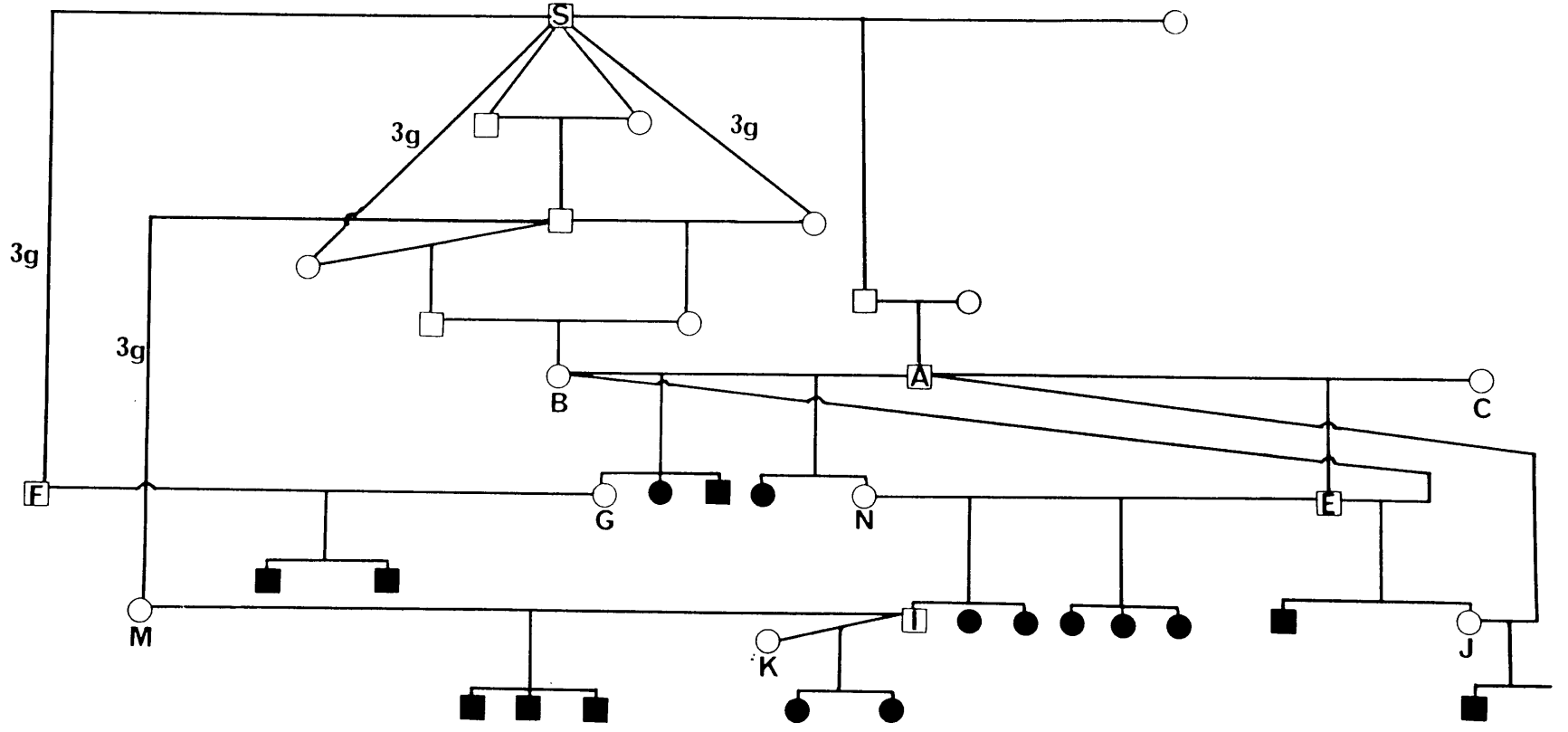
FIG. 37 SHOWING THE RELATIONSHIP BETWEEN BITCH K AND A AND B.

Fig 38 TRACING CASES DETAILED IN REPORT TO SOURCE S



3 G = 3 GENERATIONS

Fig 39 TRACING ALL AFFECTED ANIMALS TO SOURCE S



3G = 3 GENERATIONS

carrier ($P < 0.01$). Similarly bitch M produced 16 pups (litters 8, 9 and 14) from presumed carrier dogs (A and I) and none of the 16 was affected thus suggesting that bitch M was not a carrier ($P < 0.05$). It can, therefore, be argued that as the 40 pups produced by these matings had no chance of being affected they heavily biased the segregation ratio towards the unaffected. If these animals are excluded the amended ratio is 17 abnormals to 47 normals is 1:2.76 which is very close to the 1:3 ratio, which is expected in an autosomal recessive condition ($x^2 = 0.08$ $p > 0.9$).

A more appropriate method of correcting bias may be Weinberg's proband method (Stern 1973) which compensates for potential bias involved in studying litters which have been selected because they included abnormals, whereas litters which contain only normals may be excluded by the breeders. By applying Weinberg's correction the new ratio of normal to abnormal is 74:20 (Table 13). This is not significantly different from the expected 3:1 ratio ($x^2 = 0.5$, $P > 0.3$) and bears out the hypothesis that the condition is caused by an autosomal recessive gene. The possibility of imperfect penetrance cannot be absolutely precluded because the appropriate matings did not take place but from the data there is no evidence that the gene is anything but fully penetrant.

DISCUSSION

The primary purpose of this study was to define the nature and aetiology of this neurological condition in young bull mastiff dogs. Information has been obtained from 16 litters producing 98 pups, 17 of which were affected and 6 of these were examined in detail. The clinical signs suggested a central lesion affecting primarily the cerebellum but also other parts of the cerebrum and the brain stem.

The findings of the study will be discussed under three main headings: a) Clinical Aspects

b) Pathological Findings

c) Genetic Studies

(a) CLINICAL ASPECTS

The pups all demonstrated a variety of clinical signs indicative of a widespread disturbance of the brain, brain stem and particularly the cerebellum. Inco-ordination and hypermetria are suggestive of cerebellar dysfunction (Dow and Moruzzi, 1958) and many of the other signs including a visual defect have been shown to be present in animals with cerebellar pathology (Sadoff and others, 1986; Cogan and others, 1982). Not all of the clinical features are necessarily explained by a primary cerebellar lesion (Holliday, 1980). Changes in behaviour and other bizzare manifestations of a diffuse lesion in an otherwise healthy dog are seen commonly in animals with hydrocephalus (de Lahunta and Cummings, 1965; Banks and Monlux, 1952). This condition most frequent in small or brachiocephalic breeds (Frauchiger and Fankhauser, 1957) but has been described in a

number of large breeds (Selby and others, 1979). Selby and others, (1979) describe 564 individual cases of hydrocephalus in dogs involving 25 breeds but it is interesting to note that no animal of the Bull Mastiff breed is described. 64% of the animals in their survey were under 2 years of age and 36% under 6 months of age when the clinical diagnosis was made, the age range of the majority of the Mastiff pups described in this study.

The clinical signs shown by an animal with hydrocephalus vary depending on the area of the brain which has been damaged by the ventricular expansion (Chrisman, 1982). Dilation is usually due to build up of CSF pressure often as a result of a block in the ventricular system preventing removal of the fluid, whereas production continues (Jubb and others, 1985). However, in many cases no blockage exists and CSF pressure is not increased, a situation particularly common in young dogs with congenital hydrocephalus. de Lahunta (1983) suggests that this may be as a result of either inadequate aqueductal flow during a critical period of foetal development with resolution as the foetal brain enlarges; or a second possibility is that arachnoid villi malformation results in a temporary failure to remove fluid quickly enough and dilation of the ventricles results. By the time of birth both situations have resolved but the brain has been irreversibly damaged. de Lahunta (1983) described signs of disturbed consciousness, deficient motor function and sensory loss in cases of hydrocephalus. Disturbed consciousness is indicated by lethargy or in some cases severe depression, increased tendency to sleep, propulsive circling, head pressing and behavioural changes. Changes in motor function are spastic paresis, hypermetria if the cerebellum is compressed and ocular annomalies

such as strabismus. Loss of sensory functions are apparent in animals showing ataxia, visual defects with occasional blindness, mild general proprioceptive defects and slow postural reactions. These signs were usually accompanied by gross enlargement of the skull in very young animals.

