LIPID KERATOPATHY IN THE DOG

VOLUME I

SHEILA CRISPIN,

M.A., Vet.M.B., B.Sc., M.R.C.V.S., D.V.A.

MAY, 1984

DEGREE OF DOCTOR OF PHILOSOPHY,

UNIVERSITY OF EDINBURGH.

I declare that this Thesis has been composed by myself and that the work is my own.

A proportion of the material relevant to this Thesis has been published previously and appears in Appendix III.

Je l'ai vu, dis-je, vu, de mes propres yeux vu,
Ce qu'on appelle vu.

Le Tartuffe (1664) Act V, Scene 3.

Molière (Jean Baptiste Poquelin).

To my parents for their unfailing loyalty and support and in memory of my mother, Meg and Bassie.

ABSTRACT

Naturally occurring lipid keratopathy in the dog has been investigated using a variety of examination techniques. The same procedures have also been followed for a group of normal dogs matched to the clinical cases by age, sex and breed and for a third group of unmatched, normal, animals.

Investigations have included general clinical and ophthalmoscopic examination and detailed examination of the anterior segment, including tonometry, temperature measurement and fluorescein angiography. Laboratory examination has largely concentrated on serum lipid and lipoprotein analysis.

A number of microscopic methods have been applied to normal and diseased corneas. A comprehensive selection of histochemical techniques for identification of lipids have been used in conjunction with light, polarising, interference contrast and phase contrast microscopy. The physical properties of lipids have been explored using squash or imprint preparations, a heated microscope stage and polarised light (with a $\frac{1}{4}\lambda$ mica plate and a first order red gypsum accessory plate). A variety of other non-lipid methods have also been used.

Ultrastructural studies complemented those of light microscopy, employing both scanning electron microscopy and transmission electron microscopy and utilising a limited number of ultrahistochemical staining techniques with the latter.

The results of this study indicate that lipid keratopathy may be associated with a variety of conditions involving the anterior segment and that abnormalities of the serum lipids and lipoproteins can often be demonstrated in affected animals. These findings are of significance for diseases of lipid metabolism in other species.

ACKNOWLEDGEMENTS

This work was mainly carried out in the Small Animal Practice Teaching Unit and the Department of Anatomy at the Royal (Dick) School of Veterinary Studies of the University of Edinburgh. Routine laboratory examinations were carried out by the Department of Veterinary Medicine at Edinburgh University and detailed lipid and lipoprotein analyses were performed in the Department of Clinical Biochemistry at Addenbrooke's Hospital, Cambridge and the Department of Biochemistry at Edinburgh University. Periods were also spent working in the Department of Pathology at Guy's Hospital Medical School, London; the Department of Neuropathology in Southampton General Hospital and the Animal Health Trust Small Animals Centre at Kennett, near Newmarket.

A proportion of the clinical work was possible because of generous financial support from the Clinical Studies Trust Fund of the British Small Animal Veterinary Association, to whom I am grateful.

My thanks to the many owners and their dogs, who made this study possible.

My especial thanks go to Professor James Spreull for his enthusiastic support and encouragement throughout this study and also to many of my colleagues in the veterinary profession for their provision of both facilities and material. I should particularly like to thank Dr. Keith Barnett, Professor Donald Lawson, Dr. Peter Darke, Mr. Derek Lewis and Mr. Steve Carrington.

To Dr. Keith Barnett I owe a special debt, for engendering my enthusiasm for ophthalmology generally, and for this subject in particular.

I am grateful to many friends in medical fields for the help and advice they have given. Especially to the late Professor George Boyd for giving of his time and expertise so generously and for being a source of continuing encouragement. Professor Colin Adams and Professor Roy Weller were kind enough to allow me to work in their laboratories at Guy's Hospital and Southampton General Hospital respectively. Dr. Olga Bayliss High provided stimulating instruction on lipid histochemistry. Dr. John Lines of Addenbrooke's Hospital, Cambridge and the William Harvey Hospital, Kent, was kind enough to permit much of the lipid and lipoprotein analysis to be performed in his laboratories.

I have much pleasure in thanking various members of the Anatomy Department within the Faculty of Veterinary Medicine. Professor Keith Dyce for provision of facilities and valuable help with translation. Dr. Bill Stokoe for checking various aspects of the thesis. All the technical staff for their crucial and cheerful assistance; notably Gordon Goodall, Derek Denham, Derek Penman, Colin MacFarlane and Mark Patrizzio.

Kenny Thomson and Fiona Manson have given excellent photographic support.

I am delighted to acknowledge the help and encouragement given by the librarians; Mrs. Marion McIvor, Miss Eileen Panton and Mrs. Wilma Anderson.

I am most grateful to Valerie Seagrott of the Statistics Unit at the National Institute for Biological Standards and Controls for her assistance with the statistics.

I am indebted to Dr. Kate Cullen and Mrs. Marilyn Ritchie for the hours they have spent in typing the thesis.

LIPID KERATOPATHY IN THE DOG

TABLE OF CONTENTS PAGE NUMBER

LIST OF FIGURES

5.7/4 Cholesterol versus Triglyceride.

5.7/5 Cholesterol versus Phospholipid.

5.7/6 Triglyceride versus Phospholipid.

5.7/7 Densitometer Tracings of Lipoprotein Electrophoresis Patterns from Clinical Cases.

5.8/1 - 5.8/76 Light Microscopy - Clinical Cases.

5.9/1 - 5.9/67 Fine Structure - Clinical Cases.

6.9/1 Some Local and Systemic Factors which may be Inter-related in Canine Lipid Keratopathy.

LIST OF TABLES

1.0 INTRODUCTION

This study was initiated because so little information was available concerning the pathogenesis of naturally occurring lipid keratopathy and its possible relationship to systemic disturbances of lipid metabolism in any of the species in which it has been recorded.

Whilst lipid keratopathy is a relatively uncommon condition in man, it is recognised that it can occur in association with a wide variety of ocular abnormalities and a wealth of clinical observations comprise the major part of the medical literature; perhaps the very abundance of descriptive terms indicates a condition of some complexity.

The lesions of lipid keratopathy have been likened to atherosclerotic plaques. It is surprising that there has been little attempt to characterise the serum lipids and lipoproteins in most documented cases of human lipid keratopathy in view of this observation and the information available from studies of experimental hyperlipoproteinaemia with ocular involvement.

Neither the dog nor the rabbit develops arcus lipoides corneae as a normal feature of ageing; there is thus no counterpart of human arcus senilis. The fatty infiltration of the cornea which occurs in hyperlipoproteinaemic rabbits resembles human lipid keratopathy more closely than human arcus lipoides cornea.

There do not appear to be any detailed studies of naturally occurring lipid keratopathy in the dog, an animal which is known to be extremely resistant to atherosclerosis, although perusal of the veterinary literature indicates that a variety of lipid-containing corneal opacities (corneal lipidoses) have been described. In order

to avoid a confusing plethora of disparate descriptive terms the types of corneal lipidoses in which cholesterol and its esters are prominent have been classified, on the basis of the author's observations, as corneal dystrophies, arcus lipoides corneae and lipid keratopathies as set out below and illustrated in Figs. 1.0/1-1.0/6.

The lipid dystrophies which have been described in the dog have been characterised by an entirely stromal location of lipid which appears to derive exclusively from fibroblasts, so that defective fibroblast metabolism may be important in pathogenesis. The corneal opacities develop bilaterally in an apparently normal cornea and usually have a central, or paracentral, location in the anterior stroma. Occasionally, there is more diffuse corneal involvement, but in all cases there is complete absence of inflammation. Once these opacities have formed they usually remain static but they may also regress and sometimes disappear completely. Despite a strong familial tendency and evidence of inheritance in certain breeds, such as the Rough Collie and Afghan Hound, the actual mode of inheritance has not yet been demonstrated.

Investigation of serum lipids and lipoproteins in affected animals has revealed no consistent abnormalities. However, as a proportion of lipid dystrophies are first noticed in bitches during, or immediately following, oestrus, or during lactation after pregnancy, it would seem logical to consider the effects of hormones on lipoprotein patterns in these cases, particularly as alterations of lipids and lipoproteins may be both subtle and transient.

Arcus lipoides corneae is a bilateral, peripheral, lesion which, like the lipid dystrophies, develops in apparently normal

cornea. It involves the anterior portion of Descemet's membrane and the corneal stroma. The corneal lipid of early cases is probably largely derived from the perilimbal emissary vessels at all levels, although the anterior part of Descemet's membrane and the immediate sub-epithelial zone of the anterior epithelium are first to be affected. The appearance of the peripheral lesion is slightly variable but the opacity is normally most dense in the vicinity of the nictitating membrane and, to a lesser extent, near the upper and lower lids. There is frequently no lucid interval between the limbus and the opacity. Vascularisation, albeit of a rather fine and discrete nature, is a feature of established lesions and, as in the rabbit experimental arcus lesion, there is cellular involvement and some resemblance to lipid keratopathy.

In dogs where arcus lipoides corneae has been reported there is an increase of high density, (HDL), low density (LDL) and very low density (VLDL) lipoprotein, so that serum cholesterol, triglyceride and phospholipid are all raised. Hypothyroidism has been the commonest cause of the hyperlipoproteinaemia and the Alsatian (German Shepherd Dog) and Rough Collie have been the most commonly affected breeds. Treatment of hypothyroidism has been accompanied by regression of the opacity in a proportion of cases.

Lipid keratopathy, the third major category of lipid-containing corneal lesions, forms the subject of this thesis. It differs most obviously from the other two categories in being of very variable appearance and either unilateral or bilateral. Canine lipid keratopathy is always accompanied by corneal neovascularisation which may precede, or follow, lipid deposition. The way in which lipid keratopathy may be associated with ocular

disease, particularly of the anterior segment, forms an important part of this study. In a small proportion of cases arcus lipoides corneae occurred in conjunction with lipid keratopathy, a clinical feature which prompted some of the investigations into serum lipids and lipoproteins.

This study of naturally occurring lipid keratopathy is based on case material collected over a ten year period and has been designed so that clinical cases are compared with closely matched normal dogs which act as controls. It was felt important to adopt this protocol as preliminary investigations had suggested that lipid keratopathy was more prevalent in dogs of certain breed and age. Additional normal dogs form a third group of varied age, breed and sex, so as to make comparisons of normal parameters more meaningful. All three groups have been subjected to the same clinical, ophthalmoscopic and laboratory examinations.

A wide variety of microscopic techniques have been applied to corneal material from normal and affected animals, as it was of importance to establish the distribution and identity of cells and lipids in the corneas of normal animals before attempting to interpret the findings in diseased corneas.

The combination of detailed examination of the anterior segment, serum lipid and lipoprotein estimation and detailed microscopy has provided much information about canine lipid keratopathy, which may be of relevance in other species. Corneal response apparently reflects underlying systemic lipid and lipoprotein abnormalities to a considerable extent and this finding has implications for a wide variety of diseases of lipid metabolism.

2.0 LITERATURE REVIEW

2.1 DOG

2.1.1 Introduction Anterior segment disease is common in the dog, but recognisable quantities of lipid in association with anterior segment disease are rare.

The pathogenesis of naturally occurring canine lipid keratopathy has not been investigated previously, neither has the possibility of any relationship between various types of hyperlipoproteinaemia and lipid keratopathy. There are, however, a number of publications concerned with the normal canine cornea and lipid-containing corneal opacities (corneal lipidoses) have been described under a variety of names.

2.1.2 Normal Adult Cornea Slit lamp biomicroscopy of the canine anterior segment was first reported by Troncoso and Castroviejo (1936), later by Martin (1969) and Uberreiter (1970).

Aspects of gross and subgross anatomy have been described by several workers. Bayer (1914) and Donovan, Carpenter, Schepens & Tolentino (1974) cited measurements of radius, width, height and thickness; the histological appearance has been reported by Prince, Diesem, Eglitis & Ruskell (1960), Prince (1964), Spreull (1966), Anderson (1970), Ehlers (1970), Donovan et al., (1974), Slatter (1980), Martin & Anderson (1982) and Crispin & Barnett (1983). Few studies of scanning electron microscopy of the canine cornea have been published. A technique for demonstrating ocular microcirculation was described by Van Buskirk (1979), and Martin & Anderson (1982) illustrated some aspects of anterior segment anatomy with scanning electron micrographs.

Transmission electron microscopy of the canine cornea has been

reported by Shively & Epling (1970a, 1970b), Martin & Anderson (1982), Morrin, Waring, & Spangler (1982) & Crispin & Barnett (1983).

2.1.3 Ageing Changes in the Normal Eye Age changes in the canine eye were described in 1974 by Jensen (cited by Slatter, 1975) who studied 30 eyes from dogs between five and six years of age and 30 from dogs older than nine years. He noted a lack of spontaneous lipid-related corneal and scleral degeneration. Scleral calcification was noted in 11.4% of all the eyes studied, and hyalinisation of retinal blood vessels in 1.8% of the eyes from dogs older than nine years. Slatter (1975) investigated three groups of experimental American Foxhounds aged one, two and five years respectively and noted that there was no lipid accumulation in the eyes of the one and two year old dogs, but that dogs of five years contained oil red O positive material in the inner third of the sclera. The lipid was associated with scleral collagen rather than scleral fibroblasts and appeared as a fine haze which was beyond the resolution of the light microscope. It was mainly amorphous in appearance, with small quantities in particulate or droplet form. No birefringent or crystalline material was visible when polarised light was used. The lipid was mainly situated in the sclera adjacent to the pars plana and the extreme peripheral choroid; the region of the ciliary body seemed to be a preferential site for lipid deposition. No oil red O positive material was detected in either the cornea or the limbus.

It is pertinent to note that any changes which are apparently associated with ageing should be related to the breed, for the life expectancy of breeds shows considerable variation.

2.1.4 Canine Corneal Lipidosis The term corneal lipidosis signifies the presence of lipid within the cornea, be it a consequence of a corneal dystrophy, arcus lipoides corneae or lipid keratopathy. The range of descriptive terms applied by the various authors is cited and, when possible, the lesions are classified according to the categories outlined in Section 1.0.

The first publication which may be concerned with corneal lipidosis in the dog was in 1910; Schock reported three unusual cases of corneal opacity encountered over a 15 year period. He provided details of two of these cases: a two year old Pointer and a two and a half year old Pinscher. No information of the third case, a 12 year old dog, was given. The two young dogs had bilateral, similar, discoidal, grey-white opacities situated centrally to paracentrally in the corneas. There was no corneal vascularisation and the lesions had developed without inflammation or irritation. The "turbidity" was situated under the corneal epithelium and in the anterior stroma. No histology or serum lipid analyses were performed.

Schock assumed that the opacities represented keratitis disciformis, a description originally applied by Fuchs (1901) to chronic, localised, inflammatory, parenchymatous lesions of infectious origin in man. The appearance would, however, seem more consistent with central/paracentral lipid dystrophy, a topographical description suggested in a review article by Crispin (1982).

A paper by Veenendaal in 1928 described a similar bilateral, but asymmetrical, opacity in a one and a half year old Dutch Shepherd dog. In the right eye there was a small paracentral fleck, whereas in the left there was a larger, central, grey disc. Both lesions apparently consisted of fine scintillating crystals located

sub-epithelially in the anterior stroma and there was no accompanying inflammation or vascularisation. The opacity in the right eye cleared completely over a two year period, whereas the opacity in the left persisted for rather longer. The history and appearance indicate a central/paracentral lipid dystrophy, although there was no attempt to establish whether the condition was inherited.

Veenendaal considered that the possible composition of the scintillating crystals included urates and cholesterol and he compared the lesion with Went & Wibaut's (1924) description of Schnyder's central crystalline dystrophy. He disagreed with Schock's diagnosis of keratitis disciformis for opacities of this type.

In 1930 Dreyfuss reported a case of symmetrical central corneal "fatty degeneration" in a nine and a half year old Alsatian. His drawings of the lesions show a paracentral discoidal lesion in the right eye and two similarly shaped, but smaller, lesions in the left; one was central, the other in the dorso-nasal quadrant. The lesions were said to be deeply placed in the corneal stroma and had developed over several months without inflammatory accompaniment. The centre of the lesion was described as chalky-white with a less dense yellowish-gold periphery; scintillating crystals were noted within the opacity. Apart from mild senile change in the lens the eyes appeared normal in other respects.

The eyes were obtained for histological examination; they were fixed in formalin and the left eye was embedded in gelatine prior to sectioning and staining with haematoxylin and Sudan III or Nile blue sulphate. The central cornea in the region of the opacity was

thinner than normal, although there was hypertrophy and hyperplasia of the overlying epithelium. Finely granular lipid (brown-red with Sudan III) was located immediately beneath the basal epithelium producing a stippled effect at the border of epithelial cells and stroma. In the caudal two thirds of the stroma, particularly, the lamellae were loaded with fine fat droplets. In addition to granules and small droplets between lamellae there were fine needle-like crystals and, in the immediate vicinity of crystals, there were large cells containing fat droplets, identified by the author as histiocytes. There was said to be no increase in the number of fixed corneal cells, no leukocyte or round cell infiltration and absence of vascularisation. No abnormalities were noted at the corneal periphery, in Descemet's membrane or the endothelium, although a very fine line of fat was observed at the stromal border of Descemet's membrane.

Examination of the remainder of the eye revealed that the ciliary body and uveal tract were heavily infiltrated with fat - "as is to be expected in an animal of this age". With Nile blue sulphate some of the extracellular lipid droplets stained dark blue whereas the crystals were stained a light violet colour. The crystals were strongly birefringent with polarised light, lost this property when warmed to 40°C, but regained it on cooling; they were readily soluble in ether and xylol.

The right eye was embedded in celloidon and examined after sectioning and staining with Weigert's haematoxylin and Van Gieson. The morphological changes were substantially the same as those of the left eye but more obvious. Leukocytes were observed between the lamellae and areas of stromal necrosis were noted. No drawings of

the histology of the right eye were provided.

Dreyfuss came to the conclusion that this was a primary degeneration of the cornea of endogenous origin, a form of cholesterol steatosis ("cholesterinsteatose"). Unfortunately, he made no reference to the general health of the dog and stated that no serum cholesterol estimations were performed as no material from dogs of the same age, sex and breed was available for comparison. Despite the lack of laboratory investigation, he went on to postulate that the dog had such a high level of lipids in the blood plasma that the "cholesterolophilic" cornea became saturated with lipids, which led to a degenerative softening, akin to atheromatosis, with subsequent necrosis. The presence of free cholesterol was the result of cholesterol ester disintegration.

Dreyfuss also made the surprising statement that arcus senilis is known to occur in dogs, but provided no references or examples. This statement finds no corroboration in the standard veterinary texts of the time, (for example Jacob, 1920), and was specifically contradicted by Veenendaal in a paper published in 1937 as a sequel to his paper of 1928. The lesions described by Dreyfuss are of particular interest because the plaque-like appearance and some of the histological findings indicate a type of lipid keratopathy, although the apparent lack of corneal vascularisation is unusual. The staining reactions and physical properties of the crystals suggest esterified cholesterol. The presence of large quantities of lipid in the ciliary body and uveal tract are more consistent with hyperlipoproteinaemia than with the author's interpretation of normal ageing processes, and it is relevant to note that the affected animal was an Alsatian, a breed which figures

prominently in studies of corneal lipidosis and concurrent serum lipoprotein abnormalities.

Veenendaal (1937) acknowledged that "dystrophia corneae adiposa" was probably not such a rare condition in the dog as previous communications had led him to suggest in 1928. The term dystrophia corneae adiposa was first applied by Kamocki to human lesions in 1893, and was felt to be the appropriate description for canine lesions of similar appearance by Veenendaal.

Lipid deposition in one or both eyes of 24 dogs was observed by Veenendaal over a period of eight years. Several breeds were affected but it is interesting that his series included five Alsatians. The ages of affected dogs ranged from six months to 12 years, but it was not always easy to be sure how long the opacities had been present from the description provided by the author.

The corneal changes were always superficial and sub-epithelial, and were composed of fine glistening white, or mother-of-pearl, spicules. The lesions were not homogeneous, the corneal surface was smooth and shiny, and there were no signs of ocular irritation, inflammation or vascularisation. The appearance was thus inconsistent with lipid keratopathy and was more typical of central/paracentral lipid dystrophy.

In some cases it appeared that, after a considerable time lapse, spontaneous resolution took place; he cites the case of a one year old Cocker Spaniel with unilateral conjunctivitis and a corneal opacity where the opacity cleared spontaneously in a five month period.

Veenendaal was able to undertake microscopic examination of a

unilateral opacity in a nine year old Dutch Shepherd dog. In the middle of the left cornea an opacity of several millimetres diameter was present; it was composed of glistening subepithelial crystals and no irritation or inflammation was present. At the limbus there was a slightly turbid zone but no crystals. No other ocular abnormality was noted.

Microscopic examination demonstrated Sudan III positive isotropic droplets to be present at the limbus, in the turbid regions previously identified with the slit lamp biomicroscope. Investigation of the cholesterol esters was undertaken and it was concluded that birefringence was lost on warming and returned on cooling. (The form of these cholesterol esters is not specified.)

A positive reaction for cholesterol was obtained with Lugol's solution in sulphuric acid and crystals were soluble in xylol and ether. The crystals were in the form of fine needles and not rhombic plates.

On the basis of these results Veenendaal agreed with the previous publication by Dreyfuss in identifying neutral fats, cholesterol esters and free crystalline cholesterol.

Veenendaal obtained serum cholesterol measurements from eight normal dogs. They ranged from 137mg/100ml to 329mg/100ml with a mean of approximately 200mg/100ml. In animals with corneal opacities, he obtained a value of 137mg/100ml in a four year old Pointer with a unilateral lesion and a value of 322mg/100ml in a two to three year old Bouvier with bilateral lesions. He gave no values for the Dutch Shepherd dog examined histologically.

Robin α Charton (1939) reported that lipid in the dog's cornea (lipoidose cornéene) was reasonably common in France. They noted

that of about 30 cases the incidence was highest in adults and old dogs, that the lesions were bilateral and central or paracentral, of white "mica" appearance and round or oval shape. The descriptive term applied to these cases was "kératite chronique nacrée" (chronic pearly keratitis) and the lack of associated inflammatory changes indicates that the lesions were consistent with corneal dystrophy not lipid keratopathy, although the authors have not explored the possibility of inheritance.

Using extraction techniques on keratectomy specimens they were able to show that cholesterol ester and probably cholesterol were constituents of the opacity. Blood cholesterol measurements from four cases produced levels of 182, 210, 147 and 175mg/100ml respectively which they compared against a normal control value of 195mg/100ml. They mention the very great difficulties of attempting to establish whether or not the cholesterol levels were increased at the time of the original lipid precipitation in the cornea, particularly if hypercholesterolaemia was a transitory phenomenon, and when some of the patients were only seen some years after the opacities first appeared. However, they concluded that these slowly developing, non-inflammatory corneal opacities were probably not a consequence of even a transitory hypercholesterolaemia, but rather that the precipitation of fats and cholesterol within the cornea was favoured by its extremely slow interstitial circulation and by the existence of, for example, prior degeneration, senility or infection.

Waters (1959) injected various mixtures of lipids into the corneas of experimental dogs (and rabbits) and concluded that the corneal reactions (for example, ingrowth of vascular pannus) were related

more to the quantity of lipid and its position than to the type of plasma lipid injected.

Roberts, Dellaporta and Winter (1966) referred to corneal opacities in a proportion of Rough Collies with Collie Eye Anomaly. In some animals they thought the opacities were congenital. Histological examination demonstrated amorphous deposits which stained blue with haematoxylin and eosin and which were located immediately beneath the basement membrane of the epithelium. No stains for lipid were performed.

Vainisi and Goldberg writing on animal models of inherited human eye disease (1974) described the Afghan Hound, Rough Collie and Dachshund as the breeds of dog most commonly affected by a superficial, central/paracentral dystrophy of crystalline appearance. The dystrophy had been followed through three generations in the Afghan Hound and Rough Collie.

Dice (1974) described a progressive bilateral and symmetrical lipid dystrophy in three closely related male Airedale Terriers presented at between nine and eleven months of age because of a diffuse, milky opalescence of both corneas with perilimbal sparing. The topography of the opacity was designated as "diffuse" by Crispin in a later review of canine corneal dystrophies (1982). Histochemical studies of the cornea were said to demonstrate phospholipids and triglycerides. Serum lipoprotein levels were normal.

In 1975 Saunders and Rubin described corneal lipoidosis as a fatty degeneration of the cornea; they did not distinguish possible dystrophies and stated that peripheral corneal deposits differed from central deposits in provoking a mild chronic foreign body

reaction with subsequent vascularisation. Histology from an eight month old Bedlington Terrier demonstrated crystalline and non-crystalline lipid and a mild inflammatory reaction in the superficial stroma of the central cornea. No history of the case was supplied.

In a study of the effects of hyperlipoproteinaemia and ageing on the eyes of American Foxhounds, by Slatter (1975), which has been referred to earlier; a control dog was the only animal to develop oil red O positive material in the cornea itself and it was located in the anterior third of Descemet's membrane and immediately adjacent corneal stroma.

Oval corneal opacities in a closed colony of experimental Beagles were reported by Waring, Muggli α MacMillan (1977). Approximately 15% of the colony was affected and the opacities were of the same general form in different individuals; usually bilateral and symmetrical, central and avascular. According to the authors the lesions ranged from anterior stromal to the full thickness of the stroma and Descemet's membrane. In a subsequent paper (Ekins, Waring, Roth, Spangler, Sodhi α Gupta, 1979) the authors suggested that levels of cholesterol-enriched high density lipoprotein (HDL_c) were elevated in those animals with greater stromal involvement, although subsequent sampling and analysis (Roth, Ekins, Waring, Gupta α Rosenblatt, 1981) failed to substantiate this finding. The animals were followed over a four year period and Ekins, Waring α Harris, (1980) concluded that the different clinical appearances described in 1977 represented different stages of the same basic disorder - a primary lipid deposition. Investigation of the tear film was also reported in the paper of 1980 and no abnormalities were detected. Histochemical studies were performed on

a number of corneas and reported in the paper by Roth et al in 1981. The lesions were confined to the anterior stroma which was at variance with the results of ophthalmoscopic examination cited in 1977. Stains for "neutral fats", interpreted as triglycerides, were positive in all corneas. Usually intracellular lipid was in the form of large globules, whereas extracellular lipid was in finer granules. Cholesterol and phospholipid were present in all corneas with cholesterol occurring primarily in keratocytes (fibroblasts). Positive staining for free fatty acids was obtained in 12 out of 26 corneas. Other lipid stains were negative. The distribution of phospholipids and free fatty acids were not specified.

A proportion of dogs had apparently suffered natural corneal ulceration during the course of study but the authors did not distinguish these animals in presenting their results. Any variation in histological findings as a consequence of corneal ulceration would thus be conjectural. In a later paper (Spangler, Waring α Morrin, 1982) the ultrastructure of the oval opacities was described. The authors suggested that increased numbers of keratocytes or phagocytic cells were present in the anterior stroma. The stroma contained keratocytes of normal appearance away from the opacities and, within the opacities, changes ranged from hyperplasia of cell organelles to frank degeneration and necrosis. Cells with a hyperplastic Golgi apparatus and endoplasmic reticulum may have been participating in the deposition of extracellular material and cells in the various stages of degeneration were probably contributing to the extracellular material scattered throughout the stroma. Extracellular debris consisted of round or oval spaces which were surrounded by, or contained, electron-dense amorphous material. In

addition, a number of crystal-shaped clefts were also present and, whilst the majority were extracellular, others were clearly within cells.

The corneal opacities were thought to resemble Schnyder's central crystalline corneal dystrophy but no proof of inheritance was possible as the colony had been carefully outbred to maintain heterogeneity.

Waring, MacMillan, Spangler & Roth (1978) described somewhat similar crystalline corneal opacities in Siberian Huskies and they compared them with Schnyder's central crystalline dystrophy. The possibility that this was a naturally occurring animal model of Schnyder's central crystalline dystrophy was again referred to in a review article by Waring, Rodrigues & Laibson (1978) and subsequent papers by Waring, MacMillan, Roth, Spangler & Ekins (1979) and MacMillan, Waring, Spangler & Roth (1979). Some 14% of Huskies between the ages of seven months and 12 years were affected and the frequency rose from 4% in dogs of less than two years to 40% in dogs of more than nine years.

All affected eyes were free from inflammation and corneal vascularisation. The opacity was annular and the authors described five possible configurations of stromal involvement, ranging from the presence of polychromatic crystals in pre-Descemet's stroma to homogeneous deposits involving the entire stromal thickness. The morphology of these lesions was sufficiently different from central/paracentral lipid dystrophy and diffuse lipid dystrophy for Crispin (1982) to suggest that "annular" lipid dystrophy may be a more appropriate designation in the light of current knowledge; future investigations might reveal that there are similarities in

the underlying pathogenesis.

Histochemical staining revealed lipids in the sub-epithelial stroma and some keratocytes. Neutral fats were found in all six corneas examined, whereas phospholipid and cholesterol were found in four out of six. Transmission electron microscopy demonstrated needle-shaped and rhomboidal clefts in the pre-Descemet's stroma.

Single determinations of fasting serum lipids in five affected animals and five age and sex-matched controls revealed no abnormalities of serum lipoproteins.

Retrospective pedigree analysis based on affected probands did not demonstrate an hereditary pattern and, despite the inability to establish a mode of inheritance, the authors concluded that the crystalline opacities most closely resembled Schnyder's central crystalline dystrophy.

In 1978 Crispin α Barnett reported arcus lipoides corneae secondary to hypothyroidism in the Alsatian. In five animals the opacities ranged from a narrow to broad perilimbal arcus, with and without a lucid interval. The condition was bilateral but the arcus of one eye sometimes formed in advance of that in the other. The arcus was slow to form and initially non-vascularised, but later, corneal vascularisation occurred.

The results of histochemistry confirmed that early arcus consisted largely of extracellular cholesterol esters, cholesterol and phospholipid; the latter being in greatest quantity immediately beneath the basement membrane of the anterior epithelium and in the anterior portion of Descemet's membrane. Esterified cholesterol was the major lipid detected within the stroma (Crispin, unpublished observations).

In all cases hyperlipoproteinaemia was associated with an increase in high density lipoprotein (as HDL_C), low density lipoprotein and very low density lipoprotein. The diagnosis of primary acquired hypothyroidism was confirmed by laboratory investigation and subsequent histology on the thyroid gland in cases which were autopsied. The condition was further reviewed by Crispin (1982) and Crispin & Barnett (1983) and distinguished from lipid keratopathy and lipid dystrophies.

Kern & Riis (1980) reported interesting ocular manifestations of secondary hyperlipoproteinaemia associated with hypothyroidism and uveitis in a seven year old Rough Collie. The animal had bilateral irido-cyclitis with lipid aqueous effusion, bilateral glaucoma as a consequence of the uveitis and bilateral retinal detachment. There was conjunctival and episcleral congestion and the dog subsequently developed corneal arcus which was most marked in the supero-temporal quadrant of the cornea and involved the deep stromal layers. The arcus increased in width and density over a period of several weeks and was eventually five millimetres in width as a complete encircling band separated from the limbus by a lucid interval. A central focal oval area of stromal lipoidosis developed in each cornea in the ensuing months and the density of the arcus decreased.

Upon admission the patient had exhibited moderate hypercholesterolaemia, marked hypertriglyceridaemia and chylomicronaemia. Idiopathic hypothyroidism was confirmed by extremely low thyroxine (T_4) levels and an inadequate Thyroid Stimulating Hormone (TSH) challenge - T_4 response test. Hyperlipidaemia did not recur when the patient was given thyroid

replacement therapy, although the animal remained obese.

Harrington α Kelly (1980) demonstrated a more extensive incompletely arcuate, bilateral corneal opacity in a six and a half year old English Sheepdog with bilateral thyroid carcinoma. The opacities were symmetrical and broad, involving almost the whole of the dorsal (superior) aspect of each cornea but separated from the limbus by an optically clear zone. Histological examination of the cornea showed accumulations of long birefringent crystals in frozen sections examined with polarised light, although only small amounts of neutral lipid were demonstrated with oil red O. There was also a mild chronic inflammatory and fibrous reaction and the authors do not make any mention of corneal vascularisation. They termed the lesion a corneal lipoidosis and inferred that hypothyroidism with secondary hyperlipoproteinaemia was a possible cause; unfortunately, no measurements of thyroid activity or serum lipoproteins were made so the association was conjectural.

Slatter (1980) produced hyperlipoproteinaemia in experimental Beagles by a combination of surgical thyroidectomy and an atherogenic diet (thiouracil and commercial food plus cholesterol). A proportion of the dogs developed paralimbal and limbal lipid-containing corneal opacities in addition to greater lipid accumulation in other sites such as the sclera and uvea. In an animal which had recovered from unilateral corneal ulceration with vascularisation a marked lipid keratopathy developed at the previous site of the ulcer. This experimentally induced lipid keratopathy and the naturally occurring types cited by Crispin α Barnett (1978), Crispin (1982) and Crispin α Barnett (1983) provide the only examples of canine lipid keratopathy which have been examined

histologically.

From this review of the literature it would seem that, despite the great variety of names applied by other authors, there are probably three main types of corneal lipidosis in the dog:-

Lipid dystrophies may exist in their own right as inherited diseases probably involving some aspect of corneal metabolism, possibly that of the fibroblast. Central/paracentral, diffuse and annular forms have been recognised on the basis of their ophthalmoscopic and slit lamp biomicroscope appearances.

Arcus lipoides corneae exists as an ocular manifestation of a systemic problem, representing infiltration or insudation of excessive quantities of circulating lipid.

Lipid keratopathy may be a possible consequence of disorders such as anterior segment inflammation, abnormal local ocular metabolism or abnormal systemic lipids and lipoproteins - such factors acting together or independently.

The indistinct separation of clinical appearance which may be encountered makes rigid definition of the type of corneal lipidosis a slightly dangerous concept in a dynamic situation. It may be that the balance of interacting factors, or the dominance of one particular factor, determines the pathogenesis.

2.1.5 Serum Lipids and Lipoproteins An early report on the ultracentrifuge pattern of normal, hypertensive and hypothyroid dogs by Lewis, Green & Page (1952) indicated that the dog had high levels of high density lipoprotein compared with low density lipoprotein. Dogs made hypothyroid with radioactive iodine showed increased concentration of the alpha-2 lipoprotein component.

Papers by Grande & Schultz (1965) and Lindall, Grande &

Schultz (1971) demonstrated that serum levels of total cholesterol, phospholipids and triglycerides could be raised in normal dogs fed a diet with 40% of calories as coconut oil. Surgically thyroidectomised dogs given a similar coconut oil diet showed much larger increases in total cholesterol than normal dogs, but the increase in phospholipids and triglycerides was not as great, resulting in an excess of cholesterol over phospholipid.

Familial hyperlipoproteinaemia as a consequence of primary hypothyroidism was reported in Beagles by Manning, Corwin & Middleton (1973), and hyperlipoproteinaemia was marked even when diets low in cholesterol and fat were consumed. In another group of unrelated Beagles described by Wada, Minamisono, Erhart, Naito & Mise (1977) two out of five apparently normal adult dogs fed a diet low in fat and cholesterol were hyperlipidaemic as were their parents. The alpha$_2$- and beta-lipoproteins were elevated in the hyperlipoproteinaemic dogs.

Rogers, Donovan & Kociba (1975a) reported six cases of idiopathic hyperlipoproteinaemia: five in Miniature Schnauzers and one in a dog of mixed breeding. All the dogs exhibited persistent lipaemia characterised by a cream layer after refrigeration of the serum and they also had raised serum cholesterol levels and a moderate to marked increase in triglyceride concentration. Increased staining at the origin and of the beta band were the most obvious changes detected on serum lipoprotein electrophoresis and there was also a variable increase in the intensity of staining of the alpha$_2$ band. Feeding a low fat diet resulted in a decrease in serum triglycerides in all the dogs studied and serum cholesterol levels also decreased in four out of the five dogs studied.

Detailed isolation and characterisation of normal plasma proteins was performed on purebred Foxhounds and mongrels by Mahley α Weisgraber (1974). They confirmed that the distribution of plasma lipids and lipoproteins was quite unlike that in man, the dog having approximately five to six times as much high density lipoprotein as lower density lipoproteins. Approximately 85% of the total plasma cholesterol was carried by HDL_2 and approximately 50% of the plasma triglyceride was carried by very low density lipoprotein.

Very low density lipoproteins obtained by ultracentrifugation of plasma at a density of less than 1.006g/ml were triglyceride-rich particles. They varied in size from 260 to 900Å in diameter as measured using negative staining electron microscopy. Two classes of lipoprotein were contained within the density range 1.006 to 1.063g/ml: one closely resembled a low density lipoprotein with beta motility and a particle size of approximately 200Å, and the other (called HDL_1) was closely related to the high density lipoproteins with respect to immunochemical reactivity, electrophoretic mobility and apoprotein content. HDL_1 particles ranged in size from 100 to 350Å in diameter and appeared unlike any of the commonly described human lipoproteins. High density lipoproteins (called HDL_2) isolated in the density range 1.087 to 1.21g/ml were protein-rich particles ranging in size from 55 to 85Å.

A subsequent paper by Mahley, Weisgraber α Innerarity (1974) investigated the hyperlipoproteinaemia produced by feeding high cholesterol diets to surgically thyroidectomised Foxhounds. On the basis of response two groups (hyporesponders and hyperresponders) were described. In hyporesponders a non-atherogenic hyperlipidaemia

developed, characterised by an increase in the LDL and HDL_c classes (HDL_c referred to a broad spectrum of cholesterol-enriched particles which resembled high density lipoproteins - particularly HDL_1). In hyperresponders atherosclerosis developed and hyperlipidaemia was largely due to the presence of lower density lipoproteins, particulary LDL and VLDL, in addition to HDL_c. In all the hyperresponders the plasma lipoprotein patterns first passed through a stage in which they resembled those of the hyporesponders. It was suggested that HDL_c represented HDL_2 which had become overloaded with cholesterol so as to float at progressively lower densities.

Betalipoprotein (VLDL) and HDL_c both appear to have an arginine-rich apoprotein; indeed arginine-rich protein was the only protein constituent of dog HDL_c isolated by Innerarity & Mahley (1978) in experiments which demonstrated enhanced binding by cultured human fibroblasts of Apo-E containing lipoproteins such as HDL_c.

Negative staining electron microscopy indicated that the size of purified lipoproteins from both hyporesponders and hyperresponders for LDL, HDL_1 and HDL_2 were slightly larger than those of control dogs, whereas VLDL particles tended to have a smaller average diameter, particularly in the hyperresponders, as compared with controls. Thus LDL from both hyporesponders and hyperresponders ranged in size from 170 to 360Å compared with a range of 160 to 250Å for control dogs. HDL_1 ranged in size from 120 to 400Å with a peak at 230Å compared with a range of 100 to 350Å for control dogs. HDL_2 tended to be slightly larger than it was in control dogs, ranging from 70 to 100Å as compared with 55 to 85Å.

The VLDL particles from hyperresponders ranged from 240 to 550Å diameter, whereas those from the hyporesponders were seldom as small as 240Å and the control animals had VLDL diameters of 260A to 900Å.

Rogers, Donovan & Kociba (1975b) published work on lipids and lipoproteins in normal dogs and in dogs with secondary hyperlipoproteinaemia due to hypothyroidism, diabetes mellitus, or acute pancreatitis. The normal dogs used included experimental Bassett Hounds and privately owned dogs of mixed breed; the latter group had higher serum lipid concentrations than the experimental group.

Hypothyroid dogs had abnormal lipid values and lipoprotein electrophoretic patterns. Hypercholesterolaemia only was associated with increased intensity of the alpha$_2$-lipoprotein band, and in animals with more marked hyperlipidaemia, there was hypercholesterolaemia and hypertriglyceridaemia with prominent beta, prebeta and alpha$_2$-lipoprotein bands. High lipid concentrations and altered lipoprotein electrophoretic patterns were changed to near normal values after laevothyroxine administration.

In addition to the ocular manifestations of hyperlipoproteinaemia described earlier (Crispin & Barnett, 1978; Kern & Riis, 1980; Crispin & Barnett, 1983) there are limited reports of hypertriglyceridaemia (sometimes combined with hypercholesterolaemia or chylomicronaemia) producing a variety of effects: conjunctival and episcleral vessel lipaemia and congestion (Crispin, unpublished observations), lipid laden aqueous (Olin, Rogers & MacMillan, 1976; Sigler, 1977; Crispin, unpublished observations) and lipaemia retinalis (Wyman & McKissick, 1973; Crispin & Barnett, 1978; Fisher, 1979).

2.1.6 Atherosclerosis The dog may be considered an "HDL_2 animal", as such resistant to atherosclerosis whatever the method of production (Boyd, personal communication). Ocular manifestations of hyperlipoproteinaemia in naturally occurring disease has earlier been reviewed and would appear to be relatively rare. The same would appear to be true for experimentally induced hyperlipoproteinaemia to judge from the absence of any reference to ocular involvement by a number of authors working in this field (Steiner α Kendall, 1946; Bevans, Davidson α Abell, 1951; Mann α Stare, 1954; Boyd α Oliver, 1958; Guidry, Geer α Robertson, 1964; Constantinides, 1965; Geer, 1965; Suzuki, 1972).

Slatter (1975) working with American Foxhounds concluded that plasma lipid levels and the severity of atherosclerosis in the aorta, coronary and cerebral arteries correlated poorly with the presence of atheromata in the anterior ciliary arteries and with lipid accumulation in the sclera. From later work (1980) with experimental Beagles in which a mean serum cholesterol of approximately 1,000 to 1,500mg/100ml was achieved by a combination of thyroidectomy and an atherogenic diet, he suggested that "a reliable model of lipid keratopathy in the non-vascularised canine cornea could probably be developed if high levels of plasma cholesterol were maintained".

The fine structure of experimental canine atherosclerosis was studied by Geer (1965) from which he concluded that smooth muscle cells were primarily responsible for lipid accumulation with secondary involvement of macrophages. Regression of lesions in experimental canine arteriosclerosis, manifest as a reduction in aortic lipid content after stopping an atherogenic diet, was

reported by Davidson α Kendall (1951).

2.2 MAN

2.2.1 Introduction: Lipid keratopathy is a description applied to the deposition of lipid within one or both corneas. In many instances vascularisation precedes lipid deposition and, implicit in this situation, is some kind of prior damage to the cornea, or perhaps to the neighbouring tissues, which may have occurred many years before. Lipid may, however, be deposited in an apparently normal cornea, with and without a history of anterior segment inflammation. Corneal vascularisation appears to be a universal feature of lipid keratopathy, whether it occurs before, during, or following,lipid deposition.

Hyperlipoproteinaemia, particularly hypercholesterolaemia, does appear to be prevalent in patients with lipid keratopathy, although few reports have investigated this aspect. A number of authors have commented on resemblances between atherosclerotic plaques and lipid keratopathy.

2.2.2 Lipid Keratopathy. The term lipid keratopathy was first used by Cogan α Kuwabara (1958) and it was they who provided the first detailed comparison of experimental arcus lipoides with naturally occurring human senile arcus and concluded that the rabbit lesion resembled human lipid keratopathy rather than arcus senilis. Lipid-containing vascularised corneal lesions had, however, been recognised for many years and interpreted in a great many ways as the multiplicity of terms used by authors will indicate.

The review given here is not comprehensive but concentrates on those publications where microscopic examination of material was made, serum lipoproteins were estimated, or regression of opacities

was reported. It is not always easy to be sure of the type of ocular lesion from papers based purely on clinical description, particularly when details of history and laboratory examination are lacking.

One of the earliest reports of corneal fatty degeneration was made by Baumgarten (1876) who described a case secondary to sclerokeratitis, but without confirmation by histological means.

In 1893 Kamocki reported a case of fatty degeneration of the cornea with intermittent irritative phenomena in a 42 year old woman. The patient had had recurrent attacks of smarting, burning, redness, photophobia and lacrimation of either one or both eyes, with intervening periods free from ocular symptoms. She had noticed white opacities in the periphery of both corneas one year previous to examination by Kamocki. The lesions were bilateral, chalky-white, encircling, peripheral, vascularised plaques, and portions were excised for microscopical examination.

The white areas consisted of irregular refractile granules which dissolved in xylene and stained black with Flemming's fixative. There were large vacuolated and swollen cells which the author thought might have originated from the corneal fibroblasts. Hypercellularity was apparent in the regions of capillarisation. In places there was absence of Bowman's membrane and the histological picture was typical of a degenerative pannus.

Kamocki also refers to a treatise by Cuignet (1880) on "keratites parenchymateuses graisseuses" produced by a variety of ocular conditions and consisting histologically of a hypercellular and vascularised stroma with numerous fatty vesicles. There was variation of epithelial thickness and disruption of Bowman's

membrane. Cuignet comments that some of the collections of fatty vesicles were arranged exactly in the shape of normal corneal elements whereas in the regions of greatest opacity the quantity of fatty vesicles was such as to obscure underlying details of tissue morphology.

In 1907 Treacher Collins described a unilateral case of superficial corneal opacity in a 58 year old woman in which there had been recurrent pain and inflammation in the affected eye over a period of six months. The case was not examined histologically but is of particular interest as the patient was hypothyroid; she had become myxoedematous over a time of about two years. Whilst there was injection of the ocular conjunctiva, no corneal vascularisation was described; and the small discrete grey dots in the centre of the cornea, which comprised the opacity, disappeared completely over a period of six months when the patient was given thyroid treatment.

Tertsch (1911) described a case of primary fatty degeneration of both corneas ("primärer fettiger degeneration") in a 32 year old man who had had recurrent attacks of ocular redness each lasting some two to three months and at intervals of approximately three months. The right eye had been affected for two years and the left eye for five years; the right eye had the more extensive lesion. Both lesions were more or less centrally situated, appeared to involve the full thickness of the cornea and had both superficial and deep vascularisation.

Microscopic examination of a portion of the right cornea stained with Sudan showed red-brown granules of various sizes between and within the epithelial cells and an increase in the number of wandering cells in this layer, some of which contained

fat. Bowman's membrane was abnormal, being swollen, disrupted or absent.

The stromal lamellae also showed disorganisation and the spaces between lamellae, as well as the lamellae themselves, contained small and large fat globules. There was an increase in the number of cells present, although no round cells were seen. Some of the corneal fibroblasts (fixed cells) contained fat; others were degenerate. Some of the wandering cells also contained fat. Vascularisation was present and a few leukocytes surrounded the vessels. Foam cells were not mentioned and no hyaline change or calcium deposits were found.

Tertsch concluded that his case was a form of corneal dystrophy in view of the slow progress of the disease and minimal inflammatory phenomena. He suggested that death of the fixed corneal cells followed some toxic process and that this led to a fatty decomposition of the corneal lamellae. In support of this hypothesis he cited the negative results of general examination, particularly that of the vascular system, and also his belief that fat carried by the lymph would remain deposited at the corneal periphery and would not reach the centre as in his case.

Takayasu (1912) described two 16 year old girls with trachoma. The opacities were bilateral, central, greyish-white and vascularised; and when examined histologically granules and fat droplets were identified with the possibility that such fat might have originated from necrotic cells.

Whilst trachoma was very common in Japan, Takayasu believed that corneal fatty changes were very rare and that his cases represented a primary fatty degeneration (primare fettdegeneration)

of the cornea. He thought the fat deposition might be associated with the deficient nutritional status of the patients who were anaemic.

Fatty degeneration (Verfettung) of the cornea was described by Bachstez (1921) in a 36 year old man. Photophobia and bilateral circumcorneal injection preceded the development of corneal opacities; which formed first in the left eye and then in the right eye so as to cover a large area of the central cornea in both eyes, with the left eye more extensively involved. The history prior to examination by Bachstez extended over three years and included recurrent ciliary injection and iritis and the gradual extension of the lesions. Corneal vascularisation was obvious at the time of Bachstez' examination as was apparent exophthalmos. More general examination revealed an exophthalmic goitre and a blood cholesterol concentration of 162.8mg/100ml: a diagnosis of hyperthyroidism was made.

