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STUDIES ON AN INHERITED CATARACT
OF SHEEP

A thesis presented in partial fulfilment (30%) of the
requirements for the degree of Master of
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ABSTRACT

The findings presented in this thesis comprise the first report and description of an inherited cataract in sheep. The condition was first diagnosed in a number of related New Zealand Romney sheep, from a high performance Romney stud.

Clinically, cataracts which are always bilateral, are usually first visible at 2 months of age as characteristic focal opacities, confined to either the central or peripheral anterior and posterior cortex of the lens. By 8 to 10 months of age, there is more generalised lenticular opacity and total opacity usually occurs by 10 to 12 months. Two lambs born with congenital bilateral mature cataracts, showed partial lens absorption, which was obvious clinically by 8 months of age.

Controlled breeding studies, show that cataracts are inherited as a simple autosomal dominant trait. The two congenitally affected lambs, which resulted from matings between affected parents, are assumed to be homozygous for the defective gene.

Histologically, ovine cataract is characterised by the progressive degenerative swelling and lysis of lenticular fibres, beginning initially within localised areas of the anterior and posterior cortex. The distribution of early lesions correlates with the clinical appearance of early stage cataracts. With cataract progression, more of the cortical and eventually the nuclear fibres are affected and in the mature state, only a small number of attenuated equatorial fibres remain. The anterior epithelial cells become vacuolated apparently in response to cortical degeneration, and they also undergo proliferation, metaplasia to a spindle shaped cell and they migrate posteriorly beneath the posterior capsule. Beneath the posterior capsule, epithelial cells form either a single layer of flattened cells, or aggregations of large 'bladder' cells. The formation of new lens fibres at the equator continues relatively normally, throughout all stages of cataract.

Ultrastructurally, anterior epithelial cells contain two types of vacuole. Small circular vacuoles which are not membrane bound are present in small groups within the cytoplasm. These are also present and are more numerous within spindle shaped cells. The large irregular shaped vacuoles noted by light microscopy are usually membrane bound and often contain membranous or granular material. These vacuoles are interpreted as being dilated and damaged endoplasmic reticulum. Both spindle shaped and vacuolated anterior epithelial cells have increased amounts of endoplasmic reticulum and in spindle shaped cells, fibrillar material and electron dense deposits are present. Capsular material, though observed surrounding some spindle shaped cells histologically, could not be demonstrated ultrastructurally. The profound breakdown of the cellular architecture of the lens is readily demonstrated by scanning electron microscopy.

Water and electrolyte analyses of cataractous lenses, show that water and sodium content increases and potassium is lost, during cataractogenesis.

The objectives of this study, were to define the inherited cataract of New Zealand Romney sheep in clinical, genetic and pathological terms, to examine water and electrolyte shifts in cataractous lenses, and to compare the condition with other inherited cataracts of man and other animals. It is concluded, that this ovine cataract though apparently clinically unique, does in pathological and biochemical terms resemble many cataracts of man and animals of different causes. These changes are not aetiologically significant, but merely reflect the limited range of stereotyped reactions which are possible in the cataractous lens. For this reason, it is proposed that this ovine cataract would provide a useful model for fundamental studies on the pathogenesis of cataract.

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INTRODUCTION

Inherited cataracts have been recorded in man and several animal species. The objectives of this study, were to define a previously unreported ovine inherited cataract in clinical, genetic and pathological terms and to compare these findings with those reported in man and other animals.

CHAPTER I

THE DEVELOPMENT AND STRUCTURE OF THE LENS

The development and structure of the lens is similar in all mammals (Balinsky, 1970). The following description concerns the human lens and is based on descriptions by Hogan and Zimmerman (1962), Hogan *et al.* (1971), and Paterson (1979). It is augmented by observations made on the ovine lens during the present study.

DEVELOPMENT OF THE LENS

The lens is an epithelial derived structure, originating from the surface ectoderm overlying the optic vesicle (Fig. 1.1). The first evidence of its development in the human embryo is at the 4.0 mm stage, when a thickening of the surface ectoderm occurs to form the lens plate. This thickening is created by an elongation of the ectodermal cells as well as by cell division. It is believed to occur as a result of the action of inducer substances produced by the adjacent neuroectoderm. The thickened plate consists of tall cylindrical cells, which are so closely packed that the nuclei are forced to occupy different levels, giving it a multilayered appearance.

When the embryo is 5.0 mm (4 weeks), the lens plate begins to invaginate to form the lens pit. At the same time, the optic vesicle formed from the neural tube invaginates to form the optic cup, in which the embryonic lens becomes located. The lens pit deepens, and invagination proceeds until a vesicle is formed. At the 9.0 mm - 12 mm stage the lens vesicle separates from the surface ectoderm. During formation of the vesicle, the cells become inverted from their position in the surface ectoderm, so that their apical surfaces face the cavity, and their basal surfaces face outwards against the lens capsule. The capsule, is a continuous accruing basement membrane, which is formed by the lens epithelial cells.

The cells of the anterior portion of this vesicle facing the surface ectoderm remain relatively undifferentiated throughout life,

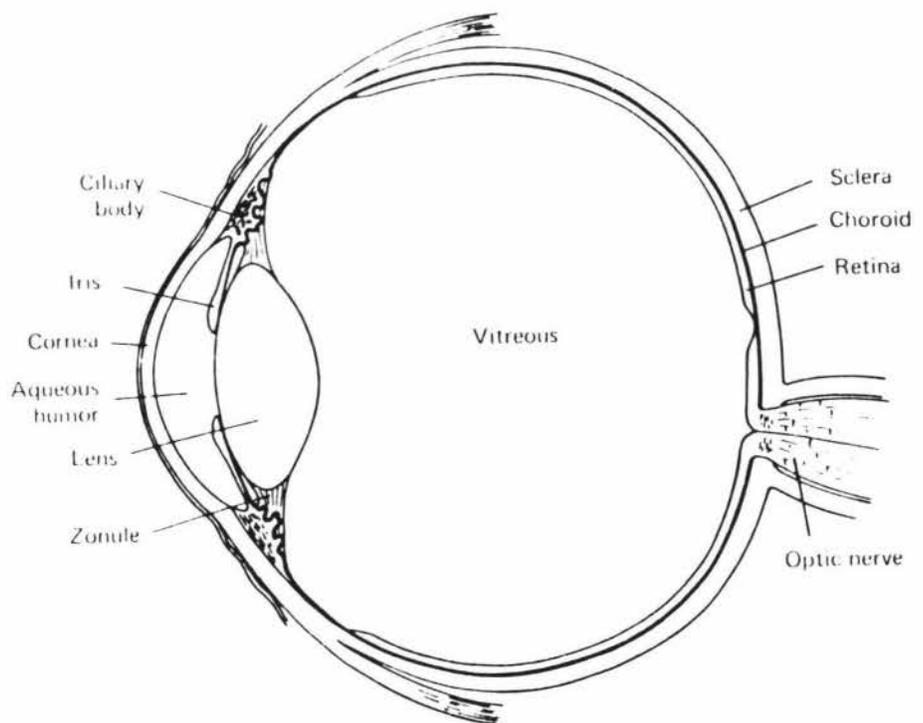
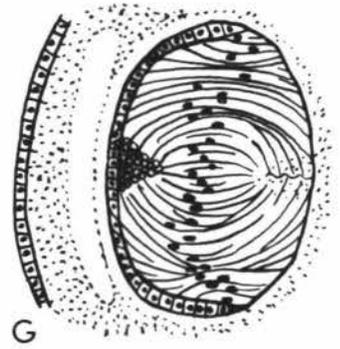
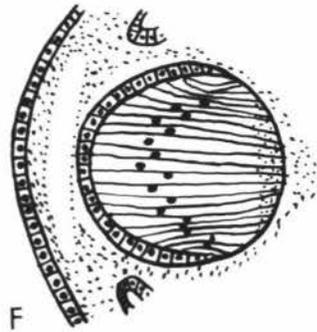
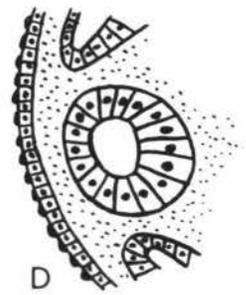
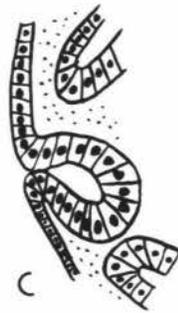
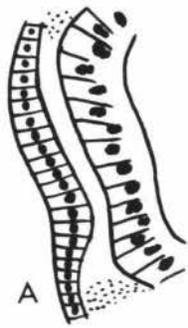
except near the equator, where they divide to form new lens cells. The posterior epithelial cells facing the optic cup elongate and completely fill the cavity of the lens vesicle by the 16 - 30 mm stage (7 weeks). These are the primary lens fibres, which eventually lose their nuclei and subsequently form the embryonic nucleus of the lens. From this stage, the cells at the equator divide and begin their lifelong function of forming secondary lens cells or fibres. These secondary fibres are displaced from the equator and become elongated; the anterior parts extend forward beneath the anterior epithelium, and the posterior parts extend backward beneath the posterior capsule, each cell becoming 'U' shaped with its nucleus near the equator. The elongated cells meet anteriorly and posteriorly, forming junctions with other cells called sutures. The continued formation of new cells displaces older cells toward the centre of the lens which becomes more dense, this area is then referred to as the lens nucleus. The more superficial layers of younger cells constitute the lens cortex. As the cells are displaced toward the interior of the lens, their nuclei form a pattern referred to as the lens bow, with further displacement they eventually lose their nuclei.

The lens capsule has its earliest origin as a basement membrane under the surface ectoderm, prior to formation of the lens plate. It becomes thicker as the lens plate develops and when the lens vesicle forms, the basement membrane is adjacent to the outer cell membranes (originally the basal portions) of all the epithelial cells. This thin basement membrane thickens as the lens develops, due to a continuous deposition of material from the adjacent epithelial cells. The capsule can be readily seen by light microscopy at the 13 mm stage in human embryos, though electron microscopic studies show it to be present much earlier.

The lens is held in position by the zonules or zonular fibres (Fig. 1.2). These fibres and the vitreous substance form at 3 months (65 mm), when the layers of the optic cup begin to grow forward to form the definitive ciliary body and iris. Zonular fibres develop within the equatorial circumlental vitreous, and extend from the inner surface of the ciliary body to the lens capsule.

Figure 1.1: A diagrammatic representation of the development of the lens. A - the surface ectoderm thickens to form the lens plate. B - the lens plate invaginates to form the lens pit. C and D - the lens pit closes and the lens vesicle separates from the surface ectoderm. E and F - the cells of the posterior wall of the lens vesicle elongate and obliterate the cavity of the vesicle. G - secondary lens fibres form from the equatorial epithelium, these fibres meet anteriorly and posteriorly to form sutures. Redrawn from Paterson (1979).

Figure 1.2: A diagrammatic section through the eye, showing the lens in relation to other structures. Reproduced from Paterson (1979).



The foetal lens is nourished by the *tunica vasculosa lentis* and the pupillary membrane. The hyaloid artery, after entering the eye through the posterior end of the foetal fissure at the 7.0 mm stage, passes anteriorly to reach the posterior pole of the lens vesicle. A net of capillaries spreads out onto the lens to form the *tunica vasculosa lentis*, which virtually encloses the lens except for the anterior portion, which is supplied with vessels spreading from the iris in a membrane called the pupillary membrane. At the end of the third month, the hyaloid system has reached its greatest development, and from this time on it atrophies. By 7 months it has completely regressed, leaving the lens without a blood supply for the rest of its life.

STRUCTURE OF THE LENS

The lens increases in size throughout life because new cells are constantly added to the cortex from the equatorial epithelium. As already mentioned, the anterior and posterior terminations of new cells meet and form junctions called sutures. At the sutures, interdigitations occur between the cells of the same generation but from different quadrants of the lens. These sutures are Y shaped when viewed from the front of the eye. The plain Y sutures delineate the foetal nucleus or embryonic lens, whilst those fibres which form subsequent to the development of the foetal nucleus, form more complicated suture lines (Fig. 1.3).

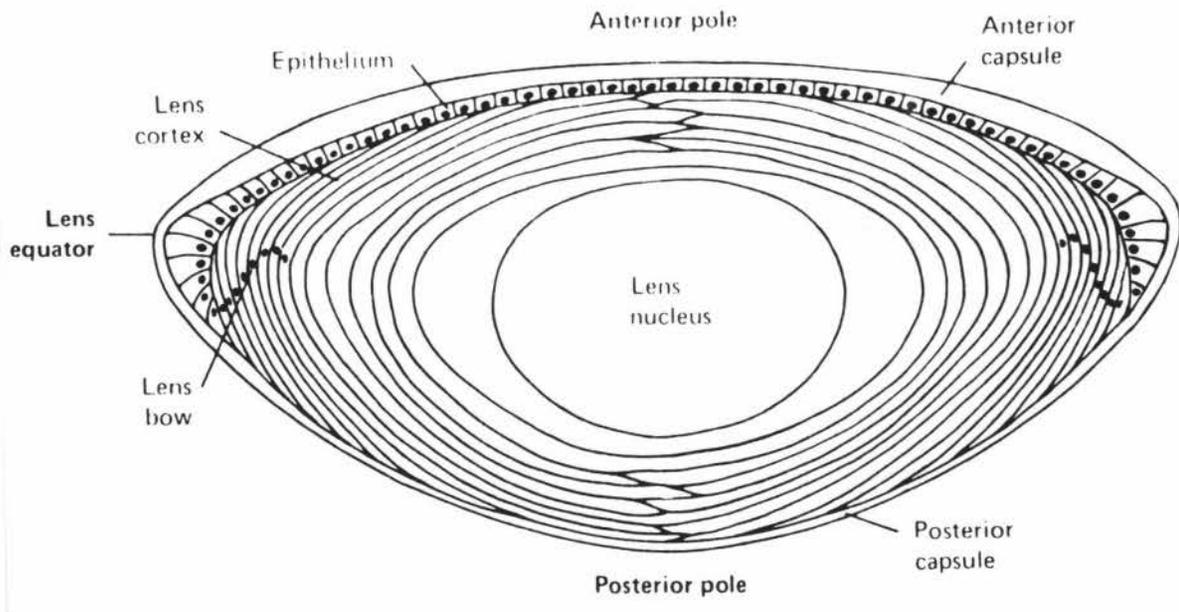
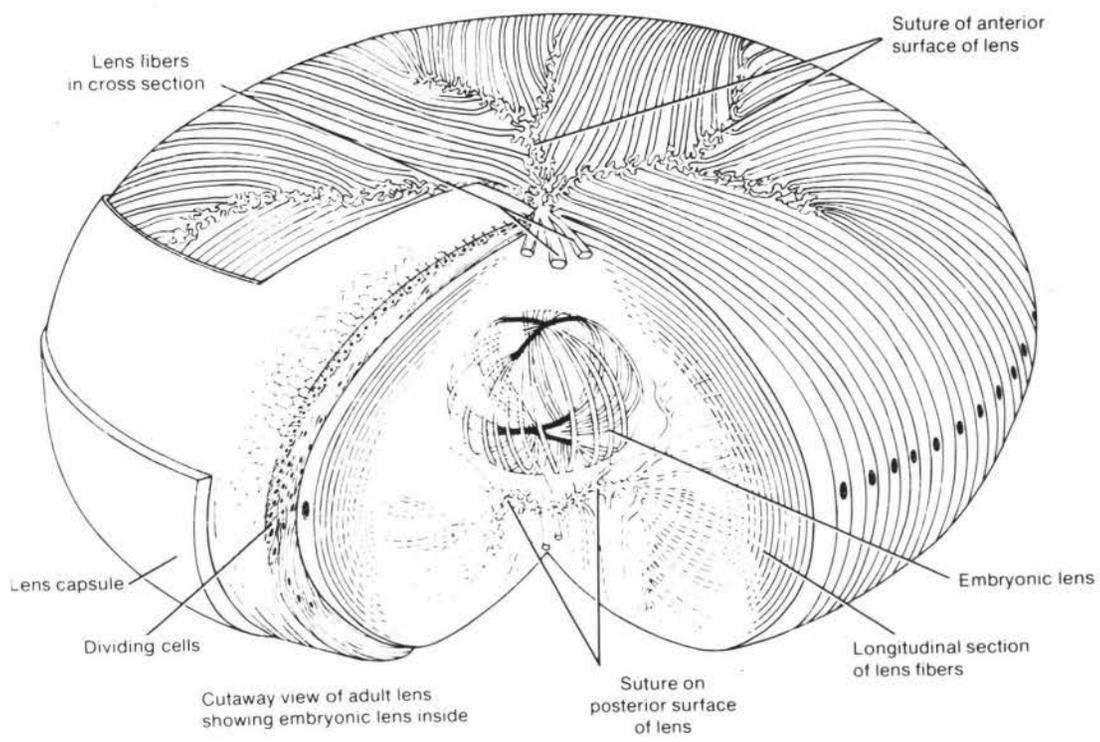
There are no sharp boundaries between the various parts of the lens, though the nucleus and cortex are readily discernible in histological sections, due to the variation in compactness of the fibres and hence their cutting qualities. The various anatomical regions of the lens are depicted in Figure 1.4.

Lens capsule

The capsule is a transparent, elastic and unusually thick basement membrane. The inner portions of the capsule are in immediate contact with the basal surfaces of the epithelial cells anteriorly and the elongated lens cells posteriorly (Fig. 1.4). The capsule contains an insoluble protein chemically similar to

Figure 1.3: A three dimensional diagram of the lens. The embryonic lens formed from the primary lens fibres, has a Y suture at both the anterior and posterior poles. Cortical fibres forming later in life, form more complicated sutures.
Reproduced from Kessel and Kardon (1979).

Figure 1.4: A diagrammatic section through the lens showing the anatomical regions of the lens.
Reproduced from Paterson (1979).



collagen, that is combined with 10% polysaccharide which renders the capsule strongly PAS positive in histological preparations. Capsular material is produced by the anterior epithelial cells and the superficial posterior cortical fibres. The elasticity of the capsule must result from the organisation of its fibrillar components, as it does not contain elastic tissue. Viewed at low magnification, the capsule is smooth, homogeneous and dense; with the polarising microscope it is birefringent, indicating a lamellar structure with the fibres arranged parallel to the surface but without orientation. In 1.0 μm sections, discontinuous 'linear densities' are visible in the anterior capsule. Pores through the capsule cannot be demonstrated.

With the electron microscope the lens capsule is found to be made up of many lamellae, each of which is composed of very fine filaments. Although relatively homogeneous, there are a number of 'linear densities' scattered throughout the anterior and equatorial portions of the capsule. These densities are composed of fine fibres, which have a periodicity of 60 nm when aligned in parallel bundles. In cross section, their diameter is the same as that of the zonular fibrils (15 nm). These linear densities do not appear to be deep insertions of zonular fibrils, but may be a similar form of collagen-like protein. Such densities are absent in the posterior capsule which suggests that they result from some activity of the epithelial cells. Zonular fibrils blend into the superficial aspects of the equatorial capsule.

The capsule is important for the integrity of the lens, and it helps regulate the transport of nutrients and waste products between the lens and the aqueous and vitreous. This is a passive process, with the capsule acting as an inert, semi-permeable membrane. Maintenance of its permeability is important for normal metabolism of the various parts of the lens.

Lens epithelium

The lens epithelium is composed of a single layer of cells present under the anterior and equatorial portions of the capsule.

The bases of the epithelial cells are in contact with the capsule, while the apices face the interior of the lens. Epithelial cell morphology varies slightly according to position from central to equatorial location (Fig. 1.5).

Central Zone: Cells in this zone appear cuboidal in meridional section and polygonal when viewed in flat section. They stain poorly with histological stains. The cells have smooth surfaces, both under the capsule and over the underlying lens cells. Their nuclei are round, located slightly apically, and mitoses are not commonly seen.

Intermediate Zone: These cells lie between those of the central zone and those at the equator (Fig. 1.6). They are smaller and more cylindrical than those in the central portion and their spherical nuclei are centrally placed. The cells form complex lateral interdigitations with adjacent cells. Mitoses are more common in this zone.

Equatorial Zone: The cells of this region divide and become elongated to form the new lens fibres (Fig. 1.7).

Electron microscopic studies have shown that there are no important differences in the structure of cells in the central, intermediate, and equatorial zones of the epithelium. Studies in many animal species and in humans, have shown the cytoplasm of epithelial cells to be granular, and to contain the usual organelles in small numbers. Mitochondria are small, and lysosomal elements are rarely noted. Adjacent epithelial cells are attached to each other by a small occluding junction at their apical border and to the underlying lens fibres by numerous intermittent occluding junctions. No cement substance or other extra cellular material is seen between the base of the epithelium and capsule, or between the apices of the epithelium and the first layer of lens fibres.

At the equator the cells retain their attachments laterally and apically, whilst spreading their widened base posteriorly

Figure 1.5: A composite drawing of the lens cortex, epithelium, capsule and zonular attachments. At (A) the cells of the central zone of the anterior epithelium are shown in flat and cross-section. The cells of the intermediate zone (B) are more columnar and within the equatorial zone (C) epithelial cells divide to form new lens fibres. The lens capsule (d) contains insertions of the zonular fibres (f) which form the pericapsular lamella of the lens (g).
Photographed from Kessel and Kardon (1979).

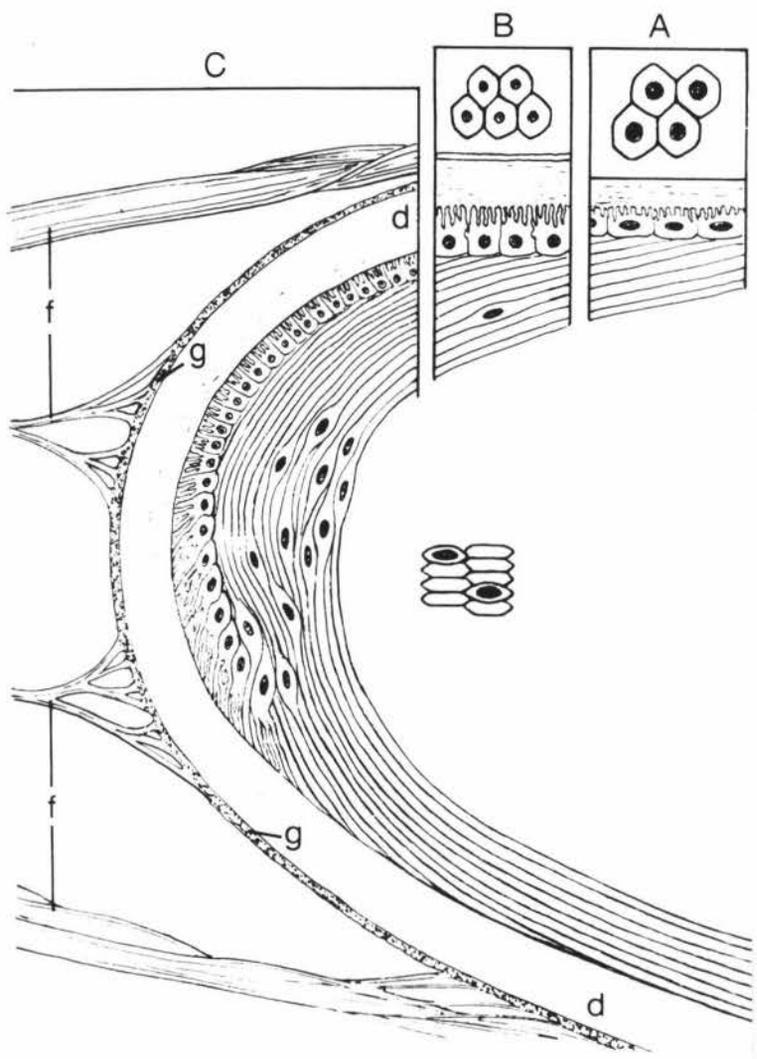
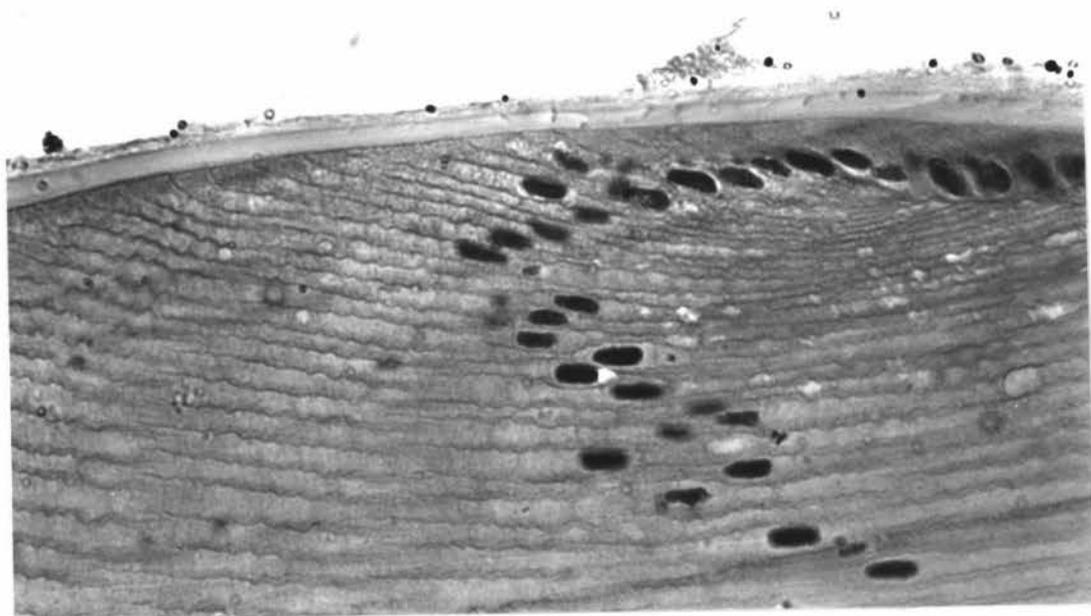
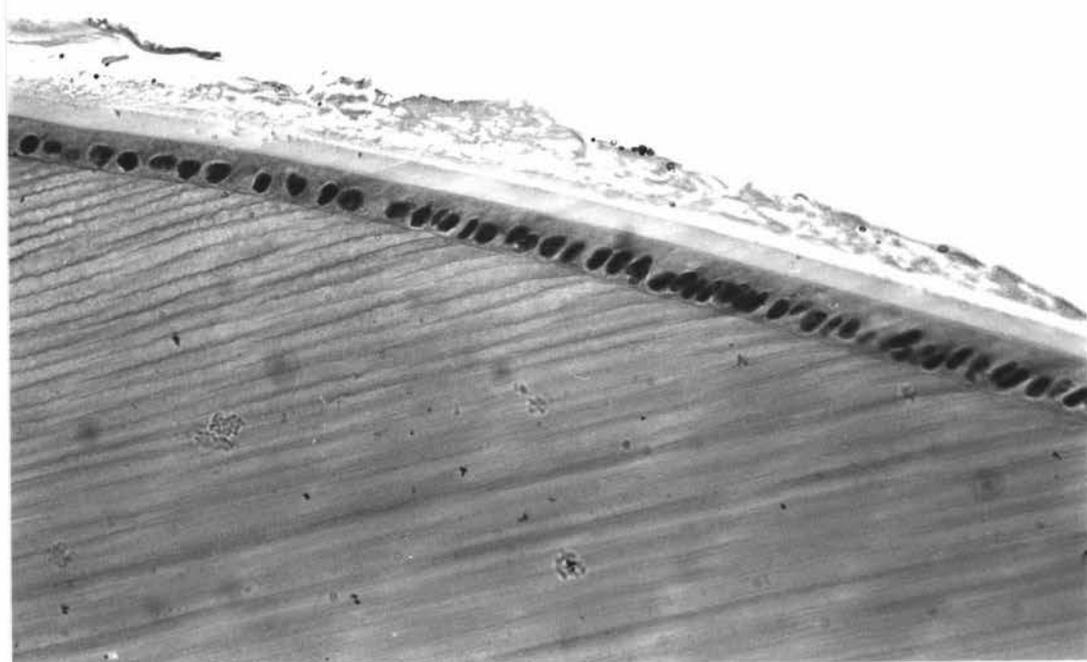


Figure 1.6: The intermediate zone of the anterior epithelium of the lens, from an 8 month old normal lamb. The cells in this zone are low columnar. Epithelial cells are in contact with the lens fibres apically and their basal surfaces are in contact with the capsule. Zonular fibres and some adherent iris tissue are present on the exterior surface of the capsule. (Paraffin section, H & E x 320)

Figure 1.7: The equatorial region of the lens of an 8 month old normal lamb. New lens fibres are forming from the anterior epithelium. As they grow their anterior processes extend beneath the anterior epithelium and their posterior processes extend beneath the posterior capsule. (Paraffin section, H & E x 500)



beneath the capsule. In addition to the normal organelles of the epithelium, equatorial cells in the process of elongation, contain cilia as well as numerous vesicles in the basal cytoplasm. There is some speculation that these vesicles are involved in the elaboration of the posterior capsule, which following the obliteration of the lens vesicle in embryonic life, has no epithelial cells on its inner surface to lay down new basement membrane.