The Bull Mastiff pups examined showed many of these signs. The breeder of the pups had indicated she could tell an affected pup before clinical signs were evident by the shape of the pup's head, the skull being more domed in appearance. This was not borne out by clinical investigation of the 6 pups examined in detail, where no appreciable enlargement of the skull was present and there was no evidence of open suture lines. None of the animals in the study demonstrated evidence of head pain, a sign described in some affected animals (de Lahunta and Cummings, 1965).

Ventriculography was carried out in one animal (Case 2) to establish whether hydrocephalus was present. Positive contrast was used to outline the entire ventricular system and demonstrate any blockage (Oliver and others, 1987). This technique demonstrated a moderate degree of hydrocephalus. No blockage was observed, the cerebral aqueduct was patent and free passage of the contrast into the sub-arachnoid space of the medulla and the cervical spinal cord was observed. This indicated the presence of a communicating hydrocephalus without blockage which is regarded as uncommon in domestic animals (Jubb and others, 1985). Unfortunately the animal deteriorated markedly on recovery from anaesthesia after this procedure and euthanasia had to be carried out. Oliver and others (1987), state that ventriculography is a safe and effective method of outlining the ventricles if performed

properly. Corticosteroids should be administered for 1 to 2 days prior to the procedure, if possible, to reduce the risk of cerebral oedema resulting from trauma to the brain (Oliver and Conrad, 1975). Air is regarded as being the safest medium to use (Oliver and others, 1987). However, this was not appropriate in our case since the purpose was to define the entire ventricular system, hence positive contrast was selected. Other workers indicate that the procedure is not without considerable risk (Hoerlein and Petty, 1961). If too much fluid is removed, ventricular collapse accompanied by sub-dural haematoma may occur. If excess contrast is injected or introduced too rapidly, considerable respiratory and cardiovascular problems can occur as a result of increased intraventricular pressure (Oliver and Conrad, 1975). The selection of contrast material is also very important as several studies have shown that different materials can result in meningitis or increased post-operative sensitivity to external stimuli (Hoerlein, 1971; Horwitz, 1956; Albert, 1967). It has also been demonstrated that Meglumine Iothalamate can cause acute toxicity when used in ventriculography (Albert, 1967; Bromage and others, 1978) making it a poor choice for this procedure. These facts perhaps explain the deterioration observed after ventriculography in our pup where a meningitis was found pathologically, a finding not duplicated in any of the other pups in the series. Non-ionic media such as metrizamide and iohexol have been proved to be safer agents when used in this procedure (Oftedal, 1973) and should have been utilised in this case.

The signs observed consistently in the cases, namely ataxia and hypermetria accompanied by a proprioceptive deficit are all

suggestive of a cerebellar lesion. Obvious tremor, another sign commonly associated with cerebellar disease, was only observed in 2 of the 6 cases. Proprioceptive signs have been shown to result when experimental lesions are produced by ablation of various parts of the cerebellum. These signs have been shown following experimental ablation of the deep cerebellar nuclei (Chambers and Sprague, 1955 a,b) in cats. Lesions produced solely in the dentate nucleus were manifest clinically as a proprioceptive loss, without alterations in postural tone, accompanied by a tremor (Chambers and Sprague, 1955 b). Proprioceptive defects observed in the mastiff pups were mainly a slowed response rather than an absence, particularly of the paw position reflex. Deficiencies in these functions after cerebellar lesions probably reflect loss of the facilitation of sensorimotor cortex or corticospinal pathways (Holliday, 1980). Marked changes in postural tone were not a feature of the presentation in the mastiff pups and tremor was observed in only 2 cases.

Compensation can occur after experimental cerebellar injuries leading to resolution of the postural defects (Holliday, 1980). This is observed over a period of several weeks to four months, and occurs even after total cerebellar ablation (Dow and Moruzzi, 1958). It is possible that similar compensation may take place in slowly developing lesions, as seen in the mastiff pups, therefore tonic changes do not feature prominently in the clinical picture. A similar mechanism may be active in preventing tremor although this has been shown to persist for long periods of time following experimental ablations of the dentate nucleus (Sperti and Zatti, 1958) and it is less easy to understand why it was not in evidence.

All of the mastiff pups were described as having a visual deficit. Blindness associated with a cerebellar lesion has been reported in Irish Setters (Palmer and others, 1973) and functional blindness as a result of inability to fix the gaze described in a baboon with cerebellar atrophy (Sadoff and others, 1986). Other ocular signs have been noted, anisorcoria and inequality of the width of the palpebral fissures were described by Chambers and Sprague (1955a,b) in association with cerebellar lesions. It is not inconceivable, therefore, that the observed visual loss in this study is due to a primary cerebellar problem. The menace response has also been described as absent in some cerebellar conditions (Palmer, Blakemore and others 1973, de Lahunta, 1983) but this did not appear to be a feature in the mastiffs. Again, as with the proprioceptive deficits it may be loss of facillitation of the cerebral cortex by cerebellum which results in the absence of the reflex (Holliday, 1980) as the response is dependent on the cerebral cortex (Rademaker and ter Braak, 1948; Sprague, 1966).

Many of the other signs observed in the mastiff pups have been described in association with cerebellar lesions. Compulsive movements may be interpreted as staggering without loss of balance. Raising of a foreleg has been associated with experimental lesions of the caudal lobe (Rijnberk, 1905) in response to auditory or mechanical stimulation. In experimental ablation of the rostal lobe, abnormal posturing was described during eating (Rothman 1913) an action which stimulated leg raising in 2 of the mastiff pups. In both of these experimental situation the changes were transient and disappeared 1 week after the ablation, as the animal compensated for the damage.

Nystagmus has been observed after ablation of the flocculonodular lobe (Holliday, 1980) and with peduncular lesions (de Lahunta, 1983). In both situations the rapid component of the eye movements is towards the side of the lesion and is accompanied by a head-tilt to the ipselateral side so constituting a paradoxical vestibular syndrome (de Lahunta, 1983).

Dysmetria and ataxia were present in all reviewed descriptions of animals with specific cerebellar lesions although they varied in onset, extent and progression. Severe tonic changes were a more common finding in animals in which abnormalities were present from birth. The signs seen in this group were often static whereas the changes observed in the second group of conditions tended to be progressive. Tremor was also observed as a clinical sign but was found in less than half the conditions reviewed, being described in Boston Bull Terrier, Beagle, Kerry Blue, Irish Setter, Rough Collie and the Rottweiler. Obvious behavioural changes were not a feature of any other condition. Visual impairment was described only in Irish setters (Palmer and others, 1973) which were completely blind with no evidence of vision, although pupillary light reflexes were still present.

A review of clinical changes observed in both naturally-occurring and experimentally-produced cerebellar disease showed that many of the signs seen in the Bull Mastiff pups could be attributed to a primary cerebellar lesion. Some of the behavioural changes, however, were difficult to explain as they have not been described in association with cerebellar abnormalities and did not present in any of the other similar conditions reviewed. These signs may have resulted from the

observed degree of hydrocephalus.

(b) PATHOLOGICAL FINDINGS

The structural changes were similar in all of the cases examined at necropsy. The brains were normal on gross examination but after sectioning a mild to moderate degree of hydrocephalus was discovered. The whole ventricular system was dilated including the cerebral aqueduct which was about twice normal diameter. No evidence of blockage was discovered in any of the cases confirming that the hydrocephalus was communicating in nature, as indicated by ventriculography. Although ventricular dilation was obvious it was not as severe as that described in dogs with a primary diagnosis of hydrocephalus (de Lahunta and Cummings, 1965; Higgins and others, 1977; Banks and Monlux, 1952) where often there was very little cerebral cortex remaining. Many dogs of brachiocephalic breeds show a mild to moderate degree of hydrocephalus in the absence of any clinical signs (Jubb and others, 1985). However, the ventricular volumes were greater than in an age-matched control which was destroyed as a result of myocarditis, therefore, this finding cannot be dismissed.