A penetrating graft of the left cornea was undertaken and the corneal specimen obtained examined microscopically. The majority of the specimen was fixed in 10% formalin, the remainder left unfixed for polarising microscopy and chemical analysis.

With the polarising microscope a large number of doubly refractile crystals in the form of plates and needles were apparent. With Sudan-type stains the sections were seen to contain many vacuolated cells in the middle and deep layers of the corneal epithelium and fat droplets were present in some of the vacuolated cells.

Bowman's membrane appeared normal, except that some very fine droplets were visible with high power in some preparations. Many

fine fat droplets were also present between the lamellae in the anterior third of the stroma but the droplets became coarser and more numerous in the deeper stroma. Bachstez identified the middle third of the stroma as necrotic because of the virtual absence of nuclei (other regions were of increased cellularity). The lamellae of the necrotic zone were thickened and typified by the presence of numerous fat droplets. The lamellae stained more palely with haemalum-eosin than those of the anterior one-third.

Beneath the necrotic zone there was a marked increase in the number of cells present and Bachstez named this the "nuclear zone" in consequence. Lymphocytes, leukocytes and lipid-laden cells were present and the latter, believed by the author to have phagocytosed the fat, were prominent in the region of the limbus.

Chemical analysis indicated that the fat consisted largely of cholesterol-fatty acid mixtures and that little glycerol ester was present.

Bachstez believed that the hyperthyroidism might have contributed to the pathogenesis in that a primary injury to the cornea, caused by the hyperthyroidism, had rendered it unable to digest the fat brought to it in normal amount by the lymph.

Kusama (1921) described a unilateral case of primary fatty degeneration of the cornea in a 55 year old woman which followed the development of corneal phlyctenulae arising at the limbus. The development of the corneal phlyctenule was associated with photophobia and lacrimation and the whitish-yellow corneal opacity developed approximately one year after the phlyctenule. The opacity was situated in the central zone of the cornea and was dense and discoidal with a vascular supply. A piece of abnormal tissue was

removed for pathological examination, fixed in 10% formalin and cut on a freezing microtome. Sections were stained with Sudan III, Nile blue and neutral red, and the Lorrain-Smith-Dietrisch method; and were also examined with a polarising micrcoscope.

The epithelium was generally normal with occasional fat-staining substances in elongated basal cells. Bowman's membrane was very disrupted and did not contain lipid material. Corneal lamellae were swollen or fragmented. Lipid droplets of varying sizes were present within the lamellae, but not in the interlamellar spaces. Both fixed corneal cells and wandering cells were of increased number in the stroma. Calcareous and hyaline degeneration were not detected.

With the polarising microscope crystalline bodies of doubly refractile nature were apparent; they dissolved readily in ether, xylene and chloroform but with difficulty in alcohol. Kusama concluded that the fat substances were predominantly cholesterol, cholesterol esters and cholesterol-fatty acid mixtures, with no glycerol esters. The condition was thought to be due to a fatty decomposition of the corneal lamellae as a consequence of primary injury to their metabolism.

In 1922 Verderame (cited by Clausen) described the anatomic and histochemical investigation of a case of symmetrical, bilateral fatty degeneration of the cornea and arcus senilis in a 38 year old woman. The patient was emaciated with acne rosacea and a chronic gastro-intestinal disturbance. Opacities developed in the infero-temporal quadrant of both eyes over a four year period without "noteworthy" inflammatory phenomena, although irritative symptoms probably occurred prior to lipid deposition. The opacities were

disc-shaped, greyish-white and vascularised; they were also sharply demarcated from an arcus lipoides which was also present. The time for which the arcus lipoides had been present was not mentioned and there were no measurements of serum lipids.

Histopathological examination of a keratectomy specimen revealed a disintegration of fibrillae with the formation of lacunae-like spaces. Fat was deposited in the basal cell layer of the epithelium, in the lamellae and the interlamellar spaces. There was a slight increase in the number of fixed cells and leukocytes. The author concluded that the fat present was of the cholesterol ester variety and that the arcus and the disc-shaped opacity were expressions of one and the same process, which was influenced by the chronic gastro-intestinal disturbance.

Elschnig (1923) reported a curious case of fat dystrophy (Fettdystrophie) at the periphery of both corneas in a 58 year old male. In this patient the central cornea was clear and the opacity was sharply demarcated centrally and became continuous with the sclera peripherally. There was intense vascularisation and the corneal surface over the opacity was uneven. A cortical cataract was the only other ocular abnormality. A single determination of blood cholesterol was apparently normal, but the patient was said to have arteriosclerosis.

Chemical examination of a keratectomy specimen indicated that it was largely composed of fat, but the type was not determined. No calcium was present.

Fatty degeneration in various parts of the globe, especially following anterior segment injury (xanthomatous bulbi) was described by Von Szily (1923). On occasions the eye was already blind, even

phthitic, from previous trauma or disease but sometimes the fatty degeneration of cornea, anterior chamber, iris and occasionally the posterior segment was directly related to an inflammation. Hypercholesterolaemia was often present.

In 1924 Meesman reported on the pathology of eyes with massive depositions of cholesterol in the anterior chamber and secondary corneal atheromatosis (sekundärer atheromatose der hornhaut). He cited the case of a 31 year old woman who had suffered a perforating injury to the left eye which was subsequently enucleated. Cholesterol crystals were present in the anterior chamber and there was a fatty opacity of the cornea. Histological examination revealed no fat in the corneal epithelium, a diffuse reaction with Sudan stains in Bowman's membrane and fat droplets in the peripheral stroma. There was also diffuse staining of Descemet's membrane. These staining reactions are more typical of an arcus lipoides than lipid keratopathy. The author goes on to suggest that the cholesterol crystals found in such profusion in the anterior chamber may sometimes destroy Descemet's membrane (and presumably also the corneal mesothelium) so that the cornea swells and crystals migrate into the parenchyma. In this patient there was diffuse stromal oedema prior to the eye being enucleated. The same author demonstrated two patients with "primary fatty degeneration of the cornea" to the Berlin Ophthalmological Society in 1924. The first case was a 54 year old woman with severe diabetes and raised serum cholesterol (360mg/100ml). Five years previously she had an acute iridocyclitis in the left eye, during the course of which a dense central corneal opacity of crystalline content developed in the middle layers of the corneal stroma. She later had an acute

iridocyclitis in the right eye with development of a similar, but larger, corneal opacity which extended over all but a narrow perilimbal zone. The extent of this opacity subsequently decreased and then became static. Microscopic examination of a trephine specimen was said to have supported the diagnosis of fatty degeneration of the cornea.

The second case was a 29 year old woman with a unilateral vascularised tongue-shaped opacity which extended from the temporal limbus nasally over the pupillary area. Over a two year period the finely punctate opacity extended centrally and simultaneously receded peripherally. "Anatomic" examination supported the diagnosis of fatty degeneration involving the middle layers of the corneal stroma. The cholesterol content of the blood was said to be raised, but no figures were provided. The cases were published in 1927.

In 1925 Landmann reported on tubercular irido-cyclitis with fatty degeneration and infiltration of the anterior segment of the eye. Denti (1926) reported a case of primary bilateral fatty degeneration of the cornea in a 40 year old woman who suffered periodic attacks of redness, photophobia and lacrimation associated with a gradual but progressive reduction in vision. The opacity was not so dense as to prevent a view of the iris and there was slight pericorneal injection.

Microscopic examination showed disruption of Bowman's membrane and some of the corneal lamellae were fragmented. There was an increase of lymphocytes in the corneal stroma. Sections stained with fat dyes revealed intracellular and extracellular fat droplets which were anisotropic when examined with the polarising microscope.

The majority of the fat droplets were identified as cholesterol esters. Denti thought that the corneal disease was a result of disturbed ovarian function as the patient had failed to menstruate after the age of 40 years; menstruation reappeared after administration of ovarian preparations which did not affect the corneal disease.

A paper by Spanlang in 1927 described a case of "dystrophia adiposa cornea" in a 53 year old woman with advanced osteomalacia, including bone atrophy and a positive Chvostek's sign. She had a raised blood glucose level of 141mg/100ml; serum lipid and calcium levels were not estimated. Her eyes were examined some seven years after she had noted a reduction in vision, at which time there was bilateral opacity of the cornea, occupying all but a perilimbal clear zone, and vascularisation was also present.

A portion of cornea was removed and fixed in formalin (with the calcium-containing Karlsbader's salt) and stained with haematoxylin and eosin and a variety of lipid stains after sectioning.

The epithelium was normal, but Bowman's membrane and the stroma were degenerate. Fatty acids and calcium were said to be present in the anterior stroma, fine granules of calcium but no fatty acids slightly deeper and then a necrotic zone even deeper before corneal stroma of more normal appearance was encountered beneath the necrotic zone. It is possible that the fixative employed (Karlsbader's Salt) may have resulted in the formation of calcium soaps and that it was these soaps, rather than naturally occurring calcium, which were detected as blue granules in haematoxylin and eosin sections. Haematoxylin and eosin sections also demonstrated the hypercellularity of the material.

Fatty acids were detected by a blue reaction (no red or pink) with Lorrain-Smith's Nile blue sulphate and Fischler's method. A delicate yellow colour in the middle layers of the stroma was obtained with Sudan III and a weakly positive lamellar reaction with neutral red.

Nitric and acetic acids produced complete extraction of lipids whereas absolute alcohol only partly extracted them. With the polarising microscope there were birefringent granules and the necrotic region contained birefringent plate-like crystals and droplets.

Jaensch (1928a, 1928b, 1933 α 1935) published a series of articles on fatty degeneration of the eye, in which he referred to lipid keratopathy occurring as a possible sequel to keratomalacia, corneal ulceration, herpes keratitis and scleritis. Rohrschneider (1927a) was of the opinion that the primary steatosis of the cornea was of two basic types: a benign peripheral type and a central more serious type.

Meyer (1928) described a case of dystrophia adiposa corneae (primäre xanthomatosis corneae) in a 65 year old woman who also had evidence of mild arteriosclerosis and cardiac insufficiency. Opacities of the central cornea developed in the right and then the left eye within a short period of each other without any associated inflammatory phenomena. When examined 15 years later there was a more marked opacity in the right eye than the left. When re-examined after eight years there was regression of the right corneal opacity and enlargement of the left. The iris and anterior chamber were apparently normal.

A keratectomy specimen was removed from the left eye and half the

tissue embedded in gelatine and stained with fat stains after sectioning; the other half was examined with the polarising microscope.

Microscopic examination revealed fine fat globules (Sudan III) in the middle layers of the epithelium which increased in number as the basal cell layer was approached. Bowman's membrane was absent in some regions and lamellated in others; and many coarse droplets were present in this area. The corneal lamellae were irregularly disposed and fat laden. Large accummulations of confluent fat droplets separated the lamellae in places. Necrotic regions were scattered throughout the corneal stroma. In vascularised areas there was increased cellularity: lymphocytes and large round fat-containing histiocytes. With Nile blue sulphate the bulk of the fat stained blue or bluish, and only a small number of fine droplets in the lamellae stained red. Polarising microscopy revealed both large and small anisotropic needles and anisotropic droplets. The needles were said to have a melting point of 47-50°C.

The author concluded that cholesterol, cholesterol esters and a small quantity of neutral fat were present in the cornea and that the pathological process was endogenous; either a result of changes in the vitality of the corneal tissue or of total body metabolism or, more probably, of both. He also remarked that regression of the opacity in the right eye might be a consequence of lipid removal by the new blood vessels.

Gilbert (1929) reported a case of xanthomatosis of the cornea and sclera in a 50 year old woman with aortic insufficiency and angina. The left eye had been affected for five years and was blind, the right eye had been affected for one year and had a brisk pupillary

light reflex. The cornea of the left eye was vascularised and opaque, there were also some large yellow masses in the inferior sclera. The anterior segment was slightly shrunken. The left eye had a yellow kidney-shaped vascularised opacity of the inferior half of the cornea which became denser towards the sclera with which it merged. In addition to the abnormalities of cornea and sclera, posterior synechiae were apparent superiorly and nasally. No tissue was taken for microscopic examination but a series of blood cholesterol examinations ranged between 194 and 200mg/100ml, which Gilbert thought high enough to account for the ocular lesions as a consequence of increased lipid being offered to, and stored by, the cornea and sclera. There was no change in the appearance of the eyes over a nine month period of observation.

Axenfield (1930) described spontaneous clearing in a case of xanthoma corneae. In 1931 Vannas published a case of fatty infiltration in an 84 year old woman in which almost complete peripheral annulae were present in both eyes. The lesions were present in the anterior stroma and superficial vascularisation was present. Histological examination revealed that the peripheral border of the corneal epithelium was thinned and contained numerous fat particles of various sizes. Bowman's membrane was atrophic and yielded a positive fat reaction. The fat was considered to be of haematogenous origin.

Brown α Katz (1932) and Katz α Delaney (1933) reported a case of dystrophia adiposa corneae in a 53 year old man with a history of bilateral ocular injection of short duration with lacrimation, photophobia and moderate redness. There was no history of ocular injury and no reason for the signs and symptoms was found. Some

months later the symptoms became more severe and the patient was described as having a "marginal keratitis" with bilateral pericorneal injection. The corneas became opaque in recurrent bouts, progression appeared to be from the periphery towards the centre. When examined by Katz and Delaney the left cornea was completely opaque and the right cornea opaque except for a kidney-shaped clear zone in the superior nasal quadrant. Plaque-like yellow-white superficial lesions were present as well as deeper opacities. Both superficial and deep vascularisation was present, no nerves were seen and corneal sensitivity was described as normal.

A number of laboratory tests were performed: blood cholesterol was usually at the high end of the normal range and blood calcium was 11.2mg/100ml. Other examinations revealed osteoporosis in the bones of the hands and feet, and a chest X-ray was interpreted as possibly tuberculosis or pneumoconiosis.

A full thickness corneal graft was performed on the left eye and the abnormal tissue obtained was subdivided. One portion was fixed in formol-Zenker solution, from a second portion frozen sections were prepared, a third portion was fixed in acetic acid, osmic acid, potassium dichromate solution (A.O.B.) and the remainder was subjected to chemical examination by the Windaus technique. The latter method revealed large amounts of both free cholesterol and cholesterol esters with the latter in greater quantity (Δ1:3 Ch:ChE).

Histological examination of haematoxylin and eosin stained material indicated increased epithelial thickness due to hyperplasia and hypertrophy and an increase in the number of wandering cells, lymphocytes and leukocytes, particularly near Bowman's membrane.

Bowman's membrane was disrupted in places, sometimes lamellated or absent. The stromal lamellae were of variable thickness, often degenerate and many contained cholesterol clefts. There was an increase in the number of nuclear elements, particularly neutrophils, but also lymphocytes, and many cells had pyknotic nuclei. Distinction of dying cells was often impossible. Histiocytes were present between lamellae. Blood vessels of various sizes were prominent and histiocytes were closely associated with them, but were not found within the blood vessel lumen.

Descemet's membrane and the endothelium were normal. Sudan stains and osmic acid were used for the detection of lipid, the former stained more elements than the latter. Lipid was detected throughout the corneal sections except for Descemet's membrane and the endothelium. The lipid of the epithelium and Bowman's membrane formed a diffuse, extremely fine granular deposit whereas that of the stroma was often coarse as a consequence of coalescence of fat droplets. The majority of Sudan positive lipid was apparently extracellular but histiocytes containing intracellular lipid were also observed. Histiocytes were also positive in sections stained with A.O.B., but their origin could not be determined.

In addition to the case cited by these authors they examined in some detail previous communications on dystrophia adiposa corneae, emphasising that only eight undoubted cases of the condition had been published previously (Kamocki, 1893; Tertsch, 1911; Bachstez, 1921; Kusama, 1921; Verderame, 1922; Elschnig, 1923; Denti, 1925 and Meyer, 1928) and that these cases "must not be confused with secondary fatty degeneration of the cornea, a well-known and frequently described pathologic process which sometimes follows an

inflammation of or injury to the cornea, sclera, or uveal tract". They concluded that dystrophia adiposa corneae was brought about by an "impairment of the physiologic process of the corneal cells" and that the abnormal cell metabolism led to disintegration of the lamellae and liberation of "invisible fat" of which cholesterol and its esters were components, with the possible addition of cholesterol and cholesterol esters from the blood stream if they were present therein in abnormal amounts. It is of interest that they decided that it was the presence of substances foreign to the cornea which produced chemical irritation manifest as circumcorneal redness, whilst in the case that they themselves cite (and others of their "confirmed" cases from other authors) irritative phenomena preceded detection of corneal opacities.

Heath (1932, 1935) indicated that "lipin interstitial keratitis" was characterised by deep interstitial fat and cellular infiltration of the cornea (usually vascular) as part of a disturbance in fat metabolism in the field of "lipореticular" disease. He claimed that the underlying pathological process in both the "mis-named" primary corneal dystrophies and those lipid changes secondary to trauma or infection were the same; namely, an overwhelming lipid cellular response to a mild activating lesion. The so-called primary types probably have a constitutional faulty metabolism of fat and a mild unrecognised uveitis as part of the underlying pathology.

The author concluded that a group of corneal lesions separately classified as distinct entities were all representative of lipin interstitial keratitis and that differences of appearance depended upon such factors as the underlying cause and whether the lesion was

in the early active phase, mature, or retrograde, according to his understanding of pathogenesis. Thus parenchymatous keratitis, keratitis centralis annularis, keratitis profunda and xanthomatosis of the cornea would all be forms of lipin interstitial keratitis.

Toth (1933) reported a case of primary annular infiltration of cholesterol in a 19 year old girl who had had recurrent attacks of redness in both eyes since the age of eight years. Slit lamp examination indicated masses of crystals within the cornea which were assumed to be cholesterol. Her blood cholesterol level was 312mg/100ml and a cholesterol-free diet decreased the inflammatory ocular changes and reduced the corneal opacification.

In 1934 Bonnet, Paufique α Bonamour reported a case in which crystals of cholesterol (as judged by their birefringence in polarised light and extraction by ether) were located in the central cornea of a 31 year old man who also had an arcus lipoides. The patient gave a history of pain, photophobia and lacrimation and the central opacity was described as a white speck, removed completely by keratectomy. The patient's serum cholesterol level was 160mg/100ml.

Wright (1936) reported on two cases of corneal degeneration, one of which was a consequence of urate keratitis, the other, in a 45 year old man, a form of lipid keratopathy. In the latter case there was a central disc-shaped lesion of the right eye and a denser annular lesion in the left eye; the conjunctiva of the left eye was said to be mildly inflamed. Bilateral pterygia were also present. Corneal sensitivity was absent in the right eye, diminished in the left and photophobia was marked, although pain and lacrimation were

absent.

A full thickness penetrating graft was performed first in the right eye and subsequently in the left. Histological appearance was similar in the two eyes. The epithelium and Bowman's membrane were normal, the stroma showed increased cellularity. Lymphocytes, large mononuclear cells and a few polymorphonuclear leukocytes accounted for the increase in nuclear elements. The leukocytes were clustered around capillaries.

Areas of foamy degeneration were present in the stroma and Sudan III and Nile blue sulphate indicated fat droplets "infiltrating the cells in this area". The endothelium and Descemet's membrane were of normal appearance. In the left eye foamy degeneration was apparently more prominent and there were very fine fatty granules which stained with Sudan III and gave a mauve colour with Nile blue sulphate. A few globules of "neutral fat" and sparse crystalline needles were also noted. The author concluded that the granules were all intercellular and probably consisted of a mixture of fatty acids.

In 1937 Szasz recorded trachomatous pannus as an unusual cause of "xanthomatosis corneae" in a 43 year old woman. Histological examination demonstrated a granulomatous reaction; plasma cells and foreign body giant cells were seen; there was Sudan III positivity and birefringence of the intracellular droplets.

In the same year Cavara undertook a survey of what he called primary adiposis of the cornea (L'adiposa primaria della cornea) which agreed in essentials with the findings of Heath. Cavara drew attention to the widely differing clinical appearances of the condition and the multiplicity of descriptions applied (fatty

degeneration, lipoid degeneration, adipose dystrophy, fatty infiltration of the cornea, fatty interstitial keratitis and xanthomatosis of the cornea). According to this author the fatty deposits consisted largely of cholesterol and its esters, but these might be associated with fatty acids and neutral fats. He observed that the fats might be present as clumps, drops or granules situated in or between the lamellae, or as a diffuse infiltration of the lamellae. In severe cases the lipid deposition produced a definite histiocytic reaction with the formation of typical foam cells.

Mann α Pullinger (1942) studied the pathology of cholesterol and fat deposition in mustard gas injuries of the cornea and compared certain of the lipid-connective tissue reactions therein to those occurring in the arterial intima in atherosclerosis.

In the same year Loewenstein (1942) published a paper on the lipoidal pathology of ocular tissue in which he described a "secondary fatty corneal dystrophy" in which there had been a previous iridocyclitis. The main quantity of lipid as demonstrated with Sudan red was in the middle layers of the corneal stroma, appearing as fatty interstitial "masses", droplets and plasma cells with fatty droplets. Cholesterol crystals, deep vessels and lymphocytic infiltrates were also present. The anterior two-thirds of Descemet's membrane and Bowman's membrane were also Sudan red positive (more suggestive of an accompanying arcus lipoides). The author also mentioned a new form of primary symmetrical fatty corneal dystrophy accompanying the systemic hyperlipidaemia of nephrosis. Further details of this patient were published in 1943 by Conway α Loewenstein and concerned a 35 year old woman who presented with arcus lipoides corneae. The lesions were

bilateral, symmetrical, non-vascularised and with no associated inflammatory changes. Blood urea and serum cholesterol were raised and the albumin:globulin ratio very low. The authors suggested that the name for this arcus lipoides corneae secondary to hyperlipidaemia might be "primary symmetrical interstitial fatty corneal dystrophy with lipoidal arc, due to nephrotic lipaemia".

Davidson (1947) described a case of primary lipid dystrophy of the cornea in a Negro of 61 years. The patient gave a history of recurrent ocular irritation first in the left eye and then in the right eye more than a year later. The irritative attacks were typified by conjunctival injection, pain and lacrimation, and the patient had noticed diminished vision in the left eye subsequent to the early irritative phenomena. When the eyes were examined there were two dense white opacities in the left eye and a more generalised grey and deeply situated diffuse opacity in the right eye. Both eyes stained with fluorescein and there was vascularisation of the lesions in the left cornea. The lesions in both eyes progressed and vascularisation became intense prior to a full thickness penetrating graft on the left eye and diagnostic scrapings from the right cornea.

The blood cholesterol was 390mg/100ml, 300mg/100ml and 250mg/100ml over a period of a month. Blood glucose was 105mg/100ml and 120mg/100ml in a three day period. The patient was put onto a low fat, low cholesterol diet prior to surgery.

Material from the left eye was fixed in Zenker's fluid and dilute formaldehyde. Sudan-stained material demonstrated normal epithelium, Descemet's membrane and endothelium. Bowman's membrane showed some staining but was disrupted in places and absent in

others. Cellular infiltration and vascularisation were prominent in the anterior two-thirds of the stroma. Numerous capillaries were present and associated with focal accumulations of macrophages and rare lymphocytes. The lamellae were thickened and not readily identified. Large and small globules of fat were present extracellularly and, to a lesser extent, within macrophages. No fatty acid crystals or cholesterol crystals were seen in plain or polarised light within the stroma but Davidson refers to doubly refractile cholesterol crystals of 5-10um length in the epithelium which "took the stain for fat" (Sudan IV). The crystals lay either in the cytoplasm or between the epithelial cells.

A most interesting series of 12 cases was published by Zeeman & Groen in 1947 under the title of lipoidosis corneae: In four of the 12 cases the lesion was not associated with inflammation, redness, pain or photophobia and, in most typical form, was yellowish, glistening and annular. In appearance it resembled the xanthoma of skin and was therefore termed xanthoma corneae. The first four cases were placed in this category although case one, a 15 year old girl, had a unilateral sickle-shaped vascularised opacity which did not quite conform with their designation. In this patient the serum cholesterol was normal (184mg/100ml); in the other 11 patients of the series cholesterol was raised and ranged from 250 to 495mg/100ml.

In eight of the 12 cases deposition of cholesterol was accompanied by corneal inflammation, usually as a keratitis or sclero-keratitis, often of unknown origin. Lesions of this type were associated with pain, photophobia, blepharospasm and lacrimation. Pericorneal injection was common and nodular scleritis

was noted in some cases. This type of lesion was characterised by a chronic course with alternating periods of remission and relapse. The authors called this form keratitis lipoides.

The majority of the 12 patients were overweight: ten were women, six were hypertensive, four had arteriosclerosis, one had diabetes mellitus and one had hypothyroidism with myxoedema.

In addition to specific treatment, where appropriate, all the patients were given a strictly vegetarian diet. If the diet was followed serum cholesterol levels fell, and three patients showed varying degrees of clearing of the corneal opacities. In one of these patients, a 36 year old woman with a unilateral lesion associated with inflammation and suggestive of tuberculous keratitis, the corneal opacity cleared completely. Serum cholesterol levels prior to treatment averaged 284mg/100ml; the corresponding figure for phospholipids was 224mg/100ml. After treatment and a vegetarian diet serum cholesterol was 192.5mg/100ml and phospholipid 169.5mg/100ml. The authors concluded that the cause of xanthoma of the cornea was unknown, but might be a partial manifestation of a general metabolic disturbance. For keratitis lipoides a general metabolic disturbance probably coincided with some local factor.

The general disturbance which manifested itself as hypercholesterolaemia could be of metabolic origin, although simple overfeeding was also a possibility. The local factor was thought to be of possible bacterial origin and the prolonged inflammation accompanying infection was a possible consequence of an inadequate cellular response. It was thought that the high cholesterol levels resulted in overloading of phagocytes like the macrophage so that

they were unable to function normally.

Van Canneyt (1948) described a case of xanthoma of the cornea in a 44 year old man. Only the right eye was affected and the opacity had developed without inflammatory symptoms and was discoidal in shape. Slit lamp examination demonstrated that the affected cornea was thickened and vascularised. The patient had numerous skin xanthomata and a serum cholesterol of 328mg/100ml. The author felt that the patient's condition might be, in part, a consequence of eating two eggs a day for ten years and treatment included a low fat, cholesterol-free diet. With this regime the serum cholesterol was reduced to 260mg/100ml over a five month period and the cornea became notably clearer.

In a paper on ocular conditions associated with idiopathic hyperlipaemia Dunphy (1949) reported a case of lipid interstitial keratitis. The history was of ocular irritation of a recurrent nature, at the time of publication present in only the left eye, whereas 3 years before it had been in the right eye.

Examination showed similar, but asymmetrical, sickle-shaped deep opacities; that of the left eye associated with minimal conjunctival congestion and photophobia. Superficial and deep vessels were apparent in the cornea of the right eye at the time of the first examination and in both eyes two weeks later. Examination of the serum lipids indicated a total cholesterol of 292mg/100ml, phospholipids 328mg/100ml and neutral fats (triglycerides) 1,360mg/100ml (a picture consistent with primary hyperlipoproteinaemia). A low fat diet reduced serum lipids slightly but hypertriglyceridaemia was marked in all samples. During the period of treatment (about seven months after the first

examination) the inflammation of the right eye recurred and two superficial infiltrates with superficial vascularisation were apparent nasally as part of this recrudescence. The inflammation subsided after a week.

Dunphy considered that idiopathic hyperlipaemia and primary lipid interstitial keratitis, although rare, represented a disturbance of fat metabolism and that it was reasonable to assume that some relationship existed between them, even though the severity of the keratitis and the lipid content of the serum could not be correlated.

During the discussion which ensued from this presentation, Heath referred to a case in a ten year old boy with hypercholesterolaemia and an ectopic cilium. When the cilium was contacting the cornea it triggered off a corneal reaction manifest as an arc of lipid crystals. When the cilium was removed the opacity diminished and when it regrew the opacity returned; thus demonstrating the importance of minor trauma in addition to a persistently high blood cholesterol.

Babel reviewed lipid deposits of the cornea in 1950. In a section entitled "steatose primaire" he described a man of 57 with bilateral corneal opacities; that of the right eye had developed at the age of 50 years and that of the left eye at 55 years. Their development was associated with recurrent inflammation and the stroma was heavily vascularised. Corneal grafts were performed on both eyes. Histological examination of the right eye revealed abundant numbers of cells in the stroma: lymphocytes, plasma cells and histiocytes which bordered necrotic areas. The necrotic region stained diffusely with Sudan-haematoxylin. Isolated droplets at the

periphery of the necrosis were also stained and there was some positive intracellular material. A large proportion of the material was birefringent.

The left eye differed from the right in having considerable quantities of needle-shaped bundles of cholesterol crystals in the necrotic regions. There was marked cellularity at the periphery of the necrotic areas with occasional giant cells. Many of the histiocytes which contained fat appeared as foam cells. The inflammation extended throughout the whole thickness of the cornea and the lesion was heavily vascularised. The patient had a serum cholesterol level of 200mg/100ml and neutral fats of 1,150mg/100ml; other laboratory examinations were normal.

The author felt that this type of corneal opacity was due to a local defect of cellular metabolism. Lipids carried to the cornea by the circulation were insufficiently oxidised, resulting in the deposition of visible fat which itself provoked local irritation. However, a more general metabolic defect might also be present in this type of primary lipid dystrophy and rather subtle pre-opacity inflammations may have contributed.

"Steatose secondaire" was subdivided into a diffuse and nodular forms. Two case histories of the diffuse type were given and one of these appears to be a lipid keratopathy following metaherpetic keratitis in a woman of 32 years. Sudan staining indicated fine extracellular lipid granules and larger intracellular lipid globules. Foam cells were also present in addition to lymphocytes and plasma cells. Intracellular lipid was partially birefringent. Vascularisation was common and deep.

As an example of the nodular type the author referred to a

bilateral case in an eight year old girl which had been described by Orzalesi in 1947. In this patient bilateral nodular opacities appeared some years after apparent recovery from trachoma. Histological examination of a paraffin embedded section stained with Mallory's demonstrated masses of foam cells in the anterior stroma with small numbers of plasma cells and lymphocytes.

The similarity of the nodular form of steatose secondaire and Saltzmann's nodular degeneration was commented on by the author. He concluded that steatose secondaire might be produced as a sequel to a wide range of inflammatory conditions of the anterior segment.

The localisation of the fat within the cornea varied, although Bowman's and Descemet's membranes were rarely involved unless an arcus lipoides was also present. Lipid might appear in droplet form between and within the corneal epithelial cells. In a case which followed a previous cinder burn only the epithelium was affected by lipid which was located around the cell nuclei. However, it was usually the stroma which was involved in lipid deposition in sites which have been described previously.

That the author was encountering a wide range of corneal and systemic disease other than lipid keratopathy is clear when he states that all types of fats were encountered: "neutral fats, cholesterol, cholesterol esters, fatty acids, cerebrosides, phosphatides etc...". Within the text he had previously cited Gaucher's disease in a description of steatose secondaire, whereas it is now recognised as a more general disturbance of cerebroside metabolism.

Forni (1951) in a review of corneal disease characterised by cholesterol deposition (and primarily concerned with Schnyder's

central crystalline dystrophy) differentiated between heredo-familial dystrophies and other corneal opacities. Conditions associated with abnormal lipid metabolism include the arcus lipoides type of lipid infiltration, steatose primaire (after Babel), xanthomatosis of the globe with corneal involvement (after Meesman, 1924) and crystalline parenchymatous degeneration ("la degenerescence cristalline parenchymateuse"), of which he observed that this type was generally secondary to anterior segment inflammation and was often unilateral.

It would seem that hyperlipoproteinaemia, whether of primary or secondary origin, is of itself an unlikely cause of lipid keratopathy. For example, Davidson, Pilz α Zeller (1951) in describing a case of generalised xanthomatosis with corneal involvement described 37 other such cases which lacked any sign of lipid keratopathy. Their case involved a 42 year old man who gave a history of keratitis at the age of 22 years. Xanthomatous plaques had developed by the age of 37 and when examined at the age of 42 the patient had a typical disc-shaped lipid keratopathy in the inferior portion of the right cornea and a diffuse haze of the whole of the left cornea. Capillary loops were present at the periphery of both corneas. The patient had a total serum cholesterol of 1,193mg/100ml and the diagnosis was of biliary cirrhosis.

Cogan (1951) described recurrent bilateral scleritis of many years duration in a 51 year old woman. The patient had had recurrent redness and pain in both eyes over a period of 18 years. Usually one eye at a time was painful and each episode lasted approximately a week and recurred at eight to fourteen day intervals. Approximately five years before she was examined by

Cogan a glistening white paralimbal opacity had developed inferiorly in the cornea of the right eye followed two years later by a similar opacity in the left eye. Both lesions were vascularised and both were gradually extending towards the pupil. Nodular elevations had occurred in the episclera two years ago, adjacent to the inferior limbus and a biopsy of this tissue had demonstrated considerable quantities of Sudanophilic fat and some birefringent crystals, together with polymorphonuclear leukocytes and a few giant cells. The serum cholesterol and phospholipids were normal and the situation remained unchanged by treatment.

Lipidosis corneae was the term employed by Cogan α Kuwabara (1955) to describe any deposition of fat in the cornea other than arcus lipidalis. They presented the histological appearance of two cases: in one, lipid deposition had occurred at the site of a previous marginal ulcer which had healed some 16 months previously leaving a vascularised scar; in the other the history was of intermittent ocular inflammation for 20 years, but the deposition of lipid was a relatively recent phenomenon.

Histological examination revealed increased cellularity and vascularisation with abundant, mainly intracellular, Sudanophilic globules, moderate quantities of birefringent crystals, areas of acellular necrosis and a macrophage response. The authors subsequently went on to publish details of the similarity showed by lipid keratopathy and atheroma in both man and hypercholesterolaemic rabbits (1958). They indicated that the form and shape of the fatty deposit was intimately connected with blood vessels and that the lesion was more likely to be reversible when it occurred in a swollen cornea, and permanent and stationary when it occurred in a

compact cornea.

The histological appearance of the fatty plaques in man was of large Sudanophilic, mainly intracellular, globules, of up to 20um diameter; and much smaller Sudanophilic granules which were situated predominantly in the acellular and hyalinised areas, and gave the impression of diffuse Sudanophilia under low power. The authors comment that "the inescapable impression is that the granular lipid is derived from the globular lipid when the cells containing the latter disintegrate".

With haematoxylin and eosin affected corneas showed an increase of cellularity in some areas and a decrease of cells or hyalinisation in others. Some of the cells had a distended or vacuolated cytoplasm but "it is impossible to state whether they are distended corneal cells or invading macrophages". In some material birefringent crystals were prominent particularly in areas of hyalinisation. In both rabbits and man the changes in the cornea were felt to be similar to those occurring in the blood vessels of the corresponding species during the development of atheromata.

Cogan (1960) discussed the lack of information available in deciding whether patients with lipid keratopathy have a hypercholesterolaemia. Ten patients in the group he had studied produced an average level of 267mg/100ml for cholesterol. In four of these the level was more than 300mg/100ml and in one the level was 500mg/100ml (with a fatty acid level of 627mg/100ml). He concluded that lipid plaques occurred in the cornea, as in the blood vessels, "predominantly but not exclusively in patients with higher than average blood cholesterol levels".

Forsius (1961) reviewed the clinical appearance and lipid

chemistry of 16 cases of lipid keratopathy. He partly followed the classification used by Busacca (1952) in which three forms of lipid keratopathy were recognised: (1) annular dystrophy which differed from marked senile arcus in, for example, its yellow colour;

(2) discoid fatty dystrophy consisting of numerous needle-shaped crystals and fat within the corneal parenchyma;

(3) other forms including fatty infiltration in keratitis and secondary adiposis of old corneal scars.

This type of distinction is helpful only in so far as it emphasises the diagnostic difficulties which have accompanied identification of corneal lipid opacities, and the multiplicity of descriptive terminologies which have been ascribed to them.

The idea that arcus senilis, or more accurately arcus lipoides, is not absolutely distinct from lipid keratopathy was not new: as early as 1931 Vannas had argued that a case of annular fatty dystrophy derived from arcus senilis, and later workers (Heath, 1935; Palich-Szánto, 1960) had drawn attention to the association of arcus lipoides with marginal conditions of the cornea.

Palich-Szánto (1960) reported 65 cases of infiltration of the cornea in the vicinity of the arcus lipoides in persons over 50. The designation of "primary" fatty degeneration for those cases in which fat deposition occurred in the cornea apparently unconnected with earlier eye disease was also discussed; careful examination may reveal signs of previous eye disease and the history obtained from the patient may be unreliable, particularly if it extends over many years. Thomas (1955) felt that only 16 published cases fulfilled

the criteria for primary lipid keratopathy and the earlier publication of Katz α Delaney (1933) has been discussed previously. At this stage of reviewing the medical literature it may be stated that there is no benefit to be gained in attempting to designate lipid keratopathies as primary or secondary - a view endorsed by Maumenee (1962): "there have been reports of primary 'lipid dystrophies' but it is doubtful if such a thing exists".

A synopsis of Forsius' sixteen cases of lipid keratopathy is as follows: 13 cases were women, three were men. The mean age was 60.2 years. Ten patients had a marked arcus lipoides in addition to the lipid keratopathy. The arcus lipoides was more obvious than in control material of the same age. Fifteen patients had a history of previous or existing ocular disease. Only two patients had a serum cholesterol level of less than 300mg/100ml (they were both 265mg/100ml). The mean cholesterol value for the 16 patients was 360mg/100ml. The series resembled that of Zeeman α Groen (1947) in having a high proportion of women and included conditions which might affect serum lipoproteins (diseases of the heart and blood vessels, nephrosis, xanthelasma, thyroidectomy for thyrotoxicosis). Only the patient with chronic glomerulonephritis (serum cholesterol 367mg/100ml) had developed a unilateral vascularised discoid lipid keratopathy without a history of prior eye disease.

Earlier investigation (Forsius, 1954) had established a good correlation in young and middle-aged persons between arcus lipoides and high serum cholesterol values, whereas a corneal factor was predominant in older people. The author concluded that the same observation seemed to be true of the development of lipid keratopathy.

Baum (1969) described a unilateral lipid keratopathy (cholesterol keratopathy) which occurred in a 72 year old Chinese woman with prior bilateral pre-irritative phenomena ("itchy" eyes). The lesion was vascularised and the opacity involved the full thickness of the stroma; the epithelium appeared normal but the involved cornea was thicker than normal. A small posterior synechia was also present. Serum lipid measurement revealed a total cholesterol of 125mg/100ml, lipid phosphorus of 12.1mg/100ml and neutral fats of 207mg/100ml.

The patient had a partial penetrating keratoplasty and the corneal button removed was placed in formalin. The total fat content was estimated by Folch's method and yielded a figure of 0.83mg of lipid. Thin-layer chromatography (TLC) and gas-liquid chromatography (GLC) indicated that cholesterol was the predominant lipid present. These results were compared with a normal cornea from a 56 year old woman whose eye had been enucleated because of malignant melanoma of the choroid. Chloroform-methanol extraction according to the method of Folch yielded 1.2mg of lipid. TLC demonstrated free sterol, sterol esters, triglycerides and phosphatides (lecithin and sphingomyelin); of these lipids the sterol esters predominated. When comparing the results from the two eyes the author acknowledged that the epithelium of the abnormal cornea may have been missing, or, that the considerable quantities of free cholesterol obscured the smaller amounts of normally occurring lipids.

It is perhaps pertinent to observe that malignant melanoma of the choroid may not be an ideal choice for control tissue; the blood flow through the tumour may present abnormal quantities of

nutrients, including lipids, to the cornea. The ages of the two patients are also widely separated (56 years and 72 years respectively).

The first report on lipid keratopathy to include ultrastructural studies was published by Jack α Luse (1970). The specimen was obtained at the time of lamellar keratoplasty on the right eye of a 51 year old woman in whom a pterygium had been excised 30 years previously. The authors did not state when the lipid appeared relative to the surgical excision but referred to "early" lipid keratopathy. Blood glucose levels were normal, serum cholesterol was 260mg/100ml and serum triglycerides 175mg/100ml.

The corneal specimen was fixed for 90 minutes in 4% glutaraldehyde in 0.1M phosphate buffer. Half of the specimen was sectioned at 15um in a cryostat. Some frozen sections were stained with oil red O and Sudan black B, others were examined unstained with a polarising microscope. The other half of the specimen was post-fixed in 1% osmium tetroxide in phosphate buffer, dehydrated and embedded in araldite for electron microscopy. Thin sections were stained with uranyl acetate and lead citrate, whereas thick sections were stained with a mixture of azure A and azure B. Thick sections demonstrated invasion of the corneal stroma by blood vessels, some of which were 50um diameter and with adjacent nerve bundles. Diffuse vacuolation was apparent in the stroma and there was abnormal cellularity.

Oil red O stains demonstrated large bright intracellular globules (up to 10um diameter) and smaller, less brightly stained, extracellular granules. Birefringent crystals and oil red O positive material were in greatest quantity in the deep corneal

stroma.

In the electron microscope the vascularity of the opacity was obvious and all vessels were abnormal in that there was marked thickening and reduplication of the basement membrane. The endothelial cells of larger vessels contained numerous apparently cylindrical dense bodies.

The regular arrangement of the corneal stroma was disrupted by spaces which apparently contained membrane remnants. Some keratocytes appeared normal but others were frankly degenerate. A proportion of cells of the corneal stroma contained many large membrane-limited intracytoplasmic vacuoles. In some regions, granules and vesicles of varying size and electron density were located extracellularly between collagen fibres following cell necrosis and breakdown of cell membranes.

Numerous abnormal nerve bundles were closely related to the neovascularisation of the corneal stroma; they lacked the usual compact organisation of peripheral nerve fibre bundles.

Macrophages were common, particularly near blood vessels, and contained abundant inclusions of variable size, shape and electron density. Lymphocytes occurred in groups or clusters between adjacent corneal lamellae, whereas plasma cells were solitary and located between stromal layers or near blood vessels.

The authors assumed that foci of extracellular lipid deposition in the corneal stroma were associated with either intact membranes or membrane remnants of intracellular origin derived from cell death. The necrosis of lipid-laden cells itself incited a chronic inflammatory reaction with further neo-vascularisation and lipid deposition, so that a positive feed-back mechanism was thereby

established. The authors did not attempt precise identification of the lipids involved.

Fine, Townsend, Zimmerman & Lashkari (1974) described an interesting exaggerated arcus lipoides under the title of primary lipoidal degeneration of the cornea. A 63 year old man gave a history of slowly progressive blurring of vision over a 15 year period, without any accompanying signs of irritation, and with no indication of previous ocular disease or trauma. In the right eye there was a complete annular opacity with no perilimbal clear zone, and with a triangular opacity which extended towards the centre of the cornea in the superior temporal quadrant with the apex of the triangle axially. In the left eye there was a superior and inferior sickle-shaped band situated paraxially to paralimbally. No vascularisation was apparent in either cornea. The fasting blood glucose level was 89mg/100ml, total serum cholesterol was 295 to 316mg/100ml and total lipids 1,304mg/100ml.

The patient underwent penetrating keratoplasty on the right eye and the corneal tissue obtained was fixed in formalin and then cut into three portions. One was embedded in paraffin, another was post-fixed in Dalton's chrome-osmium and the third was used for preparation of frozen sections. Paraffin sections were stained with H and E, PAS, Masson's trichrome and alcian blue. Frozen sections were stained with oil red O, Sudan black B, Baker's acid haematein, Landing's method for phospholipids, the Schultz method for cholesterol, Fischler's method for free fatty acids, the Mukhenji method for unsaturated lipids, the Seligman-Ashbel method for carbonyl lipids and Luxol fast blue for myelin. Polarised light was also used for examination of frozen and paraffin sections. A

sample of ordinary arcus taken from a formalin-fixed eye was used as a control specimen and processed similarly.

The most striking histological finding was marked degenerative change situated deeply in the cornea and sufficient to detach Descemet's membrane from the deep corneal stroma towards the corneal periphery; anterior to this the stroma contained a few chronic inflammatory cells and the cornea was least altered beneath the basement membrane. Blood vessels were not identified anywhere within the corneal button.

Histochemical methods demonstrated finely granular lipid which impregnated the collagen fibres diffusely, and larger globules of various sizes which were present within keratocytes and extracellularly. Many acicular cholesterol crystals were present, within cells and extracellularly, particularly in the deep necrotic cells. The granules and globules were positive with the Sudan series and phospholipids and fatty acids were also present (according to a positive result with Baker's and Fischler's method) but their location was not discussed. There were insufficient unsaturated lipids present to produce a positive reaction which the authors interpreted as a lack of cholesterol esters.

Ultrastructurally the characteristically shaped clefts occupied by cholesterol and numerous small, relatively electrolucent spaces were the most striking features.

The authors felt that the changes might be due to a primary degeneration of the keratocytes and adjacent collagen, or an unusual and exaggerated form of arcus "senilis". In favour of the latter suggestion was the similar microscopical appearance of a control arcus senilis with their case, and the additional evidence from the

clinical appearance referred to earlier. Schnyder's crystalline dystrophy was also considered a possibility but there was no indication of inheritance.

The authors concluded that the primary lipoidal degeneration of the cornea they had reported might be "a gross variation of an arcus senilis, an unusual variant of Schnyder's dystrophy, or both", thereby emphasising some of the problems associated with classification of lipid-containing corneal lesions.

Another paper on lipid keratopathy which provided ultra-structural studies was published by Tremblay α Dube in 1975. An 85 year old woman was presented because of a gradual loss of vision during the preceding 18 months. On examination she had opaque corneas with marked neovascularisation, a bullous keratopathy and severe trichiasis. She underwent a successful corneal graft on the left eye.

Part of the corneal button was immediately fixed in 2% glutaraldehyde at 4°C for 3 hours, post-fixed in osmic acid and processed routinely to epon embedding. The remaining portion of the cornea was fixed in formalin for paraffin sections.

The microscopical appearance was typical of a degenerative pannus; ulceration was also present. Neovascularisation, mononuclear inflammatory cells, foamy macrophages and lipid deposits disrupted the stromal lamellae. Oil red O revealed the presence of neutral fats and phospholipids. With polarised light numerous small collections of birefringent crystals were apparent.

With the electron microscope many osmiophilic inclusions were aggregated between the collagen fibres and were probably representative of lipid droplets. They were prominent near

capillaries and associated with disorientated collagen fibres. Fibroblasts were actively engaged in collagen synthesis in this area. In other places fibroblasts or keratocytes were seen to be hypertrophied and degenerate, sometimes cellular debris was apparent. Cholesterol crystal profiles appeared both free in the stroma and within hypertrophied and degenerate keratocytes. There were occasional macrophages containing fatty globules.

The authors concluded that the majority of the lipid accumulation was a consequence of keratocyte degeneration with modified stromal metabolism, possibly with the additional factor of neovascularisation of the corneal stroma.

Friedlander, Cavanagh, Sullivan, Gallagher & Dickersin (1977) used the title "bilateral central lipid infiltrates of the cornea" to introduce a rather unusual case involving a 55 year old woman with a history of acute onset of severe ocular pain, lacrimation and photophobia, accompanied by a diffuse erythematous skin rash of the face and chest. The symptoms cleared within a week but recurred in episodic fashion initially. Later the ocular discomfort became a constant feature whereas the skin rash remained episodic. Bilateral grey-white corneal infiltrates, central epithelial defects and stromal vascularisation had developed after the first few attacks and became denser with each recurrence so that vision became extremely limited. Total fasting serum cholesterol, determined on several occasions, was between 217 and 267mg/100ml and triglycerides were 72mg/100ml.

The patient had had a myocardial infarct at the age of 52 years and there was a family history of death at an early age from coronary artery disease.

A penetrating keratoplasty was performed in the left eye and brought immediate pain relief in this eye which lasted until she had an acute flare up of symptoms two months after discharge. A large central epithelial defect developed in the left eye and subsequently also in the right eye. The graft developed a greyish-white appearance and became progressively vascularised. The patient died suddenly, apparently from an intentional drug overdose and the eyes were obtained for post-mortem examination.

The corneal button obtained at the time of keratoplasty had been subdivided and fixed in Karnovsky's fluid for electron and light microscopy and in Tissue-Tek for immunofluorescence and histochemistry.