The epithelium is the only tissue in the lens which is capable of regeneration and it may proliferate when stimulated by injury such as trauma or inflammation in neighbouring tissues. Epithelial proliferation also occurs in many types of cataract.

Lens fibres

As the newly formed lens cells elongate, they become hexagonal in cross section and because of their exceptional length they are called fibres. Adult human cortical fibres are 8.0 to 12 mm long, 7.0 μm wide, and 4.6 μm thick. The transition from the cuboidal epithelial cell to the ribbon-like lens fibre, begins with expansion of the basal part of the cell and flattening of the nucleus. The expanded basal process moves posteriorly along the inner surface of the posterior capsule, while the apical process inserts itself under the surface of neighbouring epithelial cells (Fig. 1.7). Because of the constant formation of new cells, the preceding generations of cells are displaced deeper into the cortex carrying their flattened nuclei with them. These nuclei form a characteristic arrangement with one another called the lens bow, curving forward in crescent shape from the equator toward the nucleus (Fig. 1.8). The lens fibres splay out as they approach the sutures becoming thinner and wider and near the suture they bend rather sharply before ending. This change of direction causes them to meet the branches of the suture at wide angles, so that if meridional sections are made through the lens, the cells about to form the sutures are frequently cut in cross section, rather than longitudinally.

With transmission electron microscopy, the superficial lens fibres near the equator are found to contain most of the organelles found in the epithelial cells, the number of organelles appears reduced, but this could result from an increase in cell volume, as much as from a decreased number of organelles. The remaining cytoplasm consists of a finely fibrillar substance. At a short distance from the equator and in the deeper layers, the nucleus and organelles progressively disappear. In addition to this change, the cytoplasm of the cells becomes amorphous as a result of an accumulation of a homogeneous granular substance. It is not clear whether this results from poor fixation of the deeper layers or whether it reflects the progressive insolubility of pre-existing protein in the deeper cells of the lens. The terminal or growing ends of the cells contain numerous smooth flattened vesicles which may be utilized for expansion of the cell membrane as it grows towards the sutures.

The flattened hexagonal shape and precise alignment of cortical lens fibres is readily demonstrated by scanning electron microscopy (Fig. 1.9). The lens fibres within the cortex are held together by interlocking processes rather like ball and socket joints (Fig. 1.10). In the deeper cortex and the nucleus, interlocking processes are replaced by fine ridges (Fig. 1.11). Fewer interlocking processes are present in the superficial cortex, allowing for greater movement of cells one on another in this area. Adhesion between generations of cells is substantial in the deeper aspects of the lens.

From observations made during the course of this study using light and electron microscopy, it appears that the ovine lens is essentially similar in structure to that of man and other mammals.

Figure 1.8: The equatorial cortex of the lens from an 8 month old normal lamb. Their nuclei form the lens bow as cortical fibres are displaced toward the centre of the lens.
(Paraffin section, H & E x 125)

Figure 1.9: The anterior cortex of the lens from an 8 month old normal lamb. Cortical fibres are hexagonal in cross section and are precisely arranged in parallel rows.
(Freeze fractured, critical point dried specimen, SEM x 400)

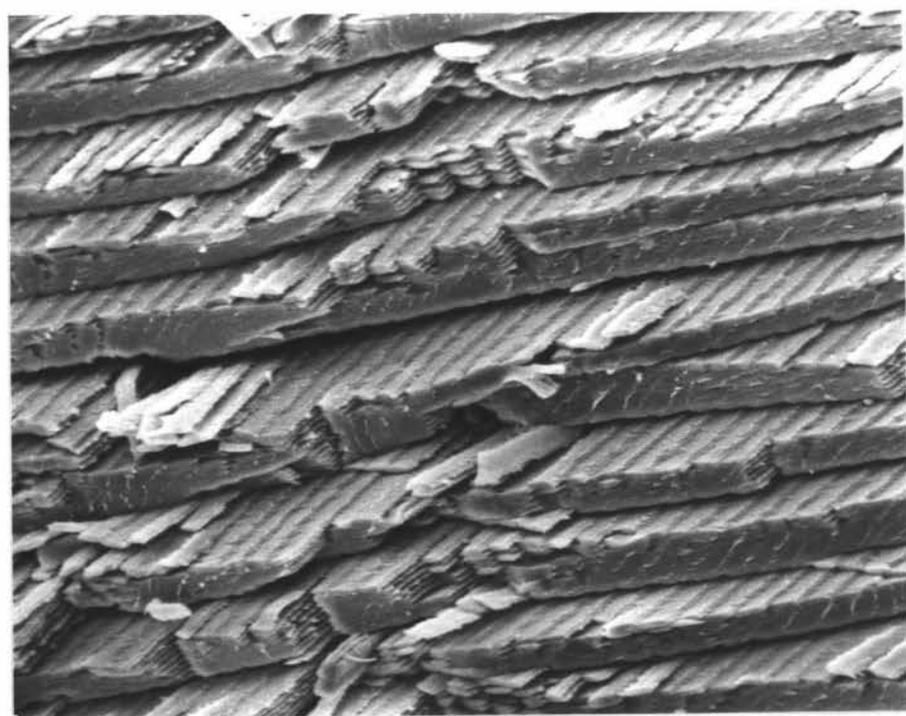
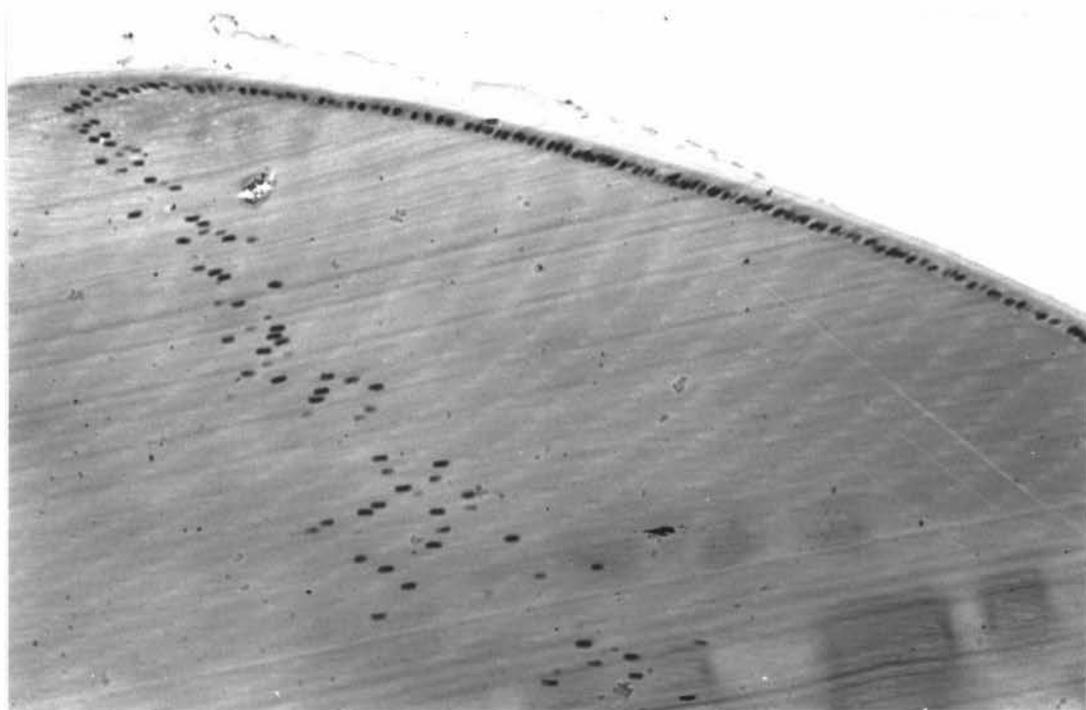
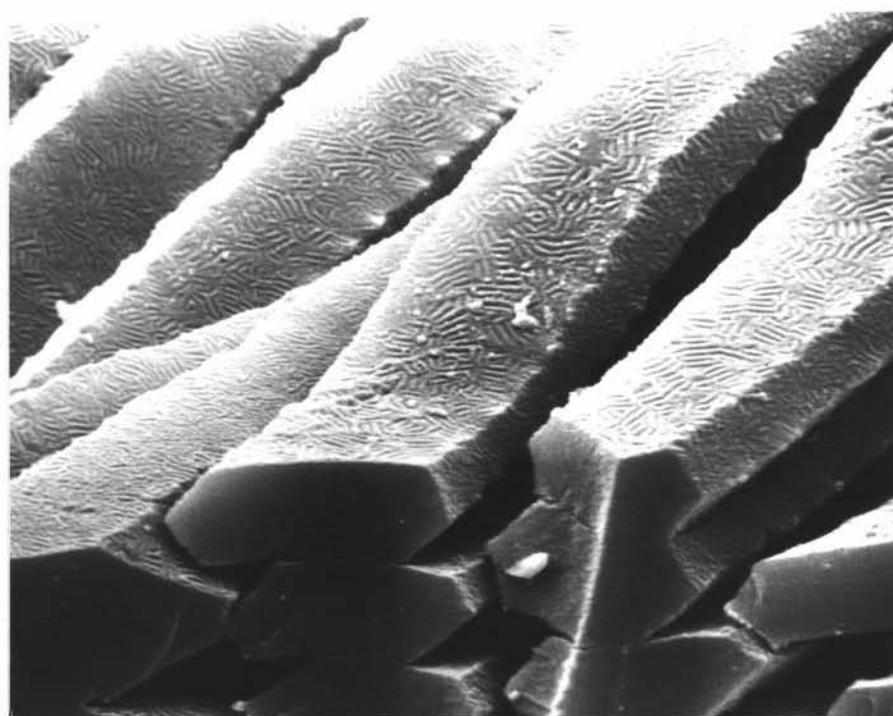
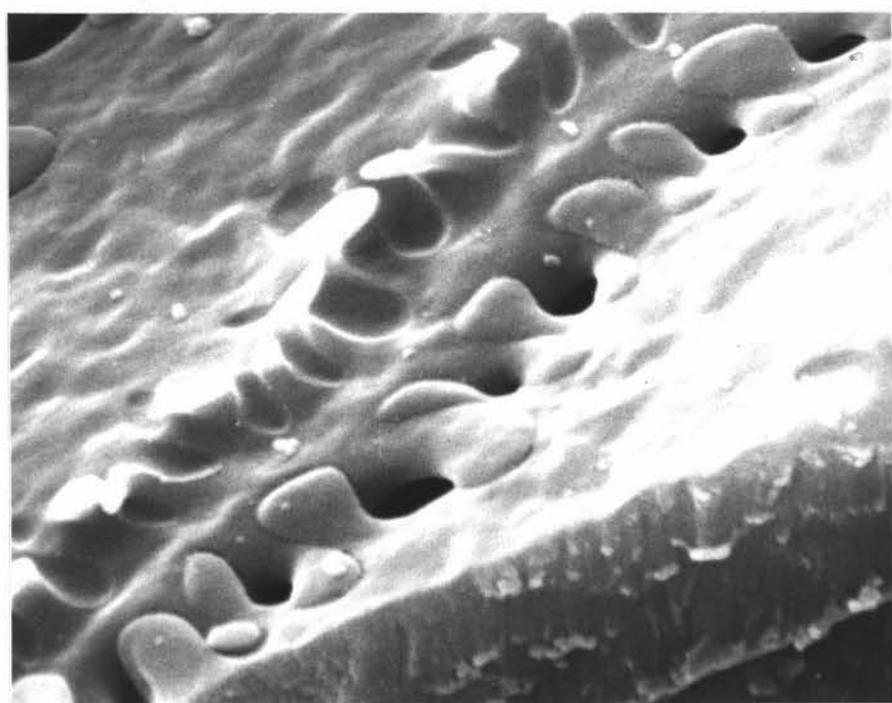


Figure 1.10: Anterior cortical fibres from the lens of an 8 month old normal lamb. The long ribbon like fibres are held together by 'ball and socket' like interlocking processes.
(Freeze fractured, critical point dried specimen, SEM x 14,000)

Figure 1.11: Deep cortical or nuclear fibres from the lens of an 8 month old normal lamb. In this region, surface ridges replace interlocking processes as the means of adhesion between fibres.
(Freeze fractured, critical point dried specimen, SEM x 4,000)



CHAPTER II

LITERATURE REVIEW : INHERITED CATARACTS

I. INTRODUCTION

A cataract may be defined as any opacity occurring in the crystalline lens (Newell and Ernest, 1974). Such opacities may be discrete and localised, or involve the whole lens. The former are usually the result of the deposition or precipitation of opaque material such as crystals or cholesterol, while more generalised changes are due to extensive changes in the lens proteins, and cation and water balance (Paterson, 1979).

There are many causes of cataract, the most important of which are summarised in Table 2.I. Those with a genetic basis have been described in man and a number of animal species. The more pertinent entities are reviewed.

TABLE 2.I

Some causes of cataract (Duke-Elder, 1969)

Mechanical injury
 Physical causes e.g. temperature and pH
 Radiation
 Changes in capsule permeability
 Interference with lens nutrition
 Anoxia
 Diseases of sugar metabolism
 Nutritional deficiencies
 Lowered blood calcium: phosphate ratio
 Toxic substances
 Endocrine influences
 Systemic infections
 Genetic

II. INHERITED CATARACTS OF MAN

Many hereditary cataracts occur in man unassociated with other ocular or systemic abnormalities. They are however, a poorly studied group due to the relative unavailability of human lenses for study. The best understood, are those occurring secondarily to the diseases of carbohydrate metabolism, galactosaemia and diabetes mellitus.

Galactosaemia is an inborn error of metabolism, characterised by either the absence of galactose-1-phosphate uridyl transferase, or a deficiency of galactokinase (Bondy and Felig, 1974). Either enzyme deficiency, results in an impairment of the conversion of galactose to glucose. Both deficient states are inherited as autosomal recessive traits. Cataract occurring shortly after birth, is a frequent complication of galactosaemia, unless sources of galactose are excluded from the diet. If not, serum and aqueous humour galactose levels become abnormally high. Within the lens, galactose is metabolised by the sorbitol pathway to its alcohol dulcitol, which accumulates, since it cannot diffuse away or be metabolised further. This causes osmotic swelling to the lens fibres with their eventual rupture. Dulcitol may also have an additional and more direct effect on other aspects of lens metabolism (Kinoshita, 1965).

Diabetic cataract, though not necessarily an inherited disease has similarities which may be conveniently discussed here. This cataract, is a rare lens opacity, seen in poorly controlled diabetics in early adulthood. Excess glucose enters the lens because of high blood and aqueous humour levels, and since it cannot all be efficiently metabolised by the glycolytic pathway or the hexose monophosphate shunt, it enters the sorbitol pathway, and is converted to sorbitol and fructose. The accumulation of these substances brings about osmotic swelling, in a manner similar to that in galactosaemia.

III. INHERITED CATARACTS OF MICE

A recessively inherited cataract of albino mice called 'lens rupture' was described by Fraser and Herer (1948, 1950). It was morphologically similar to that occurring in 'Swiss' albino mice (Smelser and von Sallman, 1949) and to that described in descendants of irradiated mice by Beasley (1963). These conditions were characterised by initial degeneration at the posterior pole, and a defect in the posterior suture with subsequent extrusion of the lens nucleus. Despite minor variations in the age of onset, the common features were small opacities at the posterior pole which histologically proved to be due either to swollen fibres, or a gap in the suture. In the cataract described by Beasley, there was a stratified anterior epithelium, which he considered produced an excess of lens fibres which lead to rupture.

A recessively inherited cataract has been extensively studied in the 'Nakano' strain of mice. Hamai *et al.* (1974), found that the cataract was first visible to the naked eye at approximately 3 weeks after birth as a pin head opacity in the posterior region of the lens. Histological changes, present earlier at 6 days, consisted of impairment of lens fibre maturation in the deep bow region and hydropic swelling of fibres near the posterior suture. By 3 weeks, these fibres had degenerated, and there were vacuoles and large swollen cells in the posterior suture region.

Sakuragawa *et al.* (1975), using scanning electronmicroscopy, showed that fibre swellings were not uniform within the affected area but were localised in small segments of the basal fibre with swollen areas interconnected with non swollen and/or shrunken and atrophied areas, rather like a 'string of sausages'. They suggested that inherent weak points in the cytoplasm, or mechanical forces, may have determined the distribution of fibre swelling, both within the fibres, and within different parts of the lens.

Prior to these morphological studies, the onset of cataract development had been shown to coincide with an increase in lens hydration and sodium content, associated with a decrease in Na-K ATPase activity (Iwata and Kinoshita, 1971). In mice heterozygous for this defect, this enzyme activity was normal, and it was postulated that in affected animals the deficient activity was brought about by an inhibitor. Kinoshita (1974) confirmed this by showing that the supernatant from cataract lens homogenates, inhibited lens ATPase activity in mouse, rat and calf lenses, and in the calf retina. The inhibitory factor was heat labile and non dialysable, and may have been an enzyme.

Iwata (1980), compared the cation changes of the 'Nakano' cataract with those occurring in another strain of mice with a dominantly inherited cataract. In the latter strain, cation changes were essentially similar, but lens ATPase inhibition did not occur. He considered that other causes were responsible for the cation changes, in this, and the many other cataracts in which marked cation shifts occur.

Cataracts in the deer mouse (*Peromyscus maniculatus*), are associated with syndactyly of the hind feed. Two forms occur, both inherited as an autosomal recessive trait (Burns and Feeney, 1975; Burns *et al.*, 1980). Type I has an onset at approximately 3 months, and is associated with persistent hyaloid vasculature and coloboma. Type II has a later onset of approximately 12 months, and is unassociated with other ocular abnormalities. The general course of development of both cataracts is similar, but more rapid in Type I animals. The earliest changes seen in both types by slit lamp examination, are grey-white opacities or small empty areas in the posterior sub capsular region, and vacuoles in parts of the equatorial cortex. The posterior opacity eventually spreads to involve the anterior cortex. Histologically, these changes consisted of enlargement and distortion of the basal processes of lens fibres, intra and extracellular clefts and migrating epithelial cells. Ultrastructural studies by Feeney -

Burns *et al.* (1980), showed that some of the swollen fibres resulted from fibre fusion and that the migratory epithelial cells were phagocytic in as much as they contained ingested protein from adjacent degenerate fibres.

The Philly mouse a recently derived strain, has a recessively inherited cataract which is first visible to the naked eye at 5 to 6 weeks of age (Kador *et al.*, (1980). Slit lamp observations, show that cataracts develop from 15 days of age as initial anterior subcapsular opacities, which subsequently involve the equatorial cortex and nucleus.

Microscopically there is disorganisation of the nuclear bow, and incomplete fibre elongation with spaces forming between fibres. Early opacity is associated with granular particles within fibres, rather than with fibre swelling.

Biochemically, there is an increase in lens hydration, sodium and calcium, and a decrease in lens dry weight and potassium. No inhibitor of Na-K ATPase has been demonstrated, and a defect in lens fibre membrane permeability is considered to be the key biochemical defect.

The Fraser cataract strain of mice develop a dominantly inherited cataract (Fraser and Schabatch, 1962; Verrusio and Fraser, 1966), which begins in prenatal life but becomes obvious to the naked eye at 3 to 4 weeks after birth. From this age the lens appears cloudy, with a more prominent central opacity, which within a few weeks extends to involve the whole lens. The developmental stages are similar in both homozygous and heterozygote mice, however in the latter, the rate of cataract development is much slower.

Microscopic (Zwaan and Williams, 1968a, 1968b, 1969) and ultrastructural studies (Hamai and Kuwabara, 1975), showed that

changes first began as early as 10 days post conception, when a greater than normal number of degenerating cells were present in the developing lens pit and vesicle. At 14 days, premature nuclear degeneration and swelling of the apical portions of the primary lens fibres occurred, these fibres and fibres forming subsequently, underwent degeneration. As a result, the central lens was replaced by an eosinophilic granular and amorphous mass. The superficial cortical and equatorial fibres, and the anterior epithelium were normal until birth. After birth, swelling and degeneration continued within the deep cortical fibres, until in the adult, only a small crescent of subequatorial fibres remained. Secondary hyperplasia and fibrous metaplasia of the anterior epithelium was observed in the later stages. No biochemical studies have been reported.

Davidorf and Eglitis (1966), studied a dominantly inherited cataract occurring in randomly bred 'Swiss' mice. The gene was lethal in the homozygous state. The cataract was present at the time of eye opening, and consisted of a dense white opacity involving all of the lens in one or both eyes.

Microscopically, premature loss of nuclei was observed in the primary lens fibres at 14 days of gestation, and these fibres underwent progressive degeneration. The surrounding secondary lens fibres were laid down abnormally, and the degenerate nucleus came to occupy a position adjacent to the anterior epithelium. In adult mice, lens degeneration had progressed to involve all regions including equatorial fibres, the posterior pole was the least affected area. The epithelium consisted of several layers of cells, and contained capillaries derived from the iris, this change was not present in embryonic cataractous lenses, and was not associated with persistent lens vasculature.

IV. INHERITED CATARACTS OF RATS

Recessively inherited cataracts in Wistar rats in a strain derived from an irradiated parent were reported by Léonard and Maisin (1965). The cataracts were associated with microphthalmia. Morphologic studies by Gorthy and Abdelbaki (1974), showed that an anterior polar cataract, and anterior displacement of the nucleus were present at birth. Some animals had mature cataracts by 3 weeks of age, whilst in others, cataracts were later developing, and were sometimes accompanied by posterior capsular rupture.