Microscopically, changes were found only in the deep cerebellar nuclei, the lateral vestibular nuclei and the inferior colliculi. These lesions were very well defined and bilaterally symmetrical in all cases. In the cerebellar nuclei and the inferior colliculus there was spongy vacuolar change and gliosis. The vacuoles were formed chiefly by intralamellar splitting of the myelin sheath. There was associated axonal degeneration and hypertrophy of astrocytes in these areas. The pathology was slightly different in the lateral vestibular nuclei where there

was little evidence of vacuolation but pronounced gliosis and axonal degeneration. The neuronal perikarya in these areas were not involved in the degenerative process. The cerebellar cortex was normal and although there was some evidence of axonal degeneration in the granule cell layer and cerebellar medulla, this was a sporadic finding. There were no other pathological changes detected. Spongy vacuolar change has been described both in naturally-occurring (Sahar and others, 1971) and in experimentally-produced hydrocephalus (James and others, 1975). The oedema is present in the periventricular area and is found mainly between the cellular components pushing them apart, oligodendroglia are not swollen nor vacuolated (McLone and others, 1971). This oedema, with an accompanying proliferation of blood vessels in the periventricular area, is thought to represent a compensatory mechanism providing an alternative pathway for CSF removal by transventricular absorbtion (Sahar and others, 1969). This feature was not noted in the cases examined nor was there any evidence of attenuation or loss of the ependymal layer or demyelination in the periventricular area (Weller and others, 1971). The conclusion is that there is very little microscopic evidence of damage as a result of the hydrocephalus.

The general distribution of the pathological changes in these animals is interesting. The cellularity of the cerebellar cortex appeared unaffected with a normal complement of Purkinje cells and no apparent decrease in granule cell population. Occasional axonal torpedoes, representing degenerating axons (Blumke and others, 1966), were observed in the granule cell layer and the cerebellar medulla. The most obvious changes in the cerebellum were present in the deep nuclei. Degenerating axons found in

these areas and the apparent preservation of adjacent neurones suggest that the basic lesion is a degeneration of the distal Purkinje axon. Changes were also observed in the lateral vestibular nuclei which are also known to receive direct projections from Purkinje cells mainly located in the flocculo-nodular area of the cortex (Gilman and others, 1981). This hypothesis does not explain the changes observed in the area of the inferior colliculus. It is possible that direct projections may exist from the Purkinje cells to the inferior colliculus but no evidence is currently available to substantiate this. A definite answer might be obtained by using horseradish peroxidase or radioactive labelled amino-acido injected into the inferior colliculus or the cerebellar cortex respectively (Kotchabhakdi and Walberg 1978). If such a connection was demonstrated a specific defect within the Purkinje system would be probable.

The vacuolation of the myelin sheath suggests a defect in the oligodendrocytes. This was confirmed on electron microscopy where fluid accumulation between the lamellae and degeneration of the inner tongue was seen. These changes rarely involved the cell body and were found in the distal terminations of the cell. There would, therefore, appear to be both an axonal and a glial lesion although it is possible that myelin changes were secondary to those in the axon. Vacuolation perhaps resulted from a failure of the Na-ion pump in the extremities of the cell. The pump mechanism is energy dependent, therefore interruption of cell metabolism or reduction of resources may lead to these changes. Vacuolation was not a prominent feature in the lateral vestibular nuclei although axonal degeneration was obvious, with an accompanying gliosis. Axonal spheroids were a common feature in

this area. The enlargement and dense staining of these structures was due to an accumulation of organelles. This suggests a disruption of axonal transport (Griffin and others, 1977) which may precede axonal degeneration. It is not known why the changes observed in the lateral vestibular nuclei were different from those seen in the other areas. One possibility is that there is a smaller population of the susceptible cell type, in this case the distal Purkinje axon. The lateral vestibular nucleus contains efferent axons from many other sources apart from the cerebellar fibres most prominently the primary vestibular fibres (Brodal, 1969). Another possibility is that the Purkinje fibres in this area are mainly derived from the flocculo-nodular formations in the cerebellum. It was predicted by the greater involvement of the dentate and interpositus nuclei that the more advanced or lateral zones of the cerebellum seemed to be more severely affected. This again may be an indication of differential cell susceptibility.

The nature and distribution of lesions found in this study are similar to those seen in certain metabolic disorders. Similar pathological changes have also been demonstrated in the cerebellar and lateral vestibular nuclei of dogs dosed with certain monoamine oxidase inhibitors (Palmer and Noel, 1963). Some of the inhibitors also caused major pathology elsewhere in the brain, notably the inferior olives (phenyl-150-propyl-hydrazine and tetra-hypronapathyl-hydrazine) or in the fore-brain (phenylezine and indanyl carbethoxy hydrazine). In the case of hepatyl hydrazine, changes were limited to the cerebellar nuclei and the lateral vestibular nuclei but no pathology was observed in the inferior colliculi. There was also a small degree of

hydrocephalus present in one of the dogs treated with this compound. The main change resulting from these substances was an accumulation of fluid within the neural ground substance, vacuolation and gliosis. The mechanism proposed was that fluid accumulation occurred following local vasoconstriction as a result of local build up of serotonin. Serotonin has been shown to be the chief substrate for monoamine oxidase activity (Corne and Graham, 1957) and elevated levels are seen in the central nervous system after monoamine oxidase inhibition in the dog (Maling and others, 1962).

Isoniazid administered at high dose levels also produces very similar vacuolation in the cerebellar roof nuclei and the lateral vestibular nuclei (Blakemore and others, 1972). In these animals there was involvement of the inferior colliculus and also the thalamus. Isoniazid does not induce vasoconstriction and therefore another pathogenesis must operate. One of the animals in Blakemore's study did not show clinical signs despite having the most marked degree of myelin vacuolation at necropsy. Suzuki (1971) was also able to produce similar pathological changes in rats without producing clinical signs. Blakemore and others conclude that clinical abnormalities may be due to neuronal and axonal disturbance rather than myelin damage. Myelin vacuolation with or without oligodendrocyte involvement is found commonly in many other toxic or metabolic conditions (Powell and others, 1980). The relevance of these observations is that a common metabolic requirement may be shared by cells in the target areas and alteration of a common metabolic pathway may result in the changes seen in the Bull Mastiffs.

Myelin vacuolation, oligodendrocyte and axonal degeneration

in the cerebellar and vestibular nuclei have been shown to occur in sub-acute thiamine deficiency (Robertson and others, 1968; Tellez and Terry, 1968) in the rat. Thiamine deficiency has been reported as an acute naturally-occuring syndrome in dogs fed on cooked meat (Read and others, 1977) and has also been investigated experimentally, by feeding dogs thiamine free diets (Zimmerman and Burrak, 1932; Read and Harrington, 1981; 1982; 1986). Feeding fresh raw fish to foxes caused the so-called Chasteck paralysis resulting from the action of thiaminase present in fish viscera (Krampnitz and Wooley, 1944). The same condition has been described in cats (Jubb and others 1956) and mink as a result of similar feeding practices. Thiamine deficiency also occurs in man as Wernicke's encephalopathy (Victor and Adams, 1961) often associated with alcoholism and poor diet.

Although pathological changes are widespread in canine thiamine deficiency, symmetrical focal degeneration within the inferior colliculi is pronounced and observed in all cases (Read and Harrington, 1986). There are also changes in other brain stem nuclei, the cerebellum and cerebral cortex. Spongy degenerative change, similar to that seen in the mastiffs, was the main pathological change. It is most improbable that the mastiff pups had a generalised thiamine deficiency as their diets were adequate and the neuropathological findings too restricted in distribution. However, it should be noted that in different species different specific sites of involvement were noted (Read and Harrington, 1986). The reason for this selective vulnerability in thiamine deficiency is uncertain (Dreyfus, 1976). It is speculated that a failure of the energy-dependent electrolyte transport system may allow an influx of fluid across

the blood-brain barrier resulting in the spongy degeneration of the oligodendrocytes (Robertson and others, 1968). A similar change has been produced in tissue culture when antimetabolites of thiamine, oxythiamine and pyrithiamine, are added in low concentrations (Yonezawa and Iwanami, 1966). The susceptible cells may have common, specific, metabolic requirements which are being compromised. In the mastiff pups it may be that a genetically-determined enzyme defect is present, producing within the affected neuronal systems an effect similar to that found in thiamine deficiency. This hypothesis might be tested by measuring relevant enzyme levels such as transketolase in tissue (Dreyfus, 1965). Although the exact pathogenesis is unknown this condition could be termed an abiotrophy implying premature degenerative change in the affected cells due to an intrinsic metabolic defect. The significant change would appear to be degeneration of the distal Purkinje axon.

