OVINE CEROID-LIPOFUSCINOSIS

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to my parents

..."Ie hebt 't good edôan"...

ABSTRACT

Ovine ceroid-lipofuscinosis is a rare inherited neurological disease which has only been diagnosed in one family of the South Hampshire breed of sheep. This breed is of relative recent origin and was established from an initial cross between Southdown and Hampshire Down sheep. Affected sheep show loss of vision and behavioural abnormalities starting at 11-12 months of age, with motor dysfunction commencing soon afterwards. The clinical signs increase in severity as the disease progresses and under field conditions affected animals are not expected to live beyond 2 years.

Ovine ceroid-lipofuscinosis is characterized histologically by the intracytoplasmic accumulation of PAS and Sudan black positive autofluorescent lipopigment material in neurons and a wide variety of other cell types. The process leading to the accumulation of lipopigment seems only to damage neurons and there is degeneration and loss of neurons, especially in the cerebral cortex and the visual neuroepithelium of the retina i.e. retinal atrophy. Grossly, affected brains show reduction in size and weigh on average 66% of those of normal sheep.

Ultrastructurally, the typical lipopigment inclusion is a round or oval body 0.2 - 5.0 µm in size, of varying electron density, in which a wide variety of membranous profiles may be seen. Some of the membranous patterns have received special names such as curvilinear, fingerprint and crystalloid.

Pathological examination of liver, skin and rectal biopsy material of lambs at 4 - 5 months of age shows the presence of accumulated lipopigment, and is a means of early diagnosis before the onset of clinical signs. This observation and the fact that lipopigment has been demonstrated in affected lambs at birth, show ovine ceroid-lipofuscinosis to be associated with a true inborn error of metabolism.

The family tree of all affected sheep and the results of sire-daughter matings of a heterozygous ram show the disease to be inherited as a simple autosomal recessive trait. The deleterious gene for ovine ceroid-lipofuscinosis is unlikely to be of economic importance to the sheep industry as the South Hampshire breed was developed to supply sires for terminal crosses associated with table lamb production.

The objects of this study were to define ovine ceroid-lipofuscinosis in clinical, pathological and genetic terms, and to compare it with similar diseases in man and domestic animals. It is concluded that the ovine disease does indeed belong to the heterogeneous group of diseases of man and domestic animals known as Batten's disease or the neuronal or generalised ceroid-lipofuscinoses. Of these the ovine entity most closely resembles the late infantile and the juvenile forms of the human syndrome, and the canine disease. It is proposed that ovine ceroid-lipofuscinosis would make a useful experimental model for Batten's disease.

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INTRODUCTION

The neuronal ceroid-lipofuscinoses, also known as the non-infantile amaurotic family idiocies and as Batten's disease, are a distinct group of inherited diseases of children. They are characterised by the progressive intracellular, and especially intraneuronal, accumulation of the autofluorescent lipopigments, ceroid and lipofuscin. Affected patients show progressive central nervous system degeneration, loss of vision, loss of mental faculties, seizures and premature death. An adult form, with milder symptomatology is also recognised. Similar diseases have also been described in a number of species of domestic animals. The disease as it occurs in the South Hampshire sheep forms the subject material of this thesis.

The neuronal ceroid-lipofuscinoses of man and domestic animals are reviewed in Chapter 1, with emphasis on clinical, pathological, biochemical and genetic findings. A brief historical account is also presented in an attempt to clarify the confusing nomenclature extant in the literature, by tracing the origins of the various eponymic and descriptive names.

The main objectives of the study reported in this thesis were to define ovine ceroid-lipofuscinosis in clinical, pathological and genetic terms and to compare these findings with those of the entities in man and domestic animals.

CHAPTER 1

REVIEW - THE NEURONAL CEROID-LIPOFUSCINOSES

1. HISTORICAL ASPECTS

Some authors (Koppang, 1973/74; Lake, 1977) consider that the first description of a neuronal ceroid-lipofuscinosis (NCL) was by Stengel in 1826. He described four siblings, born to healthy farmer parents in Norway, who showed visual failure beginning at the age of 6 years, progressing to blindness at 9 years. Epileptic seizures, speech difficulties and sensory-motor regression became increasingly severe. There was profound mental dullness at the age of 15 years. Two of the patients died at ages 20 and 21 years, the other two were alive aged 8 and 16 years at the time of his report.

Since Stengel's report was a clinical description only, the first comprehensive report of NCL is usually ascribed to Batten (1903), who described "cerebral degeneration with symmetrical changes in the maculae" in two sisters in England, beginning at ages 4 and 6 years. He noted slight pallor of the optic discs, and retinal pigmentary changes with irregular poorly defined reddish-black spots in the maculae. In 1914, Batten gave the following description of the disease:

> ....."The typical features are loss of intellectual faculties, loss of vision, loss of motor power. In some cases all three defects seem to start together, in others mental symptoms occur first, visual and motor signs remain long in abeyance, in still other cases the disease commences with visual symptoms, motor and mental deficits follow later or not at all. The disease

may begin any time during infancy and childhood and some cases pass rapidly with a fatal termination, others are slow in their progress.

Some cases show distinct changes in the macula, others retinitis pigmentosa in the peripheral retina, and still others have no fundus changes or do so only in the later stages of the disease. Clinically there is a great variation in the symptoms and this together with their time of appearance forms a basis of classification. Pathologically these cases are essentially the same and the changes in the cells are strikingly similar in all cases which have come to autopsy".....

He omitted to mention the almost invariable occurrence of seizures in his patients. However, Batten's is a lucid description of NCL and Batten's disease is a valid eponym. Three English children, produced by first cousins, who developed failure of eyesight at 6 years of age, followed by mental deterioration, were described by Mayou (1904). This has led to the eponym Batten-Mayou syndrome.

In 1905, possibly unaware of the English publications, two German authors, Vogt and Spielmeyer, independently described a disease of children which is now considered to have been a neuronal ceroid-lipofuscinosis and the eponymic designation Batten-Spielmeyer-Vogt syndrome is commonly used. However, at the time, Spielmeyer called his disease entity, "juvenile amaurotic idiocy" and Vogt called his "the juvenile form of amaurotic family idiocy".

Nosological confusion had thus begun, since amaurotic family idiocy was the name given to a disease entity of children by Sachs

(1896), in the U.S.A.; he had earlier reported on its occurrence in 1887. In England, Tay had described a similar disease of children in 1881, and Higier (1901) introduced the eponym Tay-Sachs disease. The features of Tay-Sachs disease, which has its highest prevalence in Ashkenazi Jews, are weakness beginning at 3 - 6 months of age, with mental and motor deterioration progressing rapidly after 1 year of age. Patients often exhibit cherry-red spots in the macular region. After 18 months, progressive blindness, deafness, convulsions and spasticity occur and death follows usually around 3 years of age (O'Brien, 1973).

Both Batten and Spielmeyer considered their cases to be different from Tay-Sachs disease because of differences in race predisposition, a later age of onset, a more protracted course and differences in ophthalmological findings. Vogt, on the other hand, considered his cases to be essentially similar to those of Tay and Sachs, the only difference being a later age of onset. The common findings were the familial incidence, the clinical signs of blindness and idiocy, and neuropathologically, the distention of neuronal perikarya by lipid material in both brain and retina. This characteristic neuropathological finding became known as the Schaffer-Spielmeyer process, after Schaffer who described it in cases of Tay-Sachs disease in 1905, and Spielmeyer who reported it in his own cases in the same year. Despite further differences between Batten's disease and Tay-Sachs disease, such as different staining behaviour of the intraneuronal material as well as differences in size and weight of brains at autopsy (Zeman and Dyken, 1969), a unitarian concept of amaurotic family idiocy gained widespread acceptance.

The, retrospectively, erroneous acceptance of the unitarian concept (i.e. one disease process due to the neuronal accumulation of lipid material, leading to a variety of disorders, differentiated only by age of onset) led to nosological confusion and a wide variety of "lipidoses" were classified as subtypes of amaurotic family idiocy. In the last two decades those neuronal lipidoses associated with an inborn error of lysosomal catabolism have been clearly differentiated. Tay-Sachs disease is now classified as a lysosomal storage disease according to the concept of Hers (1965). Affected patients have a deficient activity of the lysosomal enzyme hexosaminidase A, which results in the lysosomal accumulation of G_{M2} ganglioside (Okada and O'Brien, 1969), hence the term G_{M2} Gangliosidosis. The Batten-Spielmeyer-Vogt syndrome or "juvenile amaurotic family idiocy" has not yet been defined in biochemical terms. Undefined also, remain at least two further types of amaurotic family idiocy which are usually also classified as neuronal ceroid-lipofuscinoses.

In 1913, Bielschowsky, in Germany, delineated "late infantile familial amaurotic idiocy" from both Tay-Sachs disease and juvenile amaurotic idiocy as defined by Vogt. He observed severe cerebellar atrophy and considered his cases to be identical to those of Jansky, who in 1908 observed four siblings with a disease beginning during the fourth year of life. All suffered from convulsions, lost the ability to speak and walk and became blind; all were idiotic and died between ages 4 and 6 years. In 1925, Kufs, in Germany, described a disease which he called the "adult form of amaurotic family idiocy" in two siblings. The age of onset was in the late twenties, both patients were demented but neither was blind. Hallevorden described

a similar disease entity in 1938, and the eponym Kufs-Hallevorden disease is sometimes encountered in the literature.

A detailed clinical and genetic study of 115 patients with juvenile amaurotic idiocy from 50 different families in southern Sweden was reported by Sjögren in 1931. He found a range of onset from age 1 - 19 years, with the majority of patients becoming blind at age 5 - 9 years. He considered his cases to be identical with those described by Batten, Mayou, Vogt and Spielmeyer. Referring to the late infantile form of Jansky-Bielschowsky and the adult form of Kufs, he stated: "I see no reason for considering or discussing these two diseases." He proposed a recessive mode of inheritance.

Despite Sjögren's statement, a late infantile (Jansky-Bielschowsky) and adult (Kufs) form are generally classified with the juvenile (Batten, Mayou, Spielmeyer, Vogt, Sjögren) form under the general heading of neuronal ceroid-lipofuscinosis. To this list can be added an infantile form described by Haltia *et al.* (1973a, 1973b) and Santavuori *et al.* (1973). The infantile form of NCL is characterised by an onset of illness during the first or second year of life, a very rapid course and blindness developing early.

Not all disease entities classified as neuronal ceroid-lipofuscinoses fit the above categories exactly. A subgroup called "early juvenile" was described by Lake and Cavanagh (1978). A protracted form of juvenile NCL has been differentiated from the classical Kufs type by Goebel *et al.* (1976). An atypical juvenile neuronal ceroid-lipofuscinosis, showing absence of visual symptoms and retinal abnormalities was described by Greenwood and Nelson (1978).

A disease entity which is sometimes included under the neuronal ceroid-lipofuscinoses is the pigment variant of Kufs disease, which shows all the features of the latter as well as extra-neuronal pigment accumulation (Seitelberger and Simma, 1962; Koroupian and Ross, 1977; Jervis and Pullarkat, 1978).

In summary, the descriptive term "neuronal ceroid-lipofuscinosis" introduced by Zeman and Dyken (1969), has gained widespread acceptance. Realisation that cell types other than neurons were involved in lipopigment storage has led to the introduction of the term "generalised ceroid-lipofuscinosis" (Joosten *et al.*,1973). Neuronal or generalised ceroid-lipofuscinosis is synonymous with Batten's disease and some of the amaurotic family idiocies. These are umbrella terms which tend to obscure the differences between the various subtypes. Only when the chemical nature of the metabolic errors involved in this heterogeneous group of diseases is elucidated will a rational nomenclature become possible.

II. CLINICAL FINDINGS

The principal clinical findings of the human neuronal ceroid-lipofuscinoses, also called generalised ceroid-lipofuscinoses (Joosten *et al.*,1973), are blindness, loss of mental faculties, loss of motor power, seizures and a variable course inexorably leading to premature death (Zeman and Siakotos, 1973; Zeman, 1976). There exists wide variation in age of onset, presenting signs and clinical course in patients affected with NCL. However, a striking feature of the disease is the similarity in age of onset and clinical course

of affected siblings for which the terms homochronism and homotypism are used respectively.

Classification

Various attempts have been made to delineate distinct subgroups of the neuronal ceroid-lipfuscinoses, based on clinical findings, especially on the age of onset of disease. The classical division differentiates late infantile (Jansky-Bielschowsky), juvenile (Batten-Spielmeyer-Vogt) and adult (Kufs) types with respective ages of onset of from 2 - 4 years, 4 - 8 years and 20 years and over (Zeman and Siakotos, 1973). To this list can be added an infantile type with age of onset of 1 - 2 years (Santavuori *et al.*, 1973; Haltia *et al.*, 1973a, 1973b).

There is by no means universal agreement on the classification of distinct subgroups and because of the clinical variations, more recent authors (e.g. Dekaban and Herman, 1974; Lake, 1977) have adopted a multi-disciplinary approach. Based on clinical, histochemical, ultrastructural and electrophysiological findings, Lake (1977) divides his cases into infantile, late infantile and juvenile subgroups. These three types and an adult type will be used as guidelines in this review, and Table 1.I, summarises the findings of recent authors using the criteria of Lake (1977). Those entities which do not exactly fit the four subtypes as outlined in Table 1,I, such as the early juvenile form (Lake and Cavanagh, 1978), protracted juvenile form (Goebel *et al.*, 1978), and pigment variant of the adult type (Jervis and Pullarkat, 1978) will be referred to where appropriate.

TABLE 1.I

Classification of the neuronal ceroid-lipofuscinoses

Subgroup	Approximate age of onset (years)	Presenting symptoms	EEG	White blood cell appearance	Neuronal staining	Ultrastructure	Reference
	1-2	Mental and motor retardation, visual failure	Flat by 3 yrs	Normal	PAS,SB,Fℓ	Granular osmio philic deposits	Santavuori *et al.* (1973); Haltia *et al.* (1973a, 1973b)
Infantile	1	"	"	"	"	"	Hagberg *et al.* (1974)
	1	Mental and motor retardation	"	"	"	"	Lake (1977)
	2-5	Neurological and mental deterioration	Epileptiform	-	SB,Fℓ	Curvilinear or membranes in stacks	Dekaban and Herman (1974)
Late infantile	2-4	Convulsions	Dysrhythmic with spike and wave complexes	Frequent neutrophilic hypergranulation	PAS,SB,ORO Fℓ	Curvilinear, fingerprint, granular	Zeman and Siakotos (1973); Zeman (1976)
	2	Fits, speech difficulty	Response to photic stimulation	Normal	SB,LFB,Fℓ	Curvilinear	Lake (1977)

contd.

TABLE 1.I (contd.)

Subgroup	Approximate age of onset (years)	Presenting symptoms	EEG	White blood cell appearance	Neuronal staining	Ultrastructure	Reference
	7-10	Impaired vision	Epileptiform	-	SB,ORO,Fℓ	Focal densities, fingerprints	Dekaban and Herman (1974)
Juvenile	4-8	Blindness	Dysrhythmic	Lymphocytic vacuolation, neutrophilic hypergranulation	LFB,PAS,SB, ORO, Fℓ	Fingerprint, curvilinear, granular	Zeman and Siakotos (1973); Zeman (1976)
	6	Blindness	-	Vacuolated lymphocytes	PAS,SB,LFB, Fℓ	Fingerprint bodies	Lake (1977)
	31	Psychological and motor disturbance	Spikes, sharp waves, slowing	Some neutrophilic hypergranulation	PAS,LFB,Fℓ	Osmiophilic granules	Boehme *et al.* (1971)
Adult	11 and over	Myoclonic jerks, chorioathetosis	Epileptiform	-	SB, Fℓ	Lipid droplets, granular	Dekaban and Herman (1974)
	20 and over	Mental and motor disturbance	-	-	SB,PAS,ORO, Fℓ	Granular with lipid vacuoles	Zeman and Siakotos (1973); Zeman (1976)

PAS - Periodic Acid Schiff; SB-Sudan Black; ORO- Oil Red O; LFB- Luxol Fast Blue; Fℓ- Fluorescence

Visual and ophthalmological findings

Although rare in the adult form (Dom *et al.*, 1979), visual disturbance is a common and characteristic finding in the neuronal ceroid-lipofuscinoses. Lack of vision is commonly the presenting sign in the juvenile form and is considered highly characteristic by some authors (Zeman and Siakotos, 1973; Lake, 1977). In the infantile form Santavuori *et al.* (1973) reported that visual impairment was established in all their patients at 18 months of age. In the late infantile form visual disturbance usually occurs later in the disease when the patient is already severely debilitated (Zeman *et al.*, 1970). When blindness has ensued in patients with NCL , the pupillary reflex and electroretinogram are abolished.

Patients suffering visual impairment may show all or some of the following ophthalmological abnormalities: atrophy and/or waxy appearance of the optic disc, pigmentary changes of the retina and retinal atrophy. The pigmentary changes may be confined to irregular granular or "salt and pepper" patterned depositions in the macula or periphery of the retina. At other times, "bone-spicule like" changes may be seen in the periphery or other parts of the retina, the so-called *retinitis pigmentosa* (Zeman *et al.*, 1970; Zeman and Siakotos, 1973). In the infantile type of Santavuori *et al.* (1973), pigmentary change was in the form of a hypo-pigmented dystrophic retinal periphery. Retinal atrophy is a result of degeneration and disappearance of the layer of rods and cones and outer nuclear layer, permitting visualisation of the choroid vessels. There is also an accompanying attenuation of retinal vessels (Zeman *et al.*, 1970).

Mental abnormalities

The dementia of the neuronal ceroid-lipofuscinoses is non-specific, but progressive (Zeman *et al.*, 1970). In the infantile and late infantile subgroups, mental deterioration is exhibited as a retardation of normal mental development. The children become withdrawn, lose interest in their environment and stop speaking or seeking the attention of their parents. Some are apathetic, others become irritable and difficult to manage. In one to a few years, the patients become completely unresponsive to their environment (Zeman *et al.*, 1970; Santavuori *et al.*, 1973).

In the juvenile type, the earliest manifestation of mental deterioration is a loss of acquired knowledge (Sjögren, 1931). Patients lose the ability to concentrate or memorise and show lack of motivation. As the disease progresses, patients usually become more apathetic, but may show aggressive behaviour and rarely psychotic manifestation such as paranoia and hallucinations (Zeman and Siakotos, 1973). Dementia may be present in the adult form, but if encountered is less severe than in the other forms of NCL and does not affect all mental faculties. Fine *et al.* (1960), in a review of the literature recorded that mental disturbances occurred in nearly all 18 cases, but their own patient remained mentally alert.

Motor symptoms

In the infantile subgroup, motor development ceases around 18 months of age. Later, affected patients show generalised hypotonia, truncal and limb ataxia, exaggerated tendon reflexes and clonus. At a still later stage patients show hypotonia and episodes of opisthotonus

with hypertonic flexion in the arms and extension of the legs. In the terminal stage there is permanent flexor tonus in all limbs (Santavuori *et al.*, 1973).

Patients with the late infantile and juvenile forms of NCL develop motor disturbances varying from an initial partial loss of ability to perform coordinated movements to a terminal incapacitating rigidity with flexion contractures of neck, trunk and extremities. Often an initial clumsiness develops into an incoordination of gait with ataxia of the legs. Patients may show difficulty in initiating movement, but succeed in carrying out a movement once started. The upper extremities retain their use longer, despite abnormal posturing and hyperkinesia. Mastication and glutition are impeded and there is imperfect articulation of speech (Zeman and Dyken, 1969).

Motor disturbances in the adult form are usually less incapacitating. Affected patients may suffer from cerebellar ataxia and show stiffness, peculiar posture, loss of associated movements and akinesia (Zeman *et al.*, 1970).

Seizure disorders and electro-encephalogram (EEG) findings

Convulsive episodes are highly characteristic of the late infantile type of neuronal ceroid-lipofuscinosis and commonly occur in the infantile, juvenile and adult types (Zeman and Dyken, 1969; Santavuori *et al.*, 1973; Dekaban and Herman, 1974; Dom *et al.*, 1979). Seizures, which are often the presenting sign in the juvenile form, are most commonly of the *grand mal* type, but may take the form of myoclonic or *petit mal* spells or may occur as tonic spasms,

psychomotor, massive myoclonic, akinetic and cerebellar fits. In the terminal phase, regardless of age, patients become comatose with a myoclonic hyperexcitability. The frequency of convulsions varies from a few times during a year to a virtual *status epilepticus*, and when present, convulsions tend to increase in frequency towards the end of the course of the disease (Zeman *et al.*, 1970).

The EEG abnormalities consist of dysrhythmia in addition to spike and wave complexes and scattered high amplitude delta waves. Serial EEG's reflect the progressive worsening of clinical signs and symptoms in the form of deteriorating background rhythm and less pronounced paroxysmal activity (Zeman and Siakotos, 1973). Ultimately the EEG becomes isoelectric. Zeman *et al.* (1970) reported that photic stimulation evoked occipital spikes in some of their patients suffering from the late infantile and juvenile forms of NCL. No responses could be recorded in blind patients. There was no response to photic stimulation in the patients of Santavuori *et al.* (1973). Dom *et al.* (1979) reported a mild diffuse dysrhythmia as their EEG findings in two patients suffering from the adult form of NCL.

Radiological findings

X-rays and pneumoencephalography may reveal a total increase in thickness of the *calvaria* and varying degrees of cerebral and cerebellar atrophy, enlarged subarachnoid spaces and dilation of the ventricles. In general the degree of cerebral and cerebellar atrophy is more severe in entities with early onset (Hagberg *et al.*, 1974; Zeman *et al.*, 1970; Fine *et al.*, 1960). Patients with the infantile and late infantile forms of NCL also show microcephaly (Zeman *et al.*, 1970; Hagberg *et al.*, 1974).

Leukocyte abnormalities

Lymphocytic vacuolation was first observed by Bagh and Hortling (1948) in juvenile neuronal ceroid-lipofuscinosis. This observation has been confirmed by numerous authors (e.g. Rayner, 1962; Zeman and Strouth, 1967; Schwendemann , 1976; Schuurmans Stekhoven *et al.*, 1977; Noonan *et al.*, 1978; Nakano *et al.*, 1979). The percentage of vacuolated lymphocytes varies from 0.5 - 77% with a high fluctuation in individual cases at different times. Vacuolation is not present in the infantile form (Santavuori *et al.*, 1973; Lake, 1977). Although some authors (e.g. Schuurmans Stekhoven *et al.*, 1976; Lake, 1977) consider the absence of vacuolated lymphocytes a characteristic finding in late infantile NCL, others (Witzleben, 1972; Zeman and Siakotos, 1973; Schwendemann, 1976) report that lymphocytic vacuolation may be present in this subgroup. Vacuolated lymphocytes have not been reported in the adult form of NCL. Schwendemann (1976) and Schuurmans Stekhoven *et al.* (1977) emphasize that vacuolation of lymphocytes is not specific for the neuronal ceroid-lipofuscinoses.

Azurophilic hypergranulation of neutrophils has at one time or another been reported in all forms of NCL (Santavuori *et al.*, 1973; Zeman and Dyken, 1969; Boehme *et al.*, 1971). Both lymphocytic vacuolation and neutrophilic hypergranulation may be found in parents and siblings of affected patients which show these abnormalities, thus suggesting that the leukocyte abnormalities behave as markers for the mutant gene (Rayner, 1962; Zeman and Dyken, 1969).

III. PATHOLOGICAL FINDINGS

Although neuronal ceroid-lipofuscinosis manifests as a nervous disorder, visceral involvement is widespread, and some authors (e.g. Joosten *et al.*, 1973; Anzil *et al.*, 1975; Schwendemann, 1976) prefer the term generalised ceroid-lipofuscinosis. It appears that only in neurons, the formation and accumulation of autofluorescent lipopigments leads to disturbance of function with characteristic clinical manifestations.

Gross findings

Gross abnormalities due to NCL are restricted to the skull, brain and spinal cord and tend to be more severe in entities with early onset.

In the infantile form, Haltia *et al.* (1973a), Hagberg *et al.* (1974), Santavuori *et al.* (1974), reported pronounced thickening of the skull bones, with a thick layer of gelatinous tissue on the inner aspect of the *dura mater* and thickening and oedema of the leptomeninges. The brains were noted to be small, due to diffuse cerebral gyral atrophy. Brain weight varied from 305 - 420 g. The cerebral cortex was 1.0 - 1.5 mm thick, yellowish grey and gelatinous in appearance. The ventricles were dilated, while basal ganglia and thalamus were shrunken. The cerebellum was small and firm with atrophic folia. The spinal cord was less affected, but the grey matter in the anterior horns was conspicuously yellow. With the exception of atrophic optic nerves, there was no gross abnormality of cranial nerves, spinal roots and sciatic nerves.

Zeman *et al.* (1970), in their review of the neuronal ceroid-lipofuscinoses, commented that the most striking gross abnormalities were cerebral atrophy and reduced brain weight. The ranges of brain weights for the late infantile form were 254 - 800 g, and for the juvenile form 410 - 1200 g. Coronal sections of brain revealed a characteristic yellow-brown colour of the cortex and to a lesser extent, of the subcortical grey matter. Thickening of the skull is usually present in both types, while optic nerve atrophy and cerebellar atrophy are present in the late infantile, but need not be present in the juvenile form.

The adult form of NCL shows cerebellar atrophy, with gaping folia and a firm reduced central white matter at autopsy (Fine *et al.*, 1960; Boehme *et al.*, 1971). In a recent case Dom *et al.* (1979), reported a generalised cortical atrophy of cerebrum and cerebellum and a mild dilation of the ventricular system.

Histopathology

CNS and retina: The characteristic microscopic change in nerve cells of brain and retina was first described by Spielmeyer (1905). He noted an irregular distention of neurons due to the intracytoplasmic deposition of a yellow coloured granular material. This material is now considered to consist of the lipopigments, ceroid and lipofuscin (Zeman and Alpert, 1963; Siakotos *et al.*, 1970), with ceroid predominating in the late infantile (Wolfe *et al.*, 1977) and juvenile (Braak and Goebel, 1978) forms. Accumulation of lipopigments in nerve cells is the common morphologic feature of all forms of NCL. However the extent of neuronal involvement and sequelae of pigment

accumulation such as neuronal atrophy and death, as well as neuroglial and mesodermal involvement varies between the different types, albeit with considerable overlap.

The infantile form shows a qualitatively stereotyped histological picture with the only variation being the degree of tissue alteration, according to Haltia *et al.* (1973a, 1973b). They studied the microscopic pathology using brain biopsy material in twelve cases and autopsy material in three cases. In the cerebral cortex the cytoarchitecture was severely disturbed, with marked loss of neurons. The remaining neurons had large vesicular nuclei and the cytoplasm was distended by granular material or irregular homogeneous inclusions. Coarse granule containing macrophages, often with two or three nuclei were seen between the cortical neurons. In the biopsy material, neuronophagia was not a prominent feature, whereas it was common in the autopsy material. There was intense cortical fibrillary astrocytosis; the astrocytes had coarse processes and contained granular storage material similar to that of the neurons. Other changes were a total loss of neurons from the cerebellar cortex, with hypertrophy of glial cells, which contained considerable amounts of storage material. Neurons in the brain stem and basal ganglia were swollen by large amounts of characteristic storage material. The white matter showed hypertrophy and an increase in number of astrocytes with characteristic granular inclusions. In older patients there was loss of axons and myelin sheaths with an accompanying fibrillary astrocytosis. The characteristic inclusions were auto-fluorescent in ultraviolet light, were acid-fast, strongly PAS-positive and stained intensely black with Sudan black B.

The retina showed severe atrophy, with complete loss of ganglion cells, rods and cones and bipolar cells, proliferation of glial elements and large pigment laden macrophages. No myelin sheaths were left in the atrophic and gliosed optic nerves.

The late infantile form showed fine and coarse granular material within the majority of neuronal perikarya, which were mildly distended, according to a recent description of light microscopic findings by de Baecque *et al.* (1976). The cerebellum showed a reduction in the number of Purkinje cells, a striking loss of granular cells and a prominent Bergmann cell gliosis. Neurons in the spinal cord were extremely distended with frequent displacement of the nucleus and Nissl substance. The granular material showed a yellowish fluorescence under ultraviolet light. Sudan black and PAS positive material was also found in the cytoplasm of glial and endothelial cells. The white matter change consisted of mild demyelination and proliferation of astrocytes. Seitelberger *et al.* (1967) described a second type of cytoplasmic inclusion in nerve cells, these being irregularly shaped homogeneous spheroidal bodies of different sizes, which occur in addition to the granular deposits. These bodies were encountered most regularly in the *substantia nigra* but occurred elsewhere in the brain.

The retinal lesions consist of loss of rods and cones, variable accumulation of lipopigment in ganglion cells, and a variable degree of optic nerve atrophy (Zeman, 1976).

The juvenile form is characterised by moderate distention of

neuronal perikarya due to the accumulation of autofluorescent pigments. Nerve cell loss is usually not very marked, although the cells of the cerebellar granular layer may be missing or reduced in number. Pigment also accumulates in astrocytes which usually show moderate proliferation. The white matter shows a discrete astrocytic gliosis. In the retina, the ganglion cells contain considerable amounts of lipopigment. There is complete loss of rods and cones and outer nuclear layer (Zeman, 1976). While agreeing that neuronal loss in the juvenile form is moderate, Braak and Goebel (1978) report an almost complete to total loss of small pigment-laden stellate cells in the iso-cortex of an 18 years old patient. They postulated that the selective involvement of these local circuit neurons may be causally related to the functional impairment of the brain.