Microscopic examination of newborn rats, showed that the anterior polar cataract was due to epithelial cell hyperplasia and the nuclear displacement was due to incomplete closure of the anterior suture. By 3 weeks, there was extensive epithelial hyperplasia and metaplasia to a fibroblast like cell which produced capsular material. Occasionally there was duplication of the epithelium and capsule. In the anterior cortex, swollen and disorganised fibres were associated with the overlying epithelial changes, and in the posterior cortex vacuoles and swollen fibres were associated with the posterior suture. In rats older than 3 weeks, extensive degeneration of both cortex and nucleus preceeded capsular rupture, whilst in lenses which did not rupture changes were less severe. Wegner *et al.* (1980) using foetal rats, found there was retarded separation of the lens vesicle from the surface ectoderm, and considered that this lead to the anterior polar lesion and the nuclear displacement. Foetal lenses also showed retarded fibre maturation and contained large numbers of club shaped cells in the posterior region. Further histological and ultrastructural studies in neonatal rats (Gorthy *et al.*, 1980), showed that prior to the onset of hyperplasia, many cells in the epithelial monolayer were disorganised and necrotic. Such cells contained many large autophagosomes, enclosing cellular organelles in varying stages of degeneration. The presence of such active autophagy was interpreted as being a degenerative change representing an increased rate of cytoplasmic turnover. The epithelial derived fusiform cells, resembled fibroblasts ultra-structurally, and their synthetic product was identical to capsular substance.

Biochemical studies (Rossa *et al.*, 1980), showed that sodium and potassium imbalance occurred, which was in accordance with the earlier finding of reduced Na-K ATPase activity in affected lenses by Azari and Khatoon (1977).

Smith *et al.* (1969), described a recessively inherited condition of Sprague-Dawley rats, in which congenital cataracts were associated with skull malformations. The cataracts were not progressive, but their clinical appearance varied. Mild forms had anterior subcapsular and sutural opacities, water clefts in the anterior cortex and an anterior cortical haze. Nuclear and cortical changes were present in more severely affected lenses, with occasional anterior displacement of the nucleus. The most severe form, was characterised by a shrunken lens. Lenses with advanced cataracts occasionally adhered to the posterior corneal surface.

Proliferation of the anterior epithelium together with excessive capsular production was a characteristic histological finding. Cortical fibres were swollen and fibre nuclei were retained. Either the nucleus was displaced, or it liquified in advanced cases. The shrunken lenses contained large vacuoles of liquified lens material. The lenses of newborn animals, showed the same range of changes as exhibited by adults,

Another and distinct recessively inherited cataract of Sprague-Dawley rats was reported by Koch (1980). Cataracts present at eye opening, were seen as circumscribed opacities both within the nucleus, and at the anterior pole of the lens. These areas were initially interconnected by a fusiform opacity, but by 6 months of age the anterior and fusiform opacities had merged with the nucleus. The subsequently formed cortical fibres were normal, so that in adults the cataract appeared purely nuclear.

Keller *et al.* (1980) found no change in cation balance in these lenses. In human lenses, Duncan and Bushell (1979) found that sodium levels increased only when the cortex was involved.

The only dominantly inherited cataract of rats reported was that described by Smith and Barrentine (1943). Affected rats developed bilateral cataracts a few days after birth. A microscopic description was not given, neither was any difference noted between heterozygous and homozygous rats. No other gross anatomical defects were present, though affected animals had lowered fertility.

A recently reported cataract was that observed in a strain of congenitally hypertensive Wistar rats (Koch, 1980). All animals in the colony had cataracts, but the mode of inheritance had not been established. The cataract was present at birth as an opacity of the embryonic nucleus, and it remained stationary.

V. INHERITED CATARACTS OF CATTLE

Recessively inherited cataracts in Friesian cattle were reported by Detlefson and Yapp (1920), following a clinical description by Small (1919). Neither authors reported anatomical findings.

Inbred Jersey calves with recessively inherited cataracts were studied by Gregory *et al.* (1943). Immature cataracts were present at birth and progressed to maturity by adulthood. Affected calf lenses were smaller than normal and were displaced upward, histologically they consisted of a large degenerating nucleus surrounded by a thin peripheral border of normal fibres. In adults, the entire lens was degenerate and there was accompanying glaucoma with corneal bulging and enlargement of the eyeball.

Saunders and Fincher (1951), reported recessively inherited cataracts, lens ectopia, and microphakia in Jersey calves. This condition was similar to that described by Gregory *et al.* (1943), and it was thought that the sires involved in each report may have had a common ancestor. Gilman (1955), reported that the condition was widespread in Jersey bloodlines in the U.S.A. and Canada, and was present in England.

Carter (1960), described a dominantly inherited blindness in Friesian x Jersey calves sired by a partially blind Friesian bull. The condition was congenital and bilateral, and consisted clinically of varying degrees of corneal and lenticular opacity. Pathologically the cataract was shown to be associated with lens displacement. There was a variable degree of disintegration and absorption of the lens substance, the latter resulting from rupture of the posterior capsule. Additional findings were intraocular haemorrhage, retinal detachment and enlargement of the eye. It was noted that in some animals which had mature cataracts at birth, there was subsequent improvement in sight due to absorption or displacement of the lens.

Multiple ocular anomalies and hydrocephalus, were described in beef shorthorn cattle by Leipold *et al.* (1971). The inheritance though not proven was probably dominant. The condition was congenital and consisted of retinal detachment and dysplasia, Cataract, microphthalmia, persistent pupillary membrane, optic nerve hypoplasia, and vitreous haemorrhage.

Corneal opacity, cataract and other ocular anomalies, thought to be inherited, have also been recorded in Swiss Brown cattle (Winzenried, 1972; Geyer *et al.*, 1974).

VI. INHERITED CATARACTS OF DOGS

Rubin *et al.* (1969), described recessively inherited cataracts in Miniature Schnauzers. Opacities present at birth, were located in the posterior subcapsular cortex. Cataracts were usually mature within the first year. No other morphological description was given.

Yakely *et al.* (1971), found lenticular opacities in 88% of American Cocker Spaniels. These cataracts were extremely variable in morphology, and test mating suggested the condition was inherited. Yakely (1978) later described two types of cataract in the breed; a congenital nuclear cataract, which was non progressive, and not inherited, and an inherited progressive cataract, which developed after birth. The mode of inheritance of the latter was recessive,

with some variability in penetrance and expressivity. The inherited cataract was usually first apparent between 18 months and 4 years of age. Opacities were cortical, and progression varied greatly, usually with alternating periods of arrest and progression, though occasionally maturity was reached within a few months. Buskirk (1977), found that compared with those of control dogs, lenses with mature inherited cataracts showed a marked reduction of cells and mitotic activity in the central region of the anterior epithelium. In the equatorial region, affected lenses showed disorganisation of newly forming fibres.

Cataracts in Old English Sheep dogs with a probable recessive inheritance, were reported by Koch (1972). Some cataracts were congenital, but the definitive age of onset in most dogs was unknown, since most dogs examined were adult. These cataracts were nuclear, cortical, or both nuclear and cortical. Cortical opacities were either equatorially or posteriorly located. Many affected dogs also had retinal detachment, though it was not known whether the two conditions were related. Barnett (1978), reported similar cataracts in this breed.

Rubin and Flowers (1972), described recessively inherited cataracts in a family of Standard Poodles. Cataracts were present prior to 2 years of age initially in the equatorial cortex and subsequent progression involved the entire cortex. No retinal or other ocular disease was present.

Progressive cataracts with probable recessive inheritance in Afghan Hounds, were studied by Roberts and Helper (1972). The earliest changes consisted of multiple small vacuoles within the equatorial cortex and with progression, anterior and posterior subcapsular opacities developed, until finally the whole lens became opaque. Cataracts were detectable clinically between 4.5 to 23 months of age, and the rate of progression varied greatly between dogs. Histologically, early cases showed swelling, liquefaction and vacuolation of equatorial cortical fibres. Similar cataracts were reported in British Afghan Hounds by Barnett (1978).

A high prevalence of cataract in Golden Retrievers was noted by Gelatt (1972). Rubin (1974), showed that this condition was dominantly inherited and was characterised by small non-progressive posterior subcapsular opacities, however in one dog homozygous for this gene, total lens opacity occurred. Barnett (1978), found these two forms of cataract in Golden Retrievers in Britain.

Cataracts with a probable dominant mode of inheritance were described in Beagles by Anderson and Schultz (1958). A range of developmental stages were present at 2 to 3 months of age, and the condition was associated with microphthalmia and retinal folding. Histologically, lenses with mature cataracts contained masses of degenerate fibres in the posterior cortex, with occasional islands of epithelial cells at the posterior pole. Elsewhere they contained masses of ballooned fibres which became affected soon after forming from the equatorial epithelium.

Reports of dominantly inherited cataracts in German Shepherds (von Hippel, 1930) and in Pointers (Host and Sveinson, 1936), were cited by Gelatt (1972).

CHAPTER III

OVINE CATARACT : CLINICAL FINDINGS

I. INTRODUCTION

In December 1972, three related 16 month old Romney sheep were submitted to the Massey Veterinary Clinic for examination. The owner had noticed that these sheep, and a relative already culled, were blind, and commented that two closely related sheep were also noticed to be blind in the previous year. When examined, these sheep rushed into fences when hurried; if mobbed carefully with normal sheep however, they could negotiate gateways successfully. On closer examination, all three sheep were found to have bilateral cataracts which involved the entire lens.

In 1979 and 1980, a sufficient number of affected lambs were available, to enable a detailed clinical study of the lens during the process of cataract development.

II. MATERIALS AND METHODS

Animals

All affected animals were of the New Zealand Romney breed and were born during the spring of 1979 and 1980. They were the offspring of an affected sire 38/75, the son of an affected ewe, by an unaffected un-related ram. Affected animals were of both sexes.

Examination

All lambs were examined visually at or within a few days of birth, by the attending shepherd, to check for congenital cataract. More detailed eye examinations were made at 2 months of age, and at regular intervals thereafter. Drops containing 1.0% atropine sulphate were instilled into each eye prior to examination, and after effective mydriasis the lens was examined in semi-darkness with the aid of a pen light. The presence or absence of cataracts, together with an assessment of the stage of development of the

lesion were recorded. With lambs born in 1980, a sketch drawing of each lens was made during each examination.

Photography

At appropriate intervals from the age of 2 months, the eyes of typically affected animals were photographed after administering 1.0% atropine sulphate drops 1 hour, and again at 30 minutes, prior to photography. Photography was carried out with animals under general anaesthesia with intravenous pentobarbitone sodium. Bright focal illumination of the lens was achieved using a powerful fibre optic light. The camera was tripod mounted with bellows attachment and with a 50 mm auto-macro lens. High speed 400 ASA film was used.

III. RESULTS

Age of onset

In the majority of affected lambs early cataractous change was observed at 2 months of age. In two lambs fully developed cataracts were present at birth.

Early stage cataracts

Early lesions, consisted of focal anterior cortical opacities, which generally could be grouped into three patterns. Central opacity occurred in approximately one third of the cases and consisted of a wavy or irregular shaped lesion, more or less resembling the shape of a clover leaf (Fig. 3.1). The remainder of early cataracts however, consisted of peripherally disposed opacities, occurring either as multiple spherical areas within the anterior and equatorial cortex (Figs. 3.2 and 3.3), or as spoke like opacities radiating from the central anterior cortex to the equatorial cortex (Figs. 3.4, 3.5 and 3.6). Early stage lesions were present between 2 and 6 months of age.

Mid stage cataracts

By 8 to 10 months of age, there was more generalised involvement of the anterior cortex, the earlier spherical and spoke

patterns of opacity giving way to a more diffuse pattern (Figs. 3.7 and 3.8).

Late stage cataracts

At this stage, there was a diffuse opacity throughout most of the lens cortex (Figs. 3.9 and 3.10), though the equatorial cortex was still relatively clear. Just prior to cataract maturity, the opacity became very dense (Fig. 3.11). These lesions were usually present between 8 and 11 months of age.

Mature cataracts

Total lens opacity usually occurred by 10 to 11 months of age, although occasionally earlier. Opacity was evenly distributed throughout the entire lens (Fig. 3.12).

Congenital mature cataracts

In 1979, two lambs were born with mature cataracts present at birth (Fig. 3.13). In both cases partial absorption of the lens contents occurred, this being obvious clinically by 8 months of age (Fig. 3.14).

Cataracts were also observed within the posterior cortex, though only by very careful examination with the pen light. These opacities were later developing and initially less severe, though morphologically similar, to those involving the anterior cortex.

Figure 3.1: Early cataract, showing the irregular clover leaf pattern of central opacity, in the anterior cortex. Lamb 15/80 - 2 months old.

Figure 3.2: Early cataracts, showing numerous spherical opacities within the anterior cortex. Lamb 38/80 - 2 months old.

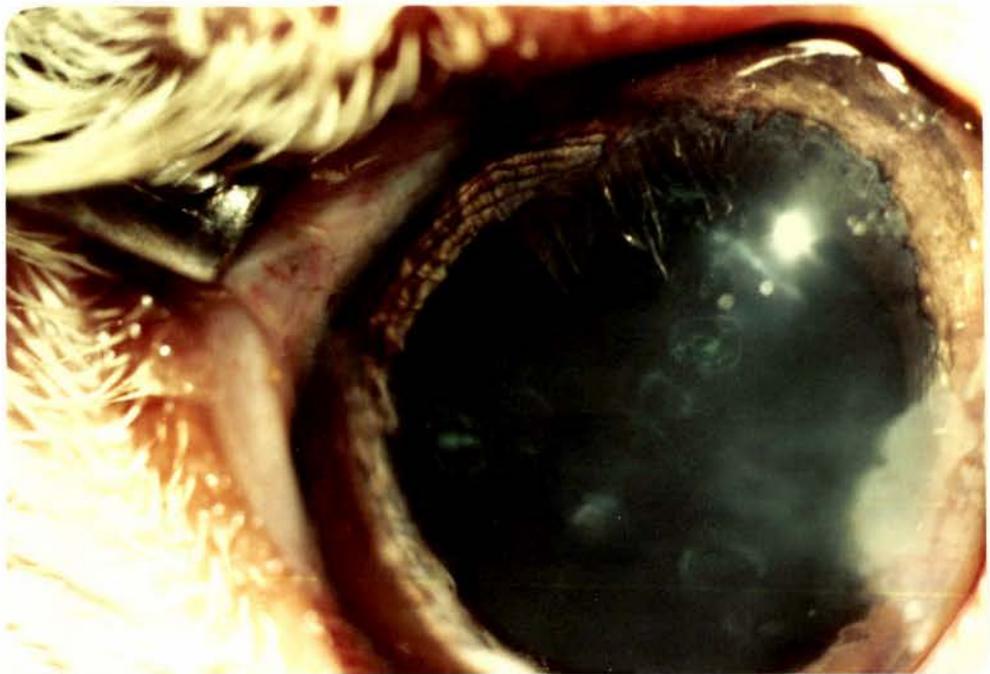
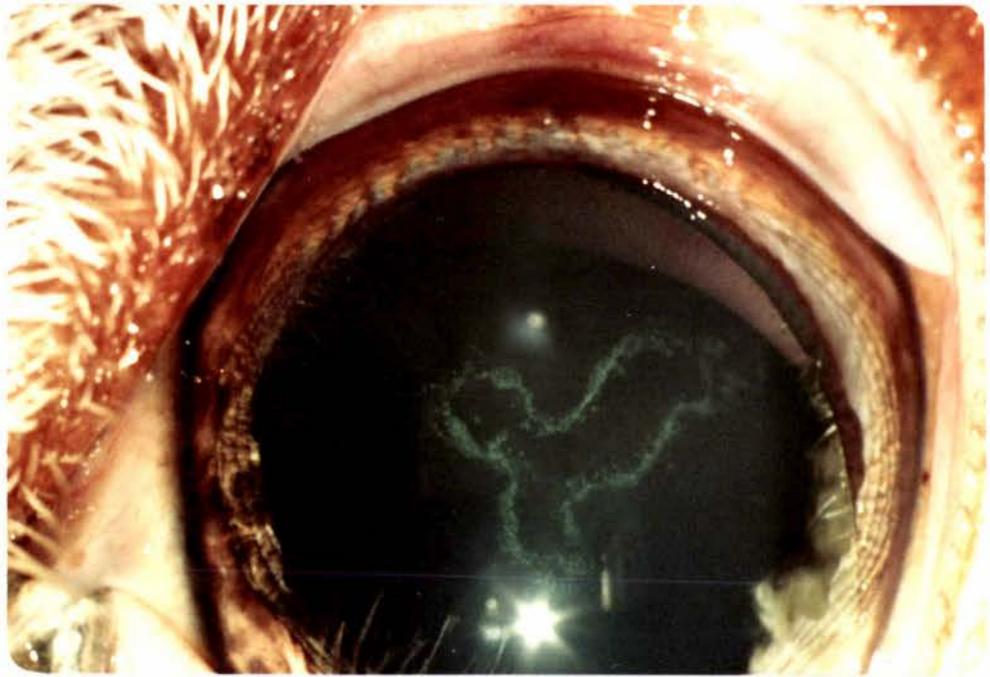


Figure 3.3: Early cataracts in which the spherical opacities are becoming confluent and more prominent.
Lamb 38/80 - 5 months old.

Figure 3.4: Early cataracts showing spoke shaped opacities in the anterior cortex.
Lamb 12/80 - 2 months old.

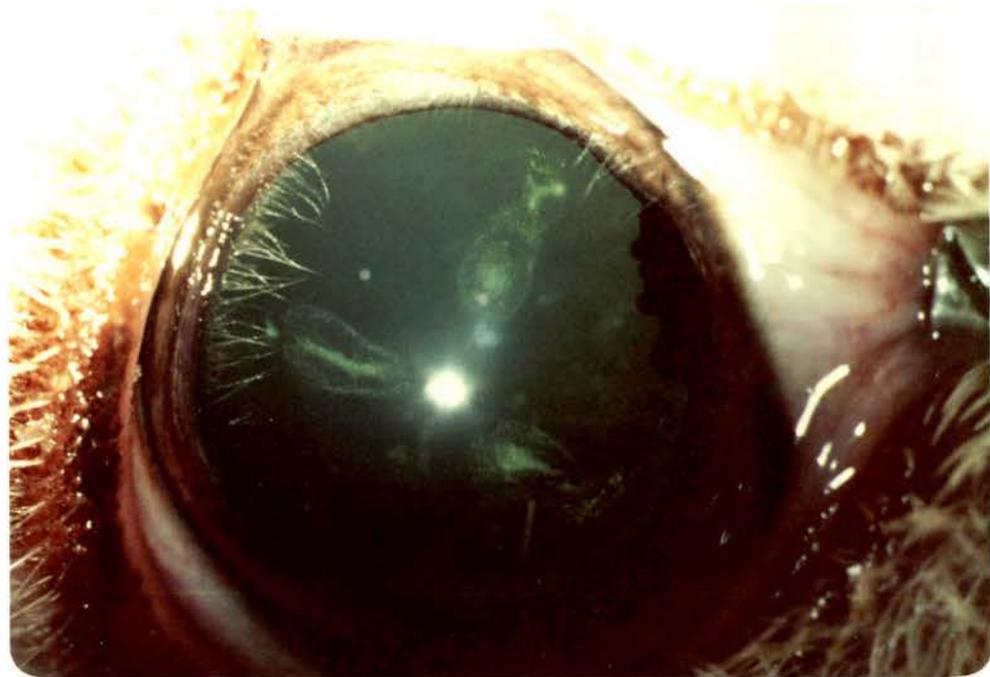
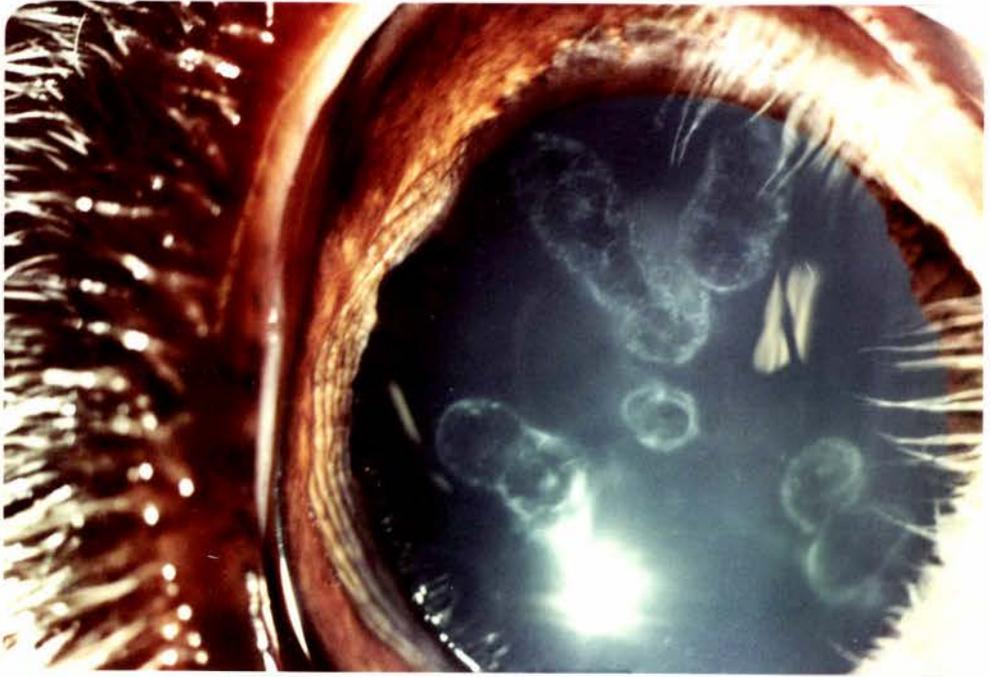


Figure 3.5: Early cataracts in which peripheral opacities have coalesced, to form radiating spoke like patterns.
Lamb 12/80 - 5 months old.

Figure 3.6: Early cataract showing the spoke like pattern of opacity.
Lamb 21/79 - 5 months old.

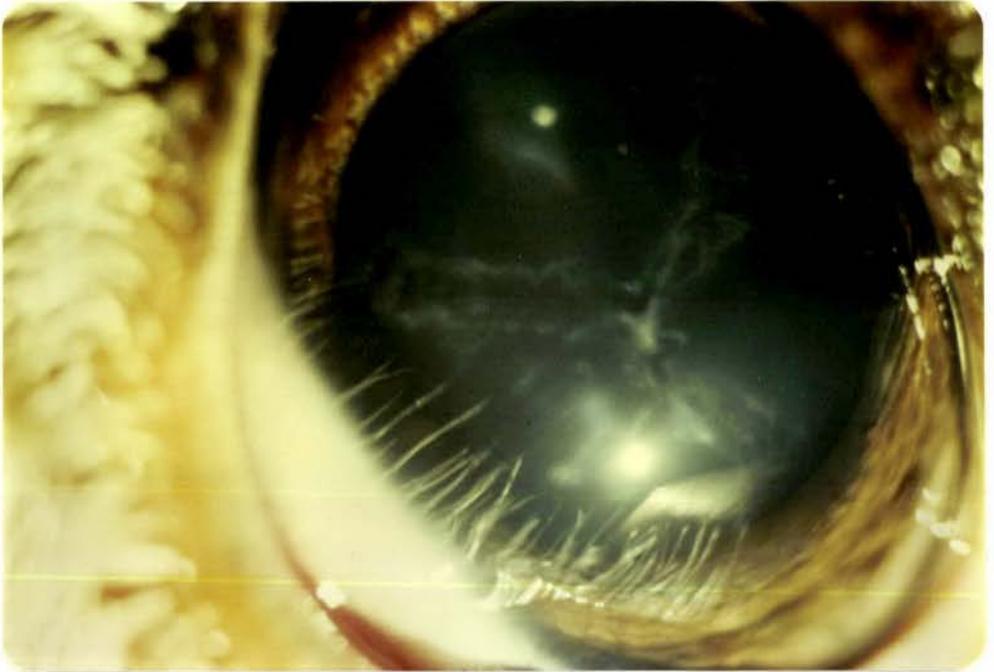
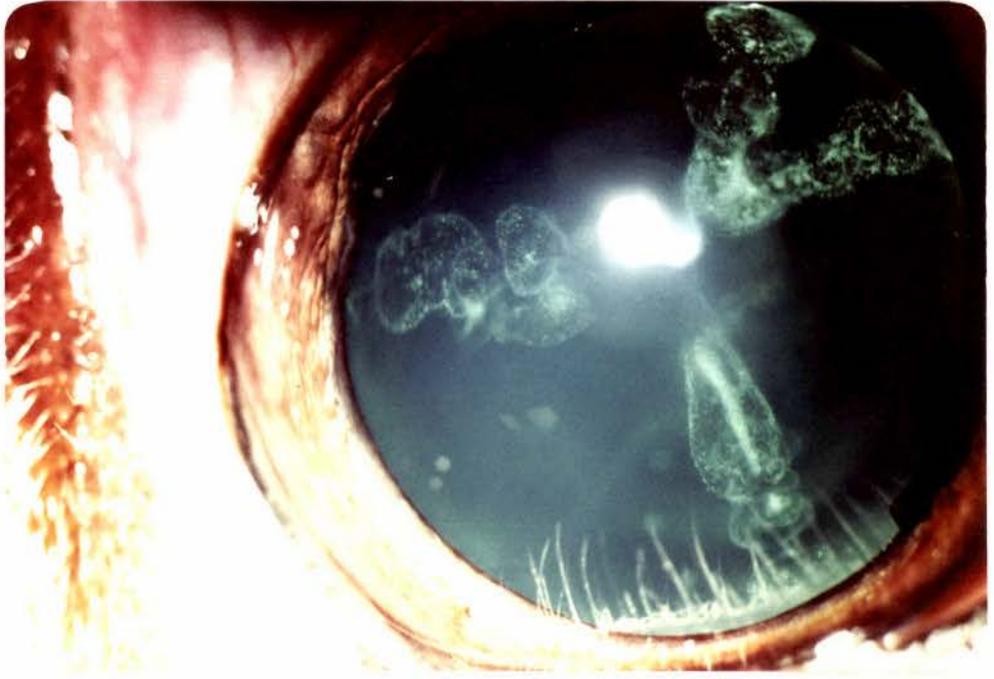


Figure 3.7: Mid stage cataract in which opacity has spread to involve more of the anterior cortex.
Lamb 10/79 - 8.5 months old.