Purkinje cells and the inferior colliculus, in addition to many other central structures, are selectively damaged by ischaemia (Palmer and Walker, 1970). There is no evidence that an episode of tissue anoxia occurred in our animals. Anoxia can occur during a convulsion but only one of the animals under investigation had a history of seizures. Therefore this is unlikely to be the cause of the pathological changes. Again as evidenced in this example the Purkinje cells and the cells within the inferior colliculus seem to be sharing a common susceptibility to damage which may be indicative of common metabolic rates or demands. In anoxia, cell types with high metabolic rates will be particularly prone to compromise. An indication of the metabolic rate of various areas of the brain can be accurately obtained by

measuring glucose utilization (Sokoloff, 1977). Quantitative autoradiography shows that the inferior colliculus, the vestibular nucleus and the cerebellar nuclei indeed do have a relatively high metabolic demands. This is particularly true of the inferior colliculus. However, other areas such as the superior olives and the auditory cortex also have high rates but did not show pathological changes in our study. Nevertheless, identifying this common factor which links the areas selectively damaged in the mastiff, again suggests that the pathogenesis is metabolic. Similar susceptibility to damage in thiamine deficiency could also be explained by this high metabolic demand since thiamine-dependent enzymes are of paramount importance in carbohydrate metabolism.

Many reports exist of conditions with specific and marked cerebellar pathology which have been reviewed in an earlier section (section 2). These conditions in young animals can be divided, depending on whether signs were present at birth or became obvious after a period of apparent normality. The described lesions were very varied and widespread but the cerebellar cortex and particularly the Purkinje cell system was frequently involved. No other condition showed the same specific distribution of lesions as found in the mastiffs. Hydrocephalus was described in four other reports in isolated cases associated with cerebellar damage (Dow, 1940; Harari and others, 1983; Pass and others, 1981; Kornegay, 1986). In all of these cases the hydrocephalus was mild in degree but there was gross abnormality of the cerebellum with partial or total agenesis. A similar combination of changes is found in Dandy-Walker syndrome in humans (Urich, 1976) where hydranencephaly is accompanied by cystic

enlargement of the roof of the posterior aspect of the 4th ventricle and agenesis or hypoplasia of the cerebellar lobes. In all of these examples cited, signs were obvious soon after birth and so do not resemble either clinical or pathological findings in the mastiff pups.

Animals developing clinical signs associated with early degenerative change are considered to be examples of neuronal abiotrophy (de Lahunta, 1980b). There is thought to be a normal complement of cells as a result of development around the time of birth but these then undergo premature degeneration. After the initial wave of destruction the signs may progress or remain static (de Lahunta, 1980b). The Purkinje system was mainly involved but degenerative changes were observed in other areas particularly in the Rough Collie (Hartley and others, 1978) and Kerry Blue Terrier (de Lahunta and Averill, 1976). The Samoyed was the only breed described which showed similar changes to those seen in the mastiffs with degeneration of the Purkinje axons but preservation of the cell bodies (de Lahunta, 1980(a)). Changes in the lateral vestibular nucleus were described only in the Rough Collie (Hartley and others, 1978) while lesions within the inferior colliculus were not reported in any of the breeds reviewed. Multiple cell types were involved in the degenerative process in certain of these conditions, as was seen in the mastiffs.

The conclusion of the pathological observations is that a metabolic disturbance results in selective degeneration of certain cell types in these pups. The cells affected are likely to be determined by common metabolic requirements which would be consistent with the description neuronal abiotrophy.

CORRELATION OF PATHOLOGY WITH CLINICAL SIGNS

Hypermetria and dysmetria, clinical signs indicative of cerebellar dysfunction, are due to the severe compromise of the cerebellar efferent system in this study as a result of degeneration of the distal Purkinje axon. The changes are most pronounced in the deep cerebellar nuclei, particularly the dentate and interpositus, suggesting that the more complex and advanced cerebellar functions may be lost with relative sparing of the more primitive vestibular actions (Holliday, 1980). The pathology noted in the lateral vestibular nucleus was mainly axonal degeneration and gliosis with little vacuolar change. The visual impairment observed in these animals is less easy to explain. Loss of vision has been described in cases of gross hydrocephalus (de Lahunta and Cummings, 1965; Higgins and others, 1977). Different reasons have been advanced for this. de Lahunta and Cummings suggest that defects could be due to optic nerve atrophy or deterioration of the optic radiation which courses from the lateral geniculate body to the visual cortex. This radiation which forms the lateral wall of the lateral ventricle has been shown to be reduced in size when dilation is present. Higgins and others suggest that as a result of expansion of the ventricular system, periventricular damage may occur and interruption of the visual pathway may result. The Irish Setters reported by Palmer and others (1973) were described as being blind but no pathology suggestive of a defect in the visual system was found. Likewise, no pathological changes were demonstrated in the optic pathways of the mastiffs. Functional blindness following failure to fix the gaze as a result of

cerebellar atrophy has been described by Cogan and others (1982). The clinical signs reported by these workers appear very similar to those encountered in the mastiff pups. Visual changes were not described in any of the other cerebellar conditions reviewed, although there was absence of the menace reflex in several as described by Palmer and others (1973).

The bizarre behavioural changes encountered in the mastiff have no parallel in the reports reviewed and it is difficult to suggest an explanation in the light of the pathological findings. Since changes were most pronounced in the dentate and interpositus nuclei one could predict that the functions most altered would be those attributable to the cerebellar hemispheres, that is the most lateral zones of the cerebellum with relative sparing of the more primitive medial zones including the vermis and the flocculo nodular formation. The more advanced functions of the cerebellum would be most markedly compromised with resultant loss of cerebellar facillitation to many areas of the cerebral cortex. It may be that loss of these cerebello-cortical discharges is responsible for some of the strange behavioural changes observed. However, these changes are commonly observed in animals with clinical hydrocephalus and although there is no pathological evidence to suggest that the degree of hydrocephalus detected in the mastiffs is significant it is impossible to exclude its potential role in producing clinical signs.

The main clinical result of damage to the vestibular nuclei is nystagmus. This was observed in only one of the six animals under investigation. The damage was confined to the lateral vestibular nucleus with preservation of the superior, medial and descending nuclei, and was less severe in extent than that

observed in the cerebellar nuclei and the inferior colliculus. These two facts may explain why nystagmus or other vestibular signs were not observed more frequently. The lateral vestibular nucleus contributes the majority of fibres to the lateral vestibulo-spinal tract. It is difficult to predict what effect the removal of Purkinje influence may have, but it is likely to result in increased facillitation on both alpha and gamma extensor neurones, so resulting in tonal abnormalities. These were not an obvious feature of the condition.

Lesions in the inferior colliculus may result in an interruption of the auditory tracts and deafness. The inferior colliculus seems to be important in the mediation of acoustic reflexes and precise localisation of sound (Masterton and others, 1967). Some of the animals under observation had great difficulty finding objects and directing themselves towards a caller. The animals were not deaf, as they responded to auditory stimuli but failure to localise the sound could lead to confusion and unpredictable movement which was a feature of the clinical condition.

(c) GENETIC STUDIES

The close relationship between many of the affected animals and the regular appearance of the condition within a breeding line both suggest that a hereditary defect exists. Many of the conditions reviewed, which present with cerebellar ataxia also have a proven or suspected hereditary basis. One of the objectives of this study was to establish if this was the case in the Bull Mastiff, by the examination of the pedigree data supplied. In any collected data there is a possibity of biased

results as litters are selected because they contain affected pups. In this study the pedigrees and litter details were the entire breeding records of a relatively small kennel and all the litters produced were presented. Two different methods of correcting possible bias of the result are presented. In the first method pups were excluded because they had no chance of being affected, by statistical determination that either their sire or dam were not carriers. Inclusion of these pups would heavily bias the result in favour of the normals. This first method is a somewhat crude elimination and a second method, considering only the litters containing affected pups and correcting for bias using the Simple Sib method (Stern 1973: Palmer and others, 1973) may be more appropriate. Both these methods of eliminating bias from the data resulted in agreement that an autosomal recessive was involved, probably being of full penetrance.