The adult form shows ubiquitous accumulation of granular lipopigment in neurons. There is loss of Purkinje cells and granular cells in the cerebellum. Spheroidal inclusions may be seen in thalamic neurons and in the *substantia nigra* (Boehme *et al.*, 1971). Retinal abnormalities had not been described (Zeman, 1976) until the recent case of Dom *et al.* (1979). In the retina of their patient they found the external segment of most rod cells to be distended with brown-yellow granules. There was also retinal atrophy and loss of ganglion cells with ballooning of persistent neurons by ceroid-lipofuscin granules.

Several authors (Seitelberger and Simma, 1962; Horoupian and Ross, 1977; Jervis and Pullarkat, 1978) have described a pigment variant of Kufs disease or adult neuronal ceroid-lipofuscinosis.

This disease resembles Kufs disease clinically, but pathologically presents additional features in the form of extra-neuronal pigment deposits which are most obvious in the *globus pallidus* and *substantia nigra*. The nature of this extra-neuronal pigment and its relation to ceroid and lipofuscin remain unknown (Jervis and Pullarkat, 1978).

Visceral lesions: A systematic examination of visceral involvement in three cases of the juvenile form of NCL using light and ultra-violet microscopy was reported by Kristensson *et al.* (1965). They found deposits with staining and fluorescence characteristics similar to those in neurons of the central nervous system in cells of the following tissues or organs: dorsal root ganglion cells, autonomic nerve cells of the intestinal wall, perinuclear region of myocardial muscle cells, epithelium of the distal part of collecting tubules in the kidneys, large reticulo-endothelial cells in spleen, thymus, lymph nodes and bone marrow, parenchymal and Kupffer cells in the liver. Variable amounts of fluorescent granular material were also found in the anterior lobe of the pituitary, pancreas, adrenals, thyroids and gonads. Inclusions in the thyroid glands were also described by Dayan and Trickey (1970). Miley *et al.* (1978) described the presence of sea-blue histiocytes in the bone marrow of a 6 years old boy with juvenile NCL.

Using the Sudan IV staining technique Dolman and Chang (1972) examined frozen and paraffin embedded sections of a large number of visceral organs in two cases of late infantile NCL. They found

intracytoplasmic Sudan IV positive staining material, varying from single small inclusions to multiple granules in cells of heart, aorta, lung, pituitary, thyroid and adrenal glands, bone marrow, lymph nodes, kidney, urinary bladder, striated muscle and skin. Nowhere was there abnormal distention of the cells as occurs in the nervous system. Similar findings were reported by de Baecque *et al.* (1976).

Visceral involvement in the infantile type of NCL was described by Haltia *et al.* (1973b). They found abundant cytoplasmic deposits in epithelial cells of the thyroid, pancreas, kidney and testis, while hepatocytes seemed not to be involved. Characteristic inclusions were also found in skeletal and cardiac muscle, and smooth muscle of the intestinal wall. Large macrophages with sudanophilic granules in their cytoplasm were seen in spleen, lymph nodes, bone marrow and *lamina propria* of intestinal muscosa. Alveoli and interstitial tissue of the lung and Kupffer cells in the liver also contained a large number of granules, while fibrocytes seemed to be free of the material.

Most reports on the adult form of NCL fail to mention visceral involvement, which could party be due to incomplete examination, although Fine *et al.* (1960) mention negative findings. Kornfeld (1972) reports involvement of Kupffer cells, glomerular podocytes and large mononuclears in spleen, bone marrow and lung. Dom *et al.* (1979) showed involvement of liver, heart and skeletal muscle. In the pigment variant of Kufs disease, Jervis and Pullarkat (1978) described the presence of lipofuscin in liver, spleen and kidney.

Ultrastructural findings

The electron microscope has been a useful tool in delineating the neuronal ceroid-lipofuscinoses from the gangliosidoses. Terry and Korey (1960) described the ultrastructure of the neuronal inclusions in Tay-Sachs disease. The first ultrastructural observations of the inclusions of juvenile amaurotic idiocy were reported by Zeman and Donahue (1963). The authors used the term "juvenile" to distinguish their cases from Tay-Sachs disease, however if the guidelines of Table 1.I are used their patients would probably be classified with the late infantile subgroup of NCL. They observed pleomorphic membrane bound lipopigment bodies of variable size in neuronal perikarya, glia and endothelial cells. They described two characteristic internal structures, i.e. the larger bodies displayed irregular aggregations of membranous material which formed round, oval or tubular structures which gave the cross-section a multilocular appearance, while the smaller bodies contained dense granular material in which tubular structures were commonly seen. Koenig *et al.* (1964) demonstrated acid phosphatase activity in inclusions of NCL, thus indicating their probable lysosomal location.

The multilocular bodies are also described as multi-lamellar cytosomes (Gonatas *et al.*, 1968), and curvilinear bodies (Duffy *et al.*, 1968). The latter described the bodies as being irregular in outline and shape and having a range in size from 0.2 - 2.6 µm. The bodies were composed of short curvilinear profiles which were either multi-directional or ran a short parallel course presenting a tubular appearance. A few profiles formed complete circles and at high magnification the profiles were in part composed of fine osmiophilic

granules. At times the curvilinear body was lined by a limiting membrane but more often the external border was formed by curvilinear profiles similar to those seen throughout the mass. The spheroidal inclusion body described by Seitelberger *et al.* (1967) was shown to consist of fine, highly electron dense granules amongst which curvilinear profiles could be discerned. Another structure rarely seen was composed of moderately broad dense parallel lines extending across the inside of an ovoid body bounded by a membrane.

Inclusions from 1.0 - 4.0 μm in diameter with a fingerprint-like pattern, consisting of a series of closely packed paired dense membranes, each pair separated from the next by a space, were described by Suzuki *et al.* (1968) in the juvenile form of NCL. "Each dense line measured about 27Å in thickness, the clear zone within the pairs measured 14 to 20Å and the space between the pairs was 25Å wide." They also found inclusions 2.0 - 5.0 μm in diameter which resembled the membranovesicular bodies first described by Gonatas *et al.* (1963). These bodies "were composed of irregularly folded wavy membranes 65 to 100Å thick and of slightly electron dense vesicular structures." Inclusions showing both fingerprint pattern and membranovesicular features were occasionally observed. Yet another type of body present in the neuronal perikarya was moderately dense, granular and lysosome-like. At times these bodies contained fine, curved or straight linear structures.

Membrane bound lipid bodies consisting of a dense granular matrix which partially surrounds large empty spaces were described by Zeman *et al.* (1970). They considered these to be very similar to the lipofuscin granules found in senescent brains.

The residual bodies of the adult form of NCL usually contain granular (Kornfeld, 1972) and occasionally additional membranous components (Chou and Thompson, 1970). Dom *et al.* (1979) found two types of neuronal inclusions in their case of Kufs disease. Some appeared like typical lipofuscin, but the majority contained curvilinear profiles. In the infantile form, the osmiophilic storage material is composed of globular particles 0.2 - 0.5 µm in diameter and of larger aggregates up to 3.0 µm in diameter. Both types of inclusions are surrounded by a unit membrane and have an electron dense homogeneous and finely granular internal structure. Occasionally, a lamellar or membranous structure may be seen within the granular matrix (Haltia *et al.*, 1973b).

Gonatas *et al.* (1968), and Duffy *et al.* (1968) attempted to correlate ultrastructural findings with clinically differentiable entities of the neuronal ceroid-lipofuscinoses. However, Towfighi *et al* . (1973), using the material of Gonatas, came to the conclusion that the pleomorphism of the reported inclusions in late infantile and juvenile amaurotic family idiocy probably reflected manifestations of a single or closely related disorders. They also confirmed that the nature of the various inclusions did not change in time. Both these observations had already been stressed by Zeman *et al.* (1970), who pointed out that in general the curvilinear type of inclusion predominates in the late infantile form, while the fingerprint patterned inclusions are more common in the juvenile form of NCL. This observation was confirmed by Goebel *et al.* (1979) who stated: "Although the classical subgroups of NCL contain electronmicroscopically well defined residual bodies, permitting distinction of the

late infantile type from the juvenile type, the ultrastructural differences are more of a quantitative than of a qualitative nature."

Numerous authors have confirmed visceral involvement electron-microscopically in virtually every organ examined in the infantile (Haltia *et al.*, 1973b), late infantile (e.g. Duffy *et al.*, 1968; Andrews *et al.*, 1971; Dolman and Chang, 1972; de Baecque *et al.*, 1976) and juvenile (e.g. Carpenter *et al.*, 1972; Towfighi *et al.*, 1973; Miley *et al.*, 1978) forms of neuronal ceroid-lipofuscinosis. Electromicroscopically, visceral involvement in the adult from has been reported by Kornfeld (1972) and Dom *et al.* (1979).

Of diagnostic significance is the occurrence of residual bodies in skin (Dolman *et al.*, 1975; Ceuterick *et al.*, 1976; Farrell and Sumi, 1977; Sipe and O'brien, 1979), sural nerve (Joosten *et al.*, 1973), autonomic ganglia and macrophages in rectal mucosa (Lake, 1977; Lake and Cavanagh, 1978) and appendix (Van Haelst and Gabreels, 1972; Rapola and Haltia, 1973), lymphocytes (Schwendemann, 1976; Schuurmans Stekhoven *et al.*, 1976, 1977; Noonan *et al.*, 1978; Baumann and Markesberry, 1978; Nakano *et al.*, 1979), urinary sediment (de Baecque, 1975; Armstrong *et al.*, 1977) and skeletal muscle (Goebel *et al.*, 1975; Dom *et al.*, 1979).

IV. BIOCHEMICAL FINDINGS

The lipopigments

The pigment found in nerve and muscle cells of aging mammalian individuals and commonly referred to as "wear and tear" pigment, was termed "lipofuscin" by Borst in 1924. A lipofuscin-like pigment

was observed in livers of rats with dietary cirrhosis by Lillie *et al.*, in 1941 and given the name "ceroid".

The distinction between lipofuscin and ceroid, based on histochemical and ultrastructural grounds, was not clear until Siakotos *et al.* (1970) isolated two distinct lipopigment fractions from human brain. Both fractions could be isolated from brains of patients with any type of neuronal ceroid-lipfuscinosis. However, one fraction was originally isolated from brains of aged but otherwise healthy individuals and was therefore termed "lipofuscin". The other pigment fraction was given the name "ceroid" as it was first obtained from the brain of a patient diagnosed as suffering from ceroid storage disease (Levine *et al.*, 1968). The pigments could be differentiated on the basis of ultrastructure, mass and ultraviolet fluorescence. Both ceroid and lipofuscin contained lysosomal hydrolases, thus revealing their nature as residual bodies (Koenig *et al.*, 1964).

Further studies (Siakotos *et al.*, 1972, 1973) showed that purified lipofuscin is a black-brown pigment with a density of 1.0 - 1.05 and is stable in salt solutions, upon chelation and ion exchange. It has a cation composition of 240 ppm of Ca, 300 ppm of Fe and 400 ppm of Zn. Two-dimensional thin-layer chromatography reveals most of the well defined phospholipids, while the major part of the soluble lipopigment phase remains at the place of application and consists of lipid polymers, which are 92% neutral and 8% acidic (Zeman, 1976). Taubold *et al.* (1975) in their study of the chemical nature of lipofuscin isolated from normal human brain,

found the molecular weight of purified lipofuscin to be between 6000 - 7000 daltons. Infra-red, ultraviolet, neutron magnetic resonance and fluorometric spectra indicated the predominantly lipid nature of lipofuscin. They suggested that lipofuscin consists mainly of polymeric lipid and phospholipid structures along with amino acids bound to the lipids or as included proteins.

Ceroid in the purified state is a yellow pigment with a density of 1.25 - 1.30. It occurs in brains of patients with NCL, livers from rats with dietary cirrhosis and is the age pigment in *Drosophilae*. In contrast to lipofuscin, ceroid is unstable in salt solution, upon chelation, and ion exchange; its cation composition is 960 ppm of Ca, 1500 ppm of Fe and 110 ppm of Zn. Two-dimensional thin-layer chromatography shows normal sphingolipids. Compared with lipofuscin a smaller amount of the soluble lipopigment phase is retained at the place of application, which in this case consists of 57% neutral and 43% acidic lipid polymers (Zeman, 1976).

The ultrastructure of the two classes of lipopigment is no longer considered a useful indication of lipopigment species, except for the characteristic "curvilinear ceroid" found in NCL (Siakotos *et al.*, 1973).

The fluorescence excitation and emission curves of ceroid and lipofuscin are similar though not identical. Lipofuscin has an excitation maximum at 360 nm and emission maximum at 450 nm, whereas ceroid has excitation maxima at 290 nm and 350 nm, and an emission maximum at 435 nm. Chio and Tappel (1969a, 1969b) showed that

Schiff-bases chemically synthesized from malonaldehyde and amino acids or other amino compounds have fluorescence spectra virtually identical to those of the lipopigments. They concluded that the fluorescence in the synthesized compounds as well as in the lipopigments is due to chromophores with the same basic structure : a 1 - amino - 3 - iminopropene (R-N=CH-CH= CH-NH-R), formed when malonaldehyde cross-links with the primary amino groups of proteins, nucleic acids and their bases or phospholipids. Since malonaldehyde is a known product of the peroxidation of unsaturated fatty acids, they suggested that lipopigment formation could be due to a disorder of peroxidation.

Recent work by Wolfe *et al.* (1976, 1977) led them to dispute the Schiff-base nature of the fluorophore in ceroid, obtained from the cerebral cortex of a child who had died from the late infantile form of NCL. The ceroid containing residual bodies were isolated by density gradient centrifugation followed by pronase digestion and showed intense fluorescence and a typical ultrastructure of curvilinear bodies. After extraction by a mixture of chloroform and methanol, 57% of the dryweight of the storage material remained as a water-insoluble amorphous fluorescent residue. According to the authors, results of infra-red spectroscopy, proton magnetic resonance spectroscopy, mass spectrometry, base hydrolysis, methanolysis and ozonolysis, indicated that the fluorescent component of the neuronal storage material is a retinoly complex and is not derived from peroxidised polyunsaturated fatty acids. In the light of their findings, the authors suggested that the chemical nature of lipofuscin be reinvestigated.

Peroxidase deficiency

The first reports that patients suffering from the late infantile, juvenile and dominant adult forms of NCL showed a peroxidase deficiency came from Armstrong *et al.* (1973, 1974a, 1974b). They found deficient myeloperoxidase activity in peripheral granulocytes by both histochemical and spectrophotometric methods using benzidine and p-phenylenediamine as hydrogen donors respectively. Armstrong *et al.* (1973) also found a decrease of leukocyte peroxidase activity in both parents of a child with NCL. Peroxidase deficiency in patients suffering from NCL has since been demonstrated in several tissues including brain, liver, kidney, thyroid, parotid and submaxillary salivary glands (Armstrong *et al.*, 1974c, 1975). Clausen and Jensen (1975) and Awasthi *et al.* (1977) confirmed the reduction in activity of granulocyte peroxidase in patients with NCL, however, the latter authors found the values of the parents of their late infantile patient to be within normal range.

On the other hand there are a number of publications reporting normal myeloperoxidase activity in granulocytes of patients with NCL (Anzil *et al.*, 1975; Wolfe *et al.*, 1976; Farrell and Sumi, 1977; Den Tandt and Martin, 1978; Pilz *et al.*, 1978). Normal values for peroxidase activity were also reported for saliva and parotid salivary gland in juvenile NCL (Pilz *et al.*, 1976a; Pilz and Goebel, 1977). When leukocyte peroxidase activity was measured as soluble and membrane-bound fractions, Pilz *et al.* (1976b) found that in two patients with the juvenile form, the activity of soluble leukocyte peroxidase was considerably reduced; in one patient with the late infantile form the activity was just below the normal range and activity was normal

in two patients with the adult form. In all patients, the activity of membrane-bound leukocyte peroxidase was not significantly altered. Only one of four obligate heterozygotes for the juvenile type had deficient values for the soluble enzyme. Pilz *et al.* (1976c) found no difference between the isoelectric enzyme patterns of leukocyte peroxidase between patients with NCL and normal controls.

In a study of granulocyte enzyme activities in patients with juvenile NCL, Tsan *et al.* (1978) found p-phenylenediamine to be unsuitable as hydrogen donor for the study of peroxidase. Using guiacol as hydrogen donor they found either normal or reduced peroxidase activity in their patients. All other enzymes studied showed normal activities. Armstrong D. (pers. comm.) points out that leukocyte peroxidase deficiency can be shown only when cetrimide (technicon WBC diluent, cat. no. T01-0486-10. Add 1 ml Technicon Brij-35 per litre of cetrimide) is used to lyse ertythrocytes, in the purification process of the leukocytes. The reason for this phenomenon is not known, but the cetrimide diluent solution contains 0.1% formaldehyde, and this additive possibly has an effect on the stability of the membrane bound peroxidase (Jolly R.D., pers. comm.). Of the authors quoted above, who found normal leukocyte peroxidase activities in patients with NCL, only Farrell and Sumi (1978) reported on the use of 0.2% cetrimide in one of their assays. They described its effect as "solubilizing the peroxidase activity from whole leukocytes".

An additional peroxidase deficiency has been described by Westermarck and Sandholm (1977) and Jensen *et al.* (1978). The

former found a decreased erythrocyte glutathione peroxidase activity in three patients with infantile and nine patients with the juvenile form of NCL. Selenium supplementation resulted in a return to normal values. Jensen *et al.* (1978) confirmed the reduced erythrocyte glutathione peroxidase activity in a group of 10 patients with Batten's syndrome. Using different peroxide donors, their patients could be divided into two groups. One group showed reduced activity using hydrogen peroxide or cumene hydroperoxide as donors, the other showed an increased activity when t-butyl hydroperoxide was used. The authors also reported an inverse relationship between erythrocyte glutathione peroxidase activity and serum eicosatrienoic acid content in their patients. Selenium content of erythrocytes was normal, but values for whole blood were reduced when compared with controls. Jensen *et al.* (1977) found normal or even increased levels of glutathione peroxidase activity in serum from patients with the late infantile form of NCL.

Acid phosphatase activity

In a study of acid phosphatase activity in granulocytes and lymphocytes of patients with NCL, Plum and Nielsen (1977) found a higher activity in patients' lymphocytes compared with normal controls.

Superoxide dismutase activity

Marklund and Plum (1978) found no difference in superoxide dismutase activity in leukocytes and erythrocytes of patients with the juvenile form of NCL, when compared to patients with neurological disease other than NCL and healthy controls.

Abnormal fatty acid concentrations

Hagberg *et al.* (1968) found remarkably low concentrations of docosahexaenoic acid, 22 : 6 (n - 3), which is the major fatty acid derived from linolenic acid in a brain biopsy from a patient with infantile NCL. There was an increase in arachidonic acid, 20 : 4 (n - 6), a major fatty acid derived from linoleic acid as well as an increase in 22 : 4 (n - 6). These findings were confirmed and elaborated upon by Svennerholm *et al.* (1975). A 54% to 93% reduction in docosahexaenoic acid was reported by Pullarkat *et al.* (1978) in leukocytes of four patients with juvenile NCL. Parents of the patients also showed a reduction in docosahexaenoic acid content but to a lesser degree. The levels of the linoleic family (n - 6) of polyunsaturated fatty acids was unchanged. Jervis and Pullarkat (1978) found a 35% depletion of docosahexaenoic acid in serine phosphoglycerides of the brain grey matter without any change in the fatty acid composition of other phospholipids. Jensen *et al.* (1978) reported an increased content of palmitic, oleic and eicosatrienoic and decreased content of linoleic acid in the serum of a group of 10 patients with Batten's syndrome.

Abnormal sialoglycoprotein concentrations

Adelman *et al.* (1974) reported an abnormal accumulation of sialoglycoproteins in the cerebrum of a patient with the late infantile form of NCL.

V. ASPECTS OF PATHOGENESIS

The metabolic disorders in the various forms of neuronal ceroid-lipofuscinosis are unknown. All types of NCL are however

characterised by the excessive accumulation of autofluorescent material within lysosomes and NCL is thus classified as a lysosomal storage disease. Most inherited lysosomal storage diseases are characterised by a deficiency of a lysosomal hydrolase (Hers, 1965). No such enzyme deficiency has been shown for NCL, however Jolly (1978) postulates that any undigestible material that accumulates in cells, due to an inherited deficiency of a non-lysosomal enzyme, would eventually end within the lysosomal system through the process of autophagy.

There are three major hypotheses concerning the formation of the autofluorescent pigments in the neuronal ceroid-lipofuscinoses.

1. The finding by Armstrong *et al.* (1974a) of peroxidase deficiency in neutrophils of patients suffering from NCL created great interest and appeared to lend support to the hypothesis of lipopigment formation due to a disturbance in peroxidation of polyunsaturated fatty acids (p. 29, Zeman and Siakotos, 1973; Zeman, 1974; Awasthi *et al.*, 1977). However, Pilz *et al.* (1976a, 1976b, 1976c, 1978), on the basis of their findings did not accept a generalised peroxidase deficiency as a causative factor in NCL. Similarly, Anzil *et al.* (1975) failed to demonstrate myeloperoxidase deficiency in their case of late infantile generalised ceroid-lipofuscinosis and suggested that myeloperoxidase deficiency could be a phenotypic marker in some types of NCL but not the genetic defect. This seems to be supported by the results obtained by Tsan *et al.* (1978). A known inherited disorder characterised by a total absence of myeloperoxidase from neutrophils and monocytes is not associated with the

symptoms found in NCL (Lehrer and Cline, 1969; Cech *et al.*, 1977).

Superoxide dismutase, an enzyme which *in vitro* inhibits lipid peroxidation was found to have normal activity in erythrocytes and lymphocytes in 10 patients with juvenile NCL (Marklund and Plum, 1978).

If the formation of the autofluorescent pigment in NCL were primarily due to a disturbance in peroxidation of polyunsaturated fatty acids, antioxidant therapy should prove beneficial. However, results of such therapy have been disappointing. Santavuori and Moren (1977) investigated the effects of antioxidant therapy in 46 patients with juvenile NCL. They found after an observation period of from 5 to 6 years, that the IQ's were higher, the neurological signs less marked and epilepsy less frequent among the patients receiving antioxidants than among non-treated control. However, the treatment did not benefit vision and was unsuccessful in advanced cases.

2. Hagberg *et al.* (1968), on the basis of their findings in infantile NCL advanced the hypothesis that the formation of the autofluorescent pigment was due to a disturbance in the metabolism of the linolenic fatty acid series. Svennerholm *et al.* (1975) proposed that this metabolic disturbance consisted of a defect of the enzyme systems that desaturate and elongate linolenic acid to docosahexaenoic acid. Low levels of docosahexaenoic acid have since been reported in leukocytes in the juvenile (Pullarkat *et al.*, 1978), and in brain grey matter of the adult (Jervis and Pullarkat, 1978) forms of NCL.

3. Wolfe *et al.* (1977), found that the fluorescent component of the storage material in their patient with late infantile NCL was a retinoyl complex. They postulated that the gene defect in the neuronal ceroid-lipofuscinoses involved an enzyme or enzymes that catabolize retinoic acid. Goebel *et al.* (1979) argue against the proposition that the matrix of ceroid consists chiefly of retinoyl compounds on the basis of the results of their neutron magnetic resonance studies of ceroid and retinoic acid.

VI. ASPECTS OF PREVALENCE AND INHERITANCE

The neuronal ceroid-lipofuscinoses are rare diseases, of worldwide distribution. They have been described in all common races and ethnic groups, and in both sexes (Zeman *et al.*, 1970). Due to difficulty in precise diagnosis, exact figures for the prevalence of the various forms are sparse.

The most extensive studies have come from Sweden and concern the juvenile type of NCL. Sjögren (1931) studied clinical aspects of the disease in 115 patients from 50 families. Genetic segregation analysis involving 133 families was consistent with an autosomal recessive monohybrid mode of inheritance. He estimated that the frequency of the disease was 1 in 25,000, giving a gene frequency of 0. 006 and heterozygote frequency of 0.012. Rayner (1962) studied 37 cases of juvenile NCL which occurred between 1950 and 1959. He also showed autosomal recessive inheritance and estimated the prevalence of disease at 1 in 50,000. All his patients showed lymphocytic vacuolation

ranging in frequency from 4.0 - 41% with a mean of 21%. In parents and siblings he found a mean frequency of 1.0% of lymphocytic vacuolation and considered this a useful marker for the heterozygous state. However, lymphocytic vacuolation is by no means specific for the juvenile form of NCL and may occur in the late infantile form and in a wide variety of other pathological conditions (Schwendemann,1976). The ultrastructure of the vacuoles has been described for patients with the juvenile and late infantile forms of NCL (Schuurmans Stekhoven *et al.*, 1976, 1977). No such information is available on the ultrastrucutre of lymphocytic vacuoles in parents or siblings, casting further doubt on the significance of their occurrence. Zeman and Strouth (1967) found vacuolated lymphocytes in eight out of fifteen cases and only occasionally in parents and healthy siblings. They considered azurophilic hypergranulation of granulocytes a more reliable and conspicuous genetic marker. Although not always demonstrable, whenever it was present in a patient, it was always found in his parents as well as in two thirds of his healthy siblings.

The prevalence of the late infantile and adult form is less well documented. No more than 30 cases of the adult type have been reported in the literature. Zeman *et al.* (1970) consider that half of their 30 patients with NCL could be classified as late infantile, thus suggesting a higher prevalence than is generally accepted. Norio *et al.* (1973) in their review of hereditary diseases in Finland state that 10 late infantile, 100 juvenile and 1 adult case are known. Santavuori *et al.* (1974) gave the prevalence of the infantile type in Finland as 7.85 per 100,000.

No extensive genetic investigations have been carried out for the infantile, late infantile and adult forms, however a recessive autosomal mode of inheritance is generally accepted (Norio *et al.*, 1973; Zeman, 1976). As an exception to the rule, Boehme *et al.* (1971) reported a dominant form of the adult type of NCL, suggesting the involvement of more than one allele or allelic pleomorphism or both.

Only two reports in the literature describe the occurrence of more than one clinical form of NCL in the same family (Zeman and Hoffman, 1962; Edgar and Post, 1963) suggesting different specific mutations, a point strongly made by Sjögren (1931) concerning the juvenile form and by Norio *et al.* (1973) concerning the infantile form of NCL. Finally the phenomena of homochronism and homotypism (p.7) led Zeman (1976) to speculate on the possibility of a dihybrid autosomal recessive mode of inheritance.

VII. THE NEURONAL CEROID-LIPOFUSCINOSES IN DOMESTIC ANIMALS

The best documented case of the occurrence of NCL in domestic animals concerns an inbred strain of English Setter dogs (Koppang, 1966, 1970). Reporting on 15 years of research into this disease, Koppang (1973/74) presented canine ceroid-lipofuscinosis as a model for human NCL and aging.

Clinically, animals affected with canine ceroid-lipofuscinosis are normal up to the age of 14 - 18 months, when reduced vision and mental dullness become obvious. From then on mental deterioration

progresses, the animals begin to stagger, there is stiffening of the extremities and convulsions set in at the age of 17 - 24 months, with death inexorably occurring before 26 months. Burcar *et al.* (1977) showed that homozygous affected dogs have abnormal EEG patterns before the onset of clinical signs.

Gross pathology, towards the end of the disease, shows a grossly atrophic, firm, yellow discoloured brain with reduced *grisea*, which weighs about 70% of that of normal controls. The lateral and fourth ventricles are dilated and the amount of spinal fluid is increased.

Light microscopically, the central and peripheral nervous systems show a gradual increase in the amount of autofluorescent PAS and Sudan B positive pigments. At ages 2 - 3 months, some 30% of neurons contain focal cytoplasmic accumulations of pigment which at age 6 months involves virtually all neurons. By the age of 12 months, pigment occupies most of the cytoplasm in a large percentage of neurons and may lead to nuclear pyknosis, rounding of soma and loss of Nissl substance. At ages of 20 -26 months, neuronal cell death becomes apparent and leads to reduction in the size of many *grisea*, especially of the cerebellar cortex. Retinal neurons also contain pigments but show little degenerative change and no cell death, while sensory elements are preserved. There is also a gradual increase of autofluorescent pigment in viscera and other tissues.

Electronmicroscopically, affected puppies, euthanased at 2 days of age show "cytoplasmic condensation" in some neurons. The condensations are less than 1.0 µm in diameter and may be granular or show membraneous profiles and are considered to be precursors of

the autofluorescent pigment. Typical membrane-bounded ceroid bodies with a fingerprint pattern are also observed at that early age showing that canine ceroid-lipofuscinosis is a true inborn error of metabolism which is present *in utero*. As a function of time, the number and size of pigment bodies increases and by the age of 12 months neuronal degeneration and death are observed. The pigment bodies are generally composed of highly organised five-layered membranous profiles and a dense, often granular matrix, sometimes interspersed with electronlucent foci. The membranous profiles may form a large number of different architectural patterns, some of which are further defined as fingerprint, curvilinear or crystalloid. The pigment bodies are also observed in extraneural tissue but significantly do not seem to give rise to cell damage.