Figure 3.8: Mid stage cataract.
Lamb 8/79 - 10 months old.



Figure 3.9: Late stage cataract in which most of the anterior cortex is affected.
Lamb 8/79 - 11 months old.

Figure 3.10: Late stage cataract.
Lamb 4/79 - 8 months old.

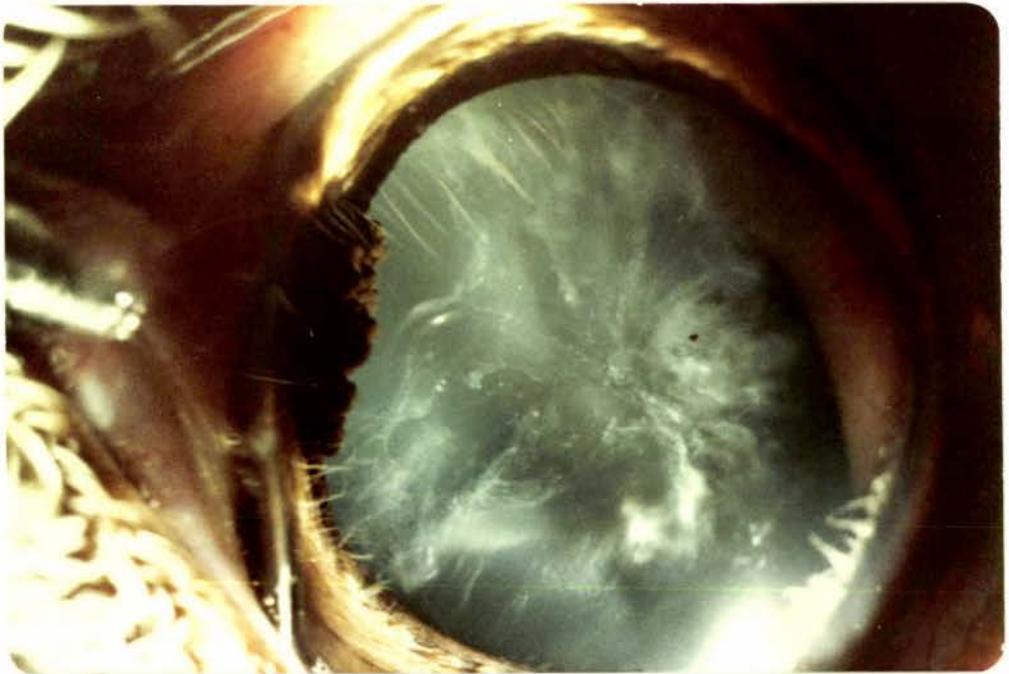
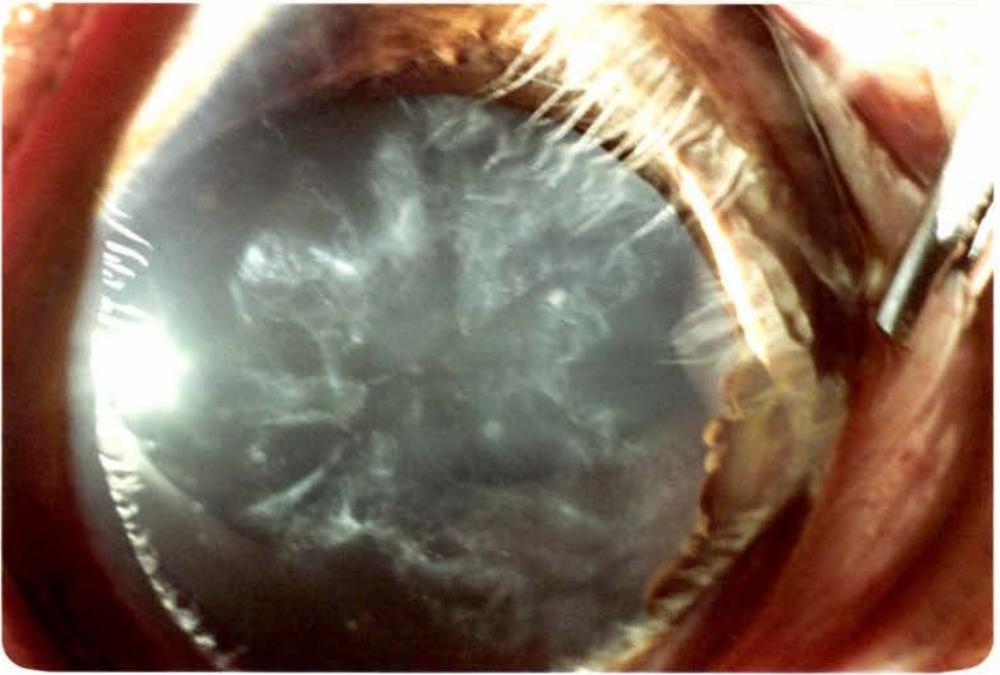


Figure 3.11: Late stage cataract in which the central lens is densely opaque, while the equatorial cortex is clearer. This cataract is close to maturity. Lamb 3/79 - 11 months old.

Figure 3.12: Mature cataract showing total lens opacity in an 11 month old lamb - 4/79.

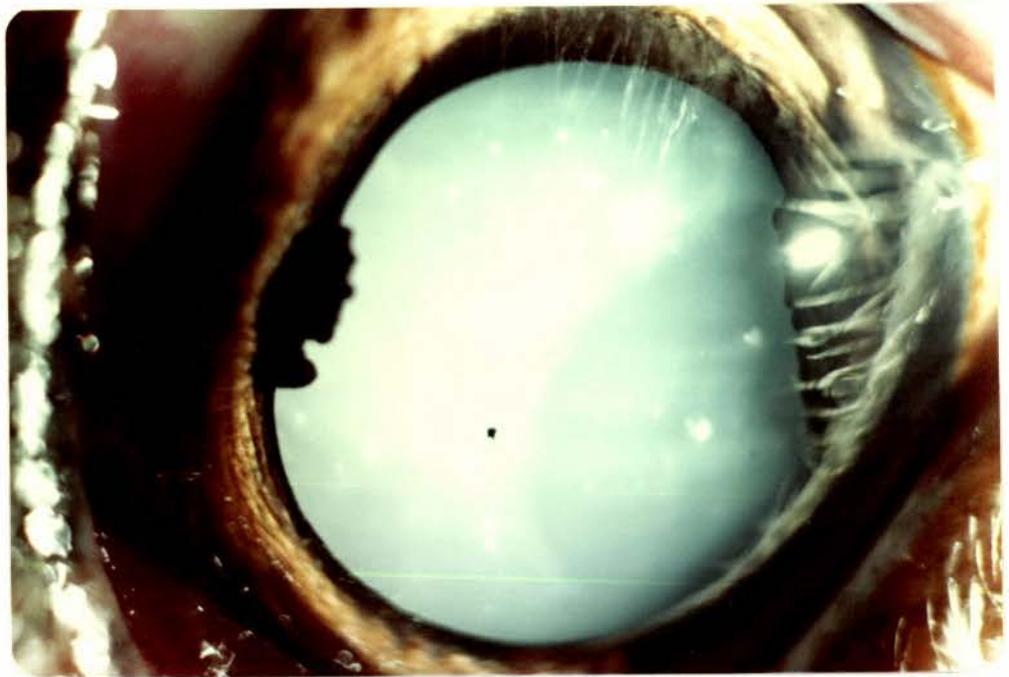
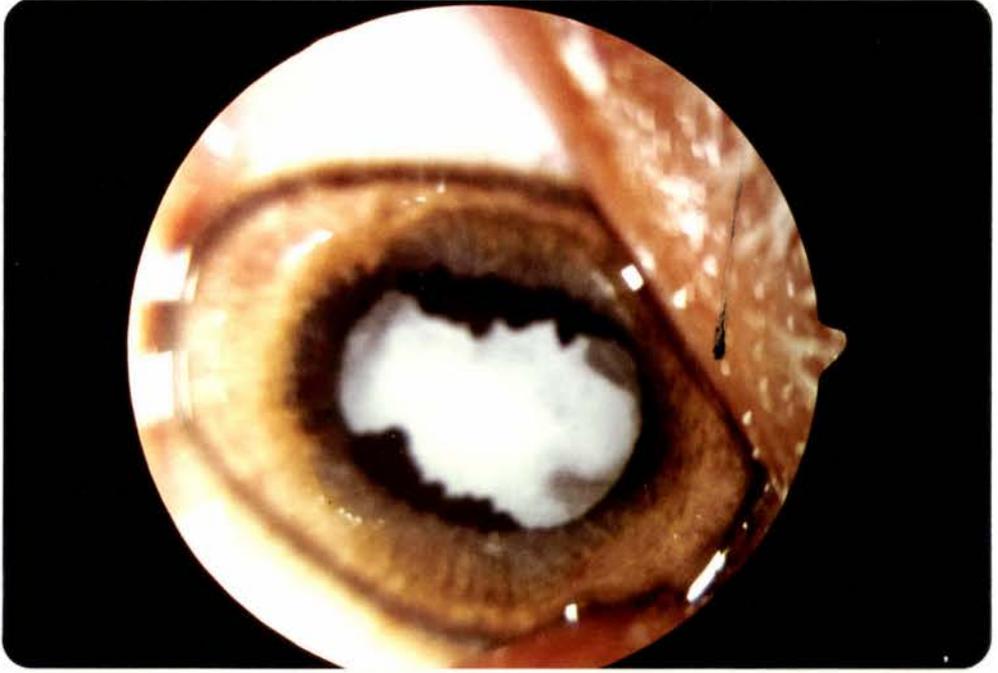


Figure 3.13: Congenital mature cataract.
Lamb 18/79 - 8 days old.

Figure 3.14: The same animal as above (Fig. 3.13), showing
partial absorption of the lens at 8 months of
age.



IV. DISCUSSION

The lenticular changes occurring in the majority of lambs are clearly progressive. Early opacities were in most cases present at 2 months of age and in some animals they were present earlier. In this study animals were not routinely examined earlier than 2 months, because it would have caused too much disturbance to the flock during lambing. Cataracts developed progressively throughout the first year of life and reached maturity at 10 to 12 months of age. Lenses remained opaque in adult life, at least during the course of this study. Progressive loss of vision was apparent from the mid stage of cataract development onward. In all cases animals were affected bilaterally, though often there was variation in appearance and rate of progression of lesions, between eyes of the same individual.

The phenomenon of lens absorption as observed in the two congenitally affected lambs, is common in man when injury to the lens is severed and generalised (Duke-Elder, 1969). Since the proteins in young lenses are predominantly soluble, the products of fibre degeneration and proteolysis are small soluble molecules, which can freely diffuse through the epithelium and capsule. Proteolysis can be so complete as to enable the whole lens to be absorbed leaving only the capsule remaining. In lenses which are older when affected, the higher proportion of insoluble protein within the lens results in a larger residue of insoluble protein remaining following fibre degeneration. This residue is resistant to enzymic proteolysis and is retained as an amorphous mass within the lens. In addition, proliferation of the anterior epithelium and thickening of the capsule may reduce the permeability of older lenses and this assists in the retention of degenerate contents (Duke-Elder, 1969). Since both the congenitally affected lambs in this series died at 8 months of age, it was not possible to determine whether in time lens absorption would have become complete. Lens absorption was also observed in calves with inherited congenital mature cataracts by Carter (1960).

CHAPTER IV

GENETICS

I. INTRODUCTION

The initial history of bilateral cataracts in several sheep from a high performance Romney stud, suggested that the condition might be inherited. As such, this could have important repercussions for the breeders and their clients. The three affected sheep initially made available for this study, were allegedly by a common sire, who himself was unaffected. Other alleged offspring of this sire also had cataracts, but they were not available for examination. This history suggested that if the cataracts had an inherited basis, inheritance would more likely be as an autosomal recessive trait since the sire was unaffected. In humans, and other animals in which cataracts have been shown to be inherited, both autosomal dominant and recessive modes of inheritance have been recorded. To date, inherited cataracts in sheep have not been described.

The present study was undertaken to confirm whether or not the ovine cataract was inherited, and if so the mode of inheritance.

II. MATERIALS AND METHODS

Animals

All sheep used in this study were of the New Zealand Romney breed. The sire 38/75 had typical bilateral cataracts and was the son of an affected ewe, from the flock in which cataracts were first diagnosed. He was by a normal unrelated ram from the Massey University flock. Ewes comprised both normal unrelated animals, and affected daughters of ram 38/75.

Breeding programme

Matings were undertaken in the 1979 and 1980 breeding seasons. In 1979, the affected sire 38/75 was mated with 19 normal unrelated ewes and 5 of his affected daughters. In 1980, he was mated with 35 normal unrelated ewes and 4 affected daughters. The birth date, sex, and parentage of each lamb was recorded, and a visual assessment of lens clarity, at or within a few days of birth, was made by the attending shepherd.

Clinical examination

Both eyes of all offspring were examined. Atropine drops were administered prior to an examination of the lens with a bright pen light in semi-darkness. Lambs were examined on at least three occasions between 2 and 8 months of age, and diagnosed as either being affected, or non affected with cataracts.

III. HYPOTHESIS

The fact that the cataractous ram used in this study had been bred from an affected ewe mated to a normal unrelated ram, implied that the disease was inherited as a dominant trait. This new hypothesis was tested by analysis of the results of the breeding programme.

IV. RESULTS

The results of mating the affected Romney ram 38/75, with unrelated normal Romney ewes are shown in Table 4.I.

TABLE 4.I

Occurrence of cataracts in the progeny of a cataractous ram mated to normal unrelated ewes.

Year	Number of Ewes	Number of Lambs				
		Total	Affected		Non-affected	
			Observed	Expected*	Observed	Expected*
1979	19	26	11	13	15	13
1980	35	47	24	23.5	23	23.5
			35	36.5	38	36.5
	Male Lambs		15	18.25		
	Female Lambs		20	18.25		

* If cataracts are dominantly inherited, half the offspring should be affected and half should be normal.

The results of mating affected ram 38/75 with his affected daughters, are shown in Table 4.II

TABLE 4.II

Occurrence of cataracts in the progeny of a cataractous ram mated to cataractous daughter ewes.

Year	Number of Ewes	Number of Lambs				
		Total	Affected		Non-affected	
			Observed	Expected*	Observed	Expected*
1979	5	5	4	3.75	1	1.25
1980	4	4	2	3	2	1
			6	6.75	3	2.25
	Male Lambs		2	3.27		
	Female Lambs		4	3.27		

* If cataracts are dominantly inherited, matings between affected animals should result in three affected and one normal lamb, for every four lambs born.

Out of nine lambs born from the sire-daughter matings, two lambs had mature cataracts at birth, whereas lambs destined to develop cataracts usually have normal lenses at birth.

V. DISCUSSION

These results strongly support the hypothesis that the ovine cataract described in this study is inherited as a simple autosomal dominant trait. With this pattern of inheritance, an affected heterozygous sire mated to normal unrelated females would be expected to produce equal numbers of affected and normal offspring. An affected heterozygous sire mated to his affected daughters would be expected to produce three affected and one normal offspring for every four lambs born. One lamb in four would be expected to be homozygous for the defective gene and should therefore show a more severe manifestation of cataract. Assuming autosomal inheritance, both sexes should appear in equal proportions in affected groups as was recorded. These criteria are closely met in the present study. Since this is clearly a dominantly inherited condition, the common ancestor should have been affected unless there was incomplete penetrance or delayed expressivity. Observations during this study would tend to rule out both possibilities. It is possible that the dams of the original cases, rather than the sire, had cataracts which were not noticed. It is apparent that the history associated with the original cases was inaccurate.

This particular cataract is unlikely to be of any economic importance to the New Zealand Romney breed, since its dominant mode of inheritance and obvious clinical manifestations enable it to be easily recognised and controlled, merely by culling affected animals.

CHAPTER V

PATHOLOGY

I. INTRODUCTION

The findings presented in this chapter, are the results of a microscopic and ultrastructural study of the lens during the progressive development of the inherited form of ovine cataract. An attempt is made to correlate pathological changes with the clinical appearance of the lens during the various stages of cataractogenesis.

II. MATERIALS AND METHODS

Animals

All sheep, both those typically affected with cataracts and normal age-matched control sheep, were of the New Zealand Romney breed. All animals were grazed at pasture under conventional New Zealand grassland farming conditions. Sheep were selected at various stages of cataract development and their lenses submitted for pathological study.

Preparation of lenses

Eyes were enucleated either under general anaesthesia, or immediately following euthanasia. The posterior section of the globe was removed by circumferential cutting of the globe just behind the lens. The cornea was perforated to allow entry of fixative into the anterior chamber. The anterior eyecup containing the intact lens with some adherent vitreous, was then placed in fixative. After 2 hours, a window was cut from the cornea to allow even better movement of fixative through the anterior chamber.

1. Light microscopy: Lenses for light microscopy were fixed in either Bouin's solution or 10% formol saline. After 24 to 30 hours in Bouin's solution, each lens was dissected out of the anterior eyecup and placed into 70% ethanol which was changed once at 24 hours. When using formol saline fixation, the lenses were removed from the anterior eyecup at 24 hours and further fixed for 7 days. After fixation by either method, the lenses were cut in half sagittally with a razor blade, and 2 - 3 mm slices cut from the exposed surfaces. When using formol saline, these slices were further fixed for another week. Routine paraffin embedding procedures were used and impregnation was done under vacuum (530 mm Hg for 15 minutes).

2. Transmission electronmicroscopy: Whole lenses were fixed in a mixture of 2.0% formaldehyde and 3.0% gluteraldehyde in 0.1 M phosphate buffer (pH 7.2) at 4°C, for 144 hours. During this time, lenses were sliced into progressively smaller pieces to improve penetration of fixative. Time intervals varied, depending on the texture of the partially fixed tissue. Following primary fixation, tissues were washed in phosphate buffer and post fixed in 1.0% osmium tetroxide for 4 hours. The tissues were then dehydrated through graded alcohols, infiltrated with propylene oxide and epoxy resin* and embedded in epoxy resin. Sections were cut on a LKB ultramicrotome**, Thick (0.5 µm) sections stained with 1.0% toluidine blue in 0.1% phosphate buffer, were used for orientation. Thin sections cut at 70 nm were mounted on both supported and unsupported copper grids. They were stained for 8 minutes with a saturated solution of uranyl acetate in 50% ethanol, and for 6 minutes with lead citrate. Sections were viewed in a Phillips 200 electronmicroscope.

* Durcupam - ACM, Fluka, Switzerland.

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

3. Scanning electronmicroscopy: Intact lenses were initially fixed in a mixture of 2.0% formaldehyde and 3.0% gluteraldehyde in 0.1 M phosphate buffer (pH 7.2) at 4°C for 144 hours. After primary fixation, thin pieces of fixed lens were washed in phosphate buffer and post fixed in 1.0% osmium tetroxide in 0.1 M phosphate buffer (pH 7.2) for 3 hours. Tissues were then dehydrated through a series of graded alcohols. From 100% alcohol, lenses were quick frozen in supercooled Freon 12 (-70°C), then placed in liquid nitrogen and cryofractured. After thawing, they were critical point dried, mounted, and gold coated. Tissues were viewed in a Cwikscan 100 scanning electronmicroscope.

III. HISTOPATHOLOGY

Early stage cataracts with central opacity

The earliest histological changes observed in affected lenses are localised areas of fibre swelling and lenticular degeneration, occurring predominantly in the anterior cortex. Similar changes are present in the posterior cortex, but these are relatively mild. The distribution of lesions within the lens is depicted in Fig. 5.1. Anterior cortical lesions involve the deep cortex adjacent to the nucleus, and to a lesser extent areas of the mid and superficial cortex (Fig. 5.2). Typically affected areas, consist of a mass of amorphous eosinophilic lens substance, surrounded by swollen fibres (Figs. 5.3 and 5.4). In the posterior cortex, lesions are restricted to a small area of fibre swelling near the posterior pole (Fig. 5.5).

The anterior epithelium is affected only in areas where it is in contact with the underlying degenerate cortex and these affected cells contain prominent cytoplasmic vacuoles (Fig. 5.6). The formation of new fibres at the equator is normal.

Early stage cataracts with peripheral opacity

In this form of early stage cataract, lesions are present in the superficial anterior nucleus and in the superficial fibres of the anterior and posterior cortex (Fig. 5.7). In the anterior cortex, the anterior extremities of many superficial cortical fibres are swollen. Many of these swollen fibres have broken down, resulting

Figure 5.1: A schematic diagram of an ovine lens affected with the central form of early stage cataracts. The cross-hatched areas depict the distribution of fibre degeneration within the lens. The deep anterior cortex (dac) and to a lesser extent the middle and superficial anterior cortex (ac), are affected. A small lesion is also present within the posterior cortex (pc). The anterior epithelial cells (ae) in close proximity to the anterior cortical lesion are vacuolated. The nucleus (n) is normal.

Figure 5.2: Amorphous areas (arrows) within the anterior cortex of the lens of a 2 month old lamb affected with the central form of early stage cataracts. Single arrows refer to the area (dac) and double arrows to the area (ac) in figure 5.1.
(Paraffin section, H & E x 35)

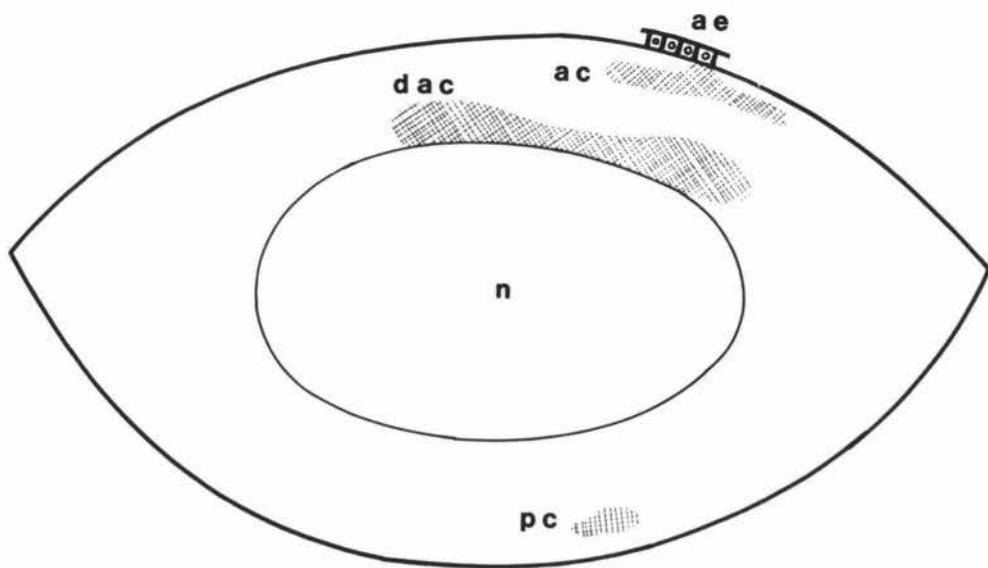


Figure 5.3: Swollen anterior cortical fibres (arrows) adjacent to an amorphous area (a) in the lens of a 2 month old lamb affected with the central form of early stage cataracts.
(Paraffin section, H & E x 400)

Figure 5.4: Swollen superficial anterior cortical fibres (arrows) in the lens of a 2 month old lamb affected with the central form of early stage cataracts. A cystic space containing a small amount of eosinophilic debris is present between the swollen fibres and an amorphous area of lenticular degeneration (a).
(Paraffin section, H & E x 400)