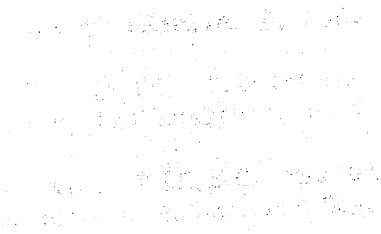
The origin of the recessive gene obviously cannot be proven but certain hypotheses can be made. If it is assumed that the condition is caused by a recessive gene, each parent of an affected pup must be a heterozygous carrier. Consideration of the geneological table indicated that all the confirmed cases and all the suspected additional cases could be traced back to a common source, dog S. All the affected carrier animals could have inherited the gene through this dog. Using this hypotheses there was not one animal whose genotype did not conform with the rules of recessive inheritance starting with dog S.

Therefore this hypothesis is the most likely. However, the spontaneous gametic mutation is unlikely to have occurred in dog S as he passed the gene to more than one line. Therefore if a

mutation has occurred it must have taken place in one of his antecedents but it is impossible to say in which generation, without more pedigree information.

Details of a hitherto undescribed condition in young Bull Mastiff dogs are presented. The clinical signs indicate there is a central neurological disturbance with involvement of the cerebellum. Pathological studies revealed distinct symmetrical areas of change primarily in the deep cerebellar nuclei, the lateral vestibular nuclei and the inferior colliculi. These changes were of spongy, vacuolar change and axonal degeneration. Histopathology determined that the degeneration was localised in the distal axons primarily of the Purkinje cells and vacuolation occurred in the myelin sheath which is a highly specialised extension of the oligodendrocyte. These specific changes were accompanied by a communicating hydrocephalus which was moderate in degree and of uncertain cause.

Genetic studies indicate that the condition has a familial basis and is probably inherited as an autosomal recessive trait.



REFERENCES

ALBERT, R.A. (1967) Canine ventriculography and encephalography. Thesis (M.S.) Auburn University, Auburn.

ANGAUT, P. and BRODAL, A. (1967) The projection of the vestibulocerebellum onto the vestibular nuclei in the cat. Arch. Ital. Biol. 105, 441.

BANKS, W.C. and MONLUX, W.S. (1952) Canine Hydrocephalus. J.A.V.M.A. 121, 453.

BLAKEMORE, W.F., PALMER, A.C. and NOEL, P.R.B. (1972) Ultrastructural Changes in isoniazid-induced brain oedema in the dog. Journal of Neurocytology 1, 263.

BLOEDEL,J.R. and COURVILLE, J. (1981) A review of cerebeller afferent systems in Handbook of Physiology, American Physiological Society, Bethesda Md.

BLUMCKE, S., NEIDORF, H.R. and RODE, J. (1966) Axoplasmic alterations in the proximal and distal stumps of transected nerves. Acta Neuropath (Berlin) 7, 44.

BRODAL, A. (1969) Neurological Anatomy - In Relation to Clinical Medicine. 2nd Edition, Oxford University Press, Inc. New York, p.263.

BROMAGE, P.R., BRAMWELL, R.S.B. and OTHERS (1978) Peridurography with Metrizamide: Animal and Human Studies. Radiology 128, 123.

CHAMBERS, W.W. and SPRAGUE, J.M. (1955)(a) Functional localization in the cerebellum. I. Organisation in longitudinal corticonuclear zones and their contribution to the control of posture, both extra-pyramidal and pyramidal. J. Comp. Neurol. 103, 105.

CHAMBERS, W.W. and SPRAGUE,J.M. (1955)(b) Functional localization in the cerebellum. II. Somatotopic organisation in cortex and nuclei. Arch. Neurol. and Psychiat. 74, 653.

CHOU, S.M. and HARTMANN, H.A. (1965) Electron microscopy of focal neuroaxonal lesions produced by B-B-iminodipropronitrile (I.D.P.N.) in rats. I. The advanced Lesions. Acta Neuropath. (Berlin) 4, 590.

CHRISMAN, C.L. (1982) Problems in small animal neurology, Lea and Febiger, Philadelphie, p.119.

CHRISMAN, C.L., CORK,L.C. and GAMBLE, D.A. (1984) Neuroaxonal dystrophy of Rottweiler Dogs. J.A.V.M.A. 184, 464.

COGAN, D.G., CHU, F.C. and REINGOLD, D.B. (1982) Ocular signs of cerebellar disease. Arch. Opthalmol. 100, 755.

CORDY, D.R. and SNELBAKER, H.A. (1952) Cerebellar hypoplasia and degeneration in a family of Airedale Dogs. J. Neuropath. Exp. Neurol. 11, 324.

CORK, L.C., TRONCOSO, J.C., PRICE, D.L., STANLEY, E.F. and GRIFFIN, J.W. (1983) Canine Neuroaxonal Dystrophy. J. Neuropath. Exp. Neurol 42, 286.

CORNE, S.J. and GRAHAM, J.D.P. (1957) J. Physiol. 135, 339.

DE LAHUNTA, A.E. and CUMMINGS, J.F. (1965) The Clinical and E.E.G. features of hydrocephalus in 3 dogs. J.A.V.M.A. 146, 954.

DE LAHUNTA, A.E. and AVERILL, D.R. (1976) Hereditary cerebellar cortical and extrapyramidal nuclear abiotrophy in Kerry Blue Terriers. J.A.V.M.A. 168, 1119.

DE LAHUNTA, A.E. (1980)(a) Diseases of the Cerebellum. Veterinary Clinics of North America 10, 96.

DE LAHUNTA, A.E. (1980)(b) Comparative cerebellar disease in domestic animals. Comp. Cont. Educat. 2, 8.

DE LAHUNTA, A.E., FENNER, W.R., INDRIERI, R.J., MELLICK, P.W. GARDNER, S and BELL, J.S. (1980) Hereditary cerebellar cortical abiotrophy in the Gordon Setter J.A.V.M.A. 177, 538.

DE LAHUNTA, A.E. (1983) Veterinary Anatomy and Canine Neurology. 2nd Edition. W.C. Saunders, Philadelphia. p.269

DOW, R.S. (1936) The fibre connections of the posterior parts of the cerebellum in the cat and the rat. J. Comp. Neurol 63, 527.

DOW, R.S. (1938) Efferent connections of the flocculo-nodular lobe in the Macaca Mulatta. J. Comp. Neurol. 68, 527.

DOW, R.S. (1940) Partial agenesis of the cerebellum in dogs. J. Comp. Neurol. 72, 569.

DOW, R.S. and MORUZZI, G. (1958) The physiology and pathology of the cerebellum. University of Minnesota Press, Minneapolis.

DREYFUS, P.M. (1965) The regional distribution of transketolase in the normal and thiamine deficient nervous system. J. Neuropath. Exp. Neurol 24, 119.

DREYFUS, P.M. (1976) Thiamine and the nervous system: an overview. J. Nutr. Sci. Vitaminol. (Tokyo) 22, Suppl. 13-16.

ECCLES, J.C. (1966) Functional organization of the cerebellum in relation to its role in motor control. In. Muscular Afferents and Motor Control, First Nobel Symposium, Stockholm, p.19. publ. John Wiley and Sons, New York.

FRAUCHIGER, E. and FANKHAUSER, R. (1957) Vergleichende Neuropathologic des Menschen und der Tiere. Berlin, Springer p.451.

GILMAN, S., BLOEDEL, J.R. and LECHTENBERG, R. (1981) Disorders of the cerebellum Contemporary neurology series, F.A. Davis Company, Philadelphia.

GOOD, R. (1962) Untersuchungen uber eine kleinhirn rindenatrophie beim hund Dissertation, University of Berne, Switzerland.

GRIFFIN, J.W., PRICE, D.L. and ENGEL, W.K. (1977) The pathogenesis of reactive axonal swellings: role of axonal transport. J. Neuropath. Exp. Neurol. 36, 214.

HARARI, J., MILLER, D., PADGETT, G.A. and GRACE, J. (1983) Cerebellar Agenesis in 2 canine littermates. J.A.V.M.A. 182, 622.

HARTLEY, W.J., BARKER, J.S.F., WANNER, R.A. and FARROW, B.R.H. (1978) Inherited cerebellar degeneration in the Rough Coated Collie. Aust. Vet. Pract. 8, 1.

HAYMAKER, W., GINZLER, A.M. and FERGUSON, R.L. (1946) Toxic effects of prolonged ingestion of D.D.T. on dogs with special reference to lesions in the brain. J. Med. Sci. 212, 423.

HIGGINS, R.J., VANDEVELDE, M. and BRAUND, K.B. (1977) Internal hydrocephalus and associated peri-ventricular encephalitis in young dogs. Vet. Path. 14, 236.