Chemically there is a normal ganglioside pattern and pigment extracted from affected English Setters is physicochemically identical with the pigment extracted from the brain of human patients with NCL (Siakotos *et al.*, 1970; 1972). In 1974, Patel *et al.* demonstrated a reduced p-phenylenediamine mediated leukocyte peroxidase activity in affected dogs. Armstrong *et al.* (1978) studied enzyme activities in whole retina and retinal pigment epithelium of affected dogs. They found markedly reduced peroxidase activity in both tissues by 2 years of age. Their findings were confirmed at the subcellular level by Siakotos *et al.* (1978).

Genetic studies show the disease to have an autosomal recessive mode of inheritance (Koppang, 1973/74).

Other reports on the occurrence of ceroid-lipofuscinosis in domestic animals are less well documented and involve only one or a few cases.

Rac and Giesecke (1975) reported on the occurrence of a disease resembling NCL in two unrelated 2 years old Chihuahua dogs. Clinically the animals showed progressive blindness, neurological disturbances and temperament changes. The most striking light microscopical observation was the presence of eosinophilic, weakly acid-fast, PAS and Sudan B positive granular material in the cytoplasm of the majority of neurons and cells of the reticulo-endothelial system. Similar cases and also involving Chihuahua dogs, have been observed in Australia (Hartley W.J., pers. comm.) and in New Zealand (Jolly R.D., pers. comm.).

Cummings and de Lahunta (1977) describe an adult case of canine neuronal ceroid-lipofuscinosis in a 4.5 years old Dachshund bitch. Clinical signs were consistent with a slowly progressive cerebellar disease. Gross pathology revealed a cerebellum two-thirds normal size, moderate enlargement of the lateral and fourth ventricle and a symmetrical distinct yellow discoloration of the cerebellar nuclei. Light microscopically there was loss of Purkinje cells, while surviving Purkinje cells, other neurons and macrophages throughout the neuraxis contained varying amounts of granular cytoplasmic material which was PAS and Sudan B positive and showed yellow-green autofluorescence under ultraviolet light. Electronmicroscopic investigation of affected neurons in the brain stem revealed large numbers of various membrane-bound cytosomes, containing membranous

profiles and granular material and ranging from 0.4 - 2.2 μm in diameter.

Neuronal ceroid-lipofuscin storage in two mature Siamese cats was described by Green and Little (1974). Clinical features were convulsions and mania in one cat, while the other showed irritability and hindleg weakness. Neuronal cytoplasmic inclusions showed similar ultrastructure and staining reactions to the curvilinear bodies described in human NCL. No differences in brain lipid quantity or quality could be detected relative to two control cats.

A disease occurring in an inbred strain of Beefmaster cattle and reported as neuronal lipodystrophy by Read and Bridges (1969) shows clinical, histopathological and ultrastructural features similar to those occurring in NCL in man. An asymptomatic condition, called Xanthosis, with a high prevalence in adult Ayrshire cattle and characterised by the excessive accumulation of a lipofuscin-like pigment in cardiac and skeletal muscle has been described in England (Hayward, 1978; Duffell and Edwardson, 1978). In one survey, the prevalence in Ayrshire cattle was 25%, suggesting that the condition could result from the inheritance of simple recessive gene (Hayward, 1978).

Jolly and West (1976) postulated on the basis of clinical, gross pathological and histopathological findings that the disease entity they had encountered in an inbred flock of South Hampshire sheep was a neuronal ceroid-lipofuscinosis. The results of investigations into clinical, histopathological, electronmicroscopical and genetic aspects of the latter disease form the subject material of this thesis.

CHAPTER II

CLINICAL FINDINGS

I. INTRODUCTION

In January 1976, two 18 months old South Hampshire rams were presented for post-mortem at the Massey University Department of Veterinary Pathology. For about 4 months the owner had noticed unusual behaviour in these rams, inasmuch as they tended to graze away from the flock and were difficult to work with sheep dogs. On clinical examination the rams appeared to be blind and they exhibited muscle tremors, which were aggravated by excitement. The salient post-mortem features were a reduction in size and weight of the brain. After histological examination of brain, eye and spinal cord, a diagnosis of neuronal ceroid-lipofuscinosis was made (Jolly and West, 1976).

Enquiries revealed that the rams originated from a small stud flock in the South Island of New Zealand. The owner of this flock was contacted and he agreed to co-operate in the investigation of this presumably hereditary disease. With sheep obtained from the South Island property an experimental flock was established at Massey University during 1976 and 1977. The original number of animals in the experimental flock were seven ewes, 41 ewe hoggets and one ram. The ram was known to be the sire of affected animals and one of the ewes had also produced an affected lamb. The remainder of the ewes

and ewe hoggets, with the exception of six animals, had been sired by either of two rams, both of which had produced affected lambs. The first lambs in the experimental flock were produced in the spring of 1977. Another crop of lambs was born in 1978.

The owner of the South Island property also undertook to forward to Massey University any of his sheep showing unusual behaviour as well as frozen brains from animals which had died suddenly.

II. MATERIALS AND METHODS

Animals

All affected animals are of the South Hampshire breed of sheep and originate either from the South Island flock described above, or from the experimental flock established with sheep derived from it. Control animals were age-matched, apparently healthy sheep from the experimental flock and a 2 years old Romney ewe. To date the combined number of confirmed affected cases from the original flock and the experimental flock totals 16, with the following case histories, which are summarised in Table 2.I.

Cases 1 and 2: Two 18 months old rams, described above on which the original diagnosis of ceroid-lipofuscinosis was made.

Case 3: A 15 months old ewe hogget, which was born on the South Island property, but had been pastured on a property near Palmerston North for 5 months. In September, 1976 the animal

exhibited signs of blindness and showed head tremors. She was hospitalised for clinical observation and euthanased in November, 1976. Tissues were collected for histological and electronmicroscopical examination, after which a diagnosis of ceroid-lipofuscinosis was made.

Cases 4 and 5: Two ram lambs 4 months old had died suddenly on the South Island property in December, 1976. The owner stored the heads in a freezer until they were sent to Massey University, where light and fluorescent microscopy of brain indicated a diagnosis of ovine ceroid-lipofuscinosis in both animals.

Case 6: A ram from the South Island property was hospitalized at Massey University in April, 1977 at 21 months of age. The animal had shown signs of blindness and exhibited head tremors for about 6 months. Electronmicroscopy of a skin biopsy indicated a presumptive diagnosis of ovine ceroid-lipofuscinosis, which was confirmed by further extensive histological and electronmicroscopical examination after the animal was euthanased in August, 1977, at the age of 25 months.

Case 7: A 15 month old ram hogget from the South Island property had shown typical symptoms for about 4 months and arrived at Massey University in November, 1977. Electron-microscopy of a skin biopsy suggested a diagnosis of ovine ceroid-lipofuscinosis. This was confirmed by light and electronmicroscopy of other tissues after euthanasia of the animal in April, 1978, at the age of 20 months.

TABLE 2.I

Case histories of South Hampshire sheep affected with ceroid-lipofuscinosis

Case No.	Sex	Origin	Age of onset (months)	Presenting signs
1	Male	Sth. Island	14	Blindness, muscle tremors
2	Male	" "	14	Blindness, muscle tremors
3	Female	" "	13	Blindness, head tremors
4	Male	" "	-	Found dead at 4 months
5	Male	" "	-	Found dead at 4 months
6	Male	" "	15	Blindness, head tremors
7	Male	" "	11	Blindess, head tremors
8	Female	Expt. flock	11	Blindness
9	Male	" "	12	Blindness, muscle tremors
10	Female	" "	11	Blindness
11	Male	" "	-	Perinatal death
12	Female	" "	-	" "
13	Male	" "	11	Blindness
14	Female	" "	11	"
15	Female	" "	11	"
16	Male	" "	11	"

Cases 8, 9 and 10: These animals were among the first crop of lambs produced in the experimental flock, in the spring of 1977. Fluorescent, light and electronmicroscopy of liver and rectal biopsies of 36 lambs, aged 4-5 months, revealed these three animals to be suffering from ceroid-lipofuscinosis. The two ewe lambs (cases 8 and 10) exhibited clinical symptoms at 11 months of age and the ram lamb at 12 months of age. Cases 9 and 10 were electively killed in October, 1978 and the original diagnosis was confirmed by further histological examination. Case 8 gave birth to a ram lamb which she successfully reared until she was euthanased in February, 1979. The original diagnosis was again confirmed by histological examination.

Cases 11 and 12: A male and female lamb in the experimental flock. Each was a member of a set of twins and died shortly after birth during the 1978 lambing season. Light and fluorescent microscopy of brain showed that both were affected with ceroid-lipofuscinosis.

Cases 13, 14, 15 and 16: Two males and two females out of a total of 51 lambs from the 1978 lamb crop of the experimental flock were diagnosed as being affected with ceroid-lipofuscinosis at 4 - 5 months of age. Diagnosis rested on the results of fluorescent microscopy of liver and skin biopsy material. All four animals exhibited clinical symptoms at 11 months of age.

Haematological techniques

1. Collection of bloodsamples: Blood samples were obtained by jugular venepuncture using 10 ml EDTA vacuum tubes. The samples were used for estimation of the haemogram and for the preparation of fresh smears.

2. Haemogram determination: The routine examination involved determinations of cell counts, differential leukocyte counts, haemoglobin concentration, packed cell volume, mean corpuscular haemoglobin concentration, total protein, fibrinogen, plasma protein to fibrinogen ratio and icteric index.

3. Staining of fresh smears for leukocyte morphology:

Fresh smears were stained with MacNeal's tetrachrome stain* as follows:

(i) Make up solution by dissolving 1.5g of stain in 500 ml of methyl alcohol

(ii) Fix by flooding smear for 3 min

(iii) Dilute with a slightly more than equal amount of phosphate buffer (pH 6.8) mixing well. Leave for 6 min and wash off under tap. Drain dry

4. Graham-Knoll technique for peroxidase activity in neutrophils:

(i) Dry smear in air

(ii) Fix in formaldehyde-alcohol (1 part of 40% formalin and 9 parts of 96% alcohol)

* George T. Gurr, Searle Scientific Services, High Wycombe, Bucks, U.K.

(iii) Rinse in water

(iv) Pour on benzidine reagent and leave for 0.5 - 5.0 min at 30 s intervals

(v) Pour off, rinse and dry

(vi) Stain with dilute Giemsa (10 drops stock solution in 10 ml distilled water) for about 45 min

(vii) Rinse and dry

Benzidine reagent - 0.2g benzidine in 6 ml of 96% alcohol
- dilute with 4 ml of water
- add 0.02 ml of a fresh 3% H_2O_2 solution

III. RESULTS

Age of onset

Only cases 8, 9, 10, 13, 14, 15 and 16 had a known date of birth and were regularly inspected by a veterinarian (i.e. the author). All these animals showed loss of vision and behavioural abnormalities at 11 or 12 months of age. Case 6 also had a known date of birth and its owner noted unusual behaviour in the animal at about 15 months of age. Cases 1, 2, 3 and 7 had no known date of birth and their ages were estimated by examination of their teeth. Based on non-veterinary observation these animals showed symptoms at an estimated 11 - 14 months of age. It therefore seems reasonable to assume that clinical signs in ovine ceroid-lipofuscinosis should be detectable at 12 months of age.

Two animals (Cases 4 and 5) died suddenly and the causes of death were not established. Cases 11 and 12 were weak at birth but appeared otherwise normal. Each had a twin lamb, which might facilitate mismothering, which combined with their own weakness led to their demise through starvation within 36 hours. Case 6 was electively euthanased at 25 months of age, when his condition deteriorated rapidly. It seems unlikely that the animal would have lived much longer even under the optimum conditions prevailing in an animal hospital.

Postural and behavioural findings

Three animals (Cases 3, 6 and 7) were observed under large animal hospital conditions, whereas seven animals (Cases 8, 9, 10, 13 14, 15 and 16) were observed under field conditions, i.e. grazing with a flock in the paddock. Under these latter conditions the affected sheep tended to graze away from the main flock. Upon disturbance due to the noise of a shouting shepherd or a barking dog they would rejoin the flock, with a high stepping gait and raised head characteristic of blind sheep, but would quickly lag behind when the flock started to move as they did not follow sudden changes in either speed or direction of movement of the other sheep. Well-trained sheep dogs could not easily control affected sheep. This was presumably due to the blindness and behavioural changes of the sheep. The senses of hearing, smell and touch seemed not be be impaired in affected animals.

Mating behaviour may be affected in rams suffering from ceroid-lipofuscinosis. At 19 months of age one ram (Case 7) was put with

cycling ewes and although the ewes exhibited normal mating behaviour, the ram failed to respond. On the other hand a 13 months old affected ewe gave birth to a healthy lamb and reared it successfully. Left undisturbed, her maternal behaviour was normal, however sudden noise would cause her to run about erratically, constantly bleating for her lamb, which she obviously could not see. If the noise continued, she would come towards its source and adopt a threatening posture.

The three animals hospitalised (Cases 3, 6 and 7) were in reasonable nutritional condition upon arrival. Initially the easier access to food led to weight gain, however as the disease progressed, weight loss became apparent. The animals were blind and had widely dilated pupils, which gave them a somewhat anxious expression (Fig. 2.1). There was no eye preservation reflex, but the corneal and palpebral reflexes were present and the pupillary reflex could be slowly elicited under strong artificial light. In the standing resting position there was a moderate extension and slight lowering of the head. In case 6 there was also a slight rotation and flexion to the left and a drooping left ear. There was frequent twitching of ears, eyelids, muzzle and lips, less frequently accompanied by episodes of head nodding and champing of the jaws. These bouts of involuntary movement increased both in frequency and severity upon excitement and as the disease progressed. Fine tremors of the musculature of the back, rump and flanks were also occasionally noticed. When moving around the pen, the animals would constantly nudge the wall, presumably for orientation; case 6 also showed compulsive circling usually to the left. This became less

Figure 2.1: South Hampshire ram, 24 months of age, affected with ceroid-lipofuscinosis.

Note
- dilated pupil despite strong photographic lights
- slight lowering and extension of the head
- straw adhering to lower jaw which is wet from sham drinking
- marks on pen wall caused by animal's nudging, presumably for orientation
- peculiar to this case: a drooping left ear and a slight longitudinal rotation of the head to the left.

pronounced as the disease progressed and apart from the normal standing resting position, he would often rest on his carpal joints, with head extended and lower jaw resting on the ground. The affected animals spent a long time eating, with which they appeared to have difficulty and the wool on the lower jaw was constantly wet with water from prolonged periods of sham drinking. All affected animals strongly resisted being handled, struggling continuously and they proved difficult to shear or examine clinically.

Haematology

(i) Haemograms: Bloodsamples were collected from all affected animals, except cases 13, 14, 15 and 16. Case 6 was bled at fortnightly intervals over a period of 4 months prior to euthanasia. Haemograms were determined on the same day that collection of samples took place. All bloodsamples submitted had values within normal ranges.

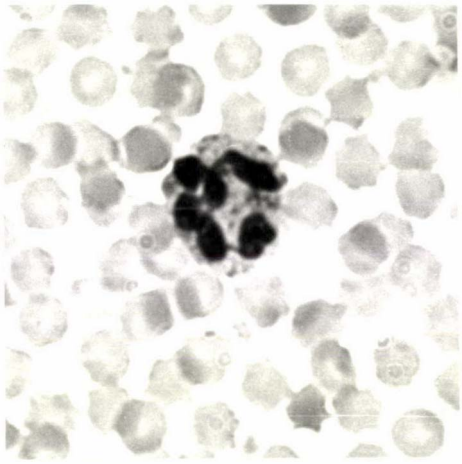
(ii) Fresh smears: Air dried smears were stained by MacNeal's stain (p.48) and examined. Lymphocytic vacuolation and neutrophilic hypergranulation were not observed. Hypersegmented neutrophils, i.e. neutrophils with more than 6 nuclear lobes, were not a feature in bloodsmears of affected animals when compared to those of controls. No differences were apparent between fresh smears of affected and control animals, when stained with the PAS, Oil-Red-O or Sudan black methods. When viewed under blue light unstained smears did not show fluorescence.

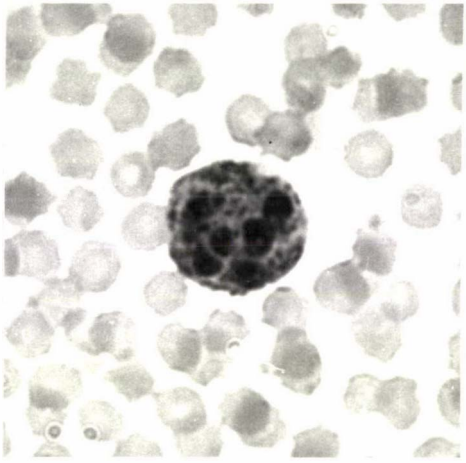
(iii) Peroxidase activity in neutrophils: Fresh air dried blood smears were stained according to a modified Graham-Knoll technique (p.48), with incubation times of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 min. Reactions in neutrophils were scored as non-reacted, partially reacted and completely reacted; the latter two are represented in Fig. 2.2. Although considerable time was spend standardizing test conditions, such as using the same freshly prepared solutions for both control and affected animals, and keeping the time interval between collection of blood and preparation of smears standard at 10 min, the technique proved erratic. Results from the same animal would vary depending on day of collection, and the same bloodsample would give varying results when treated with different freshly prepared solutions. However at any one time, using the same freshly prepared solution, no difference was apparent between affected animals and controls. Smears were examined 'blind', and two-hundred neutrophils were counted, one hundred on either edge of the smear. In general the first partially reacted cells would appear at 30 - 90 s, at 2 - 4 min there would be a varying mixture of partially and completely reacted cells, and at 4 - 5 min nearly all neutrophils were fully reacted.

Radiography

Routine lateral X-rays of the head of affected animals showed slight thickening of the skull bones (*ossa frontalia* and *ossa parietalia*) overlying the cerebrum relative to control sheep. The rostral part of the cranial vault harbouring the cerebrum appeared

Figure 2.2: Graham-Knoll technique to demonstrate peroxidase activity in sheep neutrophils. A partially reacted cell (left), and a completely reacted cell (right) are shown.





slightly flattened dorso-ventrally, thus giving it an elongated rather than a rounded shape. Attempts at quantifying these differences, by making tracings or radiographs, failed. The major difficulty lay in placing the head of the anaesthetised sheep in such a position that the dorso-ventral median plane of the cranial vault was exactly parallel to the X-ray plate, and at right angles to the X-ray beam.

Ophthalmology

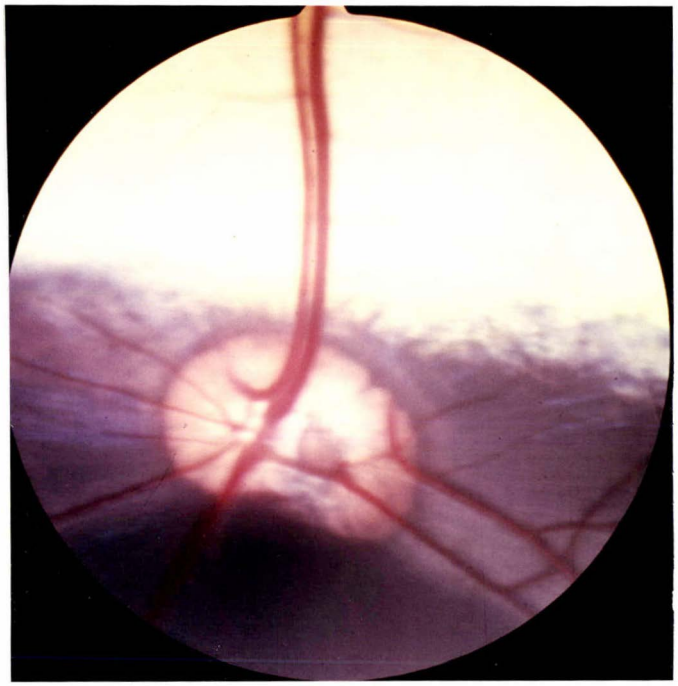
The retinae of cases 3, 6, 7, 9 and 10 and age-matched controls were examined by funduscope* while the sheep were under general anaesthesia. Affected animals showed attenuation and straightening of retinal vessels (Fig. 2.3). The attentuation became more enhanced as the disease progressed. Thus, in general, the older the animal, the more severe the retinal atrophy and in case 6 the retinal atrophy was more severe at 24 months than at 21 months of age. Funduscopy did not reveal any other retinal abnormalities.

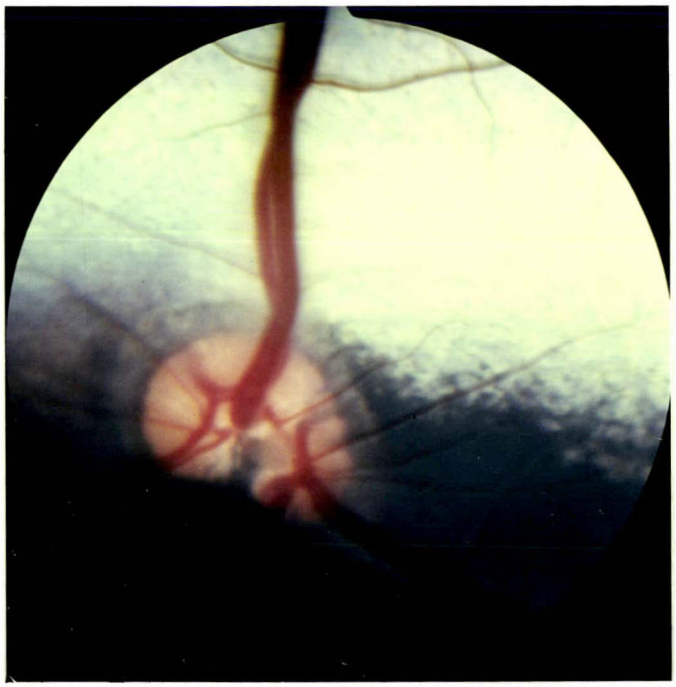
IV. DISCUSSION

The most prominent clinical findings in ovine ceroid-lipofuscinosis are loss of vision and behavioural abnormalities, starting at 11 - 12 months of age with motor dysfunction starting soon afterwards and increasing in severity as the disease progresses.

* Kowa Co. Ltd., Nagaio, Japan.

Figure 2.3: Retinographs of the ovine eye showing attenuation and straightening of retinal vessels in an affected 24 months old ram (above), when compared to a control sheep (below).





Zeman and Siakotos (1973) noted that despite wide variation in ages of onset and a varying clinical course within the various types of human neuronal ceroid-lipofuscinoses, affected individuals from the same family showed a remarkable similarity in both age of onset and clinical course. Ovine ceroid-lipofuscinosis has only been diagnosed in one family of the South Hampshire breed, and affected individuals have shown a close similarity in age of onset and clinical course.

That the disease is present before clinical symptoms appear is illustrated by cases 4, 5, 11 and 12, and especially by cases 8, 9, 10, 13, 14, 15 and 16. The latter showed no clinical abnormalities at 4 - 5 months of age when pathological examination of biopsy material showed them to be affected with ovine ceroid-lipofuscinosis. Out of 87 lambs, only these seven were so diagnosed and only these seven duly developed clinical signs at the predicted age of onset at about 12 months. The cause of death in cases 4 and 5 was not established, however feed was in short supply due to drought conditions. Cases 11 and 12 were weak at birth and each had a twin lamb. They died of starvation presumably due to mismothering as a result of their own weakness and competition from their twins. It is suggested that ovine ceroid-lipofuscinosis may have been a contributory factor in the deaths of these last four animals insofar as it rendered them less 'fit' to deal with adverse conditions. Perinatal mortality associated with the lysosomal storage disease mannosidosis has been reported in Angus cattle in New Zealand by Jolly and Thompson (1967) and cases 11 and 12 possibly represent a similar situation in ovine ceroid-lipofuscinosis.

As all live cases of ovine ceroid-lipofuscinosis, with the exception of cases 13, 14, 15 and 16, have been electively euthanased, it remains unknown how long the animals would survive under optimum conditions. Case 6 was euthanased at 25 months of age, when, even while hospitalised his condition started to deteriorate rapidly. It is doubtful whether this ram could have survived to this age under field conditions and indeed would have survived much longer under hospital conditions. It appears reasonable therefore to put the expected survival time at up to 2 years which is very similar to that of English Setter dogs with ceroid-lipofuscinosis, whom Koppang (1973/74) reports as not living beyond 26 months of age.

It would appear that the blindness in affected sheep has a retinal and a cerebral component. Clinical evidence consists of retinal atrophy, noted in fundoscopy, and reduction in size of the cerebrum, shown radiographically as a reduction in size of the rostral cranial vault. Pathological examination of both retina and cerebrum supports and adds to the clinical evidence (Jolly and West, 1976; Chapter III). It is likely that the behavioural abnormalities are due to a combination of blindness and central neuronal impairment of function. The observation that affected sheep under field conditions lose contact during fast or erratic movement of the flock could be explained by their blindness alone. The excessive struggling of all affected sheep when being handled, as well as the change in mating behaviour in case 7 suggest a contributing central component. The occurrence of muscle tremors, head nodding, champing of the jaws and the compulsive circling of case 6 further indicates central neuronal

damage. The weight loss which is invariably associated with this disease is probably caused by many factors such as difficulty in prehension, mastication and deglutition of food and difficulty with drinking.

Haemograms of sheep with ceroid-lipofuscinosis are normal, as is their leukocyte morphology. In this, the disease in sheep resembles the canine entity (Koppang 1973/74). It differs from human neuronal ceroid-lipofuscinosis, where lymphocytic vacuolation is a characteristic finding in the juvenile form and may less frequently occur in the infantile form (Zeman and Siakotos, 1973; Schwendemann, 1976). The latter two human entities also commonly show neutrophilic hypergranulation and increased numbers of hypersegmented neutrophils (Zeman and Dyken, 1969). These phenomena have not been observed in bloodsmears of affected sheep. The histochemical test used by Armstrong *et al.* (1974a) to demonstrate reduced neutrophil peroxidase activity in patients with the late infantile and juvenile form of human neuronal ceroid-lipofuscinosis, did not reveal a reduction in peroxidase activity in ceroid-lipofuscinosis sheep when compared to normal controls. The significance of this finding cannot be evaluated until more is known about the underlying metabolic error in the ceroid-lipofuscinoses in both man and domestic animals.

The radiographic findings of thickening of the skull bones, overlying the cranial cavity and dorso-ventral flattening of the rostral part of the cranial vault as well as the ophthalmological finding of retinal atrophy are useful additional facts in the clinical

diagnosis of ovine ceroid-lipofuscinosis. However radiography and funduscopy appear to have little value in early diagnosis.

The clinical picture of ceroid-lipofuscinosis is highly characteristic and a provisional diagnosis should not be difficult to make, at least within the South Hampshire breed of sheep. The compulsive circling, drooping ear and lateral rotation and flexion of the head shown by case 6, resembled some of the clinical signs associated with the encephalitis due to infection with *Listeria monocytogenes* in sheep, however, the severe depression, usually followed by prostration, coma and death were absent. Progressive retinal atrophy has been encountered in one family line of New Zealand Romney sheep (West D., pers. comm.). These sheep are normal at birth and show loss of vision by 9 - 12 months leading to complete blindness by 2 - 3 years. They have a normal life span and there are no motor or behavioural disturbances except those directly related to the blindness. This condition is therefore readily distinguishable from ovine ceroid-lipofuscinosis. Similarly sheep affected with progressive retinal degeneration observed in Great Britain and reportedly due to ingestion of bracken *(Pteris aquilina)*, only show clinical signs relating to their blindness (Watson *et al.*, 1972).

From the viewpoint of comparative medicine, ovine ceroid-lipofuscinosis has features in common with both the late infantile and juvenile forms of human neuronal ceroid-lipofuscinosis (Zeman *et al.*, 1970), and with canine ceroid-lipofuscinosis (Koppang 1973/74). In all

three species the disease is characterised clinically by blindness, behavioural abnormalities and motor dysfunction . In affected children there is also a loss of intelligence often leading to dementia. The intelligence of sheep affected with ceroid-lipofuscinosis has not been measured, but reduced intelligence is implied from the reduction in size of the cerebrum.

CHAPTER III

PATHOLOGY

I. INTRODUCTION

The original report by Jolly and West (1976), established that the disease entity they had encountered in an inbred flock of South Hampshire sheep resembled the ceroid-lipofuscinoses of man and other animals. These diseases are characterized by the widespread intracytoplasmic accumulation of autofluorescent lipopigments in neurons and a wide variety of other cell types. It is the purpose of this chapter to describe the pathology of ovine ceroid-lipofuscinosis in greater detail and to compare it with the other entities.

II. MATERIALS AND METHODS

Animals

Sections III, IV and V of this chapter are based on observations of cases 1, 2, 3, 6, 7, 8, 9 and 10 (Table 3.1). These animals had all shown clinical signs of ceroid-lipofuscinosis and were electively euthanased between the ages of 13 - 25 months. Age-matched control animals were three healthy male flock mates and a healthy female Perendale. Fresh brain weights from an additional six sheep of various breeds, and all over 12 months of age, were recorded to establish a range of normal values. Section VI deals with cases 8, 9, 10, 13, 14, 15 and 16, while 80 healthy flock mates served as age-matched control animals.