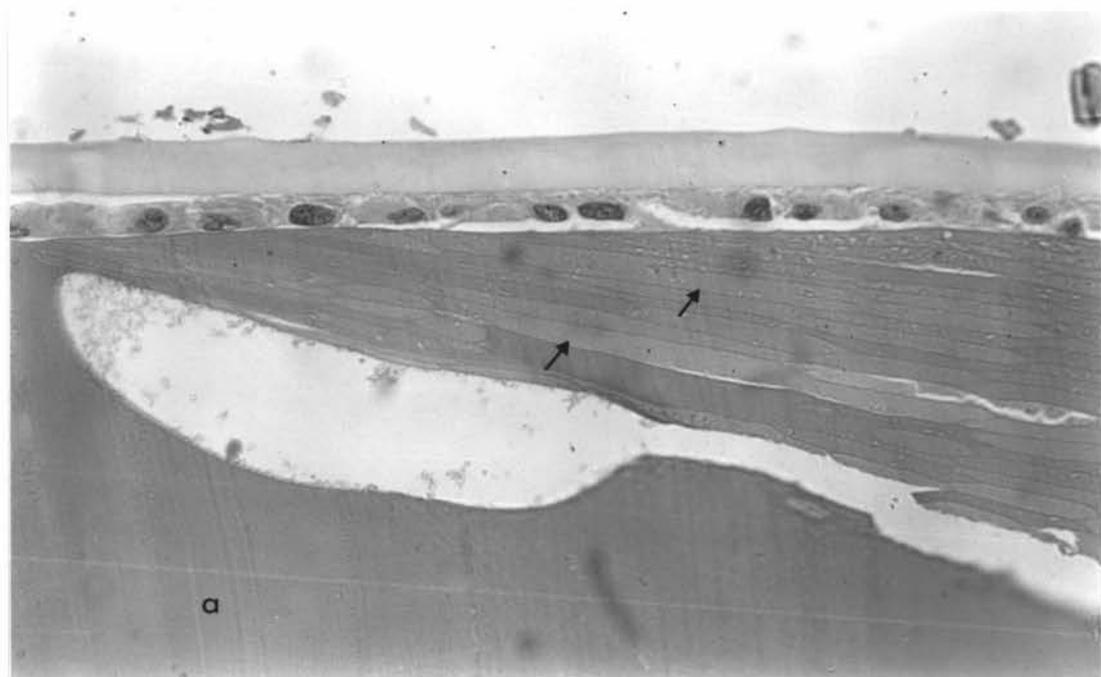
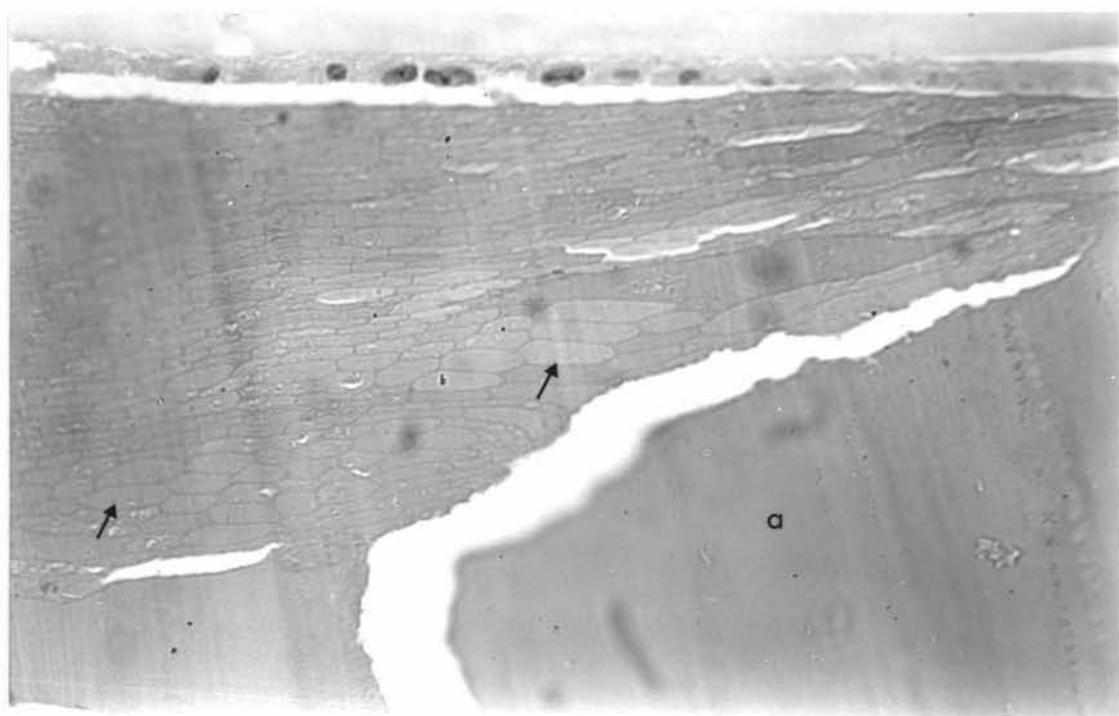


Figure 5.5: Swollen posterior cortical fibres in the lens of a 2 month old lamb affected with the central form of early stage cataracts.
(Paraffin section, H & E x 260)

Figure 5.6: Vacuolated anterior epithelial cells overlying an area of cortical degeneration in the lens of a 2 month old lamb affected with the central form of early stage cataracts.
(Paraffin section, H & E x 500)

in the formation of large intercellular spaces, often containing proteinaceous debris (Fig. 5.8). In the posterior cortex, the posterior extremities of the superficial fibres are swollen where they terminate against the posterior capsule.

The anterior nuclear lesion consists of fibre swelling and degeneration. The anterior epithelial cells are occasionally vacuolated where they overlies cortical lesions, otherwise the epithelium appears normal. The formation of new fibres at the equator is normal, but just anterior to the nuclear bow, the anterior extremities of some young cortical fibres are slightly swollen (Fig. 5.9).

Mid stage cataract

By mid stage, most of the anterior and to a lesser extent the equatorial and posterior lens cortex is affected, as depicted in (Fig. 5.10). A small area of the anterior nucleus is degenerate, whilst the remainder of the nucleus is normal. Degenerate areas are present at all levels within the anterior cortex (Fig. 5.11). Rounded up globular shaped fibres are prominent at the interface between intact fibres and the degenerate lens substance, both in the anterior and posterior cortex. In the latter, many swollen fibres are present posterior to the nuclear bow and these fibres often contain abnormally retained pyknotic nuclei. Near the posterior pole, the degenerate lens substance is lightly calcified. A single layer of flattened cells is abnormally present beneath most of the posterior capsule, these appear to be derived from epithelial cells migrating posteriorly from the equator (Fig. 5.12). There is extensive cytoplasmic vacuolation of the anterior epithelium and in some areas a few spindle shaped cells are interposed between the epithelium and the degenerate cortex. In association with the epithelial changes are small clusters of round nucleated foamy cells within the underlying degenerate cortex (Fig. 5.13), these cells appear to derive from the proliferating epithelium. The production of new lens fibres at the equator is relatively normal, although the nuclear bow is quite disorganised at this stage.

Late stage cataract

At the late stage of cataract development, all areas of the lens are severely affected (Fig. 5.14), except for the equatorial zone of the anterior epithelium, the first few superficial cortical fibres near the equator and a small part of the nucleus. Most of the anterior and posterior cortex is a lake of amorphous eosinophilic material, in which aggregations of swollen and rounded-up fibres are located (Fig. 5.15). There are numerous nucleated swollen cells present beneath the posterior capsule (Fig. 5.16), and in association are many intercellular cystic spaces, some of which contain proteinaceous material. The formation of new lens fibres at the equator is relatively normal, but at a short distance within the cortex, these elongating fibres are swollen, particularly within their posterior extremities (Fig. 5.17), and the configuration of the lens bow is disrupted. Most cells of the anterior epithelium show cytoplasmic vacuolation, except in those parts of the intermediate and equatorial zones, beneath which the cortex is only mildly affected. In several areas, there is epithelial metaplasia to a spindle shaped cell (Fig. 5.18).

Mature cataract

At the stage of cataract maturity, there is complete loss of structural integrity throughout all of the lens, except for the most superficial aspects of the equatorial cortex. The lens is virtually a mass of amorphous, eosinophilic, proteinaceous material, enclosed by the epithelium and capsule. A few layers of relatively normal fibres are present at one equator, whilst at the other equator, partially elongated fibres are displaced posteriorly beneath the posterior capsule (Fig. 5.19). Further toward the posterior pole these fibres are associated with large intercellular cystic spaces containing proteinaceous material (Fig. 5.20). In many areas the anterior epithelium is hyperplastic (Fig. 5.21), and occasionally is duplicated with fibrous capsular material present between the two epithelial layers (Fig. 5.22). This epithelial response is particularly apparent beneath capsular wrinkles, produced by lens shrinkage during cataract development. The fibrous inter-epithelial material, stained positively for a collagen-like substance, with Van Gieson's stain. Similar capsule-like extracellular material is sometimes abnormally present beneath the posterior capsule.

Figure 5.7: A schematic diagram of an ovine lens affected with early stage cataracts of peripheral distribution. The cross-hatched areas depict the distribution of fibre degeneration within the lens. Lesions are present in the anterior nucleus (an), the superficial anterior cortex (ac) and posterior cortex (pc). The nucleus is marked (n)

Figure 5.8: Sub epithelial clefts, intercellular cystic spaces and swollen fibres, within the anterior cortex of the lens of a 3 month old lamb affected with a peripheral (spoke) form of early stage cataract. (Paraffin section, H & E 320)

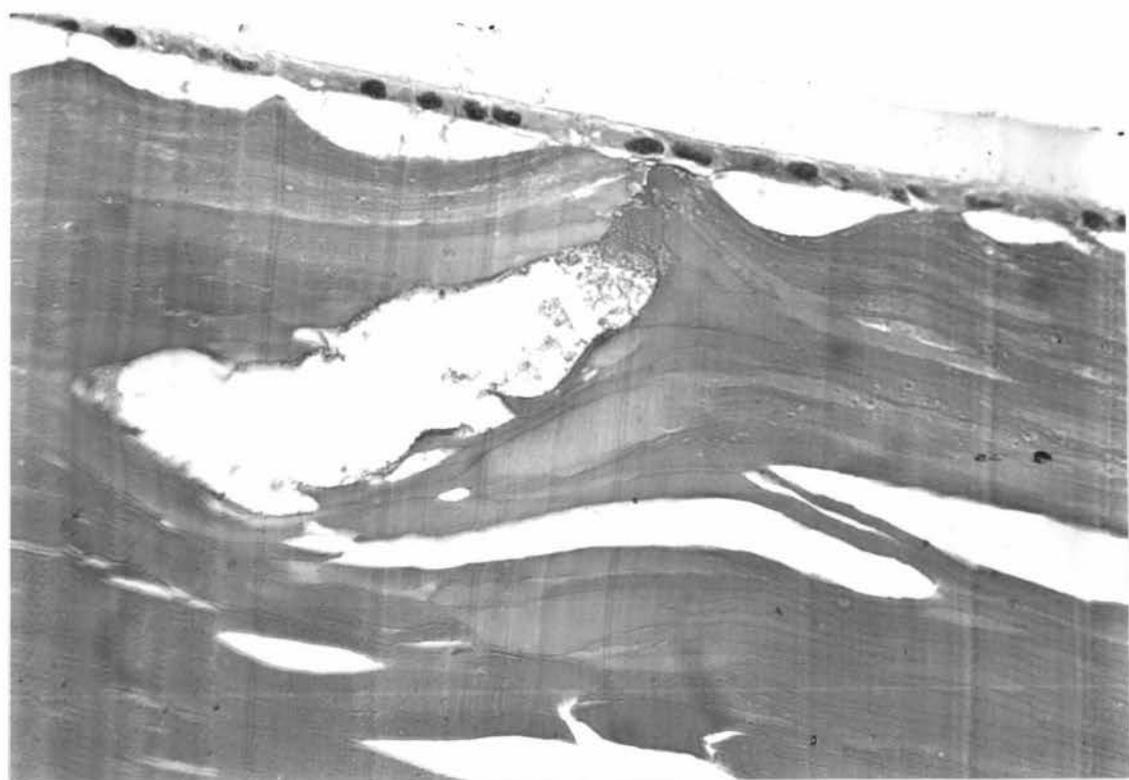
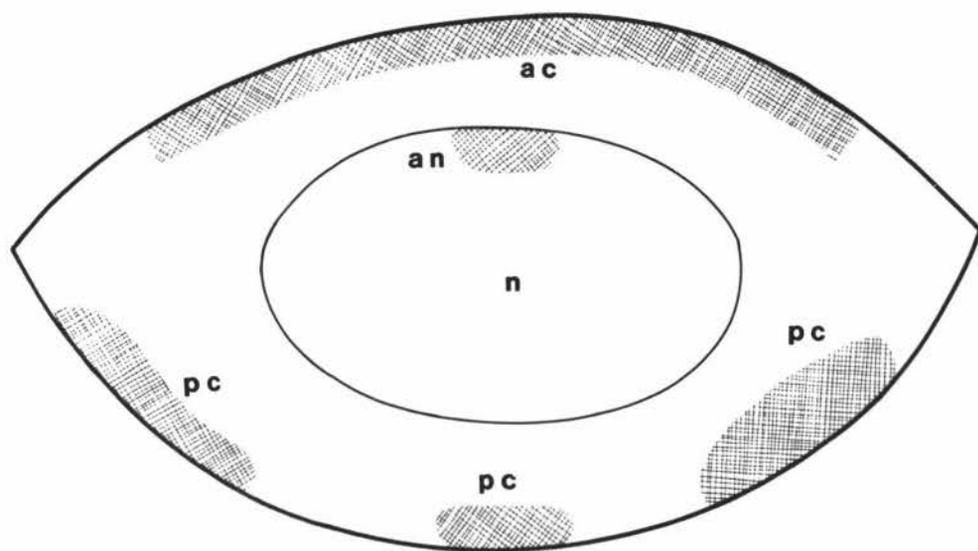


Figure 5.9: Swollen and pale staining anterior extremities of some of the recently formed cortical fibres in the lens of a 3 month old lamb affected with the peripheral (spoke) form of early stage cataracts. (Paraffin section, H & E 275)

Figure 5.10: A schematic diagram of the ovine lens affected with mid stage cataracts. The cross-hatched areas depict the distribution of fibre degeneration within the lens. Lesions are present in the anterior cortex (ac), the equatorial cortex (ec), the posterior cortex (pc) and the anterior nucleus (an).

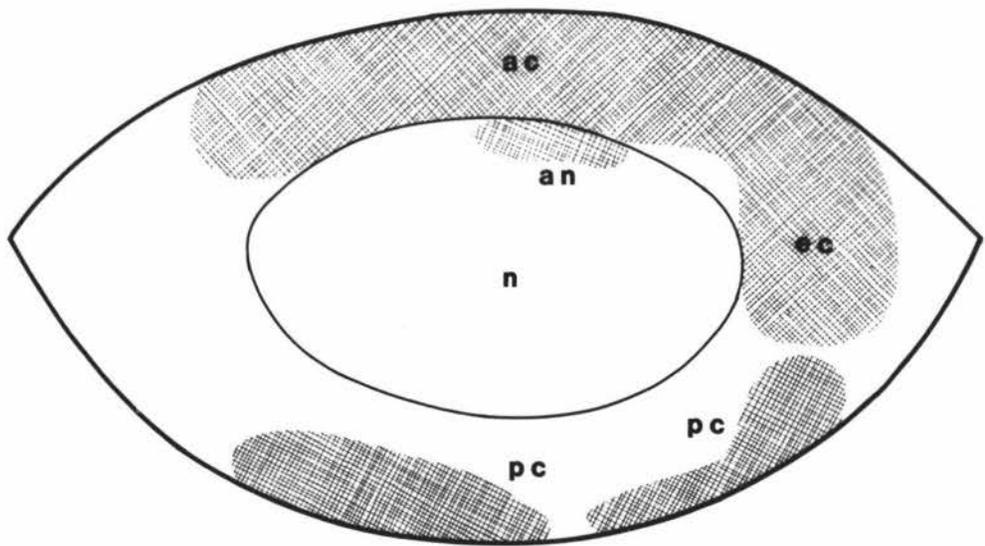
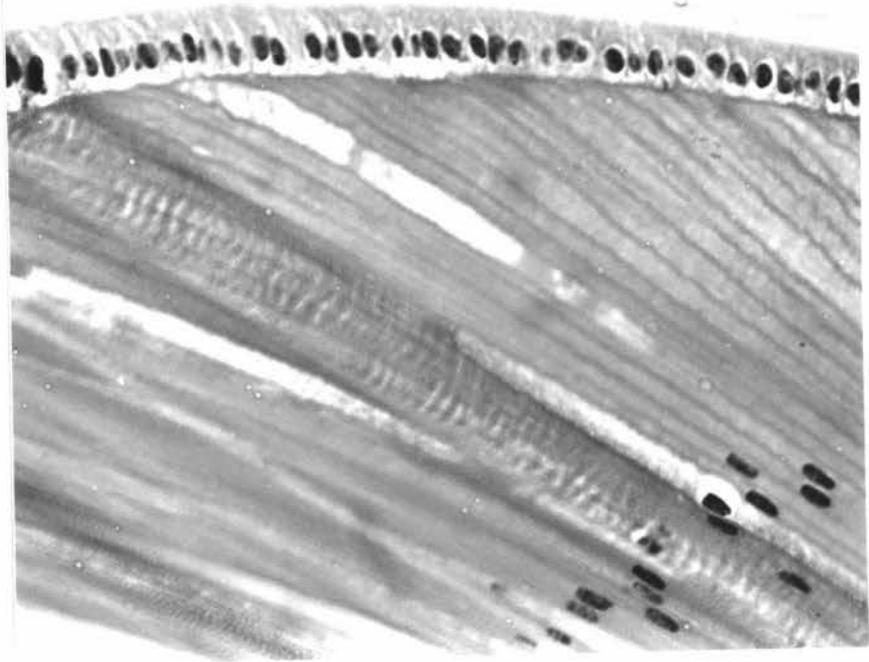


Figure 5.11: Degenerate amorphous areas within the anterior cortex of the lens of an 8.5 month old lamb affected with mid stage cataracts. The overlying anterior epithelial cells are extensively vacuolated. (Paraffin section, H & E x 320)

Figure 5.12: Epithelial cells (arrows) migrating from the equator beneath the posterior capsule in the lens of an 8.5 month old lamb affected with mid stage cataracts. (Paraffin section, H & E x 250)

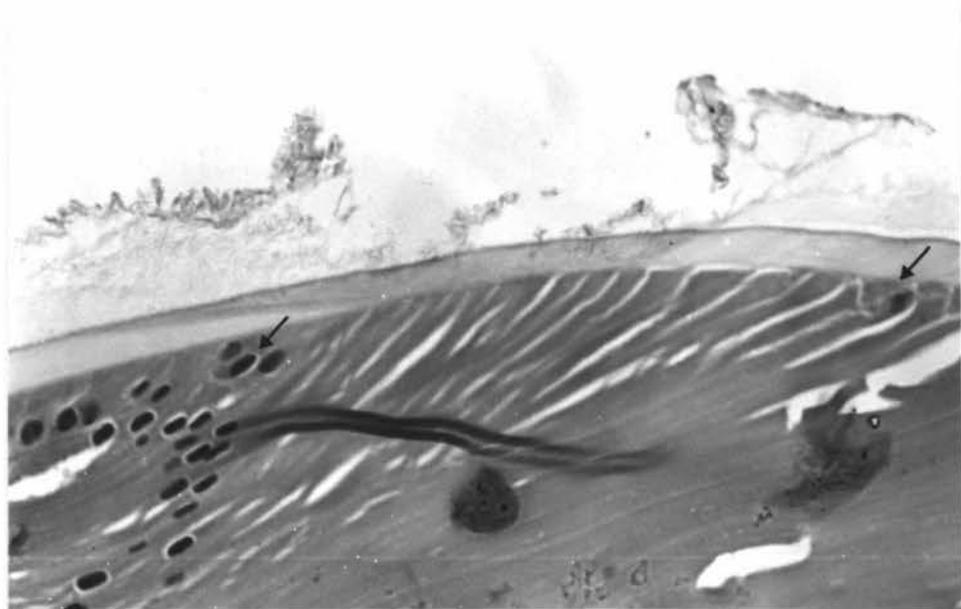
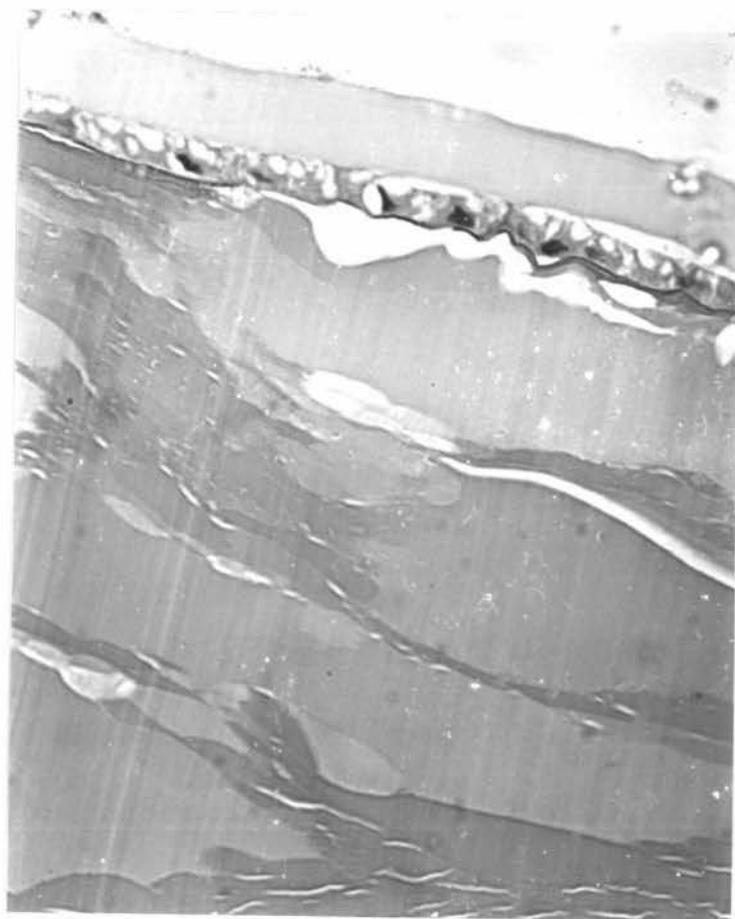


Figure 5.13: Vacuolated anterior epithelial cells which appear to be the source of the round foamy cells lying free in the degenerate cortex, in the lens of an 8.5 month old lamb affected with mid stage cataracts.
(Paraffin section, H & E 500)

Figure 5.14: A schematic diagram of an ovine lens affected with late stage cataracts. The cross-hatched areas depict the distribution of degeneration within the lens. The only unaffected areas are the superficial equatorial cortex (ec) and a small area within the nucleus (n).

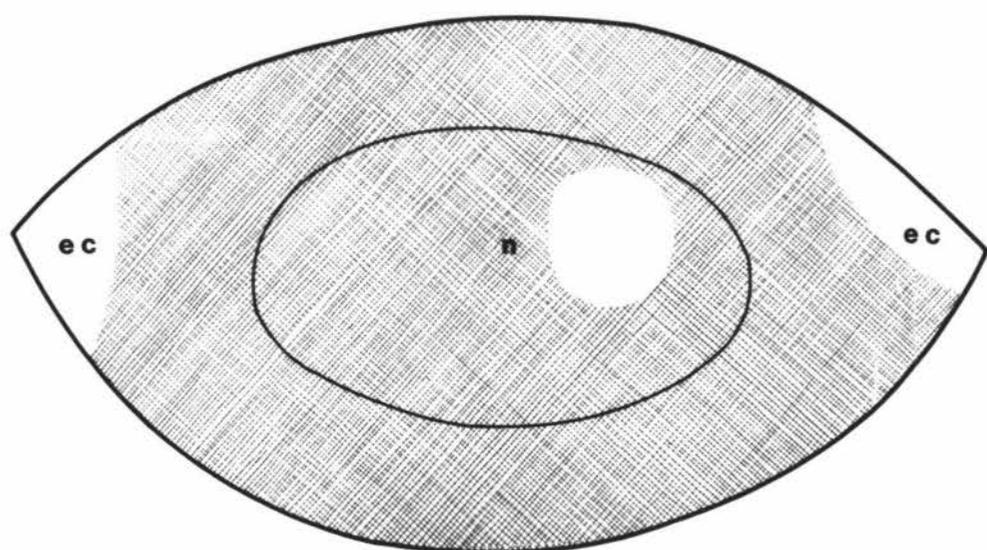
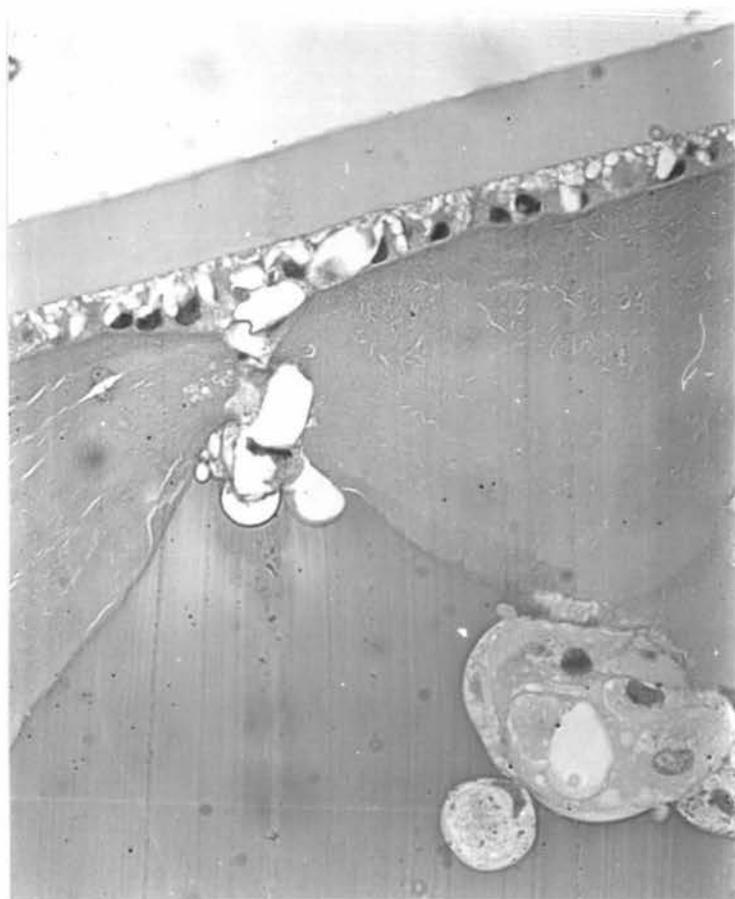


Figure 5.15: Rounded up and swollen fibres within the degenerate amorphous anterior cortex, in the lens of an 8.5 month old lamb affected with late stage cataracts.
(Paraffin section, H & E x 145)