The post-mortem eyes were obtained by post in 3% glutaraldehyde; they were stored in 0.1M cacodylate buffer after subsectioning. The histology of the corneal button and the right eye were similar except that the right eye was ulcerated. In both specimens there was disruption of Bowman's membrane and the stroma contained "vacuolated and granulated fibroblasts" and similarly affected macrophages. Capillarisation was prominent and Descemet's membrane and the endothelium normal. In the epithelium of the corneal button many of the superficial cells were extensively vacuolated, those of the basal layer were hyperplastic and some contained dark granules.

Frozen sections stained with Baker's acid haematein revealed deposits of blue-black material throughout the entire epithelium and stroma, which were most intense in the areas of greatest inflammation beneath Bowman's membrane. The authors interpreted this staining as positive for phospholipids and galactolipids. A Sudan IV and Schultz stain were negative, as was a normal control

cornea with all histochemical methods. Immunofluorescent techniques demonstrated sparse stainings for fibrin in the epithelium basement membrane, stroma and the walls of new blood vessels.

Ultrastructural examination revealed a number of medium-sized electron-dense "lipid droplets" in the epithelial cells. Grey and black "droplets" as well as clear vacuoles were present in fibroblasts and histiocytes. Some of the more deeply situated fibroblasts also contained a moderate number of osmiophilic droplets. Bowman's membrane was frequently displaced from the epithelium and surrounded on both sides by fibrovascular tissue.

The authors concluded that deposition of lipid in this patient may have been due to excessive lipid production or an inability to mobilise it, aided by inflammation with increased corneo-scleral limbal vascular permeability. They were unsure of the exact relationship between the patient's skin condition and the corneal abnormalities.

Shapiro α Farkas (1977) reported a case of lipid keratopathy in a 40 year old man with type IV hyperlipoproteinaemia. The patient had had a pterygium excised from the right eye on two occasions with beta radiation therapy after each. A year later he presented with a two day history of redness in the left eye. Initial examination indicated conjunctivitis but the patient returned five days later complaining of a sudden decrease in left visual acuity. A circular rupture of Descemet's membrane was discovered and punctate staining of the central cornea was apparent. About one week later the patient developed multiple patches of stromal oedema in the right eye with small breaks in Descemet's membrane underlying them. The

following week discrete white deposits were seen in the area of hydrops in the left eye and in the areas of the breaks in Descemet's membrane in the right eye.

Serum lipoprotein examination indicated elevation of pre-beta lipoproteins and a normal beta band. The serum cholesterol ranged between 267 and 310mg/100ml and serum triglycerides were 252mg/100ml.

A penetrating keratoplasty was performed on the left eye and no deposits were present in the donor cornea a year after the operation.

Histological examination demonstrated no abnormalities in the epithelium, but thickening of the epithelium basement membrane and Bowman's membrane. The stromal oedema was more marked posteriorly and there was a decrease in keratocyte number from anterior to posterior. The fibrillar structure of the deep stromal lamellae was lost and a few giant cells and scattered inflammatory cells were present. The edges of Descemet's membrane appeared curled up in the region of the break. With oil red O, lipid globules deposited in and between the corneal lamellae were apparent; immediately above Descemet's membrane the deposit was particularly dense. The lipid material did not stain with alcian blue or PAS.

The authors concluded that the cause of the spontaneous hydrops was unclear as was the lipid "dystrophy" which followed. They speculated that the lipid may have gained access to the cornea from the aqueous humour and that the patient's condition represented an unusual form of corneal degeneration.

Corneal lipid deposition was recognised as a complication of corneal damage and limbal guttering by Watson α Holt-Wilson (1974)

when they considered corneal involvement in episcleritis and scleritis. The close association of the cornea with the sclera and anterior uveal tract may help to explain their combined involvement in sclerokeratitis and keratouveitis and Donaldson (1980) provided several examples of the widely differing conditions which may result in lipid keratopathy. He emphasised the association of lipid deposition with vascularisation and the possibility of systemic lipid disturbances, whilst recognising that there was much variety in the pathogenesis. Similar observations were made by Klintworth (1982) who theorised that a number of factors might be involved in pathogenesis, for example: hyperlipidaemia; increased vascular permeability; an inherent metabolic defect in which the cornea is unable to catabolise normal serum-derived lipids; and possibly also the local synthesis of lipids by corneal cells rather than plasma derived lipid infiltration.

In summary, human lipid keratopathy is an unusual condition. The cause is not always apparent and the lesion may regress. Cholesterol in free and esterified form has been the most commonly identified lipid in diseased corneas, but there have been few detailed investigations of either corneal lipids or serum lipids in affected patients. The importance of anterior segment inflammation in lipid keratopathy is reviewed in Section 2.3.5.

2.3 RABBIT

2.3.1 Introduction There have been numerous studies on the effects of high fat diets in rabbits. As early as 1913 Anitschkow and Anitschkow and Chalatow demonstrated that feeding rabbits a cholesterol-enriched diet led to the development of atheroma.

The aortic lesion produced by short term high level cholesterol

feeding is essentially a lipoidosis with minimal intimal fibrosis: such gross lipid accumulation has been attributed to the remarkable degree of hypercholesterolaemia (up to 10,000mg/100ml as compared with normal levels of less than 35mg/100ml) that develops in the cholesterol-fed rabbit. With prolonged cholesterol feeding at a lower dietary level the lesions are more fibrous and less cellular (Adams, Miller, Morgan α Rao, 1982). The early, predominantly fatty, atheromatous lesion is thus converted into a fibro-fatty, atherosclerotic lesion over a period of time.

Ocular manifestations of hypercholesterolaemia in the rabbit have been reported by a number of workers and some of this work is reviewed in the next section. The association was accurately described and illustrated many years ago (Versé, 1913; Rohrschneider, 1925a, 1925b).

It would appear that the rate at which plasma lipid levels rise, the severity of ocular lesions and the manner in which they develop, depend upon the type and quantity of high fat diet consumed and the time for which it is fed.

2.3.2 Ocular Manifestations of Hypercholesterolaemia From the considerable literature relating to the lipid-fed rabbit it would appear that ocular fat deposition is early and profuse in the region of the ciliary body, where it occurs initially as fine droplets in the stroma surrounding vessels, particularly near the origin of the ciliary processes (Janes, 1964). Lipid deposition is probably initially an insudative process involving the passage of lipid or lipoprotein from blood into tissues, although Leary (1941) proposed that all lesions containing lipid-laden cells in the cholesterol-fed rabbit arose from permeation of lipid-laden macrophages into these

sites.

From the ciliary body lipid deposition extends centripetally towards the pupillary margin successively involving the numerous ciliary processes attached to the posterior margin of the iris as well as the posterior iris (Walton α Dunkerley, 1974).

Xanthomatous plaques (Cogan α Kuwabara, 1959; Francois α Neetens, 1964) form on the posterior aspect of the iris; occasionally fatty cystic lesions covered by a thin layer of iris epithelium project into the posterior chamber (Loewenstein, 1942; Gwin α Gelatt, 1977).

In albino rabbits the fatty plaques which formed on the posterior surface of the iris were often the most striking ophthalmoscopic manifestation of ocular involvement (Cogan α Kuwabara, 1959; Francois α Neetens, 1964). Lipid deposition in the iris precedes that in the cornea and is more conspicuous (Versé, 1924); this involvement could be very pronounced when corneal changes were mild (Francois α Neetens, 1964).

Lipid deposits occurred within the anterior part of the choroid, particularly next to the lamina vitrea, although in longstanding cases the full thickness of the choroid is involved. The lipid engorgement of the ciliary body region becomes continuous with the lipid of the choroid (Janes, 1964).

Early changes in the cornea and sclera indicate that vessels in the region of the limbus and deep scleral plexus are dilated and hyperaemic. The vessels contain lipid and are surrounded by fine perifibrous lipid deposits in the corneal and scleral stroma (Walton α Dunkerley, 1974). The lipid is invariably confined to the anterior stroma, although Janes (1964) described lipid droplets in

the basal epithelial cells and the mesothelial (endothelial) cell layer. Other authors described the lesions as subepithelial and mainly anterior stromal and all are agreed that Descemet's membrane contains no visible fat droplets.

The ophthalmoscopic appearance of the corneal lesion changed throughout the period of lipid ingestion and following cessation of feeding (whether due to dietary alteration or inappetance). Francois α Neetens (1964) noted peripheral paralimbal white-yellow spots which increased in size and density, so that a complete broad annulus formed in some cases and there was progressive opacification from peripheral towards central cornea. Occasionally a type of lucid interval in the paralimbal circumference was discernable in early lesions, but in many cases, and certainly in more advanced lesions, opacification adjoined the sclera. Complete corneal opacification was seen towards the fourth month in the experiments conducted by Francois and Neetens (1964), and it was not correlated with the severity of lesions in other organs. The extent of corneal involvement was graded by Roscoe α Vogel (1968) and found to correlate closely with the cholesterol concentration in mg/g wet weight at all times during the period of study. Using cholesterol concentration as a measure of atherosclerotic involvement they found no significant correlation between corneal and aortic disease although there was significant correlation between iris and aorta cholesterol concentration. The experimental period in this study varied between one and three months and the authors concluded that there was a good degree of correlation ($p < 0.01$) between the severity of iridic and aortic involvement during the period of study.

Whilst lipid deposition was largely confined to structures bounding the anterior chamber in the early stages of feeding a high fat diet, later changes included enhanced deposition in the posterior segment. In addition to more extensive full thickness involvement of the choroid a greater quantity of lipid than usual was said to be present in the retinal photoreceptors and pigment epithelial cells, and retinal detachment was not uncommon. Myelinated structures such as the optic nerve sometimes stained more strongly with Sudanophilic dyes. Lipid was usually present in the entire circumference of the sclera, particularly its deep portion next to the choroid; however, Francois & Neetens (1964) described an absence of scleral involvement other than thinning and fusion with the affected choroid to form a solid sclerochoroidal block as a consequence of xanthomatous alterations in the choroid.

The accumulation of lipid produced a gross but irregular thickening of all affected regions which was particularly marked in the posterior segment.

Verse (1916) described the corneal lesion of the cholesterol-fed rabbit as an "arcus lipoides" and compared the pathogenesis with the presenile arcus sometimes encountered in young persons with hyperlipoproteinaemia.

Experimental arcus lipoides corneae in the rabbit differs from human arcus senilis in a number of ways which are summarised below from the work of Cogan & Kuwabara (1959), Walton (1973) and Walton & Dunkerley (1974).

1. The lipid producing arcus in hypercholesterolaemic rabbits is usually restricted to the superficial cornea and never extends throughout the corneal thickness to involve Descemet's membrane. In

man the lesion usually forms in the deeper layers of the cornea with early involvement of Descemet's membrane. At a later stage of development there is a separate area of lipid deposition superficially and Bowman's layer is involved.

2. In the rabbit there is no sparing of the paralimbal region; in man, although the lucid interval of Vogt is a well documented ophthalmoscopic feature, histochemical techniques have demonstrated that lipid is actually present in this region.

3. The rabbit arcus consists primarily of intracellular globular lipid accompanied by considerable histiocytosis; less obvious granular Sudanophilia may be detected extracellularly as may amorphous masses in later lesions. In man lipid is almost exclusively extracellular, in the form of fine pericollagenous droplets in the stromal regions and hyaline Sudanophilia of Descemet's membrane and Bowman's layer.

4. Advanced lesions in the rabbit contain abundant quantities of acicular birefringent crystals in addition to numerous fat-filled cells. Xanthomatous masses of lipid-filled cells and cholesterol crystals or clefts may involve posterior portions of the eye as well as those which border the anterior chamber. Acicular cholesterol crystals are not seen in the human lesion and cellular involvement is minimal.

5. Arcus lipoides in the rabbit is always vascularised; not so in man.

The slightly variable manifestations of canine arcus lipoides corneae have been referred to earlier and, whilst it was noted that a proportion of early canine lesions resembled the human arcus lesion in having involvement of the whole stromal thickness and part

of Descemet's membrane, later lesions and a proportion of early material bore a much closer resemblance to the experimental arcus of the rabbit. The differences observed seem at least partly due to the cellular involvement which is present in the rabbit and dog, but not in man.

A comparison of differences in both the cornea and other parts of the eye were tabulated by Janes (1964) when he compared his own findings for lipid distribution in the eyes of hypercholesterolaemic rabbits with those of Kolen (1927) for human arcus senilis.

Some regression, even disappearance, of corneal opacities has been reported when the rabbit was returned to a normal diet (Verse, 1924; Rohrschneider, 1927b; Rodger, 1971 and Walton & Dunkerley, 1974).

Rodger (1971) noted that, within six months of reversing a high cholesterol diet fed for approximately twelve months, only small quantities of amorphous osmiophilic material and occasional crystals remained beneath Bowman's membrane in the peripheral cornea. The majority of keratocytes appeared normal, with only scanty vacuoles, and collagen had reformed wherever necessary. This type of resolution contrasted strongly with those of animals in which he had incised the limbus and sutured the incision with virgin silk; the influence of trauma will be discussed more fully below. In the rabbits reported by Walton & Dunkerley (1974) which were returned to a standard diet after four months of cholesterol feeding there was progressively less extracellular lipid and more complete uptake of lipid by cells. After four months of a standard diet extracellular lipid was absent or sparse and there were fewer lipid-filled cells. Lipid resorption appeared to progress in the same sequence as that

in which it originated as lipid deposition; thus extracellular lipid disappeared first from the ciliary body and its processes leaving only scattered lipid-filled cells, the iris at this stage contained substantial lipid deposits. In the cornea extracellular perifibrous lipid disappeared rapidly whereas amorphous extracellular deposits and lipid-filled cells persisted longer. In those regions in which crystalline cholesterol deposits and a surrounding fibrotic reaction were apparent, such as the iris and ciliary processes, minimal resolution was apparent.

These findings are of interest when compared with the equivocal results obtained for regression, or otherwise, of aortic lipid lesions in cholesterol-fed rabbits. The confusing literature concerning changes in aortic lipid content after cessation of cholesterol feeding was summarised by Adams, Morgan α Bayliss (1973). Further work by Adams α Morgan (1977) indicated that there was no evidence of regression of advanced atherosclerosis in the rabbit and they concluded "At best slow partial regression may occur under some undefined circumstances". Mild atheroma in the rabbit, by contrast, does seem to regress, as illustrated by the short-term cholesterol feeding used in the experiments of Bortz (1968) and Adams, Knox α Morgan (1975).

It is known that atheromatous lesions may be slowly converted into fibrous atherosclerosis even during a period of apparent regression and that the presence of crystalline cholesterol within the aortic wall renders it relatively inaccessible to physico-chemical exchange. Adams (1972) suggested that at least two lipoprotein-associated cholesterol pools were present in the normal artery and that a third pool, formed by deposited crystalline

cholesterol which had become dissociated from its lipoprotein vehicle, arose at the moment that atherosclerosis developed. Thus whilst exchange and nett transfer of cholesterol could occur rapidly between Pools 1 and 2, the cholesterol of Pool 3 was neither in the correct physical state for exchange nor accessible to intracellular metabolic processes because of its extracellular situation (Adams, 1973).

2.3.3 Serum Lipids and Lipoproteins Hypercholesterolaemia in the rabbit was recognised as a prerequisite for ocular lipid lesions and lesions elsewhere (xanthomata, atherosclerosis) from the early experiments of feeding high cholesterol diets (Anitshkow, 1933). However, quantification of serum lipids and lipoproteins was not possible with any degree of accuracy and even modern studies of hyperlipoproteinaemia in animals with ocular lesions are not comprehensive.

The level of hyperlipoproteinaemia and the classes of lipoprotein particularly affected depend upon such factors as the time for which an atherogenic diet is fed, the type of diet and individual variation.

Janes (1964) fed 0.75% cholesterol and 2.5% cottonseed oil to rabbits for two to six months and recorded serum cholesterol levels of up to 2000mg/100ml. Francois α Neetens (1964) conducted a four month experiment, although the rabbits ceased to eat their diet of normal food plus cholesterol dissolved in olive oil (3g cholesterol/ 15ml olive oil) after two and a half to three months. Measurements taken from six animals at the four month stage showed cholesterol levels which ranged from 1750mg/100ml to 6,706mg/100ml; phospholipids which ranged from 780 to 1,873mg/100ml and total lipids which

ranged from 3,250 to 13,400mg/100ml. As the animals had stopped eating at the time of these measurements the serum lipid levels would actually be decreasing as compared with those at the two and a half to three month stage.

Parker & Odland (1966 & 1968) induced experimental atherosclerosis and xanthoma in rabbits by feeding a 4% cholesterol diet. Cholesterol (both free and esterified) rose rapidly over a two week period reaching a peak at four weeks with levels of approximately 2,400mg/100ml. Phospholipids increased by four weeks to a level of approximately 500mg/100ml and they too remained elevated. Changes in triglycerides and free fatty acids were not significant when compared with normal controls.

In a study of lipid changes in the eye and aorta Roscoe & Vogel (1968) fed a 1% cholesterol diet for one to three months. In fourteen animals the cholesterol level ranged from 1200 to 3276mg/100ml at one month and reached a final range of values from 2374 to 3031mg/100ml by the end of the third month. The plasma phospholipid concentration ranged from 580 to 815mg/100ml at the end of the three month period.

In a publication on lipid accumulation and clearance in the cholesterol-fed rabbit cornea (Rodger, 1971) where the animals were fed approximately 800mg cholesterol daily five days a week (0.5% of the daily diet) an increase in the beta-lipoprotein band, often with chylomicron staining at the origin, was observed after a month or more. The beta band comprised 38% cholesterol ester, 22% phospholipid and 8% free cholesterol as part of its constitution. Increased serum cholesterol level resulted in an even more marked staining of the beta band and staining in the albumin region also

became obvious, as did chylomicron staining at the origin. On returning to a normal diet the lipoprotein levels fell rapidly to pretreatment values and the electrophoretic pattern was also typical in having faint staining of albumin only, or of albumin combined with a very faint band in the beta position. In fifteen animals studied the serum cholesterol levels ranged from approximately 500 to 1600mg/100ml after about eight months of cholesterol feeding.

In studies of experimental xanthoma in rabbits Walton, Thomas & Dunkerley (1973) and Walton (1973) fed a semi-synthetic beef diet with no added cholesterol, as described by Gresham & Howard (1962) and a 2% cholesterol diet to separate groups of rabbits. Animals on the beef-fat diet had triglyceride levels of 80mg/100ml, phospholipid levels of 280mg/100ml and cholesterol levels of 215mg/100ml after 11 weeks, whereas the corresponding figures for those on the cholesterol diet were 200, 761 and 1434mg/100ml respectively. The distribution of serum cholesterol in the two groups was also analysed by ultracentrifugation. Animals on the beef-supplemented diet had raised HDL (alpha-lipoproptein) levels with a cholesterol concentration of 66mg/100ml (normal 24) and beta-lipoproteins at a cholesterol concentration of 96 in the S_f 20-400 fraction and 52 in the S_f 0-20 fraction. For animals on the cholesterol diet lipoprotein cholesterol concentration was 34 and beta-lipoprotein was 1184 in the S_f 20-400 fraction and 216 in the S_f 0-20 fraction. The authors concluded that the marked turbidity in the serum from cholesterol-fed animals was therefore not due to exogenous lipid particles (chylomicra), but to a marked increase of endogenous particles (subchylomicra) abnormally rich in

cholesterol. Their results agreed with those of Gresham α Howard that arterial lesions in animals on the beef-fat diet were characterised by more extensive extracellular lipid deposition in the intima and fewer foam cells than the corresponding lesions in cholesterol-fed animals in which foam cells were abundant.

Walton α Dunkerley (1974) extended their studies of the two different diets in relation to ocular lipid in the rabbit. They confirmed that both diets caused plasma cholesterol and beta-lipoprotein (LDL) to rise abruptly after a few days or weeks of feeding and then to attain a less steeply ascending plateau. The speed with which the plasma levels rose and the eventual height of the plateau region varied with the diet, being much more marked on the cholesterol diet.

Ocular lesions also developed more quickly and became more severe in the cholesterol-fed animals which showed the earlier and more pronounced hyperlipoproteinaemia. A progressive accumulation of mononuclear cells occurred in the region of the limbus and deep scleral plexus, in addition to perifibrous lipid droplets, in both groups of animals; but lipid deposition was faster, more severe, and fat-filled cells more abundant, in the cholesterol-fed animals. In more advanced lesions crystalline material was more profuse in animals on the cholesterol diet than in those on the beef-fat diet.

The most extreme ocular changes were seen in a cholesterol-fed rabbit which had been on the diet for 10 months. In addition to extensive involvement of the regions bordering the anterior chamber this animal showed an overall increase in the size of the eyeball. There were extensive xanthoma-like masses of lipid-filled foam cells in the retina, which was detached, surrounding the optic nerve and,

most strikingly, in the choroidal and sub-choroidal tissues where numerous sheaves of cholesterol clefts and cholesterol crystals were also present. A blood sample taken just before this animal was killed indicated a serum cholesterol level of 2750mg/100ml, of triglyceride 350mg/100ml and of beta-lipoprotein 5800mg/100ml.

Francois & Neetens (1964) had earlier noted the lack of a degenerative corneal lesion in rabbits fed olive oil alone rather than cholesterol dissolved in olive oil. The "xanthomatous degeneration of the cornea" with the latter diet has already been described. With the olive oil diet the authors refer to a "nacreous peripheral ring of the cornea" with no lucid interval, which appeared after about three to four weeks of olive oil feeding and thereafter changed little when observed over an eight month period. They do not mention whether or not this opacity was vascularised, but they do say that no other macroscopic lesions were observed in the eye or elsewhere in the body.

The influence of the type of lipid fed on the type of ocular lesion produced is of particular interest in view of the earlier observations concerning the clinical appearances of arcus lesions in man, dog and rabbit.

Occasionally bizarre diets administered to pet rabbits may produce abnormal infiltration of lipid within the eye. Gwin & Gelatt (1977) reported bilateral lipid infiltration of the corneal stroma, sclera and uvea in a two year old cottontail rabbit which had been fed an all-milk diet for two months. They noted Sudan IV positive material and many inflammatory cells which were apparently neutrophils. Xanthomatous masses were prominent in the anterior uvea with focal lipid depositions in the sclera and choroid.

Carrington (personal communication) was kind enough to supply details of a five year old Dutch Rabbit which over a two to three year period had been fed approximately half a pound of cheese each week plus quantities of buttered toast. This animal had a serum cholesterol level of 11.7mmol/l and extensive ocular involvement. There was obvious infiltration of the iris, a vascularised corneal arcus, diffuse but mild opacification of the aqueous humour and possibly cholesterol crystals within the vitreous. The ocular lesions had developed gradually over a period of months or years (the owners were not very observant). Unfortunately the animal died suddenly and was not available for post mortem examination. In view of the circumstances of its death it would have been of interest to know the extent of any atheromatous or atherosclerotic lesions.

2.3.4 Identification of Ocular Lipids The majority of microscopic examinations in the rabbit, other animals and man have been made on formalin-fixed paraffin material stained with haematoxylin and eosin or on Sudan stained material prepared in a number of ways. Polarising microscopy has also been frequently employed. Chuma (1923), Versé (1924, 1940), Rohrschneider (1925b), Loewenstein (1942), Cogan & Kuwabara (1958), Gwin & Gelatt (1977) and others were able to demonstrate Sudanophilic droplets, birefringent crystals and round cell infiltration. However, clarification of the sequence whereby death of cells containing globular lipid with subsequent necrosis produced granular lipid and cholesterol crystals was largely due to the work of Cogan and Kuwabara.

Janes (1964) fixed eyes in 10% formalin or Flemming's solution (osmic acid). Formalin fixed sections were cut frozen and some were

stained with Sudan black and others examined unstained with a polarising microscope. The osmic-acid fixed material was embedded in paraffin, sectioned, mounted and examined unstained.

Sudanophilic lesions were found in the cornea, iris, ciliary body and choroid both extracellularly and in lipid-filled macrophages.

The Schultz reaction produced a positive result in parts of the lesion where anisotropic crystals and droplets were present and these observations were interpreted as indicative of cholesterol. It was assumed that the Sudanophilic material was either a degradation product of cholesterol or that cholesterol was closely associated with some lipid which would take up the stain.

Francois & Neetens (1964) did not explain their preparative techniques for histological material. They mention the presence of birefringent lipid associated with xanthoma in various sites. The presence of neutral fats at the base of the iris was also referred to as was the absence of acid or neutral mucopolysaccharides "at the sites of spreading". The only stains specifically mentioned were haematoxylin and eosin, PAS, Reticulin Lagnesse and Masson's trichrome. In relation to the latter they report deep red staining on the scleral side of the limbus which they took to indicate a change in the lipoprotein contents of this region.

Roscoe & Vogel (1968) and Roscoe & Riccardi (1969) employed extraction procedures to analyse ocular lipids. Roscoe & Vogel determined a nine-fold increase in cholesterol concentration of the cornea after one month of cholesterol feeding. By the third month the increase of cholesterol concentration was 21-fold as compared with control animals. Phospholipid concentration doubled after one

month of feeding and thereafter a small but constant increase in phospholipid concentration occurred with time. For the iris and ciliary body the changes in cholesterol concentration were more pronounced than those for iridic phospholipid although both increased. The biggest increase for iridic phospholipid was found during the third month of cholesterol feeding, and the phospholipids of iris and ciliary body were essentially the same during the course of the experiment.

Roscoe α Riccardi (1969) fed rabbits 1% cholesterol for two to three months and compared the composition of the tissue phospholipid which accumulated with that of plasma. In both diseased cornea and iris they found an increase in sphingomyelin over the three month period. However, as the cornea also contained a high percentage of lecithin they assumed that the distribution of phospholipid in the diseased cornea was essentially the same as that found in the plasma. Conversely, the sphingomyelin content of diseased iris and atherosclerotic intima contained a higher percentage of sphingomyelin and a lower percentage of lecithin than did the plasma suggesting that in situ synthesis might be of importance rather than simple lipid infiltration from the plasma. The time for which the study was performed may also be of relevance.

Rodger (1971) was the first author to perform ultrastructural studies as well as cytological ones. He gives details of processing for the electron microscopy and infers that all examination techniques for cytochemistry were applied to unfixed sections cut on a freezing microtome.

Using polarised light and a first-order red quartz plate the author observed solid plate-like crystals of free cholesterol within

the cornea together with birefringent "Maltese Cross" liquid crystal droplets interpreted as mainly cholesterol combined with phospholipid. Isotropic droplets were also present and were identified as largely cholesterol esters.

With PAN and digitonin-PAN it was possible to ascertain the proportions of esterified and free cholesterol. OTAN with an alcian blue counterstain produced a heavy black stain around blood vessels at the corneal periphery, and less dense, more diffusely distributed small black droplets in the subendothelial stroma. On the basis of OTAN staining only, as no specific stains for triglyceride were employed, it was suggested that the perivascular lipid comprised triglyceride and the less dense, smaller, droplets cholesterol ester.

The early invasion by lipid was apparently dominated by the presence of droplets of free cholesterol along with cholesterol ester, triglyceride and phospholipid, the latter usually combined with free cholesterol. With prolonged cholesterol feeding a proportion of the free cholesterol droplets became crystalline and there was an increase in the amount of crystalline cholesterol ester.

With the electron microscope lipid was first detectable at the corneal periphery after about five months of cholesterol feeding and appeared as mainly large empty vacuoles or smaller osmiophilic ones occupying the paranuclear cytoplasm of the anterior stromal fibroblasts. The vacuoles were intermingled later with several free cholesterol crystalline spaces. In later stages occasional monocytes were seen at the corneal periphery; in early material they were "conspicuous by their absence".

Walton α Dunkerley (1974) carried out their histolgical procedures on frozen unfixed material supported in gelatine and snap frozen in isopentane cooled with liquid nitrogen, and on material fixed in neutral formalin and embedded in polyethylene glycols.

Frozen sections of unfixed material were examined by the fluorescent antibody technique. In some instances where precise comparison of LDL distribution (as revealed by the fluorescent antibody technique) with that of lipid (as shown by oil red O) was required, the immunological and lipid staining was performed sequentially on the same section, comparing photographs of the same field as previously described by Walton, Williamson α Johnson (1970).

When haematoxylin and eosin sections were examined there was a progressive accumulation of mononuclear cells in the region of the deep scleral plexus and limbus and the vessels were dilated and hyperaemic. Oil red O sections indicated intracellular lipid and extracellular lipid in the form of fine dark red droplets of perifibrous distribution and confined to the superficial cornea. In more advanced lesions extracellular lipid was present as bright-red amorphous masses.

Fine droplets of lipid were also detected in the ciliary processes and iris and the tendency to xanthoma formation has been referred to earlier. In all regions intracellular and extracellular acicular crystals, presumed to be cholesterol, became a feature of advanced lesions.

Specific fluorescence for LDL was found to correspond closely with the distribution of extracellular lipid as delineated by oil red O. Antisera to both fibrinogen and LDL gave particularly vivid

specific fluorescence in the walls of small arteries and arterioles in the cornea, ciliary processes and iris. Occasionally brilliant fluorescence with anti-LDL was obtained in iridal arterioles which lacked perivascular lipid as judged by oil red O staining, and the endothelial cells of such vessels may be involved in active pinocytosis of plasma lipid as lipoprotein.

Lipid-laden cells in close proximity to the extracellular lipid showed variable reactions for LDL, but generally the more advanced lesions, in which lipid was predominantly or exclusively within cells, were negative for specific fluorescence with either fibrinogen or LDL. The authors concluded that intact LDL permeated the tissues by an insudative process from plasma and that it was selectively entrained at sites rich in acidic mucopolysaccharides resulting in the formation of LDL-AMPS complexes. Intracellular lipid was largely a consequence of phagocytosis of LDL-AMPS concentrates with subsequent degradation of the lipoprotein molecule by intracellular proteases to leave a lipid residue detectable by conventional fat stains. Degradation of the complex may give rise to the release of cholesterol and other lipids into the tissues, thus promoting an inflammatory hyperaemia which exacerbated the process and later produced a sclerotic reaction. In the manner in which LDL-AMPS complexes form the pathogenesis is similar to that previously proposed for man, for both arcus formation and atherosclerosis (Walton α Williamson, 1968; Iverius, 1972; Walton, 1973).

However, results from others studying atherosclerosis and human arcus formation raise doubts about both the form in which lipoprotein is deposited and the extent to which glycosaminoglycans

might be involved in LDL retention. In addition, there are extracellular components other than glycosaminoglycans which could interact (Camejo, 1982). Smith α Slatter (1973) and Tschetter (1976) demonstrated that normal human peripheral cornea which lacked an arcus contained lipid similar to LDL and Winder, Sheraidah α Fielder (1978) showed that the single lipid-rich component present in saline extracts from human arcus material was of lower molecular weight and had a lower cholesterol/protein ratio than plasma LDL. Most of the extracts failed to react with antibody raised against plasma LDL and they neither contained, nor became bound to, glycosaminoglycans (Sheraidah, Winder α Fielder, 1981). Smith (1977) was able to show a direct linear relation between the relative concentrations of various plasma proteins and their molecular weight in the normal and diseased aortic intima. The continuity between LDL and other proteins supported the concept that LDL accumulated in the intima because of molecular sieving and not as a consequence of specific binding to glycosaminoglycans. As might be expected from its different properties and size, plasma levels of HDL do not apparently influence human arcus development (Winder, 1983).

2.3.5 Vascular Permeability and Ocular Lipid A relationship between trauma, vascularisation and fat deposition in the cornea was observed as early as 1924 by Versé and Rohrschneider who demonstrated that injury to the peripheral cornea produced a local increase in the arcus of the hypercholesterolaemic rabbit whereas injury to the centre of the cornea did not.

Van Herwaarden (1937) caused a marked increase of the arcus in an area of traumatised cornea in an experimentally

hypercholesterolaemic rabbit.

Versé (1940) described a rabbit with a heavily vacularised disciform lipid lesion in the right eye and an annular lipid lesion more typical of experimental arcus lipoides in the left.

Cogan α Kuwabara (1959) produced trauma in the eye of experimental rabbits with a variety of techniques. They used diathermy of the sclera, thermal cautery of the cornea and manipulation of a magnetic "flea" within the anterior chamber to traumatise the posterior aspect of the cornea. The authors compared the plaque produced by injury to a xanthoma. Corneal plaques were often associated with abnormal vascularity; they were most marked if neo-vascularisation occurred when the rabbit was hypercholesterol-aemic, but also resulted in less marked form as a consequence of corneal vascularisation prior to hypercholesterolaemia.

The effect of a magnetic "flea" in the anterior chamber was to produce a local increase in the arcus of the superficial portions of the cornea and additional xanthomatous plaques on the posterior aspect of the iris of the sector corresponding to the area of manipulation. The authors suggested that the effects on the iris were a consequence of altered blood vessel permeability rather than a direct result of injury.

Friedman α Byers (1959) investigated the effects of implantation of either normal aortic tissue or polyethylene discs in the iris of rabbits fed cholesterol and cottonseed oil and they concluded that deposition of lipid (including cholesterol) was enhanced by this procedure. In an additional study they found that intermittent exposure of the eyes of cholesterol-fed rabbits to intense light also provoked an earlier and more intense deposition

of lipid in the iris than was observed in the eyes of similarly fed rabbits kept under either ordinary laboratory conditions or in absolute darkness. The authors postulated that vasodilatation of the iridal vasculature as a consequence of their experimental procedures was the factor responsible for the greater deposition of lipid in the iris; in further support of this concept they demonstrated that if cholesterol feeding was delayed until the acute inflammation which followed intraocular implantation subsided, then iridic infiltration was less severe.

The same authors (1962) extended their investigations to the properties of newly formed capillaries in rabbits fed a 2% cholesterol:2% cottonseed oil diet. In all the types of very young vascular tissue they examined there was excessive permeability to lipid and cholesterol, with or without the presence of inflammation.

Rodger (1971) produced considerable modification of the experimental arcus lesion in the rabbit by performing limbotomy, and occasionally iridectomy, in animals maintained on a 0.5% cholesterol diet. Following limbotomy lipid droplets and crystals collected rapidly in the keratocytes a millimetre or two within the traumatised periphery and in the anterior stroma of the cornea. The arcs so produced were associated with "lucid intervals" and the arcs themselves broadened and in six months spread widely over the cornea.

Ultrastructural examination of the traumatised cornea indicated that the cytoplasm of the keratocytes was densely infiltrated by lipid vacuoles and crystalline spaces were present in the cell processes. Some of the keratocytes disintegrated to be replaced by amorphous osmiophilic material; there was also disorganisation of the collagen with loss of normal periodicity, and fragmentation or

condensation of the fibres. Even when the animal had been returned to a normal diet there was minimal repair or clearance. After a year on a normal diet monocytes were present in greater quantity and were observed digesting swollen keratocytes. It could not be ascertained whether the monocytes had been mobilised from the keratocytic syncytium or from the limbus. The author makes no mention of the presence of vascularisation in the damaged and undamaged corneas.

Walton (1973b) investigating experimental xanthoma production in the rabbit suggested that both hyperlipoproteinaemia and altered vascular permeability were important factors in the pathogenesis of xanthomata. Walton & Dunkerley (1974) suggested that in the rabbit, as in man, there was interdependence between hyperbetalipoproteinaemia and vascular permeability in determining the sites of LDL deposition in the tissues of the eye. In this respect it is worth reemphasising the more vascular arrangement of the iris and extensive ciliary processes in the rabbit as compared with man.

Walton & Dunkerley also postulated that the relative ease with which gross hyperlipidaemia can be induced in the rabbit, particularly with cholesterol based diets, may explain the rate of deposition and the degree of severity attained in the rabbit lesion as compared with man. The most severely affected animals had serum lipids and lipoprotein levels between 40- and 50-fold the normal value. In man, increases of serum lipids more than 5-fold over normal values are rarely encountered.

From the review of the literature pertaining to lipid-containing ocular lesions in the rabbit it is obvious that the quantity and type of fat in the diet and the period for which it is

eaten do indeed affect the development of the ocular lesion, but that in addition the anatomical arrangement of emissary vasculature and factors which influence vascular permeability must also be considered of importance.

In being initially a peripheral lesion in undamaged cornea the lesion resembles arcus lipoides corneae, in all other respects it is a form of lipid keratopathy.

2.4 OTHER ANIMALS

Lipid keratopathy has been reported in a neutered male cat (Carrington, 1983) in which the lesion developed at the site of a vascularised corneal ulcer. The animal had a raised serum cholesterol level of unknown cause.

2.5 SUMMARY OF LITERATURE

1. In the rabbit an association between hyperlipoproteinaemia and ocular involvement has been established. The appearance of the lesion can be modified by local and systemic factors. The corneal lesion produced in the cholesterol-fed rabbit is more like human lipid keratopathy than human corneal arcus.
2. In man, lipid keratopathy is rare. The higher incidence in women apparent in the literature has not been studied. There has been little characterisation of serum lipids and lipoproteins in affected patients, whereas corneal arcus has been associated with low density lipoprotein in some studies. Human arcus is typified by its peripheral location, persistence and lack of inflammatory phenomena such as vascularisation. Lipid keratopathy has been described in conjunction with a variety of anterior segment diseases, is always associated with vascularisation and is of variable location, usually more

central than peripheral. Corneal arcus and lipid keratopathy can be present together, or separately.

3. In other animals there is little information concerning the factors which may be important in the pathogenesis of lipid keratopathy.

4. Most studies indicate that cholesterol, in free and esterified form, is an integral feature of lipid keratopathy in all the species in which it has been recorded. Histological methods for lipid fixation, preservation and identification have generally been somewhat limited however, and the role of other lipids in lipid keratopathy has not been critically evaluated. The manner in which cholesterol can accumulate in, or be mobilised from, affected corneas, is imperfectly understood.

3.0 MATERIALS AND METHODS

3.1 Introduction

3.1.1 Source of Material The normal and clinical material used in this study was obtained through the courtesy of clients, veterinary surgeons in practice, British Veterinary Schools, the Animal Health Trust Small Animals Centre and a number of commercial organisations.

3.1.2 Method of Study Material for microscopic examination was obtained from a proportion of the clinical cases as part of their treatment and from a group of unwanted pets and unclaimed stray dogs presented for euthanasia.

Whenever possible, a clinical case was matched with a control animal of similar age, breed and sex, and the same procedures were followed for both.

3.1.3 Investigations Performed

General clinical and ophthalmological examination

Detailed examination of the anterior segment

Charting and photography

Temperature measurement (not all cases)

Electronic applanation tonometry (not all cases)

Anterior segment fluorescein angiography (not all cases)

Venous blood samples for routine analysis and serum lipid estimations

Lipoprotein electrophoresis

Keratectomy on some of the clinical cases (matched controls processed similarly)

Whole eye studies (clinical material at post mortem and normal eyes)

3.2 General Clinical and Ophthalmoscopic Examination

Full physical examination of the animal was followed by ophthalmic examination.

The eye and adnexa were examined briefly under artificial light and then in more detail in a darkened room. A focal light source and binocular loupe, with and without a condensing lens, and a direct ophthalmoscope were used for this examination. Particular attention was paid to abnormalities which might influence the integrity and normality of the cornea.

Any relevant diagnostic procedures such as intravital staining and the Schirmer tear test were also performed at this stage. When mydriatics were employed as part of the ophthalmic examination they were applied following detailed examination of the anterior segment. In occasional animals, the filtration angle was also viewed following topical application of local analgesic drops (Ophthaine, Squibb). For this procedure a Barkan low vacuum gonioscopy lens was used.

3.3 Detailed Examination of the Anterior Segment

This was performed using a slit lamp biomicroscope of the fixed (Gambs 1000 B) or hand-held (Kowa) variety, the methods employed being mainly those described by Duke Elder (1962).

Intraocular pressure was recorded indirectly using an electronic applanation tonometer (Mackay Marg) and the mean of two recordings used. The measurements were made on conscious non-sedated animals following topical application of local analgesic drops.

3.4 Charting and Photography

The extent of any corneal opacity was measured with corneal

callipers. A standard chart (Fig. 3.4/1) was used to record the appearance of the cornea and surrounding tissue, employing a scheme highly modified from Bron (1973) α Waring α Laibson (1977). In the en face diagram of the cornea given in Fig. 3.4/1, the central zone is denoted by (a), the paracentral zone by (b), the paralimbal zone by (c), and the sclera by (d).

Photography was used routinely as a means of recording ophthalmoscopic appearance. For this purpose a beam focussing camera (Rayner Wray), fundus camera (Kowa RC 2) and various 35mm single lens reflex cameras were used. Both the slit lamps employed had facilities for photography.

An operating microscope (Carl-Zeiss) was employed occasionally for patients undergoing keratectomy; this also had facilities for photography.

Kodacolour II 828 was used in the beam focussing camera and Kodachrome 25 in the other cameras.

3.5 Temperature Measurement

The surface temperature of specific areas of the anterior segment was measured using a rapidly responding thermocouple (Digitron Instrumentation) as sensor. The thermocouple was in the form of a spring-loaded surface probe of 3mm diameter and either an exposed glass-fibre insulated or PTFE insulated bead tip of between 0.3 and 0.5mm diameter. The probes were calibrated in a water bath with a reference mercury thermometer graduated to 0.01°C and were found to read with an accuracy of $\pm$ 0.3°C within the range 15°C to 45°C.

Temperatures were recorded at an ambient temperature of between 15°C and 20°C and at a relative humidity of between 50 and 60 per

Fig. 3.4/1

RECORD OF EYE EXAMINATION

Identification Number(s) | Synopsis of Ocular Complaint

Classification | Synopsis of Systemic Disease

Breed | Sex | Date of Birth

Dog's Name | Owner's Name

Previous Examination | Present Examination

Examination Technique: Direct/Indirect ophthalmoscopy | Pen torch/Loupe | SL Biomicroscopy | Other

Special Investigations

Regions Examined:

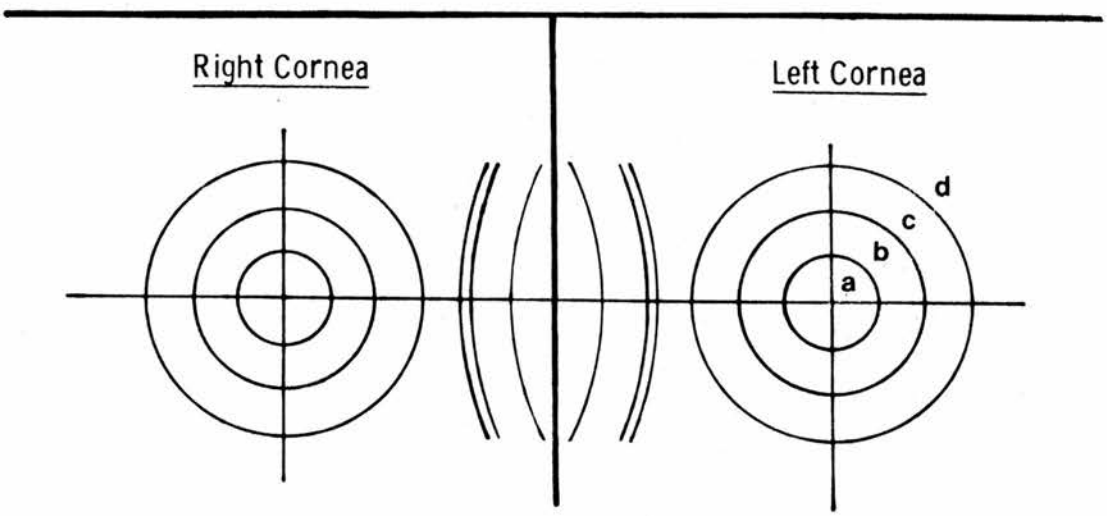
Fundus	Vitreous	Uvea	Pupil & Lens
Lens	Drainage angle	Cornea	Conjunctiva
Episclera	Sclera	Lacrimal	Eyelids

Photography

Intravital Stains | Cytology | Microbiology

Keratectomy/Other

Microscopy



cent. Analgesic drops were applied 10 to 15 minutes before measurements were made and the readings were taken after a 15 minute period of equilibration in a draught-free room.

3.6 Tonometry

An electronic applanation tonometer (Mackay Marg) was used to measure the intraocular pressure. The mean of two measurements was recorded and the recordings were made between 10.30 a.m. and 12.30 a.m. in fasted, resting, animals, following the application of local analgesic drops to each cornea.

3.7 Fluorescein Angiography of the Anterior Segment

This was performed on five normal Beagles as well as on a proportion of the clinical cases.

The technique was performed under general anaesthesia, using a thiopentone (Pentothal, Abbot), oxygen, halothane (Fluothane, I.C.I.) sequence, following premedication with acepromazine (Acetyl promazine, C.Vet.).

A 10 per cent, or occasionally a 20 per cent, solution of sodium fluorescein was administered as a bolus by rapid intravenous injection into the cephalic vein at a dose rate of approximately 25mg/kg and, in a proportion of cases, rapid serial photographs were taken using Kodak Tri-X film and a Fundus Camera (Kowa RC 2 Fluorescein Camera) with a Kodak Wratten 47A blue excitation filter in front of the flash source and a Kodak Wratten 15 yellow barrier filter in front of the camera. Whilst this method represented something of a compromise in that a slit lamp fitted with an orange barrier filter is the usual equipment employed, the results were acceptable. Choromokos (1982) has reported a similar technique in man. The photography was usually carried out on the right eye with

the animal in left lateral recumcency.

Rapid serial photographs were taken five to seven seconds after the injection had been made. Once fluorescence was observed approximately four to six photographs were taken rapidly before any fine focussing adjustments were made whereupon photography continued. Serial photography was performed over a three minute period and individual photographs were also taken at four minutes, five minutes and ten minutes.

3.8 Venous Blood Samples

3.8.1 Introduction The importance of standardisation and technique in the examination of blood lipids and lipoproteins have been emphasised by Cramp (1972) and Lewis (1976).

Accordingly, after a standard 15 hour fast, venous blood samples were taken from the external jugular or cephalic vein. Samples were analysed for cholesterol, triglyceride and phospholipid, and those for serum lipoprotein estimation were examined visually following centrifugation and also by serum electrophoresis.

In the majority of dogs routine haematology, blood glucose and blood urea estimations were also performed and, in a proportion of the clinical cases, more specific examinations were required to determine the underlying cause of secondary hyperlipoproteinaemia. The results of these tests are only given when relevant, with the case histories, and are not presented in detail.

3.8.2 Lipid Analysis Both serum and plasma samples were used for determination of cholesterol, triglyceride, phospholipid and lipoprotein electrophoresis.

Serum Samples Blood was taken into a plain glass tube and the serum separated by centrifugation at not less than 1,500G for 5

to 15 minutes. The sample was usually analysed immediately, or stored for a short period in a refrigerator at 4°C prior to analysis. Visual examination of the serum was performed following the short storage period and prior to other analytical procedures.

Plasma Samples Blood was taken into E.D.T.A. or heparin and centrifuged immediately after cooling to 4°C. The plasma extracted was analysed immediately or stored temporarily at 4°C. The residue of samples were stored at -20°C.

Cholesterol estimations were performed on duplicate samples using the Technicon-N-24a semi-automated photometric method.

Triglyceride estimations were performed on duplicate samples using the Technicon-N-78 semi-automated method with fluorometric analysis.

Phospholipid estimations were performed on duplicate samples using a colorimetric method based on the molybdate/vanadate reaction (Boehringer).

Lipoprotein Electrophoresis Paper electrophoresis was performed according to the method of Lehman α Lines (1972) and agarose gel electrophoresis after Lines (personal communication). Electrophoretic patterns were scanned by a densitometer (Beckman microsome densitometer: Beckman Instruments) for qualitative evaluation of the lipoprotein patterns.

3.8.3 Other Estimations In addition to routine haematology and analysis of blood glucose and blood urea other tests included estimations of thyroid, pituitary, adrenal and hepatic function, as and when they were deemed necessary.

The procedures followed were those of the Department of Veterinary Medicine at Edinburgh University as set out by Arslan

(1980) and, in addition, those reported by Crispin α Barnett (1978), Crispin α Langslow (1980), Lavelle α Yoxall (1980), Kaneko (1980), Lorenz α Stiff (1980), Ross α Valori (1982), Eckersall α Williams (1983), α Graham (personal communication).