TABLE 3.1

List of South Hampshire sheep with ceroid-lipofuscinosis that were examined pathologically

Case No	Sex	Age at death or biopsy (months)	Tissues examined	Microscopy
1	Male	18 (E)	Brain, cord, retina	Light, F, EM
2	Male	18 (E)	Brain, cord	Light, F, EM
3	Female	15 (E)	Brain, cord, retina, viscera	Light, F, EM
4	Male	4 (D)	Brain	Light, F
5	Male	4 (D)	Brain,retina	Light, F
6	Male	25 (E)	Brain, cord, retina, viscera	Light, F, EM
7	Male	20 (E)	Brain, cord, retina, viscera	Light, F, EM
8	Female	5 (B), 18 (E)	Brain, cord, retina, viscera	Light, F, EM
9	Male	5 (B), 13 (E)	Brain, cord, retina, viscera	Light, F, EM
10	Female	5 (B), 13 (E)	Brain, cord, retina, viscera	Light, F, EM
11	Male	0 (D)	Brain	Light, F
12	Female	0 (D)	Brain	Light, F
13	Male	5 (B)	Liver, skin	F
14	Female	5 (B)	Liver, skin	F
15	Female	4 (B)	Liver, skin	F
16	Male	4 (B)	Skin	F

B - Biopsy; D - Died; E - Euthanasia; F - Fluorescent; EM - Electronmicroscopy

Preparation of tissues

1. Light microscopy: Tissues for light microscopy were fixed in either 10% formol saline or in Bouin's solution, and routine procedures for paraffin embedding and sectioning were employed. Sections were stained with haematoxylin and eosin (H & E), Sudan black B and according to the periodic-acid-Schiff (PAS) method. Selected sections were also stained with the Schiff reagent and by the long Ziehl-Neelsen technique. A number of sections of brain were treated according to Cajal's gold sublimate method for astrocytes. Some fresh frozen sections were stained with the Sudan IV and Oil-Red-O reagents.

Sections of 0.5 - 1.0 µm in thickness were also cut for light microscopy from epoxy resin embedded tissues prepared as for electron-microscopy. These sections were stained with 1% toluidine blue in 0.1M phosphate buffer (pH 7.2) on a hot plate at 80^{o}C for 10s, and counter-stained with 4% basic fuchsin for 4 s at the same temperature.

2. Fluorescent microscopy: To demonstrate autofluorescence, unstained fresh frozen sections or unstained deparaffinised sections were examined in a Reichert Immunopan microscope, fitted with a quartz halogen lamp, using excitor filter 30, 8x1 FITC3 and blue barrier filter 18x3 OG515 GG9.

3. Electronmicroscopy: Tissues for electronmicroscopy were obtained within 10 min of euthanasia and fixed overnight in an ice-cold mixture of 2% formaldehyde and 3% glutaraldehyde in 0.1M phosphate buffer (pH 7.2). For leukocyte ultrastructure, buffy coats or lymphocytes purified on lymphoprep*, were fixed for 8 h in 3% phosphate buffered

*Nyegaard & Co., Oslo, Norway.

(pH 7.2) glutaraldehyde at 4°C. Biopsy specimens were stored in a phosphate buffered (pH 7.2) mixture of 4% formaldehyde and 1% glutaraldehyde at 4°C until required. After primary fixation, tissues were washed in phosphate buffer and post-fixed in 1% osmium tetroxide in phosphate buffer (pH 7.2). The tissues were then put through a graded series of ethanol and propylene oxide and embedded in epoxy resin*. For light microscopy sections 0.5 - 1.0 µm in thickness were cut on a LKB III ultramicrotome**. Thin sections for electronmicroscopy were cut at 70 nm and mounted on unsupported copper grids. The sections were stained in 50% ethanol with saturated uranyl acetate for 8 min, and in lead citrate for 6 min and examined in a Philips 200 electron microscope.

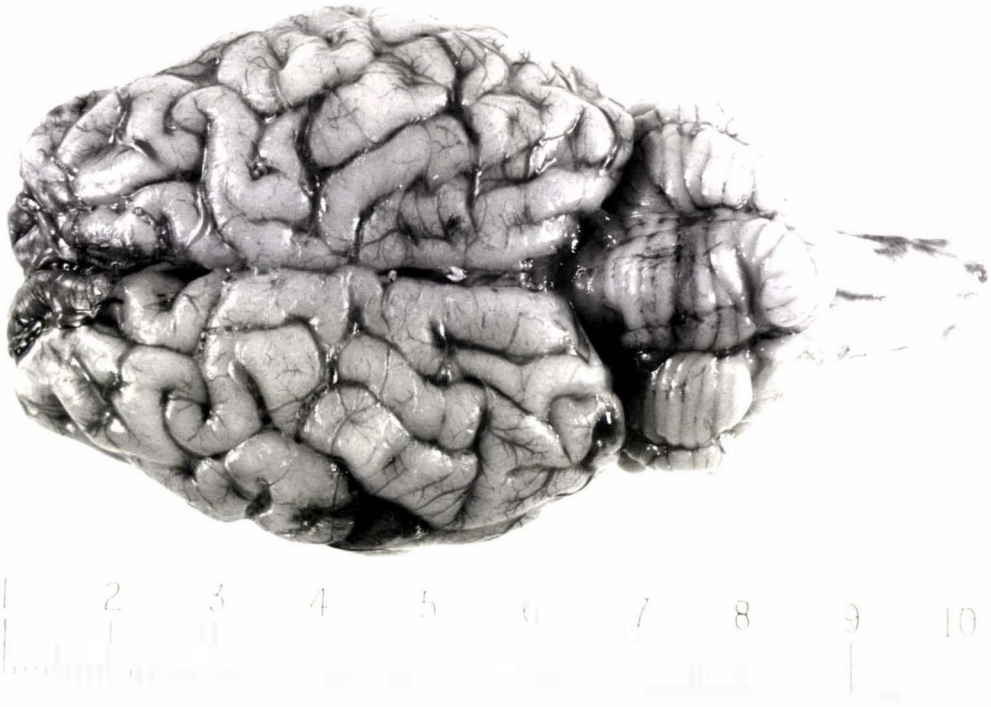
III. GROSS PATHOLOGY

All affected animals were in good nutritional condition but invariably lighter than their peers. Case 9 weighed 33.1 kg, whereas breed, age and sex-matched controls weight 42.6, 38.6 and 42.6 kg respectively. In all affected cases the skull bones overlying the brain were thickened and upon removal revealed an atrophied brain. When the brains were removed their weights varied from 53.2 - 71.0 g, compared to weights of 86.2 - 102.5 g of 10 similarly aged normal sheep. Examination of the affected brains showed that the atrophy mostly affected the cerebrum, which showed uniform thinning of the gyri, was slightly flattened dorso-ventrally and was of a firm consistency (Figs. 3.1 & 3.2). Cross-section of the cerebrum revealed slightly enlarged lateral ventricles and thinning of the cortex (Fig. 3.3). The

* Durcupam -ACM, Fluka, Switzerland

** LKB-Produkter AB S-161 25 Bromma 1. Sweden.

Figure 3.1: Dorsal view of brains from two 13 months old ewe hoggets. The brain of the affected sheep (above) is reduced in size and shows thinning of the cerebral gyri. The brain weight of the affected animal is 57.2g, compared with 86.2g for the normal control sheep (below). (Formalin fixed. Scale in cm).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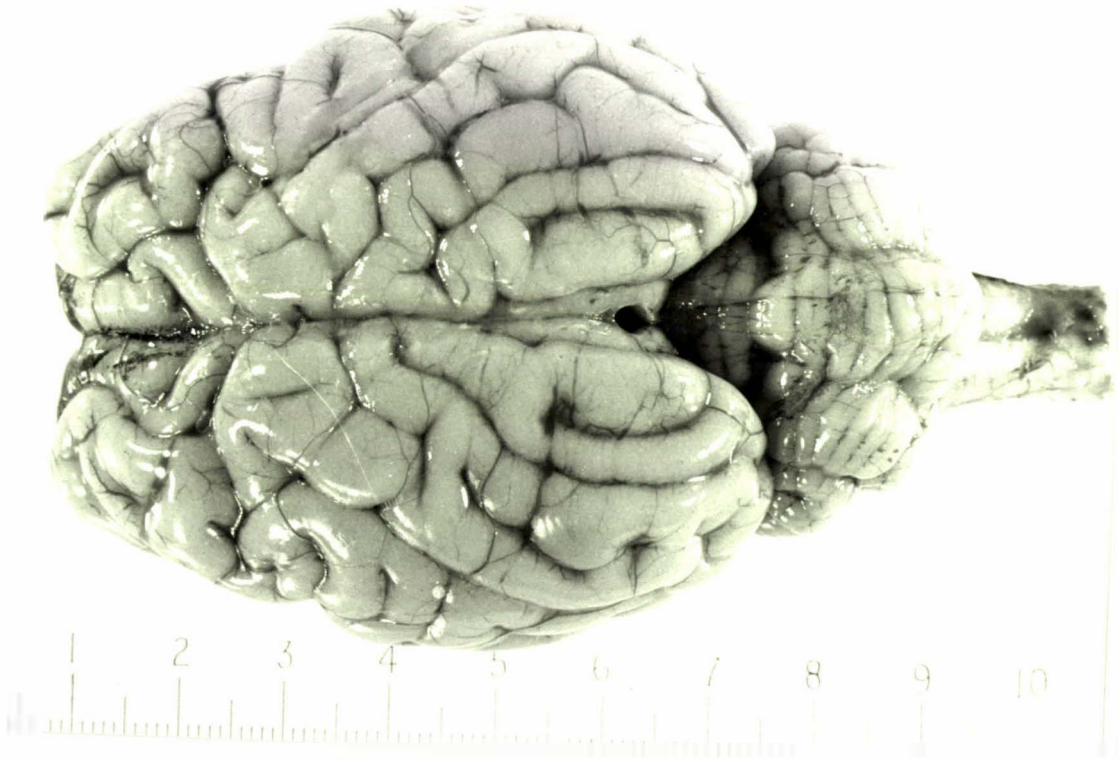
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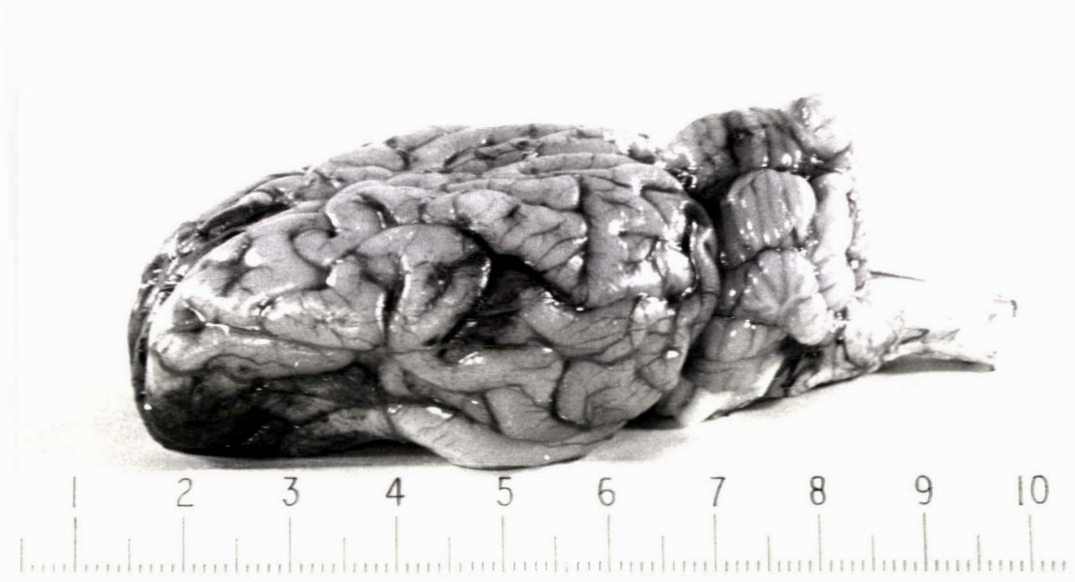
Figure 3.2: Lateral view of brains from two 13 months old ewe hoggets. The cerebrum of the affected animal (above) shows reduction in size, thinning of the cerebral gryi and a slight dorso-ventral flattening, when compared with the brain of the normal control sheep (below).
(Formalin fixed. Scale in cm)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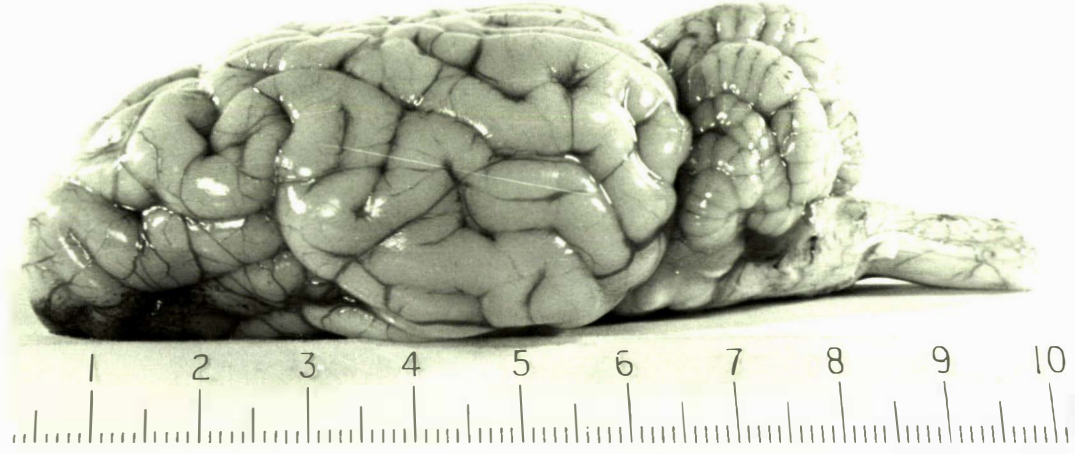
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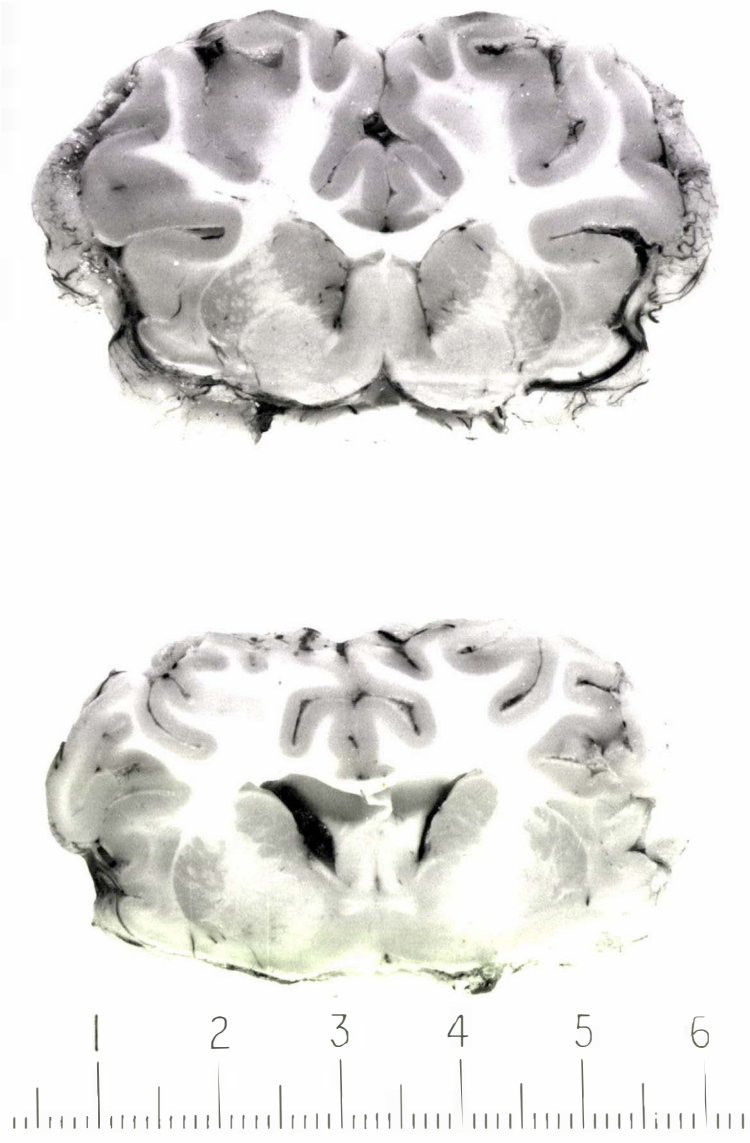
Figure 3.3: Transverse section of the cerebrum of an affected 13 months old ewe hogget (below) and of an age and sex-matched normal control sheep (above). Note reduced size of cerebrum, marked thinning of cortex, thinning of *corpus callosum* and *septum pellucidum* and enlargement of lateral ventricles in the affected sheep. (Formalin fixed. Scale in cm)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white matter also revealed some atrophy, espicially of the *corpus callosum*, and *septum pellucidum*. Brain stem and cerebellum of affected brains appeared essentially normal on gross examination.

Other gross findings were unremarkable in all cases and consisted merely of parasitic lesions affecting lungs and liver. Cases 8, 9 and 10 showed scar tissue consequent to liver biopsy.

IV. HISTOPATHOLOGY

The characteristic light microscopic finding in ovine ceroid-lipofuscinosis is the occurrence of intracytoplasmic lipopigment inclusions in neurons and a wide variety of other cells in the body (Table 4.II). These inclusions are not constantly seen in H & E stained paraffin sections, but they are consistently PAS and Sudan black B positive and show autofluorescence when unstained sections are viewed under blue light. This latter characteristic is the most useful method of demonstrating the inclusions, particularly in non-nervous tissues. The distribution and staining characteristics of the lipopigments in various organ, tissue and cell types are described below.

Brain and spinal cord

In H & E stained paraffin sections, virtually all neuronal perikarya, at all levels of the brain and spinal cord, contain single or multiple slightly eosinophilic refractile inclusions ranging in size from fine granules to bodies up to 15 µm (Fig. 3.4). The inclusions stain slightly with the Schiff reagent but more strongly when this is preceded by oxidation with periodic acid (PAS + ve): small

TABLE 3.II

Distribution of lipopigment in ceroid-lipofuscinosis of sheep over 12 months of age

Organ, tissue or cell type	Light microscopy					Electron microscopy
		Paraffin sections			Fresh frozen sections	
	H&E	PAS	SB	AF	AF	
Brain and spinal cord	++	+++	+++	+++	+++	+++
Retina	+	++	++	++	++	++
Peripheral nervous system	+	++	++	++	++	++
Mononuclear phagocytic system	+	++[2]	++[2]	++[2]	++[2]	++
Skin	0	++[1,2]	++[1,2]	++[2]	++[2]	++
Skeletal muscle	0	0	0	0	0	0
Gastro-intestinal tract	0	+	+	+	+	+
Salivary glands	0	+	++[1]	+	n	+
Liver	0	++[1]	++	++	++	++
Pancreas	0	++[1]	++[1]	++	n	++
Lung	0	+[3]	+[3]	0	n	0
Heart	0	+[1]	+	+	n	+
Kidney	0	++[1]	++[1]	++	n	++

Contd.

TABLE 3.II (Contd.)

Organ, tissue or cell type	Light microscopy					Electron microscopy
	Paraffin sections				Fresh frozen sections	
	H&E	PAS	SB	AF	AF	
Bladder	0	+	+	+	n	n
Pituitary gland	0	++[1]	++[1]	+	n	n
Thyroid gland	0	+[1]	+	+	n	+
Adrenal gland	0	++	++	++	n	++
Ovary	0	+	+	+	n	n
Testis	0	+	+	+	n	++
Seminal vesicles	0	++[1]	+	+	n	++
Blood cells	0	0	0	0	0	0
Fibrocytes	0	0	0	0	0	+
Pericytes	0	0	0	0	0	+

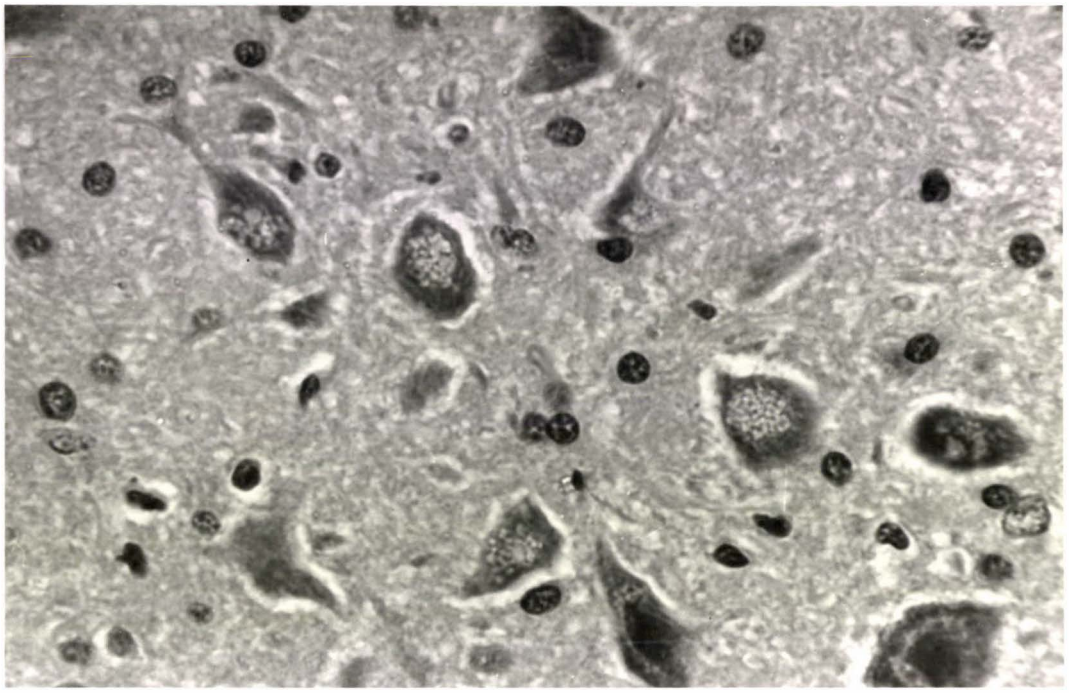
H&E - Haematoxylin and eosin; PAS - Periodic-acid-Schiff; SB - Sudan black; AF - Autofluorescence under blue light

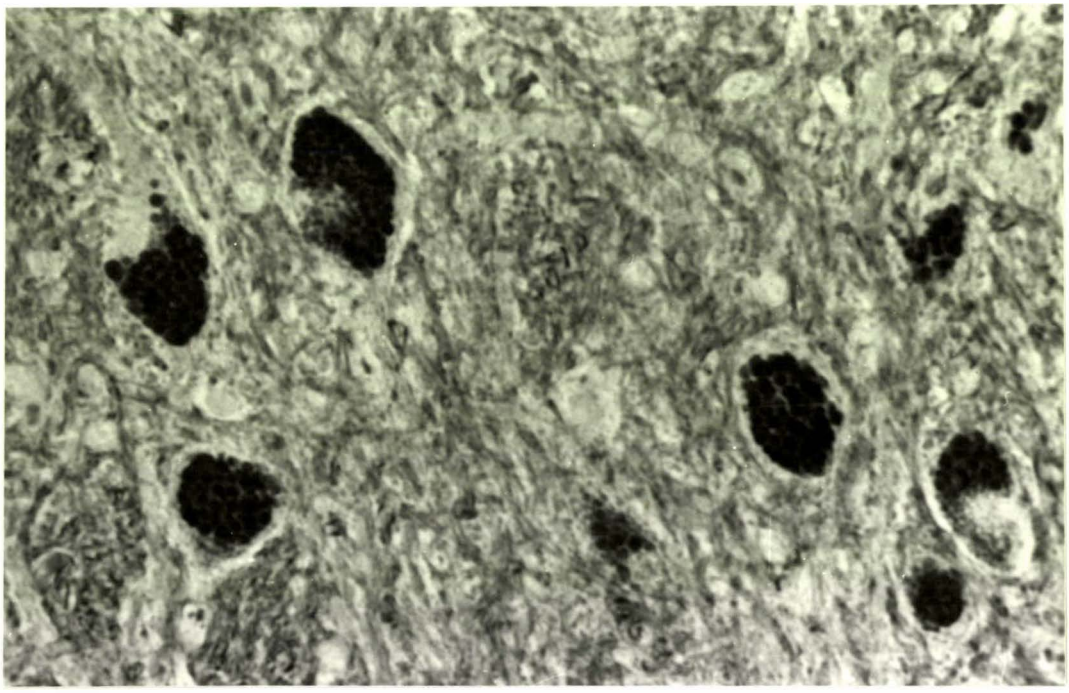
n - not done; 0 - not present; + - some positive cells; ++ - many positive cells; +++ - many strongly positive cells

1 - partially due to pigment; 2 - also in controls, but to a lesser extent; 3 - not due to lipopigment.

Figure 3.4: Cerebral cortex of a 15 months old South Hampshire ewe hogget with ceroid-lipofuscinosis showing neurons laden with lipopigment granules.
(Paraffin section, H&E x500)

Figure 3.5: Thalamus of a 25 months old affected ram.
Lipopigment granules stain black and in some neurons appear to almost completely fill the cytoplasm.
(Paraffin section, Sudan black B x500)



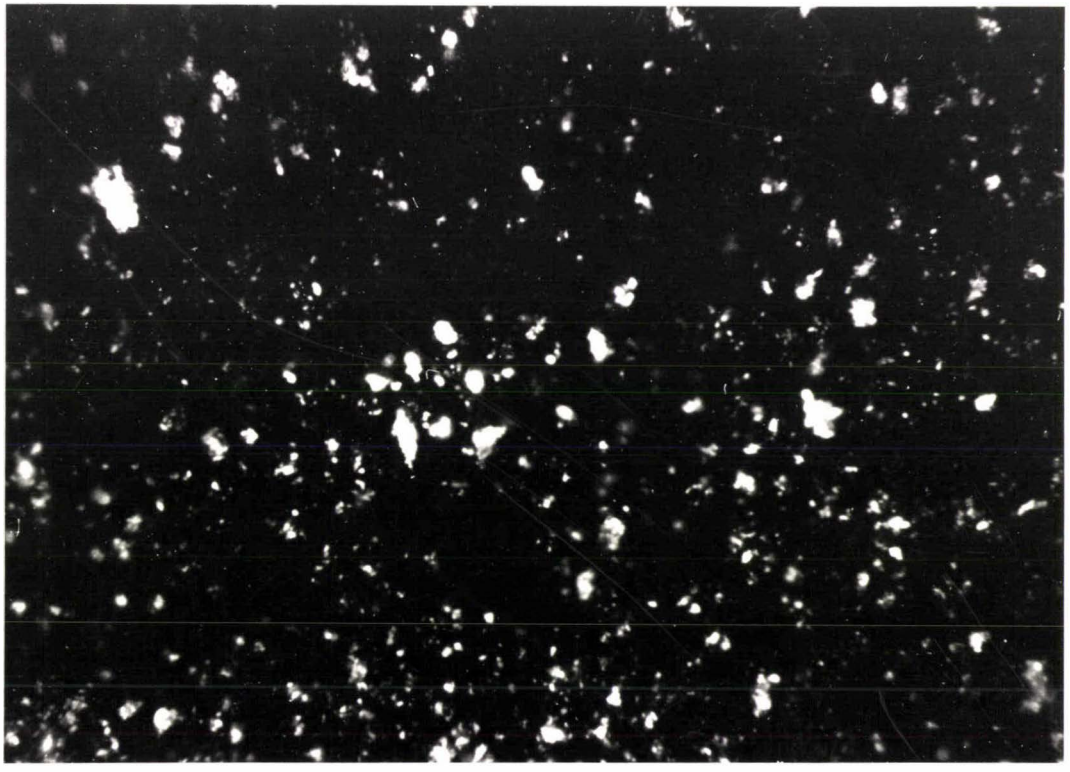


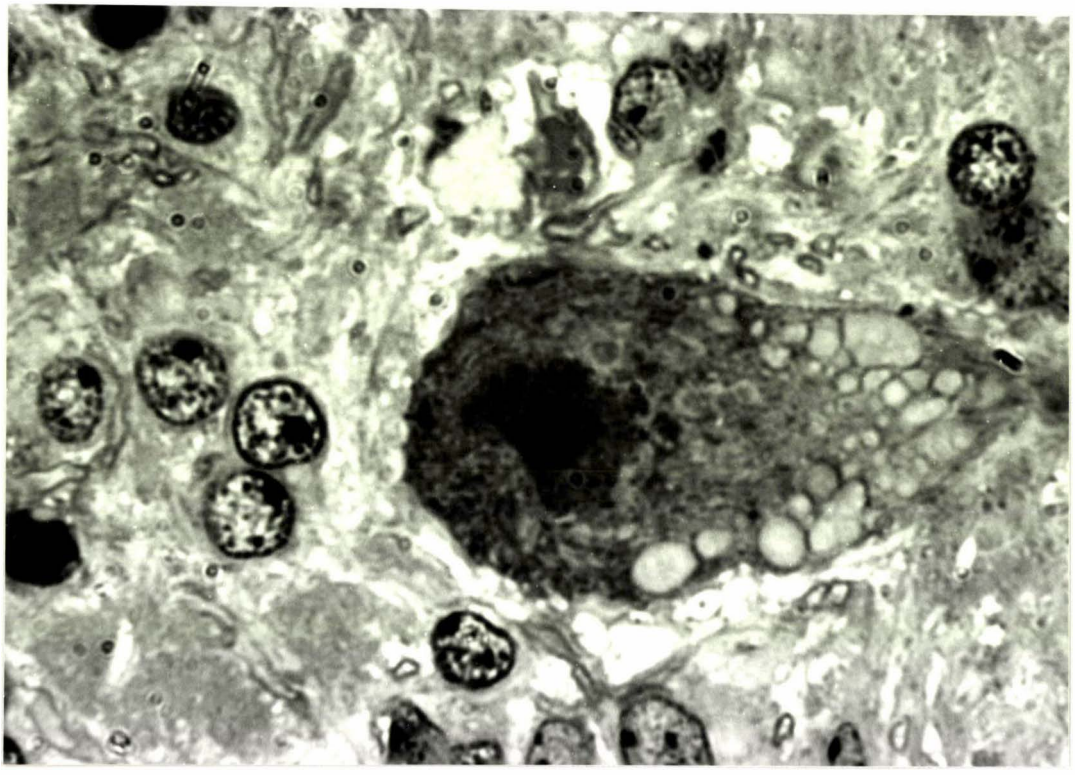
granules are brightly red whereas the larger bodies stain less intensely. With the Sudan black stain the granular inclusion bodies are coloured an even black independent of size and number (Fig. 3.5). This stain is the most useful in demonstrating the ubiquity of the inclusions in neurons, brain macrophages and neurectodermal cells. With the Sudan IV and Oil-Red-O stains on fresh frozen sections the inclusions stain pink and red respectively, and they may stain faintly red in the long Ziehl-Neelsen stain. When unstained fresh frozen or unstained deparaffinised sections are viewed under blue light, the inclusions show a bright yellow-green autofluorescence (Fig. 3.6). There is a slight loss of intensity of fluorescence in deparaffinised sections compared with that in fresh frozen sections. When H & E stained paraffin sections are viewed under blue light the fluorescence appears to be enhanced while it is quenched in PAS and Sudan black stained sections. Unstained sections viewed under blue light after 3 years still show fluorescence, indicating the stability of this lipopigment characteristic.