Figure 5.16: Swollen cells and intercellular cystic spaces beneath the posterior capsule, in the lens of an 8.5 month old lamb affected with late stage cataracts.
(Paraffin section, H & E x 152)

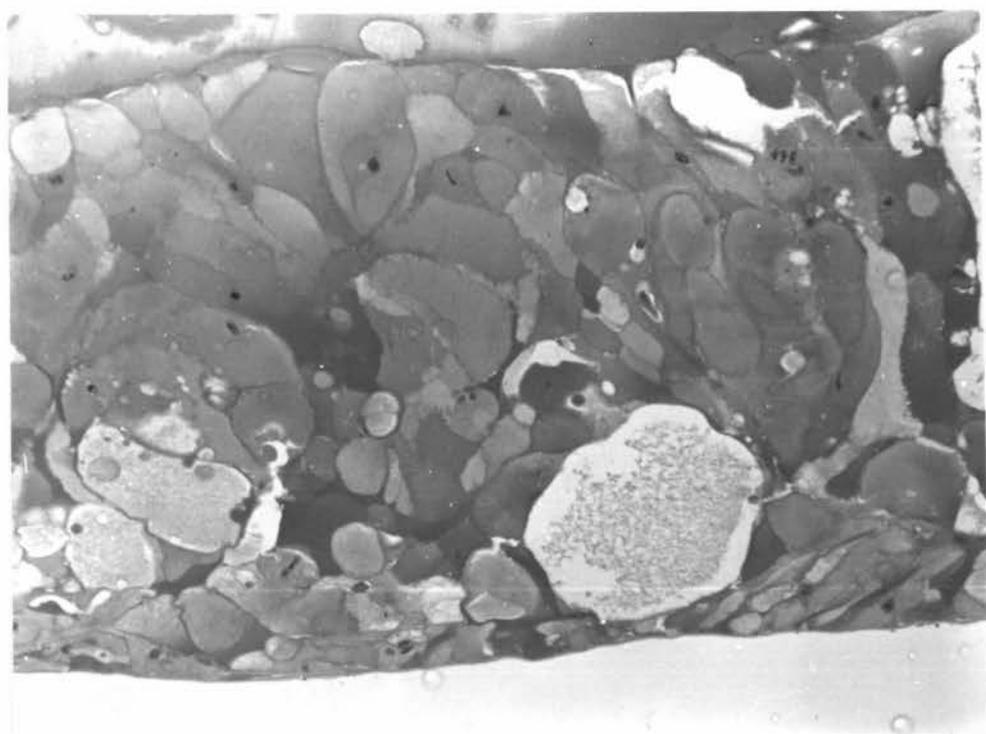
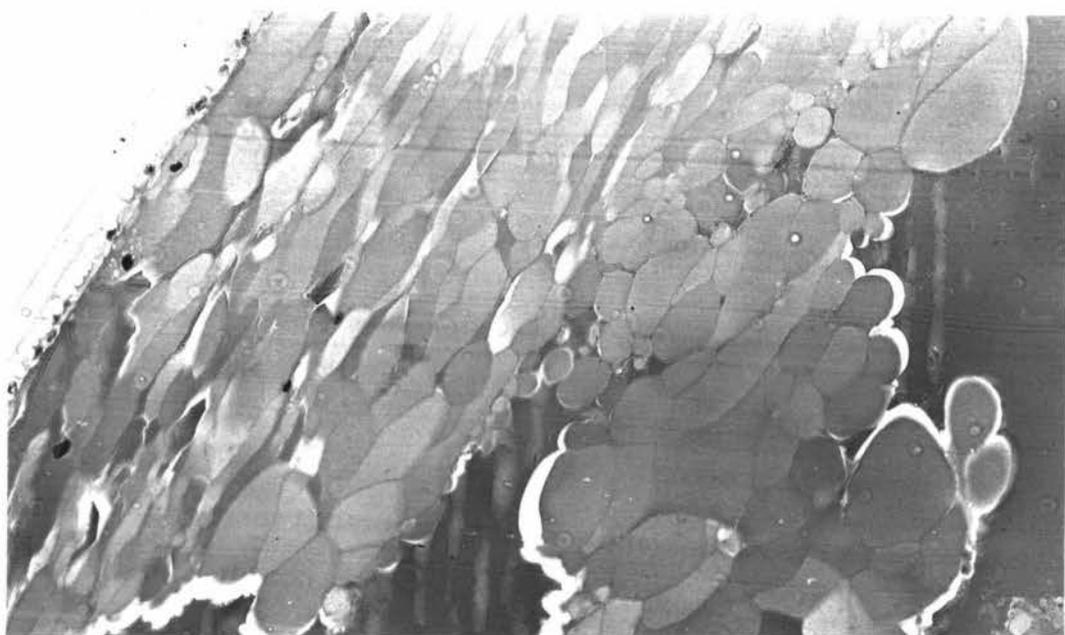
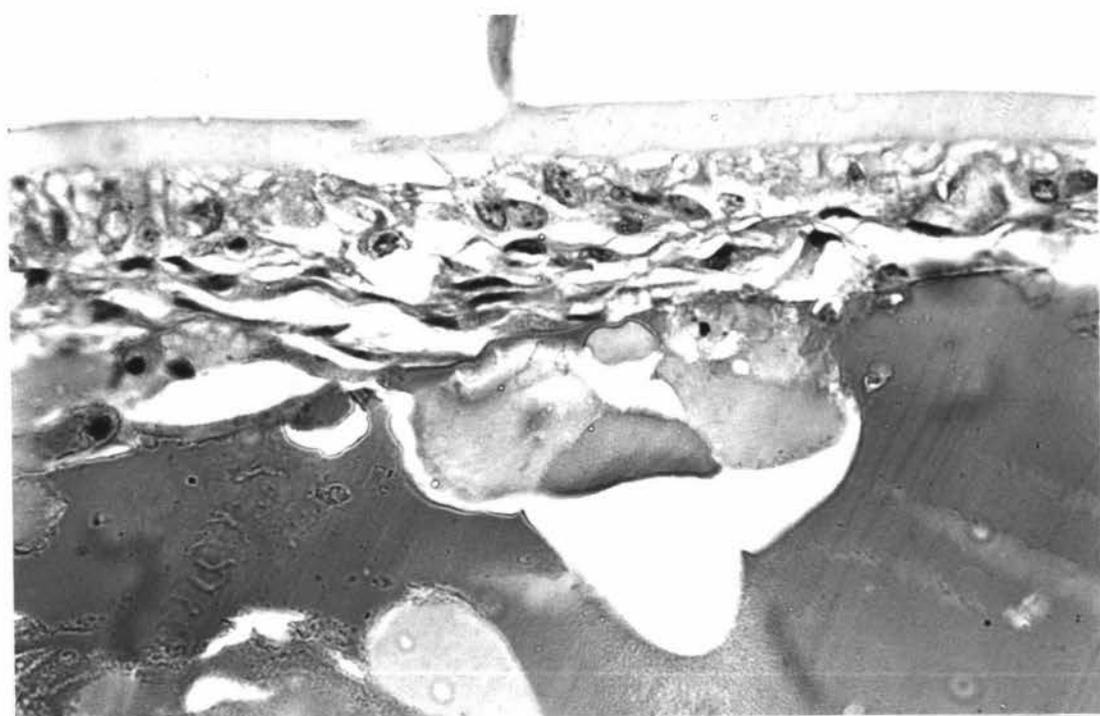
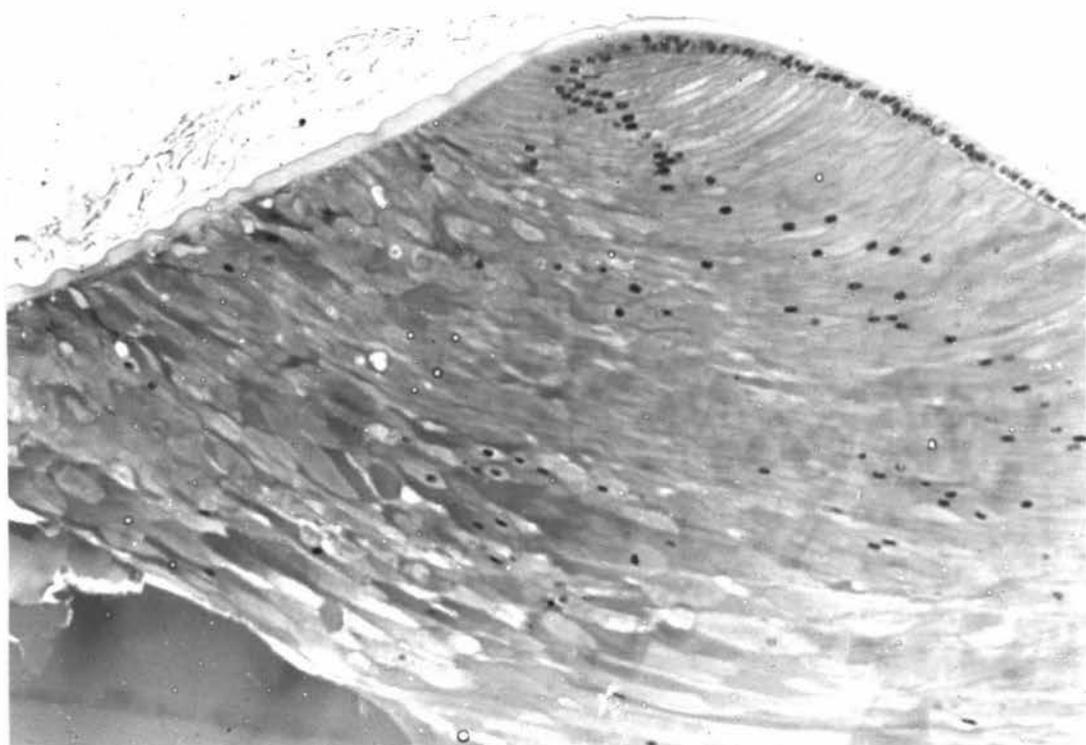


Figure 5.17: Disorganisation of the nuclear bow and swelling of the posterior extremities of young cortical fibres, in the lens of an 8.5 month old lamb affected with late stage cataracts.
(Paraffin section, H & E x 112)

Figure 5.18: Metaplasia of the anterior epithelium from cuboidal to spindle shaped cells, in the lens of an 8.5 month old lamb affected with late stage cataracts.
(Paraffin section, H & E x 336)



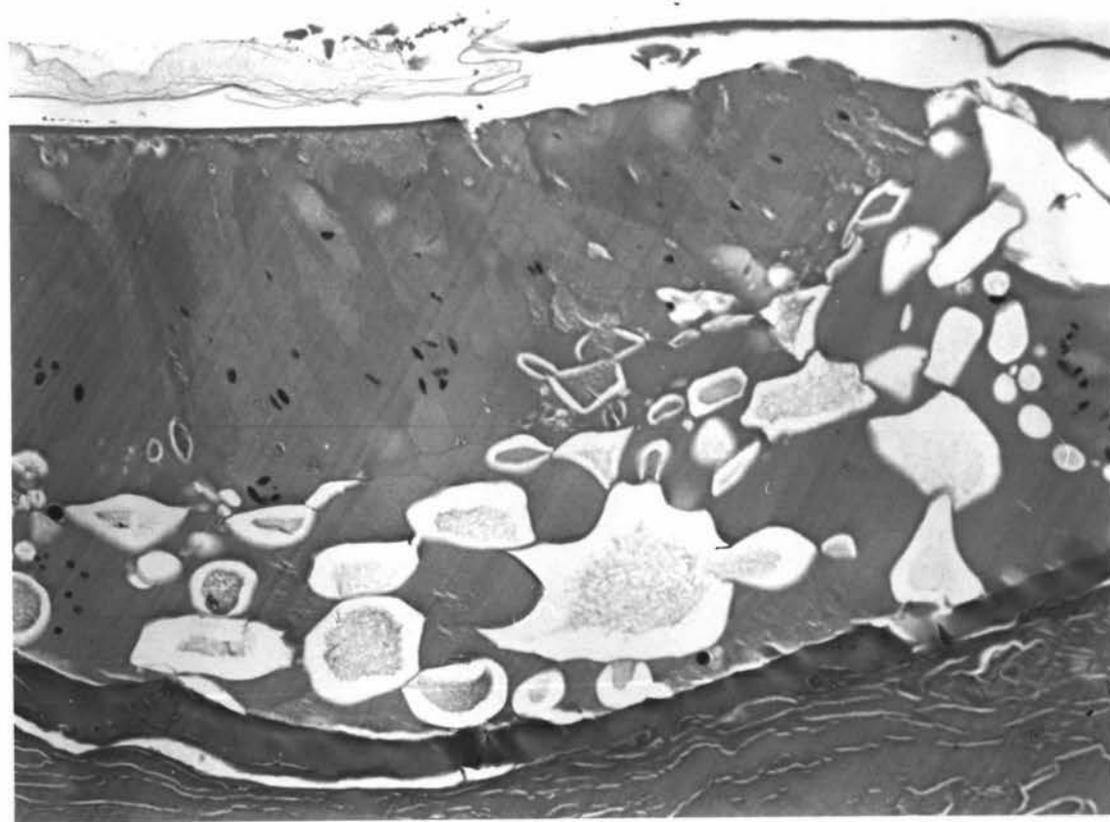
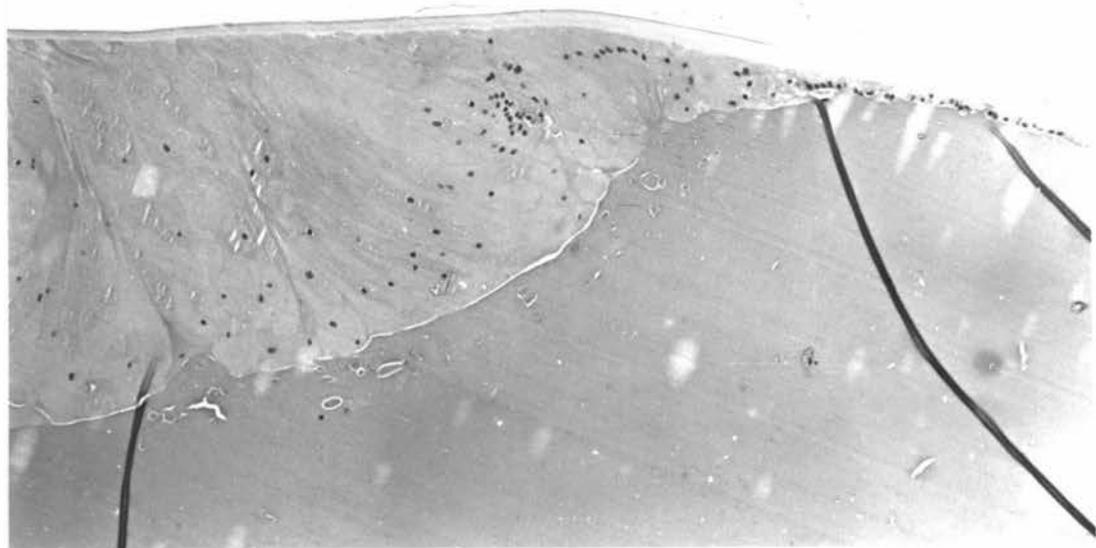
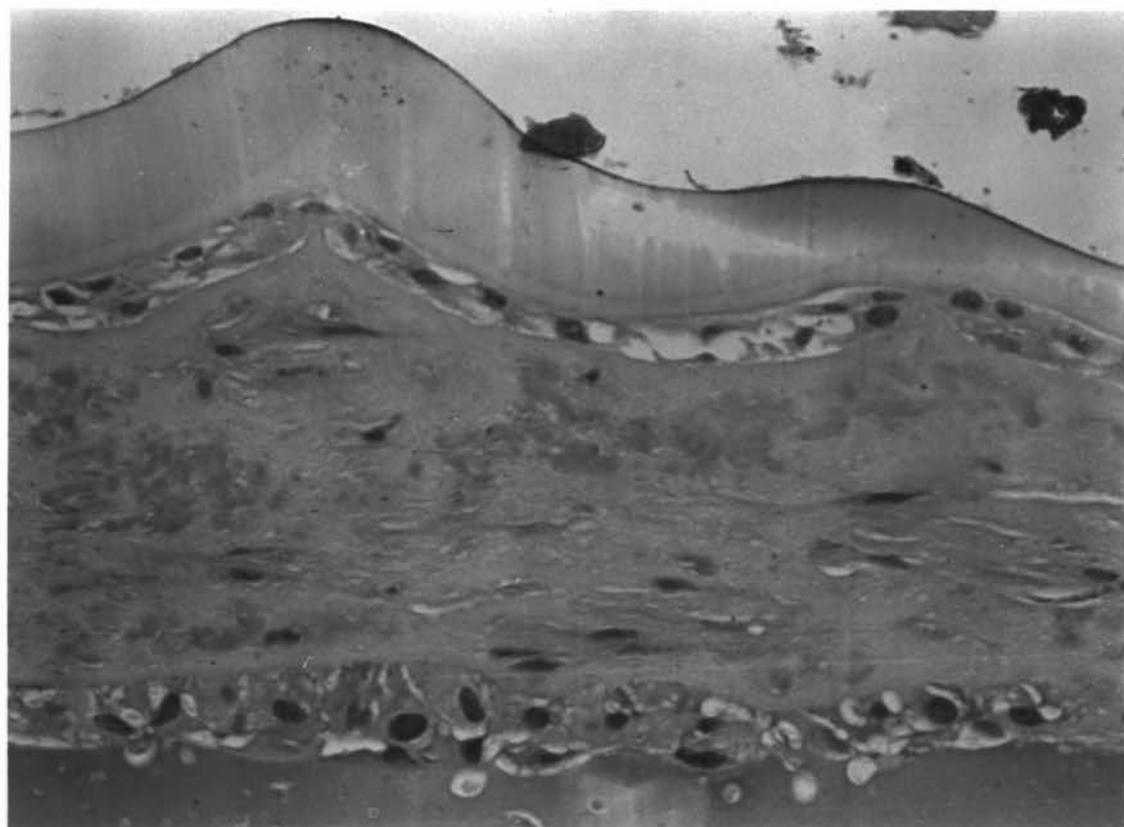
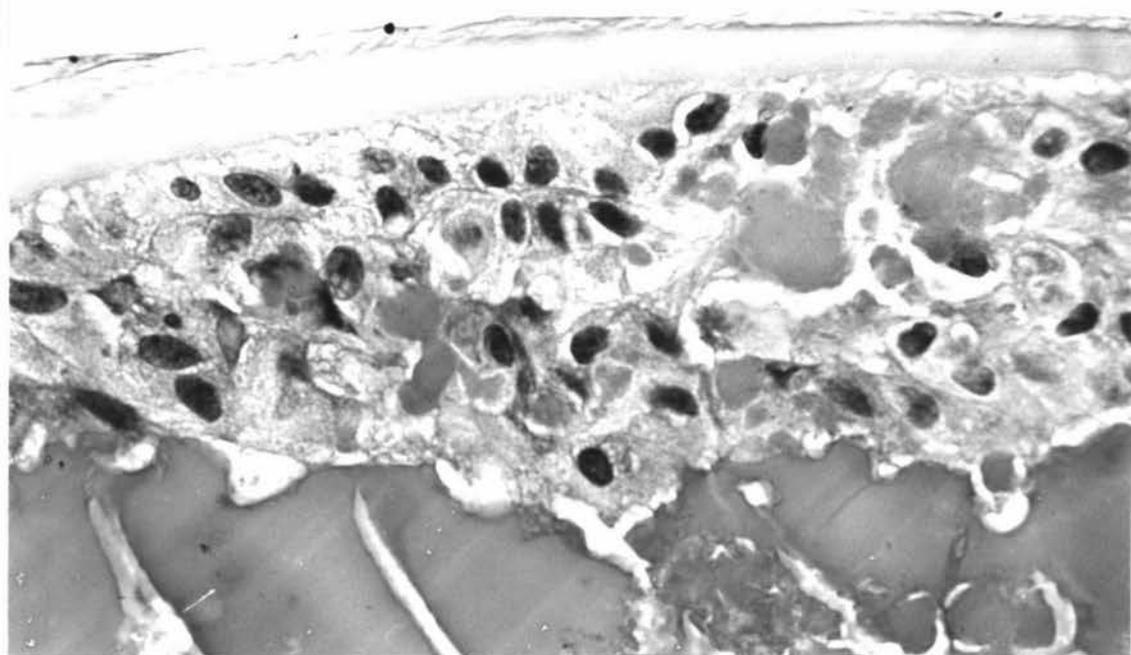


Figure 5.21: Anterior epithelial cell hyperplasia in the lens of a 15 month old sheep affected with mature cataracts.
(Paraffin section, H & E x 400).

Figure 5.22: Duplication of the anterior epithelium and the formation of capsular material beneath the wrinkled capsule, in the lens of a 15 month old sheep affected with mature cataracts.
(Paraffin section, H & E x 250)



IV. TRANSMISSION ELECTRON MICROSCOPY

The anterior epithelial and spindle-shaped cells were examined ultrastructurally. The epithelial cells contain two types of vacuole (Fig. 5.23). Numbers of small circular vacuoles, for which a membrane cannot be demonstrated, are sometimes present, usually grouped together within the cytoplasm. These small vacuoles are more numerous within the spindle shaped cells. Larger, irregularly shaped vacuoles previously seen by light microscopy, could sometimes be shown to have a lining membrane (Fig. 5.24). The occasional association of these vacuoles with dilated membrane systems (Fig. 5.25), suggests that they may be dilated endoplasmic reticulum or Golgi apparatus. They are mostly empty, other than for a few membranous fragments but in some instances, electron dense amorphous and granular material is present (Fig. 5.26). This type of vacuole is only occasionally seen within spindle shaped cells. In addition, the spindle shaped cells contain electron dense deposits (Fig. 5.27), which in parts are enclosed by a membrane, possibly part of the endoplasmic reticulum or Golgi system (Fig. 5.28). Fibrillar material is also present in the spindle shaped cells (Fig. 5.29). Both the anterior epithelial and spindle shaped cells from severely cataractous lenses contain abundant rough endoplasmic reticulum, whereas normal control anterior epithelial cells have a homogeneous cytoplasm containing only a small amount of rough endoplasmic reticulum (Figs. 5.30 and 5.31).

V. SCANNING ELECTRON MICROSCOPY

Cortical fibres in the normal sheep lens, are long ribbon-like cells with a flattened hexagonal cross section. They are arranged precisely in regular rows, and are joined to one another by interlocking tongue and groove processes (Figs. 5.32 and 5.33).