HOLLIDAY, T.A. (1980) Clinical signs of acute and chronic experimental lesions of the cerebellum. Vet. Science Communications 3, 259.

HORLEIN, B.F. and PETTY, M.F. (1961) Contrast encephalography and ventriculography in the dog - preliminary studies. Am. J. Vet. Research 22, 1041.

HORLEIN, B.F. (1971) Canine Neurology, 2nd Edition, Saunders, Philadelphia.

HORWITZ, N.J. (1956) Positive contrast ventriculography - critical evaluation. J. Neurosurg. 13, 388.

INNES, J.R.M., RUSSELL, D.S. and WILSDON, A.J. (1940) Familial cerebellar hypoplasia and degeneration in Hereford Calves. J. Path. and Bacter 50, 455.

JAMES, A.E., BURNS, B., FLOR, W.F., STRECKER, E.P., MERZ, T., BUSH, M. and PRICE, D.P. (1975) Pathophysiology of chronic communicating hydrocephalus in dogs. J. Neurol. Sci. 24, 151.

JANSEN, J. and BRODAL, A. (1940) Experimental studies on the intrinsic fibres of the cerebellum. II. The Conticonuclear Projection. J. Comp. Neurol. 73, 267.

JUBB, K.V.F., KENNEDY, P.C. and PALMER, N. (1985) Pathology of Domestic Animals IIIrd Edition, Vol. 1. Academic Press Inc., Orlando, p.209.

JUBB, K.V.F., SAUNDERS, L.Z. and COATS, H.V. (1956) Thiamine deficiency encephalopathy in cats. J. Comp. Path. 66, 217.

KARNOVSKY, M.J. (1965) A formaldehyde-gluteraldehyde fixative of high osmolarity for use in electron microscopy. Journal of Cell Biology 37, 137.

KAY, W.J. and BUDZILOVICH, G.N. (1970) Cerebellar hypoplasia and agenesis in the dog. J. Neuropath. Exp. Neurol. 29, 156.

KNECHT, C.D., LAMAR, C.H., SCHAIBLE, R. and PFLUM, K. (1979) Cerebellar Hypoplasia in Chow Chows. J.A.A.H.A. 15, 51.

KORNEGAY, J.N. (1986) Cerebellar vermian hypoplasia in dogs. Vet. Path. 23, 374.

KOTCHABHAKDI, N. and WALBERG, F. (1978) Cerebellar afferent projections from the vestibular nuclei in the cat. An experimental study with the method of retrograde axonal transport of horseradish peroxidase. Exp. Brain Res. 31, 591.

KRAMPNITZ, L.O. and WOOLEY, D.W. (1944) The manner of inactivation of thiamine by fish tissue. J. Biol. Chem. 152, 9.

LAMPERT, P.W. (1967) A comparative electron microscopic study of reactive, degenerating, regenerating and dystrophic axons. J. Neuropath. Exp. Neurol. 26, 345.

LEWEY, F.H. (1950) Neuropathological changes in nitrogen trichloride intoxication of dogs. J. Neuropath. Exp. Neurology 9, 396.

MASTERTON, B., JANE, J.A. and DIAMOND, I.T. (1967) Role of brainstem auditory structures in sound localisation. 1. Trapezoid body, superior olive and lateral leminiscus. J. Neurophysiol 30, 341.

MALING, H.M., HIGHMAN, B. and SPECTOR, S. (1962) J.Pharm. Exp. Ther. 137, 334.

McLONE, D.G., BONDAREFF, W. and RAIMONDI, A.J. (1971) Brain oedema in the hydrocephalic H_y3 mouse - sub-microscopic morphology. J. Neuropath. Exp. Neurology 30, 627.

OFTEDAL, S.I. (1973) Intraventricular application of water soluable contrast media in cats. Acta Radiol. (Suppl.) 355, 125.

OLIVER, J.E. Jnr. and CONRAD, C.R. (1975) Cerebral Ventriculography. In ed Ticer, J.W. Radiographic technique in small animal practice. publ. W.B. Saunders & Co., Philadelphia, p.271.

OLIVER, J.E., HORLEIN, B.F.and MAYHEW, I.G. (1987) Veterinary Neurology, publ. W.B. Saunders & Co., Philadelphie, p.97.

PALMER, A.C. and NOEL, P.C. (1963) Neuropathological effects of prolonged administration of some hydrazine monoamine oxidase inhibitors in dogs. J. Path. and Bact. 86, 463.

PALMER, A.C. and WALKER, R.G. (1970) The neuropathological effects of cardiac arrest in animals: A study of 5 cases. J.S.A.P. 11, 779.

PALMER, A.C., BLAKEMORE, W.F., COOK, W.R., PLATT, H. and WHITEWELL, K.E. (1973) Cerebellar hypoplasia and degeneration in the young arab horse. Clinical and neurological features. Vet. Rec. 93, 62.

PALMER, A.C., PAYNE, J.E. and WALLACE, M.E. (1973) Hereditary quadriplegia and amblyopia in the Irish Setter. J.S.A.P. 14, 343.

PALMER, A.C. (1976) Introduction to Animal Neurology. Publ. Blackwell Scientific Publications, Oxford.

PASS, D.A., HOWELL, J.M. and THOMSON, R.R. (1981) Cerebellar malformations in 2 dogs and 1 sheep. Vet. Path. 18, 405.

PERCY, D.H., CARMICHAEL, L.E., ALBERT, D.M., KING, J.M. and JONAS, J.M. (1971) Lesions in puppies surviving infection with canine herpes virus. Vet. Path. 8, 37.

PHEIMISTER, R.D. and YOUNG, S. (1968) The post natal development of the canine cerebellar cortex. J. Comp. Neurol. 134, 243.

POWELL, H.C., MYERS, R.R. and LAMPERT, P.W. (1980) in Experimental and clinical neurotoxicology, p.118 ed Spencer P.S. and Schaumberg, H.H. Williams and Wilkins, London.

RADEMAKER, G.G.J. and ter BRAAK, J.W.G. (1948) On the central mechanisms of some optic reactions. Brain 71, 48.

READ, D.M., JOLLY, R.D. and ALLEY, M.R. (1977) Polioencephalomalacia of dogs with thiamine deficiency. Vet. Path. 14, 103.

READ, D.H. and HARRINGTON, D.D. (1981) Experimentally induced thiamine deficiency in Beagle Dogs - Clinical Observations. Am. J. Vet. Research 42, 984.

READ, D.H. and HARRINGTON, D.D. (1982) Experimentally induced thiamine deficiency in Beagle Dogs - Clinicopathological Findings. Am. J. Vet. Research 43, 1258.

READ, D.H. and HARRINGTON, D.D. (1986) Experimentally induced thiamine deficiency in Beagle Dogs - Pathological changes of the central nervous system. Am. J. Vet. Research 47, 2281.

RIJNBERK, G. VAN (1905) Tentativi di localizzazioni funzionali nel cervelletto. Arch. Fisiol 1, 569.

ROBERTSON, D.M., WASAN, S.M. and SKINNER, D.B. (1968) Ultrastructural features of early brain stem lesions in thiamine deficient rats. Am. J. Path. 52, 1081.

ROTHMAN, M. (1913) Die funktion des mittelappens des kleinhirns, Monatschur Psychiat. U. Neurol 34, 389.

SADOFF, D.A., GIDDENS, W.E. and MORTON, W.R. (1986) Cerebellar cortical atrophy in a baboon. Vet. Path 23, 335.

SAHAR, A., HOCHWALD, G.M. and RANSOHOFF, J. (1969) Alternative pathway for cerebrospinal fluid absorption with experimental obstructive hydrocephalus. Exp. Neurol. 25, 200.

SAHAR, A., HOCHWALD, G.M., KAY, W.J. and RANSOHOFF, J. (1971) Spontaneous canine hydrocephalus: cerebrospinal fluid dynamics. J. Neurol. Neurosurg. Psychiat 34, 308.

SELBY, L.A., HAYES, H.M. and BECKER, S.V. (1979) Epizootiologic features of canine hydrocephalus. Am. J. Vet. Research 40, 411.

SILVER, M.L. (1949) Canine epilepsy caused by flour bleached with NCI_3. J. Neuropath. Exp. Neurol. 8, 441.

SOKOLOFF, L. (1977) Relation between physiological function and energy metabolism in the central nervous system. J. Neurochemistry 29, 13.