The number of discrete granules in the plane of section varies from none to approximately 40 per neuron. Sometimes the granular material is less discrete and appears to almost completely fill the cytoplasm (Fig. 3.5). The lipopigment granules show most variation in size and are most numerous in the larger neurons of the cerebral cortex, the subcortical nuclei, dorsal and ventral horns of the spinal cord, and in Purkinje cells of the cerebellum (Fig. 3.7). However PAS and Sudan black positive material is also clearly visible in smaller neurons, glial cells, neuropil, cells of the choiroid plexus and sometimes in endothelial cells of blood vessels. Examination of coronal sections of the brain at 4 - 5 mm intervals reveals no obvious pattern of neuronal involvement at the different levels.

Figure 3.6: Cerebral cortex of a 25 months old affected ram. The white structures are autofluorescent lipopigment granules, single or in clusters. (Unstained deparaffinised section viewed under blue light, x400)

Figure 3.7: Cerebellar cortex of a 25 months old affected ram. Inclusions in a Purkinje cell are numerous and show variation in size. (Epoxy resin embedded section, toluidine blue-basic fuchsin x1250)





The overall architecture of the brain appears intact although loss of cerebral cortical neurons is noted. There is a moderate microgliosis but a marked astrocytosis, with astrocyte nuclei sometimes occurring in clusters of three or four and exhibiting unusual shapes (Fig. 3.8). With the Cajal stain the astrocytosis appears to be confined mainly to the cerebral cortex and is seen to consist of an increase in both number and size of astrocytes (Fig. 3.9). Many neurons show swelling of cell processes which in the cerebellar cortex leads to staghorn formation of swollen Purkinje cell dentrites.

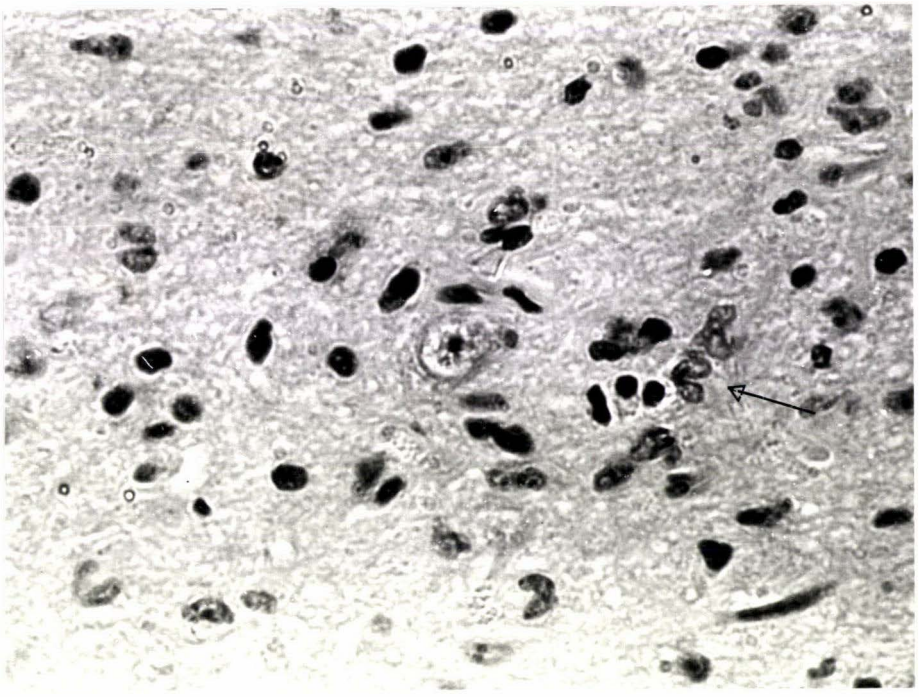
Eye

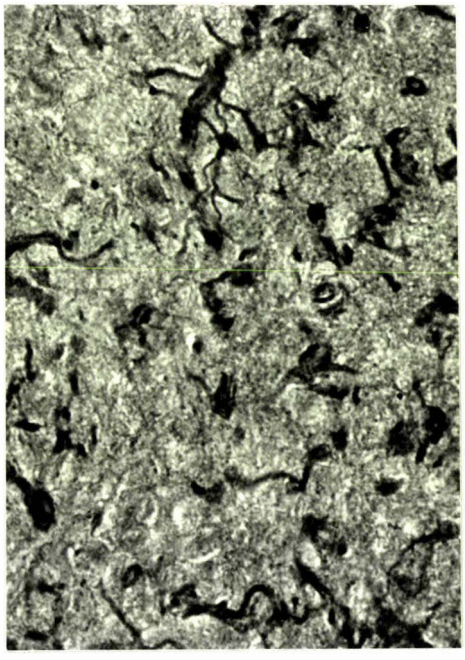
Histopathology of the eye in ovine ceroid-lipofuscinosis is mainly confined to the retina. Intracytoplasmic inclusions with the same staining and fluorescent characteristics as those described for brain and cord are present in the layer of rods and cones, the outer and inner nuclear layers and particularly in the large neurons of the ganglion cell layer. As well as lipopigment accumulation there is retinal atrophy, the severity of which varies with the age of the animal (Fig. 3.10). In its mildest form (Cases 9 and 10) there is some degeneration of the layer of rods and cones and some loss of nuclei from the outer nuclear layer. When the disease has been present longer (Case 6) the retinal atrophy is more severe and there is almost complete loss of the layer of rods and cones and the outer nuclear layer is reduced to a single row of nuclei, while the number of nuclei in the inner nuclear layer also appears to be reduced.

The retinal pigment epithelium underlying the tapetum lucidum of the fundus is free of melanin pigment and shows PAS and Sudan black

Figure 3.8: Cerebral cortex of a 25 months old ram with ceroid-lipofuscinosis, showing a neuron surrounded by an increased number of glial cells. Astrocytes predominate and may show unusually shaped nuclei (arrow). (Paraffin section, H&E x500)

Figure 3.9: Cerebral cortices of two approximately 15 months old South Hampshire ewe hoggets. The affected animal (left) shows an increase in number and size of astrocytes, compared to the control sheep (right). (Fixed frozen sections, Cajal x230)





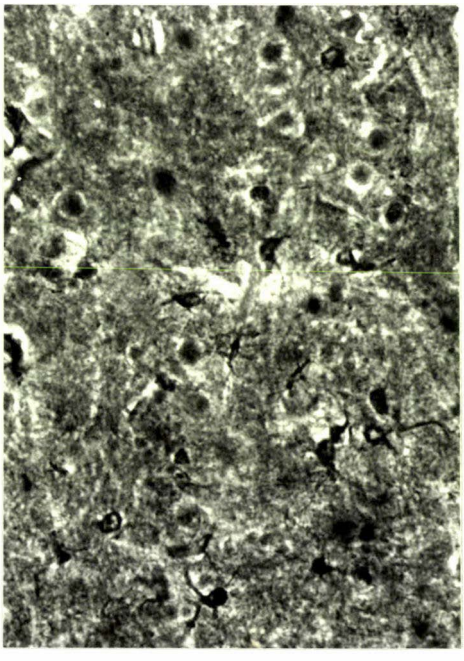
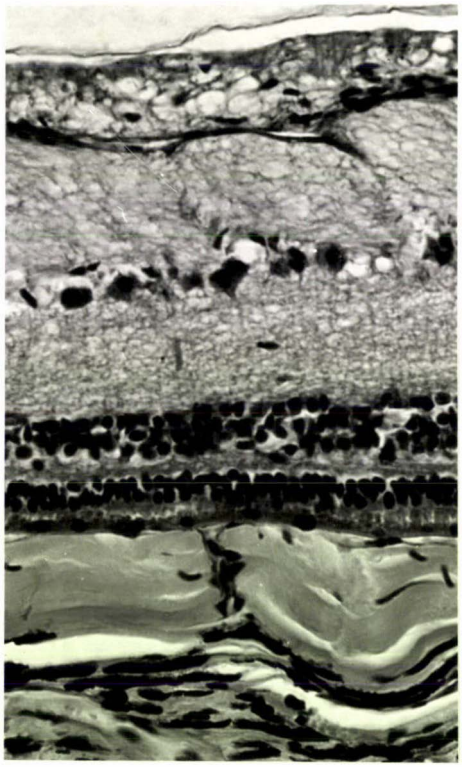
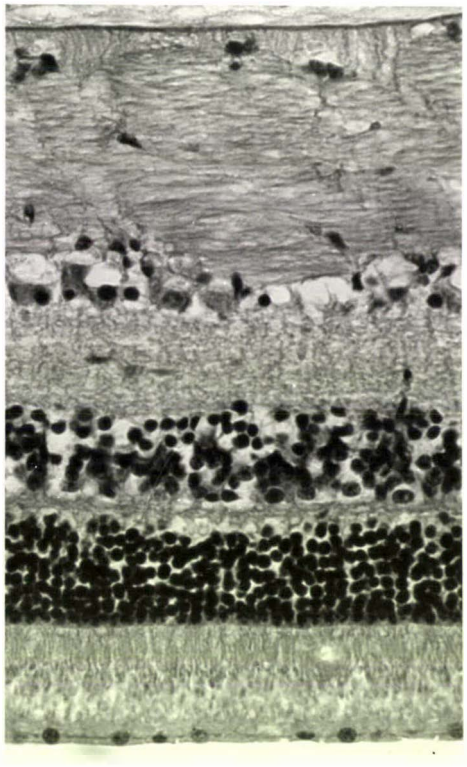


Figure 3.10: The histology of the retina of an 18 months old South Hampshire ewe with ceroid-lipofuscinosis (left), compared with that of a normal sheep (right). The affected animal shows severe atrophy of the layer of rods and cones (LRC) and the outer nuclear layer (ONL). The retinal pigment epithelium (RPE), inner nuclear layer (INL) and ganglion cell layer (GCL) appear normal. (Paraffin sections, H&E x230)




GCL
INL
ONL
LRC
RPE

positive autofluorescent material. The intensity of the autofluorescence varies from case to case. In the non-tapetal area, the presence of melanin granules obscures the staining reactions of other material in the retinal pigment epithelium.

Peripheral nervous system

Neurons of the sympathetic ganglia in the intestinal wall display varying numbers of characteristic inclusions. They are weakly PAS positive, but stain strongly with Sudan black and autofluoresce under blue light. Lipopigment granules are also seen in other neurons of the peripheral nervous system wherever encountered in sections. These inclusions are not observed in Schwann cells or in peripheral nerves.

Mononuclear phagocytic system

Material with similar staining and fluorescent characteristics as described for neurons is present in large mononuclear cells of lymphoid follicles in lymph nodes, spleen and intestinal wall. It also occurs in fixed macrophages of bone marrow, the medulla of lymph nodes and spleen, and in Kupffer cells and free tissue macrophages. Some age-matched control sheep also show PAS and Sudan black positive, autofluorescent material in the same cells, but to a lesser extent.

Skin

Fine PAS and Sudan black positive autofluorescent material is present in glandular and ductal cells of sweat glands. Control sheep of the same ages as affected animals contain similar material but to a much smaller degree. The squamous epithelium and sebaceous glands appear to be free of lipopigment.

Skeletal muscle

Skeletal muscle fibres are apparently free of pigment inclusions characteristic for ovine ceroid-lipofuscinosis.

Gastrointestinal tract

Characteristic inclusions are found in large mononuclear cells and in neurons of the sympathetic plexus. Smooth muscle cells sometimes show very fine PAS and Sudan black positive autofluorescent material. The covering epithelium of the oral cavity, oesophagus, forestomachs, abomasum and intestine is free of lipopigment at least as seen by light microscopy.

Salivary glands

Glandular and ductal cells of the submaxillary and to a lesser extent of the parotid salivary glands show lipopigment accumulation.

Liver

Autofluorescent, PAS and Sudan black positive material is found in hepatocytes and Kupffer cells, but not in biliary epithelium. In hepatocytes the material is finely granular and appears to involve most cells. Normal sheep may show small numbers of autofluorescent granules in Kupffer cells and hepatocytes.

Pancreas

Most acinar cells show fine granular inclusions with characteristic autofluorescence and staining reactions. In their normal physiological state islet cells are PAS and Sudan black positive,

but in sheep with ceroid-lipofuscinosis some of this material autofluoresces, indicating the presence of lipopigment.

Lung

No lipopigment is seen in any of the epithelial cells of the lower respiratory tract.

Heart

Both cardiac muscle fibres and Purkinje fibres contain PAS and Sudan black positive autofluorescent material in fine granular form. Normal sheep may exhibit small amounts of similar material in cardiac muscle fibres.

Kidney

Material with characteristic staining reactions and showing autofluoresence is present especially in tubular cells and in epithelial cells lining the collecting ducts. These cells also show PAS and Sudan black positive material in control animals, however it does not fluoresce. Some pinpoint autofluorescence is also seen in glomeruli and in pelvic epithelium.

Bladder

The muscular wall shows some fine PAS and Sudan black positive autofluorescent material, but this is not apparent in epithelial cells.

Pituitary gland

There is autofluorescent material present in the *pars glandularis* only. Material which stains positively with Sudan black in the *pars intermedia* and neurohypophysis does not fluoresce when viewed under blue light.

Thyroid glands

Some follicular cells show small amounts of autofluorescent lipopigment granules.

Adrenal glands

Autofluorescent lipopigment granules are present in many medullary and cortical cells.

Ovary

Only tissue macrophages show characteristic lipopigment inclusions.

Testis

Both spermatagonia and Sertoli cells show PAS and Sudan black positive autofluorescent granules.

Seminal vesicles

The glandular epithelium shows the presence of autofluorescent material with characteristic staining reactions. In the normal physiological state the epithelium also contains PAS positive material.

Blood

No characteristic inclusions are seen in the cells of blood. Lymphocytes do not show vacuolation (Fig. 3.11), nor is hypergranulation or hypersegmentation a feature of neutrophils in sheep affected with ceroid-lipofuscinosis.

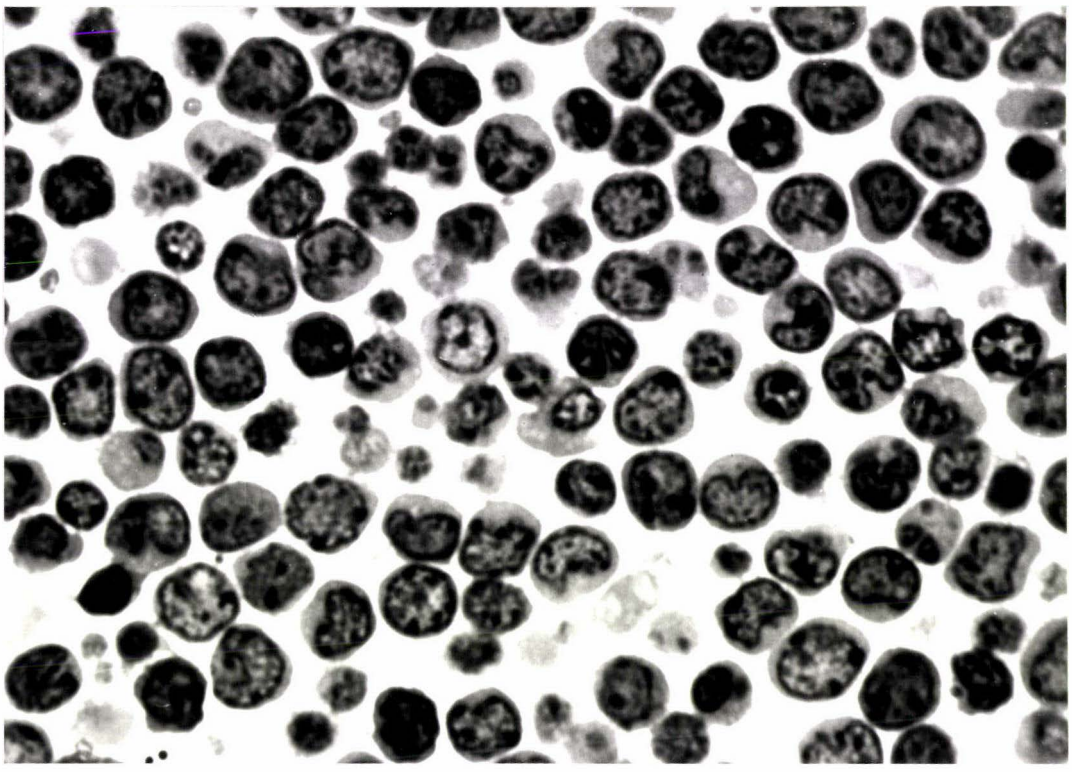
V. ELECTRONMICROSCOPY

Ultrastructurally, the autofluorescent storage material in ovine ceroid-lipofuscinosis consists of pleomorphic intracytoplasmic bodies which show great variation in size, electron density and internal morphology. The size varies from less than 1.0 μm to 15 μm. The smaller bodies are usually round or oval, while the large accumulations may be round, oval or have an irregular sometimes lobulated outline, giving the impression of having been formed by confluence of smaller bodies (Fig. 3.12). The internal structure varies between and within bodies and consists of a granular matrix of varying electron density almost invariably associated with membranous profiles sometimes interspersed with electronlucent foci.

In many profiles the membranes measure approximately 12 nm across and under high magnification show a five-layered structure, a central dense line separated from two outside less dense lines by intervening light layers (Figs. 3.13 & 3.14). There is some suggestion that the five-layered membranes may result from the fusion of two tripartite membranes (Fig. 3.13). The five-layered membranes may run singly in various directions or may run parallel to each other which sometimes leads to a myelin pattern of alternating dense and less dense lines

Figure 3.11: Lymphocytes from a 25 months old affected ram. Note absence of lymphocytic vacuolation. (Epoxy resin embedded section, toluidine blue-basic fuchsin x1250)

Figure 3.12: Residual body in cortical neuron showing lobulated shape. (EM x17,500)



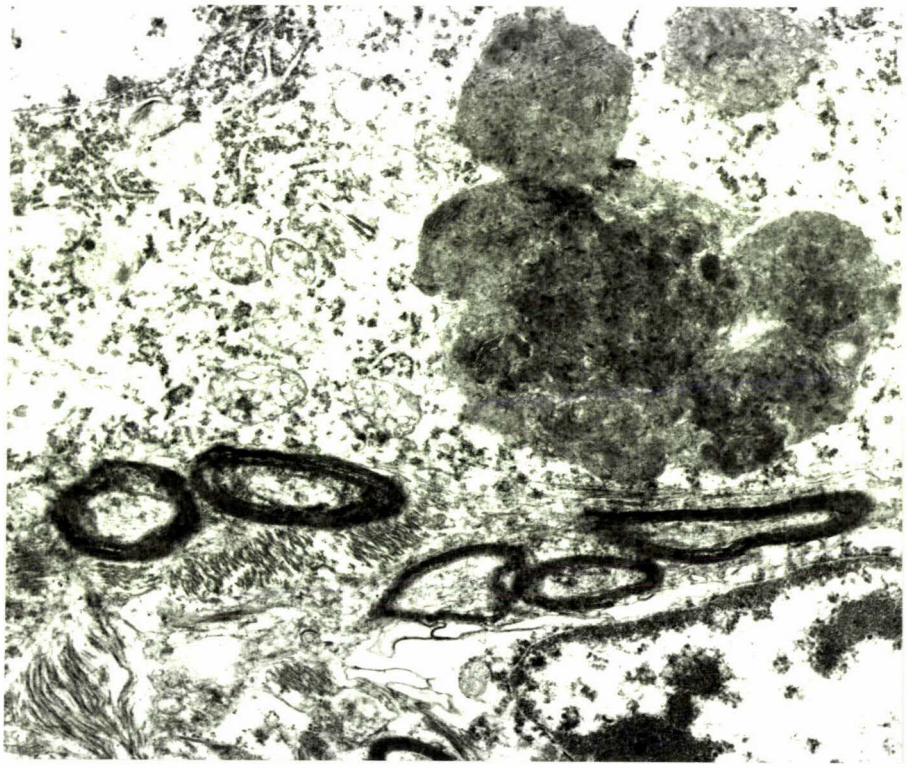
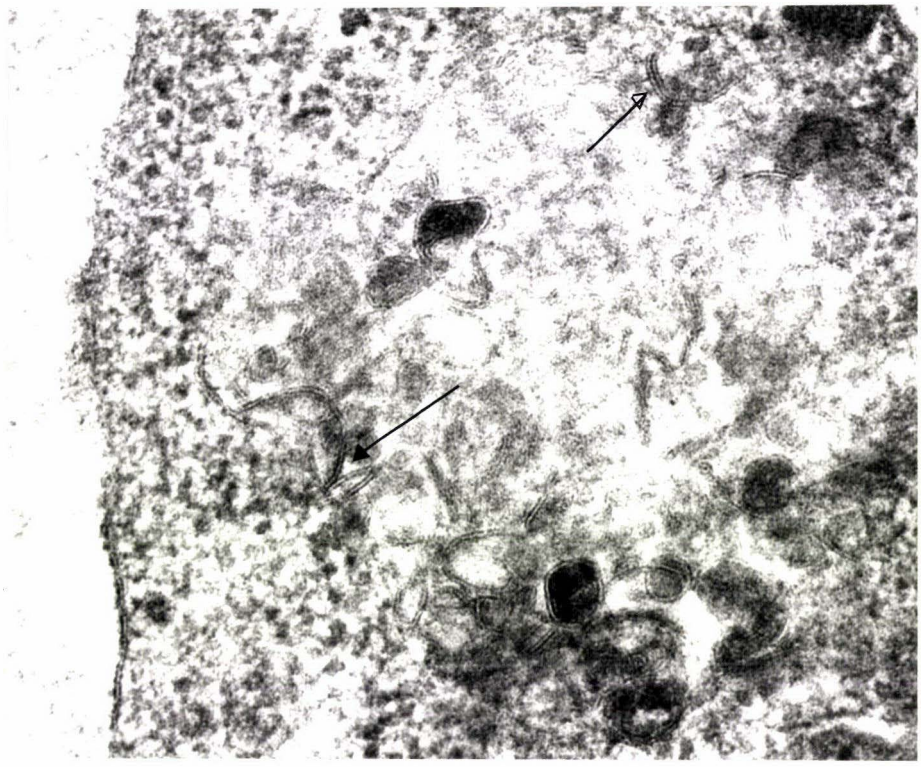
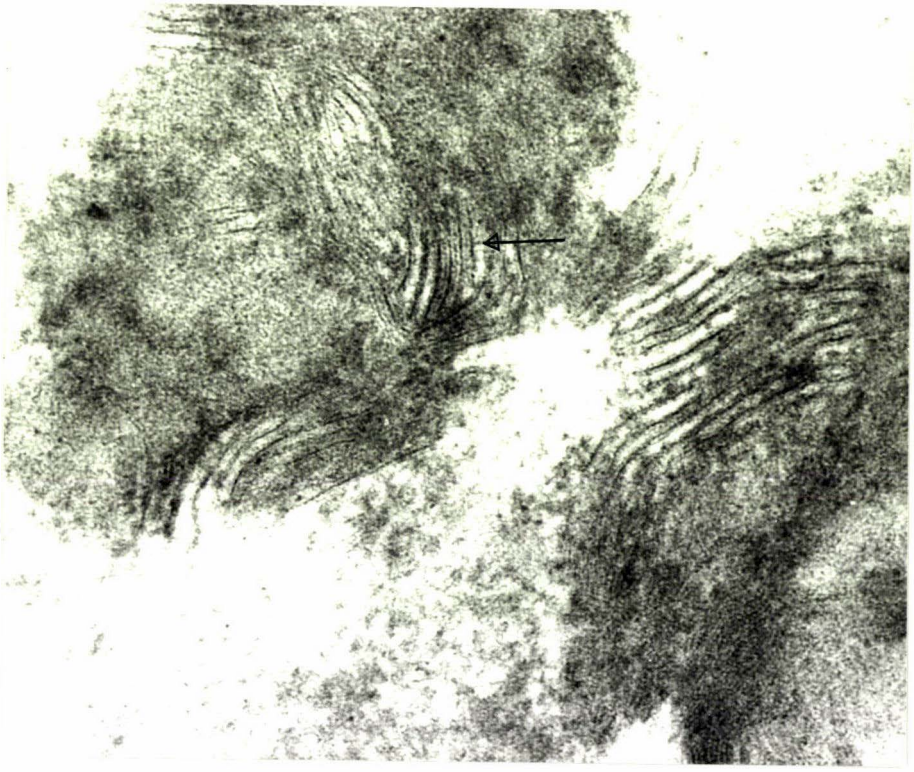


Figure 3.13: Storage material apparently free in the cytoplasm of a meningeal capillary endothelial cell. Short arrow points to a five-layered membrane, which at long arrow seems to be formed by the fusion of two tripartite membranes.
(EM x110,000)

Figure 3.14: Part of lipopigment body in retinal neuron showing granular matrix of varying electron density, and five-layered membranes. The arrow indicates a myelin pattern.
(EM x128.000)





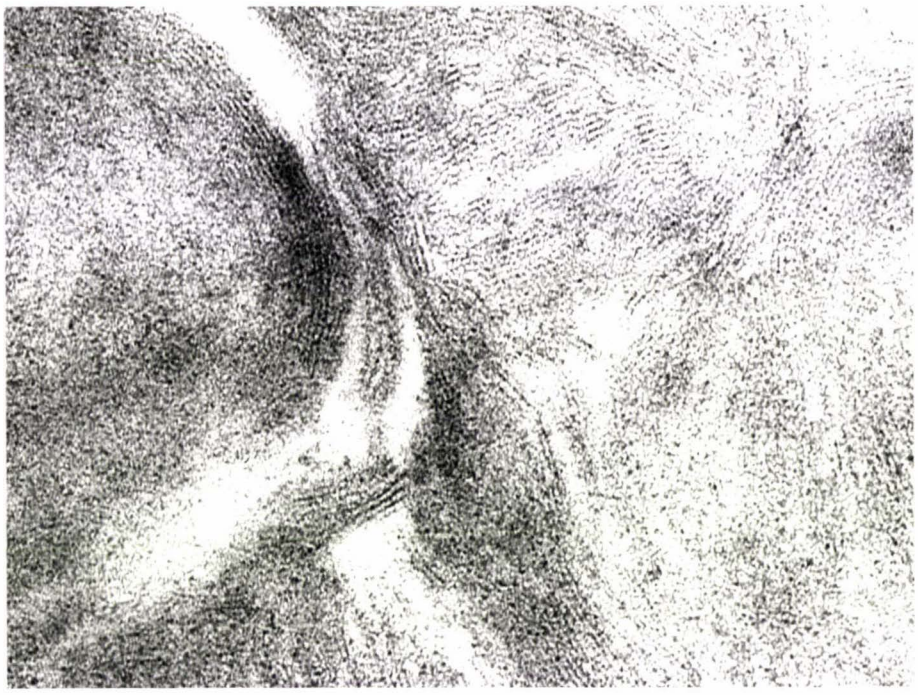
separated by intervening light layers (Fig. 3.14). Another common membranous configuration consists of stacks of alternating dense and light lines with a periodicity of approximately 5.2 nm (Fig. 3.15). These various membranous profiles may be short or long, straight or curved and form a wide variety of architectural patterns. Some of the patterns have been given special names in the literature such as curvilinear (Fig.3.16), fingerprint and crystalloid (Fig. 3.17). The latter occurs apparently as the result of intersecting membranes. Tubules approximately 30 nm across are frequently seen amongst the membranous profiles at least in neuronal inclusions (Fig. 3.18). The structures described above may be enclosed by a unit membrane, others may show a five-layered limiting membrane, while some appear not to be enclosed by a limiting membrane.