3.9 Microscopic Studies

3.9.1 Keratectomy and Whole Eye Studies.

Keratectomy. This procedure was undertaken as a means of treatment with the advantage of providing material for study.

The quantity of material available from keratectomy was rarely sufficient to allow more than light microscopy and transmission electron microscopy. When more material was available it was also examined with a scanning electron microscope.

Anaesthetic Technique. Animals in good general health were usually premedicated with acepromazine and atropine (Atropine, M α B).

An ultra-short acting barbiturate such as 2.5% thiopentone or 1% methohexitone (Brietal, Eli Lilly) was used for induction.

A cuffed endotracheal tube was passed and anaesthesia was maintained with halothane or methoxyflurane (Penthrane, Abbot), delivered from a calibrated vaporiser and volatilised by oxygen and nitrous oxide. A semi-closed anaesthetic circuit was employed.

Following surgery, post-operative recovery took place in warm and quiet conditions.

In those animals in which intercurrent disease was present, the anaesthetic technique was modified appropriately (Crispin, 1980); for example, the lowered basal metabolic rate associated with hypothyroidism renders affected animals particularly sensitive to drugs of any type.

Keratectomy Technique The lesion was either removed intact within a surrounding rim of tissue which appeared normal (as viewed with naked eye or operating microscope), or representative portions of more extensive lesions were biopsied along with macroscopically normal tissue. For vascularised lesions, dissection was directed towards the origin of the blood supply at the edge of the lesion.

The aim in performing the keratectomy, and indeed subsequent fixation and embedding, was to disturb the normal corneal architecture as little as possible. To this end the eye was gently irrigated with balanced salt solution to prevent inadvertent drying of the cornea whilst the sample was being excised; and the tissue was placed on non-absorbent card of about 100μm thickness, usually with the posterior aspect of the keratectomy specimen against the card.

Whole Eye Studies For pairs of normal eyes, one was usually fixed whole or after hemi-section, whereas the other was dissected into much smaller pieces at the outset or sampled by keratectomy.

Eyes fixed whole were attached to pieces of cork by the optic nerve and suspended in the fixative; usually the aqueous humour was replaced by fixative, care being taken to preserve the normal corneal profile during this procedure.

When there was asymmetry between the eyes or within the lesion itself, the approach was modified to ensure that material for light microscopy and transmission electron microscopy was received from each relevant area. If possible, the tissue was bisected in situ or ex situ to provide mirror image portions for the two techniques.

Controls The positive and negative controls employed will be

described with the various techniques.

3.9.2 Examination of Material for Light Microscopy. This was undertaken using a Leitz Ortholux-II, Ortholux-Pol or Reichert-Jung Polyvar microscope, all of which had facilities for photomicrography. Measurements were usually made using an eyepiece graticule calibrated with a stage micrometer graded in 0.01mm divisions (Graticules Ltd., Kent).

The Ortholux-II microscope was used with a microscope stage which could be heated to 80°C; the microscope also had facilities for polarisation, fluorescence and phase contrast microscopy as well as for standard brightfield illumination.

Examination in polarised light was also possible with the Ortholux-Pol microscope and polarisation studies were undertaken with the aid of a $^1/_4\lambda$ mica plate and a first-order red gypsum accessory plate.

The Ortholux-Pol and Polyvar microscopes also had facilities for Nomarski differential interference contrast microscopy.

Black and white photographs were taken with Ilford FP4 and colour photographs with Kodak High Speed Ektochrome.

3.9.3 Lipid Studies In an attempt to overcome some of the problems inherent in lipid histochemistry, the optical and physical properties of lipids obtained from fresh imprint preparations were examined, in addition to using the standard histochemical methods which have been comprehensively reviewed by Baker (1946a), Adams (1965, 1969a) and Bayliss High (1972, 1982).

Unfixed Material. Whilst hydrophobic apolar lipids are retained well within tissue sections cut on the freezing microtome, this is not the case with hydrophilic polar lipids (Adams,1969). Using thin

layer chromatography Roozemund (1967) demonstrated that water extracts a large part of the polar lipids from unfixed sections.

Fixed Material. Formaldehyde alone when used for fixation may affect hydrophilic and hydrophobic lipids and is not an ideal fixative for protein (Halliday, 1949; Baker, 1958; Jones α Gresham, 1966; Adams, Knox α Morgan, 1975; Bayliss High, 1982). Polar lipids, notably phosphoglycerides, may be degraded to their water-soluble derivatives whereas sphingomyelin is little affected. Unsaturated fatty acids react with formaldehyde. Hydrophobic lipids appear to be less susceptible to the effects of formaldehyde although prolonged fixation may produce crystallisation.

Baker (1945, 1946a) showed that the addition of calcium ions to formalin improved lipid preservation, particularly of hydrophilic phospholipids, possibly by the formation of insoluble calcium-phosphate-lipid complexes. Formol-calcium is the best general fixative for lipid histochemistry (Bayliss-High, 1972). Fixation times should not exceed two to three days for blocks of tissue and one hour for cryostat sections of fresh tissue, as longer fixation impairs the staining of phosphoglycerides (Bayliss-High, 1982). Calcium ions derived from the fixative form calcium soaps with free fatty acids so that desaponification is required for fatty acid regeneration.

Temperature. The physical form of some lipids may also be affected by changes of temperature. High temperatures are required for certain histochemical techniques and low temperatures are employed in cutting cryostat sections. Adams α Bayliss (1975) investigated atherosclerotic lesions from rabbit and man and confirmed that true crystals were present at 37°C. As such crystals often increase in

size and numbers on cooling, they suggested that a proportion were derived from enlargement of existing crystals and that others were actually newly formed crystals.

Physical and Optical Properties It was because of the preceding considerations that standard histochemical techniques on cryostat sections were supplemented by the investigation of optical and physical properties as described. The properties were investigated using fresh unfixed material which was imprinted or squashed on to a degreased microscope slide warmed previously to 33°C and maintained at this temperature whilst being transported to the heated microscope stage. The stage had been equilibrated at 33°C for 30 minutes prior to the examination. A proportion of the material was examined without further preparation whilst some was mixed gently with 80% glycerol at 33°C (Hata, Hower α Insull, 1974). The results from this technique were felt to relate more closely to the situation within the cornea than those obtained from standard histochemical methods. A polarising system with a ¼λmica plate and first order red gypsum accessory plate was used in order to visualise isotropic droplets in addition to anisotropic droplets and crystals. Examination of the squash or imprint preparations included an estimate of the size and relative proportions of the various lipid phases (solid crystals, liquid crystals, lipid droplets) and their thermotropic and lyotropic properties. Some preparations were also stained with a variety of lipid stains if sufficient material was available.

Thermotropism. The effects of heating and cooling were assessed by heating the microscope stage at about one to two °C per minute to the maximum temperature available of 80°C. The temperature of any

phase change (phase transition) was recorded. After each phase change the temperature was reversed at a rate of approximately 1° per minute to assess the reversibility of the transition.

Following heating, the specimen was allowed to cool slowly to room temperature and observed for phase changes during this process.

If sufficient material was available, a slide was placed immediately onto dry ice at the time of keratectomy, as a way of rapid quenching to simulate the conditions under which some specimens were taken for lipid histochemistry. This material was then examined without further preparation at room temperature.

Lyotropism. Those specimens not mounted in glycerol were dried and then 0.9% saline or distilled water was added to test the effects of dehydration and rehydration.

Finally, the specimen was heated to 155°C in an oven and examined at approximately 10°C intervals during this procedure and when cooled again to room temperature.

Lipid Histochemistry Samples for lipid histochemistry were divided into two portions: one portion was frozen in liquid nitrogen with Arcton 12, and 10μm sections were cut unfixed at a temperature of -20°C to -30°C on a cryostat. The other portion was fixed for a short period in calcium-cadmium-formol (Baker, 1944), or 1% calcium acetate-4% formol (Lillie, 1954), or 2% calcium acetate-2% trichloracetic acid-10% formalin (Adams, Knox α Morgan, 1975), before being cut on the cryostat at 10-15μm. Fixed sections were attached to degreased slides or coverslips which had been coated with chrome-gelatin, whereas unfixed sections were attached directly to degreased slides or coverslips.

If samples had to be transported any distance before sectioning

was possible, the unfixed material was supported in either a 20% gelatin or a 7% gelatin-in-Azide freezing mixture in a freezing tube, prior to immersion in liquid nitrogen. It was then transported in liquid nitrogen, or on dry ice, and either stored en bloc in a deep freeze at -20°C, or sectioned on the cryostat prior to staining or storage. Fixed material was infiltrated by a gelatin-glycerol-thymol mixture and transported in this fashion. The material could then be either sectioned as above, or trimmed into small blocks and stored in calcium-cadmium-formol. These supporting mixtures were also useful when dealing with very small specimens.

The fixed and unfixed cryostat material was examined as soon after removal from the animal as possible. Any material remaining was either stored temporarily in the cryostat cabinet at -20°C to -30°C, or in a deep freeze at -20°C until required.

Wherever possible, normal material from a dog of similar age and breed was processed at the same time as the clinical material, together with known positive controls.

Controls The positive controls employed for lipid histochemistry were as follows:

human atherosclerotic aorta and coronary arteries for cholesterol and cholesterol ester,

canine adrenal gland for cholesterol ester,

canine fatty liver for triglycerides and fatty acids,

canine brain, myelinated nerve, and red blood cells for phospholipids.

Methods: Extraction Techniques. Chloroform-methanol (2:1 v/v) at 20°C for one hour was used as a general lipid solvent. Occasionally acetone-ether (3:1 v/v) was employed in similar fashion.

Anhydrous acetone (acetone at 4°C dried over anhydrous calcium chloride) for one hour was used for extraction of apolar (hydrophobic) lipids (Keilig, 1944; modified by Elleder & Lojda, 1971).

Hydrochloric acid (1%) (LeBaron & Folch, 1956) and 25% aqueous acetic acid (Kiernan, 1981) were used to disrupt lipoprotein bands, and hydrochloric acid was combined with either chloroform-methanol or anhydrous acetone to effect additional lipid extraction.

Pyridine extraction (Baker, 1946a) was used for phospholipid extraction in the early part of this work but chloroform-methanol-hydrochloric acid (66:33:1 v/v/v) was substituted as it was found to be more efficacious.

Bromine Sudan Black B Method (Bayliss & Adams, 1972) was used as a screening method for any type of lipid, a blue-black colouration being obtained. When combined with anhydrous acetone extraction, the technique was used to detect phospholipids alone (Bayliss High, 1981).

Standard Sudan Black B Method (Lison & Dagnelie, 1935) in 70% ethanol was used, with which unsaturated cholesterol esters and triglycerides stain blue-black and some phospholipids stain grey-blue. The phospholipids of myelin exhibit a bronze dichroism in polarised light with this technique.

Oil Red O Method (after Lillie & Ashburn, 1943): this dye stains unsaturated hydrophobic lipids red while some phospholipids appear pink. The oil red O was made up in 70% ethanol. Sudan III and IV were also used in the early stages of this work but offered no advantages over oil red O and were discontinued.

Nile Blue Sulphate Method (Cain, 1947, 1948): this technique utilises an oxazone component which dissolves in neutral lipids so that unsaturated hydrophobic lipids stain red or pink; and an oxazine component which is basic, so that any phospholipid present usually stains blue. Free fatty acids stain pink to blue with this method. A mixture of hydrophobic and hydrophilic lipids may produce a mauve colouration.

Acetone - Nile Blue Sulphate (Dunnigan, 1968) was used for the detection of phospholipids which stain blue.

Schultz Reaction for Cholesterol (Schultz, 1924): this is an adaptation of the Liebermann-Burchardt reaction which can be used to demonstrate cholesterol in tissue sections. As modified by Feigin (1956) and Schnabel (1964), it allows detection and differentiation of cholesterol and cholesterol esters. The reaction, however, was found to produce complete disruption of the tissue; accordingly PAN and digitonin-PAN methods were substituted at an early stage in the study.

Perchloric acid-naphthoquinone (PAN) method (Adams, 1961): this method produces a more precise location of the reaction products and is a more sensitive way of detecting cholesterol (Adams α Bayliss High, 1980). Using PAN, preceded by oxidation with ferric chloride, cholesterol and its esters were stained blue or red.

Digitonin-PAN method (Adams α Bayliss, 1974; after Schnabel, 1964): this technique precipitates free cholesterol and thus allows its detection with the PAN method, following acetone extraction of cholesterol esters. Free cholesterol is stained blue or red. The two methods are used in conjunction to detect and differentiate cholesterol and cholesterol esters. Precipitation of cholesterol

with digitonin and acetone extraction of esters without PAN staining was sometimes used in sections which were to be photographed as this combination produced less distortion.

Osmium tetroxide Method (Adams, Abdulla α Bayliss, 1967) was used, wherein unsaturated lipids stain brown to black, whereas saturated lipids and crystalline cholesterol do not react.

The Osmium tetroxide alpha-naphthylamine (OTAN) Method (Adams, 1959) has fallen into disfavour in recent years (Adams α Bayliss, 1968, 1974) because the differentiation of unsaturated hydrophilic polar lipids (orange-red-brown) and unsaturated non-polar lipids (brown to black) is not always clear. However, provided the limitations of the technique are appreciated when interpreting the results, the method is of value for the conditions being investigated (Bayliss-High, personal communication). The method was also used in modified form with hydrolysis in 2N-NaOH for the detection of sphingomyelin (Adams, 1965).

Acid Haematein Method (after Baker, 1946b) stains choline-containing phospholipids blue-black; when combined with the oil red O method hydrophobic non-polar lipids are also stained, allowing simultaneous demonstration of polar and non-polar lipids. The method was also used with sodium hydroxide as a means of alkaline hydrolysis to allow detection of sphingomyelin, which stains blue (Adams α Bayliss, 1963).

The method was used on parallel sections in conjunction with cold anhydrous acetone for extraction of non-polar lipids.

Copper Rubeanic Acid Method (Holczinger, 1959; modified by Elleder α Lojda, 1972). Using this technique, free fatty acids are stained dark green to black. Control sections were extracted with cold

anhydrous acetone to ensure that positive results were due to free fatty acids and not to phospholipids.

Calcium lipase-lead sulphide Method (Adams, Abdulla, Bayliss α Weller, 1966): this enzyme method stains triglycerides brown to black. A duplicate control section which was not subjected to enzyme treatment was included as a matter of routine.

In material which had reacted positively for free fatty acids potassium hydroxide-dioxan extraction was performed prior to the calcium lipase-lead sulphide technique as recommended by Archibald α Orton (1970). Otherwise the presence of free fatty acids would produce a positive reaction in both the lipase-treated sections and the controls making specific identification of triglycerides unreliable.

3.9.4 Enzyme Histochemistry The lipases are generally held to be enzymes capable of hydrolysing long chain fatty acid esters, possibly with some predilection for the unsaturated ester compounds, whereas esterases hydrolyse short chain fatty acid esters (Pearse, 1960; Abe, Kramer α Seligman, 1964). However, Brockerhoff (1970) showed that purified pancreatic lipase was able to hydrolyse short chain fatty acid esters and that esterases could hydrolyse long chain fatty acid esters. Lake (1972) reasoned that to differentiate lipases from esterases on the basis of the chain length of the fatty acid ester was not valid and that any enzyme hydrolysing fatty acid esters under specified conditions should be described as a fatty acid hydrolase, rather than preserving an artificial distinction between esterases and lipases as though they were unrelated enzymes.

The multiformity of esterase activity has also been examined by a number of workers (Choudhury, 1972; Lake, 1973; Hashimoto α

Dayton, 1974; Wolman, 1974; Bayliss, 1975). Holmes & Masters (1968), in a comparative study of the multiplicity of mammalian esterases, derived two groups of amylesterases, three groups of cholinesterases and five groups of carboxylesterases. The situation is further complicated by the suggestion that in some animals microsomal esterases may be able to act as hydrolases as well as synthetases (Hashimoto & Dayton, 1974).

The application and pitfalls of enzyme histochemistry have been reviewed by Adams (1969b).

Methods Because of the limited amount of material available, these techniques were restricted to identification of non-specific esterase, so-called lipase, and acid phosphatase.

Two methods were used for the demonstration of non-specific esterase, and parallel sections were treated with the inhibitor diethyl-paranitrophenyl phosphate (E600).

The alpha naphthyl acetate esterase method (Gomori, 1950; modified by Davis & Ornstein, 1959 and Lake, 1971) was used on short-fixed, formol-calcium acetate material. A reddish-brown colour indicated sites of esterase activity and either methyl green, Carrazzi's haematoxylin haemalum, or no counterstain was used. The specificity of the reaction was checked on parallel sections with 10^{-5}M diethyl-paranitrophenyl phosphate (E600) which inhibits non-specific B esterases. E600 sensitive esterases appear to reside in the microsomal cell fraction and positive results with alpha naphthyl acetate alone show a diffuse intracytoplasmic distribution. E600 resistant acid esterases are of lysosomal origin (Lake & Patrick, 1970; Bayliss, 1975). Incubations were performed at pH 5.8 and occasionally at pH 5.8 and 7.5.

In a proportion of the material the technique was also applied to parallel sections delipidised with chloroform-methanol.

For paraffin sections, a chloracetate method with Fast Blue RR was used, after Burstone (1962). Sites of esterase activity were indicated by varying shades of blue. Mayer's haemalum was somtimes employed as a counterstain and E600 as inhibitor at 10^{-5}M concentration and pH 5.8.

The Tween Method (Gomori, 1952) was used for detection of lipase activity which is denoted by a yellow to brown-black colouration. Pearse (1960) considered this an insensitive and unsatisfactory method, but supposedly capable of distinguishing enzymes which hydrolyse long-chain fatty acid esters (lipases) from those which hydrolyse short chain fatty acid esters (esterases). The validity of this artificial distinction has been discussed earlier.

Acid phosphatase. Short fixed cryostat sections were used for light microscopy. The Gomori lead method (Gomori, 1941) and the naphthol As-BI phosphate method (Burstone, 1958; modified by Barka, 1960 and Barka α Anderson, 1962) were both used with methyl green as a nuclear counterstain. Sites of acid phosphatase activity were black with the Gomori lead technique and red with the naphthol As-BI phosphate method.

Positive control material, usually canine pancreas, was processed in parallel.

Two lead capture methods were used for the ultrastructural localisation of acid phosphatase activity; both produced a black reaction product. One method employed 3% glutaraldehyde in 0.1M cacodylate buffer as the prefixative, after Etherton α Botham (1970)

whereas the other used paraformaldehyde in phosphate buffer (Pease, 1964). Tissue prefixed in glutaraldehyde produced superior results and became the method of choice.

3.9.5 Other Staining Methods For these and other methods where frozen sections were not routinely employed, the tissue was usually fixed in neutral formal saline, formal-calcium or Millonig's phosphate-buffered formalin (Carson, Martin α Lynn, 1973). After processing, it was embedded in paraffin wax and celloidin and 5-15μm sections were cut on a rotary microtome (American Optical 820).

Methods Haematoxylin and Eosin. In addition to its use as a general histological stain, haematoxylin was a widely employed counterstain for a number of the techniques used on frozen material. Ehrlich's, Mayer's, Harris's, Carazzi's and Weigert's or Heidenheim's haematoxylins were used according to the method employed.

Neutral Red-Fast Green F.C.F. (Monroe α Frommer, 1967) was occasionally used as an alternative to haematoxylin and eosin.

A number of trichrome techniques were employed but were not applied to all the material; they included Masson's (Masson, 1929), the Phosphotungstic acid haematoxylin (PTAH) Method (Mallory, 1938), the Van Gieson Technique (Van Gieson, 1899) and the Martius, Scarlet, Blue (MSB) Technique for fibrin (Lendrum, Fraser, Slidders α Henderson, 1962).

Protein Methods. Millon Reaction (Baker, 1956) was used to detect tyrosine-containing proteins which should stain red or pink. Performic acid-Alcian blue Method (Adams α Sloper, 1955) was used to locate disulphides such as cysteine or cystine, which stain blue

with this technique.

D.M.A.B.-nitrite Method (Adams, 1957) was used for detection of tryptophan which stains dark blue. Unfortunately fixation in formaldehyde for more than a day or two prevents the reaction of tryptophan with other aldehydes such as the para-dimethylamino-benzaldehyde of the stain. Accordingly, tissues unsuitable for the DMAB method were stained with the glycerol-ferric chloride technique of Adams (1960). With this method tryptophan produces a mauve colouration.

The modified Sakaguchi Reaction (Baker, 1947) was used to detect arginine which produces an orange-red colouration in 12-15μm sections. The positive controls employed were canine pancreas for tryptophan and tyrosine, canine skin for cystine/cysteine, and canine lymphoid tissue or testis for arginine.

Carbohydrate Methods. (Glycogen and Mucins) These techniques were usually applied to formalin-fixed paraffin wax embedded material prepared as previously described. Lake (1976) has indicated the inadequacy of some fixation methods for the demonstration of acid mucins.

The Periodic acid-Schiff (PAS) Reaction (McManus, 1946) is widely applied in carbohydrate histochemistry, staining material because of its hexose content (Garner, 1976). The range of PAS positive material includes glycoproteins, glycolipids, some phospholipids and neutral mucins. Glycogen can be readily demonstrated by this method, usually employing a parallel diastase digested control. Acid mucins are essentially PAS negative. The complex relationships of the PAS reaction have been discussed by Pearse (1968).

Periodic acid-Schiff combined with Alcian Blue (Mowry, 1956) stains both neutral (magenta) and acid (blue) mucins.

Alcian Blue Solutions of varying pH (after Steedman, 1950) aid differentiation of acid mucins. In practice, a pH of 0.5 and 2.5 was usually employed, achieved by dissolving 1g of alcian blue in 100ml of N/5 hydrochloric acid or 100ml of 3% acetic acid respectively. The more strongly sulphated mucins stain well at pH of 1.0 or less, whereas the weakly sulphated mucins stain reasonably at a pH of 2.5. A 0.5% aqueous neutral red counterstain was used.

Alcian Blue combined with Aluminium Sulphate (Heath, 1961) was sometimes used for staining sulphated mucins which appear blue whereas nuclei are stained red.

Colloidal ferric hydroxide Method (Hale, 1946; Kiernan, 1981) stains proteoglycans and glycoproteins a strong Prussian blue colour; iron deposits, if present, also stain dark blue, and the colloidal ferric hydroxide stage was omitted to provide suitable controls.

In practice the colloidal ferric hydroxide, PAS methods and alcian blue of varying pH were the carbohydrate methods routinely employed.

Proteoglycans were visualised in the electron microscope following incubation of some material in 3.2% glutaraldehyde and 0.05% ruthenium red in 0.1M cacodylate buffer at pH 7.4 according to the method of Ausprunk, Boudreau α Nelson (1981). Ultrathin sections were examined unstained, or after staining with uranyl acetate and lead citrate.

Highman's Congo Red Technique (Highman, 1946) was used in conjunction with polarising microscopy. In plane light, amyloid

stains pink whereas in polarised light there is a characteristic apple green fluorescence.

Perls' Prussian Blue Reaction (Perls, 1867) for ferric iron was used to demonstrate haemosiderin which appears blue. Canine spleen was used as a positive control.

Sudan Black B Method (previously described) was used on paraffin and celloidin material to demonstrate early lipofuscin and possible lipoproteins. Both lipofuscin and lipoprotein may stain black with this method.

Pigments such as lipofuscin were investigated according to methods described by Stevens (1982), for example, Schmorl's Method was used for the detection of late lipofuscin although other substances such as melanin and haematoidin also stain blue.

Lipofuscin may also be demonstrated by its staining with PAS and its inherent primary fluorescence. In practice, light microscopy proved a convenient way of confirming the findings of transmission electron microscopy whereby lipofuscin is easily identified.

Melanin methods were applied as controls for some of the techniques given above. The most useful characteristic of melanin in this respect being its removal by bleaching in 0.25% potassium permanganate followed by 1% oxalic acid (Pearse, 1960). Bleaching of melanin was also necessary in a proportion of the normal material with heavy pigmentation of the limbus.

Von Kossa's Method for calcium salts (Von Kossa, 1901) was also employed routinely. With this technique calcium phosphates and calcium carbonates appear black.

3.9.6 Scanning Electron Microscopy (SEM) Scanning electron microscopy was used to study normal eyes and a proportion of keratectomy specimens using techniques after Hayat (1973) & Robinson & Terras (1982).

Fixation. Whole eyes, parts of the anterior segment and keratectomy specimens were usually rinsed in balanced salt solution before immersion in ice-cold 4% glutaraldehyde in 0.1M cacodylate buffer.

For whole eyes the aqueous humour was replaced by fixative and sometimes the eyes were transected in an equatorial plane at various distances behind the limbus halfway through fixation. Material was fixed for a minimum of four hours, and up to 18 hours for larger specimens, at 4°C, with a change of fixative approximately halfway through the schedule.

Following fixation, the material was trimmed to the required size, rinsed several times in cacodylate buffer and then post-fixed for two hours in 1% osmium tetroxide.

Dehydration. Graded acetones were used for dehydration and on occasions, material was stored in anhydrous acetone at 4°C for examination at a later date. The acetone was removed by critical point drying with liquid carbon dioxide (after Anderson, 1951).

Coating. The dried specimens were mounted onto aluminium stubs with silver DAG and coated with a 20nm layer of gold in a coating unit (Polaron E5100). The specimens were viewed in a scanning electron microscope (ISI 60 and Philips 505) at approximately 30 kV. Coated specimens were stored for short periods in the clean dry atmosphere of a dessicator.

Microvascular Replicas. Microvascular castings of the ocular

vessels were prepared by cannulation of the vortex veins or long posterior ciliary arteries according to the technique described by Van Buskirk (1979 and personal communication). Batson's methyl methacrylate corrosion compound No. 17 was used for the microvascular casts and the preparations were examined in the scanning electron microscope.

3.9.7 Transmission Electron Microscopy Transmission electron microscopy was used for the examination of normal and abnormal material, following principles and methods reviewed by Glauert (1965a & 1965b), Hayat (1973 & 1981) & Robinson (1982).

In most cases, pieces of tissue no greater than 0.5mm to 1.0mm were used, but for some of the normal material whole eyes were fixed for approximately one hour before being subdivided. When whole eyes were fixed, the aqueous was replaced by fixative without producing any distortion or deviation from the normal appearance. Material was always subdivided using single clean cuts from an extremely sharp and long razor blade so that deformation of the tissue was avoided; subdivision was performed with the specimen covered in fixative or buffer.

Prolonged fixation was avoided because of its tendency to produce such artifacts as myelin figures and extraction of cellular materials (Hayat, 1973 & 1981).

Methods.

4% glutaraldehyde in 0.1M Cacodylate buffer (with calcium chloride) (after Robinson, 1982): specimens were immersed in room temperature or ice-cold (0-4°C) fixative immediately after removal, and fixed at the same temperature. After several washes in buffer, they were post-fixed in 1% osmium tetroxide, rinsed in distilled water,

dehydrated in graded acetones and embedded in araldite.

3% glutaraldehyde in 0.12M Millonig's phosphate buffer (with calcium chloride (after Hayat α Giaquinta, 1970). This procedure is very much shorter than the usual processing methods; fixation, staining, dehydration, infiltration and embedding, including polymerisation, being completed within four hours as compared with approximately 36 hours using conventional methods.

Specimens were fixed at room temperature for 30 minutes or for longer periods at 0-4°C. After several washes in buffer they were post-fixed in osmium tetroxide and then stained en bloc with 2% aqueous uranyl acetate. Dehydration through graded acetones was followed by embedding in Epon 812.

Minor modifications applied to the techniques given included the use of graded alcohols followed by transitional stages in 1:2 epoxypropane (propylene oxide) instead of graded acetones and slight variations in the time schedule.

Occasionally Millonig's phosphate-buffered formalin and neutral buffered formalin were used for specimens obtained from other centres.

After embedding in Epon and Araldite and following polymerisation, the block face was trimmed to a trapezium preparatory to sectioning.

Thick Sections. 1um sections were cut with a glass knife on an ultramicrotome (Reichert Ultracut OMU4) and stained with 0.5% toluidine blue in 1% borax at 60°C.

Ultrathin Sections. 60-70nm sections were cut with a diamond or glass knife on an ultramicrotome and mounted on uncoated copper grids. The sections were stained with saturated uranyl

acetate in 50% alcohol followed by lead citrate (Reynolds, 1963) and viewed in an electron microscope (Philips 400).

4.0 RESULTS - NORMAL ANIMALS

4.1 Introduction

Animals included as normal controls were free from signs of systemic disease and anterior segment abnormalities. They are summarised in Table 4.1/1. It was possible to match 24 normal controls with clinical cases by age, sex and breed during the course of this study and the analyses are presented in the next Section.

A subjective assessment of normal size and shape was made for each dog. In addition its weight was recorded (Table 4.1/1).

All animals were being fed a diet based on commercially manufactured pet foods of known standard and satisfactory composition. Individuals were usually fed the same brand and amount from day to day. A proportion were given other foods such as table scraps, tripe, offal, bones, vegetables, dairy products, biscuits, sweets, bread and cereals, either regularly or occasionally. Some dogs received vitamin and mineral supplements. The diets of individuals are summarised together with serum lipid values in Table 4.5/1.

4.1.1 General Examination: Summary and Discussion Whilst there is information available as to the average size and weight of pedigree dogs, most authorities would emphasize the considerable individual variation, particularly in relation to energy needs (Walker, 1983).

Subjective assessments of physical condition are routinely employed as part of the clinical examination and were of value in selecting the "average" dogs which formed the normal control group. The weight of the animal served largely to emphasize the very great differences between breeds of dog but was useful as a basis for

Table 4.1/1 Summary of Normal Animals Used as Controls

Identification Breed/Number		Age Years.Months	Sex M/F/Mn/Fn	Weight Kg	Investigations a	b	c	d	e	f	g	h	i
Alsatian	21	5.2	M	32	+	+	+	-	-	-	+	+	+
"	23	4.0	F	26	+	+	+	-	-	-	+	+	+
"	81	6.1	F	33	+	+	+	-	+	-	+	+	-
"	102	5.4	M	42	+	+	+	+	-	-	+	+	-
"	103	7.6	F	40	+	+	+	+	-	-	+	+	-
"	109	4.3	M	34	+	+	+	-	+	-	+	+	-
"	119	3.2	M	33	+	+	+	-	-	-	+	+	-
"	147	2.4	M	34	+	+	+	+	-	-	+	+	-
"	156	3.9	F	28	+	+	+	-	-	-	+	+	-
"	157	2.11	F	25	+	+	+	-	+	-	+	+	-
"	159	3.10	F	32	+	+	+	-	-	-	+	+	+
"	163	4.2	F	35	+	+	+	+	-	-	+	-	-
Alsatian X	35	3.2	M	30	+	+	+	-	-	-	-	-	+
"	68	5.7	F	28	+	+	+	-	-	-	-	-	+
"	69	1.11	F	29	+	+	+	-	+	-	+	-	+
Golden Retriever	41	6.1	F	27	+	+	+	-	-	-	-	-	+
"	80	4.0	F	28	+	+	+	+	+	-	+	+	-
"	97	4.3	F	29	+	+	+	-	+	-	+	-	-
"	100	3.8	F	26	+	+	+	+	+	-	+	-	-
"	123	7.11	F(n)	30	+	+	+	-	-	-	+	-	-
"	161	5.1	F	31	+	+	+	+	-	-	+	+	-
"	162	13.6	F	33	+	+	+	-	-	-	+	-	-
"	168	2.9	F	25	+	+	+	-	-	-	+	-	-
"	169	3.6	F	30	+	+	+	-	-	-	+	+	-
"	176	3.3	F	29	+	+	+	-	-	-	+	-	-

Retriever X	181	3.5	F	26	+	+	+	-	-	-	-	-	+
Labrador Retriever	34	11.2	M	34	+	+	+	-	+	-	+	-	+
"	41	6.5	F	31	+	+	+	-	-	-	+	-	+
"	48	4.1	M	28	+	+	+	-	+	-	+	+	-
"	50	3.7	M	26	+	+	+	+	+	-	+	+	-
Labrador X Collie	44	12.4	M	28	+	+	+	-	-	-	+	-	+
Labrador X	55	2.0	F	21	+	+	+	-	-	-	+	-	+
"	51	2.8	F	20	+	+	+	-	-	-	-	-	+
English Springer Spaniel	85	4.1	M	23	+	+	+	-	+	-	+	+	-
"	106	5.0	F	18	+	+	+	+	-	-	+	-	-
"	146	3.8	M	24	+	+	+	+	-	-	+	-	-
"	164	2.5	F	25	+	+	+	-	-	-	+	+	-
"	165	7.9	F	22	+	+	+	-	-	-	+	-	-
"	166	2.0	F	20	+	+	+	-	-	-	+	-	-
Cavalier King Charles Spaniel	22	5.6	F	11	+	+	+	-	-	-	+	-	+
English Cocker Spaniel	32	3.7	M	13	+	+	+	-	-	-	-	-	+
"	92	3.4	F	12	+	+	+	-	+	-	+	-	-
"	108	4.0	M	14	+	+	+	+	-	-	+	+	+
"	171	2.9	F	13	+	+	+	-	-	-	+	-	-
Spaniel X	30	1.4	F	9	+	+	+	-	-	-	-	-	+
Rough Collie	47	16.3	M	28	+	+	+	-	-	-	+	-	+
"	127	3.2	F	24	+	+	+	+	+	-	+	-	+
"	175	3.10	M	25	+	+	+	-	-	-	+	+	-
Old English Sheepdog	96	5.0	M	40	+	+	+	-	-	-	+	+	-
"	126	3.10	F	32	+	+	+	-	-	-	+	+	-
"	148	1.7	M	31	+	+	+	+	-	-	+	-	-

Great Dane	135	4.0	M	66	+	+	+	-	-	-	+	+	-
Bearded Collie	46	3.2	M	28	+	+	+	-	+	-	+	-	+
"	67	15.5	F	20	+	+	+	-	-	-	+	+	+
"	120	7.8	F(n)	18	+	+	+	+	+	-	+	-	-
Collie X	12	3.3	F	19	+	+	+	-	+	-	+	-	+
"	49	17.4	M	26	+	+	+	+	-	-	+	-	+
"	62	5.8	M	14	+	+	+	-	-	-	+	+	+
Shetland Sheepdog	87	4.2	F	9	+	+	+	+	+	-	+	-	-
Jack Russell Terrier	86	5.4	F	6	+	+	+	+	+	-	+	-	-
Terrier X	18	16.2	F	13	+	+	+	-	-	-	+	-	+
"	52	5.6	F	16	+	+	+	-	+	-	+	+	+
"	57	1.0	F	7	+	+	+	-	-	-	-	-	+
Border Terrier	107	1.3	M	8	+	+	+	+	-	-	+	-	-
German Wire Haired Pointer	116	4.4	M	32	+	+	+	+	-	-	+	-	-
Beagle	110	2.3	M	12	+	+	+	-	+	+	+	-	-
"	111	3.4	F	11	+	+	+	-	+	+	+	-	-
"	112	3.4	M	13	+	+	+	-	+	+	+	+	-
"	113	2.8	F	10	+	+	+	-	+	+	+	-	-
"	114	3.10	F	12	+	+	+	-	+	+	+	+	-
"	152	4.3	F	14	+	+	+	+	-	-	-	-	-
Greyhound	54	1.0	F	19	+	+	+	-	-	-	-	-	+
"	117	3.2	F	28	+	+	+	+	+	-	+	+	-
Miniature Poodle	16	12.7	F	8	+	+	+	+	-	-	+	-	+
"	29	9.0	F	7	+	+	+	-	+	-	-	-	+
Afghan Hound	84	4.5	F	26	+	+	+	-	+	-	+	+	+
Whippet X	53	4.0	M	15	+	+	+	-	-	-	-	-	+
Airedale	125	4.7	M	33	+	+	+	-	+	-	-	-	-

Doberman Pinscher	43	1.0	M	27	+	+	+	-	-	-	+	+	+
Min. S. H. Dachs.	64	10.2	M	6	+	+	+	-	-	-	-	-	+
Keeshound	39	7.2	F	21	+	+	+	+	-	-	+	+	+
Lurcher X	40	2.4	M	26	+	+	+	-	-	-	-	-	+
Corgi (Cardigan)	20	11.4	F(n)	13	+	+	+	-	-	-	+	-	-
Mongrel	19	3.0	M	17	+	+	+	-	-	-	-	-	+
"	56	1.1	F	10	+	+	+	-	-	-	-	-	+
"	58	1.2	F	13	+	+	+	-	-	-	-	-	+
"	61	11.0	M	23	+	+	+	+	-	-	+	-	+
"	63	16.3	F	21	+	+	+	+	-	-	+	-	+

Key to Table 4.1/1

Age in years and months

Sex (n = Neutered)

Weight in kilograms

a General clinical and ophthalmological examination

b Detailed examination of the anterior segment

c Charting and photography

d Temperature measurement

e Electronic applanation tonometry

f Anterior segment fluorescein angiography

g Venous blood samples

h Lipoprotein electrophoresis

i Keratectomy or whole eye studies

comparison of normal and abnormal groups particularly when matched by age and sex.

The diet of normal controls was considered satisfactory as regards balance, composition and quality. The possible influence of non-proprietary additions is discussed in Section 5.0.

All the animals used as controls, were of a size, shape and weight considered within normal range for their age, sex and breed.

4.2 Detailed Examination of the Anterior Segment

4.2.1 Vascular Plexuses The bulbar conjunctival plexus derived from palpebral and anterior ciliary vessels and was located in freely mobile conjunctiva. At various distances from the limbus ciliary emissary veins emerged from more deeply situated scleral emissary foramina and ran backwards over the surface of the globe to join the anterior ciliary veins. The perilimbal arteries were difficult to identify in the normal eye being finer, straighter, more sparse and less branched than the veins. They too emerged at episcleral level and ran in the direction of the limbus before bending acutely to pass superficially into the conjunctiva (Fig. 4.2/1).

In the majority of normal eyes episcleral vessels could not be traced at all, or were only apparent over short distances. Episcleral veins appeared darker with less bright red contents than conjunctival veins.

Near the limbus the radial arrangement of portions of the intrascleral venous plexus (of Hovius) could be observed in some dogs.

At x20 to x30 magnification the pattern of vascular flow in superficial conjunctival vessels was readily observed. Its most striking feature was the multiple choice of venous pathways

available for blood draining backwards over the surface of the globe away from the limbus. As one vessel emptied another would fill, and this constantly changing and dynamic arangement emphasized the richness of the superficial vascular pattern.

4.2.2 Pigmentation The amount of pigmentation in the perilimbal regions varied and was always accentuated nasally and temporally when present. Some dogs lacked perilimbal pigment, but all those examined displayed a prominent ring of pigment within the middle and deep epithelial cell layers of the clear cornea, usually attenuated or absent nasally, but often prominent and incursive temporally, sometimes extending for about 0.5mm beyond the superficial edge of the limbus in these regions (Fig. 4.2/1).

In a proportion of the animals examined, a grey zone was apparent at the transition of opaque sclera to clear cornea. The amount of pigment present rendered the transition zone less abrupt in the majority of animals, especially as the external scleral sulcus was not marked.

4.2.3 Fluorescein Angiography

Results of Fluorescein Injection The results of fluorescein angiography in normal Beagles are summarised in Figs. 4.2/2 - 4.2/4 (Beagle 110). All the dogs presented similar results.

Diffuse fluorescence of the anterior segment was detected approximately ten seconds after the injection of fluorescein (Fig. 4.2/2). The fluorescence was not detected in individual superficial conjunctival vessels, but more deeply within the sclera itself. Superficial conjunctival arteries were not clearly delineated but the venous system (fluorescein negative at 10 seconds) was boldly outlined by the diffusely fluorescent background. The pattern of

superficial conjunctival vascularisation changed during the period of photography, a feature previously noted during ophthalmic examination of the anterior segment.

The filling of superficial conjunctival arteries may be so transient that it is not readily observed, or it may be that the very richness of episcleral fluorescence obscures fluorescence in the conjunctival arteries, particularly when a yellow, rather than an orange, barrier filter is employed.

Very slight fluorescence at the limbus and just within clear cornea was first noted approximately 15 seconds after initial fluorescein injection. Corneal fluorescence had usually formed a complete annulus by 20 seconds after injection (Fig. 4.2/3) but never extended more than 0.5 to 1.0mm into clear cornea in normal eyes. By 20 seconds the diffuse fluorescence from the sclera was beginning to diminish and once this occurred it was possible to discern some fluorescein positive superficial conjunctival veins (Fig. 4.2/4). A laminar flow pattern was characteristic as small venous tributaries emptied into larger veins.

A certain amount of multifocal leakage was apparent in superficial vessels and was most striking in the conjunctiva of the nictitating membrane; it occurred to a lesser extent in other conjunctival and episcleral vessels. More than five minutes after injection, the intensity of fluorescence had diminished overall but a diffuse positive reaction was present in the conjunctiva, episclera, sclera, corneo-scleral zone and in the aqueous humour of the anterior chamber.

4.2.4 Luminal Castings of Ocular Vessels Microvascular castings of the ocular vessels indicated the intricate rich and anastomotic

blood supply of the canine eye. The extent of anastomosis was such that injection of a single vessel produced filling of the whole globe. Technically this was more difficult to demonstrate in arteries than veins; the arteries were much finer vessels and were more readily damaged during injection of the methacrylate compound.

Venous drainage was effected by a number of well-developed interconnecting plexuses of which the intrascleral venous plexus (venous circle of Hovius, plexus venosus sclerae) was the most prominent (Figs, 4.2/5 α 4.2/6).

The intrascleral venous plexus was formed by large circumferential veins which communicated directly with veins of the conjunctiva and episclera at the limbus, the choroidal vortex veins, the ciliary plexus, and the aqueous collector channels draining the filtration angle.

In all the eyes examined, the intrascleral venous plexus drained into the orbit by the anterior ciliary veins and choroidal vortex veins.

The anterior ciliary veins were the principal route for venous drainage from the limbus and the anterior ciliary arteries the major supply. These were the vessels most frequently cannulated for methacrylate luminal castings.

The anterior ciliary arteries supplied all levels of the anterior segment. In the region of the superficial marginal plexus of the cornea, the arteries bent acutely to pass from episcleral to conjunctival level as recurrent vessels; they also passed forward as terminal vessels to anastomose with conjunctival veins. There were rich arterio-venous anastomoses with the intrascleral venous plexus. In conjunction with the long posterior ciliary arteries, the

anterior ciliary arteries formed the major arterial circle and supplied the iris, the ciliary body, and, in part, the choroid.

In view of the rich anastomotic pattern encountered in the anterior segment, the propensity for inflammation of one region to extend to others is not surprising.

4.2.5 Vasculature of the Canine Anterior Segment: Summary and Discussion The results confirm that the cornea is surrounded by a rich and complex vascular system present in the conjunctival, episcleral, scleral and uveal tract tissue.

Methacrylate luminal castings were of particular value in studying the arrangement of perilimbal vessels, especially when combined with SEM, examination of the eye, and anterior segment fluorescein angiography.

The vasculature of the canine eye possessed a number of interesting features of which the extensive anastomosis of all vessels was most noteworthy. The circumferential venous plexus of Hovius was particularly well defined in the dog and it apparently serves as a common intrasceral pool for drainage of aqueous humour and uveal venous blood as reported by Van Buskirk (1979). The choroidal vortex veins were shown to communicate directly with either the venous circle of Hovius or its drainage vein. These findings compare with those of Van Buskirk (1979) in which he described five to six vortices in the canine eye which usually extended anteriorly from their ampullae. In some cases the vortical veins extended as a single channel for a short distance before branching and entering the Hovial veins, whereas in others, they merged with a similar ampulla arising posteriorly from the Hovial veins and passed through the sclera into the orbit posterior

to the equator of the globe. In one of the 31 specimens examined by Van Buskirk the choroidal veins arose from their vortex ampullae and passed posteriorly and exteriorly without anterior anastomoses, as is the common pattern in the primate eye.

The results of fluorescein angiography indicated that there is no rapid transit of fluorescein into the normal cornea, although there is a slow passage into a narrow zone of clear cornea at the limbus. Intense fluorescence of the episclera and sclera was an early and prominent feature and multifocal leakage from conjunctival and episcleral vessels was both diffuse and localised. Background fluorescence demonstrated the intricate pattern of more superficial fluorescein negative vessels which appeared as dark silhouettes against the fluorescein-rich background.

Further work is required to explore the possibilities of anterior segment fluorescein angiography in the dog and this will be undertaken as soon as a suitably equipped slit lamp is obtained. There appear to be no veterinary publications on fluorescein angiography of the corneo-limbal region, although there has been limited study of fluorescein injection in dogs with albinotic and subalbinotic irises (Gelatt, personal communication). The advantages of fluorescein angiography of the anterior segment have been reported in man by a number of authors, including Brunn-Jensen (1969), Bron α Easty, (1970), Matsui α Justice (1982), Martonyi (1982) α Choromokos (1982).

4.3 Temperature Measurement

The means of two recordings from 26 normal dogs are given in Table 4.3/1.

Table 4.3/1 Temperature Measurements From Normal Animals

Identification Number	Environmental Temp. °C	Rectal Temp.°C	Inf. Conj. Sac Temp.°C	Central ant. Cornea Temp.°C
102	17.4	38.5	37.8	29.5
103	17.9	39.0	38.5	29.6
147	17.3	38.3	36.8	29.9
163	15.8	38.4	36.6	29.5
69	19.5	38.3	37.0	31.4
80	17.2	38.8	38.2	30.2
100	18.9	38.4	37.4	30.6
161	19.3	38.6	38.0	31.5
50	18.6	38.8	37.2	31.3
106	15.9	38.2	37.6	29.2
146	19.9	38.8	37.0	30.3
108	18.5	38.6	37.8	30.6
127	20.0	38.8	37.4	30.9
148	17.5	38.5	37.6	29.9
120	15.2	38.4	36.6	28.7
49	15.6	38.7	36.2	28.8
87	16.9	38.6	36.9	29.0
86	15.4	38.4	37.0	28.8
107	19.8	38.8	37.4	30.7
116	18.2	38.5	37.8	30.4
125	16.4	38.8	36.9	29.2
117	15.3	38.7	36.8	28.5
16	15.0	38.6	37.2	28.9
43	16.9	38.4	37.7	30.0
39	15.1	38.5	37.0	29.2
63	16.3	38.4	36.8	29.4
Mean ± SEM	17.3 ± .322	38.6 ± .04	37.3 ± .107	29.8 ± .174
n=26				
Range	15.0-20.0	38.2-39.0	36.2-38.5	28.5-31.5

4.3.1 Temperature Measurement: Summary and Discussion The results indicate that the temperature of the inferior conjunctival sac approached the rectal temperature, whereas the surface temperature of the exposed central cornea was influenced by the external environment as well as the internal environment of adjacent ocular and extraocular tissues.

The effects of the lids on corneal and pericorneal temperature have been studied by Schwartz (1964) in rabbits and by Mapstone (1968) in man. Changes of environmental temperature were investigated in rabbits by Schwartz (1965) and in man by Mapstone (1968). They concluded that a linear drop in corneal temperature was produced by a fall in environmental temperature over a specified range, when the cornea was exposed. The temperature of the inferior fornix approached that of the rectum (in rabbits) and varied quantitatively, like the rectal temperature, with a decrease in environmental temperature. The results obtained for the dog indicate that the same determinants of corneal temperature operate and that a temperature gradient exists between the inferior conjunctival fornix and the central anterior cornea when the lids are open. The presence of a temperature gradient may have important implications for the phase-transition temperature of lipids within membranes and free within the cornea, and this topic is discussed more fully in Section 6.7.

4.4 Tonometry

The results of indirect measurement of intraocular pressure on 28 normal non-sedated conscious dogs are given in Table 5.6/1 along with those recorded for clinical cases. They are discussed with the results for clinical cases.

4.5 Results of Laboratory Examination

4.5.1 Lipid Analysis The results of cholesterol, triglyceride and phospholipid estimations appear in Table 4.5/1 together with a synopsis of each animal's diet.