A profound breakdown in the cellular architecture of the lens during cataractogenesis is readily demonstrated by scanning electronmicroscopy. Mildly affected anterior cortical fibres are swollen and are almost circular in cross-section (Fig. 5.34), and

Figure 5.23: Large irregular vacuoles and small circular vacuoles within anterior epithelial cells (ae), in the lens of a 10 month old lamb with mature cataracts. Small vacuoles are also present within the spindle shaped cells (s). (TEM x 4,700)

Figure 5.24: Large irregular vacuoles which are partly membrane bound (arrows) within an anterior epithelial cell, in the lens of a 10 month old lamb with mature cataracts. (TEM x 17,500)

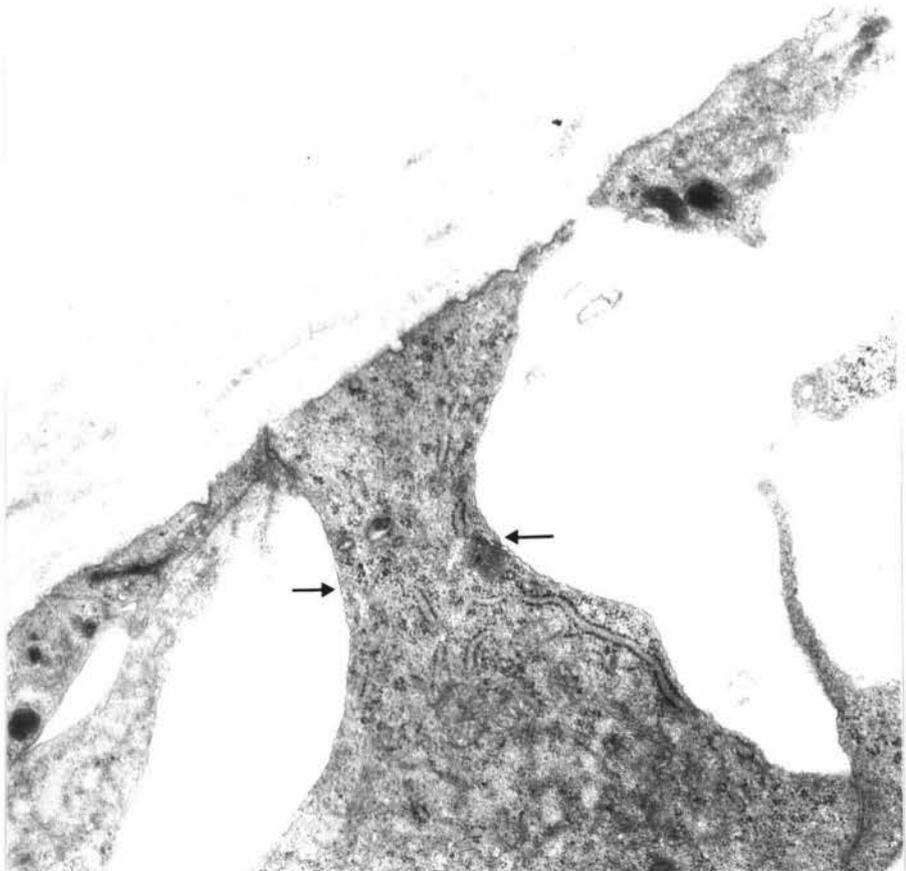
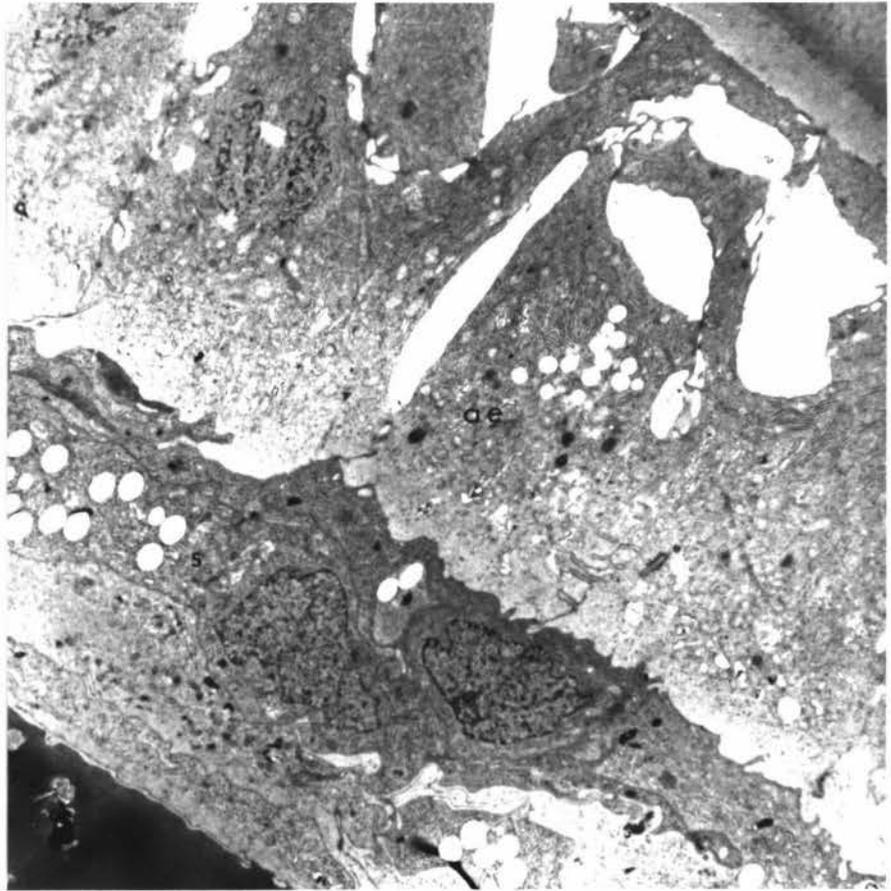


Figure 5.25: Membranous material within the large vacuoles of an anterior epithelial cell in the lens of a 10 month old lamb with mature cataracts. Mildly dilated membrane systems (arrows), interpreted as smooth endoplasmic reticulum, are often associated with these large vacuoles. (TEM x 10,500)

Figure 5.26: Electron dense granular and amorphous material within a large vacuole of an anterior epithelial cell, in the lens of a 10 month old lamb with mature cataracts. Numerous small circular vacuoles (arrows) are also present. (TEM x 10,500)

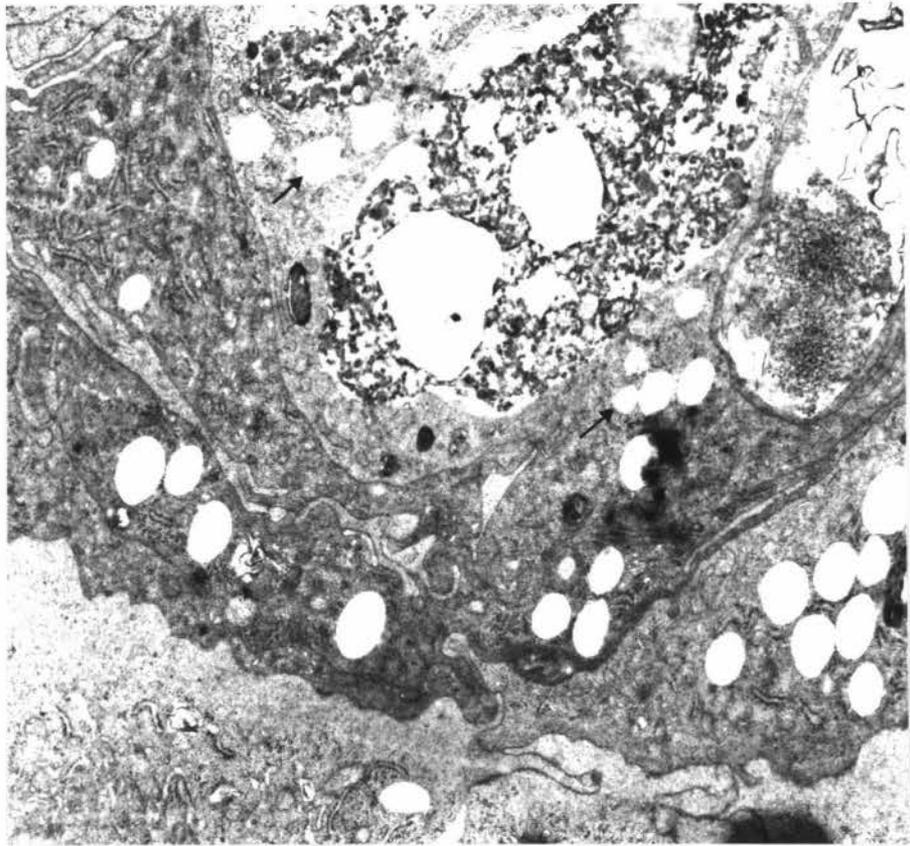
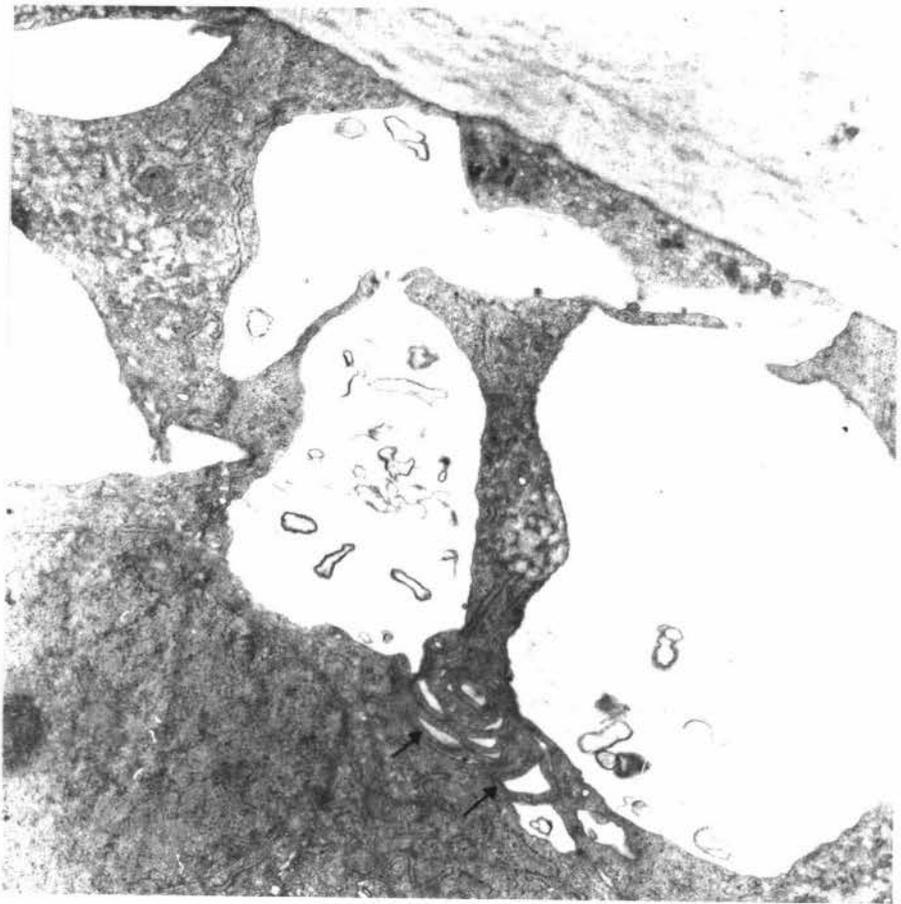


Figure 5.27: Electron dense deposits and small circular vacuoles, within a spindle shaped cell in the lens of a 10 month old lamb with mature cataracts. (TEM x 6,400)

Figure 5.28: Electron dense deposits surrounded by a limiting membrane within a spindle shaped cell, in the lens of a 10 month old sheep with mature cataracts. The shape of the enclosed deposits suggests that they may be accumulating within a membrane system, such as the endoplasmic reticulum or golgi apparatus. (TEM x 34,000)

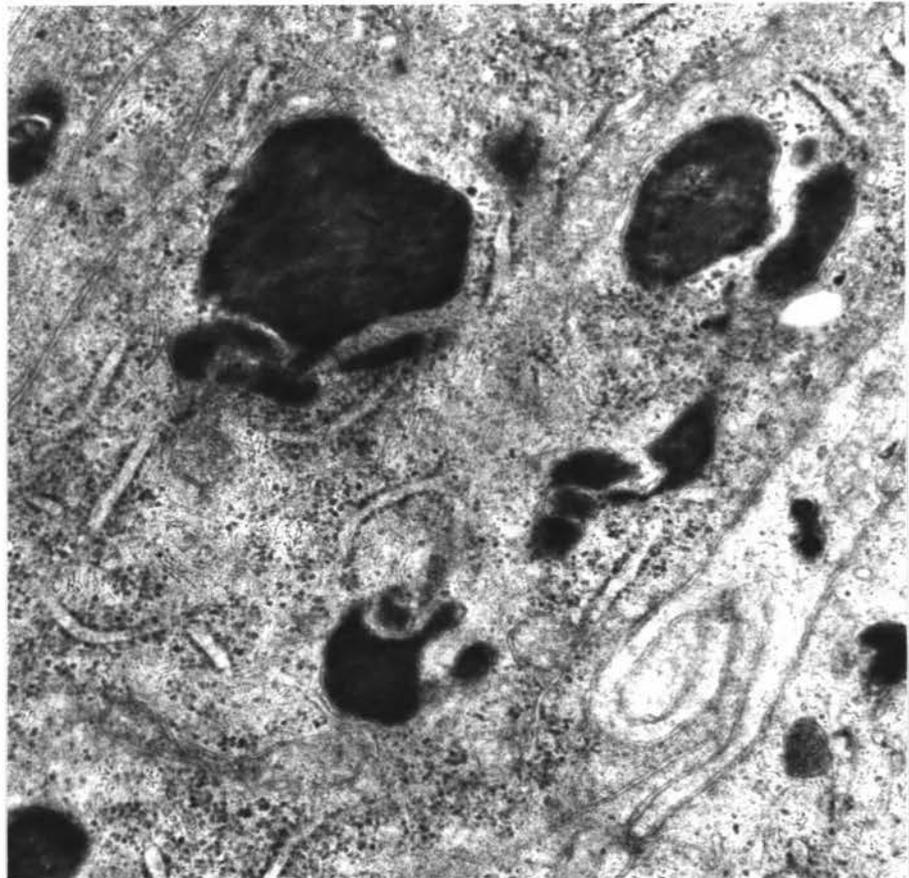
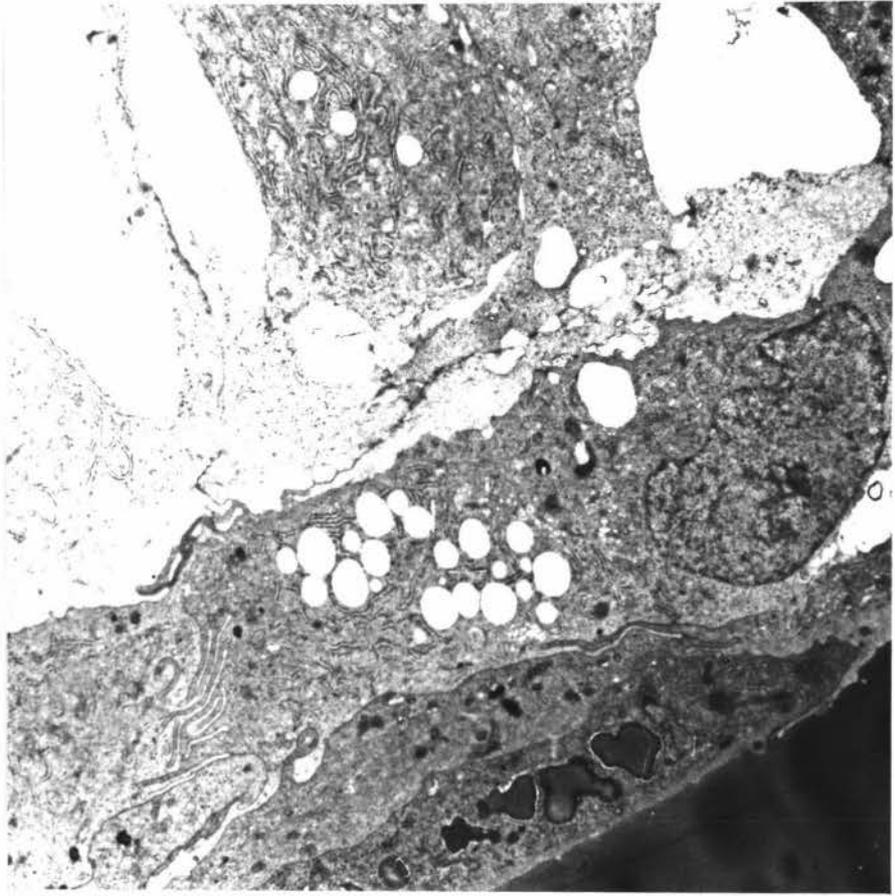


Figure 5.29 Unlined circular vacuoles, abundant rough endoplasmic reticulum and fibrillar material (arrow), within the cytoplasm of a spindle shaped cell, in the lens of a 10 month old lamb with mature cataracts.
(TEM x 34,000)

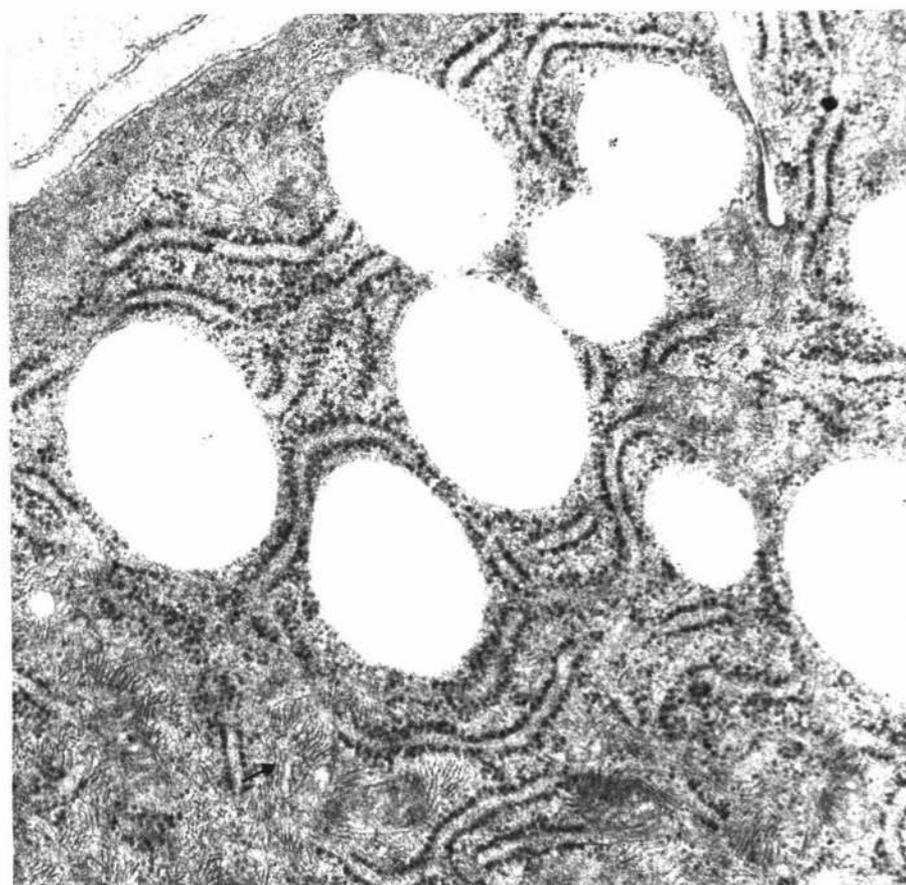


Figure 5.30: The anterior epithelium of the lens of a 5 month old normal lamb. The capsule is marked (c) and the cortical fibres (f).
(TEM x 6,400)

Figure 5.31: An anterior epithelial cell in the lens of a 5 month old normal lamb. The cytoplasm is homogeneous and contains numerous mitochondria. The lens capsule is marked (c).
(TEM x 10,500)

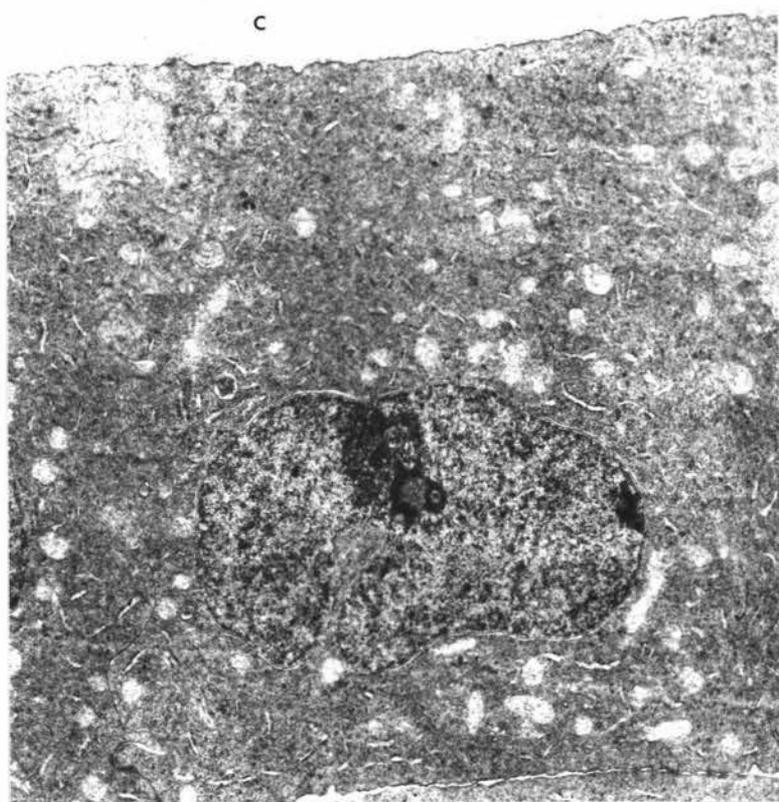
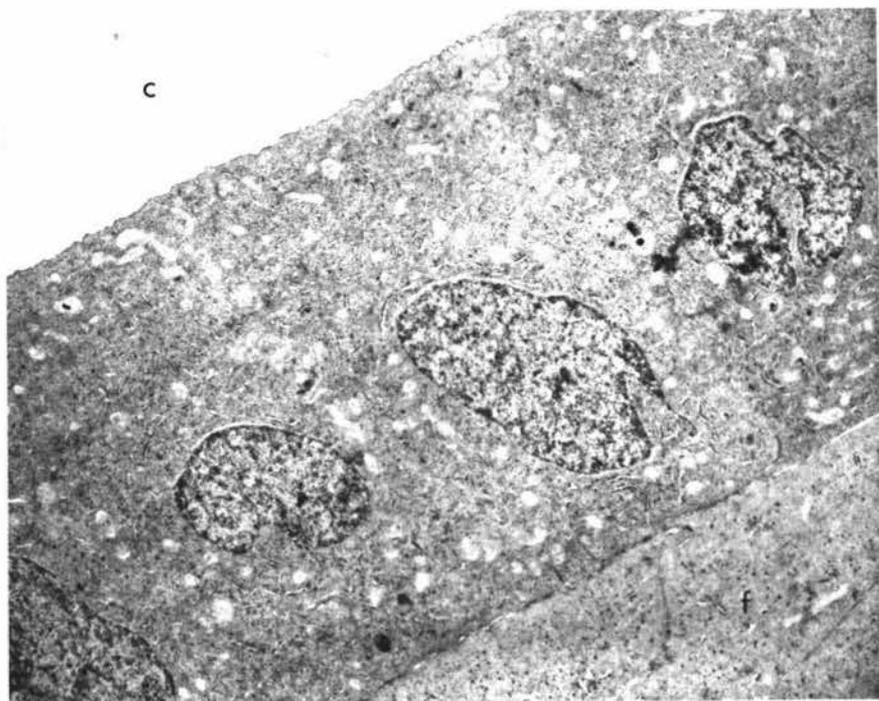


Figure 5.32: Anterior cortical fibres in the lens of a 2 month old normal lamb. Note the flattened hexagonal shape of the fibres and their alignment in rows.
(SEM x 1600)

Figure 5.33: Anterior cortical fibres in the lens of a 2 month old normal lamb. Note the precise interlocking of the hexagonal fibres and their tongue and groove processes.
(SEM x 4,000)

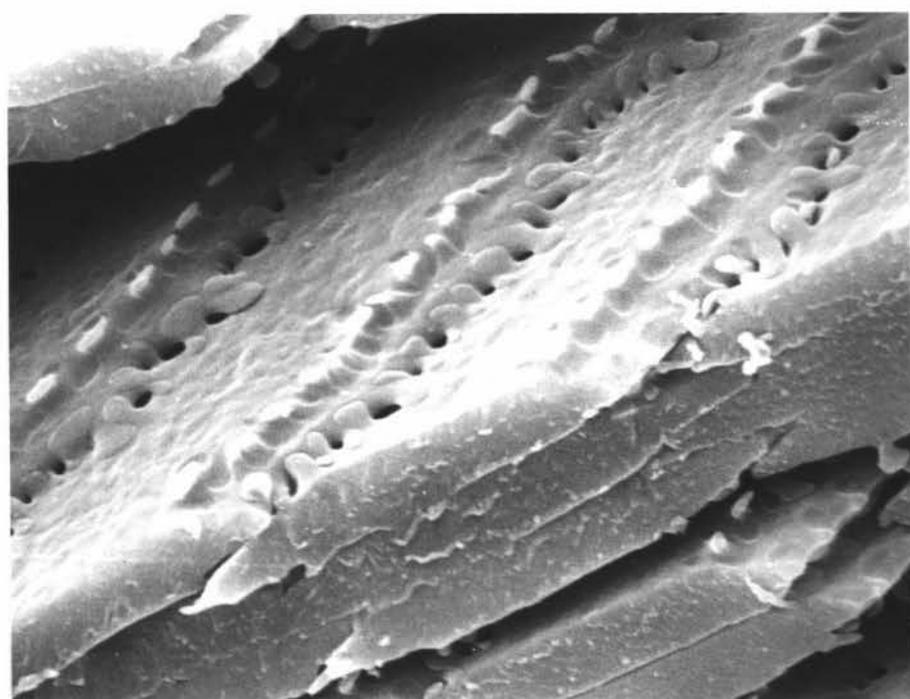
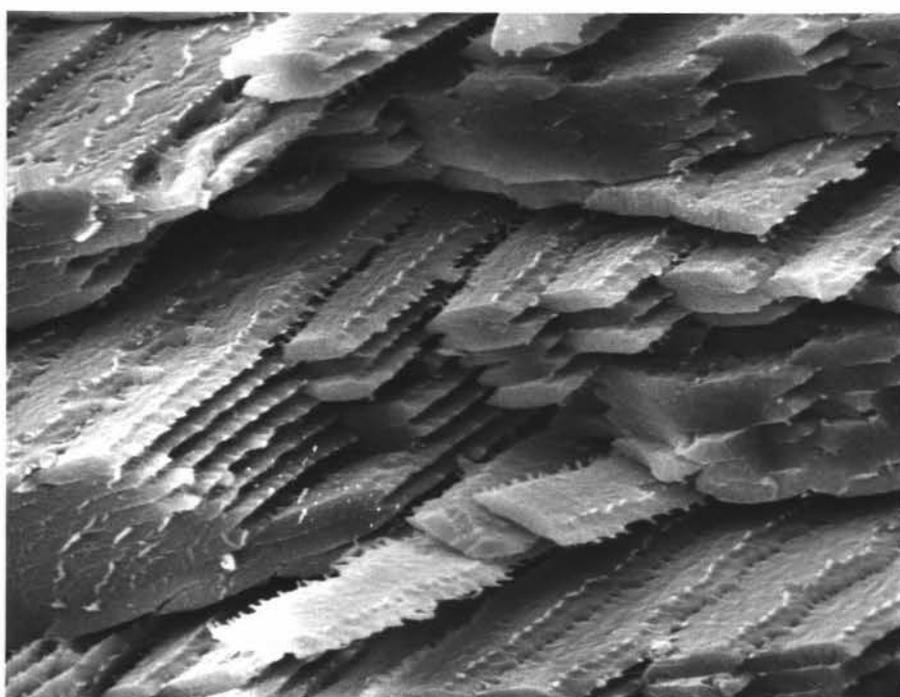