The neuronal and glial cell inclusions present the widest variation in membranous profiles and are the only ones showing tubular arrays, whereas the visceral inclusions are predominantly, but not exclusively of the curvilinear type. In the brain there is no obvious difference in topographical distribution of type of pigment bodies. Purkinje cells and ventral horn neurons throughout the spinal cord show large numbers of inclusions, with up to 40 being counted in the plane of sectioning (Fig. 3.19). Most neurons of brain, spinal cord and retina as well as many glial cells show one or more inclusion and endothelial cells are frequently involved.

Storage bodies are encountered in most visceral organs and tissues examined. In glandular epithelial organs such as liver, pancreas (Fig. 3.16), kidney, adrenal (Fig. 3.20), testis, seminal

Figure 3.15: Part of a residual body in a ventral horn neuron showing stacks of alternating dense and light lines, with a periodicity of approximately 5.2 nm. (EM x166,500)

Figure 3.16: Pancreatic acinar cell with curvilinear inclusion bodies. The cellular architecture appears normal. (EM x25,000)



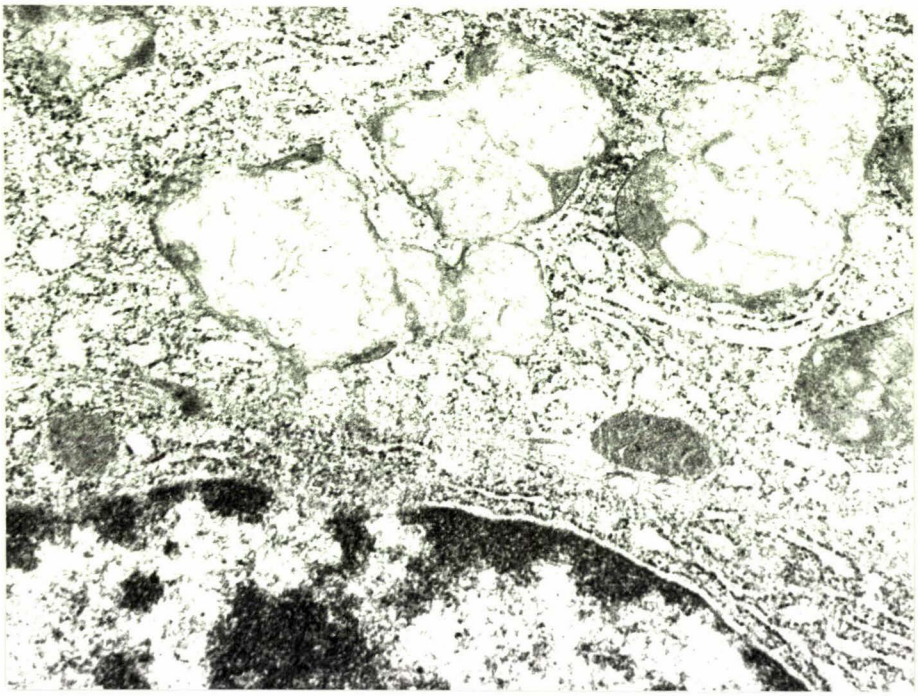
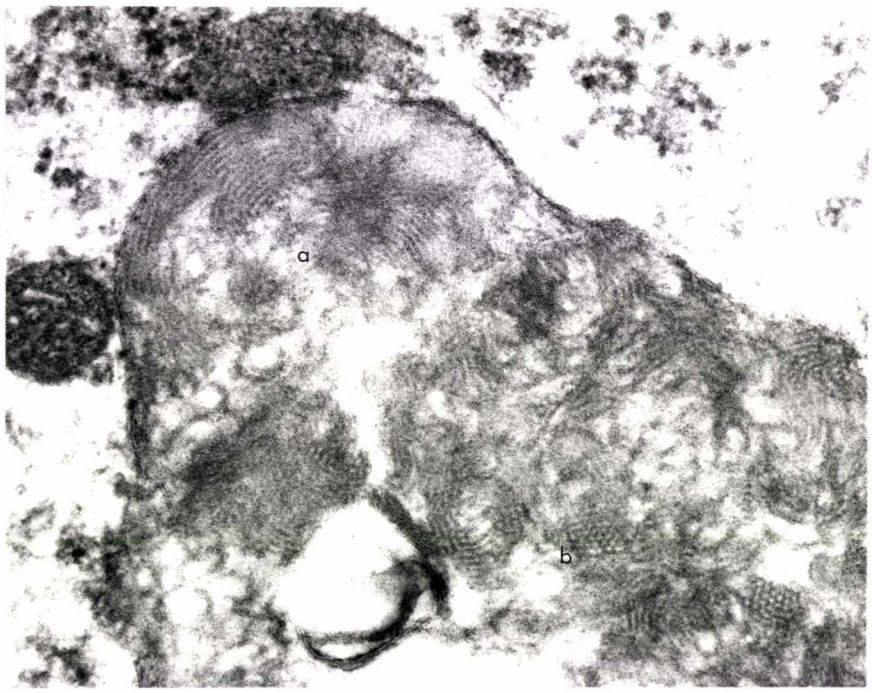


Figure 3.17: Inclusion in cerebral cortical neuron showing fingerprint (a) and crystalloid (b) patterns. (EM x74,000)

Figure 3.18: Storage body within a cerebral cortical neuron showing membranous profiles and tubular arrays. (EM x30,600)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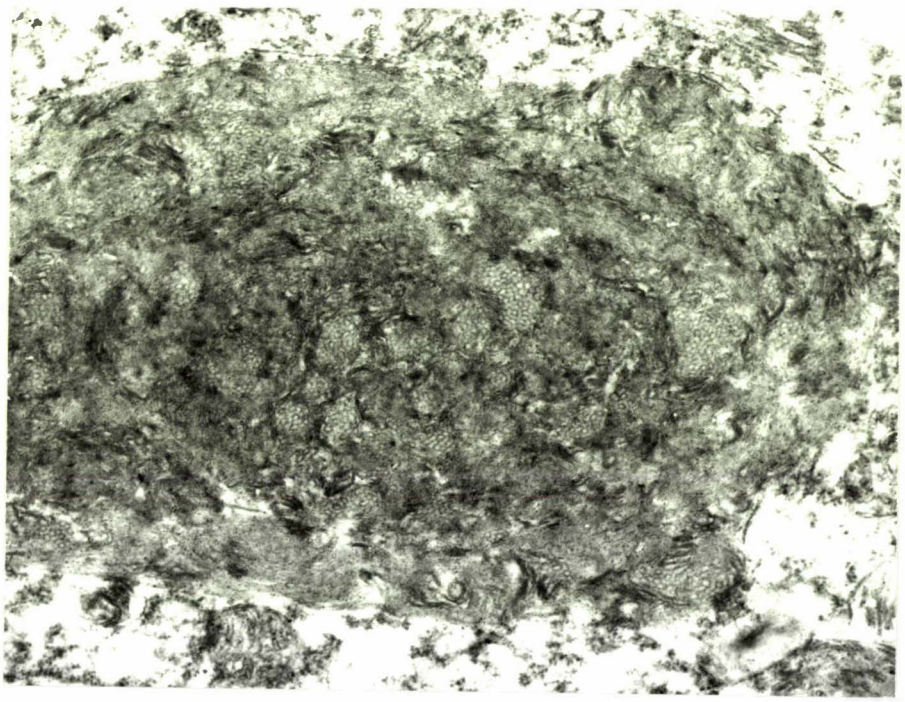
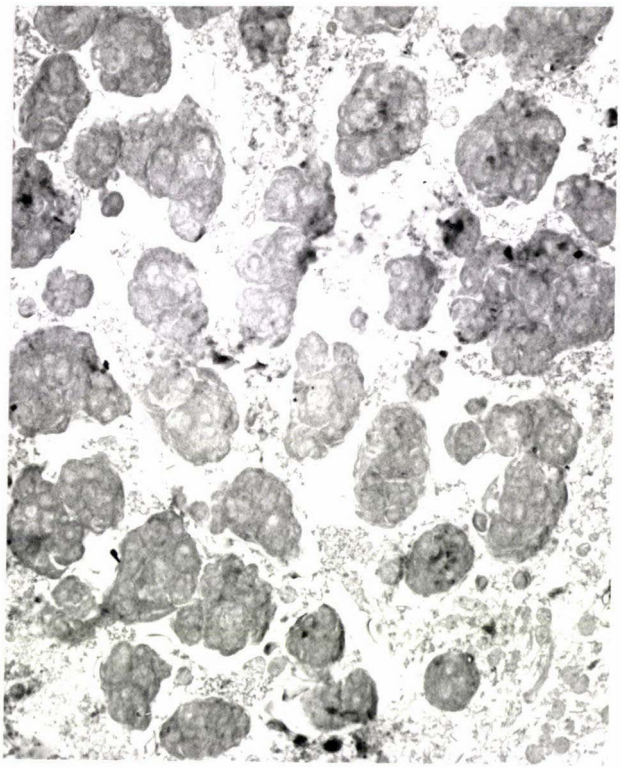
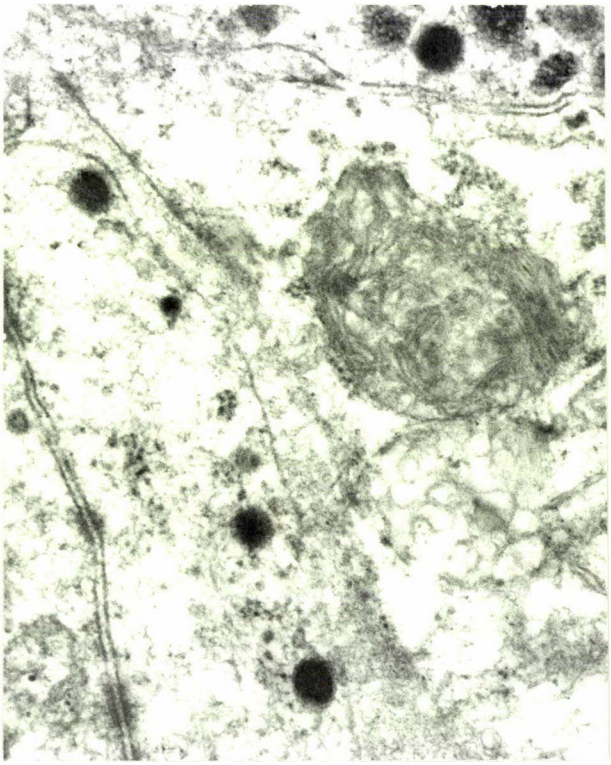


Figure 3.19: Part of ventral horn neuron of lumbar cord showing a large number of inclusions. The cytoplasm shows sparsity of normal organelles. (EM x4,700)

Figure 3.20: Membranous inclusion body in cell at the cortico-medullary junction of the adrenal gland. (EM x41,400)





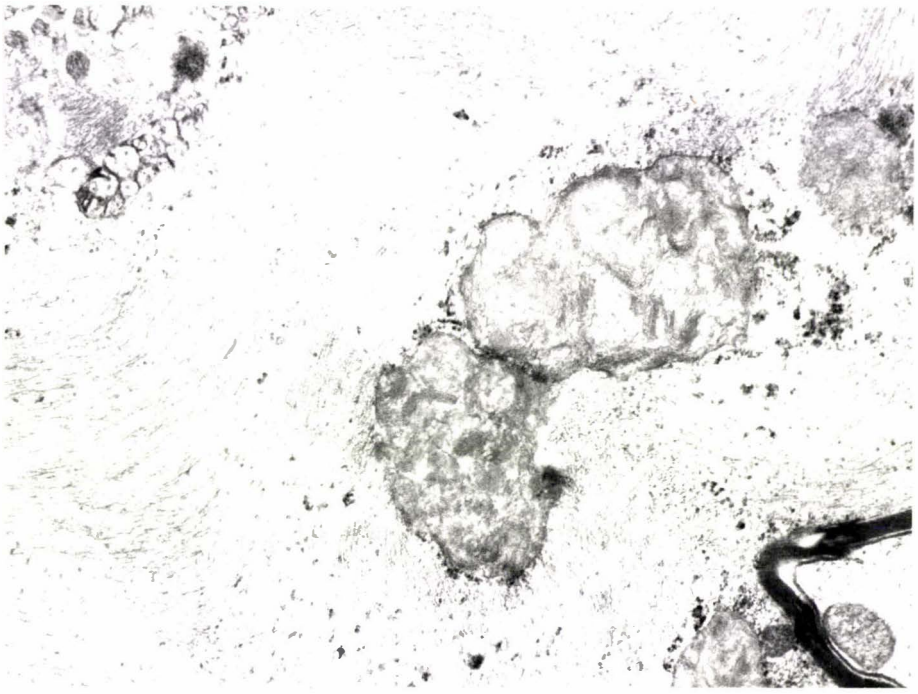
vesicle and sweat glands inclusions are numerous but they are also moderately common in parotid, thyroid and pituitary glands. Large mononuclear cells of spleen, lymph nodes, bone marrow as well as macrophages in mucosa and submucosa of gut and Kupffer cells in the liver frequently harbour lipopigment bodies. Cardiac muscle, intestinal smooth muscle, neurons of the autonomic nervous system, Schwann cells of peripheral nerves, endothelial cells, pericytes and fibrocytes also reveal typical inclusion bodies. Storage bodies are not seen in squamous epithelium of skin, bronchial epithelium, epithelium of intestinal tract and bladder, skeletal muscle, lymphocytes and neutrophils.

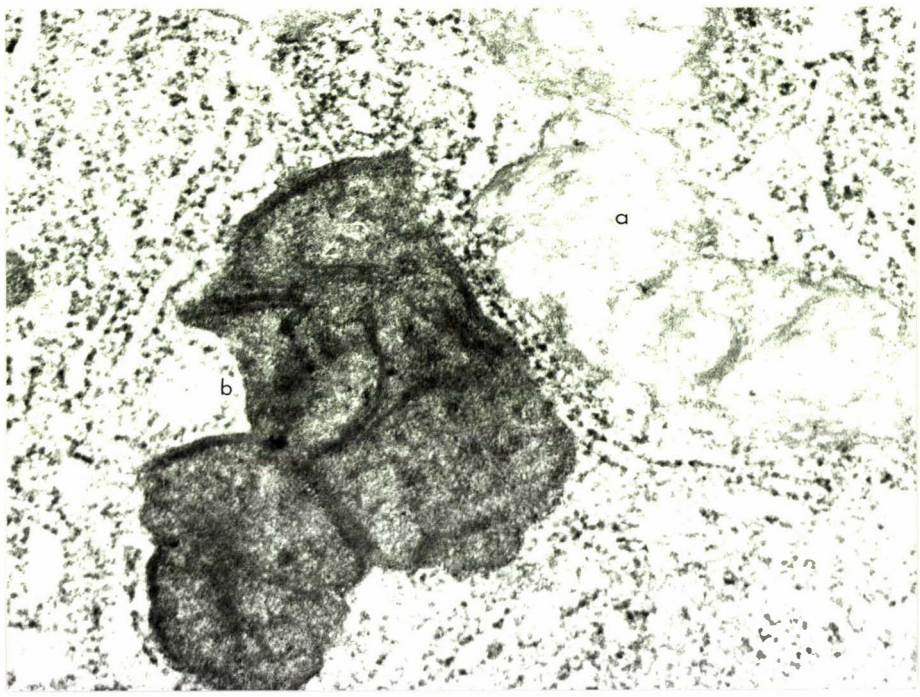
In non-neuronal cells, the pigment inclusions appear not to damage the cell (Fig. 3.16). Neurons, particularly if the inclusions are numerous, may show rarefaction of cytoplasm and sparsity of normal organelles (Fig. 3.19). They may also show thickening of cell processes, which is most obvious in larger neurons such as Purkinje cells. The fibrous astrocytosis noted under light microscopy is evident mainly in the cerebral cortex (Fig. 3.21).

While typical membranous bodies are only encountered in sheep affected with ceroid-lipofuscinosis, they and normal sheep exhibit another type of residual body which consist of a granular matrix of varying electron density often containing fat globules but without characteristic membranous profiles (Fig. 3.22). These are most frequently encountered in cells of the mononuclear phagocytic system, and also in brain, liver, pancreas, heart, male sex glands and skin adnexa.

Figure 3.21: Membranous inclusion in a thick bundle of astrocyte fibres in the cerebral cortex of a 25 months old ram with ceroid-lipofuscinosis.
(EM x22,500)

Figure 3.22: Pancreatic acinar cell showing two types of residual body. A curvilinear body typical for ovine ceroid-lipofuscinosis (a), and a granular, more electron dense, body (b) which may also be seen in normal sheep.
(EM x46,000)




a
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VI. EARLY DIAGNOSIS

Introduction

In order to establish whether sheep affected with ceroid-lipofuscinosis could be diagnosed before the appearance of clinical signs, the surviving lambs from the 1977 lamb crop of the experimental flock were subjected to liver and rectal biopsies at 4 - 5 months of age. The biopsy samples from the 36 lambs were processed for fluorescent, light and electron microscopy. The surviving 51 lambs of the 1978 lamb crop were subjected to skin biopsy. In addition, 21 of these lambs also had liver biopsies taken. The biopsy samples were processed for fluorescent microscopy only. Anaesthetic and surgical procedures during the taking of biopsy samples and post-operative care of the lambs were under the direction and supervision of staff from the Department of Veterinary Surgery.

Results

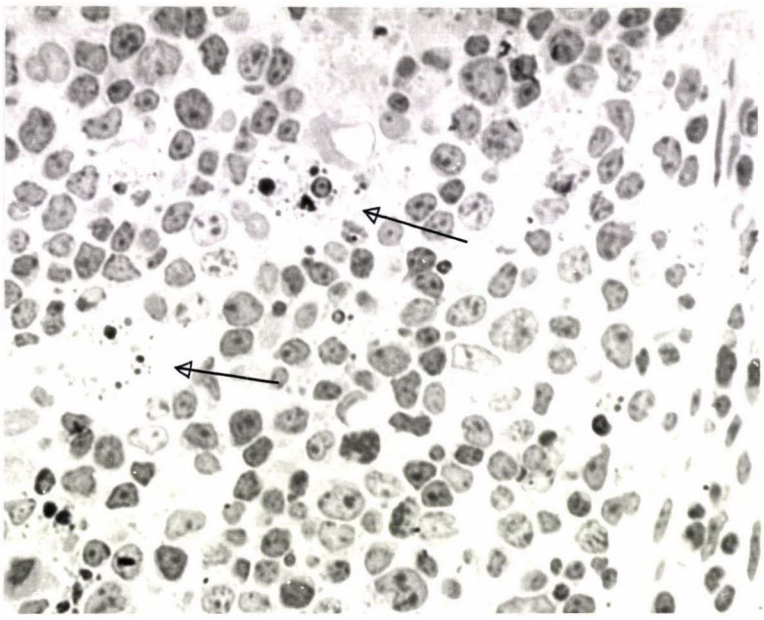
Three of the 36 lambs born in 1977 showed inclusions typical for ceroid-lipofuscinosis in both liver and rectal biopsy samples taken at 5 months of age. For diagnostic purposes the most useful characteristic of the inclusions was their autofluorescence when viewed under blue light. Autofluorescent inclusions were seen in hepatocytes and in macrophages of the *lamina propria* and lymphoid follicles of the rectal wall. Neurons of the autonomic nervous system were present in about 15% of rectal biopsies, but not in the biopsies from the three lambs

diagnosed as affected. The PAS and Sudan black stains were not as strongly positive as in affected animals over 12 months of age. When 1.0 µm epoxy resin embedded sections were stained with toluidine blue, inclusions could be seen in macrophages of the rectal wall (Fig. 3.23). Electronmicroscopically, curvilinear inclusion bodies were encountered in each of the three affected animals in hepatocytes, Kupffer cells and in macrophages of the rectal wall (Fig. 3.24). Biopsy samples from three animals diagnosed as normal, after histopathological examination, were also examined electronmicroscopically. No inclusions typical of those in ovine ceroid-lipofuscinosis were seen. Granular electron dense inclusions without membranous profiles were seen quite commonly in macrophages of the rectal wall (Fig. 3.25) and sometimes in Kupffer cells and hepatocytes of both affected and control sheep. The three lambs diagnosed as affected subsequently developed clinical signs of ceroid-lipofuscinosis at 11 - 12 months of age. After euthanasia the original diagnosis was confirmed by gross and histopathological examination (Cases 8, 9 and 10). None of the 33 lambs diagnosed as non-affected had developed clinical signs of ceroid-lipofuscinosis by the age of 2 years. None of the animals from this group which have been examined pathologically after slaughter or fatal illness have shown findings consistent with ovine ceroid-lipofuscinosis.

Four out of the 51 lambs from the 1978 lambing crop were diagnosed as being affected with ceroid-lipofuscinosis at 4 - 5 months of age on the basis of the presence of autofluorescent inclusions in glandular and ductal cells of sweat glands (Fig. 3.26). Liver biopsies

Figure 3.23: Lymphoid follicle in rectal wall of a 5 months old ewe lamb. Macrophages (arrows) contain nuclear remnants and lipopigment inclusions.
(Epoxy resin embedded section, toluidine blue-basic fuchsin x500)

Figure 3.24: Ultrastructural detail of part of macrophage showing typical curvilinear bodies. Thin section obtained from same tissue as shown in figure 3.23.
(EM x17,500)



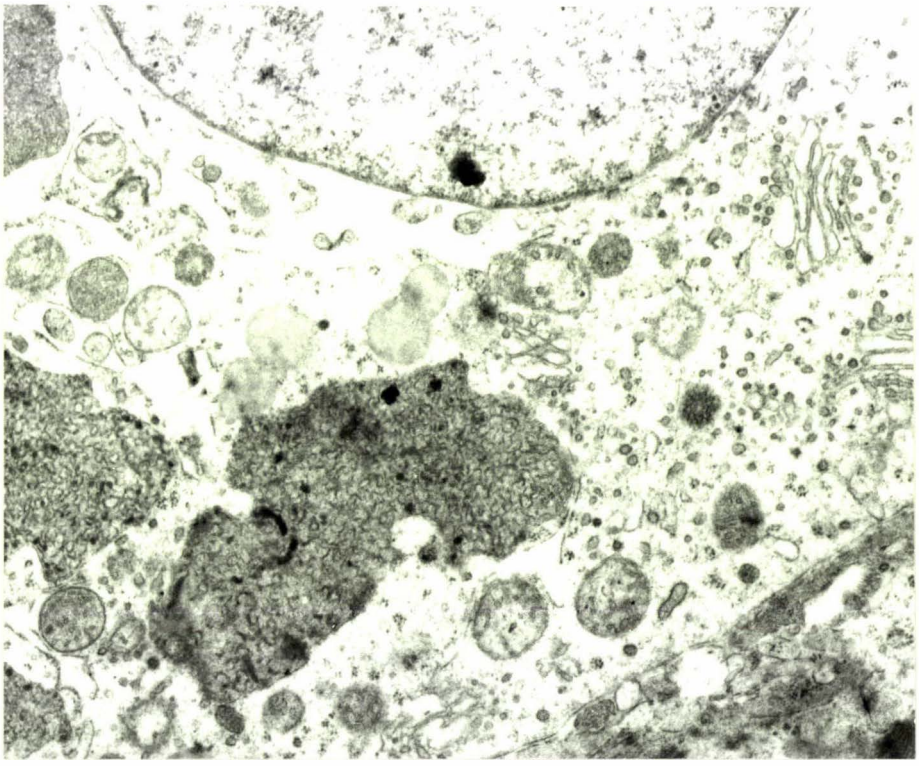
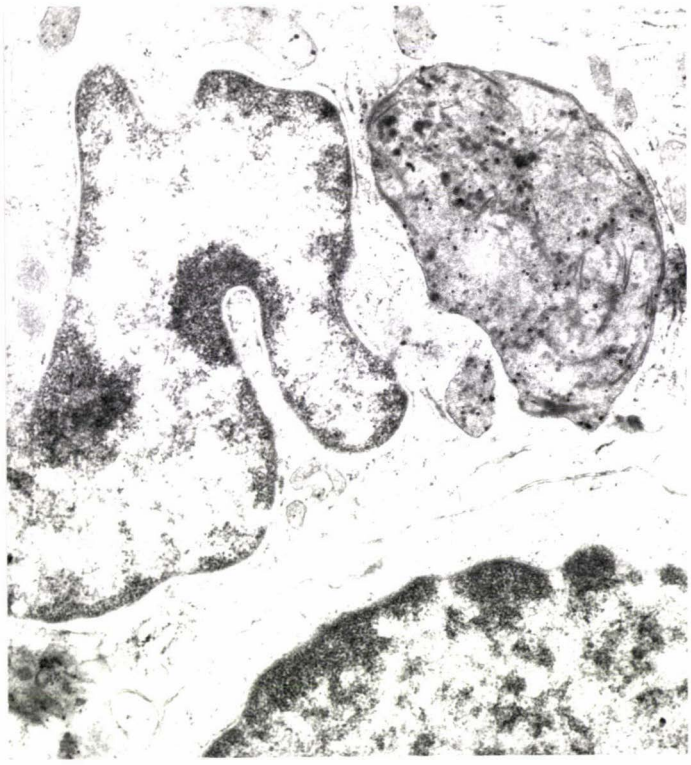
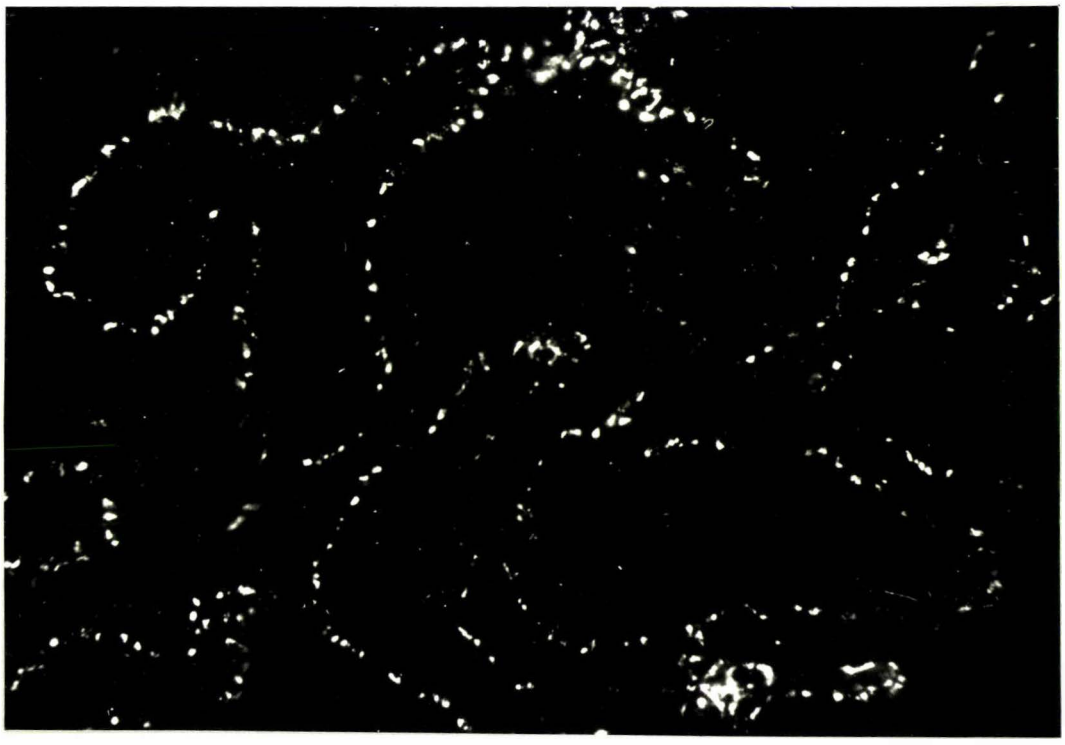


Figure 3.25: Granular electron dense inclusion in a macrophage in the rectal wall of a 5 months old normal lamb. These inclusions are encountered in both normal and affected sheep, and carry no significance in the diagnosis of ovine ceroid-lipofuscinosis. (EM x17,500)

Figure 3.26: Autofluorescent material outlines the sweat glands in the skin of a 25 months old ram affected with ceroid-lipofuscinosis. Before the onset of clinical signs, this autofluorescence is already present in affected lambs at 4 - 5 months of age. (Unstained deparaffinised section viewed under blue light, x160)





were taken from three of the four affected animals and the hepatocytes also contained autofluorescent material. Only those animals diagnosed as affected showed symptoms of ovine ceroid-lipofuscinosis at the predicted age of 11 - 12 months.

VII. DISCUSSION

The pathological findings in ovine ceroid-lipofuscinosis present a characteristic picture, but with the severity of lesions increasing with age. In this, the severity of the pathological changes closely follows the clinical course of the disease.