Age The ages of dogs used as normal controls ranged from just over a year to more than 17 years. The animals were initially examined by breed as four groups (Alsatians, Spaniels, Retrievers, Others) and scatter diagrams of age versus cholesterol, triglyceride and phospholipid respectively were drawn and compared with similar diagrams for the clinical cases (Figs. 4.5/1, 4.5/2 & 4.5/3).

As there were no obvious differences between breeds the animals were grouped together for calculation of correlation coefficients. The normal dogs were placed as a single group and as a group of between one and less than nine years of age to facilitate direct comparison with the clinical cases which lay in this age range. The results for normal controls and clinical cases are presented together in Table 5.7/1. The control group showed a correlation of cholesterol, triglyceride and phospholipid with age, significant at the 1% level, which was largely a consequence of dogs over nine having higher serum lipid values. When a control group aged between one and less than nine years was examined, only serum phospholipid with age gave a significant value.

Sex No significant differences were found for serum lipid values in entire females and entire males, aged less than nine years. The results appear with those for clinical cases (Table 5.7/2).

Breed The earlier scatter diagrams (Figs. 4.5/1, 4.5/2 & 4.5/3) had indicated no obvious differences of serum lipid values in

Table 4.5/1 Serum Lipid Values and Diets of Normal Animals

Identification Number	Diet	Cholesterol in mmol/l	Triglyceride in mmol/l	Phospholipi in mmol/l
21	Cm Cb ± ds	5.20	0.61	4.84
23	Cm Cb	3.86	0.51	3.02
81	Cm Cb ± dmg	4.16	0.73	3.80
102	Cm Cb + mg ± s	5.85	0.69	4.24
103	Cm Cb ± mg	5.17	0.62	4.83
109	Cm Cc	4.50	0.70	3.76
119	Cm Cb	3.92	0.61	3.63
147	Cm Cb ± mg	4.74	0.42	3.96
156	Cm Cb + d	5.46	0.58	4.04
157	Cm Cb ± dmg	4.76	0.61	3.96
159	Cm Cb ± mg	4.81	0.50	4.24
163	Cm Cc	3.64	0.69	3.64
69	Cm Cb Cc	3.48	0.54	3.44
80	Cm Cb	4.93	0.65	4.84
97	Cm Cb ± dmg	4.97	0.37	4.20
100	Cm Cb ± d	4.03	0.42	3.46
123	Cm Cb	2.68	0.46	3.80
161	Cm Cb + dmg	6.32	0.64	3.98
162	Cm Cb ± d	4.14	1.29	6.61
168	Cm Cb	3.82	0.45	3.04
169	Cm Cb ± dmg	4.22	0.39	3.90
176	Cm Cb	3.95	0.61	4.03
34	Cm Cb ± mg	5.64	0.38	6.04
41	Cm Cb Cc ± s	4.36	0.69	4.02
48	Cm Cb	3.62	0.33	3.76
50	Cm Cb	3.99	0.46	3.82

44	Cm Cc ± dmg	6.28	0.56	5.16
55	Cm Cb	3.49	0.44	3.21
85	Cm Cb Cc ± dms	4.73	0.53	3.33
106	Cm Cb ± mg	4.04	0.45	3.66
146	Cm Cb	3.87	0.79	3.87
164	Cm Cb + dmg	7.76	0.30	5.36
165	Cm Cc ± mg	4.54	0.48	4.47
166	Cm Cb + d	5.14	0.31	3.49
22	Cm Cb	3.79	0.54	3.82
92	Cm Cc	4.75	0.61	3.42
108	Cm Cb ± dmg	5.36	0.75	4.63
171	Cm Cb	3.68	0.52	3.98
47	Cm Cb Cc ± s	5.86	0.69	6.80
127	Cm Cb	3.54	0.52	3.10
175	Cm Cb ± d	4.01	0.39	3.87
96	Cm Cb + dmg	6.03	0.61	4.81
126	Cm Cb Cc	4.84	0.54	4.12
148	Cm Cb + d	4.94	0.50	4.20
135	Cm Cb	3.43	0.42	3.07
46	Cm Cb Cc	3.26	0.39	3.26
67	Cm Cc	5.68	0.59	5.84
120	Cm Cb Cc ± d	2.96	0.61	3.74
12	Cm Cb	3.07	0.38	3.99
49	Cm Cb Cc ± ds	6.83	0.68	6.28
62	Cm Cb Cc	4.66	0.29	3.89
87	Cm Cb	3.65	0.52	3.04
86	Cm Cb Cc ± d	3.48	0.56	3.83
18	Cm Cb ± s	6.23	0.58	6.04

52	Cm Cb	3.66	0.49	3.88
107	Cm Cb Cc	2.77	0.59	2.63
116	Cm Cb	3.04	0.53	3.0
110	Cm Cb ± d	4.52	0.45	3.71
111	Cm Cb ± d	4.80	0.59	3.94
112	Cm Cb ± ds	4.24	0.57	3.48
113	Cm Cb ± d	3.91	0.52	3.84
114	Cm Cb ± dmg	4.76	0.34	4.16
117	Cm Cb	3.97	0.34	2.93
16	Cm Cc +	4.63	0.68	5.21
84	Cm Cb ± d	4.83	0.56	3.95
43	Cm Cb Cc	2.86	0.63	3.21
39	Cm Cb ± d	4.49	0.53	3.84
20	Cm Cb ± dmg	5.68	0.68	5.08
63	Cm Cb ± d	6.02	0.56	4.99

Cholesterol: Range 2.77-7.76 Mean 4.50 SD 1.03

Triglyceride: Range 0.30-1.29 Mean 0.54 SD 0.15

Phospholipid: Range 2.63-6.80 Mean 4.10 SD 0.90

n = 69

Key to diets

\+ = regularly ± = occasionally

Cm = Commercial Meat, Cb = Commercial Biscuit, Cc = Commercial Cereal] Proprietary dog foods

d = dairy products, m = meat products, g = vegetables and cereals, s = sweets] Mainly human foods

Figure 4.5/1

Relationship between Age and Cholesterol-Controls

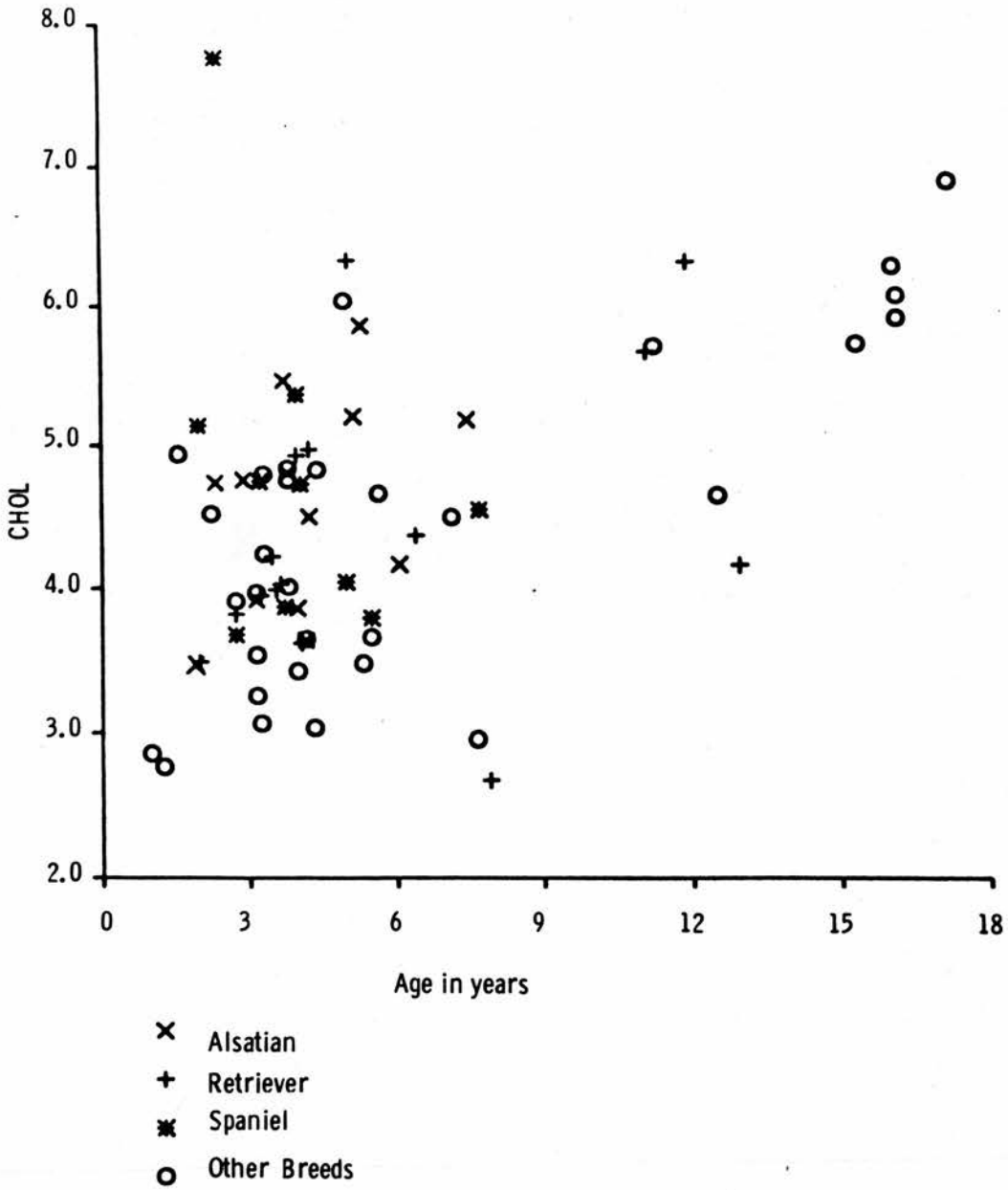


Figure 4.5/2

Relationship between Age and Triglyceride-Controls

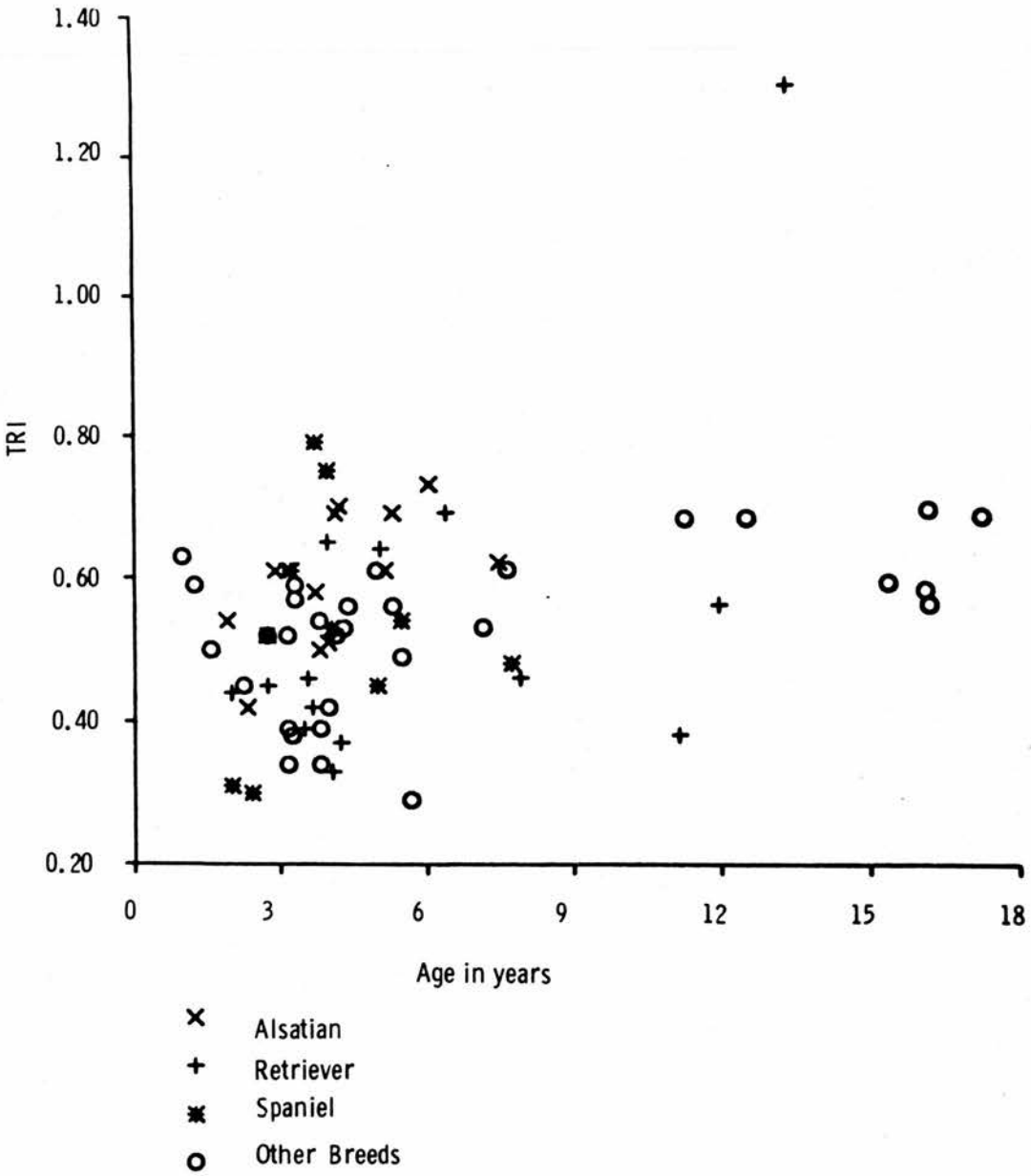
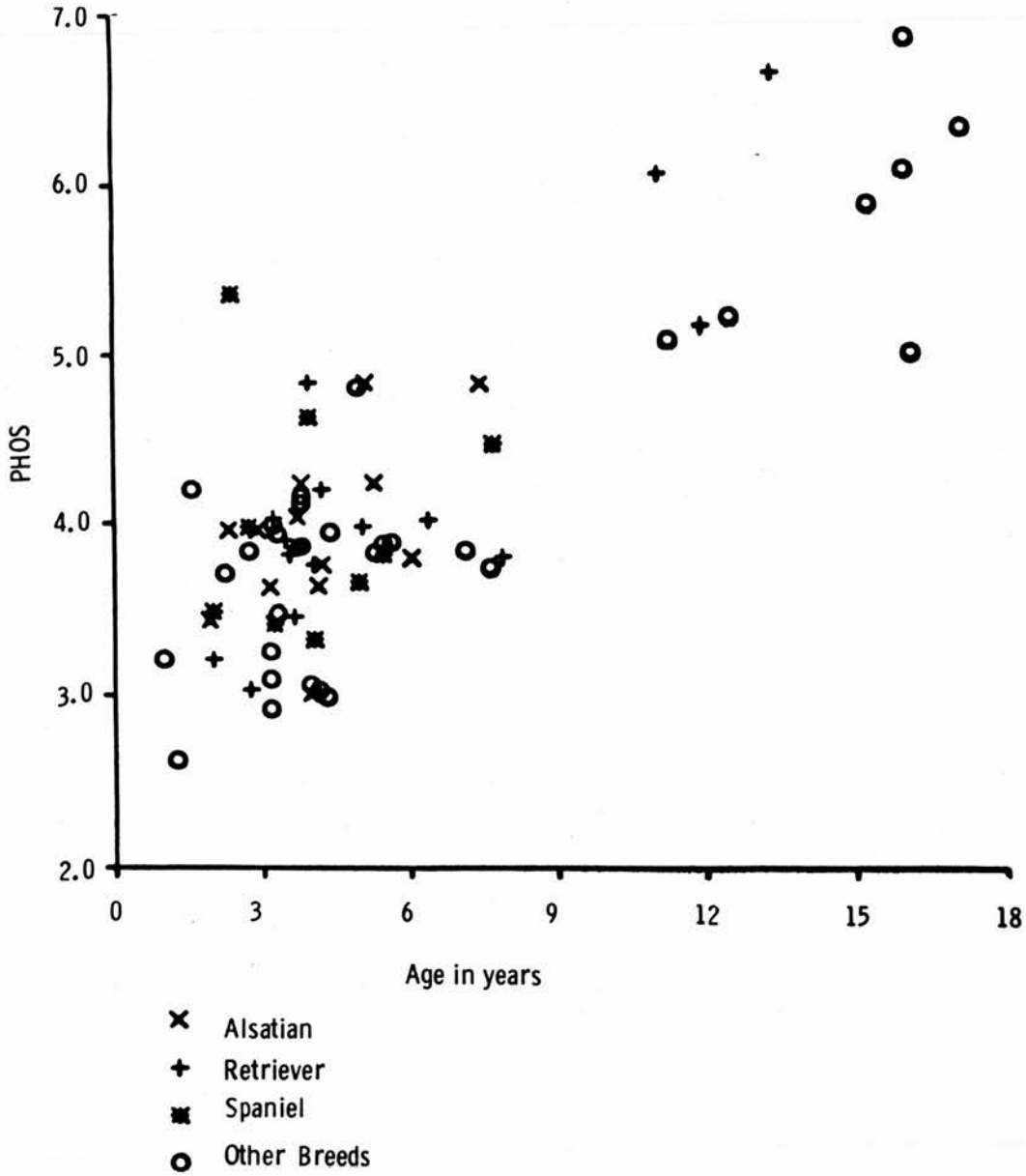


Figure 4.5/3

Relationship between Age and Phospholipid-Controls



various breeds. Analysis of variance confirmed that there were no significant differences between normal dogs of different breeds.

Diet Animals used as normal controls had been selected to the extent that they were of a size, shape and weight considered average for their breed, age and sex.

The owners described the dogs' appetites as ranging from "choosy" to "greedy". The majority were reported to have good appetites.

All dogs were fed commercially prepared foods as the basis of their diet; some exclusively so, others with a variety of substitutions or additions. Individual diets were assessed in relation to the needs of the animal and a summary of the diets is given with Table 4.5/1.

It was not possible to analyse the effects of diet in relation to exercise and individual variation with any precision; but in general it appeared that diets enriched with dairy products and/or animal fats produced higher serum lipids in some animals than did diets of purely commercial origin.

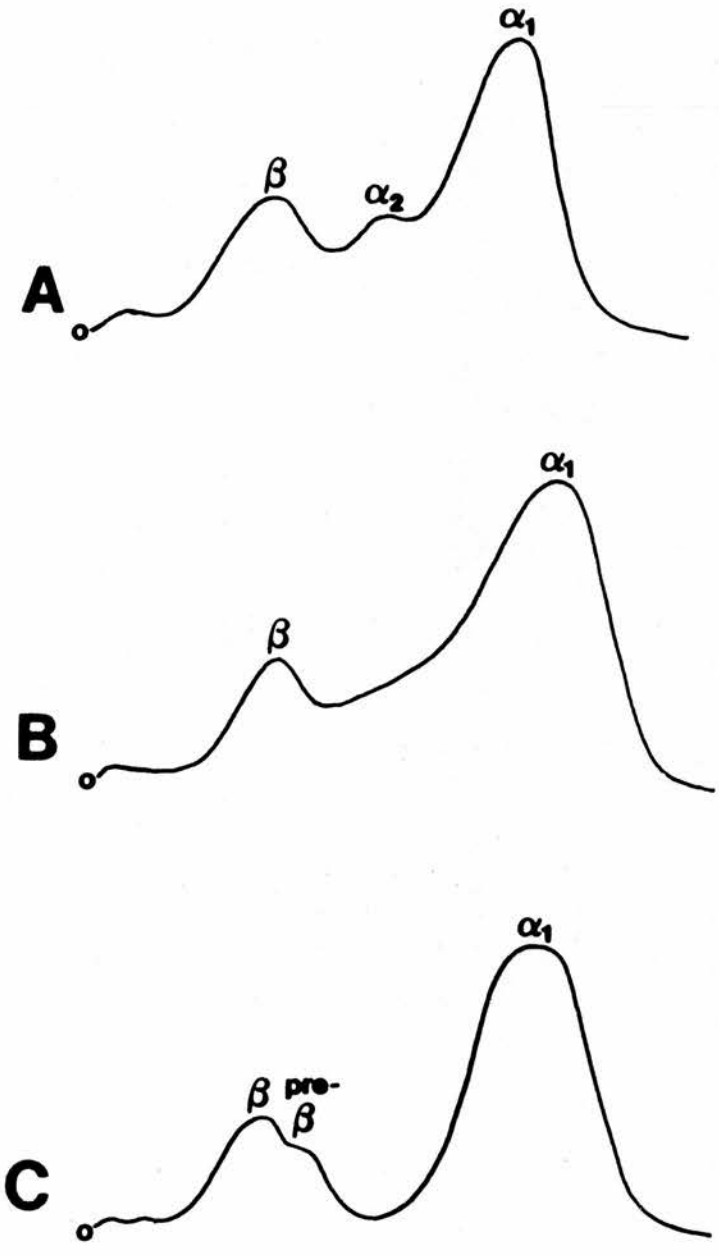
4.5.2 Visual Inspection Normal fasting serum was clear and without a cream band in all dogs except 162, in which it was faintly opalescent.

4.5.3 Lipoprotein Electrophoresis Paper and agarose gel electrophoresis resulted in up to four partly or completely separated bands although all four were not evident on the same strip.

The commonest types of electrophoretic densitometer tracing are summarised in Fig. 4.5/4. In the majority of normal controls, three distinct bands migrated to the alpha$_1$ (HDL_2)

Figure 4.5/4

Densitometer Tracings of Lipoprotein Electrophoresis Patterns from Normal Dogs.



alpha$_2$ (HDL_1) and beta (LDL) positions.

Variations in the basic pattern seemed to be largely a consequence of different mobility of components between the alpha$_1$ and beta bands so that separation was less complete. A distinct band close to the beta position, as prebeta (VLDL) lipoprotein, was not seen without concentration of plasma prior to electrophoresis.

There was no chylomicron band at the origin of any samples, all dogs having been fasted for at least 15 hours.

In only three dogs in which lipoprotein electrophoresis was performed, was there a reduction in the intensity of staining of HDL_2 and an increased intensity of staining of HDL_1. This variation was marked in 164 and less evident in 161 and 96.

4.5.4 Other Venous Samples The results of other laboratory examinations are summarised together with those from clinical cases in Table 5.7/6. There were no significant differences between the two groups.

4.5.5 Serum Lipids and Lipoproteins: Summary and Discussion The results of lipid analysis indicated that privately owned dogs in normal health, being fed a balanced diet of the correct quantity, had slightly higher values, of cholesterol particularly, than those obtained by other workers for kennelled experimental dogs. These results are in accordance with Hoe & Harvey (1961), Schiller, Berglund, Terry, Reichlin, Trueheart & Cox (1964) and Rogers et al. (1975b) when two such groups were compared.

Diet Schiller et al. (1964) commented that there are many differences between kennelled experimental dogs and pet dogs which do not lend themselves to detailed study, whereas differences in the dietary levels of total fat and cholesterol do. Commercially

manufactured diets are generally low in cholesterol in comparison with supplements such as table scraps and dairy products, and the authors felt that pet dogs were receiving additional fat and cholesterol by way of these supplements, sufficient to account for their higher cholesterol levels. Other workers (Hoe & Harvey, 1961; Rogers et al., 1975b) reached similar conclusions. However, experiments in which purified commercial diets supplemented with various fats (or cholesterol) have been fed produce somewhat equivocal results, and these apparent differences may relate to the time for which a particular diet is given. Steiner & Domanski (1941) were unable to produce even a moderate hypercholesterolaemia in dogs fed a cholesterol-rich diet. Steiner & Kendall (1946) and Boyd & Oliver (1958) stated that in the dog, hypercholesterolaemia will result from cholesterol feeding only if the animals are rendered hypothyroid, and Shull, Mann, Andrews & Stare (1954) reviewing previous studies, indicated that hypercholesterolaemia is induced in the dog only by the creation of abnormal or atypical dietary regimes or physiological states; for example, massive cholesterol feeding, protein deficiency plus high fat feeding, or hypothyroidism and hypothyroidism plus cholesterol feeding. Slatter (1975 & 1980) was able to achieve high serum cholesterol levels in experimental dogs fed an atherogenic diet (cholesterol and propyl thiouracil) after surgical thyroidectomy. Mahley et al. (1974) divided experimental dogs which had been similarly treated into hyperresponders and hyporesponders on the basis of the type of hyperlipidaemia and the extent of atherosclerosis produced. They related the differences between the groups to variations in total lipolytic serum enzyme activity.

Lindall et al. (1971) fed a variety of fat enriched diets to experimental dogs over a short period and proposed that saturated fats achieved an increase in low density (beta) lipoprotein in normal dogs. If the ultracentrifugation cuts they used are directly compared with the more complete separation achieved by later workers (Mahley & Weisgraber, 1974; Mahley et al., 1974) it can be seen that the cholesterol enrichment is actually occurring in high density lipoprotein rather than low density lipoprotein. Lindall et al. (1971) have, in effect, demonstrated the cholesterol-rich lipoprotein HDL in the lower density lipoproteins. Their results are also of interest in that they show little change in total cholesterol as a result of feeding diets containing 94% saturated fats or 65% monounsaturated fats, but rather a shift of cholesterol away from HDL_2 towards HDL_1 and lower density lipoproteins. With an equal mixture of saturated, monounsaturated and polyunsaturated fats, or one of 76% polyunsaturated fats, they achieved a reduction in total serum cholesterol relative to normal controls.

Individual variation as it relates to the effect of diet on serum lipids and lipoproteins complicates interpretation, particularly in a species of such diverse breeds. Whilst variations may be indicative of individual differences, or underlying disorders, it may also be a feature within breeds of apparently normal dogs. Kronfeld, Johnson & Dunlap (1979) found that in a proportion of racing huskies, there was an inherited predisposition to hypercholesterolaemia induced by diet.

Age The influence of age on serum lipids has received little investigation in the dog and many of the available publications make

no reference to the age or breed of the animals investigated, which is unfortunate as it makes meaningful comparisons difficult. For example, the life span of different breeds is variable so that the effects of age must be related to breed. Also, serum cholesterol values are high in young growing dogs, as observed by Schiller et al. (1964) and as is evident from the data supplied in publications by Slatter (1975 α 1980), Rogers et al. (1975) and Arslan (1980).

The serum cholesterol values of young growing animals probably reach a peak before one year of age, thereafter declining to adult levels. For 12 dogs of ten months or less in Arslan's series serum cholesterol had a mean value of 7.7mmol/l (range 5.5-10.93 SD ± 1.636) whereas the mean value was 4.7mmol/l for 12 dogs between one year and 14 years of age (range 2.58-7.92 SD ± 1.418).

In this series, the ages were chosen to correspond with the ages of clinical cases, and also to provide information from a proportion of aged dogs, so that an analysis of serum lipids in normal adult dogs of different ages could be obtained. The results indicated that serum phospholipid increased gradually with age, whereas there were less regular changes in serum cholesterol and serum triglyceride until the dog was nearing the end of its natural life span, a time when other influences, such as the amount of exercise, or food in relation to exercise, may complicate interpretation.

There are no directly comparable references available for such a wide range of ages in pet dogs, although Manning et al. (1975) obtained similar results in two groups of experimental Beagles (1.5 to 2.5 years and 5.5 to 8.5 years).

Sex There were no obvious differences in serum lipids of entire

anoestrous or metoestrous females and entire males. As a variety of hormones may influence serum lipids this is an area worthy of investigation; particularly in relation to the effects of neutering, pro-oestrus, oestrus, pregnancy and lactation on the pattern of lipoproteins relative to serum lipid levels.

The results of lipoprotein electrophoresis obtained in this study agreed with those of others (Mahley & Weisgraber, 1974; Rogers et al., 1975b) in demonstrating that paper or agarose electrophoresis of serum from normal dogs revealed three bands; HDL_2, HDL_1 and LDL as the commonest patterns. Plasma concentration indicated VLDL in a prebeta position in a proportion of normal dogs and there was considerable overlap in the speed of migration of components between HDL_2 ($alpha_1$) and LDL (beta) positions. In animals with above average levels of serum cholesterol the lipoprotein pattern indicated a shift away from HDL_2 into HDL_1 and occasionally towards the beta band.

4.6 Light Microscopy

4.6.1 Introduction The canine cornea consists of an outer epithelium, stroma (or substantia propria), Descemet's membrane and mesothelium (or endothelium). The stroma forms the bulk of the cornea and contains fibroblasts as the principal type of cell (Figs. 4.6/1-4.6/3).

The staining reactions of the normal non-swollen epithelium and stroma in the central region of the adult cornea are summarised in Fig. 4.6/3. Swelling may affect the staining reactions of the cornea (Ashton, 1959). The lipid histochemistry and enzyme histochemistry or normal adult epithelium and stroma is described prior to more detailed analysis of fine structure.

4.6.2 Lipid Histochemistry Positive staining was obtained with bromine Sudan black B adjacent to the precorneal tear film, in the surface cells of the corneal epithelium, at epithelial cell boundaries and in the epithelial cell basement membrane (Fig. 4.6/4). Staining of the surface epithelial cells was accentuated towards the limbus.

The fibroblasts were lightly stained and, in occasional dogs, there was diffuse amorphous staining of the anterior stroma (Fig. 4.6/5). This reaction was most commonly seen in older animals. However, it was not present universally in old dogs and, as it was also detected in the occasional younger animal, no firm conclusions as to its significance can be reached, although the possible influence of serum lipids and lipoproteins is discussed later.

Myelinated nerves were clearly demonstrated with bromine Sudan black B (Fig. 4.6/6) as they were also with Sudan black B. Myelinated nerves stained with Sudan black B exhibited a typical bronze dichroism in polarised light. Sudan black B produced a similar pattern to bromine Sudan black B but of reduced staining intensity (Fig. 4.6/7). In paraffin embedded sections stained with Sudan black B only myelinated nerves were stained blue-black.

With Nile blue sulphate (Fig. 4.6/3), the epithelium and stroma stained uniformly blue with the epithelium appearing slightly darker. Melanin gave an acetone-resistant staining reaction with Nile blue sulphate. Baker's acid haematein (Fig. 4.6/8) produced a rather weak and inconstant positive reaction in the superficial epithelium, most readily observed near the limbus. Occasionally the epithelial cell membranes and basement membrane were stained and the basal and intermediate cell layers had a

faintly granular appearance. Fibroblasts sometimes stained grey, which does not necessarily denote a positive reaction. Red blood cells were strongly positive.

With OTAN the epithelium stained orange-brown, the colour being accentuated towards the surface and at the limbus. The basement membrane and cell membranes were sometimes delineated, staining brown. The stroma was stained blue by the alcian blue counterstain and fibroblasts were not clearly distinguished. This technique also stained myelinated nerve fibres and red blood cells a rich brown (Fig. 4.6/ 9).

With the calcium lipase-lead sulphide method there was a clearly defined dark brown staining of the superficial epithelium which was only slightly diminished by prior extraction with potassium hydroxide in dioxan and very reduced in the duplicate control section not treated with enzyme (Fig. 4.6/3).

Holczinger's copper rubeanic acid method stained light green with a slightly darker green in the superficial epithelial cells and individual cells could be distinguished in all layers, as could fibroblasts in the stroma, when carmalum counterstain was used (Fig. 4.6/10). An acetone extracted control section stained pink with carmalum counterstain; no green colour was apparent as in the non-extracted section.

Osmium tetroxide stained the whole thickness of the epithelium brown and the stroma a paler brown. Individual fibroblasts were not readily identified.

Oil red O stained the section a pale orange-yellow colour, red blood cells usually stained orange (Fig. 4.6/11).

Lipofuscin was not identified in the normal cornea with such

stains as PAS, Sudan black B and Schmorl's Method (Figs. 4.6/3; 4.6/12). PAS stained the cell boundaries of epithelial cells and the epithelial basement membrane distinctly. The walls of blood vessels were also clearly delineated.

4.6.3 Lipid Histochemistry: Summary and Discussion The results indicate that the normal cornea and perilimbal regions do not contain lipid as visible droplets or crystals. The only cells which stain readily with some evidence of a detectable lipid content are those of the superficial epithelium in the region where desquamation occurs, the reaction often appearing darker towards the limbus.

The epithelial basement membrane and the cell membranes of epithelial cells were sometimes delineated by lipid stains as were intracellular organelles such as mitochondria.

Lipid staining within the cornea and perilimbal regions did not appear to increase as a direct consequence of ageing. However, in animals in which serum lipids were above average, there was a tendency for bromine Sudan black B to stain grey-blue in the anterior stroma with increased staining towards the limbus and in the sclera. There do not seem to be any histochemical or biochemical studies of corneal stroma lipids in the dog available for comparison. However, ageing dense connective tissues in man, including those of the peripheral cornea, accumulate esterified cholesterol which is probably derived from plasma low density lipoprotein (Crouse, Grundy & Ahrens, 1972; Adams & Bayliss, 1973; Broekhuyse, 1975 & 1976). Arcus senilis is regarded as a normal feature of the ageing human eye (Duke-Elder and Leigh, 1965) and the lack of any comparable condition in the normolipoproteinaemic dog may reflect innate differences in the eyes or lipoprotein patterns

of the two species.

4.6.4 Enzyme Histochemistry Results with tween lipase were negative in epithelium and stroma.

Non-specific esterase activity was present in cells of the superficial and intermediate cell layers of the epithelium and cell membranes of the basal cell layer stained more darkly. The reaction was accentuated towards the limbus (Fig. 4.6/13). Activity was not detected within the corneal stroma, although occasional cells at the limbus gave a weakly positive reaction.

With methods for acid phosphatase, a granular cytoplasmic reaction was obtained in all cell layers, but was more marked in the surface layers and towards the limbus (Fig. 4.6/14). Rarely, a granular reaction was also obtained within fibroblasts of the corneal stroma and, less rarely, the cells of perilimbal regions.

There do not appear to be any references to lipid enzyme histochemistry of the canine stroma, but Ehlers (1970) obtained similar results to those given here with methods for non-specific esterase and acid phosphatase in the epithelium of the dog's cornea.

4.6.5 Other Methods The results of staining with other techniques are summarised in Fig. 4.6/3. The negative results obtained with the Sakaguchi Method, Alcian Blue/PAS and Perls' Prussian Blue Reaction are shown in Figs. 4.6/15, 4.6/16 α 4.6/17.

4.7 Fine Structure of the Normal Canine Cornea

4.7.1 Epithelium The epithelium of the central cornea consisted of some 10 to 20 cell layers of overall thickness between 55 and 75μm (Fig. 4.7/1). Towards the corneal periphery there was often some attenuation of the corneal epithelium before it underwent an increase of thickness. At the corneal limbus the corneal epithelium

thinned abruptly to continue as the corrugated conjunctival epithelium which was only of four to eight cell layers thick (Figs. 4.7/2).

The most superficial aspect of the cornea was formed by several layers of flattened surface cells, some five to fifteen layers thick in the central cornea, about half this at the corneo-limbal junction. The surface cells contained free ribosomes, well developed Golgi complexes and tonofilaments (Fig. 4.7/3). The cytoplasm of the epithelial cells became increasingly rich in vesicles and vacuoles as the corneal surface was approached and there appeared to be constant desquamation of the most anterior cells which were often of degenerate appearance.

The adjacent plasma membranes of the squamous epithelial cells interdigitated and there were well developed desmosomal attachments. Surface projections, interpreted as microplicae and microvillae, were seen at the anterior aspect of the cornea with the transmission and scanning electron microscopes (Figs. 4.7/3 α 4.7/4).

Polygonal, or wing, cells formed an intermediate series of cell layers between the surface cells and those of the basal epithelium; there were some three to four layers of these cells in the central cornea and up to three layers at the corneo-limbal junction. The interdigitations and desmosomes of the polygonal cells are best demonstrated in tangential section (Fig. 4.7/5) which also shows the rather sparse organelles.

A single layer of columnar cells formed the basal epithelium. Desmosomes connected the lateral cell membranes of neighbouring cells in the same fashion as the polygonal cells, but the connections were not prominent and lacked the complex

interdigitations of other layers. Hemidesmosomes were regularly arranged along the basal cell membrane (Fig. 4.7/6).

Tonofilaments were diffusely distributed within the cell cytoplasm and were the most prominent intracytoplasmic feature of the basal cell layer as other organelles were sparse. Free ribosomes, profiles of rough endoplasmic reticulum and mitochondria were evenly distributed in the cell cytoplasm; Golgi complexes were located near the prominent oval nuclei. Occasional mitotic figures were observed in the basal cell layer.

Pigment granules appeared in cells other than the superficial squamous cell layer towards the limbus. Other cells found in the corneal epithelium included lymphocytes, usually in the basal cell layer between epithelial cells (Fig. 4.7/7).

Unmyelinated nerves were also noted between epithelial cells, but without the Schwann cell which may invest them within the stroma. Occasional cells had previously been observed with the light microscope which stained in a different way from the majority, usually by staining more darkly. Light and dark cells were also seen with the electron microscope and the variation of cell appearance was interpreted as reflecting the amount of cellular hydration (Perera, 1969). Darkly staining cells were probably indicative of damage and cellular dehydration. As they were more prominent in pathological material they are illustrated in Section 5.0.

4.7.2 Basal Lamina The basal lamina (basement membrane of light microscopy) stained positively with PAS, silver-methanamine and various lipid methods, including osmium tetroxide for T.E.M.. In the electron microscope it was seen to consist of a lamina lucida

and a lamina densa which comprised the osmiophilic basal lamina and a reticular lamina of fine collagen fibrils. In the normal canine cornea the basal lamina was of approximately 100 to 140nm thickness. The thickness of the basal lamina did not change between the centre and the periphery (4.7/6).

Towards the limbus the basal lamina and the epithelial cells which overlay it became more convoluted, sometimes giving a spurious impression of variation in basal lamina thickness. Filamentous extensions radiated from the posterior aspect of the basal lamina into the subepithelial zone in places, and these extensions were darker and more extensive peripherally.

4.7.3 Beneath the Basal Lamina ("The Subepithelial Zone")

Bowman's layer has a distinctive appearance under the light microscope in primates. The dog has no directly comparable structure although there is a hypocellular region of approximately 10µm immediately beneath the basal lamina where the collagen fibrils have a less regular arrangement than those more deeply placed (Figs. 4.7/6 & 4.6/7).

In the central cornea the collagen fibrils of the subepithelial zone were approximately 30nm in diameter; loosely arranged and randomly orientated. The apparent space between fibrils was variable, but was often in excess of the fibril diameter. Peripherally, the apparent interfibrillar separation was even greater as the less ordered arrangement of the limbus was encountered. In some animals, electrolucent, round spaces increased the interfibrillar separation in the subepithelial zone.

Apart from occasional nerve fibres with their investing perineural cells, and rare fibroblasts, this region was acellular and consisted

of ground substance and collagen fibrils.

4.7.4 Corneal Stroma The collagen fibrils of the cornea are unusual in that they undergo no change of transverse diameter throughout their length. They were arranged as sheets or lamellae of densely packed fibrils in an amorphous ground substance matrix. The lamellae appeared to cross the entire cornea parallel to the corneal surface and to other lamellae (Figs. 4.7/8 & 4.7/9). The arrangement of lamellae in the anterior one third of the stroma was slightly more oblique than those of underlying lamellae with some antero-posterior intermingling, which was most marked in tangential or oblique sections towards the limbus (Fig. 4.7/10). In the posterior two thirds of the stroma the lamellar pattern was more orthogonal. There was therefore a transition from random orientation of the collagen fibrils in the subepithelial zone to a regular arrangement in deeper layers. The periodicity of stromal collagen fibrils was approximately 64 to 66nm.

In the majority of the stroma of the central cornea the average diameter of individual collagen fibrils was 28 to 35nm and the apparent interfibrillar separation in the mid-stromal region varied from approximately half the fibril diameter to just greater than the fibril diameter; a more compact arrangement than that of the subepithelial zone and limbus.

The true space available for free diffusion of substances between fibrils is obviously much less than interfibrillar measurements suggest, because of the glycoprotein and mucopolysaccharide coating which invests each fibril. Ruthenium red preparations indicated regularly arranged perifibrillar proteoglycan particles.

The transition between the stromal collagen and Descemet's membrane was rendered less abrupt by the presence of fine filaments of approximately 10nm diameter which resembles "nests" of Descemet's fibrils within the corneal stroma (Fig. 4.7/11).

The corneal fibroblasts (keratocytes or fibrocytes) appeared as extraordinarily attenuated cells which were located between, and ran parallel with, the lamellae (Fig. 4.7/12 α 13). They had extensive horizontally branching processes which appeared to merge with those of neighbouring cells although there was actually space between the cell processes. In older animals the cell processes were usually more obvious than in younger dogs of the same breed.

The cell broadened slightly to accommodate the elongated oval nucleus which showed few, if any, indentations, contained several nucleoli and had an electro-dense marginal zone of heterochromatin surrounding a much greater quantity of euchromatin (Fig. 4.7/14).

The organelles contained within the cytoplasm largely reflected the role of the cell as a producer of ground substance and collagen. Usually the rough (granular) endoplasmic reticulum with its long interconnecting cisternae was the most obvious feature. Some of the cisternae appeared to communicate directly with the nuclear envelope and the cell membrane. There were also numerous free ribosomes, a Golgi zone in the perinuclear region and rather small sparse mitochondria. A number of vesicles and vacuoles were present within the cytoplasm as well as micropinocytotic vesicles connected with the cell membrane. The cisternae of the granular endoplasmic reticulum, the intracytoplasmic vesicles arising from the Golgi zone and the cell membrane vesicles of active cells contained a fine granular material, apparently identical to that appearing in the

immediate vicinity of the cell, and usually contained within an invagination of the cell membrane. Dark staining granules of approximately half the diameter of the collagen fibril were also seen in the immediate vicinity of the cell. The fine granular material and the larger granules are usually held to represent precursors of ground substance and collagen respectively. Lipid droplets were identified within the cytoplasm of some fibroblasts.

Lysosomes were not a feature of inactive corneal fibroblasts. Lysosome-containing cells were of undifferentiated appearance and were commoner in peripheral than central cornea. They differed from normal inactive fibroblasts most notably by their pale cytoplasm, lack of prominent granular endoplasmic reticulum and lysosome content. Lysosome-containing cells were often closely associated with normal inactive fibroblasts (Fig. 4.7/15). Enzyme histochemistry had indicated non-specific esterase and acid phosphatase activity in cells at the limbus and cells with these enzymes may correspond with the lysosome-containing cells seen in the transmission electron microscope. Cells of this type were thought to represent degenerating fibroblasts rather than macrophages; they were not seen to be engaged in phagocytic activity.

Other cells which probably derived from fibroblasts were typified by quantities of membranous bodies. Such cells were rarely seen in normal cornea, but were common in diseased cornea. They did not seem to be associated with poor fixation or processing, as cells of normal appearance were found in the vicinity, and it seems more likely that such cells were undergoing a type of *in situ* necrosis, or that the fibroblast possesses the ability to discharge matrical

lipidic debris via its extruded cytoplasmic processes as has been suggested for other cells in avascular situations (Ghadially, 1975). Fibroblasts associated with membranous bodies or matrical lipidic debris will be described more fully in Section 5.0.

Unmyelinated nerve bundles were encountered quite frequently in the superficial stroma, as were unmyelinated nerves enwrapped by a Schwann cell sheath (Fig. 4.7/16).

Lymphocytes, tissue macrophages and leukocytes were rarely found within the corneal stroma and then only at the periphery. Vascular and lymphatic channels were absent. Electrolucent round spaces were sometimes found in the anterior stroma.

4.7.5 Descemet's Membrane Descemet's membrane (posterior limiting lamina) is the basal lamina of the mesothelium (endothelium). It was PAS positive and neither Sudanophilic nor osmiophilic in the normal canine eye.

It was composed of regularly arranged stratified layers of filamentous collagen, but the periodic structure of the fibrils was not as obvious in the dog as in man; there was no banding in the anterior zone and the membrane was of more uniform and homogeneous appearance (Figs. 4.7/17 α 4.7/18).

Descemet's membrane terminated near the insertion of the primary pectinate fibres and, in this region, and less frequently elsewhere, broad spacing 110nm collagen fibrils were sometimes seen, particularly in older animals. In the majority of dogs the primary pectinate fibres were heavily pigmented, arising as they do from the similarly pigmented aspect of the base of the iris (Fig. 4.7/19).

In older dogs Descemet's membrane was thicker than in young dogs, sometimes in excess of 20μm, suggested that, as in other

species, it was produced continuously by the mesothelium in life. Hassal-Henle bodies were not seen and the junction between Descemet's membrane and the mesothelium was smooth with no junctional complexes.

4.7.6 Mesothelium (Endothelium) This was a single cell layer of approximately 2μm thickness in young adult dogs, which thinned slightly with age (Fig. 4.7/17 α 4.7/20).

The cells were richly endowed with organelles suggesting an active metabolism. The nucleus contained both heterochromatin and euchromatin and there were a number of nuclear pores in the nuclear envelope. The Golgi apparatus was prominent and perinuclear in position. Mitochondria with longitudinally arranged cristae were large and plentiful. Numerous free ribosomes were present in addition to those associated with the endoplasmic reticulum; agranular endoplasmic reticulum was also present.

Lysosomes, multivesicular bodies and pinocytotic vesicles were also found within the cell and at the cell membranes. The lateral cell membranes pursued a tortuous course. There was a gap of approximately 200 Å between membranes except at the sites of junctions, which were probably maculae occludentes between cells and, close to the anterior chamber, possible zonulae occludentes. There were marginal folds at the latter junctions. The cell surface in contact with aqueous humour had short cellular projections or microvillae. A terminal web, free of organelles, lay immediately beneath the posterior cell membrane (Fig. 4.7/21).

The mesothelium continued into the ciliary cleft as a sheet of cells covering the inner surface of the corneoscleral trabecular meshwork (Fig. 4.7/22).

4.7.7 The Limbus The limbus marks the transition between the ordered avascular austerity of the cornea and the richly cellular, vascular profusion of the tissues which surround it. The limbus consists of conjunctiva, Tenon's capsule, episclera, corneoscleral stroma and part of the anterior uveal tract (Figs. 4.7/2; 4.7/20; 4.7/22; 4.7/23).

The vascular arrangement of this region was complex with extensive anastomoses at all levels which have been described earlier. The position of blood vessels was clearly defined in meridional sections. Lymphatic vessels appeared to be limited to the conjunctiva. Myelinated and non-myelinated nerves were present at all levels.

In general the fibroblasts of the limbal region, particularly those close to the uveal tract, differed from those of the cornea in possessing occasional lysosomes and cilia. Their shape was modified according to the looseness of surrounding connective tissue.

4.7.8 Limbal Conjunctiva The epithelial cells of limbal conjunctiva were similar to those of the cornea. On light micrographs the basal portion of the epithelium appeared serrated (Fig. 4.7/23), mitochondria and tonofilaments were more prominent; in the electron microscope the latter were often closely associated with desmosomes (Fig. 4.7/24).

Lymphocytes and melanocytes occurred frequently in the basal and suprabasal layers and melanin granules were also found (Fig. 4.7/25). There was quite pronounced folding of the conjunctiva with formation of rete pegs and papillae. Blood vessels, nerves and lymphatics (Fig. 4.7/26) were common in the loose conjunctival tissue along with fibroblasts, melanocytes, macrophages, mast cells

and lymphocytes. Plasma cells, polymorphonuclear leukocytes and eosinophilic leukocytes were less frequently encountered.

4.7.9 Tenon's Capsule and Episclera Both Tenon's capsule and the episclera consisted of denser connective tissue than the conjunctiva and were rich in fibroblasts. Tenon's capsule terminated in the limbal episclera.

Episclera differed from the underlying sclera in being of looser structure. Fibroblasts in this region were less flattened than those of the cornea and sclera because of the looser arrangement of the connective tissue. Episcleral vessels, particularly veins, were obvious (Figs. 4.7/27; 4.7/28). Macrophages, lymphocytes and leukocytes were uncommon.