SPERTI, L. and ZATTI, P. (1958) Osservazzioni sul comportamento di cani con lesioni cronica di uno a piu nuclei cerebellari. Atti. Soc. Med. Chir., Padova 31, 437.

SPRAGUE, J.M. (1966) Interaction of cortex and superior colliculus in mediation of visually guided behaviour in the cat. Science 153, 1544.

STEINBERG, S., TRONCOSO, J.C., CORK, L.C. and PRICE, D.L. (1981) Clinical features of inherited cerebellar degeneration in Gordon Setters. J.A.V.M.A. 179, 886.

STERN, C. (1973) Principles of human genetics, 3rd edition. Publ. Freeman, San Francisco.

SUZUKI, K. (1971) Some new observations in triethyl-tin intoxication of rats. Exp. Neurol. 31, 207.

TELLEZ, I. and TERRY, R.D. (1968) Fine structure of the early changes in the vestibular nuclei of the thiamine deficient rat. Am. J. Path. 52, 777.

URICH, H. (1976) Malformations of the nervous system, perinatal damage and related conditions in early life, p.361. In Greenfield's Neuropathology, ed Blackwood and Corsellis, Publ. Edward Arnold, London.

VICTOR, M. and ADAMS, R.D. (1961) on the aetiology of the alcoholic neurologic diseases: with special reference to the role of nutrition. Am. J. Clin. Nutrition 9, 379.

VOOGD, J. (1969) The importance of fibre connections in the comparative anatomy of the mammalian cerebellum in Llinas, R. ed Neurobiology of Cerebellar Evolution and Development, A.M.A. Chicago, p.493.

WELLER, R.O., WISNIEWSKI, H., SCHULMAN, K. and TERRY, R.D. (1971) Experimental hydrocephalus in young dogs. Histological and ultrastructural study of brain tissue damage. J. Neuropath. Exp. Neurol. 30, 613.

YONEZAWA, T. and IWANAMI, H. (1966) An experimental study of thiamine deficiency in nervous tissues using tissue culture techniques. J. Neuropath. Exp. Neurol. 25, 362.

ZIMMERMAN, H.M. and BURACK, E. (1932) Lesions of the nervous system resulting from deficiency in Vit. B. Complex. Arch. Pathol. 13, 207.

TABLE 1: DISTRIBUTION OF FIBRE TRACTS IN THE CEREBELLAR PENDUCLES

	Afferent Tracts	Efferent Tracts
Rostral	Tectocerebellar Rubrocerebellar (Ventral Spinocerebellar)	Interpositus Dentate
Middle	Pontocerebellar (Reticulocerebellar)	
Caudal	Dorsal Spinocerebellar	Purkinje Projection to Vestibular Nuclei
	Cuneocerebellar Reticulocerebellar Olivocerebellar	Fastigial

TABLE 2: EFFERENT PROJECTIONS OF CEREBELLUM

Origin			Termination
1. Cerebellar Cortex			Vestibular Nucleus
2. Cerebellar Nuclei	I)	Fastigial	Vestibular Nucleus
			Reticular Formation
	II)	Interpositus	Red Nucleus
			Reticular Formation
	III)	Dentate	Red Nucleus
			Reticular Formations
			Ventral Lateral Nucleus of Thalamus
			Globus Pallidus

TABLE 3: SUMMARY OF INHERITED OR SUSPECTED INHERITED CEREBELLAR DISEASE WITH CLINICAL SIGNS PRESENT AT BIRTH

Breed	Age at Onset	Main Clinical Findings	Pathological Findings	Inherited	Reference
1. Beagle	Birth	Severe cerebellar ataxia. Tremor	Loss of Purkinje neurones.	?	de Lahunta (1980)
2. Berne Running Dog	Birth	Cerebellar Ataxia.	Loss of Purkinje cells, degeneration of olivary nuclei.	?	Good (1962)
3. Boston Bull Terrier	Birth	Dysmetria with hyperextension of hind legs. Tremor	Internal hydrocephalus Cerebellar cortical atrophy. Cerebellar nuclei atrophy. Inferior Olives atrophy.	?	Dow (1940)
4. Chow	Birth	Cerebellar ataxia.	Atrophy of cerebellar cortex.	Autosomal Recessive	Knecht and others (1979)
5. Irish Setter "	Birth	'Swimmers' unable to stand, propelled on bellies. Severe visual defect, almost blind. Nystagmus, tremor	Purkinje cell loss Granule cell loss in older animals.	Autosomal Recessive	Palmer and others (1973)
6. Irish Setter	Birth	Non-progressive cerebellar ataxia	Cerebellar hypoplasia cerebral lissencephaly.	?	de Lahunta (1980)

Breed	Age at Onset	Main Clinical Findings	Pathological Findings	Inherited	Reference
7. Poodle	Birth	Dysmetria, inability to stand.	Cerebellar agenesis	?	Kay & Budzilovich (1980)
8. Samoyed	Birth	Hypermetria and Hypertonia of pelvic limbs which were forward under body.	Purkinje axonal degeneration Purkinje neurones intact. Swollen axons in granule layer.	?	de Lahunta (1983) (1980(a))
9. Wire Haired Fox Terrier	Birth	Non-progressive cerebellar ataxia.	Cerebellar hypoplasia Cerebral lissencephaly	?	de Lahunta (1980)

TABLE 4: SUMMARY OF INHERITED OR SUSPECTED INHERITED CEREBELLAR DISEASE WITH CLINICAL SIGNS DEVELOPING SOME TIME AFTER BIRTH

Breed	Age at Onset	Main Clinical Findings	Pathological Findings	Inherited	Reference
Airedale	Under 12 weeks	Severe cerebellar ataxia marked hypermetria.	Slight decrease in cerebellar size. Severe Purkinje loss and degeneration.	Hereditary but mode unknown.	Cordy & Snelbaker (1952)
Cairn	8-12 weeks	Progressive cerebellar ataxia.	Purkinje loss and degeneration.	?	de Lahunta (1983)
Cocker Spaniel	8-12 weeks	Progressive cerebellar ataxia.	Purkinje loss and degeneration.	?	de Lahunta (1983)
Gordon Setter	6-24 months	Slow progressive cerebellar ataxia. Hypermetriaof thoracic limbs. Stiff dysmetric gait.	Purkinje loss and degeneration. Some granule cell loss. Gliosis and axonal swelling in cerebellar nuclei.	Autosomal Recessive	de Lahunta and others (1980) Steinberg and others, 1981
Great Dane	8-12 months	Progressive Cerebellar ataxia.	Purkinje loss and degeneration.	?	de Lahunta (1983)
Golden Retriever	8-12 weeks	Progressive Cerebellar ataxia	Purkinje loss and degeneration.	?	de Lahunta (1983)

Breed	Age at Onset	Main Clinical Findings	Pathological Findings	Inherited	Reference
Kerry Blue Terrier	9-16 weeks	Severe Cerebellar ataxia then unable to stand. Hypertonia and Hypermetria. Mild head tremor.	Purkinje cell degeneration Granule cell depletion. Degeneration of Cerebellar nuclei, olives, caudate and substantia nigra. Changes in medial thalamic nucleus, lateral geniculate optic tract.	Autosomal Recessive	de Lahunta (1976) de Lahunta & Averill (1976) Mettler & Goss (1946)
Labrador	8-12 weeks	Progressive Cerebellar ataxia.	Purkinje loss and degeneration.	?	de Lahunta (1983)
Rough Collie	6 weeks	Most sable pups. Progressive cerebellar ataxia. Falling, Intention. Tremor	Cerebellar cortex degeneration Loss of neurones Cerebellar Nuclei Lateral vestbular nuclei Olivary nuclei, ventral roots. Wallerian degeneration Reticular formation Medial longitudinal fascicle brain stem All levels cord except dorsal columns.	Autosomal Recessive	Hartley & others (1978)
Rottweiler	Under 1 year	Ataxia, hypermetria.	Axonal speroids indicating degeneration widespread throughout the CNS.	Autosomal recessive.	Chrisman & Others (1984). Cork & others (1983)