The most striking gross pathological finding in affected animals is the reduction in size and weight of the brain. The average brain weight of affected sheep is 66% of that of normal sheep. This closely approximates the figure of 70% reported by Koppang (1973/74) for canine ceroid-lipofuscinosis. Cross-section of human and canine brains affected with ceroid-lipofuscinosis reveals a yellow discolouration (Zeman *et al.*, 1970; Koppang, 1973/74). This was not observed in the ovine cases. Thickening of skull bones overlying the brain in ovine ceroid-lipofuscinosis is presumably due to lack of remodelling associated with the atrophic cerebrum. The firm consistency of affected cerebral hemispheres is probably due to the astrocytosis noted histologically.

Histological examination reveals the generalised nature of ovine ceroid-lipofuscinosis. In this it resembles the canine disease

(Koppang, 1973/74). Although emphasis has been placed on the neurological consequences of the human syndromes, there is growing awareness of widespread visceral involvement of the four main human syndromes as well (Haltia *et al.*, 1973b; De Baecque *et al.*, 1976; Zeman, 1976; Kornfeld, 1972). Following the suggestion of Joosten *et al.* (1973) the alternative name "generalised ceroid-lipofuscinosis" has been used by some (Anzil *et al.*, 1975; Schuurmans Stekhoven *et al.*, 1976, 1977; Schwendemann, 1976). However Goebel *et al.* (1979) point out that the process which leads to the increased accumulation of lipopigment bodies damages the neurons in particular and almost exclusively. This observation is also true for ovine ceroid-lipofuscinosis.

In the central nervous system the neuronal accumulation of lipopigment inclusions is often accompanied by neuronal degeneration in the form of swelling of cell processes and sparsity of normal cytoplasmic organelles (Fig. 3.19). As expected from the gross findings the number of cerebral cortical neurons is less than in control brains. However neuronal death and accompanying neuronophagy is not commonly observed. It would appear therefore that neuronal loss is a gradual process and starts early in the disease possibly before the onset of clinical signs. To resolve this question, a longitudinal study such as described by Koppang (1973/74) in canine ceroid-lipofuscinosis is indicated for the ovine disease. Braak and Goebel (1978) reported selective loss of local circuit neurons in human juvenile neuronal ceroid-lipofuscinosis. This was not

apparent in the present study because a quantitative investigation of types of neurons at various levels of the brain was not carried out. The mild microgliosis and marked astrocytosis observed in the cerebral cortex of sheep with clinically advanced ceroid-lipofuscinosis is presumably a reaction to the neuronal degeneration and neuronal loss (Zeman *et al.*, 1970).

The retinal atrophy in ovine ceroid-lipofuscinosis closely resembles that of the juvenile form of human neuronal ceroid-lipofuscinosis (Zeman, 1976), but differs from the canine disease , where sensory elements of the retina are preserved even in animals that die from the disease at an age of 26 months (Koppang 1973/74). In this regard the ovine disease would make a useful model for the study of retinal atrophy in general and the retinal atrophy associated with the juvenile form of the human disease in particular.

The usefulness of the autofluorescent characteristic of the stored material becomes apparent in visceral organs and tissues as a number of cell types may contain PAS and/or Sudan black positive material in their normal physiological state. A pertinent finding is the absence of lipopigment inclusions in epithelium of skin, lower respiratory tract, gastro-intestinal tract and urinary bladder. It seems reasonable to assume that this is due to rapid turnover of these cells. On the other hand, of the long-lived postmitotic cells, pigment accumulation is apparently absent in skeletal muscle cells, scant in smooth muscle and cardiac cells, but plentiful in neurons. This would point to pigment

accumulation being due to the specialised biochemistry of the cell rather than its longevity. Finally the widespread involvement of the cells of the mononuclear phagocytic system would appear to be a result of their phagocytic function.

Ultrastructurally, the typical inclusion body in ovine ceroid-lipofuscinosis is a round or oval body 0.2 - 5.0 µm in size, with a granular matrix of varying electron density in which a wide variety of membranous profiles may be seen. Large conglomerates of these bodies may measure up to 15 µm. Under high magnification a five-layered membrane 12 nm across appears to be the common component of many membranous profiles. Exceptions are the tripartite membranes (Fig. 3.13), the tubular arrays (Fig. 3.18) and the membranous stacks (Fig. 3.15). The latter consist of alternating dark and light lines with a periodicity of 5.2 nm. In canine ceroid-lipofuscinosis, Koppang (1973/74) provides morphological evidence that the membranous stacks are derived from fusion of adjacent less dense outer lines of five layered membranes. This fusion was not observed in the ovine cases but the measurement of the structures involved does not preclude the possibility of its occurrence.

Much has been written about the significance of the ultra-structural morphology of the inclusion bodies in relation to clinically differentiable entities of human neuronal ceroid-lipofuscinosis. There is general agreement that the inclusions in the infantile form are granular osmiophilic deposits (Haltia *et al.*, 1973b; Zeman, 1976;

Lake, 1977). Characteristic for the adult type are similar deposits often with lipid vacuoles (Zeman, 1976). In this latter disease, Dom *et al.* (1979) also reported on the occurrence of curvilinear bodies. There is difference of opinion on the significance of the ultrastructural morphology of inclusions in the late infantile and juvenile forms. One group of authors consider curvilinear bodies typical for the late infantile and fingerprint patterns typical for the juvenile form (e.g. Gonatas *et al.*, 1968; Duffy *et al.*, 1968; Lake, 1977; Lake and Cavanagh,1978). Others consider that the ultrastructural differences of the inclusion bodies in these two forms of neuronal ceroid-lipofuscinosis are more quantitative than qualitative (Towfighi *et al.*, 1973; Anzil *et al.*, 1975; Goebel *et al.*, 1979). In a recent comprehensive study of the ultrastructure of the residual bodies in the late infantile and the juvenile forms of neuronal ceroid-lipofuscinosis, Goebel *et al.* (1979) stressed the diversity of the patterns. They found that of the two more common types of inclusion bodies, the curvilinear pattern predominated in the late infantile form, and the fingerprint pattern occurred more commonly in the juvenile form. Lake (1977) acknowledged that curvilinear bodies may be found in some tissues of patients with the juvenile type. However in neurons and smooth muscle cells of rectal biopsy material of their patients, inclusions were consistently of the curvilinear type in the late infantile form and consistently showed fingerprint patterns in the juvenile form. On this basis Lake and Cavanagh (1978) delineated a further "early juvenile" subgroup which contained inclusions with a fingerprint pattern in rectal biopsy material but

the patients showed other features characteristic for the late infantile form.

In ovine ceroid-lipofuscinosis the ultrastructural patterns of the residual bodies show widest variation in the central nervous system. Fingerprint and curvilinear patterns are equally common, followed by crystalloid and tubular patterns and a variety of configurations which have not received special names. Curvilinear bodies predominate in visceral organs and tissues. These findings are similar to those in canine ceroid-lipofuscinosis (Koppang, 1973/74). Rectal biopsy material of affected lambs showed mainly curvilinear bodies and the ovine entity therefore resembles the late infantile form of the human syndrome according to Lake (1977). The neuronal inclusions show features similar to those encountered in late infantile and juvenile neuronal ceroid-lipouscinosis in the human (Goebel *et al.*, 1979).

Peculiar to ovine ceroid-lipofuscinosis, when compared to the latter two entities, is the absence of inclusion bodies in lymphocytes (Schuurmans Stekhoven *et al.*, 1976; 1977) and in skeletal muscle Goebel *et al.*, 1975).

During the course of this study, electronmicroscopy resolved the initial confusion created by the presence of PAS and Sudan black positive, autofluorescent inclusions in cells of the mononuclear phagocytic system in normal sheep. These inclusions were shown to

be morphologically different from the typical curvilinear bodies of ovine ceroid-lipofuscinosis. They occurred in both affected and control sheep and were already present in 4 - 5 months old lambs. It was noted that these inclusions occurred infrequently in hepatocytes and sweat gland epithelium. Since many of these cells harbour typical lipopigment inclusion bodies in affected sheep over 12 months, liver and skin became the biopsy tissues of choice for early diagnosis of ceroid-lipofuscinosis in lambs at 4 - 5 months of age. Diagnosis rested on the demonstration of autofluorescent inclusions in fresh frozen sections. With the three lambs born in 1977, diagnosis was confirmed after electronmicroscopy revealed the presence of curvilinear bodies in both liver and rectal biopsies. With the 1978 lambs, it was felt that the demonstration of autofluorescent material in sweat gland epithelium was sufficient for a diagnosis of ceroid-lipofuscinosis. Only the four animals so diagnosed developed typical clinical signs at the predicted age of 11 - 12 months.

The finding that curvilinear bodies are already present at 4 - 5 months of age and the occurrence of PAS and Sudan black positive autofluorescent neuronal inclusions in two lambs which died perinatally (Cases 11 and 12), strongly suggest that ovine ceroid-lipofuscinosis is indeed caused by an inborn error of metabolism. This agrees with the findings of Koppang (1973/74) in the canine disease.

In the biopsy material of the affected lambs at 4 - 5 months of age, there was a discrepancy between the amount of lipopigment seen

by fluorescent microscopy of fresh frozen sections and that seen by light microscopy of PAS and Sudan black stained formalin fixed sections. There was less extensive evidence of lipopigment accumulation with the latter stains, when compared with fluorescent microscopy. In affected animals over 12 months of age, this discrepancy does not exist to such a noticeable degree. This tends to suggest that the lipopigment is less stable in the younger animals and that part of the fluorescent component is removed by the processes of fixation and/or dehydration. Koppang (1973/74) observed the same phenomenon in tissues of his affected dogs up to 6 months of age and speculated that part of the fluorescent material was not yet polymerized or crosslinked to the same degree as in residual bodies.

CHAPTER IV

GENETICS

I. INTRODUCTION

The four main subgroups of the human neuronal ceroid-lipofuscinoses are generally considered to have an autosomal recessive monohybrid mode of inheritance (Norio *et al.*, 1973; Zeman, 1976), although this has only conclusively been shown for the juvenile form (Sjögren, 1931). There is one recorded exception to this general rule. Boehme *et al.* (1971) reported on a family in which the adult form of neuronal ceroid-lipofuscinosis was inherited as a Mendelian dominant with full penetrance and no variability of expressivity. Canine ceroid-lipofuscinosis is also inherited as a simple autosomal recessive trait (Koppang, 1973/74).

The results of investigations into the mode of inheritance of ovine ceroid-lipofuscinosis are presented and discussed in this chapter. The investigations consisted firstly of the tracing of the pedigrees of all affected individuals and secondly of the analysis of sire-daughter matings in the experimental flock. The sire used in the latter experiment was known to have produced affected lambs.

II. RESULTS

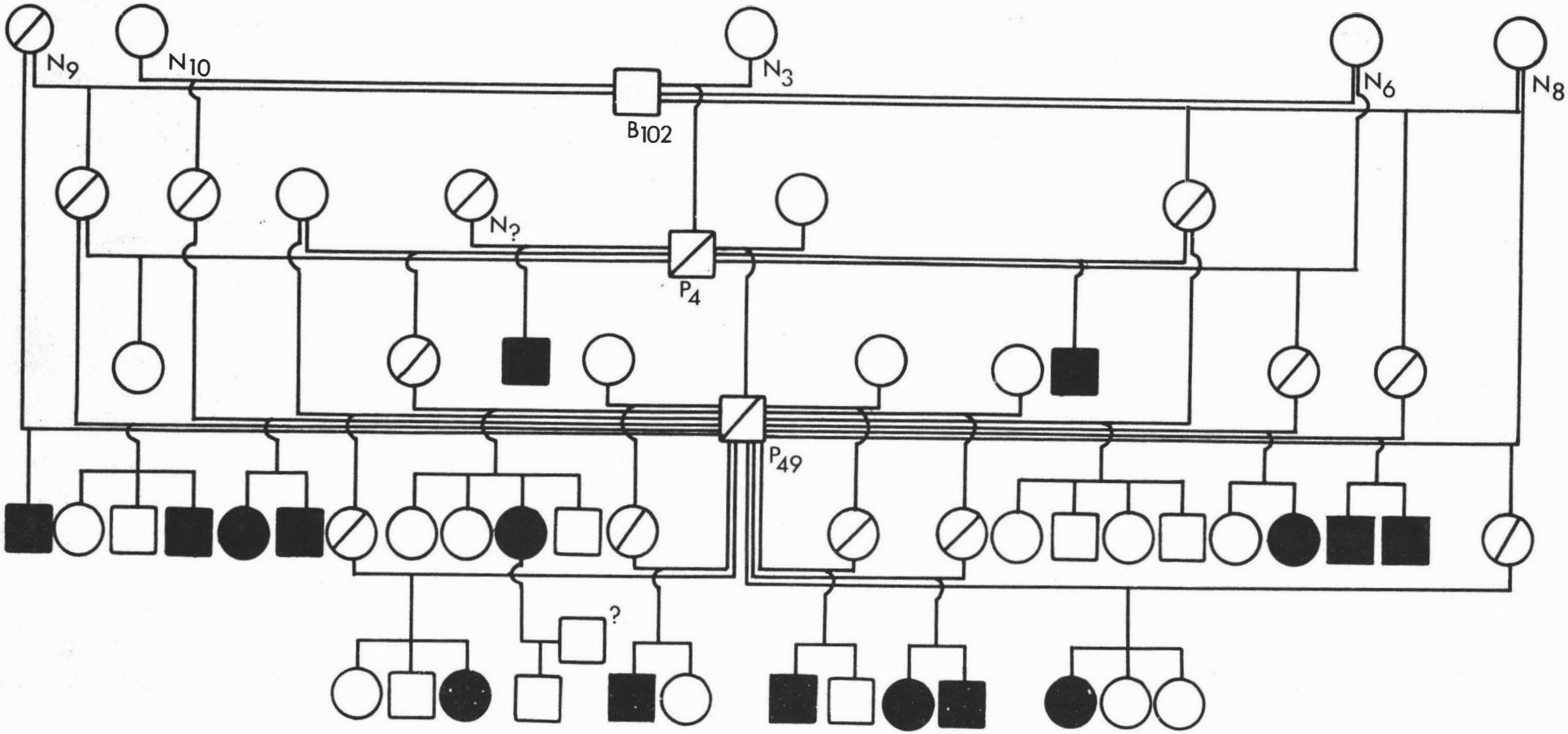
All cases of ovine ceroid-lipofuscinosis diagnosed to date have occurred in one closely interrelated group of the South Hampshire breed of sheep. The first seven cases occurred in a small inbred stud flock

in the South Island of New Zealand; the remaining nine cases were diagnosed in an experimental flock derived from it and located at Massey University. The family tree of affected individuals (Fig. 4.1) shows that both male and female sheep may be affected, indicating a probable autosomal mode of inheritance. The recessive nature of the trait is indicated by the observation that matings between heterozygous males and females tend to produce more normal than affected individuals. One affected ewe was mated by an unknown ram and produced a healthy ram lamb, providing further evidence for an autosomal recessive mode of inheritance.

Originally the sire B102 was thought to be the common heterozygous ancestor. However the mating of P49 and N9 resulted in an affected ram lamb. It appears likely therefore that the deleterious gene was introduced by the ewes identified by the letter N. Their exact relationship is not known, but there is reason to believe that they could be half sisters, thus making their sire the probable common heterozygous ancestor.

The results of sire-daughter matings of a South Hampshire ram, heterozygous for ceroid-lipofuscinosis are shown in Table 4.I.

Figure 4.1: Family tree of affected individuals. Only matings of heterozygotes and other pertinent individuals are shown.



CLINICALLY NORMAL MALE & FEMALE

DISEASED MALE & FEMALE

OBLIGATE HETEROZYGOUS MALE & FEMALE

TABLE 4.I

Results of sire-daughter matings of a South Hampshire ram heterozygous for ceroid-lipofuscinosis

Year	Number of ewes	Number of lambs				
		Total	Affected		Non-affected	
			Observed	Expected	Observed	Expected
1977	23	30*	2	3.8	28	26.2
1978	29	37	4	4.6	33	32.4
			6	8.4	61	58.6

(X^2_1 = 0.48; 0.50 > p > 0.25)

* Three lambs were not available for diagnosis, but were classified as being non-affected.

With the exception of three lambs, all offspring of these matings were examined pathologically and diagnosed either as affected or non-affected with ceroid-lipofuscinosis. Chi-squared analysis, incorporating Yates correction, of this data (Table 4.I) is compatible with the hypothesis of a simple autosomal recessive mode of inheritance. With this type of inheritance, a heterozygous sire mated to his daughters would be expected to produce one homozygous affected individual in eight such matings.

III DISCUSSION

The South Hampshire breed is of relatively recent origin having been developed from an initial cross of Southdown and Hampshire Down sheep. It can be assumed that a relatively small number of individuals from either breed would have formed the founding nucleus of the new breed and that subsequent breeding policies, such as line and inbreeding, would have been employed to establish a more or less homogeneous breed. If the recessive gene for ovine ceroid-lipofuscinosis is not a recent mutation, it must also be present in either the Southdown or Hampshire Down breeds. There are no reports on the occurrence of the disease in either breed, suggesting a low frequency of the deleterious gene. The ovine disease therefore illustrates that through the founder effect, an extreme case of genetic drift, and certain animal breeding policies, a rare gene may become incorporated in a new population at a much higher gene frequency than in the population of origin. If the rare gene is also deleterious it may emerge, in the new population, in the homozygous state with manifest disease as a consequence.

Ovine ceroid-lipofuscinosis is unlikely to be of economic importance to the sheep industry, except to the pure bred flocks involved. The South Hampshire sheep are relatively uncommon and are used as a specialist meat breed with the purpose of supplying sires to be crossed with the much more common indiginous New Zealand Romney breed, for table lamb production. As this is a terminal cross the use of heterozygous sires is of negligible importance.

CHAPTER V

GENERAL DISCUSSION

Ovine ceroid-lipofuscinosis is characterised clinically by loss of vision and behavioural abnormalities starting around 12 months of age. Motor disturbances in the form of episodes of muscle twitching, head nodding and champing of the jaws commence soon afterwards, and increase in severity as the disease progresses. It it unlikely that under field conditions, affected sheep would live much longer than 2 years. Funduscopy of the eye and radiography of the skull may reveal retinal atrophy and thickening of the skull bones overlying the brain respectively. On the basis of these findings, a presumptive clinical diagnosis of ovine ceroid-lipofuscinosis should not be difficult to make, at least in the South Hampshire breed of sheep. From the viewpoint of comparative medicine, the clinical signs of the ovine disease resemble most closely those of the juvenile form of the four main entities in the human syndrome (Santavuori *et al.*, 1973; Zeman *et al.*, 1970; Zeman and Siakotos, 1973; Dom *et al.*, 1979), and those of the canine disease (Koppang, 1973/74). Lymphocytic vacuolation, a common finding in juvenile neuronal ceroid-lipofuscinosis, is not observed in either the ovine or the canine disease.

The salient gross pathological features of ovine ceroid-lipofuscinosis are a reduction in size and weight of the brain, slight dilation of the lateral ventricles and thickening of the skull bones overlying the cerebrum. The reduction in size mainly affects the cerebral cortex and to a lesser extent, the cerebral white matter.

Cerebral cortical atrophy is also seen in the infantile, late infantile and juvenile forms of the human syndrome (Haltia *et al.*, 1974; Zeman *et al.*, 1970). However in the infantile and late infantile types, this is accompanied by cerebellar cortical atrophy, which need not be present in the juvenile form. In the canine disease the atrophy affects both the cerebral and cerebellar cortex, but is more marked in the latter (Koppang, 1973/74). The adult human form is characterised by cerebellar atrophy (Fine *et al.*, 1960; Boehme *et al.*, 1971).

Histologically, ovine ceroid-lipofuscinosis, like its human and canine counterparts, is characterised by the intracytoplasmic accumulation of autofluorescent PAS and Sudan black positive lipopigment in neurons and a wide variety of other cell types. From morphological observations, it appears that the process which leads to increased accumulation of lipopigment bodies only damages neurons. In affected sheep, lesions other than pigment accumulation are mainly confined to the cerebral cortex and retina. The cerebral cortex shows degeneration and loss of neurons, a mild microgliosis and a marked astrocytosis. The retina show degeneration and loss of the layer of rods and cones and associated outer nuclear layer, which becomes more severe as the course of the disease progresses. Compared to the infantile and late infantile human forms, the ovine disease shows relatively mild neuropathological changes which resemble those found in the juvenile form (Haltia *et al.*, 1973a; de Baecque *et al.*, 1976; Zeman, 1976). The neuronal loss in the adult form and in canine ceroid-lipofuscinosis is most obvious in the cerebellar cortex (Boehme *et al.*, 1971; Koppang, 1973/74). Retinal atrophy is not present in the canine form and has only been reported in one case of the adult human disease (Dom *et al.*,

1979). The visceral involvement in lipopigment accumulation reflects the generalised nature of the metabolic disturbance associated with ovine ceroid-lipofuscinosis. In this it resembles the human and canine entities (Haltie *et al.*, 1973b: de Baecque *et al.*, 1976; Zeman, 1976; Kornfeld, 1972; Koppang, 1973/74).

Ultrastructurally, the typical lipopigment inclusion in ovine ceroid-lipofuscinosis is a round, oval or irregularly shaped body 0.2 - 5.0 μm in size, with a granular matrix of varying electron density in which a wide variety of membranous profiles may be seen. Some of the profiles have received special names such as fingerprint, curvilinear and cystalloid. The membranous inclusion bodies are similar to those found in late infantile and juvenile neuronal ceroid-lipofuscinosis, in the canine disease and in bovine neuronal lipodystrophy (Goebel *et al* 1979; Koppang, 1973/74; Read and Bridges, 1969). The infantile and adult human forms are characterised by granular osmiophilic deposits (Haltia *et al.*, 1973b; Zeman, 1976), although recently, Dom *et al.* (1979) reported the presence of curvilinear bodies in the adult type. The predominance of curvilinear bodies, at least viscerally, which is noted in the ovine disease would make it most closely resemble the late infantile human form according to Lake (1977) and Lake and Cavanagh (1978).

Pathological examination of biopsy specimens from lambs at 4 - 5 months of age shows that lipopigment accumulation is present before the onset of clinical signs, and allows for an early diagnosis of ovine ceroid-lipofuscinosis to be made. The autofluorescent characteristic of the lipopigment makes diagnosis relatively simple

by examining fresh frozen unstained sections under blue light. However, this characteristic is not unique to the storage material of ovine ceroid-lipofuscinosis. In normal sheep, cells of the mononuclear phagocytic system, and to a lesser degree other cell types, often harbour PAS and Sudan black positive autofluorescent inclusions. Electronmicroscopy reveals granular structures of varying electron density but without membranous profiles. These inclusions are also found in affected sheep amongst the typical membranous ceroid bodies. The nature of this material is not known, but it is already present in biopsy specimens of 4 - 5 months old lambs.

The mode of inheritance of the ceroid-lipofuscinoses of man and domestic animals is generally considered to be autosomal recessive (Norio *et al.*, 1973; Zeman, 1976; Koppang, 1973/74). This is well documented for the juvenile human form and the canine disease, both of which are inherited as a monohybrid autosomal recessive trait (Sjögren, 1931; Rayner, 1962; Koppang, 1973/74). A dominant autosomal mode of inheritance in the adult human form was reported in one family by Boehme *et al.* (1971) suggesting the involvement of more than one allele or allelic pleomorphism. The phenomena of homochronism and homotypism (p. 7) led Zeman (1976) to speculate on the possibility of a dihybrid autosomal recessive mode of inheritance. The family tree of affected sheep and the results of sire-daughter matings of a heterozygous ram, show ovine ceroid-lipofuscinosis to be inherited as a simple autosomal recessive trait. If the deleterious gene is not a recent mutation, its frequency in the founding breeds of the South Hampshire sheep is expected to be low, and ceroid-lipofuscinosis has not been diagnosed in either the Southdown or Hampshire Down sheep.

Ovine ceroid-lipofuscinosis illustrates the dangers inherent in the practice of establishing a new breed from a relatively small number of founders and the use of line and inbreeding to increase homogeneity of the new breed. Rare deleterious genes in the original populations may become established at a much higher frequency in the new breed, with emergence of the homozygous state and manifest disease as a consequence.

The findings presented in this thesis firmly establish the ovine disease as belonging to the heterogeneous group of diseases of man and domestic animals generally known as Batten's disease or neuronal or generalised ceroid-lipofuscinosis. When compared to the other entities, the ovine disease has many features in common with both the late infantile and juvenile form of the human syndrome,i.e. with Batten's disease in its narrower sense as originally defined by Batten (1903, 1914), and elaborated upon by Zeman and Donahue (1963) and Zeman and Dyken (1969). It also has features in common with the canine disease. The physical characteristics of the storage material in ovine ceroid-lipofuscinosis (I. Morrison, pers. comm.) and the ultra-structual morphology are those of "ceroid" as defined by Siakotos *et al.* (1970,1972) and Wolfe *et al.* (1977). It would seem appropriate to refer to the disease as "ovine ceroidosis" however for comparative purposes, the term, "ovine ceroid-lipofuscinosis" has been used. A definitive nomenclature of all the entities will only become possible after their biochemical elucidation.

Ovine ceroid-lipofuscinosis is unlikely to be of economic importance to the sheep industry except to the pure bred flocks involved. The South Hampshire breed of sheep is relatively uncommon and is used to supply sires to be crossed with the common indiginous New Zealand

Romney breed for table lamb production. As this is a terminal cross the use of heterozygous sires is of neglible importance. The ovine disease could however serve as a useful experimental model for the human syndrome.

Of the inherited human neuronal storage diseases, the ceroid-lipofuscinoses remain the largest and most common group of those that have not been defined in biochemical and pathogenic terms. The ovine model lends itself for longitudinal biochemical study of the process of lipopigment formation. The retinal atrophy associated with the ovine disease closely resembles that of the juvenile human entity. Further study of this abnormality should aid in the understanding of retinal atrophy in general and that associated with juvenile neuronal ceroid-lipofuscinosis in particular. In the inherited diseases of man, the detection of heterozygotes is of importance, so that the occurrence of the homozygous recessive state can be avoided by genetic counselling. The availability of known heterozygotes in the experimental flock of South Hampshire sheep provides an opportunity for evaluating methods of heterozygote detection. As more becomes known about the underlying metabolic anomaly and pathogenic pathways of the ceroid-lipofuscinoses, the ovine disease would present a valuable model for the evaluation and monitoring of therapeutic trials. The range and scope of such experiments are more limited in human patients, on moral and ethical grounds.

REFERENCES

Adelman, L.S.; Young, E.; Bass, N.H. (1974): Abnormal accumulation of sialoglycoproteins in a case of late infantile amaurotic idiocy. *Neurology*, 24: 1045-1050.

Andrews, J.M.; Sorenson, V.; Cancilla, P.A.; Price, H.M.; Menkes, J.H. (1971): Late infantile neurovisceral storage disease with curvilinear bodies. *Neurology*, 21: 207-217.

Anzil, A.P.; Blinzinger, K.; Harzer, K.; Reither, M.; Zimmermann, G. (1975): Cytosome morphology and distribution of generalized ceroid-lipofuscinosis in a twenty-eight month old boy with normal myeloperoxidase activity. *Neuropädiatrie*, 6: 259-283.

Anzil, A.P.; Martinius, J.; Blinzinger, K. (1976): Follow-up study of a case of generalized ceroid-lipofuscinosis of childhood with special reference to the findings of an abnormal serum lecithin fatty acid pattern. *Neuropädiatrie*, 7: 362-367.

Armstrong, D.: personal communication. Department of Neurology, University of Colorado Medical Centre, Denver, Colorado.

Armstrong, D.; Dimmitt, S.; Grider, L.; Van Wormer, D. (1973): Deficient leukocyte peroxidase activity in the Batten-Spielmeyer-Vogt syndrome. *Trans. am. neurol. Assoc.*, 98: 3.

Armstrong, D.; Dimmitt, S.; Van Wormer, D.E. (1974a): Studies in Batten disease. I. Peroxidase deficiency in granulocytes. *Arch. Neurol.*, 30: 144-152.

Armstrong, D.; Dimmitt, S.; Boehme, D.H.; Leonberg, S.C.; Vogel, W. (1974b): Leukocyte peroxidase deficiency in a family with a dominant form of Kufs disease. *Science*, 186: 155.

Armstrong, D.; Siakotos, A.; Koppang, N.; Connole, E. (1978): Studies on the retina and the pigment epithelium in hereditary canine ceroid-lipofuscinosis. I. The distribution of enzymes in the whole retina and pigment epithelium. *Invest. Ophthalmol. vis. Sci.*, 17: 608-617.

Armstrong, D.; Van Wormer, D.E.; Dimmitt, S.; Barber, D.; Neville, H. (1974c): Tissue peroxidase deficiency of brain and viscera in Batten's disease. *Trans. am. Soc. Neurochem.*, 5: 135.

Armstrong, D.; Van Wormer, D.; Neville, H.; Dimmitt, S.; Clinigan, F. (1975): Thyroid peroxidase deficiency in Batten-Spielmeyer-Vogt disease. *Arch. Pathol.*, 99: 430-435.

Armstrong, D.; Wehling, C.; Van Wormer, D. (1977): Diagnosis of Batten disease from urinary sediment: a brief report. *Pathology*. 9: 39-41.