Figure 5.34: Anterior cortical fibres in the lens of a 3 month old lamb with early stage cataracts. Compared with normal fibres (Fig. 5.33), these fibres are swollen and almost rounded in cross section. (SEM x 1300)

Figure 5.35: Stunted, atrophic, interlocking processes of anterior cortical fibres, in the lens of a 3 month old lamb affected with early stage cataracts. (SEM x 8,000)

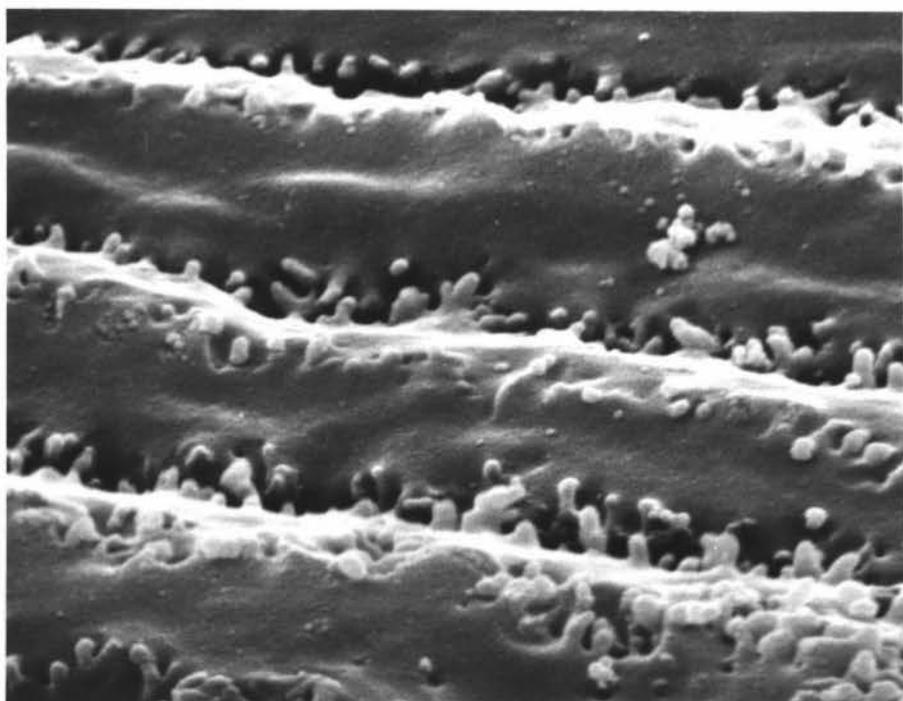
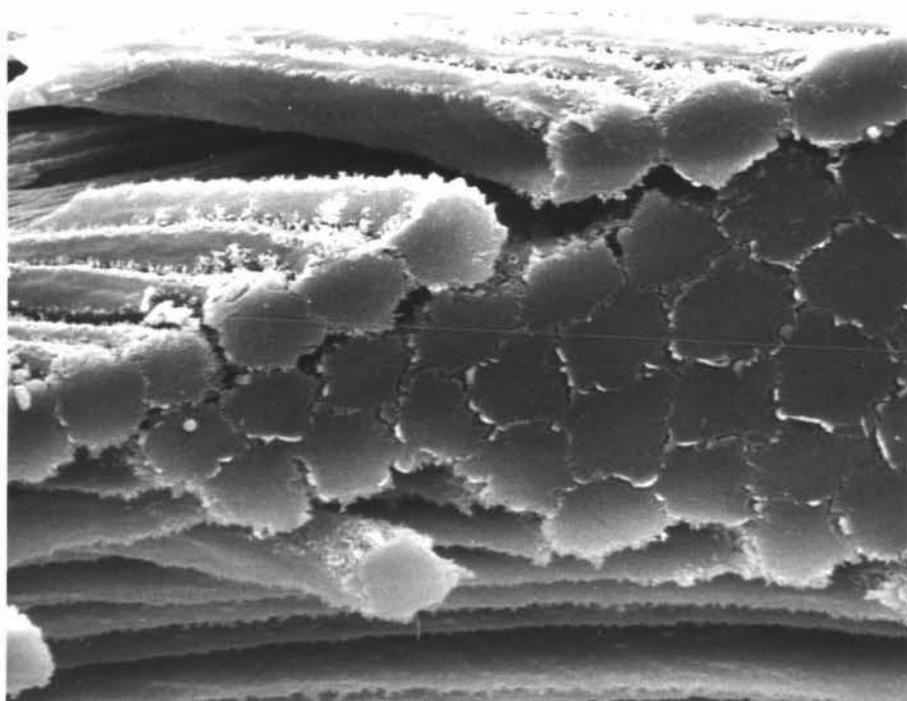


Figure 5.36: Mishapen convoluted anterior cortical fibres, in the lens of a 4 month old lamb affected with late stage cataracts.
(SEM x 1600)

Figure 5.37: Anterior cortical fibres with a nodular surface and atrophic interlocking processes, in the lens of a 4 month old lamb affected with late stage cataracts.
(SEM x 4000)

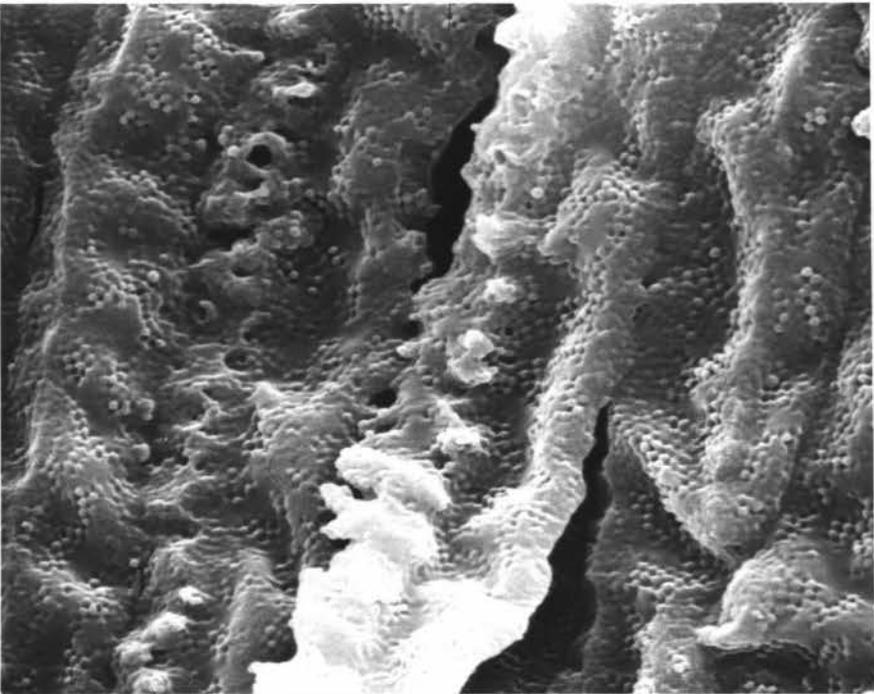
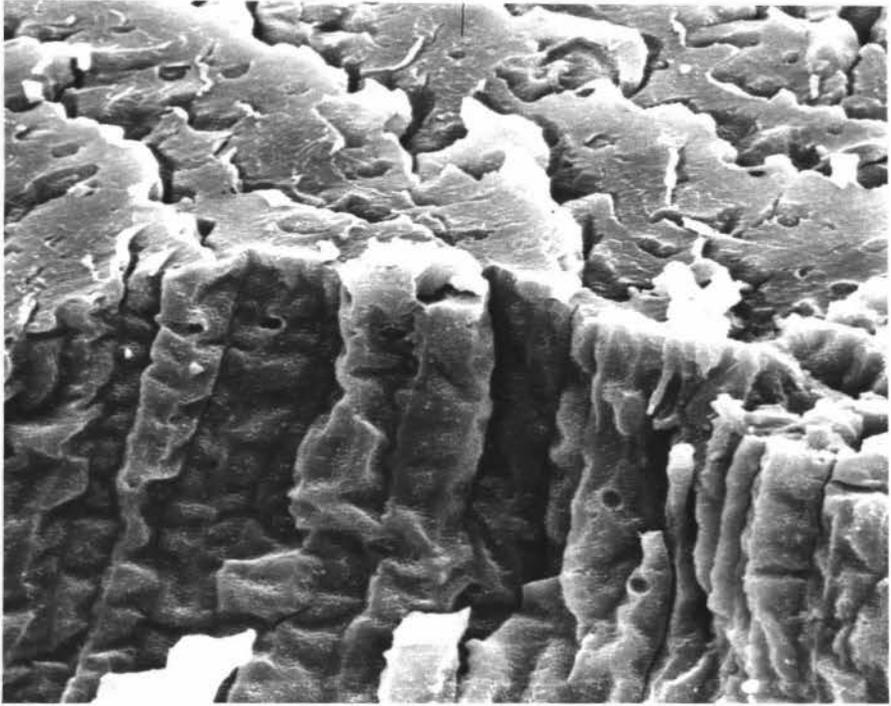
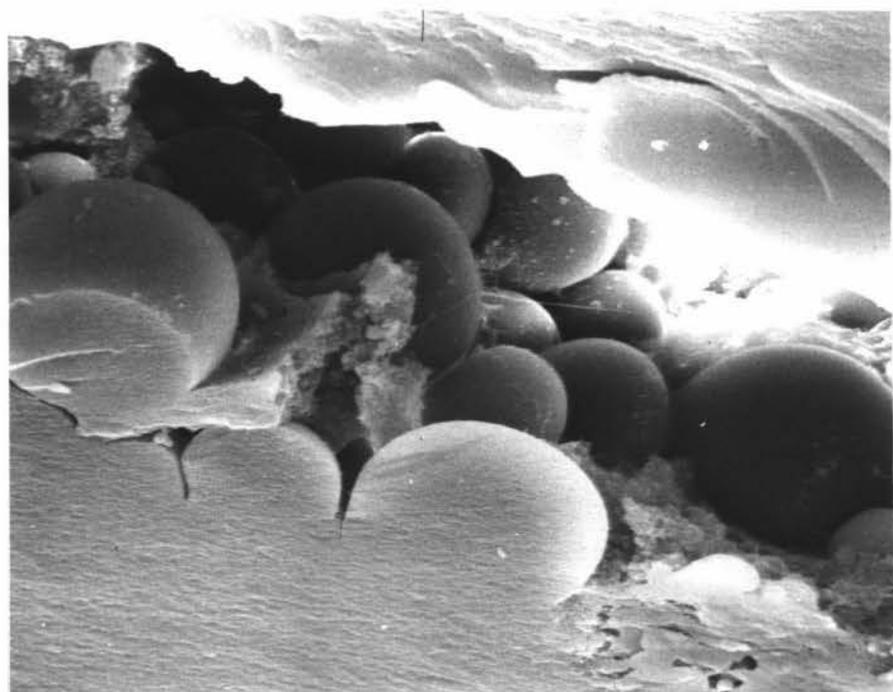
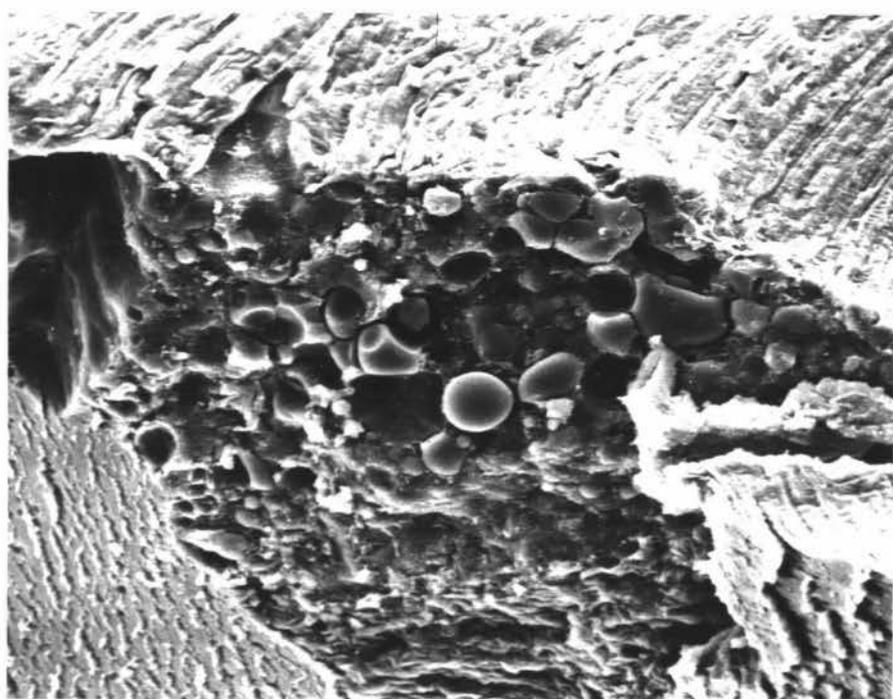


Figure 5.38: Degenerate globular shaped fibres, within rows of degenerate cortical fibres in the lens of a 4 month old lamb affected with late stage cataracts. (SEM x 400)

Figure 5.39: Degenerate globular fibres within the anterior cortex, in the lens of a 4 month old lamb affected with late stage cataracts. (SEM x 1200)



their tongue and groove processes are atrophic and irregular (Fig. 5.35). In lenses with late stage cataract, surviving anterior cortical fibres are severely convoluted and mishapen, their surface is nodular and their interlocking processes are extremely atrophied (Figs. 5.36 and 5.37). Rounded globular shaped fibres are often noted within rows of degenerating cortical fibres (Fig. 5.38) and at the edge of degenerate amorphous areas (Fig. 5.39);

VI. DISCUSSION

The processing of sheep lenses for histopathological and ultrastructural study was always difficult. The poor penetration of fixatives and embedding media, together with the compact lamellar structure of the lens, made trimming and section cutting extremely difficult. Torn and shattered sections and areas of artifactual separation within sections were therefore a common result, and accordingly many sections were rejected. Many of the photomicrographs included in this thesis contain these processing faults, but they represent the best of the material available and to have excluded them would have substantially reduced the understanding of the text. Photography of lens tissue is also disappointing since it is almost uniformly eosinophilic, the only nuclei present to provide basophilic contrast, being those in anterior epithelial cells and the nuclear bow.

Pathologically, ovine cataract is a progressive condition in which lenticular lesions begin as localised areas of fibre degeneration occurring initially within the anterior cortex. Posterior cortical fibres show essentially similar changes, though these are later developing. During the first year of life, fibres throughout the lens become progressively affected and by cataract maturity there is almost complete loss of cellular architecture within the lens. Such lenses are virtually bags of coagulated protein enclosed by the epithelium and capsule. The anterior epithelium shows a variety of pathological changes, the most consistent of which is cytoplasmic vacuolation. This occurs in the earliest stages of cataract development and becomes more generalised as the cortex is progressively affected. In the latter stages of

cataract development, epithelial proliferation and metaplasia occurs and epithelial cells migrate beneath the posterior capsule. The epithelium continues to produce new lens fibres at the equator throughout all stages of cataract development.

The microscopic changes occurring within affected lenses correlate well with the clinical findings described earlier in Chapter III, the two clinical forms of early stage cataract being readily distinguished histologically. In early stage cataracts with central opacity, the central areas only of the anterior cortex are affected, whilst in lenses with peripheral opacity, the lesions are very superficial within the anterior cortex and extend from the equator, centrally toward the anterior pole. This linear distribution causes the spoke-like patterns of opacity observed clinically.

The reaction of the lens fibres is in all cases quite stereotyped, lesions vary from mild fibre swelling and loss of eosinophilia, to more severe swelling, and finally lysis. Often swollen fibres round up into globular forms, before undergoing lysis. Areas of fibre lysis appear amorphous in histological sections, since the proteinaceous contents of the lysed fibres cannot escape from such sites.

The pathological changes occurring within the anterior epithelium consist of cytoplasmic vacuolation, proliferation, metaplasia and migration beneath the posterior capsule. The vacuolation of these cells is a consistent finding in cataractous lenses whenever the underlying cortex is degenerate. This suggests that substances may be released from degenerating cortical fibres which are toxic to the epithelium. Gorthy *et al.* (1980), described identical lesions in rats with an inherited cataract and claimed that the large irregular vacuoles were secondary lysosomes. They considered that the vacuolated cells were undergoing greatly increased autophagic activity as a degenerative process. Histochemical identification of acid phosphatase reaction product within vacuoles was not attempted in this present study, however the evidence presented by Gorthy

et al. (1980) should be regarded as somewhat speculative due to the inaccuracy of such techniques. From the ultrastructural studies presented in this thesis, it seems more likely that the vacuoles are dilated and damaged endoplasmic reticulum. There seems little doubt in either case, that vacuolation is a degenerative process. The nature and role of the small circular vacuoles within the epithelial and spindle shaped cells remains unknown. The greatly increased amount of rough endoplasmic reticulum within vacuolated epithelial cells indicates increased synthetic activity.

Proliferation of anterior epithelial cells is regularly observed in the latter stages of cataract development. Cells tend to proliferate as nests of large round foamy cells and occasionally these cells detach and lie free in the degenerate anterior cortex. Duke-Elder (1969), considers that epithelial proliferation is due to the liberation of abnormal chemical products by the pathological lens. Epithelial proliferation is described in many of the inherited cataracts reviewed in this thesis (Beasley, 1963; Zwaan and Williams, 1969; Davidorf and Eglitis, 1966; Gorthy and Abdelbaki, 1974; Smith *et al.*, 1969). A separate form of proliferation is that associated with wrinkling of the capsule, subsequent to lens shrinkage during advanced cataract development. This is a common response seen whenever the capsule becomes wrinkled, whether as a result of trauma or lens shrinkage (Duke-Elder, 1969).

Epithelial metaplasia from a single layer of cuboidal cells, to a multilayer of spindle shaped cells, is commonly noted in the later stages of ovine cataract. This is a common epithelial response in cataracts (Duke-Elder, 1969; Hogan and Zimmerman, 1962) and the fibroblast-like spindle shaped cells usually elaborate capsular material which encircles them locally. Gorthy *et al.* (1980) show abundant capsular material surrounding fibroblast-like cells in their rat cataract, whilst in this study, capsular material is not readily identified. The abundant rough endoplasmic reticulum, fibrillar material and electron dense deposits within the spindle shaped cells, does however indicate synthetic activity. A functional role for epithelial metaplasia is not defined, but it may have a strengthening effect.

The migration of epithelial cells beneath the posterior capsule is a common finding whenever posterior cortical fibres degenerate (Hogan and Zimmerman, 1962). Once beneath the posterior capsule, these cells exist either as a monolayer of flattened cells or as aggregations of large swollen vesicular cells, referred to as bladder or 'Wedl' cells. These cells represent abortive attempts to form lens fibres, such cells are very prominent in later stage ovine cataracts.

In comparison with other body tissues the lens is anatomically very simple. The uniformity of cell type and the avascular nature of the lens, permits only a limited range of pathological changes to occur, regardless of the aetiology of the cataract. It is not surprising therefore, that the majority of the pathological changes described in this inherited ovine cataract are similar to those reported in many cataracts of man and other animals.

CHAPTER VI

WATER AND ELECTROLYTE CHANGES IN CATARACTOUS LENSES

I INTRODUCTION

Transparency of the normal lens is considered to be due to the tightly packed nature of the lens fibres, the arrangement of lens proteins and the regulation of intralenticular ion and water balance (Paterson, 1979). The precise mechanism underlying most forms of naturally occurring cataract is not understood, but the lens proteins, water and electrolyte shifts and intralenticular active transport systems have been most studied. In most naturally occurring forms of cataract, lens potassium is reduced and sodium, water and calcium content are increased. In this study these substances were measured in a small number of cataractous ovine lenses.

II MATERIALS AND METHODS

Animals

Cataractous New Zealand Romney sheep from the experimental flock were used.

Preparation of lenses

Sheep were killed and their lenses dissected from enucleated eyes as rapidly as possible. Care was taken to ensure that the capsule was not damaged during lens dissection and that any adherent vitreous was removed from the posterior lens surface. Lenses were placed into preweighed plastic vials and reweighed prior to rapid freezing at -70°C . They were transported by air in dry ice to the U.S.A. and analysed by Dr. C.A. Paterson of the University of Colorado Medical School.

III RESULTS

The water content and the cation concentrations of affected lenses are shown in Table 6.I.

TABLE 6.I

Percentage water and cation concentrations in cataractous lenses at different stages of development, from six sheep.

Stage of lens opacity	Sheep	% water*	Concentration of cations**			
			K ⁺	Na ⁺	Ca ⁺²	Mg ⁺²
Early stage	1	66.78	114.4	17.9	0.43	5.21
	2	68.06	113.9	18.9	0.36	5.67
Early - mid stage	3	65.67	131.28	23.6	0.53	4.58
Mature	4	79.58	17.47	135.9	5.58	2.38
	5	82.62	10.72	116.0	5.56	1.99
	6	82.30	8.34	147.5	5.60	1.75

* by weight

** mEquiv/kg lens water

IV DISCUSSION

Though the number of lenses sampled in this preliminary study is small, it is apparent that sodium is concentrated and potassium is lost during cataractogenesis. Similar shifts in sodium and potassium ions have been found to occur in a variety of human and animal cataracts (Iwata and Kinoshita, 1971; Iwata, 1980; Kador *et al.*, 1980; Rossa *et al.*, 1980). In the majority of cases such changes are probably the result of cataract formation rather than the cause (Paterson, 1979). Iwata and Kinoshita (1971), found that an increase in lens water and sodium, and a decrease in potassium, coincided with the onset of cataract in the Nakano mouse. Kinoshita (1974),

demonstrated the presence of an inhibitor of Na-K ATPase in such affected lenses. It was not determined whether this inhibitor was responsible for initiating the cataractous process and it remains possible that a more fundamental lesion is present in this cataract. In the Philly mouse (Kador *et al.*, 1980) and in a strain of mice studied by Iwata (1980), electrolyte shifts similar to those occurring in the Nakano mouse were recorded, but no inhibitor of Na-K ATPase was detected.

The mechanism of lens electrolyte homeostasis, has been shown by Kinsey and Reddy (1965) to be due to a balance between the activity of a metabolically dependent transport system in the anterior epithelium, which extrudes sodium and takes up potassium, and the passive movement of these ions by diffusion, mainly through the posterior capsule. Any substantial damage to the anterior epithelium could thus be expected to seriously upset electrolyte balance within the lens.

In this study, pathological changes have been consistently observed within the anterior epithelium of cataractous lenses (page 36). In early stage cataracts, cytoplasmic vacuolation of epithelial cells is confined to small localised areas of the epithelium but epithelial cell involvement becomes more generalised with increasing degeneration of the lens cortex. This could suggest that the epithelial cell lesions are primary and aetiologically significant in this cataract. Several observations tend to discount this hypothesis however. In some early stage cataracts quite large areas of the deep cortex are affected without any obvious epithelial change (page 31) and epithelial lesions are only seen in those areas which are in contact with the products of cortical fibre breakdown. This latter observation suggests that the products of cortical fibre breakdown may be toxic to epithelial cells. It may be that when fibre breakdown occurs deep in the cortex, the overlying intact fibres may prevent toxic products diffusing out and reaching the epithelium, or that the deeper fibres being less active metabolically do not contain substances potentially damaging to the epithelium. Although the epithelial lesions are most likely secondary the fact is that as more epithelial cells become affected

so will the cation and water balance of the lens become even more severely disturbed, since the cation pump is located in the anterior epithelium.

CHAPTER VII

SUMMARY AND CONCLUSIONS

The findings presented in this thesis comprise the first report and description of an inherited cataract in sheep. Controlled breeding studies showed that cataracts were inherited as a simple autosomal dominant trait.

The majority of lambs had clear lenses at birth and bilateral cataracts were first apparent at one to two months of age. Cataracts developed progressively throughout the first year of life, usually becoming mature by 10 to 12 months of age. Two lambs had bilateral mature cataracts at birth, and since both parents of each of these lambs were themselves affected, it was concluded that these lambs were homozygous for the gene causing cataract.

Early forms of cataract appeared clinically as either a single irregular area of opacity in the central anterior cortex, or as several spots or spokes extending from the equator towards the anterior pole. These morphological features correlated with the distribution of early microscopic changes within the lens. With cataract progression, all lenses became similar in appearance and showed increasingly diffuse opacity. Lenses with mature cataracts were densely opaque and remained so for at least five years in animals kept that long. However in the two lambs affected congenitally, lens absorption occurred within six months of age.

Microscopically, lens fibres showed a stereotyped pattern of degenerative swelling and lysis. The anterior epithelial cells became vacuolated, apparently in response to adjacent cortical fibre degeneration. Ultra-structurally the vacuoles were provisionally interpreted as being dilated and damaged endoplasmic reticulum. In the latter stages of cataract development, anterior epithelial cell hyperplasia and metaplasia to a spindle shaped fibroblast-like cell occurred. These latter cells produced

capsular-like material. The migration of epithelial cells beneath the posterior capsule was a frequent finding in advanced cataracts. These cells either formed a single layer of flattened cells, or aggregations of swollen 'bladder' cells beneath the posterior capsule.

Biochemically, cataractous lenses showed an increase in sodium ion concentration and water content and a loss of potassium.

The pathological and biochemical changes occurring in sheep lenses affected with inherited cataract, are similar to those recorded in a variety of cataracts of man and animals of different causes. These changes are not aetiologically significant, but merely reflect the limited range of stereotyped responses which are possible in the lens.

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