4.7.10 Corneoscleral Stroma The corneoscleral stroma consisted of a dense connective tissue framework of interweaving and branching collagen bundles. As in other limbal zones the collagen fibrils had tapered ends and the maximum diameter of the fibril increased slightly in progressing from conjunctiva to deep sclera. The diameter of fibrils in the deep sclera exceeded 100nm (Fig. 4.7/29).

In addition to collagen, sparse elastic tissue was also present. Fibroblasts were reasonably common, as were melanocytes in some regions; other cells such as macrophages, lymphocytes and polymorphonuclear leukocytes, less so (Fig. 4.7/30). Blood vessels were present at all levels and often lay close to bundles of myelinated and unmyelinated nerves (Fig. 4.7/31).

Capillaries of the bulbar conjunctiva were mainly non-fenestrated, occasionally fenestrated. Non-fenestrated capillaries were also found in the episclera, sclera and iris. A limited number

of survey sections of the ciliary body and its processes, and the choriocapillaries of the choroid indicated that fenestrated capillaries were present.

The intimate association of the deep cornea and sclera with parts of the anterior uveal tract, linked as it were by the trabecular meshwork of the ciliary cleft, is shown in Figs. 4.2/7; 4.6/7; 4.7/2 α 4.7/22).

4.7.11 Microscopy: Summary and Discussion. This study has established the normal lipid histochemistry of the canine cornea in dogs of different ages and is in agreement with the results of other workers with regard to normal structure. It also confirms the lack of age-related lipid deposition in the corneas of normolipoproteinaemic animals suggested by the work of Jensen (1974) and Slatter (1975).

Using oil red O with plane or polarised light as the sole method of lipid identification Slatter was able to demonstrate lipid deposition in the inner third of the sclera in hyperlipoproteinaemic American Foxhounds of one, two and five years and in some of the untreated control animals of the five year old group. In addition, a five year old bitch from this latter group was the only animal to develop oil red O positive material in the cornea, affecting the anterior part of Descemet's membrane and the immediately adjacent corneal stroma, the intensity of staining was greatest near the limbus. The author does not comment on the fact that this control animal had slightly abnormal serum lipid values. For example, one sample indicated cholesterol 290mg/100ml, triglyceride 101mg/100ml and phospholipid 27mg/100ml. Mean values for the whole five year old control group were 215, 54 and 17 respectively and for the five

year old treated group 825, 325 and 29 respectively. The high phospholipid value is noteworthy as there is some evidence (Crispin, unpublished observations) that phospholipid-rich lipid has a predilection for the anterior portion of Descemet's membrane in the dog. The results are also of general interest in endorsing the findings of this study that lipids and lipoprotein patterns may be of importance in determining the distribution and type of lipid observed in the canine cornea.

Bromine Sudan black B was the most useful screening method for corneal lipid and the detection of small quantities of lipid in the anterior stroma with this stain may correlate with the presence of occasional electrolucent round spaces in material from the same animals examined with the electron microscope. It is also possible that the matrical lipidic debris infrequently detected in the normal cornea, and associated with degenerate changes in the fibroblast, may be responsible for the enhanced staining of occasional corneal fibroblasts with Bromine Sudan black B.

Ageing changes in the canine cornea appeared to be limited to a progressive increase in the thickness of Descemet's membrane and a gradual attenuation of the mesothelium. In the electron microscope the cell processes of stromal fibroblasts became more obvious in the older dogs, possibly because they branched into adjacent collagen lamellae instead of remaining between lamellae. Broad spacing collagen fibrils were found in Descemet's membrane and were more frequent in the region of insertion of the pectinate ligaments; their quantity appeared to increase with age. There was no broad spacing collagen zone in the anterior part of Descemet's membrane and Hassall Henle warts were not observed, although they are a

ubiquitous feature of adult human cornea.

The vascular system of limbal regions has been discussed earlier; the other obvious feature of this region was the richness of lymphatic vessels in the conjunctival stroma and the absence of lymphatic vessels deep to conjunctiva. Trabecular veins (or small aqueous collector channels) showed some similarities to lymphatic vessels and were also distinctive in that they lacked a continuous basal lamina.

5.0 RESULTS - CLINICAL CASES

5.1 Introduction

The clinical material referred to in this study is summarised in Table 5.1/1 and the appearance of clinical cases at the time of first examination in Fig. 5.1/1. The range of clinical appearances is given in Figs. 5.1/2 to 5.1/25 and as indicated elsewhere in the text. Individual case histories, together with details of the photographs, are presented as Appendix I; general aspects of history and physical examination appear below.

5.2 Case History and Physical Examination

For each clinical case details of diet and feeding habits were recorded as previously outlined for normal controls. The diets of individual cases appear with serum lipid levels in Table 5.7/1. The animals were weighed at the time of first examination and at subsequent re-examinations and the owners also kept a monthly record of weight in a proportion of cases.

On the basis of the case history and physical examination the animals could be tentatively assessed as normal, normal but overweight, or abnormal because of systemic disease. According to the diagnosis the animals were untreated, or treated by a change of dietary regime, or were given specific therapy for the underlying systemic disorder. Most animals were re-examined at regular intervals, and in a few cases it was necessary to revise treatment in the light of subsequent developments; although it was of interest that "subclinical" disease had been suspected at the time of first examination.

In all animals, which were overweight but without demonstrable disease as a cause, a change of diet was achieved simply by feeding

Table 5.1/1 Summary of Clinical Cases

Identification Breed/Number	Age when corneal opacity first noticed Yrs.Mths.	Age when animal first presented Yrs.Mths.	Sex M/F/Mn/Fn	Weight Kg	Investigations a	b	c	d	e	f	g	h	i
Alsatian	23 3.10	4.3	M	43	+	+	+	+	-	-	+	+	+
"	36 3.1	3.10	M	38	+	+	+	-	+	-	+	+	+
"	153 3.5	3.9	F	31	+	+	+	-	-	-	+	-	-
"	160 3.11	4.0	F	36	+	+	+	-	+	-	+	+	-
Alsatian X	150 1.0	1.8	F(n)	32	+	+	+	+	+	-	+	-	+
Golden Retriever	1 2.3	2.5	F	25	+	+	+	-	+	-	+	-	-
"	4 3.1	4.1	F	33	+	+	+	-	+	-	+	+	+
"	6 3.3	3.7	F	30	+	+	+	-	+	-	+	+	+
"	9 3.11	4.3	F	47	+	+	+	-	+	-	+	+	+
"	14 3.7	3.9	F	32	+	+	+	-	-	-	+	+	-
"	79 2.10	3.5	M(n)	37	+	+	+	+	-	-	+	+	-
Golden Retriever	151 4.9	5.3	F	36	+	+	+	-	+	-	+	+	-
"	154 3.1	3.2	F	30	+	+	+	-	-	-	+	-	-
"	155 3.5	3.6	F	31	+	+	+	-	-	-	+	+	-
English Springer Spaniel	65 2.10	3.1	M	23	+	+	+	-	-	-	+	+	+
"	66 2.2	2.4	F	24	+	+	+	-	-	-	+	+	+
"	131 1.6	1.7	M	22	+	+	+	-	+	-	+	+	-
"	142 8.7	8.7	F	25	+	+	+	-	+	-	+	+	-
Welsh Springer Spaniel	42 6.0	6.1	F	26	+	+	+	+	+	+	+	+	+
English Cocker Spaniel	24 2.9	3.0	F(n)	18	+	+	+	-	+	-	+	+	+
"	83 4.3	4.3	M	16	+	+	+	+	-	-	+	+	-
"	133 2.2	2.3	F	15	+	+	+	+	-	-	+	+	-
Rough Collie	73 2.0	2.9	F(n)	22	+	+	+	-	+	+	+	+	+
"	134 4.8	4.8	M	24	+	+	+	+	-	-	+	+	-

"	149	3.0	3.2	F	20	+	+	+	-	-	-	+	+	-
Old English Sheepdog	134	4.7	4.11	M	39	+	+	+	-	+	-	+	+	+
"	38	4.1	5.1	M	44	+	+	+	-	+	-	+	+	+
Great Dane	74	3.2	4.2	M	68	+	+	+	+	+	-	+	-	+
"	130	3.0	3.8	M	58	+	+	+	-	-	-	+	+	-
Bearded Collie	26	5.4	5.8	M	24	+	+	+	+	+	-	+	+	+
Jack Russell Terrier	132	5.6	6.2	F	7	+	+	+	+	+	-	+	+	+
German S. H. Pointer	75	5.6	6.6	F	30	+	+	+	-	+	+	+	+	-
Basset Hound	77	4.1	4.4	M	27	+	+	+	-	+	-	+	+	-

Key to Table 5.1/1

Age in years and months

Sex (n = Neutered)

Weight in kilograms

a General clinical and opthalmological examination

b Detailed examination of the anterior segment

c Charting and photography

d Temperature measurement

e Electronic applanation tonometry

f Anterior segment fluorescein angiography

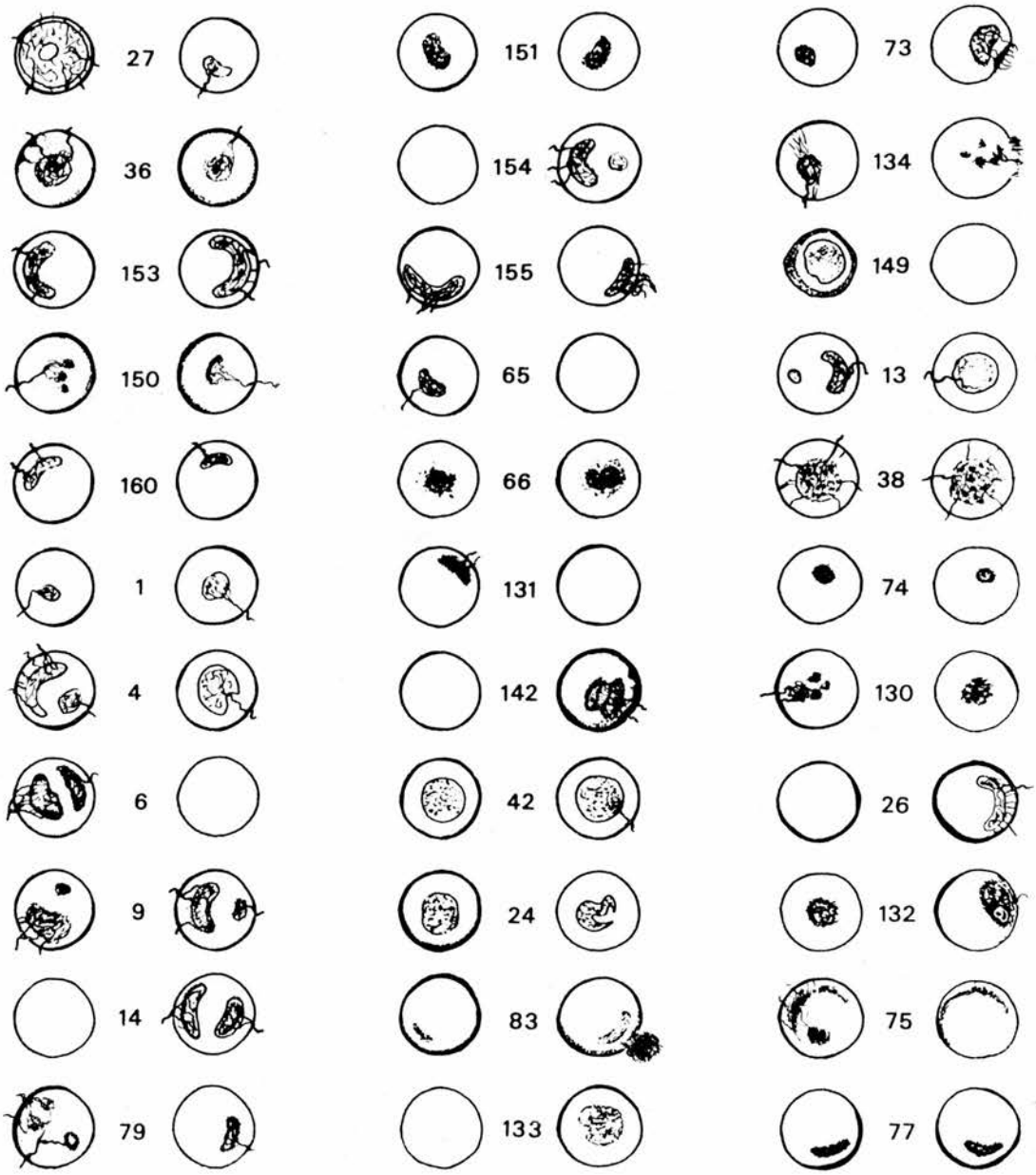
g Venous blood samples

h Lipoprotein electrophoresis

i Keratectomy or whole eye studies

Figure 5.1/1

Appearance of Clinical Cases at the Time of First Examination.



Details of the external eye photographs 5.1/2 - 5.1/25 which appear on subsequent pages are given with individual case histories in Appendix I.

commercial dog food low in saturated fats; usually "Chappie" (Pedigree Petfoods). Whilst recognising that individual variation was possible, if a low fat diet, coupled with appropriate exercise, was ineffective in producing weight reduction and in lowering serum lipid levels, further investigation of the animal was required, particularly in those cases in which "subclinical" hypothyroidism was suspected.

Hypothyroidism was the commonest systemic disease producing secondary hyperlipoproteinaemia and it was of interest that the majority of cases were seen in the early stages of the disease and clinical signs were minimal. Hypercholesterolaemia, mainly due to cholesterol-rich high density lipoprotein, was the only serum lipid abnormality in early cases, whereas cholesterol, triglyceride and phospholipid levels were increased in more advanced cases.

The other systemic diseases identified in this study were pancreatitis, intrahepatic cholestasis and iatrogenic Cushing's syndrome. No specific therapy was given for these conditions, other than a slow withdrawal of cortisone treatment for the animal with Cushing's syndrome.

5.3 Detailed Examination of the Anterior Segment

5.3.1 Vasculature Detailed examination of the anterior segment indicated that mixed conjunctival and ciliary injection was the most frequently encountered abnormality and the history and clinical examination indicated this was often an intermittent phenomenon. Conjunctival hyperaemia alone was observed in chronic superficial keratoconjunctivitis (pannus) and corneal ulceration. Ciliary injection alone was observed in a case of anterior uveitis and two cases of scleritis. Abnormalities of limbal vessels were undetected

in only three cases (Fig. 5.3/1).

Lipaemia of the limbal vessels was only seen in animals in which all the serum lipids were raised and where hypertriglyceridaemia was prominent (Fig. 5.3/2). Peripheral arcus lipoides corneae was likewise associated with an increase of all serum lipids rather than hypercholesterolaemia alone.

Corneal neovascularisation was not always present in the early stages of lipid keratopathy; in some cases neovascularisation preceded the appearance of corneal lipid, in others it was a sequel to the appearance of lipid and in yet others keratectomy was performed, or treatment instituted, before neovascularisation developed. Neovascularisation was often both superficial and deep. Superficial vascularisation alone was observed in chronic superficial keratoconjunctivitis and corneal ulceration. Deep vessels from the perilimbal vascular network were sometimes found prior to superficial vascularisation in progressive opacities. In most cases, however, vascularisation was both superficial and deep with no connections within the cornea between vessels derived from conjunctival vessels and those derived from the deeper perilimbal vessels.

The presence of lipid within the cornea in this study was most commonly associated with episcleral and scleral inflammations, although uveal tract inflammations and a variety of more superficial insults were also recorded. In only three cases was there no history of any form of anterior segment inflammation.

The range of clinical appearances at the time the animals were referred for second opinion has been summarised in Fig. 5.1/1 and the variety may be partially correlated with time for which the

lesions have been present, the presence or absence of neovascularisation, the level of conjunctival, episcleral, scleral, or uveal, inflammation and whether or not the cornea itself was normal or abnormal.

5.4 Fluorescein Angiography

Fluorescein angiography of the anterior segment was performed on a limited number of clinical cases and proved a technique worthy of further investigation. In animals with vascularised lipid keratopathy the extensive nature of corneal neovascularisation was apparent after fluorescein injection whether the eye was quiet (Fig. 5.4/1) or actively inflamed (Fig. 5.4/4). Circulation of fluorescein was rapid and leakage from new vessels conspicuous, particularly from superficial vessels. Extensive leakage into the corneal stroma produced a "negative" vascular phase whereby the new vessels appeared as finely detailed dark silhouettes against a fluorescent background. (Figs. 5.4/2 and 5.4/3).

The amount of fluorescein entering the corneal stroma was markedly different in one animal (42) according to whether the eye was quiet or suffering a recrudescence of episcleritis and scleritis. In both situations superficial vessels derived from the conjunctiva were fluorescein positive and there was leakage from their advancing tips. During a phase of active inflammation in which there was conjunctival and ciliary injection and mild corneal oedema, fluorescein leakage was so intense within parts of the corneal opacity that it could be photographed without special facilities. (Fig. 5.4/4).

5.4.1 Fluorescein Angiography of the Anterior Segment: Summary and Discussion

There do not appear to be any references to fluorescein angiography of the abnormal cornea and limbus in the dog although inflammations of the iris have been studied using the technique (Gelatt, personal communication) and are typified by marked increases in vessel permeability and fluorescence of the aqueous humour. Fluorescein angiography of the anterior segment has proved a valuable technique for studying anterior segment disease in man (Mitsui,Matsubara α Kanagawa, 1969; Bron α Easty, 1970; Easty α Bron, 1971). The individual variation between the amount of fluorescein leakage and the intensity of intravascular fluorescence in cases of lipid keratopathy was remarked on by Easty α Bron (1971).

5.5 Temperature Measurement

The measurement of ocular temperature in dogs with lipid keratopathy indicated that the temperature recorded could be correlated with the amount of anterior segment inflammation seen clinically. Inflamed eyes or specific areas of inflammation (such as nodular scleritis and nodular fasciitis) were compared with the same eye when quiet, the opposite eye, or area, in unilateral cases, or the results obtained from normal controls under the same conditions. Both the central anterior corneal temperature and the temperature beneath the nictitating membrane were found to increase in cases of anterior uveitis, scleritis, episcleritis and keratoconjunctivitis. Temperatures recorded in the cornea in the immediate vicinity of new blood vessels were also raised compared with avascular areas, or those in which only ghost vessels were present. The results are summarised in Table 5.5/1.

Table 5.5/1 Temperature Measurements from Clinical Cases

Identification Number	Environmental Temp °C	Rectal Temp °C	Inf. Conjunctival Sac Temp °C		Central ant. Cornea Temp °C	
			R Eye	L Eye	R Eye	L Eye
27	18.0	37.9	36.6	35.9	29.7	28.9
Hypothyroid - prior to treatment. Cornea vascularised.						
27	17.2	38.4	36.9	36.8	28.8	28.9
Hypothyroid - two months after treatment started.						
79	18.8	38.7	38.4	38.3	33.3	32.0
Sclerosing keratitis - active. Cornea vascularised.						
79	18.5	38.6	36.8	36.8	31.5	31.4
Sclerosing keratitis - eye quiet. Ghost vessels.						
42	16.6	38.8	39.0	38.9	33.0	33.2
Episcleritis/scleritis - Active. R. eye non-vascularised, L. eye vascularised						
42	16.3	38.7	37.0	36.9	30.4	30.3
Episcleritis/scleritis - eye quiet. One month after keratectomy of R. eye.						
83	19.3	38.5	38.2	38.4	32.6	32.8
Scleritis/Nodular scleritis - active. Non-vascularised.						
83	19.5	38.3	36.9	36.7	31.9	31.4
Scleritis/Nodular scleritis - eye quiet. Non-vascularised.						
134	15.8	38.7	37.4	37.6	30.7	31.2
Benign nodular fasciitis L. eye. Vascularised both eyes.						
134	16.0	38.8	36.7	36.8	29.8	29.9
Benign nodular fasciitis - one month after keratectomy.						
74	17.7	38.8	38.6	39.8	32.4	32.5
Active anterior uveitis. Non-vascularised.						
74	18.2	38.6	36.8	36.8	29.9	30.0
After anterior uveitis - eye quiet. Non-vascularised.						

26	19.2	38.6	37.0	38.2	30.4	32.7
Active anterior uveitis - vascularised. Unilateral.						
26	18.6	38.5	36.2	36.3	29.9	30.1
After anterior uveitis - eye quiet.						
132	17.2	38.5	36.8	37.9	30.2	31.4
Bilateral lens luxation, previous corneal ulceration. Vascularised L. eye.						
32	17.8	38.5	36.7	36.8	30.3	30.5
After lendectomy and lesion regression. No vascularisation three years later						

5.5.1 Temperature Measurement: Summary and Discussion

These results are similar to those obtained by Mapstone (1968) for man, in which he demonstrated that the inflammatory vaso-dilatation of anterior segment inflammation could increase the corneal temperature up to 2.4°C relative to the opposite cornea. It would be of interest to see if the temperature rose even higher in patients with anterior segment neoplasia where increased local blood supply and increased local tumour tissue metabolism might both operate. In the one animal of this series with a limbal based lesion resembling nodular fasciitis (134) the temperature of the tumour was 35.3°C and that of the same region of the other eye 34.6 °C, but this type of connective tissue disturbance may have a less rich vascular supply and metabolic rate than would say a malignant melanoma at the same site. The dog with nodular scleritis (83) had an isolated nodule in the sclera of the left eye and a generalised scleral inflammation of both eyes. The temperature recorded from the nodule was the same as that of a similar region of the opposite eye at 35.7°C.

Variations of corneal temperature may affect enzyme-mediated activities and lipid melting points, and this subject is examined in greater detail in Section 6.7.

5.6 Tonometry

Measurement of intraocular pressure demonstrated no significant differences between the clinical cases and normal controls, with the exception of lower than normal values in active anterior uveitis (26 α 74) scleritis (42) and increased values in bilateral lens luxation (132). The recordings for normal controls and clinical cases are presented together in Table 5.6/1.

Table 5.6/1 Tonometry in Controls and Cases

Normal Dogs

Identification Number	R. Eye mm Hg	L. Eye mm Hg
81	17	18
109	17	17
157	16	17
69	16	16
80	17	17
97	17	16
100	16	16
34	21	21
48	17	16
50	15	16
85	16	17
92	18	18
127	15	15
46	17	16
120	20	20
12	14	15
87	13	14
86	16	15
52	20	20
110	19	19
111	18	18
112	19	18
113	17	18

114	19	19
117	14	14
29	21	20
84	19	18
125	13	13

Clinical Cases

Identification Number	R. Eye mm Hg	L. Eye mm Hg	Comment
36	18	18	Episcleritis
160	17	18	Sclerosing keratitis
150	16	17	Pannus
1	16	16	No clinical signs reported
4	17	16	Sclerosing keratitis
6	16	16	Sclerosing keratitis
9	18	18	Sclerosing keratitis
151	17	17	Recurrent ocular redness reported
131	17	16	Healed corneal ulcer right eye
142	18	17	Healed corneal ulcer left eye
42	14	15	Active episcleritis.scleritis
42	20	21	Eye quiet
73	16	16	Nodular fasciitis
13	16	16	Recurrent perilimbal redness
38	19	20	Episcleritis
74	10	11	Active anterior uveitis both eyes
26	19	12	Active anterior uveitis left eye
132	27	54	Bilateral lens luxation
75	17	17	Previous panuveitis and pannus
77	20	20	Distichiasis

Normal Dogs		Clinical Cases
n = 56	n = number of eyes	n = 40
Mean (± SEM): 17 (± 0.279)		Mean (± SEM): 18 (± 1.023)
Range: 13-21		Range: 10-54

Differences between normal dogs and clinical cases not significant ($p>0.05$) by student's t-test.

5.7 Laboratory Examination

5.7.1 Lipid Analysis The results of serum cholesterol, triglyceride and phospholipid estimation are given in Table 5.7/1 together with a synopsis of each animal's diet. The key to dietary components is given with the Table.

Age Scatter diagrams for cholesterol, triglyceride and phospholipid levels against age demonstrated no obvious pattern or differences between breeds (Figs. 5.7/1, 5.7/2, 5.7/3). There was no correlation between serum lipid values and age in the clinical cases (Table 5.7/2) and in this they differed from the normal controls of a similar age range, in which phospholipid levels showed some correlation with age.

Sex No significant differences were found between the serum lipid values of entire females and entire males (Table 5.7/3).

Breed The scatter diagrams (Figs. 5.7/1 - 5.7/3) had indicated no obvious differences of serum lipid values between breeds. Analysis of variance confirmed that there were no significant differences between clinical cases of different breeds.

Serum Lipids The relationship between cholesterol, triglyceride and phospholipid was also examined for individual animals. Plots of cholesterol against triglyceride and phospholipid are shown in Figs. 5.7/4 - 5.7/6 and the correlation coefficients for cases and controls are listed in Table 5.7/4. For both cases and normal controls the cholesterol and phospholipid levels were highly correlated. For the controls, triglyceride against cholesterol and against phospholipid were not correlated, whereas for the cases they were. This apparent difference was partly due to a proportion of the clinical cases with high values of triglyceride also having high values of cholesterol

Table 5.7/1 Serum Lipid Values and Diets of Clinical Cases

Identification Number	Diet	Cholesterol in mmol/l	Triglyceride in mmol/l	Phospholipid in mmol/l
27	Cm Cb + dmg	6.98	0.62	5.24
36	Cm Cb + dmg	7.47	0.42	5.98
153	Cm Cb Cc	5.63	0.38	3.76
160	Cm Cb ± dmg	6.84	0.49	4.24
150	Cm Cb ± dmg	6.30	0.44	5.80
1	Cm Cb Cc ± s	3.68	0.30	2.65
4	Cm Cb Cc ± s	6.20	0.39	4.39
6	Cm Cb	3.15	0.42	3.70
9	Cm Cb Cc ± d	11.20	1.0	7.27
14	Cm Cb ± dmg	6.39	0.58	4.88
79	Cm Cb Cc ± dmg	7.41	0.36	4.93
151	Cm Cb Cc	8.34	0.63	6.83
154	Cm Cb	4.23	0.36	3.86
155	Cm Cb ± d	6.0	0.48	4.83
65	Cm Cb ± dmg	6.28	0.44	4.00
66	Cm Cb	9.93	0.98	6.38
131	Cm Cb ± dmg	6.52	0.69	4.88
142	Cm Cb ± s	10.28	1.26	7.84
42	Cm Cb + dmg	6.46	0.18	4.74
24	Cm Cb + dmg	9.99	0.64	6.21
83	Cm Cb + dmg + s	9.39	0.86	7.16
133	Cm Cb ± dm	6.16	0.39	4.51
73	Cm Cb ± mg	4.98	0.16	4.45
134	Cm Cb ± dmg	6.22	0.28	3.67
149	Cm Cb Cc	9.14	1.10	6.76
13	Cm Cb ± d	7.60	0.48	5.08

38	Cm Cb + dmg	8.21	0.79	6.39
74	Cm Cb + dmg	5.34	0.34	3.58
130	Cm Cb Cc	5.54	1.42	2.85
26	Cm Cb	5.00	0.44	3.86
132	Cm Cb Cc ± d	6.66	0.73	5.44
75	Cm Cb ± s	5.17	0.89	4.90
77	Cm Cb ± mg	9.15	1.02	7.63

Cholesterol:	Range 3.15-11.2	Mean 6.94	SD 1.95
Triglyceride:	Range 0.28-1.42	Mean 0.61	SD 0.31
Phospholipid:	Range 2.65-7.84	Mean 5.11	SD 1.37

n = 33

Key to diets

+ = regularly ± = occasionally

Cm = Commercial Meat
Cb = Commercial Biscuit
Cc = Commercial Cereal
] Proprietary dog foods

d = dairy products
m = meat products
g = vegetables and cereals
s = sweets
] Mainly human foods

Figure 5.7/1

Relationship between Age and Cholesterol-Cases

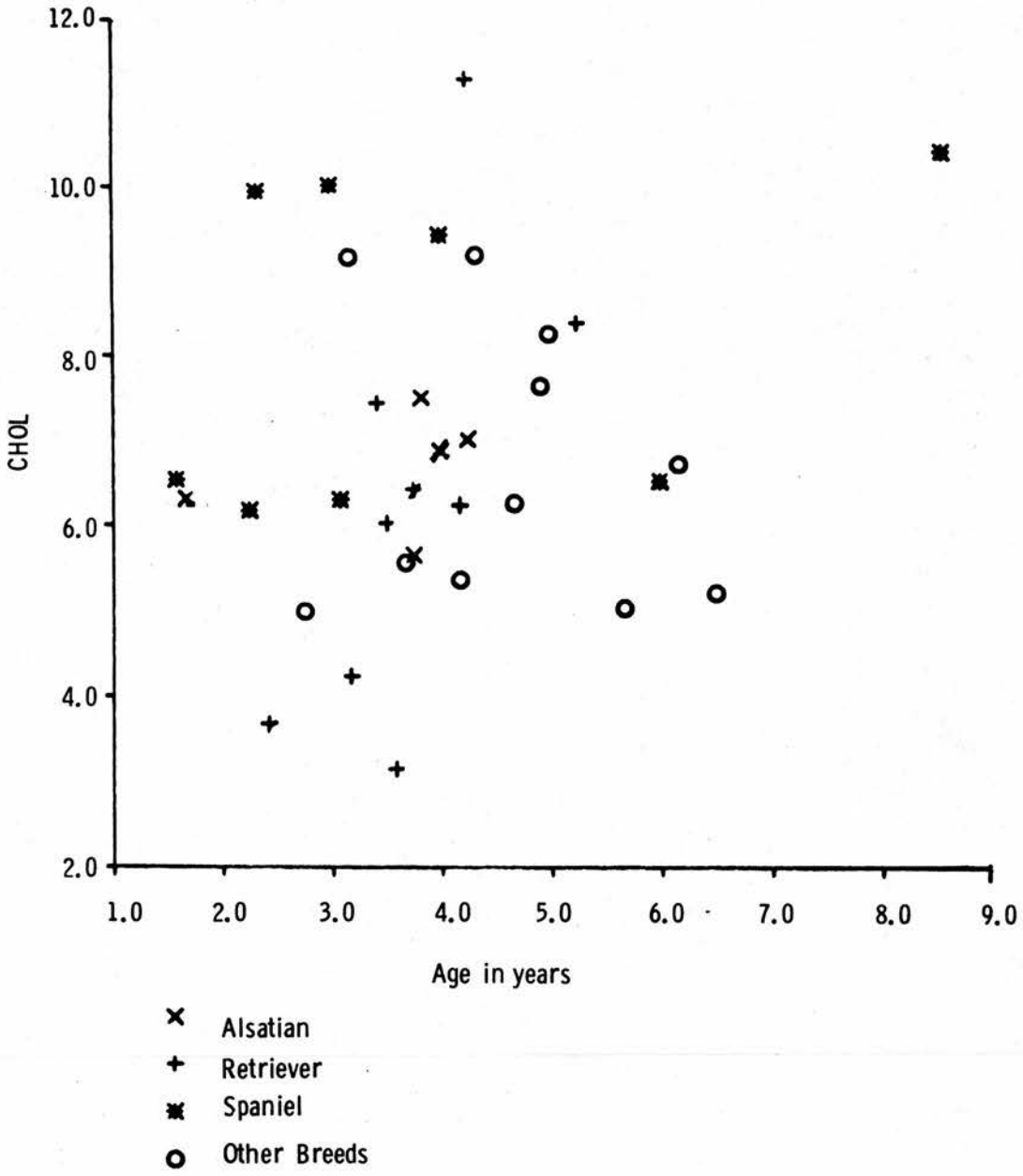


Figure 5.7/2

Relationship between Age and Triglyceride-Cases

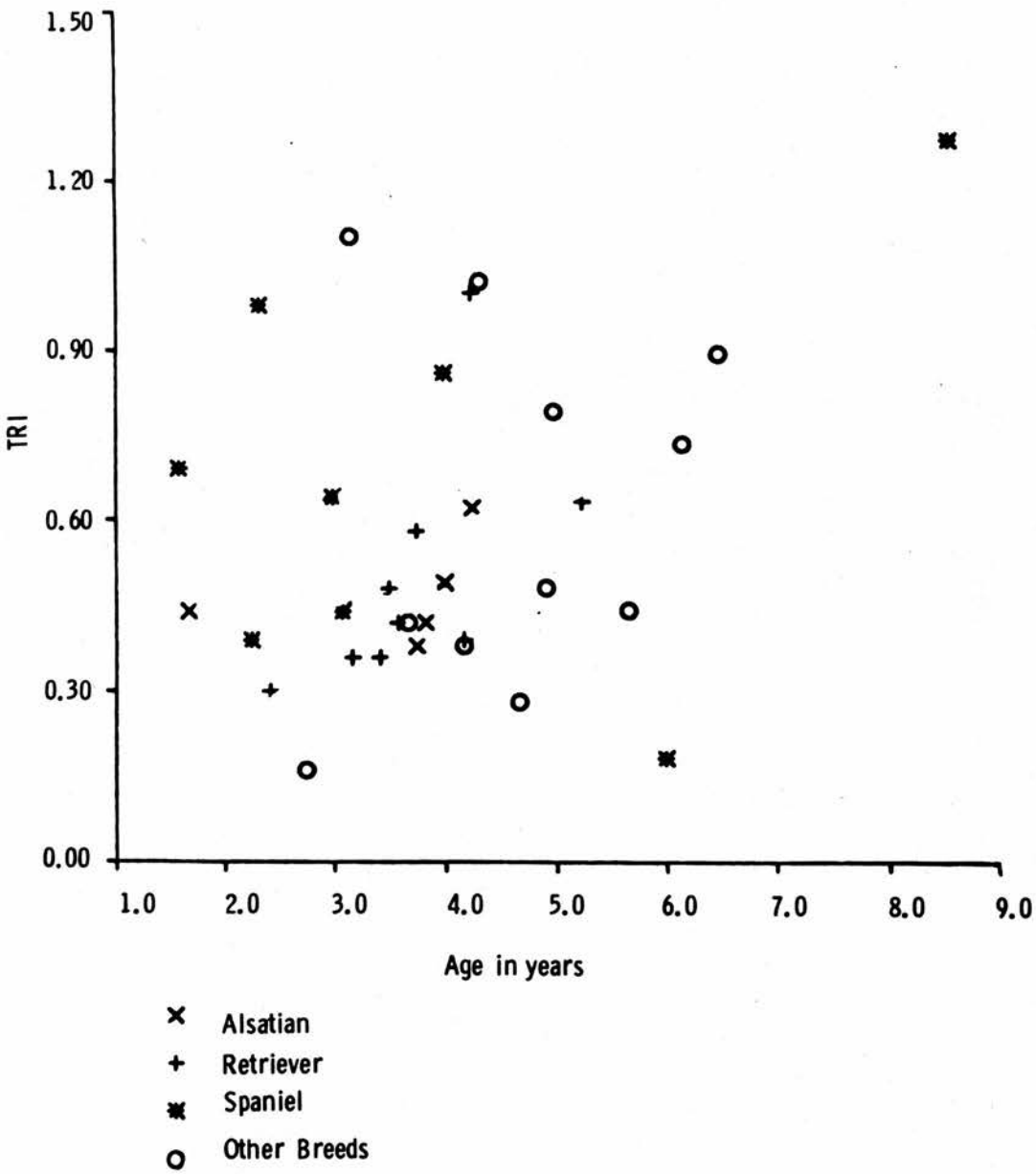


Figure 5.7/3

Relationship between Age and Phospholipid-Cases

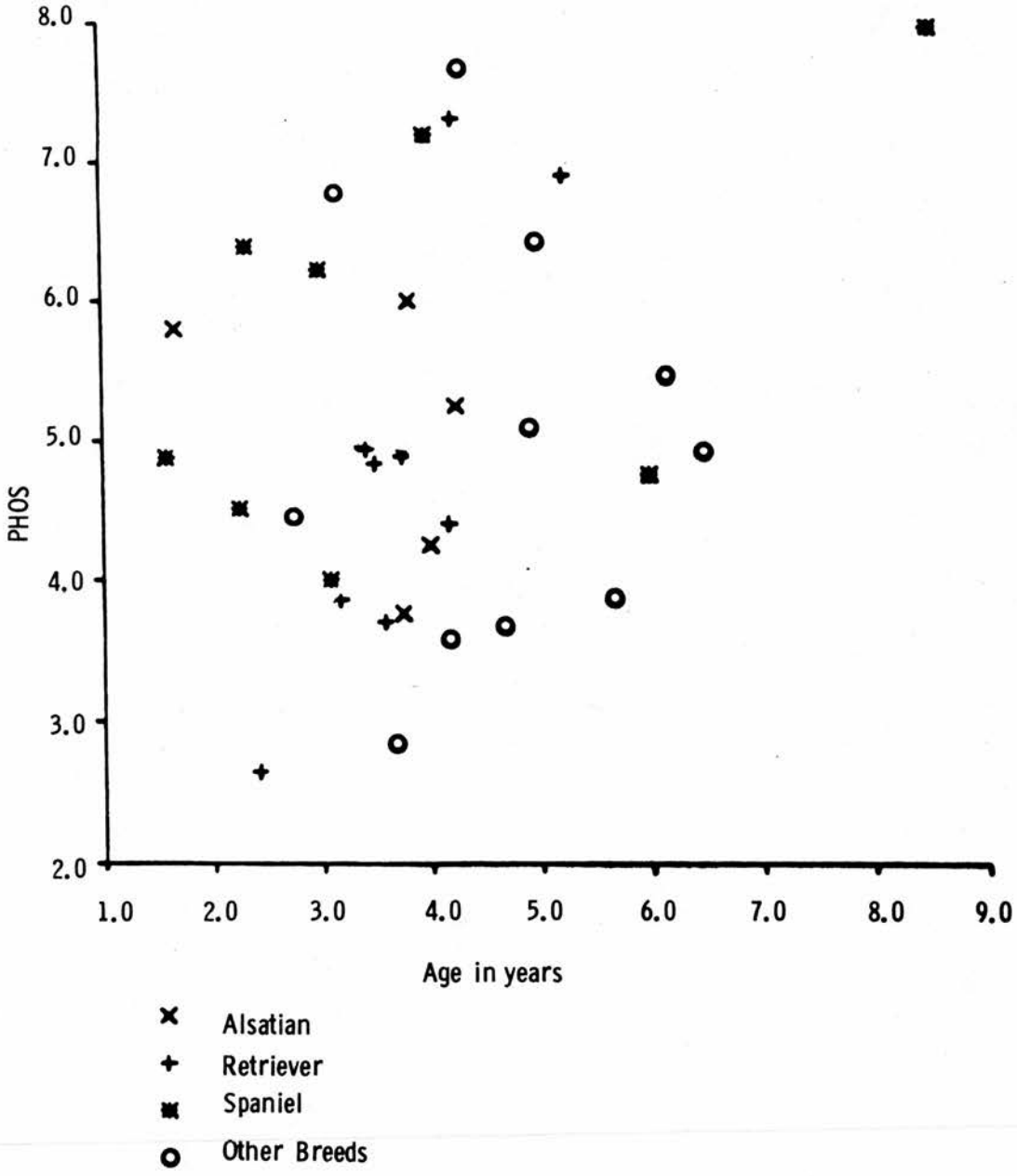


Table 5.7/2 Correlation of Serum Cholesterol, Triglyceride and Phospholipid Levels with Age for Controls and Cases

		No. of Animals	Ch v Age	Tr v Age	Ph v Age
Controls	All ages	68	.56***	.29**	.80***
Controls	aged 1-9 years	58	.12 ns	.20 ns	.40***
Cases		33	.19 ns	.33 ns	.27 ns

** , *** Denote significance at 1% and 0.1% levels respectively.

ns: not significant ($p>0.05$).

Table 5.7/3 Comparison of Cholesterol, Triglyceride and Phospholipid Levels in Female and Male Animals Aged <9 Years for Controls and Cases

		No. of animals	Ch mean (SD)	Tr mean (SD)	Ph mean (SD)
Controls	females	35	4.38 (.75)	.51 (.12)	3.84 (.54)
	males	20	4.07 (.79)	.53 (.12)	3.66 (.41)
	difference		+.31	-.02	+.18
Cases	females	16	6.57 (2.18)	.58 (.28)	4.95 (1.29)
	males	12	6.97 (1.43)	.65 (.33)	5.03 (1.51)
	difference		-.40	-.07	-.08

Differences not significant (>0.05) by student's t-test.

Table 5.7/4 Correlation of Cholesterol, Triglyceride and Phospholipid Levels for Controls and Cases Aged <9 Years.

	No. of animals	Ch v Ph	Tr v Ch	Tr v Ph
Controls	59	.70***	.041 ns	.13 ns
Cases	33	.87***	.70***	.76***

*** Denotes significance at 0.1% level
ns: not significant.

Figure 5.7/4

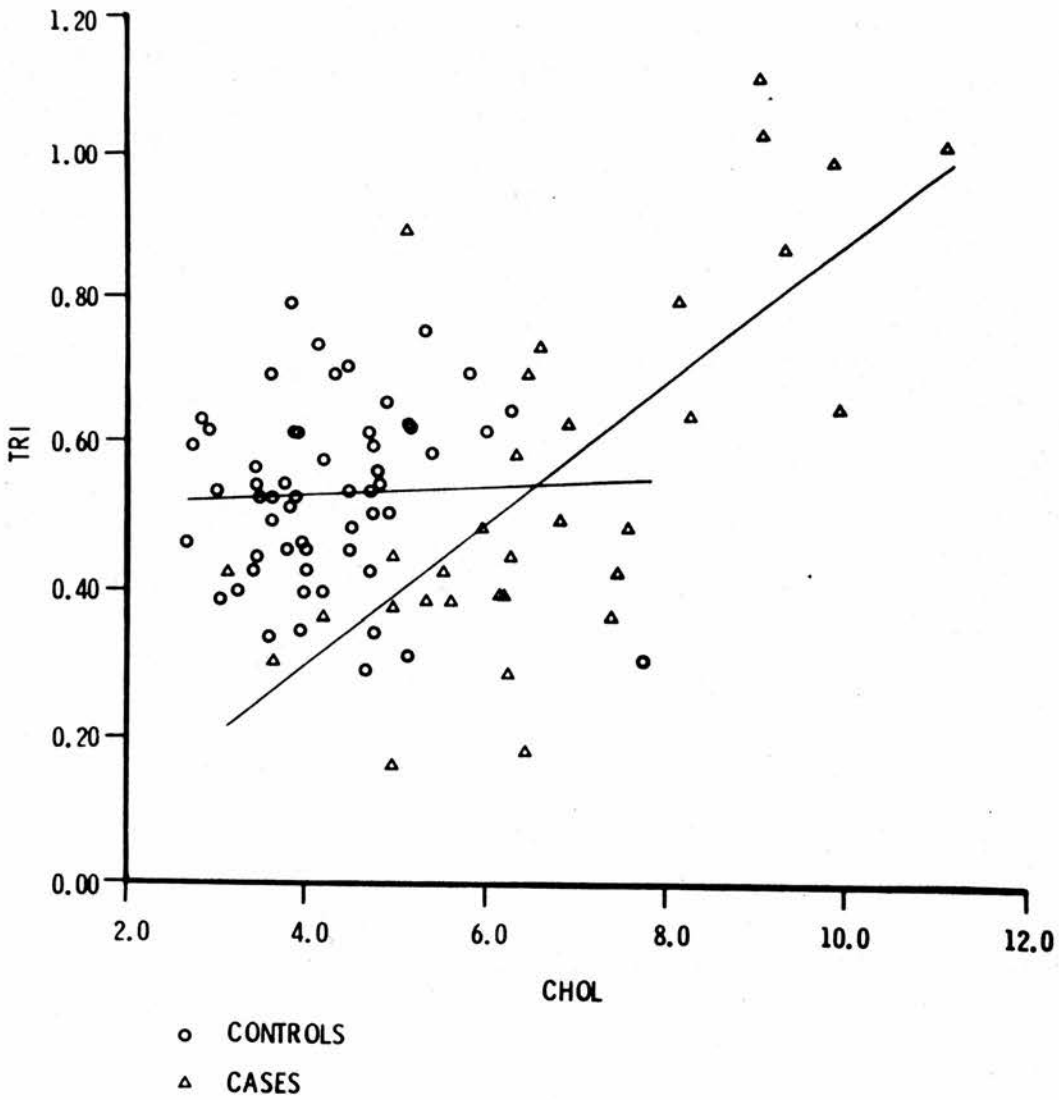

CHOL VS TRI
1.20
1.00
0.80
0.60
0.40
0.20
0.00
TRI
2.0
4.0
6.0
8.0
10.0
12.0
CHOL
CONTROLS
CASES

Figure 5.7/5

CHOL VS PHOS

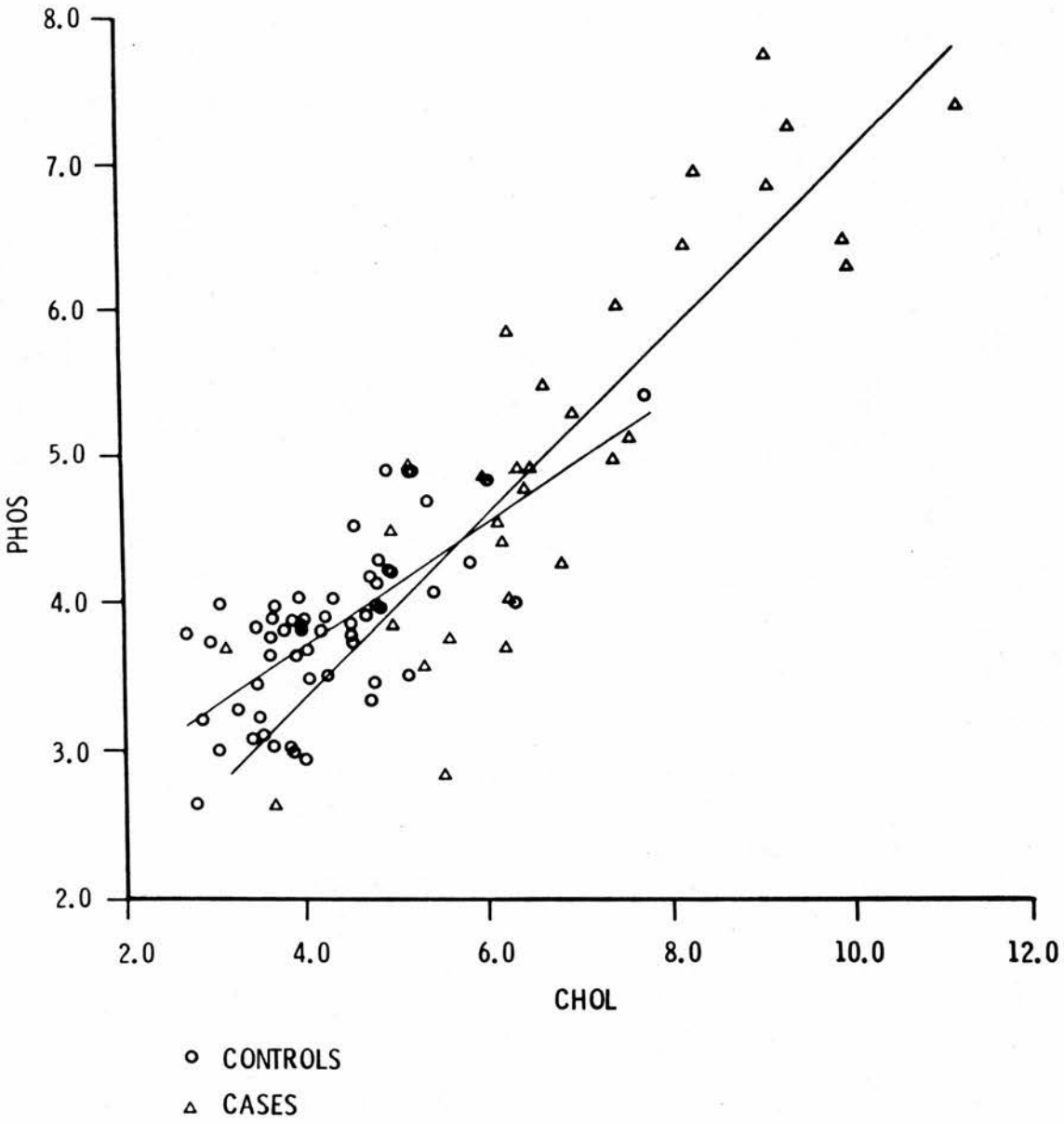
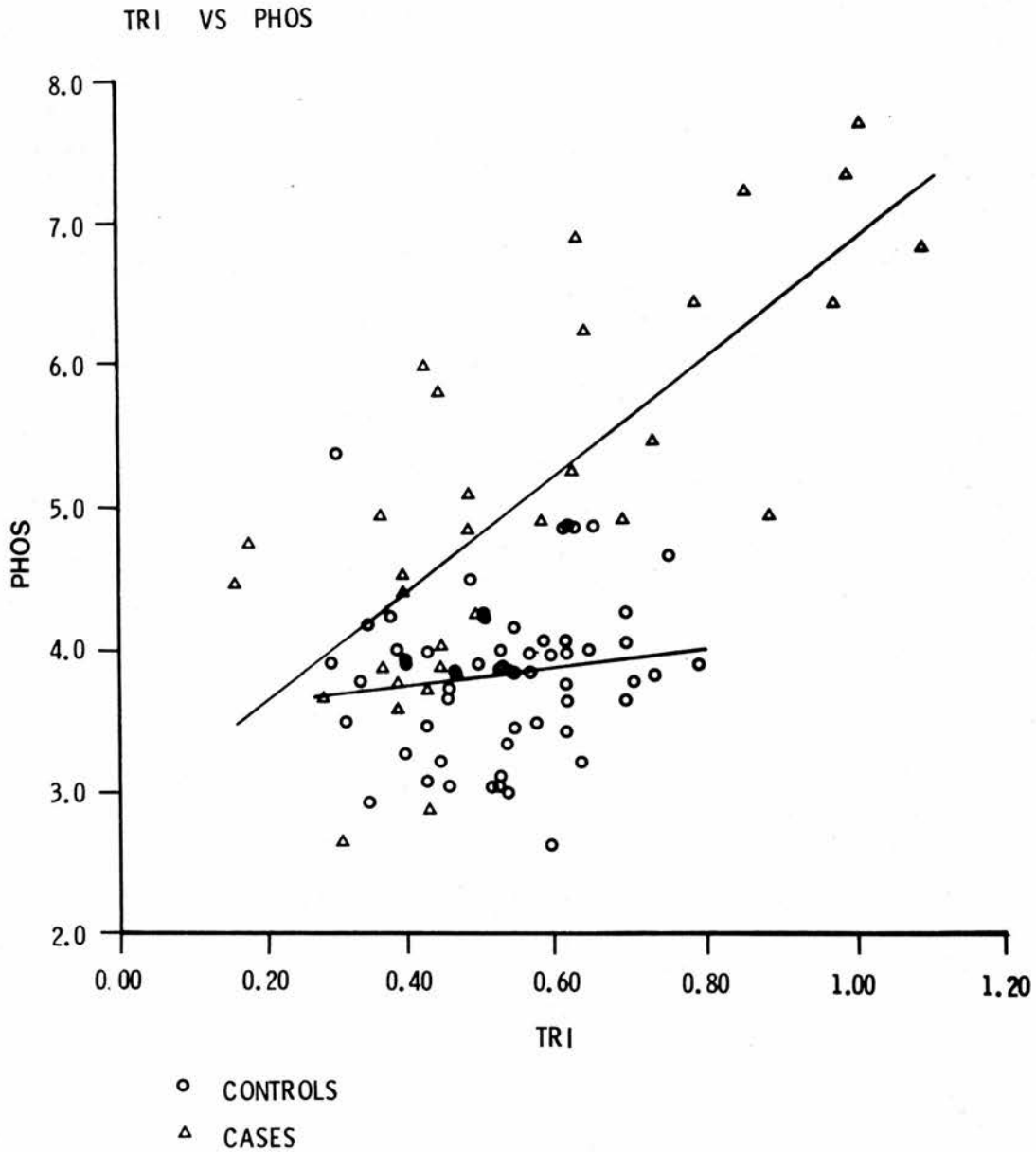


Figure 5.7/6


TRI VS PHOS
8.0
7.0
6.0
5.0
4.0
3.0
2.0
PHOS
0.00
0.20
0.40
0.60
0.80
1.00
1.20
TRI
CONTROLS
CASES

and phospholipid, whereas only one of the controls (162) had a triglyceride level in excess of one and she was thirteen and a half years old. Six of the 33 clinical cases (9, 66, 142, 149, 130, 77) had high triglyceride values, but a number of cases also had low values, so that overall the mean was close to that of the controls. The variation of triglyceride levels was much higher among cases than controls, with a standard deviation of 0.25 and 0.12 respectively. The effect of serum lipid values on the lipoprotein electrophoresis pattern is discussed below.