Awasthi, Y.C.; Morris, H.H.; Schochet, S.S.; Powell, G.F.; Schmalstieg, F.C.; Srivastava, S.K. (1977): Studies in neuronal ceroid-lipofuscinosis: leukocyte peroxidase deficiency in a patient with neuronal ceroid-lipofuscinosis (Jansky-Bielschowsky type). *J. Lab. and clin. Med.*, 89: 770-780.

Bagh, K.; Hortling, H. (1948): Blodfynd vid juvenil amaurotisk idioti. *Nord. Med.*, 38: 1072-1076. (Cited by Schuurmans Stekhoven *et al.*, 1977).

Batten, F.E. (1903): Cerebral degeneration with symmetrical changes in the maculae in two members of a family. *Trans. ophthalmol. Soc. U.K.*, 23: 386-390 (Cited by Zeman, 1970).

Batten, F.E. (1914): Family cerebral degeneration with macular change (so-called juvenile form of amaurotic family idiocy). *Quart. J. Med.*, 7: 444-454. (Cited by Zeman *et al.*, 1970).

Baumann, R.J.; Markesberry, W.R. (1978): Juvenile amaurotic idiocy (neuronal ceroid-lipofuscinosis) and lymphocytic fingerprint profiles. *Ann. Neurol.*, 4:531-536.

Bielschowsky, M. (1913): Über spät-infantile familiäre amaurotische Idiotie mit Kleinhirnsymptomen. *Dtsch. Z. Nervenheilk.*, 5: 7-29. (Cited by Zeman, 1970).

Blogg, J.R. (1975): Diseases of the fundus. In *The Eye in Veterinary Practice*, pp. 513-581. V.S. Supplies Ltd., North Melbourne.

Boehme, D.; Cottrel, J.; Leonberg, S.; Zeman, W. (1971): A dominant form of neuronal ceroid-lipofuscinosis. *Brain*, 94: 745-760.

Borst, M. (1922): *Pathologische Histologie*. Vogel, Leipzig. (Cited by Zeman and Siakotos, 1973).

Braak, H.; Goebel, H.H. (1978): Loss of pigment-laden stellate cells: a severe alteration of the isocortex in juvenile neuronal ceroid-lipofuscinosis. *Acta neuropath.*, 42: 53-57.

Burcar, P.; Armstrong, D.; Koppang, N.; Lewis, J.; Johnson, S.; Neville, H. (1977): Detection of canine Batten disease with the EEG. *Electroencephalography clin. Neurophysiol.*, 42: 120-124.

Carpenter, S.; Karpati, G.; Andermann, F. (1972): Specific involvement of muscle, nerve and skin in late infantile and juvenile amaurotic idiocy. *Neurology*, 22: 170-186.

Cech, P.; Stalder, H.; Boreux, G.; Papathanasiou, A.; Widmann, J.J.; Roth, P.; Rohner, A.; Miescher, P.A. (1977): Déficience héréditaire en myéloperoxidase. Etude clinique, biologique et génétique. *Schweitz. med. Wschr.*, 107: 1458.

Ceuterick, C.L.; Martin, J.J.; Casaer, P.; Edgar, G.W.F. (1976): The diagnosis of infantile generalized ceroid-lipofuscinosis (type Hagberg-Santavuori) using skin biopsy. *Neuropädiatrie*, 7: 250-260.

Chio, K.S.; Tappel, A.L. (1969a): Synthesis and characterization of the fluorescent products derived from malonaldehyde and amino acids. *Biochemistry*, 8: 2821-2827.

Chio, K.S.; Tappel, A.L. (1969b): Inactivation of ribonuclease and other enzymes by peroxidizing lipids and by malonaldehyde. *Biochemistry*, 8: 2827.

Chou, S.M.; Thompson, H.G. (1970): Electron microscopy of storage cytosomes in Kufs' disease. *Arch. Neurol.*, 23: 489-501.

Clausen, J.; Jensen, G.E. (1975): Acid proteinase and peroxidase activity in Spielmeyer-Vogt's syndrome (Batten's syndrome - Stengel's syndrome). *Clin. chim. Acta*, 65: 283-289.

Cummings, J.F.; deLahunta, A. (1977): An adult case of canine ceroid-lipofuscinosis. *Acta neuropath.*, 39: 43-51.

Dayan, A.D.; Trickey, R.J. (1970): Thyroid involvement in juvenile amaurotic family idiocy (Batten's disease). *Lancet*, 2: 296-297.

DeBaecque, C. (1975): Diagnosis of neuronal ceroid-lipofuscinosis by electron microscopy of urinary sediment. *N. Engl. J. Med.*, 292: 1408.

DeBaecque, C.M.; Pollack, M.A.; Suzuki, K. (1976): Late infantile neuronal storage disease with curvilinear bodies. Systemic pathologic features. *Arch. Path. Lab. Med.*, 100: 139-144.

Dekaban, A.S.; Herman, M. (1974): Childhood, juvenile and adult cerebral lipidoses. Are these different nosological entities, *Arch. Path.*, 97: 65-73.

Den Tandt, W.R.; Martin, J.J. (1978): Peroxidase in ceroid-lipofuscinosis. *J. neurol. Sci.*, 38: 191-193.

Dolman, C.L.; Chang, E. (1972): Visceral lesions in amaurotic familial idiocy with curvilinear bodies. *Arch. Path.*, 94: 425-430.

Dolman, C.L.; MacLeod, P.M.; Chang, E. (1975): Skin punch biopsies and lymphocytes in the diagnosis of lipidoses. *Canad. J. neurol. Sciences*, 2: 67-73.

Dom, R.; Brucher, J.M.; Ceuterick, C.; Carton, H.; Martin, J.J. (1979): Adult ceroid-lipofuscinosis (Kufs' disease) in two brothers. Retinal and visceral storage in one; diagnostic muscle biopsy in the other. *Acta neuropath.* (Berl.), 45: 67-72.

Duffell, S.J.; Edwardson, R. (1978): Xanthosis in cattle. *Vet. Rec.*, 102: 269-270.

Duffy, P.E.; Kornfeld, M.; Suzuki, K. (1968): Neurovisceral storage disease with curvilinear bodies.*J. Neuropath. exp. Neurol.*, 27: 351-370.

Edgar, G.W.F.; Post, P.J.J. (1963): Amaurotic idiocy and epilepsy. *Epilepsia* (Boston), 4: 241-260. (Cited by Zeman *et al.*, 1970).

Farrell, D.F.; Sumi, S.M. (1977): Skin punch biopsy in the diagnosis of juvenile ceroid-lipofuscinosis. A comparison with leukocyte peroxidase assay. *Arch. Neurol.*, 34: 39-44.

Fine, D.I.M.; Barron, K.D.; Hirano, A. (1960): Central nervous system lipidosis in an adult with atrophy of the cerebellar granular layer. *J. Neuropath. exp. Neurol.*, 19: 355-369.

Goebel, H.H.; Pilz, H.; Gullotta, F. (1976): The protracted form of juvenile neuronal ceroid-lipofuscinosis. *Acta neuropath.* (Berl.), 36: 393-396.

Goebel, H.H.; Zeman, W.; Pilz, H. (1975): Significance of muscle biopsies in neuronal ceroid-lipofuscinosis. *J. Neurol. Neurosurg. Psychiatry*, 38: 985-993.

Goebel, H.H.: Zeman, W.; Patel, V.K.; Pullarkat, R.K.; Leonard, H.G. (1979): On the ultrastructural diversity and essence of residual bodies in neuronal ceroid-lipofuscinosis. *Mech. Ageing and Devt.*, 10: 53-70.

Gonatas, N.K.; Gambetti, P.; Baird, H. (1968): A second type of late infantile amaurotic idiocy with multilamellar cytosomes. *J. Neuropath. exp. Neurol.*, 27: 371-389.

Gonatas, N.K.; Terry, R.D.; Winkler, R.; Korey, S.R.; Gomez, C.J.; Stein, A. (1963): A case of juvenile lipidosis: Electron microscopic and biochemical observation of a cerebral biopsy. *J. Neuropath. exp. Neurol.*, 24: 318-340.

Green, P.D.; Little, P.B. (1974): Neuronal ceroid-lipofuscin storage in Siamese cats. *Can. J. comp. Med.*, 38: 207-212.

Greenwood, R.S.; Nelson, J.S. (1978): Atypical neuronal ceroid-lipofuscinosis. *Neurology*, 28: 710-717.

Hagberg, B.; Sourander, P.; Svennerholm, L. (1968): Late infantile progressive encephalopathy with disturbed polyunsaturated fat metabolism. *Acta paediatr. scand.*, 57: 495-499.

Hagberg, B.; Haltia, M.; Sourander, P.; Svennerholm, L.; Eeg-Olafson, O. (1974): Polyunsaturated fatty acid lipidosis. Infantile form of so-called neuronal ceroid-lipofuscinosis. I. Clinical and morphological aspects. *Acta paediatr. scand.*, 63: 753-763.

Hallervorden, J. (1938): Spätfalle von amaurotischer Idiotie. *Verh. dtsch. path. Ges.*, 31: 103-107. (Cited by Zeman, 1970).

Haltia, M.; Rapola, J.; Santavuori, P.; Keränen, A. (1973a): Infantile type of so-called neuronal ceroid-lipofuscinosis. Part 2. Morphological and biochemical studies. *J. neurol. Sciences*, 18: 269-285.

Haltia, M.; Rapola, J.; Santavuori, P. (1973b): Infantile type of so-called neuronal ceroid-lipofuscinosis. Histologic and electron microscopic studies. *Acta neuropath.*, 26: 157-170.

Hartley, W.J.: personal communication. Department Veterinary Pathology, Sydney University, Sydney.

Hayward, A.H.S. (1978): Xanthosis, an abnormal pigmentation of cattle. *Vet. Rec.*, 102: 96-97.

Hers, H.G. (1965): Inborn lysosomal disease. *Gastroenterology*, 48: 625-633.

Higier, H. (1901): Weiteres zur Klinik der Tay-Sachs'schen familiären paralytisch-amaurotischen Idiotie. *Neurol. Cbl.*, 20: 843-851. (Cited by Zeman, 1970).

Horoupian, D.S.; Ross, R.T. (1977): Pigment variant of neuronal ceroid-lipofuscinosis (Kufs' disease). *Can. J. neurol. Sci.*, 4: 67-75.

Jansky, J. (1908): Dosud neposaný případ familiárni amauroticke idiotie komplikovanés hypoplasic mozeckovou. *Sborn. lek.*, 13: 165-196. (Cited by Zeman, 1970).

Jensen, G.E.; Clausen, J.; Melchior, J.C.; Konat, G. (1977): Clinical, social and biochemical studies on Batten's syndrome, alias Spielmeyer-Vogt or Stengel syndrome. *Eur. Neurol.*, 15: 203-211.

Jensen, G.E.; Shukla, V.K.S.; Gissel-Nielsen, G.; Clausen, J. (1978): Biochemical abnormalities in Batten's syndrome. *Scand. J. clin. Lab. Invest.*, 38: 309-318.

Jervis, G.A.; Pullarkat, R.K. (1978): Pigment variant of lipofuscinosis. *Neurology*, 28: 500-503.

Jolly, R.D.: personal communication. Department of Veterinary Pathology and Public Health, Massey University, Palmerston North.

Jolly, R.D. (1978): Lysosomal storage diseases. *Neuropath. appl. Neurobiol.*, 4: 419-427.

Jolly, R.D.; Thompson, K.G. (1976): Mannosidosis - a cause of neonatal mortality in Angus calves. *N.Z. vet. J.*, 24: 184-185.

Jolly, R.D.; West, D.M. (1976): Blindness in South Hampshire sheep. A neuronal ceroid lipofuscinosis. *N.Z. vet. J.*, 24: 123.

Joosten, E.; Gabreels, F.; Stadhouders, A.; Bolmers, D.; Gabreels-Festen, A. (1973): Involvement of sural nerve in neuronal ceroid-lipofuscinosis: report of two cases. *Neuropädiatrie*, 4: 98.110.

Koenig, H.; McDonald, T.; Nellhaus, G. (1964): Morphological and histochemical studies of neurolipidosis by light and electron microscopy. *J. Neuropath. exp. Neurol.*, 23:191.

Koppang, N. (1966): Familiäre Glykosphingolipidose des Hundes (juvenile amaurotische Idiotie). *Ergebn. allgem. Path. pathol. Anat.*, 47: 1-43.

Koppang, N. (1970): Neuronal ceroid-lipofuscinosis in English Setters. Juvenile amaurotic familiar idiocy (A.F.I.) in English Setters. *J. small Anim. Pract.*, 10: 639-644.

Koppang, N. (1973/74): Canine ceroid-lipofuscinosis. A model for human neuronal ceroid-lipofuscinosis and aging. *Mech. Ageing Devt.*, 2: 421-445.

Kornfeld, M. (1972): Generalized lipofuscinosis (Generalized Kufs' disease). *J. Neuropath. exp. Neurol.*, 31: 668-682.

Kristensson, K.; Rayner, S.; Sourander, P. (1965): Visceral involvement in juvenile amaurotic idiocy. *Acta neuropath.* (Berl.), 4: 421-424.

Kufs, J. (1925): Über eine Spätform der amaurotischer Idiotie und ihre heredofamiliären Grundlagen. *Z. Ges. Neurol. Psychiat.*, 95: 169-188. (Cited by Zeman, 1970).

Lake, B.D. (1977): Histochemical and ultrastructural studies in the diagnosis of inborn errors of metabolism. In *Records of the Adelaide Children's Hospital*, 1:337-345.

Lake, B.D.; Cavanagh, N.P.C. (1978): Early-juvenile Batten's disease - a recognisable subgroup distinct from other forms of Batten's disease. *J. neurol. Sci.*, 36: 265-271.

Lehrer, R.L.; Cline, M.J. (1969): Leukocyte myeloperoxidase deficiency in disseminated Candiasis: the role of myeloperoxidase in resistance to Candida infection. *J. clin. Invest.*, 48: 1478-1488.

Levine, A.S.; Lemieux, B.; Brunning, R.; White, J.G.; Sharp, H.L.; Stadlan, E. (1968): Ceroid accumulation in a patient with progressive neurological disease. *Pediatrics*, 42: 483-491.

Lillie, R.D.; Ashburn, L.L.; Sebrell, W.H.J.; Daft, F.S.; Lowry, J.V. (1942): *Publ. Health Rep.*, 57: 502. (Cited by Zeman and Siakotos, 1973).

Marklund, S.; Plum, C.M. (1978): Superoxide dismutase in juvenile neuronal ceroid-lipofuscinosis (Spielmeyer-Vogt-Batten's disease). *J. Neurochem.*, 31: 521-523.

May, N.D.S. (1964): *The Anatomy of the Sheep.* University of Queensland Press, St. Lucia, Brisbane.

Mayou, M.S. (1904): Cerebral degeneration with changes in the maculae in three members of a family. *Trans. opthalmol. Soc. U.K.*, 24: 142-145. (Cited by Zeman, 1970).

Miley, C.E.; Gilbert, E.F.; France, T.D.; O'Brien, J.F.; Chun, R.W.M. (1978): Clinical and extraneural histologic diagnosis of neuronal ceroid-lipofuscinosis. *Neurology*, 28: 1008-1012.

Morrison, I.: personal communication. Department of Veterinary Pathology and Public Health, Massey University, Palmerston North.

Nakano, T.; Sakai, H.; Kinoshita, J.; Yagishita, S.; Itoh, Y. (1979): The fine structure of blood cells in ceroid-lipofuscinosis (Spielmeyer-Vogt's disease). *Neuropädiatrie*, 10: 56-66.

Noonan, S.M.; Desousa, J.; Riddle, J.M. (1978): Lymphocytic ultrastructure in two cases of neuronal ceroid-lipofuscinosis. *Neurology*, 28: 472-477.

Norio, R.; Nevanlinna, H.R.; Perheentupa, J. (1973): Hereditary diseases in Finland: rare flora in a rare soil. *Ann. clin. Res.*, 5: 109-141.

O'Brien, J.S. (1973): Tay-Sachs' disease and juvenile G_{M2}-gangliosidosis. In *Lysosomes and Storage Diseases* (Eds. Hers, H.G., Van Hoof, F.), pp. 323-345. Academic Press, New York, London.

Okada, S.; O'Brien, J.S. (1969): Tay-Sachs' disease: generalized absence of a beta-D-N-hexosaminidase component. *Science*, 165: 698-700.

Patel, V.; Koppang, N.; Patel, B.; Zeman, W. (1974): p-Phenylenediamine-mediated peroxidase deficiency in English Setters with neuronal ceroid-lipofuscinosis. *Lab. Invest.*, 30: 366-368.

Pilz, H.; Goebel, H.H. (1977): Peroxidase activity in the parotid gland in juvenile neuronal ceroid-lipofuscinosis. *Neuropath. appl. Neurobiol.*, 3: 93-101.

Pilz, H.; Goebel, H.H.; O'Brien, J.S. (1976c): Isoelectric enzyme patterns of leukocyte peroxidase in normal controls and patients with neuronal ceroid-lipofuscinosis. *Neuropädiatrie*, 7: 261-270.

Pilz, H.; O'Brien, J.S.; Heipertz, R. (1976a): Human saliva peroxidase: microanalytical isoelectric fractionation and properties in normal persons and in cases with neuronal ceroid-lipofuscinosis. *Clin. Biochem.*, 9: 85-88.

Pilz, H.; O'Brien, J.S.; Heipertz, R. (1976b): Human leukocyte peroxidase: a ctivity of a soluble and membrane-bound enzyme form in normal persons and patients with neuronal ceroid-lipofuscinosis. *Metabolism*, 25: 561-570.

Pilz, H.; Schwendemann, G.; Goebel, H.H. (1978): Diagnostic significance of myeloperoxidase assay in neuronal ceroid-lipofuscinosis (Batten-Vogt syndrome). *Neurology*, 28: 924-927.

Plum, C.M.; Nielsen, A. (1977): Acid phosphatase activity in lymphocytes from patients with Spielmeyer-Vogt-Batten's syndrome. *Clin. Chem. clin. Bioch.*, 15: 645-648.

Porta, E.A.; Hartroff, W.S. (1969): Lipid pigments in relation to aging and dietary factors (lipofuscins). In *Pigments in Pathology* (Ed. Wolfan, M.), pp. 191-235. Academic Press, New York.

Pullarkat, R.K.; Patel, V.K.; Brockerhoff, H. (1978): Leukocyte docosahexaenoic acid in juvenile form of ceroid-lipofuscinosis. *Neuropädiatrie*, 9:127-130.

Rac, R.; Giesecke, P.R. (1975): Lysosomal storage disease in Chihuahuas. *Aust. vet. J.*, 51: 403-404.

Rapola, J.; Haltia, M. (1973): Cytoplasmic inclusions in the vermiform appendix and skeletal muscle in two types of so-called neuronal ceroid-lipofuscinosis. *Brain*, 96: 833-840.

Rayner, S. (1962): Juvenile amaurotic idiocy in Sweden. Institute for medical genetics, Uppsala, Sweden. (Cited by Zeman and Siakotos, 1973).

Read, W.K.; Bridges, C.H. (1969): Neuronal lipodystrophy. Occurrence in an inbred strain of cattle. *Path. vet.*, 6: 235-243.

Sachs, B. (1887): On arrested cerebral development with special reference to its cortical pathology. *J. nerv. ment. Dis.*, 14: 541-553. (Cited by Zeman, 1970).

Sachs, B. (1896): A family form of idiocy, generally fatal and associated with early blindness (amaurotic family idiocy). *N.Y. med. J.*, 63: 697-703. (Cited by Zeman, 1970).

Santavuori, P.; Haltia, M.; Rapola, J.; Raitia, C. (1973): Infantile type of so-called neuronal ceroid-lipofuscinosis. Part I. A clinical study of 15 patients. *J. neurol. Sci.*, 18: 257-267.

Santavuori, P.; Haltia, M.; Rapola, J. (1974): Infantile type of so-called neuronal ceroid-lipofuscinosis. *Developm. Med. and Child Neurol.*, 16: 644-653.

Santavuori, P.; Moren, R. (1977): Experience of antioxidant treatment in neuronal ceroid-lipofuscinosis of Spielmeyer-Sjögren type. *Neuropädiatrie*, 8: 333-344.

Schaffer, K. (1905): Zur Pathogenese der Tay-Sachs'schen amaurotischen Idiotie. *Neurol.Cbl.*, 24: 437-448. (Cited by Zeman, 1970).

Schwendemann, G. (1976): Lymphocyte inclusions in the juvenile type of generalized ceroid-lipofuscinosis. *Acta neuropath.* (Berl.), 36: 327-338.

Schuurmans Stekhoven, J.H.; van Haelst, U.J.G.M.; Joosten, E.M.G.; Gabreels, F.J.M. (1976): Ultrastructural study of so-called curvilinear bodies and fingerprint structures in lymphocytes in late-infantile amaurotic idiocy. *Acta neuropath.* (Berl.), 35: 295-306.

Schuurmans Stekhoven, J.H.; van Haelst, U.J.G.M.; Joosten, E.M.G.; Loonen, M.C.B. (1977): Ultrastructural study of the vacuoles in the peripheral lymphocytes in juvenile amaurotic idiocy. Juvenile form of generalised ceroid lipofuscinosis. *Acta neuropath.* (Berl.): 38: 137-142.

Seitelberger, F.; Simma, K. (1962): On the pigment variant of amaurotic idiocy. In *Cerebral Sphingolipidoses* (Eds. Aronson, S.M.; Volk, B.W.)., pp. 1324-1328. McGraw-Hill, New York.

Seitelberger, F.; Jacob, H.; Schnabel, R. (1967): The myoclonic variant of cerebral lipidosis. In *Inborn disorders of Sphingolipid Metabolism* (Eds. Aronson, S.M.; Volk, B.W.), pp. 43-74. Pergamon Press, Oxford.

Siakotos, A.N.; Armstrong, D.; Koppang, N.; Connole, E. (1978): Studies on the retina and the pigment epithelium in hereditary canine ceroid lipofuscinosis. II. The sub-cellular distribution of lysosomal hydrolases and other enzymes. *Invest. Ophthalm. vis. Sci.*, 17: 618-633.

Siakotos, A.; Goebel, H.; Patel, V.; Watanabe, I.; Zeman, W. (1972): The morphological and biochemical characteristics of ceroid isolated from cases of neuronal ceroid-lipofuscinosis. In *Sphingolipids, Sphingolipidoses and allied Disorders* (Eds. Volk, B.W.; Aronson, S.M.), pp. 53-61. Plenum Press, New York.

Siakotos, A.N.; Watanabe, I.; Saito, A.; Fleischer, S. (1970): Procedures for the isolation of two distinct lipopigments from human brain: lipofuscin and ceroid. *Biochem. Med.*, 4: 361-375.

Siakotos, A.N.; Watanabe, I.; Pennington, K.; Whitfield, M. (1973): Procedures for the mass isolation of pure lipofuscin from normal human heart and liver. *Biochem. Med.*, 7: 25-38.

Sipe, J.C.; O'Brien, J.S. (1979): Ultrastructure of skin biopsy specimens in lysosomal storage diseases: Common sources of error in diagnosis. *Clin. Genetics*, 15: 118-125.

Sjögren, T. (1931): Die juvenile amaurotische Idiotie. Klinische und erblichkeitsmedezinische Untersuchungen. *Hereditas* (Lund) 14: 197-426. (Cited by Zeman, 1970).

Snedecor, G.W.; Cochran, W.G. (1967): *Statistical Methods*, 6th edtn. Iowa State Univ. Press, Ames. Iowa, U.S.A.

Spielmeyer, W. (1905): Über familiäre amaurotische Idiotien. *Neurol. Cbl.*, 24: 620-621 and 1131-1132. (Cited by Zeman, 1970).

Stengel, C. (1826): Beretning om et maerkelight sygdomstilfaelde hos fire sødskendei naer heden af Røraas. *Eyr* (Kristiana), 1: 347-354. (Cited by Koppang, 1973/74).

Suzuki, K.; Johnson, A.B.; Marquet, E.; Suzuki, K. (1968): A case of juvenile lipidosis: electron microscopic, histochemical and biochemical studies. *Acta neuropath.*, 11: 122-129.

Svennerholm, L.; Hagberg, B.; Haltia, M.; Sourander, P.; Vanier, M.T. (1975): Polyunsaturated fatty acid lipidosis. II. Lipid biochemical studies. *Acta paedriatr. scand.*, 64: 489-496.

Taubold, R.D.; Siakotos, A.N.; Perkins, E.G. (1975): Studies on the chemical nature of lipofuscin (age pigment) isolated from normal human brain. *Lipids*, 10: 383-390.

Tay, W. (1881): Symmetrical changes in the region of the yellow spot in each eye of an infant. *Trans. ophthalm. Soc. U.K.*, 1: 55-57. (Cited by O'Brien, 1973).

Terry, R.D.; Korey, S.R. (1960): Membranous cytoplasmic granules in infantile amaurotic idiocy. *Nature* (London), 188: 1000-1002.

Towfighi, J.; Baird, H.W.; Gambetti, P.; Gonatas, N.K. (1973): The significance of cytoplasmic inclusions in late infantile and juvenile amaurotic idiocy. An ultra-structural study. *Acta neuropath.* (Berl.), 23: 32-42.

Tsan, M.F.; Gale, A.N.; Murphy, E.A.; McIntyre, P.D. (1978): Neuronal ceroid-lipofuscinosis. Studies of granulocyte enzyme activities. *J. neurol. Sci.*, 36: 13-24.

Van Haelst, U.J.G.M.; Gabreels, F.J.M. (1972): The electron microscopic study of the appendix as early diagnostic means in Batten-Spielmeyer-Vogt disease. *Acta neuropath.* (Berl.), 21: 169-172.

Vogt, H. (1905): Über familiäre amaurotische Idiotie und verwandte Krankheitsbilder. *Mschr. Psychiatr. Neurol.*, 18: 161-171 and 310-357. (Cited by Zeman, 1970).

Watson, W.A.; Barnett, K.C.; Terlecki, S. (1972): Progressive retinal degeneration (Bright Blindness) in sheep. A review. *Vet. Rec.*, 91: 665-670.

West, D.M.: personal communication. Department of Veterinary Clinical Sciences, Massey University, Palmerston North.

Westermarck, T.; Sandholm, M. (1977): Decreased erythrocyte glutathione peroxidase activity in neuronal ceroid lipofuscinosis (NCL) - corrected with Selenium supplementation. *Acta pharmacol. et toxicol.*, 40: 70-74.

Witzleben, C.L. (1972): Lymphocyte inclusions in late-onset amaurotic idiocy. Value as a diagnostic test and genetic marker. *Neurology*, 22: 1075-1078.

Wolfe, L.S.; Baker, R.R.; Ng Ying Kin, N.M.K.; Carpenter, S. (1976): Isolation and chemical nature of curvilinear bodies from Batten's disease. *Canad. J. neurol. Sci.*, 3: 153-154.

Wolfe, L.S.; Ng Ying Kin, N.M.K.; Baker, R.R.; Carpenter, S.; Andermann, F. (1977): Identification of retinoyl complexes as the autofluorescent component of the neuronal storage material in Batten disease. *Science*, 195: 1360-1362.

Zeman, W. (1970): Historical development of the nosological concept of amaurotic familial idiocy. In *Handbook of Clinical Neurology* (eds. Vinken, P.J.; Bruyn, G.W.), pp. 212-232. North Holland Publ. Co., Amsterdam.

Zeman, W. (1974): Studies in the neuronal ceroid-lipofuscinoses. *J. Neuropath. exp. Neurol.*, 33: 1-12.

Zeman, W. (1976): The neuronal ceroid-lipofuscinoses. In *Progress in Neuropathology*, vol. III (Ed. Zimmerman, H.M.), pp. 203-223. Grune and Stratton, New York.

Zeman, W.; Alpert, M. (1963): On the nature of the "stored" lipid substances in juvenile amaurotic idiocy (Batten-Spielmeyer-Vogt). *Ann. Histochim.*, 8: 255-258.

Zeman, W.; Donahue, S. (1963): Fine structure of the lipid bodies in juvenile amaurotic idiocy. *Acta neuropath.*, 3:144-149

Zeman, W.; Dyken, P. (1969): Neuronal ceroid-lipofuscinosis (Batten's disease): Relationship to amaurotic family idiocy? *Pediatrics*, 44: 570-583.

Zeman, W.; Hoffman, J. (1962): Juvenile and late forms of amaurotic idiocy in one family. *J. Neurol. Neurosurg. Psychiatr.*, 25: 352-362.

Zeman, W.; Siakotos, A.N. (1973): The neuronal ceroid-lipofuscinoses. In *Lysosomes and Storage Diseases* (Eds. Hers, H.G.; Van Hoof, F.), pp. 519-551. Academic Press, New York - London.

Zeman, W.; Strouth, J.C. (1967): Leukocytic hypergranulation *versus* lymphocytic vacuolation as markers for heterozygotes and homozygotes with Batten-Spielmeyer-Vogt disease. In *Inborn Errors of Sphingolipid Metabolism* (Eds. Aronson, S.M.; Volk, B.W.), pp. 475-484. Pergamon Press, Oxford.

Zeman, W.; Donahue, S.; Dyken, P.; Green, J. (1970): The neuronal ceroid-lipofuscinoses (Batten-Vogt syndrome). In *Handbook of Clinical Neurology* (Eds. Vinken, P.J.; Bruyn, G.W.), pp. 588-679. North Holland Publ. Co., Amsterdam.