TABLE 5: DETAILS OF PATHOLOGICAL INVESTIGATION

Case	Mode of Fixation	Brain Removed	Cord Removed	Details of other Tissue Examined
1	Perfusion Fixation 10% Formol-Saline	+	+	Duodenum, Jejunum, Colon, Liver Spleen
2	Immersion Fixation 10% Formol-Saline	+	-	-
3	Perfusion Fixation Glutaraldehyde	+	+ Lumbar ganglia	Duodenum, Jejunum, Colon, Liver Spleen, Kidney, Pancreas, Mesenteric Node
4	Immersion Fixation 10% Formol-saline	+	-	Duodenum, Jejunum, Liver, Kidney, Pancreas
5	Immersion Fixation 10% Formol-saline	+	-	-
6	Immersion Fixation 10% Formol-saline	+	-	-

TABLE 6: PROCEDURE FOR PROCESSING BRAINS FOR PARAFFIN SECTIONS

Step	Agent	Time
1	70% Spirit + 5% Phenol	6 hours
2	Methylated Spirit	8 & 1/2 hours
3	Absolute Alcohol + 5% Phenol	4 & 1/2 hours
4	Repeat Step 3	4 & 1/2 hours
5	Equal Parts Absolute Alcohol/Amylacetate	2 & 1/2 hours
6	Amyl Acetate I	8 & 1/2 hours
7	Amyl Acetate II	8 & 1/2 hours
8	1% Celloidin in Methyl Benzoate I	18 & 1/2 hours
9	1% Celloidin in Methyl Benzoate II	18 & 1/2 hours
10	Xylene	2 & 1/2 hours
11	Wax I	12 & 1/2 hours
12	Wax II	20 hours

Tissues are then blocked in fresh wax and 8um sections cut for staining.

TABLE 7: DETAILS OF SEX, AGE AND DURATION OF SIGNS IN EACH CASE

Case	Sex	Age at Initial signs	Age at Euthanasia	Duration
1	M	7 weeks	17 weeks	10 weeks
2	M	6 weeks	17 weeks	11 weeks
3	M	7 months	10 months	12 weeks
4	F	4 weeks	16 weeks	12 weeks
5	F	6 weeks	23 weeks	17 weeks
6	F	4 weeks	12 weeks	8 weeks

TABLE 8: SUMMARY OF CLINICAL SIGNS INDICATING THE FREQUENCY OF EACH IN 6 DOGS

	Clinical Signs	Frequency
1	Ataxia	6/6
2	Hypermetria	4/6
3	Proprioceptive Defect	5/6
4	Visual Defect	6/6
5	Hysterical Behaviour	2/6
6	Tremor	2/6
7	Paresis	1/6
8	Compulsive Movements	2/6
9	Lifting Leg	2/6
10	Circling	2/6
11	Dull	2/6
12	Nystagmus	1/6

TABLE 9: DETAILS OF CLINICAL SIGNS EXHIBITED BY EACH CASE

Clinical Sign	Case Number					
	1	2	3	4	5	6
1. Ataxia	+	+	+	+	+	+
2. Hypermetria	+	-		+	+	+
3. Paresis	-	-	+	-	-	-
4. Fits/Hysterical Behaviour	+	-	+	-	-	-
5. Proprioception						
Paw Position	slow	slow	slow	slow	slow FL	+
Sway	slow	slow	-	slow	+	+
Reflex Stepping	slow	slow	slow	slow	slow FL	+
Placing	slow	slow				
6. Tremor	+*	-	+	-	-	-
7. Visual Defects						
i) Bumping into objects	+	+	+	+	+	+
ii) Difficulty finding objects	+	-	-	+	-	+
8. Compulsive Movements	+ back	+ forward				
9. Lifing Leg	+	-	-	+	-	-
10. Circling	-	+ (right)	-	-	-	? (right)
11. Dull	+	+	-	-	-	-
12. Nystagmus	-	-	+	-	-	-

*Tremor induced on moving head

TABLE 10: RESULTS OF BLOOD, CSF AND URINE ANALYSES IN EACH CASE

Parameter	Units	Case 1 Samples (i)	(ii)	Case 2	Case 3	Case 4 Samples (i)	(ii)	Normal
Blood								
White cell count	$10^9/1$	13	12	14.1	15.9	13.9	16.6	
Red cell count	$10^{12}/1$	5.03	4.82	4.82	5.91	4.41	5.48	
Haemaglobin	g/dl	10.5	12.0	11.1	14.5	8.7	10.4	
Haematocrit		0.33	0.35	0.34	0.42	0.25	0.35	
M.C.V.	f/1	66	65	71	72	58	65	

Samples		(i)	(ii)	(iii)			(i)	(ii)	(iii)	
Urea	m.mol/1	2.1	3.9	5.8	3.3	3.5	4.5	2.2	2.2	
Sodium	m.mol/1	153	139	159	139	140	135	150	152	(136-160)
Potassium	m.mol/1	5.0	4.8	4.5	4.6	4.7	4.6	4.5	5.8	(3.4-5.8)
Chloride	m.mol/1	110	107	108	107	106	107	107	109	(95-115)
Calcium	m.mol/1	2.79	3.37	2.89	-	3.09	2.83	2.86	2.24	(2.34-3.03)
Magnesium	m.mol/1	0.75	0.61	0.62	-	0.69	0.66	0.61	0.59	(0.61-2.19)
Phosphate	m.mol/1	3.09	1.72	2.9	2.89	1.81	3.27	2.38	2.17	(1.29-2.9)

Parameter	Units	Case 1 Samples (i)	(ii)	(iii)	Case 2	Case 3	Case 4 Samples (i)	(ii)	(iii)	Normal
Sugar	m.mol/1	7.6	6.3	-	-	5.0	-	-	6.0	(2.49-4.99)
Bilirubin	u.mol/1	1	1	8	2	1	0	8	1	(0-10)
Alkaline phosphatase	i.u.	492	646	349	250	317	541	488	389	
A.L.T.	i.u.	24	32	36	20	102	71	29	24	
A.S.T.	i.u.	31	32	25	38	26	24	29	30	
Total Protein	g/1	45	53	60	53	62	56	55	58	(50-78)
Albumin	g/1	34	34	33	33	33	29	-	27	(31-40)
Globulin	g/1	11	29	27	21	29	27	-	31	
Creatinine	u.mol/1	-	-	-	71	-	-	-	-	(44-132)
Bromosulphalein Dye					1.8% 0.8%					
Cerebrospinal Fluid										
Protein						60		180		
Urine										
Protein					+8					
Urea					11.3					
pH					8.5					
Specific Gravity					1.02					

TABLE 11: SUMMARY OF PATHOLOGY

Area	Pathological Findings
1. CEREBELLUM	
a) Cortex	Normal compliment of Purkinje cell bodies. Occasional 'torpedo' in otherwise normal granule cell layer
b) White matter	Occasional degenerating axons
c) Cerebellar Nuclei	Vacuolation, Gliosis Axonal Spheroids
2. LATERAL VESTIBULAR NUCLEUS	Gliosis, Spheroids Vacuolation - less severe
3. INFERIOR COLLICULUS	Vacuolation, Gliosis

In addition there was a moderate internal hydrocephalus in all cases.

TABLE 12: DETAIL OF ALL MATINGS AND OFFSPRING

Litter No.	Sire	Dam	Total Pups	Normal		Abnormal	
				M	F	M	F
1	A	B	5	1	3	-	1
2	A	C	4	4	-	-	-
3	A	B	6	2	2	1	1
4	D	N	8	3	5	-	-
5	E	N	5	3	-	-	2
6	E	B	3	1	1	-	1
7	F	G	3	-	1	2	-
8	A	H	9	5	4	-	-
9	I	H	3	2	1	-	-
10	D	N	7	5	2	-	-
11	E	N	10	4	3	-	3
12	A	J	0	2	6	1	-
13	D	K	9	3	6	-	-
14	I	H	4	1	3	-	-
15	I	K	9	3	4	1	1
16	I	L	4	2	2	-	-
17	I	M	6	-	3	3	-
Total	5	9	104	41	46	8	9

TABLE 13: CORRECTION FOR BIAS BY WEINBERG'S SIMPLE SIB METHOD

Litter Ref.	Total	Normal	Affected	Corrected	
				Normal	Affected
1	5	4	1	4	0
3	6	4	2	8	2
5	5	3	2	6	2
6	3	2	1	2	0
7	3	1	2	2	2
11	10	7	3	21	6
12	9	8	1	8	0
15	9	7	2	14	2
17	6	3	3	9	6
	56	39	17	74	20