Comparison of Clinical Cases and Normal Controls In planning the method of study in the early part of this work, it was felt that there might be differences in serum lipids related to age, breed or sex and that it would therefore be sensible to match clinical cases with a normal control of the same, or similar, age, breed and sex, wherever possible. Apart from correlation between age and phospholipid levels in the control group, neither clinical cases nor controls have demonstrated general differences due to age, breed or sex within the age range one to nine years. There is, however, a great deal of individual variation and comparison of matched pairs probably offers the most accurate method of analysis. Of the 33 clinical cases, 24 were paired as indicated in Table 5.7/5 which also gives the weights of individual animals.

The weights of clinical cases and normal controls were compared (Table 5.7/5) and the differences were of significance using a paired t-test ($p < 0.01$). Comparison of serum cholesterol, triglyceride and phospholipid in paired animals demonstrated that differences in the levels of cholesterol and phospholipid were significant ($p < 0.001$: paired t-test) whereas those for

Table 5.7/5 Comparison of the Weights of 24 Paired Controls and Cases

Identification of clinical case	Weight in kg	Identification of normal control	Weight in kg	Difference
27	43	109	34	+10
36	38	119	33	+5
153	31	156	28	+3
160	36	163	35	+1
150	32	69	29	+3
1	25	168	25	0
4	33	80	28	+5
6	30	100	26	+4
9	47	97	29	+18
151	36	161	31	+5
154	30	176	29	+1
155	31	169	30	+1
65	24	146	24	0
66	26	164	25	+1
131	22	166	20	+2
142	25	165	22	+3
24	18	92	12	+6
83	16	108	14	+2
133	15	171	13	+2
134	24	175	25	-1
149	20	127	24	-4
38	44	96	40	+4
74	68	135	66	+2
132	7	86	6	+1

t-test applied to the mean of differences between pairs, $p < 0.01$. Significant at 1% level.

triglyceride were not (Table 5.7/6). The variation in triglyceride levels found in the clinical cases is discussed further below.

5.7.2 Visual Inspection A control serum in which lipoproteins and lipids were known to be normal was included. Clear samples were present in animals with normal lipid values and those with an excess of alpha lipoprotein or beta lipoprotein. Turbidity or opalescence of the sample was observed in animals which had an increase of pre-beta lipoprotein, or a more generalised broad beta band.

A cream layer was almost invariably associated with chylomicronaemia and was rare. It accompanied a subnatant opalescence in one sample from 149 (subacute pancreatitis). The commonest reason for a cream layer in conjunction with a cloudy or clear subnatant was the presence of chylomicra in a non-fasting sample and feeding could also produce opalescence of the serum without a cream layer. Such samples were not included in the analysis.

5.7.3 Lipoprotein Electrophoresis A synopsis of the abnormal lipoprotein patterns of a proportion of the clinical cases is presented in Fig. 5.7/7. In the majority of animals the most obvious abnormality was an increased intensity of staining in the $alpha_2$-lipoprotein (HDL_1) band. In these animals levels of serum cholesterol, and usually phospholipid, were raised, whereas triglyceride levels were lower than normal, or normal.

It appeared that the $alpha_2$-lipoprotein moved progressively more slowly between beta and $alpha_1$ lipoprotein as the serum cholesterol level increased. Examination of densitometer tracings indicated that $alpha_2$-lipoprotein had become overloaded with cholesterol and migrated with less speed according to the amount of

Table 5.7/6 Comparison of Cholesterol, Triglyceride and Phospholipid in 24 paired Controls and Cases

	Cholesterol mmol/l	Triglyceride mmol/l	Phospholipid mmol/l
Mean of cases	7.09	.60	5.22
Mean of controls	4.53	.54	3.91
Mean of paired differences	2.56***	.065 ns	1.31***
(SE)	(.38)	(.065)	(.26)

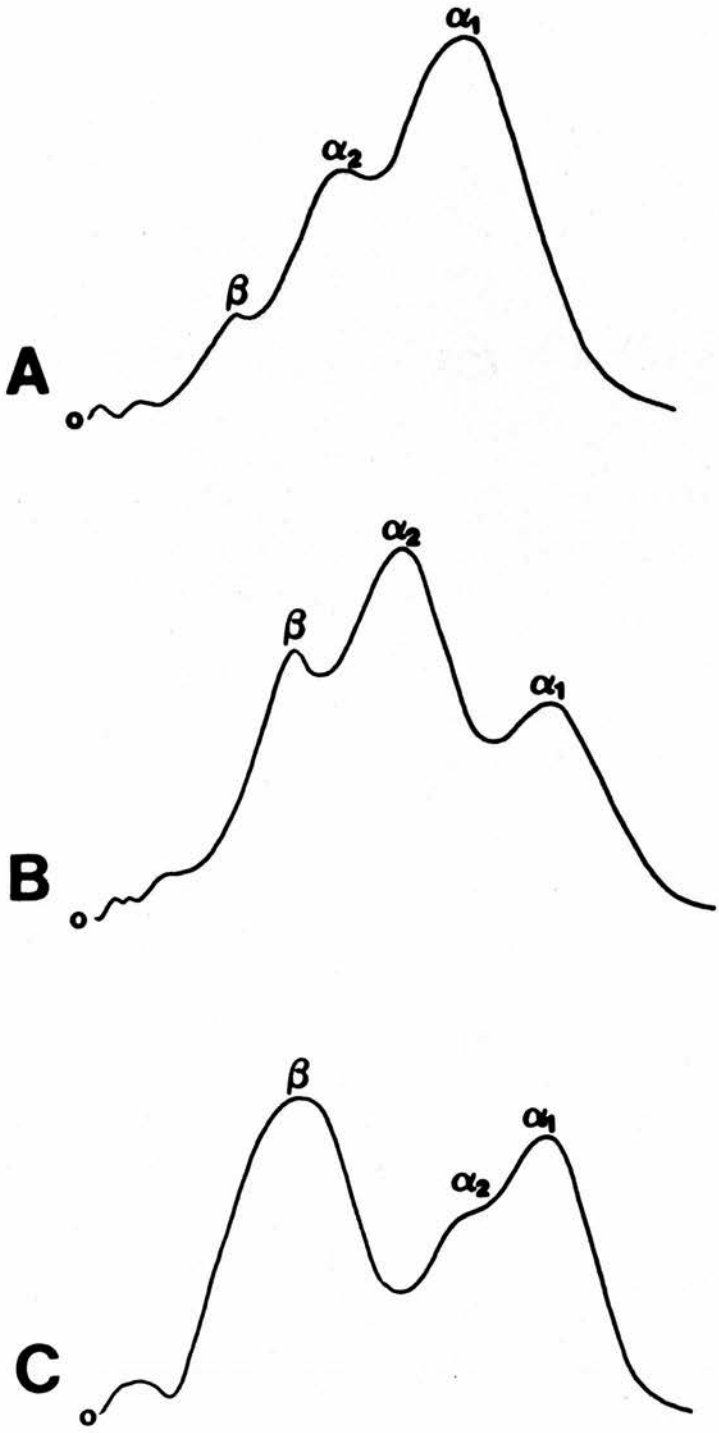
*** Denotes significant at 0.1% level by paired t-test

ns: not significant

S.E. = Standard Error of mean differences.

Figure 5.7/7

Densitometer Tracings of Lipoprotein Electrophoresis Patterns from Clinical Cases.



A and B were typical of animals in Groups II, III and IV
C was typical of animals in Group V

cholesterol carried. Mahley et al (1974) suggested that the least confusing designation for cholesterol-rich HDL_1-like particles was HDL_C and that has been the approach adopted here.

With progressive slowing of migration the beta lipoprotein band was encroached upon and increased intensity of staining in the beta, prebeta and alpha regions produced a broad beta band. Broad beta and possible pre-beta bands were only found in animals with raised triglyceride levels, irrespective of whether cholesterol was also raised.

Animals treated for diseases such as hypothyroidism and pancreatitis showed a return to a more normal serum lipoprotein pattern which correlated with reduction of high serum lipid levels. In Alsatian 27 there remained a mild increase of serum cholesterol with increased intensity of staining from HDL_C on paper electrophoresis.

5.7.4 Laboratory Examination: Summary and Discussion. The results of some aspects of laboratory examination were similar between the clinical cases and normal controls as summarised in Table 5.7/7. Individual values for particular clinical cases appear with the case histories, where relevant, and in this section the results for serum lipids and lipoproteins will be summarised and discussed.

The serum lipid and environmental values and lipoprotein patterns ranged from normal to an increase of all, or most, serum lipids measured. Genetic factors may influence some of the variations. The majority of animals form a series as regards their serum lipid levels and serum lipoprotein electrophoresis: they have been placed in four groups in Tables 5.7/8 & 5.7/9. A fifth group comprises dogs in which hypertriglyceridaemia, was the major lipid

Table 5.7/7 Results of Laboratory Examination for Controls and Cases

Values	Controls n = 40 Mean ± SEM	Cases n = 33 Mean ± SEM
Red Blood Cells x 10^{12}/l	6.5 ± 0.087	6.9 ± 0.126
	Range 5.5 - 7.4	5.8 - 8.2
White Blood Cells x 10^{9}/l	10.73 ± 0.366	9.68 ± 0.261
	Range 6.5 - 16.6	7.3 - 14.2
Blood Glucose mmol/l	3.86 ± 0.131	3.77 ± 0.162
	Range 2.4 - 5.7	2.3 - 5.9
Blood Urea mmol/l	4.27 ± 0.193	3.98 ± 0.185
	Range 2.8 - 8.0	2.4 - 6.2

No significant difference ($p > 0.05$) between controls and cases.

Table 5.7/8 Classification of Lipid Patterns in Clinical Cases

Group I	II	III	IV	V
Ch < 6	Ch > 6	Ch > 6.5	Ch > 8	Ch - variable
Tg < 0.5	Tg 0.7 or less	Tg 0.7 or less	Tg 0.8 or more	Tg > 0.8
Ph < 5	Ph > 4	Ph > 4	Ph > 6	Ph - variable
n = 7	n = 7	n = 10	n = 5	n = 4
Ch = 4.6 (.9)	6.2 (.13)	7.4 (1.07)	9.8 (1.11)	7.3 (2.23)
Tg = .3 (.08)	.4 (.10)	0.5 (.15)	1.0 (0.19)	1.1 (0.22)
Ph = 3.7 (.54)	4.6 (.68)	5.3 (.78)	7.0 (0.61)	5.6 (2.09)

Mean value (SD) n = number of dogs

Ch = Cholesterol in mmol/l

Tg = Triglyceride in mmol/l

Ph = Phospholipid in mmol/l

Table 5.7/9 Summary of Principal Findings in Clinical Cases

Animal	Systemic Disease	Ocular Disease/Signs	Group
27	Hypothyroidism	Conjunctivitis/episcleritis reported	III
36	Hypothyroidism	Episcleritis	III
153	NAD	Sclerosing keratitis	I
160	NAD	Sclerosing keratitis	III
150	? Hypothyroidism	Chronic superficial keratitis/pannus	II
1	NAD	No clinical signs reported	I
4	NAD	Sclerosing keratitis	II
6	NAD	Sclerosing keratitis	I
9	Hypothyroidism	Sclerosing keratitis	IV
14	NAD	Sclerosing keratitis	III
79	NAD	Sclerosing keratitis	III
151	Hypothyroidism	Recurrent ocular redness reported	III
154	NAD	Sclerosing keratitis	I
155	NAD	Scleritis	II
65	NAD	Anterior uveitis	II
66	? Hypothyroidism	None reported	IV
131	NAD	Corneal ulceration	III
142	Hypothyroidism	Corneal ulceration	IV
42	NAD	Recurrent episcleritis/scleritis	III
24	Hypothyroidism	Mild keratoconjunctivitis sicca	III
83	? Hypothyroidism	Scleritis/nodular scleritis	IV
133	NAD	Retrobulbar abscess/exposure keratitis	II
73	NAD	Benign nodular fasciitis left eye	I
134	NAD	Benign nodular fasciitis left eye C.E.A.	II
149	Subacute Pancreatitis	Phthisis bulbi post lendectomy. C.E.A.	V
13	NAD	Conjunctival/episcleral injection reported	III

38	Hypothyroidism	Episcleritis	IV
74	NAD	Anterior uveitis	I
130	? Chronic Pancreatitis	None reported	V
26	NAD	Unilateral anterior uveitis	I
132	NAD	Lens luxation and previous corneal ulceration	III
75	Intrahepatic Cholestasis	Panuveitis and pannus	V
77	Cushing's Syndrome	Distichiasis	V

abnormality (Tables 5.7/8 & 5.7/9).

The commonest pattern for clinical cases with normolipoproteinaemia comprised three distinct bands in the alpha$_1$ (HDL_2) alpha$_2$ (HDL_1) and beta (LDL) positions, an arrangement identical to that found in the majority of normal controls where alpha$_1$-lipoprotein predominated. As in normal controls any differences in electrophoretic patterns were largely a consequence of the position of the alpha$_2$-lipoprotein band with reference to alpha$_1$ and beta lipoprotein. Animals with normal serum lipids and lipoprotein patterns were placed in Group I.

Animals with mild hypercholesterolaemia and detectable quantities of HDL_c on lipoprotein electrophoresis were arbitrarily placed in two groups (II and III) and probably form a transition series between normal (Group I) and abnormal (Group IV). A similar pattern of serum lipids was also observed in occasional control animals, most notably 164, a Springer Spaniel. Group II and III animals were of interest because their triglyceride levels were normal or lower than normal; a situation comparable with the hyporesponders described by Mahley et al. (1974).

More severe hypercholesterolaemia (Group IV) was associated with a concomitant increase in the levels of both phospholipid and triglyceride. In these animals there was often a marked reduction in the alpha$_1$-, lipoprotein band and intense staining of the alpha $_2$-, pre-beta and beta regions which sometimes produced a broad beta band.

The possible association of the type of hyperlipoproteinaemia in Groups II, III and IV with hypothyroidism is of interest. Of 33

dogs, seven were treated for hypothyroidism and three were suspected of subclinical hypothyroidism; one of the three subsequently became clinically hypothyroid. Thyroid function tests in the dog are complicated by the very low levels of total thyroxine present (some four times less than human values). Estimations of "free" thyroxine (T_4) would seem to be a more promising alternative (Ross α Valori, 1982; Eckersall α Williams, 1983). The most valuable method of diagnosis currently available in dogs is the response to thyroid stimulating hormone (Lorenz α Stiff, 1980) but it is an expensive technique. Preliminary investigation of "free" triiodothyronine (T_3) in man suggests another possible way of assessing thyroid function which may have application in the dog (Lines, personal communication).

Analysis of suspected familial hypothyroidism in a Springer Spaniel (66) and her relatives was, unfortunately not possible in this study, particularly as earlier work with the relatives of severely hypothyroid Alsatians (Crispin, unpublished observations) had suggested that, in this breed, primary acquired hypothyroidism could show a strong familial tendency. One Alsatian bitch with moderately severe hypothyroidism had a mother with no signs of clinical hypothyroidism other than lethargy, which had been ascribed to her age of ten years. Laboratory examination of the mother indicated cholesterol 8.19mmol/l, triglyceride 1.1mmol/l and phospholipid 7.42mmol/l and lipoprotein electrophoresis showed a diminution in HDL_2 and an increase in HDL_1 (as HDL_c).
The majority of the excess cholesterol was in the region of the $alpha_2$-lipoprotein band. Thyroid activity was subnormal and she responded to thyroxine orally.

Clinical and laboratory examination of the affected bitch's other relatives: a sister, a son, and a daughter, revealed no abnormalities at this time. Re-examination just over one year later was performed at the owner's request, as she had noticed that the five year old daughter had become dull and not come into heat as expected. Examination confirmed hypothyroidism. Thyroid function tests were subnormal and her blood cholesterol was 10.04mmol/l, triglyceride 1.23mmol/l, and phospholipid 8.14mmol/l. Increased alpha band staining was the most prominent abnormality on serum lipoprotein electrophoresis, with increased intensity of staining in the beta band as well. Thyroid replacement therapy resulted in marked clinical improvement and a return to normal serum lipids and lipoproteins after one month's treatment. Oestrus occurred within two months of thyroxine treatment and she subsequently became pregnant and had a litter of five puppies. The only animal to exhibit ocular signs of hyperlipoproteinaemia in this family was the original affected bitch (Case 3; Crispin α Barnett, 1978).

Familial hyperlipoproteinaemia was associated with hypothyroidism in three generations of Beagles by Manning et al (1973) and they observed that susceptible animals developed high serum lipid levels, of cholesterol particularly, even when fed a low fat diet. The age at which lipid levels became raised was variable and the abnormal dogs were traced to a single stud dog. The manner in which lipids and lipoproteins changed from normal to abnormal resembled the pattern for clinical cases in this study, except that a higher proportion of the Beagles had both hypercholesterolaemia and hypertriglyceridaemia as they were more severely hypothyroid. Rogers et al (1975b) have also indicated similar

alterations of lipids and lipoproteins in naturally occurring cases of hypothyroidism.

In an experimental study of surgically thyroidectomised Foxhounds fed cholic acid, propylthiouracil and lipid equivalent to 1% cholesterol by weight, Mahley et al (1974) were able to divide the dogs into two groups based on the type of hyperlipidaemia and the extent of atherosclerosis produced. In one group, referred to as hyporesponders, hyperlipidaemia was characterised by cholesterol levels which ranged from 5.8mmol/l to 19mmol/l whereas phospholipids were approximately 2.5 to 7.0mmol/l and triglycerides 0.17 to 0.56mmol/l. These dogs developed only minimal fatty streak lesions or no gross atherosclerosis. The other group of dogs were termed hyperresponders and they showed gross elevation of cholesterol and triglyceride particularly, with a less marked elevation of phospholipid. These dogs had grossly visible atherosclerosis after only four months and extensive disease by six months. The authors suggested that the difference between groups might be determined by the activity of two post-heparin lipases. Hyporesponders apparently had a significantly elevated total lipolytic activity primarily due to lipoprotein lipase, whereas the hyperresponders had reduced total lipolytic activity with very low levels of protamine-resistant hepatic lipase. The pattern of lipids and lipoproteins in the hyporesponders is very similar to that demonstrated for many of the clinical cases presented in this study, particularly as regards the presence of cholesterol-rich high density lipoprotein. The possible significance of mild hypercholesterolaemia, HDL_C and lipid keratopathy will be discussed more fully in Section 6.0.

Of the remaining animals, Group V of the present study (149,

130, pancreatitis; 75, intrahepatic cholestasis; 77, iatrogenic Cushing's syndrome) all had a prominent beta lipoprotein band on paper lipoprotein electrophoresis with normal or reduced staining of alpha$_1$-lipoprotein and increased or normal staining of alpha$_2$-lipoprotein. The separation of alpha$_1$ and alpha$_2$-lipoprotein was poor in 130 and 149. A pre-beta band of VLDL was apparently present in 77; however, caution is required when attempting to establish the contribution of VLDL to a prominent beta lipoprotein band because of similar rates of migration. Ideally ultracentrifugation combined with block electrophoresis is necessary to separate lipoproteins into pure classes.

5.8 Light Microscopy

5.8.1 Introduction. The predominant serum lipoprotein abnormality in one group undergoing keratectomy was a raised level of cholesterol-rich high density lipoprotein (27/1, 160, 4, 151, 65, 42, 13). Another group consisted of normolipoproteinaemic animals in which no major lipoprotein abnormality had been detected initially (150, 1, 6, 73, 74, 26, 132) or where treatment had restored serum lipoprotein levels to normal (9, 14, 38, 42/3). A third group consisted of patients in which a more generalised alteration of serum lipoproteins was present at the time of the first (36, 66, 24) or second (27/2) keratectomy.

Description of the histopathology of central or paracentral lesions was arbitrarily divided into three phases in this section, according to whether the portion of opacity being examined appeared to be in the process of formation, progression, or regression.

Phase I In an attempt to isolate some of the corneal changes associated with the initiation of the lesion this phase was

restricted to non-vascularised lesions in which opacification was developing and where the opacity had been present for a limited period. The lesions had a rather granular or amorphous appearance with the slit lamp biomicroscope. Vascularised lesions were placed in Phase II.

Phase II The majority of material was of this category. It correlated clinically with lesions, or parts of lesions, which were of variable appearance, progressive, rather than regressive, and in which crystals were prominent on slit lamp examination. Such opacities enlarged centrifugally, and could be modified by corneal neovascularisation. The fibroblast was the predominant cell type revealed by microscopy.

Phase III In those cases in which possible contributory factors could be isolated and controlled there was sometimes regression of the opacity. Slit lamp biomiscroscope examination demonstrated regions of clear cornea of almost normal appearance between isolated islands of opacity. Capillarisation and haematogenous macrophages were the most obvious features of this phase.

5.8.2 Examination of fresh material from imprint and squash preparations This method revealed the presence of isotropic droplets, anisotropic droplets and solid crystals. The relative proportions varied in different types of lesion. They were all examined initially at 33°C and measurements were made with an eyepiece graticule.

In Phase I occasional isotropic droplets were the dominant type of lipid although some anisotropic droplets were seen. In Phase II necrotic lesions were rich in isotropic droplets and the size of such droplets was very variable. In late Phase II, lesions, or

parts of lesions, with numerous cells but little necrosis, contained predominantly anisotropic droplets; these were generally smaller than isotropic droplets and showed no tendency to coalesce prior to the application of staining techniques. Anisotropic droplets were also the major type of droplet in Phase III.

Solid crystals were not apparent in Phase I but were prevalent in Phase II, particularly in necrotic material, they became less obvious and less aggregated in Phase III.

The solid crystals were of two basic types; large (at least 10μm) and tiny (less than 2μm), and whilst the large type was always found in Phases II and III the small types were sometimes not apparent.

Isotropic droplets These droplets appeared as homogeneous grey spheres when viewed with 80°crossed polarisers, or yellow rings on a red background with a first-order red gypsum accessory plate (Fig. 5.8/1). When suspended in distilled water, physiological saline or 80% glycerol in water a proportion of the droplets showed an initial rapid swelling, but there was no change in their optical properties. The diameters of the droplets in fresh preparations ranged from approximately 0.5μm to 4μm with occasional much larger droplets (up to 10μm). Their average diameter was about 2μm in fresh preparations. The droplets were temperature stable between 25°C and 35°C and they appeared to possess the physical properties of a true liquid with little intermolecular order.

Isotropic droplets stained red with oil red O and blue with PAN, they were negative with copper rubeanic acid, acid haematein, and not precipitated by digitonin. The calcium lipase-lead sulphide technique was usually negative, but occasionally a

positive reaction was obtained in heavily vascularised material. The positive reaction was limited to the rim of large droplets which exceeded 2.5μm diameter.

Whilst triglyceride might occasionally be present in small quantity, the bulk of isotropic droplets contained cholesterol ester as their main component.

Anisotropic droplets When viewed with 80° crossed polarisers these droplets appeared as bright whitish-grey spheres divided into equal quadrants by a black formée cross, which remained centred over the droplets without change in the orientation of its arms when the microscope stage was rotated. The anisotropy of individual droplets appeared to be an optical property independent of any axis.

With a ¼λ mica retardation plate the commonest pattern was white in the upper left and lower right quadrants and dark blue in the upper right and lower left quadrants; whereas with a first order red gypsum accessory plate (retardation 550μm) blue was obtained in the upper left and lower right quadrants and yellow in the upper right and lower left. The location of the interference colours was altered by rotating the microscope stage through 180° (Figs. 5.8/2 & 5.8/3).

Anisotropic droplets were resistant to deformation; pressure applied to the coverslip produced distortion of the formée cross image, which returned, along with the spherical shape, when the pressure was released. Severe deformation, especially in preparations of a few days standing, could produce permanent loss of the formée cross image with the production of brilliantly birefringent irregular fragments within the sphere.

Most anisotropic droplets, particularly the larger ones,

underwent initial rapid swelling when suspended in distilled water, physiological saline or 80% glycerol in water. In the presence of excess water anisotropic droplets continued to swell and developed isotropy. Isotropic changes started centrally and progressed centrifugally, usually as a series of concentric birefringent rings which sometimes broke up into more irregular fragments (Fig. 5.8/1). Collections of anisotropic droplets remained separate and showed no tendency to coalesce.

The size of anisotropic droplets ranged from less than 0.5µm to approximately 3µm in fresh preparations. Isotropic changes were usually present in the larger anisotropic droplets. The majority of droplets were 2µm or less.

When anisotropic droplets were dried they lost their spherical shape, formée cross image and interference colours to become whitish, amorphous, brilliantly birefringent masses. Water appeared to be an essential constituent for maintenance of their spherical form and optical properties.

The droplets were temperature stable over the range 25°C to 35°C. Approaching 40°C and above a proportion of anisotropic droplets were transformed into isotropic ones and the transition was reversible.

In material which contained a large proportion of anisotropic droplets histochemical studies indicated that they were oil red O and PAN positive, whereas the calcium lipase-lead sulphide and copper rubeanic acid methods were negative. With OTAN the droplets stained red-brown to dark-brown; with acid haematein, unstained or grey-blue and with Nile blue sulphate, pink, mauve or purple. Acid haematein combined with oil red O varied between red

and dark red to black. With digitonin it was not possible to interpret the effect of the solution on anisotropic droplets alone as larger crystals and other debris were certainly precipitated in the immediate vicinity. Similar interpretative problems obtained with the PAN method when solid crystals were present in quantity.

The results indicated that the anisotropic droplets consisted largely, or exclusively, of hydrophobic lipid, probably as esterified cholesterol. Smaller quantities of hydrophilic lipid, as phospholipid, appeared to be part of many anisotropic droplets. Free cholesterol, if present, was a minor constituent not readily detected by histochemical means; although the melting point of some anisotropic droplets (precipitated as crystals by drying and observed directly on a heated microscope stage) was apparently greater than 75°C, which would suggest highly saturated or monounsaturated cholesterol esters, free cholesterol or mixtures of free and esterified cholesterol.

Histochemical, physical and optical methods did not suggest the presence of other lipids in any quantity within anisotropic droplets.

The anisotropic droplet possessed properties consistent with organisation as a lyotropic liquid crystal mesophase. Mesomorphic states occur in some organic compounds in their transition from a true liquid (isotropic) state to a crystalline (anisotropic) state and the possible significance of mesomorphic forms is discussed in greater detail in Section 6.0.

Solid Crystals These took the form of tiny irregular clusters of birefringent crystals of variable shape and much larger acicular, parallelogram, and rhombic types, the latter were

frequently notched. They were all temperature stable between 25°C and 35°C.

Each form was birefringent and exhibited yellow and blue interference colours depending on thickness and orientation when viewed with a first order red gypsum accessory plate.

Addition of water did not affect the larger crystals, some of the smaller ones lost their birefringence in the presence of water. Birefringence was increased by drying the specimens but no other changes were noted.

Heating the specimens, with direct observation, on a heated microscope stage produced melting of the small irregular crystals at temperatures ranging from 40°C to 75°C. It was not possible to correlate the variation of melting point within this range with any particular type of lesion, except that in lesions where small crystals were present in any quantity melting was predominantly at the upper end of the range and the opacity was white and plaque-like on clinical examination. The crystals went directly from solid state to isotropic melt and no mesophase was detected. These properties are consistent with mixed, saturated, and possibly unsaturated, lipids.

The larger parallelogram, rhombic and acicular crystals melted at temperatures of 135°C to 155°C but were otherwise similar to the smaller crystals. Measurement of the angles of rhombic plates gave figures approaching 100°70' and 79°30'. These properties are those of free cholesterol (Fig. 5.8/4).

The PAN method was positive for both types of crystal and the large forms were precipitated by digitonin. It was not possible to state the effect of digitonin with any accuracy on the small

forms because of the presence of other lipids.

From these results it would appear that some of the crystals, particularly the larger ones, were free cholesterol; whereas the smaller more irregular types were cholesterol esters, or mixtures of cholesterol esters with other lipids or cholesterol.

5.8.3 Light Microscopy - Phase I The appearance of thick sections prepared for transmission electron microscopy was of value in demonstrating the morphology of early lesions. In a proportion of this material no abnormalities were apparent, whereas in the remainder, the epithelium and basement membrane were of normal appearance and only the fibroblasts appeared affected within the stroma. The fibroblasts were usually of normal number and shape but some of them contained intracytoplasmic vacuoles (Figs. 5.8/5). In other material the fibroblasts were larger than usual and of a variety of bizarre shapes (Fig. 5.8/6).

When very dense opacities were present there was some disruption of the lamellar arrangement of the collagen, a greater number of vacuolated fibroblasts of abnormal shape and size, and varying numbers of extracellular translucent spaces. Whilst the overall number of cells did not deviate from normal cornea in early cases with little stromal disruption, this was not the case in damaged corneas with lamellar separation to be described in Phase II.

Unfixed and short-fixed cryostat sections were examined stained and unstained. Material examined with Nomarski differential interference contrast microscopy and polarised light confirmed the presence of droplets within a proportion of the fibroblasts (Fig. 5.8/7). In some of the material there were very fine, apparently

pericollagenous droplets, in other material rather coarser extracellular droplets were apparent (Fig. 5.8/8). These droplets retained their shape and form between 25°C and 35°C and were predominantly isotropic; less commonly anisotropic droplets were also present. Old sections (i.e. stored for several weeks or months) sometimes contained crystalline material at sites previously occupied by droplets.

Bromine Sudan black B was used as a general screening method for lipids and was also valuable in demonstrating the extent and site of lipid within the keratectomy specimen. Two basic patterns were demonstrated with this stain; in one the fibroblasts alone stained more darkly than normal and, in the other, the fibroblasts and surrounding stroma were diffusely stained as had been observed previously in occasional corneas of the control series.

In material in which lipids occupied a subepithelial position the basement membrane stained more heavily than usual and the epithelial cells often appeared rather darker than usual (Fig. 5.8/9).

The apparently extracellular lipid may have come from fibroblasts, or may be representative of lipid which had infiltrated the cornea, or both sources may be involved. It was of interest that extracellular lipid was more frequent in cases with anterior segment inflammation as one of the initiating factors, than in those in which corneal opacity developed without any apparent preceding inflammation.

Identification of Specific Substances

Lipoprotein Lipoproteins should be insoluble in non-polar solvents and most polar solvents, so they should still be present in

paraffin wax embedded material. They were investigated in paraffin sections, fixed and unfixed cryostat sections and delipidised or acid-hydrolysis-treated cryostat sections.

No intact lipoprotein was detected using bromine Sudan Black B or Sudan Black B on paraffin sections. Prior acid hydrolysis did not enhance the staining reaction with solvent dyes on cryostat or paraffin sections.

Protein methods for the amino acids of plasma proteins (tryptophan, cysteine, tyrosine) or the arginine-containing apoprotein of HDL_c were also negative (Figs. 5.8/10 α 5.8/11).

On the basis of these results it appeared that intact lipoprotein was probably absent, or that the methods used for its detection were insufficiently sensitive, particularly if only small quantities were involved.

Free Fatty Acids In all the material examined, the epithelium and basement membrane overlying affected stroma stained dark green to black with the Holczinger technique using copper rubeanic acid, whereas staining reactions were as for normal cornea away from the opaque region.

Fibroblasts, in affected regions, particularly at the periphery of lesions, also stained darkly, although in those cells containing intracytoplasmic droplets it was clear that the droplets were unstained (Fig. 5.8/12). In a proportion of the material there was also a fine, diffuse, rather granular haze associated with fibroblasts in the affected area. Acetone extracted sections were uniformly negative. From these staining reactions it appeared that greater quantities of free fatty acid than normal were associated with fibroblasts and epithelial cells in abnormal areas of the

specimen and that occasionally there was also some free fatty acid in an apparently extracellular location near fibroblasts.

Triglyceride Sections treated with calcium lipase-lead sulphide stained dark brown in identical regions to those which had stained positively for free fatty acids, except that they lacked the diffuse stromal reaction which was occasionally observed as described above. The reaction was substantially reduced in non-enzyme treated control sections and totally, or partially, extinguished in sections which had been extracted with potassium hydroxide-dioxan prior to enzyme treatment.

It was concluded that triglycerides were either absent from the material, or present in minimal quantities in comparison with free fatty acids.

Cholesterol Ester and Cholesterol The perchloric acid-naphthoquinone (PAN) reaction was used initially to detect cholesterol and its esters. In all the material studied a positive result was obtained. The blue staining droplets appeared related to vacuolated fibroblasts, although on other occasions they were more widely distributed within the stroma. The epithelium and basement membrane was not involved in any of the material examined (Fig. 5.8/13).

Sections treated with digitonin showed no typical cholesterol-digitonin complexes and examination of such sections with polarised light confirmed that any droplets present were unaffected. Extraction of digitonin-treated sections with acetone followed by PAN were negative.

From these results it was inferred that the intracytoplasmic lipid droplets contained cholesterol ester and, that when

pericollagenous lipid droplets were present, they too consisted largely of cholesterol ester.

Free cholesterol was not detected with digitonin-PAN and crystalline cholesterol was not observed. It was concluded that free cholesterol was either absent, or present in quantity too small for detection.

Phospholipid With acid haematein there was enhanced diffuse staining of fibroblasts, epithelium and basement membrane in the affected region. This reaction was diminished, but not extinguished, by prior extraction with cold anhydrous acetone. Chloroform-methanol and acidified chloroform-methanol extraction also produced a diminished reaction. Sections subjected to alkaline hydrolysis before chromation were lightly stained with acid haematein. Acetone-Nile blue sulphate produced a reaction which was only marginally darker than control cornea and the unaffected tissue included with the keratectomy specimen. Combining acid haematein with oil red O demonstrated that fibroblasts containing intracytoplasmic droplets stained bright red and that only occasional fibroblasts of normal size and shape stained grey or black. The much finer pericollagenous droplets, or amorphous haze, also stained bright red when present (Fig. 5.8/14). The epithelium and basement membrane in affected and normal areas usually stained grey with this method.

These results indicated that phospholipid was not an important constituent of early lesions.

Other Lipid Methods With OTAN lipid droplets were stained dark brown, as was the epithelium and basement membrane, whereas fibroblasts stained orange brown.

Osmium tetroxide stained a faint brown colour in similar regions to OTAN, indicating that unsaturated lipids were not present in detectable quantities.

Lipofuscin was not detected with any of the methods employed.

Enzyme Histochemistry Non-specific esterases were detected in the outer epithelium, as for normal cornea; no activity was detected elesewhere in keratectomy specimens from the central or paracentral cornea.

Acid phosphatase activity in the epithelium and stroma of the central cornea was identical to that obtained in normal control tissue.

No lipase activity was detectable within the epithelium or stroma.

The results of enzyme histochemistry in early central or paracentral lesions were essentially those of normal control tissue.

5.8.4 Light Microscopy - Phase II This phase was accompanied by many changes in the affected regions of cornea and mixtures of all three phases were sometimes present in predominantly Phase II material (Figs. 5.8/15 - 5.8/21). The most characteristic feature was fibroblast death and disintegration, one consequence of which was liberation of intracellular lipid contents as extracellular lipidic debris, so that the ratio of extracellular to intracellular lipid was high in this phase. Such changes resulted in zones of necrosis surrounded by less severely affected cells; the necrotic zone increasing in size with involvement of the surrounding cells. Whilst the necrotic areas were relatively acellular, there was often hypercellularity of the surrounding regions. In early

material there was an increase in the number of fibroblasts, and, sometimes, polymorphonuclear leukocytes. Later there were cells such as melanocytes, lymphocytes, plasma cells and occasional mast cells in a proportion of cases. In vascularised lesions mononuclear phagocytes were prominent in addition to cells associated with the incursive capillary bed. Non-myelinated nerve fibres were sometimes present, often lying close to new blood vessels. The appearance of the collagen lamellae was variable; ranging from apparently unaffected, to complete disruption, disintegration and necrosis.

In opaque regions the epithelium and basement membrane were also altered. The basement membrane was frequently corrugated and irregularly thickened, occasionally it was discontinuous. The epithelium often contained an increased number of adventitial cells such as polymorphonuclear leukocytes and lymphocytes and melanin granules were sometimes abundant, especially in the deeper epithelium. Mitotic figures were evident in a proportion of the epithelial cells, particularly those of the basal layer. There was both hypertrophy and hyperplasia with variable thickness. In some cases acanthosis was superceded by epidermalisation, rete peg formation and parakeratosis. Superficial blood vessels produced considerable epithelial disruption, as did the occasional erosion of crystalline material through the basement membrane.

Unfixed, unstained, cryostat sections contained varying quantities of isotropic and anisotropic droplets and birefringent crystals. Isotropic droplets were contained within fibroblasts and also distributed extracellularly. Anisotropic droplets were commoner in older lesions and their situation was similar, but they were particularly prominent in extracellular location, especially

immediately beneath the basement membrane.

Solid crystals were of varying sizes; the smallest were irregular and barely discernable at light microscope level, whereas the largest were acicular, rectangular or rhombic in shape. Very rarely, rosettes of feathery crystals were seen in frozen sections examined immediately after storage in the cryostat chamber.

The small highly irregular birefringent crystals were usually extracellular in location, closely associated with dying and dead fibroblasts. The large rhombic and acicular crystals were also associated with dying and dead fibroblasts and accumulated in acellular necrotic regions. Feathery rosettes were randomly distributed extracellularly and were in limited quantity in the cases in which they were found.

Identification of Specific Substances

Lipoprotein No evidence of intact lipoprotein was found (Fig. 5.8/22).

Free Fatty Acids A strong reaction was obtained within and surrounding many fibroblasts, some of which were apparently disintegrating (Fig. 5.8/23). In later lesions with fibroblasts and lysosome-containing fibroblast-like cells the cells showed more discrete positivity (Fig. 5.8/24). In necrotic acellular regions, no free fatty acids were detected (Fig. 5.8/25). In cholesterol granulomata a reaction was obtained in a proportion of the cells near cholesterol crystals and this will be described with Phase III.

The epithelium in the region of corneal opacity was stained dark green to black, with the Holczinger technique, sometimes the entire epithelium was involved, in other cases the nuclei of the anterior or posterior portions stained more darkly (Fig. 5.8/23,

5.8/24, 5.8/25).

Acetone extracted sections were negative (Fig. 5.8/26).

Triglycerides With calcium lipase-lead sulphide the majority of material was negative. False positives were frequent if free fatty acids were not extracted and parallel sections untreated by enzyme were always included with the calcium lipase-lead sulphide method to aid interpretation.

In a proportion of cases brown-staining granules were detected in the nuclei and the perinuclear cytoplasm of epithelial cells. The reaction was reduced, but not extinguished, in non-enzyme treated controls and sections subjected to free fatty acid extraction (Figs. 5.8/27 α 5.8/28).

In the stroma of most cases brown staining granules were found in identical situations to those described for free fatty acids. They were a little less obvious in sections not subjected to enzyme and they were absent from sections subjected to fatty acid extraction (Figs. 5.8/27 α 5.8/28).

Much less commonly, and only in animals with extensive neovascularisation and raised serum triglyceride levels, there was brown staining of the lumen and possibly the walls of new blood vessels, with occasional diffuse patches in the stroma and infrequent large droplets which stained brown at their periphery (Fig. 5.8/29). These staining reactions were absent in parallel sections not treated with enzyme and persisted following fatty acid extraction. In these cases it had also been possible to detect feathery crystals in unfixed cryostat material and squash preparations examined below 15°C.

From these results it was concluded that triglyceride could be

detected with the calcium lipase-lead sulphide technique in the corneal epithelium, particularly anteriorly, whereas its presence in the stroma was less frequent. Of the available histochemical methods only the calcium lipase-lead sulphide technique, combined with suitable controls and fatty acid extraction gave specific identification of triglyceride. The results obtained with the calcium lipase-lead sulphide technique were compared with other methods which demonstrated esters of fatty acids.

Cholesterol Ester and Cholesterol Sections stained with PAN were always strongly positive but the form and site of the material varied. Parallel sections were examined unstained with Nomarski differential interference contrast to assist in determining the localisation of lipid droplets and crystals as some of the staining techniques affected their precise location.

Neither cholesterol ester nor cholesterol was found in normal epithelium, or abnormal epithelium where the basement membrane was intact, although occasional lipid droplets were detected in adventitial cells within the epithelium.

Cholesterol Ester This was mainly contained within fibroblasts and fibroblast-like cells in Phase II, although some adventitial cells may also have been involved (Fig. 5.8/30). The quantities of cholesterol ester were assessed in relation to a variety of techniques performed on parallel sections; notably PAN, digitonin, Sudan black B, oil red O, copper rubeanic acid and calcium lipase-lead sulphide. The results correlated with earlier examination of squash preparations. The fatty acid esters of early Phase II were in the form of isotropic droplets, anisotropic droplets or solid crystals. Considerable quantities of cholesterol

esters were sometimes present and it was not always possible to locate their intracellular or extracellular origin with accuracy (Fig. 5.8/30; 5.8/31 α 5.8/32).

Cholesterol The presence of cholesterol in cryostat sections was confirmed by its characteristic appearance and by precipitating it with digitonin (Fig. 5.8/32 α 5.8/33). In addition PAN was applied to acetone extracted sections which had been treated with digitonin, so as to demonstrate the quantities of free cholesterol remaining.

The amount of crystalline cholesterol present in sections correlated with the crystal aggregates observed with the slit lamp biomicroscope at the time of clinical examination (Figs. 5.8/32 to 5.8/37). In animals with hyperlipoproteinaemia at the time of keratectomy there was considerable lipid present in vascularised regions with accompanying tissue disruption (Fig. 5.8/33).

Solid crystals became more obvious in stored sections; they sometimes developed at sites rich in cholesterol esters. In order to reduce artifacts, keratectomy specimens were examined and photographed within twenty four hours of collection wherever possible.

Phospholipid The staining reactions were as for Phase I in some material, but in other material, and particularly in lesions established for some months, there was enhanced staining extracellularly and in fibroblasts and fibroblast-like cells. Red cells, when present, were always strongly positive for phospholipid.

The most useful histochemical methods were acid haematein, acid haematein combined with oil red O, Nile blue sulphate and OTAN. For the last two methods parallel sections were also submitted to acetone extraction and, in most cases, alkaline

hydrolysis.

Intracellular phospholipid appeared as droplets or finer granules in those cases where masses of extracellular staining did not obscure examination (Fig. 5.8/38). Similar granular phospholipid was present within the stroma and particularly beneath the basement membrane of the epithelium. It appeared that the extracellular granular lipid derived from disintegrating fibroblasts. In some cases the basement membrane appeared more darkly stained than usual and the posterior cells of the epithelium showed a distinct granularity or fine, more diffuse, stippling (Fig. 5.8/39). When alkaline hydrolysis was performed prior to acid haematein, or OTAN, the reaction was marginally reduced, or unaffected, indicating sphingomyelin as a major component (Fig. 5.8/40).

In a limited number of cases in which raised serum phospholipids and neovascularisation were present there was, in addition to granular phospholipid, a strong reaction within blood vessels and a more diffuse staining reaction in the region of newly formed capillaries. The reaction was sometimes sufficiently strong to hide structural details (Fig. 5.8/41). Following alkaline hydrolysis this phospholipid could not be detected with acid haematein or OTAN suggesting that phosphoglycerides such as lecithin formed a more important constituent and that sphingomyelin was absent or undetectable. These results may indicate a vascular origin for the diffuse phospholipid and active in situ synthesis for the droplet and granular types.

There was a close association between intracellular and extracellular stromal droplet and granular phospholipid and the

other lipids of fibroblast degeneration already detected (cholesterol ester and cholesterol).

Acid haematein combined with oil red O was a useful way of demonstrating the relative proportions of cholesterol ester (as hydrophobic lipid) and phospholipid within the cells. In material classed as early Phase II, hydrophobic lipid predominated and disintegrating oil red O positive cells were often associated with crystalline cholesterol. (Fig. 5.8/35). The amount of phospholipid detected in Phase II was quite variable (Figs. 5.8/41 - 5.8/43). Frequently cells of normal fibroblast shape were strongly phospholipid positive, whereas distended foam cells were positive for hydrophobic lipids and there were gradations between these two extremes. In degenerate cells hydrophobic lipid (both droplets and crystals) were sometimes coated by phospholipid. Cryostat sections stained with acid haematein and examined at 33°C demonstrated that large quantities of anisotropic droplets were present which coincided with the extracellular and some of the intracellular hydrophobic lipid droplets and phospholipid (Fig. 5.8/44). Isotropic material was less evident, but small quantities were present both intracellularly and extracellularly.

Parallel sections confirmed that the anisotropic droplets positive with acid haematein and acetone-Nile blue sulphate were the same as those which were strongly positive with PAN. It therefore seems likely that the anisotropic droplets, of material adjudged to be late Phase II, contain phospholipid and cholesterol ester as major constituents. Cholesterol may also be present, but its physical properties preclude incorporation in anything other than small quantity, probably insufficient for detection by histochemical

methods.

Many of the anisotropic droplets apparently accumulated beneath the epithelial basement membrane (Fig. 5.8/44), a region in which acid mucins were also sometimes present.

With a number of phospholipid staining methods there was darkening of the epithelium and basement membrane in affected regions and the presence of diffuse, droplet or granular, phospholipid positive material apparently in the posterior epithelial layers has been illustrated previously. If there was transit of phospholipid from stroma to tear film via the basement membrane and epithelium it was likely to be of minor significance when the anatomy of this region was normal. Increased quantities of lipid, particularly phospholipid, were detected when the integrity of the basement membrane was breached.

Other Lipid Methods OTAN stained lipids red-brown to black. In general, hydrophilic lipids appeared red-brown, whereas hydrophobic lipids were darker brown or black. Mixtures of hydrophilic and hydrophobic lipids may account for some variation in the colours. Comparison as to the relative quantities of both types of lipid were made by extracting a parallel section with cold anhydrous acetone.

Sections stained with osmium tetroxide generally contained insufficient quantities of unsaturated lipid to give a positive reaction, intracellular lipid droplets were either unstained or pale brown in a proportion of stromal cells, whereas the epithelium and basement membrane stained a darker, uniform brown.

Lipofuscins were demonstrated in older lesions with cholesterol granulomata using Sudan black B, PAS or Schmorl's method on paraffin sections (Figs. 5.8/45 - 5.8/46). Early lipofuscins were

probably detected more readily with Sudan black B and PAS and late lipofuscins by Schmorl's method. Schmorl's method was also employed with parallel bleached sections to remove melanin in heavily pigmented material.

Enzyme Histochemistry Tween lipase gave a uniformly negative reaction to all Phase II material studied.

Non-specific esterase activity was limited or absent in early Phase II material (Fig. 5.8/47). Activity was usually detected at the periphery of well developed cholesterol granulomata later in Phase II (Fig. 5.8/48) and non-specific esterase activity was always marked in the round cells pallisading around cholesterol clefts, particularly when giant cells were involved. The role of esterases in cells apparently involved in cholesterol resorption will be described in Phase III.

Acid phosphatase activity was often striking in the vicinity of fibroblasts and, in such cases, the enzyme was not retained within the cell; suggesting poor localisation of enzyme with the technique employed, liberation of hydrolytic enzymes upon cell death, or release of hydrolytic enzymes prior to fatty acid uptake (Fig. 5.8/49). Parallel sections indicated close coincidence of free fatty acids and acid phosphatase activity in these cells (Figs. 5.8/23 α 5.8/49).

Acid phosphatase activity was common in Phase II particularly in those cases in which phospholipid containing cells were a prominent feature. Such cells were rich in acid phosphatase activity but contained little or no esterase activity. Cholesterol granulomata were usually ringed by occasional acid phosphatase positive cells (Fig. 5.8/50) and greater numbers of lipid positive

cells.

In summary, there was more acid phosphatase than esterase activity, in most Phase II material, but examination of parallel sections indicated that in some cells activity was apparently coincident. This situation was in contrast to that in which round cells and giant cells surrounded cholesterol crystals and appeared to infiltrate between them, as was more typical of Phase III; in such material there was marked esterase activity and diminished, or very limited, acid phosphatase activity.

Other Methods With haematoxylin and eosin the variations in cellularity were clearly displayed (Fig. 5.8/51). In addition to demonstrating lipid- containing residual bodies the PAS technique was notable for its ability to emphasize any thickenings or irregularities in the epithelial basement membrane and for staining the walls of new blood vessels (Fig. 5.8/52).

Perls' method was negative except in some material with extensive neovascularisation amidst disorganised corneal architecture. In such cases haemosiderin was present.

Hale's dialysed iron method indicated acid mucin positive material in a proportion of the fibroblasts, between lamellae and, apparently, accumulating beneath the basement membrane of the corneal epithelium (Fig. 5.8/53).

Trichrome Methods such as Masson's and the MSB technique indicated variation in the staining reaction of collagen in extensive lesions, particularly when cholesterol granulomata were present (Fig. 5.8/54 α 5.8/55). Fibrin was also a minor component of some established vascularised lesions, although the majority of material contained little, or none (Fig. 5.8/55).

5.8.5 Light Microscopy- Phase III The typical features of this phase included considerable neovascularisation with many round cells. The round cells were apparently derived from blood monocytes. Multinucleate giant cells were seen in lesions which contained large quantities of crystalline cholesterol. Both types of cell accumulated lipid without consequent cell death. In this phase there was thus an increased ratio of intracellular lipid to extracellular lipid. Lipid containing macrophages were frequently located close to blood vessels and large globules, similar to those within the stroma or within the macrophage, were sometimes seen within the lumen of blood vessels. It also seemed likely from study of thick sections prepared for T.E.M. that mononuclear phagocytes were capable of penetrating the wall of newly formed capillaries. In areas devoid of extracellular lipid the cornea was of more normal appearance.

The range of appearances is summarised in Figs. 5.8/56 - 5.8/58. Unfixed, unstained, material differed from that of other phases in containing tiny droplets of strongly birefringent lipid which were located in presumed phagocytic cells. Such cells were often located in clusters around birefringent cholesterol crystals or within the stroma close to new blood vessels. In material in which crystalline cholesterol was apparently absent giant cells were not seen and macrophages were distributed diffusely without aggregation in any particular area. Small numbers of polymorphonuclear leukocytes were sometimes present, particularly in this type of material or relatively young lesions. Whereas the presence of crystalline lipid was associated with formation of cholesterol granulomata, the response to non-crystalline lipid was

more typical of a modified granulomatous, or xanthomatous, reaction (Figs. 5.8/59 α 5.8/60).

Identification of Specific Substances

Lipoproteins As in the other phases it was not possible to demonstrate intact lipoprotein. Sudan black B positive granules in the vicinity of cholesterol granulomata were interpreted as lipofuscin (vide infra). Methods for individual proteins also gave essentially normal results.

Free Fatty Acids There were some positive cells in affected regions but the majority stained with carmalum counterstain only.

Darkly staining cells appeared to consist largely of fibroblasts and fibroblast-like cells, with mononuclear phagocytes and giant cells staining less strongly or not at all. Extracellular free fatty acids were not detected (Fig. 5.8/61 α 5.8/62).

Triglycerides Results were as in Phase II in that the majority of material was negative. Occasional cells within the stroma contained large, often single, globules of lipid which stained dark brown at their periphery with the calcium lipase-lead sulphide technique. There was also occasional granular staining of the corneal epithelium (Fig. 5.8/63).

Cholesterol and Cholesterol Ester Material viewed with polarised light consisted of birefringent crystals and anisotropic droplets with limited quantities of isotropic droplets (Fig. 5.8/63). Birefringent crystals were seen free in the stroma and also surrounded by cells. Many cells contained anisotropic droplets (Fig. 5.8/64). Anisotropic droplets were also seen free in the stroma and within the lumen of blood vessels. Isotropic droplets had similar locations to anisotropic droplets but were far less

numerous; they were also uncommon in the cells which surrounded isolated groups of, or individual, cholesterol crystals.

The PAN method was strongly positive early in Phase III, less so later, as the proportion of extracellular lipid reduced.

Digitonin precipitated free cholesterol and the quantity of free cholesterol detected diminished between early and late material (Fig. 5.8/65). Concomitant with a decrease in crystalline cholesterol there appeared to be greater quantities of anisotropic intracellular lipid in cells located close to, or surrounding, cholesterol crystals. Disaggregation of free cholesterol was noted as a probable consequence of macrophage and giant cell activity.

Phospholipids These were detected both intracellularly and extracellularly in the material examined; the former predominated in Phase III material.

With acetone-Nile blue sulphate (Fig. 5.8/66) or acetone-OTAN there was a strong blue and red-brown reaction respectively in the stromal cells and corneal epithelium. There was also a rather diffuse reaction in the region of vascularised cholesterol granulomata.

With Nile blue sulphate a mauve reaction was obtained in a proportion of the cells (Fig. 5.8/67) and this produced a finely granular effect throughout mononuclear phagocytes whereas the stain was disposed peripherally in giant cells. It was interpreted as representing the presence of hydrophilic and hydrophobic lipid.

Acid haematein stained red blood cells strongly black and a proportion of the cells close to cholesterol crystals were also stained. When acid haematein was combined with oil red O the proportion of hydrophilic to hydrophobic lipid was clear. This

technique also demonstrated discrete darkly staining granules lining a proportion of cholesterol clefts. The epithelium and basement membrane usually stained more darkly than normal in affected areas, particularly so if its integrity had been disrupted (Fig. 5.8/68).

Alkaline hydrolysis prior to staining for phospholipids reduced the reaction in cells interpreted as macrophages and giant cells, indicating that sphingomyelin was probably a minor component of the phospholipids present.

Methods for lipofuscin were positive in material with cholesterol granulomata and positive granules were located immediately surrounding cholesterol crystals (Fig. 5.8/46).
Other lipid methods Oil red O demonstrated that in Phase III the positive lipid was more discrete than in Phase II. Phase II material was typified by cell death and disintegration with large quantities of poorly emulsified bulky isotropic intracellular lipid droplets, and extracellular lipid in the form of both crystals and droplets. Phase III material contained proportionately less extracellular lipid. Phagocytic cells were predominantly mononuclear, but multinucleate giant cells were common when large cholesterol crystals were present. The intracellular lipid was typically anisotropic with each lipid droplet clearly separated from neighbouring droplets, suggesting adequate emulsification, and the cells themselves appeared healthy and viable (Fig.5.8/64).

OTAN stained hydrophobic lipids brown to black and phospholipids red-orange, mixtures of lipids produced intermediate shades. In material where OTAN produced a black colour osmium tetroxide also produced a strong black reaction suggesting the presence of unsaturated lipids (Figs. 5.8/69 & 5.8/70). For both

OTAN and osmium tetroxide the black reaction was produced by the phagocytic cells.

Sudan black B stained lipid-containing cells strongly and was useful as a screening method to demonstrate the differences between Phases II and III (Figs. 5.8/71 α 5.8/72).

Enzyme Histochemistry The Tween lipase method was occasionally positive in material which consisted largely of mononuclear phagocytes as the major phagocytic cell. Even in this material the method was somewhat capricious, but the consensus from repeated stains indicated that a proportion of the mononuclear phagocytes were positive and that enzyme activity was also present within the corneal epithelial cells (Fig. 5.8/73).

Non-specific esterase activity was present in a high proportion of the mononuclear phagocytes and multinucleate giant cells of Phase III (Fig. 5.8/74). Positive cells were found surrounding cholesterol crystals and infiltrating aggregates of cholesterol. Mononuclear phagocytes also demonstrated esterase activity in material which lacked multinucleate giant cells.

In both mononuclear phagocytes and giant cells, microsomal (E600 sensitive) and lysosomal (E600 resistant) activity was demonstrated (Figs. 5.8/75 α 5.8/76). In a proportion of cases lysosomal activity was shown in the granules which lined cholesterol clefts; the granules were thus positive for hydrophilic lipid, hydrophobic lipid and lipofuscin.

Acid phosphatase activity was much less marked in Phase III and had a rather sporadic distribution, which did not apparently coincide with mononuclear phagocytes and giant cells engaged in esterase activity, whether synthetic or hydrolytic.

Other Methods In general, the results obtained with these methods were similar to those reported previously for Phase II material and normal cornea, with a range of intermediate variations. PAS Material stained similarly to that of Phase II except that the stroma and basement membrane were of more normal appearance in regions of lipid clearance in which numerous round cells were apparent.

Hale's dialysed iron In regions free of extracellular lipid with a preponderance of round cells, acid mucins were present between lamellae; in general there was less acid mucin positive material in Phase III than Phase II. Perls' Method was usually negative in Phase III but was strongly positive in cases in which there was prominent neovascularisation with eroding cholesterol granulomata with free haemorrhage. Melanin was also a feature of some vascularised material and was present within epithelium and stroma.

5.8.6 Light Microscopy: Summary and Discussion. Examination of fresh squash and imprint preparations at the temperatures commonly encountered in the central, anterior, cornea has not, apparently, been reported previously. It proved a most useful technique as the material may be examined free from the influences of fixatives and organic solvents and at a temperature close to that of the naturally occurring lesion. Other workers have used similar methods, most notably in the study of atheroma. Their results indicate that both isotropic and anisotropic droplets consist largely of cholesterol ester, whereas solid crystals are mainly of free cholesterol (Stewart, 1961; Lang α Insull, 1970; Hata, Hower α Insull, 1974; Adams α Bayliss, 1975). Phospholipids can be decisive for the anisotropic state in lipid mixtures (Chapman, 1967

α 1973) and their possible role in this, and other respects, will be discussed in Section 6.0. Few detailed studies by other workers of the histopathology of naturally occurring canine corneal lipidoses are available for comparison with the results presented in this thesis. Fixation of material has often been inadequate for lipid histochemistry, particularly in relation to phospholipid retention. Identification of non-crystalline lipid has usually been restricted to the "neutral fats" indicated by positive Sudan staining. Birefringence in polarised light has been the most frequent method of identifying solid crystals as free cholesterol. There have been no attempts to equate histopathology with the progression, regression, or static nature of a lesion.

There are two reports of dystrophies in dogs where adequate phospholipid fixation with calcium formal was obtained. MacMillan et al. (1979) described crystalline corneal opacities in Siberian Huskies. Histochemical stains on frozen sections identified neutral fats in all the corneas examined, whereas cholesterol and phospholipids were found in four of the six corneas examined. They did not specify the location of the lipids identified, nor the methods which were used for actual identification. Micrographs accompanying their article indicated acicular and rhomboidal clefts, characteristic of free cholesterol, and mainly situated extracellularly. In addition there were less obvious round spaces, presumably indicative of non-crystalline lipid, in both intracellular and extracellular locations. The authors felt that this condition might be an animal model of Schnyder's central crystalline dystrophy.

The same analogy with Schnyder's central crystalline dystrophy

was made by Roth et al (1981) when they reported an oval corneal opacities in Beagles. This group used adequate fixation and a variety of histochemical stains for lipids. Unfortunately, in presenting their results they did not differentiate between animals with normal corneas and those in which "corneal ulceration had occurred in conjunction with the oval opacities, and the corneas vascularised secondarily". There are illustrations which demonstrate granular extracellular lipid probably derived from the disintegration of fibroblasts containing larger globules of lipid. Methods for cholesterol indicated its presence within the fibroblasts; free and esterified cholesterol were not differentiated. Phospholipids were present in all the corneas and free fatty acids were detected in 12 out of 26. The locations of phospholipids and free fatty acids were unspecified; neutral fats detected were assumed to be triglycerides.

It is of interest that an initial analysis of serum cholesterol in affected dogs had suggested elevated cholesterol-rich HDL, although this finding was not substantiated by a subsequent blood sample and further statistical analysis. More recently (Crispin, 1982 and unpublished observations, Crispin and Barnett, 1983) it has been suggested that oestrus, and possibly pregnancy and lactation, may modify the evolution of central/paracentral lipid dystrophy. The ways in which hormones may influence lipoprotein patterns in normal dogs are currently being investigated; it is known, for example, that a variety of gynaecological conditions and disorders in bitches can affect the serum lipids and lipoproteins (Fisher, 1979; Arbeiter α Lorin, 1981) and such changes may be transient rather than maintained.

In central/paracentral lipid dystrophy (Crispin, 1982 and unpublished observations) a proportion of the fibroblasts in opaque areas, particularly in the anterior cornea, become distended by intracellular globules. With death of the cell there is release of the cell contents as finer granules of crystalline and non-crystalline lipid. The major lipids detected have been free and esterified cholesterol and phospholipid in identical situation to that described for lipid keratopathy.

The manner in which the fibroblast accumulates free and esterified cholesterol and releases it upon cell death appears similar for central and paracentral lesions whether the lesion has been diagnosed clinically as central/paracentral lipid dystrophy, lipid keratopathy, or modified arcus lipoides corneae. Perhaps the fibroblast in this situation has little potential for variation when it is of the mature resting type. The rather discrete disintegration of individual fibroblasts which, together with a complete lack of inflammatory changes, typifies central/paracentral lipid dystrophy, may indicate a primary defect of the fibroblast which only becomes apparent because of secondary and subtle alterations in the corneal nutrients derived from perilimbal vessels.

In studying the effects of experimentally induced extreme hypercholesterolaemia on the canine eye, Slatter (1980) used plane or polarised light and oil red O on formalin-fixed tissue, and was able to demonstrate both lipid droplets and solid crystals in the arcus lipoides corneae which developed in a proportion of the Beagles. In one animal the arcus was considerably modified became of a previously vascularised interstitial keratitis and uveitis; in

effect, an experimentally produced lipid keratopathy.

An association between hyperlipoproteinaemia and arcus lipoides corneae had previously been reported in Alsatians with naturally occurring hypothyroidism (Crispin and Barnett, 1978). Subsequent work (Crispin, unpublished observations, Crispin and Barnett, 1983) has confirmed that the early arcus lesion is, as in man, most obvious in the anterior part of Descemet's membrane and beneath the anterior epithelium. It differs from the human senile arcus, and resembles the experimentally induced rabbit arcus, in becoming, in untreated animals, a more cellular lesion at stromal level associated with low grade inflammatory changes. Whilst phospholipid predominates in Descemet's membrane and beneath the anterior epithelium, it is cholesterol, in esterified and free form, which is most readily detected in the corneal stroma. Much of the stromal lipid is in the form of fine perifibrous droplets, or an amorphous haze, which may have gained entry via perilimbal blood vessels. In areas of heavy lipid infiltration and away from the limbus, solid cholesterol crystals and membranous debris of esterified cholesterol and phospholipid are present in association with degenerate fibroblasts. At the edge of the lesion, within less dense regions of lipid infiltration and near the limbus, however, many fibroblasts appear healthy, and a proportion shows signs of increased synthetic activity including numerous pinocytotic vesicles at the cell border. There are also numbers of actively phagocytic cells in these areas.

There are thus obvious similarities between the degenerative changes which can develop in the fibroblast in a variety of naturally occurring corneal lipidoses in the dog. Some of the

factors which may determine whether or not inflammatory changes accompany fibroblast degenerations will be discussed in Section 6.7. The presence of free and esterified cholesterol in the corneal stroma of patients with lipid keratopathy was suggested by early studies in both man and the dog. Dreyfuss (1930) thought that neutral fats, including esterified cholesterol, fatty acids and free cholesterol might all be present in the bilateral lesion he examined. Drawings accompanying his paper indicate acicular crystals of free cholesterol, fibroblasts containing lipid droplets and some fine extracellular lipid droplets. The crystals which melted at 40 C were probably composed of saturated or monounsaturated cholesterol esters, possibly as mixed esters.

The inability to detect phospholipid in histological sections has been interpreted as largely due to inadequate fixation techniques for phospholipid preservation and the use of non-specific general methods for lipid histochemistry. However, Roscoe and Vogel (1968) and Roscoe and Riccardi (1969) used extraction methods to estimate lipids in hypercholesterolaemic rabbits and their results are of interest. Roscoe and Vogel showed that cholesterol, and to a lesser extent, phospholipid increased within the cornea of rabbits maintained on 1% dietary cholesterol for one to three months, whereas they were unable to detect significant quantities of triglyceride even after three months of feeding. Roscoe and Riccardi extended this work and showed that there was a significant correlation between the cholesterol and phospholipid contents of corneas, irises and atheromas of animals fed cholesterol for two or three months. They also produced evidence to show that diseased irises and atherosclerotic intimas contained a higher

percentage of sphingomyelin and a lower percentage of lecithin than did plasma at the end of the three month period, thus suggesting active synthesis of phospholipid in these sites. For the cornea the distribution of phospholipid was essentially the same as that found in the plasma, the inference being that the mechanism of phospholipid accumulation in the cornea was one of simple infiltration from the plasma. However, the authors also noted that there was a significant increase in sphingomyelin as compared to control corneal tissue and, that as the cornea does not have a direct blood supply, it was difficult to compare the results obtained in corneal tissue with that of the aorta. They did not mention whether any corneal vascularisation occurred during the study. To these findings may be added the difference in cellularity between the cornea and iris, or aorta, and the relatively short period of the experiment. In naturally occurring canine lipid keratopathy significant numbers of sphingomyelin-rich cells were only found in vascularised corneal lesions which had been established for several months. It does appear that the phospholipid detected in established canine lesions is derived in part from the blood vessels of vascularised corneas, and that sphingomyelin-rich phospholipid is present within a proportion of the stromal cells as a result of synthesis.

Whilst neutral fats were frequently equated with triglycerides before their specific identification was possible, with modern techniques, it seems unlikely that triglycerides contribute significantly to central or paracentral corneal lesions in lipid keratopathy, as triglycerides were only detected in vascularised corneas from animals with raised triglyceride levels

and the densest staining occurred withinthe lumen of blood vessels. Rodgers (1971) drew similar conclusions concerning the corneal lesions produced by experimental hypercholesterolaemia in the rabbit on the basis of OTAN staining, which is not as specific as the calcium lipase-lead sulphide technique. It is pertinent to note that in describing a form of stromal lipid dystrophy in the Airedale, Dice (1974), identified triglycerides and phospholipids on the basis of oil red O and Sudan black B staining respectively, whereas these methods will detect all lipids other than those in a solid state and are certainly not specific for triglycerides and phospholipids (Bayliss High, 1982).

Free fatty acids were mainly located within, or close to, corneal fibroblasts in lipid keratopathy. These free fatty acids may be liberated with cell death and disintegration, or they may represent fatty acid prior to cell uptake and subsequent esterification. In either event, parallel sections stained for acid phosphatase demonstrated that enzyme and fatty acids were similarly disposed in some fibroblasts. Whilst the general impression was that such staining reactions were common in areas where fibroblasts were dying and disintegrating, they were also encountered at the periphery of opacities, and it is well known (Ghadially, 1982) that many cells hydrolyse fatty acid esters by release of hydrolytic enzymes, so that they can assimilate the simpler fatty acids for re-esterification. Francois and Victoria-Troncoso (1978) reported abnormal lysosome development in a variety of human corneal dystrophies, with discharge of acid hydrolases from the lysosomes. Berman (1982) has described direct extracellular secretion of acid hydrolases by histiocytes and lymphocytes as well as fibroblasts.

It is unlikely that plasma-derived free fatty acids contributed significantly to the positive staining reaction, for in most cases the positive areas were discrete, and associated with fibroblasts, rather than diffuse. Furthermore, fatty acids whether bound to albumin, or free, are of small size, have a high flux rate, and a short half life, and are therefore unlikely to accumulate within the cornea (Boyd, personal communication) although there is nothing to prevent their transit by free diffusion through the corneal substance.

The failure to detect plasma proteins within the cornea in most cases of lipid keratopathy may well indicate that the amounts present were too small for standard histochemical techniques, and the results were disappointingly ambiguous, even in heavily vascularised corneas. The Millon reaction for tyrosine was difficult to interpret because the sections became disrupted, and stains for tryptophan and cysteine differed little between normal and abnormal corneas, although tryptophan was occasionally detected within fibroblasts in vascularised lesions.

Identification of the arginine-rich apoprotein concerned with the transport of cholesterol-rich high density lipid was generally unrewarding, in that both normal and affected corneas gave similar orange-red colouration. In Section 6.3 the possibility that cholesterol-rich high density lipoprotein dissociates into lipid and protein as it passes through the blood vessel wall and enters the cornea is discussed more fully. If such dissociation does occur it would explain the apparent lack of arginine within the stroma; being soluble, the protein would not accumulate.

Similar considerations apply to the other plasma proteins, and

the more damaged the cornea the greater the potential speed of disappearance. In addition, there may be loss during specimen preparation, for there will be poor retention of soluble protein in frozen sections and formalin-based fixations are not particularly good protein precipitants (Adams, Knox α Morgan, 1975).

There do not seem to be any detailed reports of the role of phagocytic cells in naturally occurring lipid keratopathy, although the presence of such cells has been noted by a number of workers. Dreyfuss (1937) referred to the presence of lipid-filled cells, which he thought were histiocytes, in the case he recorded in the Alsatian. Harrington and Kelly (1980) mentioned the presence of a few macrophages and fibroblasts around the cholesterol clefts of their case in an Old English Sheepdog. In neither of these cases was vascularisation reported. Crispin and Barnett (1983) noted the presence of inflammatory cells, including macrophages, in lipid keratopathy and arcus lipoides corneae, whereas central/paracentral lipid dystrophy was typified by absence of any inflammatory response.

The tendency for histiocytes to be located near blood vessels was clearly shown for human lipid keratopathy by Brown and Katz (1933). Their illustrations show fat laden histiocytes grouped about blood vessels, and small histiocytes within the wall of an arteriole. The authors postulated that the histiocytes originated from the cells of the perivascular sheaths which accompanied the newly-formed corneal blood vessels. Jack and Luse (1970) confirmed the close association of macrophages with blood vessels using light and transmission electron microscopy. Tremblay and Dubé (1975) described neovascularisation, mononuclear inflammatory cells and

"foamy" macrophages in a case of lipid keratopathy.

Rodger (1971) studied experimental hypercholesterolaemia in the rabbit and concluded that monocytes were rare in early lesions, except towards the corneal periphery. He assumed a phagocytic role for keratocytes during this early phase, whereas later, in animals where limbotomy had been performed, monocytes were of frequent occurrence and appeared to be digesting swollen keratocytes. The author assumed that the free monocytes might have come from the limbus, or have been mobilised from the keratocyte syncytium, although he could not ascertain which. He makes no comment concerning the effects of vascularisation.

Waters (1959) observed active phagocytosis of lipid in dogs' corneas which had vascularised secondary to direct injection of various lipid mixtures into the central corneal stroma. He commented that the lipophagocytosis was accompanied by large mononuclear cells and by spindle cells of fibroblast type and that the latter cell type diminished in number as Sudanophilia decreased. Eventually a dense corneal scar with little or no residual lipid was all that remained some three to six weeks from the commencement of capillary ingrowth from the limbus.

In summary; the results obtained in this section, in conjunction with those obtained by other workers, indicate that cholesterol, in free and esterified form, is an important component of progressive lesions and that the majority of cholesterol apparently derives from the fibroblast. Phospholipid associated with corneal cells, and probably with extracorneal cells as well, may be a modifying influence on the evolution of corneal opacities. Corneal neovascularisation and round cell infiltration may be

necessary for effective lipid clearance.

Vascularisation apparently provides direct access for lipid into the cornea in hyperlipoproteinaemic patients; presumably it can also provide a means of exit, most easily detected in normolipoproteinaemic patients when deposited lipid crystals have been solubilised by effective phagocytosis.

5.9 Fine Structure of Lipid Keratopathy

5.9.1 Introduction The ultrastructural changes associated with lipid keratopathy were studied in relation to the form and type of lipid demonstrated with the light microscope and the results were correlated where possible. The range of appearances of epithelium, basal lamina and stroma have been illustrated earlier using thick sections prepared for TEM. In all cases there was early or later involvement of the epithelium and basal lamina and accumulation of fine spaces of granular appearance in the sub-epithelial zone. The extent of this involvement was probably related to the site of the corneal lesion as well as the time for which it had been present. Lipid keratopathy associated with chronic superficial keratitis, for example, showed earlier and more severe changes in the epithelium and anterior stroma than lipid keratopathy associated with scleritis; a difference which probably related to the level of anterior segment inflammation. (Figs. 5.9/1 & 5.9/2). The quantity of lipid deposition was enhanced in a damaged or vascularised cornea in the presence of systemic hyperlipoproteinaemia (Fig. 5.9/3).

5.9.2 Epithelium Fig.5.9/4 is of an early case of lipid keratopathy in which the epithelium and basal lamina of central cornea appear relatively normal. In some material there was, in comparison with normal epithelium, an increase in the number of

clear spaces, spaces with lamellated remnants and superficial desquamation (Fig 5.9/5). Considerable quantities of tonofilaments were sometimes present in animals with problems such as chronic superficial keratitis.

Tangential sections in a proportion of material demonstrated clear spaces, particularly perinuclearly, increased numbers of dark cells and various wandering cells (Fig. 5.9/6). In meridional section, epithelial melanosis was common in vascularised corneas and mitotic figures were frequent in the basal layers. The number of cell layers was sometimes doubled, but could also be reduced, full thickness epithelial loss was observed occasionally. Adventitial cells were mainly polymorphonuclear leukocytes and lymphocytes (Figs. 5.9/1 α 5.9/7).

5.9.3 Basal Lamina The appearances ranged from normal through mild irregularities and variations in thickness to complete disruption and absence (Figs.5.9/7 - 5.9/10). In general, however, the basal lamina remained intact even when considerably deformed by the material which accumulated beneath it and provided an effective barrier to material such as hydrophobic lipids.

Irregular thickening of the basal lamina resulted in lack of definition in its posterior border and sometimes hemidesmosomes were less regularly disposed than normal, particularly if there were any breaks. Duplication of the basal lamina was sometimes a feature of advanced lesions. The plane of sectioning could give the impression of discontinuity in a tortuous and convoluted basal lamina, but genuine breaks were usually accompanied by changes in the cells overlying them, such as darkening relative to neighbouring cells.

5.9.4 Stroma In this region the normal architecture was often

lost because of separation of collagen fibrils with intervening clear spaces (Fig. 5.9/11); less frequently fragmentation and necrosis were apparent, so that the regular interfibrillar spacing of normal cornea was lost. Whilst there was sometimes no structured appearance to the clear spaces they were often circular, defined by a finely granular rim and of very variable size. Some were empty and others contained lamellated remnants. The circular spaces were probably representative of the sites of lipid droplets, the undefined spaces were possibly more typical of separation due to oedema fluid. Ruthenium red positive proteoglycan particles were less regularly disposed than in normal cornea (Fig. 5.9/11).

There were also a variety of irregular, rectangular, hexagonal, rhomboidal and acicular spaces more suggestive of previous occupancy by crystals (Fig. 5.9/8 - 5.9/12).

In regressing lesions collagenesis was apparent. Newly formed collagen was embedded in abundant ground substance and the fibrils were often of larger diameter and with broader spacing than those of undamaged cornea.

Fibroblast The diverse appearances included normal resting fibroblasts, immature undifferentiated fibroblasts, activated fibroblasts, degenerate and dead fibroblasts. Evidence of mitosis in corneal fibroblasts was not found but increased numbers of fibroblasts or fibroblast-like cells were present in much of the material. (Fig. 5.9/13). It is possible that this population derived from in situ fibroblasts and extra-corneal cells.

Fibroblasts classified as activated were uncommon in most of the material examined, particularly in regions of obvious corneal opacity where the majority of fibroblasts were degenerate. They

were, however, more common at the periphery of lesions, towards the limbus and in corneas observed to be clearing. Activated fibroblasts showed evidence of micropinocytotic activity and microgranular deposits were often found close to the cell (Fig. 5.9/14). Mitochondria and intracytoplasmic vesicles were usually more prominent than those of normal adult resting corneal fibroblasts and other changes included hypertrophy of Golgi elements and sometimes distension of the cisternae of granular endoplasmic reticulum.

Centrioles, presumably associated with cilia, were occasionally observed (Fig. 5.9/15) and many of the fibroblasts had extremely attenuated cytoplasmic processes with close association between one cell and another. The cytoplasmic processes branched extensively between, and apparently within, distorted collagen lamellae.

The most varied appearances were exhibited by degenerate fibroblasts (Fig. 5.9/16). The shape of the cell was variable; frequently of convoluted outline, with a highly indented crenellated dark nucleus or, at the other extreme, a much more rounded cell with pale cytoplasm and an oval nucleus (Fig. 5.9/13). Dilatation of the cisternae of the granular endoplasmic reticulum was sometimes accompanied by degranulation. In degenerating cells disaggregated polyribosomes usually lay free in the cytoplasmic matrix but occasionally intracisternal sequestration and extrusion were apparent (Fig. 5.9/17).

Increased numbers of vesicles and vacuoles were characteristic of degenerate cells. A proportion of vesicles and vacuoles seemed to derive from the granular endoplasmic reticulum-Golgi complex route, and others seemed to arise directly by budding off from the

endoplasmic reticulum membrane to produce single-layered droplets. The endoplasmic reticulum was also unusual in that some cisternae appeared to communicate directly with the outside of the cell (Fig. 5.9/16). In some specimens flocculent electro-dense material was found within the elements of the Golgi complex, the granular endoplasmic reticulum and a proportion of the vesicles and vacuoles. Ruthenium red positive material in the same sites probably corresponded with the electro-dense material (Fig. 5.9/18). This same material was also seen in the immediate vicinity of the fibroblasts and accumulated beneath the basal lamina of the corneal epithelium but not in such great quantity as within the cell. Outside the cell the electro-dense material was located within electrolucent circular spaces, at the periphery of such spaces (Fig. 5.9/18) and, possibly, as a fine osmiophilic rim at the border of spaces left by crystals dissolved out during processing (Fig 5.9/19).

Mitochondria ranged from the normal dark and inconspicuous types to smaller and larger forms, the latter being the most common. In some mitochondria the cristae appeared to be tubular and vesicular rather than the usual lamellar, and there was sometimes a close association between mitochondria and endoplasmic reticulum. Clear spaces developed within some mitochondria; occasionally cristae and even the mitochondrial inner membrane had apparently disappeared leaving only a shell of outer membrane (Figs. 5.9/14, 5.9/16, 5.9/20 & 5.9/21).

A very characteristic feature of dying fibroblasts was the quantity of membranous debris derived from the breakdown of cellular components which was present as small vesicles, larger vacuoles and

whorled membranous structures or membranous cytoplasmic bodies. (Fig. 5.9/21).The quantity of whorled membranous structures was considerable in some of the material examined and some cells may have been actively engaged in their synthesis (Fig. 5/9/22).

The presence of crystal-shaped clefts was common within necrotic fibroblasts (Fig. 5.9/23). The proportions of crystal-shaped spaces and vesicles and vacuoles varied within dying cells. Tangential and meridional sections demonstrated the extent of some crystals (Figs. 5.9/24 α 5.9/25). Membrane-like material was sometimes associated with the crystal clefts (Fig. 5.9/26).

Whilst lysosomes were not a feature of adult resting fibroblasts in the normal cornea they were prominent in the fibroblasts of established lesions (Figs. 5.9/27 α 5.9/28). Using a lead capture method for acid phosphatase as a lysosomal marker it was possible to detect strong activity in round or ovoid organelles with a finely granular matrix which probably represented primary lysosomes (Fig. 5.9/29). Structures which were interpreted as secondary lysosomes contained tightly whorled osmiophilic lamellae, which were more separated in larger organelles and sometimes absent in the larger translucent ones, save for a single membrane at the rim. Acid phosphatase activity was less strong as the size of the secondary lysosome increased and in the largest structures only the osmiophilic rim gave a positive reaction (Figs. 5.9/28 α 5.9/29). The possible phagocytic role of fibroblasts and fibroblast-like cells is discussed, together with that of macrophages, later in this section and section 5.9.5.

Membranous lamellae were thus a prominent feature of many fibroblasts whether they were of microsomal or lysosomal origin. In

both sites the osmiophilic line was of some 30 to 40 A thickness and the space between concentric dark lines was variable according to the closeness of packing. Extracellular membranous lamellae were of similar structure and appeared to derive directly from dying and dead fibroblasts (Fig. 5.9/30). In material from cases in which the opacities were becoming less dense fibroblasts were of healthier appearance (Fig. 5.9/31) and were apparently engaged in collagenesis.

Vascularisation Vascularisation occurred at the level of lipid deposition and was also frequent in the superficial cornea, whatever the original level of corneal involvement. Free haemorrhage was sometimes apparent but was less frequent in stromal vessels than the superficial, conjunctival-derived vessels. Superficial vessels branched dichotomously from their origin peripherally, whereas stromal vessels grew in radially from the limbus parallel to the lamellae unless disruption of stromal architecture was such as to allow a less disciplined invasion. The fragile and leaky nature of superficial vessels was best appreciated in the scanning electron microscope. (Figs. 5.9/32 - 5.9/34). It should be noted that preparation for SEM and cryostat sections for light microscopy may both result in loss of the rather delicate endothelium of the new vessels.

Capillaries were the commonest vessel encountered, they had a diameter of approximately 25μm, and newly formed vessels sometimes contained rather bulging endothelial cells with quantities of free ribosomes, dilated granular endoplasmic reticulum and occasional mitotic figures. Micropinocytotic vesicles were often prominent and were probably an important means of transferring substances between

the vessel lumen and corneal stroma. Within the lumen, red blood cells, polymorphonuclear leukocytes, platelets and macrophages were the most common cells encountered. The macrophages and polymorphs sometimes appeared to be adherent to the luminal surface of endothelial and it is possible that these cells gained access to the corneal stroma by migrating between the endothelial cells. Intraluminal macrophages were undifferentiated; they had the appearance of blood monocytes. In older lesions venules and arterioles were also present, but were not common. Translucent spheres were sometimes observed in the blood vessel lumen and these will be described in greater detail later.

In the transmission electron microscope the convoluted appearance of young endothelial cells was apparent; a single layer of endothelial cells formed a continuous endothelial cell lining (Fig. 5.9/35). Endothelial cells at the growing tips of the capillaries lacked a basal lamina whereas older vessels had a basal lamina of variable thickness which was closely applied to the basal surface of the endothelial cell in normolipoproteinaemic animals. The basal surface was smooth and non-convoluted in older vessels (Fig. 5.9/36).

Pericytes were usually associated with capillaries; they were identified by the presence of large numbers of cytoplasmic filaments, an encircling basal lamina which was absent in places where pericyte and endothelial cell made contact, and dense bodies attached to the cytoplasmic aspect of the cell membrane (Fig. 5.9/37). Micropinocytotic vesicles were often numerous. Smooth muscle cells were of similar appearance to pericytes; they formed a continuous layer, up to two cells thick, in arterioles, and a

discontinuous single layer in venules (Fig. 5.9/37). Pericytes and smooth muscle cells apparently accumulated lipid and were almost certainly a possible source of foam cells, both near the blood vessels and within the stroma itself.

In some animals, particularly those with hyperlipoproteinaemia, electrolucent particles of various sizes were encountered in the subendothelial space and beyond. Many of the circular translucent spaces were bordered by a fine osmiophilic rim. Particles in the immediate vicinity of the basal endothelium were smaller and more discrete than those further away. Darkly staining, more granular, elements were also present and they appeared to reach further into the stroma than the translucent particles, although such distinctions may have been influenced by resolution difficulties at high magnification.

There seemed to be accumulation of many electrolucent particles between the endothelial cell and its basal lamina and there was frequent reduplication of the basal lamina which appeared to be very convoluted and sometimes even discontinuous. The characteristic appearance of reduplicated basal lamina was particularly evident in ruthenium red preparations (Fig. 5.9/38). Similar separation was effected between endothelial cells and pericytes, or smooth muscle cells, by the electrolucent particles and their basal laminae were similarly affected (Figs. 5.9/38 α 5.9/39).

The electrolucent particles with an osmiophilic rim which were situated immediately beneath the basal endothelium showed a range of sizes, most commonly of 30 to 60nm diameter. Particles contained by the basal laminae of pericytes or smooth muscle cells were approximately twice as large and, in general, particle size

increased with increasing distance from the blood vessel lumen (Figs. 5.9/36, 5.9/38 & 5.9/39).

It is relevant to observe that the size of particles situated immediately beneath the endothelium encompassed the range of 7 to 55nm diameter determined by negative staining electron microscopy for both normal and abnormal canine lipoproteins by Mahley et al (1974). Thus it could be concluded that whilst small particles were of a size compatible with intact lipoprotein, many particles were of too great a size to merit inclusion. In conjunction with the results of light microscopy it would seem that small particles could represent either intact lipoprotein or lipid dissociated from its apoprotein vehicle. Larger particles might be modified lipoprotein or lipid without apoprotein. (Fig. 5.9/39).

Red blood cells New blood vessels in damaged stroma leaked all vascular components directly into the surrounding tissue but this situation was uncommon. Superficial vessels associated with the corneal epithelium were generally leaky (Figs. 5.9/40 & 5.9/41).

Fibrin Fibrin was occasionally identified in the vicinity of new blood vessels or, more easily, remaining within the stroma as a legacy of previous vascularisation. It had a characteristic 22nm cross-striational periodicity (Fig. 5.9/42).

White blood cells Polymorphonuclear leukocytes were quite commonly encountered in the epithelium and stroma as well as within the lumen of blood vessels. They sometimes contained clear spaces some of which were apparently membrane bound, others not (Figs. 5.9/43 & 5.9/44). Within the stroma, and to a lesser extent within the epithelium, these cells were often degenerate. Eosinophils were identified in some material within the stroma, whereas

lymphocytes were commoner between epithelial cells as illustrated previously. Plasma cells were found within the stroma of a proportion of cases, particularly in long standing lesions; they were usually degenerate with very dilated cisternae of the endoplasmic reticulum and pyknotic nuclei. (Fig. 5.9/45).

Nerves Unmyelinated nerves and their associated perineural cells were not uncommon, particularly close to blood vessels, a situation reminiscent of the nerve-vascular bundles encountered in the dermis of skin. Whilst there were sometimes clear spaces, with and without an osmiophilic rim, in a proportion of the nerves, they seemed largely unaffected, even when surrounded by death and destruction. It thus seemed unlikely that they contributed in any measure to the lipid deposition in affected corneas (Fig. 5.9/46). Ultrastructural studies endorsed the findings of light microscopy that the majority of lipid in affected corneas appeared to derive directly from the death of fibroblasts, irrespective of whether such lipid was in the form of solid crystals, liquid crystals, or liquid droplets (Figs. 5.9/46 - 5.9/50).

Melanocytes Epithelial melanosis was common, particularly in superficial lesions associated with lipid keratopathy. Melanocytes were also found within the stroma, sometimes associated with blood vessels and sometimes as isolated cells, or groups of cells, in the stroma (Figs. 5.9/51 - 5.9/54).

Macrophages - Mononuclear phagocytes Some macrophages were clearly recognisable as of haematogenous origin, whereas others had less clearly defined features and may have derived from other mesenchymal cells or sites such as the limbus (Figs. 5.9/55 α 5.9/56). A proportion of the fibroblasts in established lesions

were also phagocytic and showed characteristics consistent with their mesenchymal origin, they were usually referred to as fibroblast-like cells (Fig. 5.9/57).

All the cells classified as mononuclear phagocytes had the following ultrastructural characteristics. They lacked a basal lamina, although a cell coat, or glycocalyx, could be demonstrated with ruthenium red on the outer surface of the cell membrane of some cells. The cell membrane was often irregular and lamellipodia, or more extensive cell processes, were common. Neighbouring cells were often closely linked by these projections. Subplasmalemmal linear densities were occasionally present subjacent to the inner leaflet of the plasma membrane. Numerous pinocytotic invaginations (both smooth and coated) were present on the cell surface and the cell contained varied quantities of vesicles, vacuoles, numerous lysosomes of various sizes, and occasional residual bodies. The Golgi zone was usually prominent and both smooth and coated vesicles were apparent. The granular endoplasmic reticulum of active cells consisted of numbers of short chains sparsely populated by polyribosomes.

Phagocytosis Small particles (whether of round or crystalline shape) were observed in the various phases of phagocytosis by fibroblast-like cells and macrophages in numerous micrographs. Sometimes particles were also observed trapped between the processes of contiguous cells. The particle was ingested by invagination of the cell membrane so that a single membrane bound vesicle or vacuole derived from the cell membrane and containing the ingested material was formed. The pinosomes and phagosomes formed in this manner may have fused with each other before fusing

with primary lysosomes, others appeared to fuse with primary lysosomes direct. In either event secondary lysosomes were formed and these were either solitary or multiple (Figs. 5.9/56 - 5.9/58).

In some cases residual bodies were common and the phagocytic cells contained large quantities of poorly digested, poorly segregated material of crystalline and non-crystalline appearance (Figs. 5.9/58 - 5.9/60). Cells of this type were apparently derived from the fibroblast-like cells with limited numbers of large lysosomes as a characteristic feature.

In other cases digestion seemed more complete and the phagocytic cells were more typically macrophages which contained well segregated round spaces. The spaces usually had a grey halo and an osmiophilic rim or they contained quantities of osmiophilic lamellated remnants. Similar material to that observed within the cells was also seen within the lumen of some blood vessels and may represent one way in which lipid droplets, or "liposomes" can return to the general circulation (Figs. 5.9/61, 5.9/62 & 5.9/63).

Giant Cells These were seen in material which had presumably contained crystals too large for ingestion by a single macrophage. In this situation a number of macrophages were found ranged around the characteristic cholesterol profile and there was a most complicated interdigitation of cytoplasmic processes. Membranous lamellae and discrete or conglomerate osmiophilic circles were common within the profiles and the surrounding cells contained quantities of segregated clear spaces. The composite giant cell formed by this process of circumfusion was rich in organelles; the Golgi complex and numerous vesicles and vacuoles being notable (Figs. 5.9/64 & 5.9/65).

The way in which giant cells disposed of the ingested lipid was not entirely clear, dying cells were not seen and neither was extracellular lipid in their vicinity. It would seem that they probably dissociated into the more mobile mononuclear cells once their task was completed, unless they were sufficiently close to a blood vessel for mobility not to be a problem. Giant cells in the immediate vicinity of blood vessels contained clear and osmiophilic vesicles which were similar to vesicles in the blood vessel and its lumen and it is possible that this appearance was compatible with discharge of "liposomes" into the general circulation (Fig. 5.9/66).

Lipid clearance was thus associated with intracellular lipids in healthy cells and vascularisation. Ultrastructural examination endorsed the findings of light microscopy that free and esterified cholesterol and phospholipid were prominent, whereas other neutral fats, such as triglycerides, were absent or rare (Fig. 5.9/67).

5.9.5 Fine Structure: Summary and Discussion. There are no published studies of the pathogenesis of canine lipid keratopathy available for comparison. However, there has been one ultrastructural study of naturally occurring corneal opacities in Beagles (Spangler et al. 1982) and another study, which included ultrastructure, of experimentally induced hyperlipoproteinaemia in the same breed (Slatter, 1980). The changes which Spangler et al. reported in the stromal fibroblast were identical to those reported from the broader classification of central/paracentral lipid dystrophy (Crispin, 1982; Crispin & Barnett, 1983; Crispin, unpublished observations), and the fibroblast changes resemble those illustrated for Schnyder's central crystalline dystrophy. Degenerative alterations of the fibroblast observed in canine

corneal dystrophies appear remarkably similar to those encountered in naturally occurring canine lipid keratopathy and strengthen the hypothesis that the fibroblast response to a variety of insults involving cholesterol is rather limited.

Slatter (1980) illustrated his account of the effects of experimentally induced hypercholesterolaemia in Beagles with five electron micrographs of affected corneas from two dogs, but without indicating which part of the lesion was being displayed. Two micrographs concerned a dog in which the eye had become deeply ulcerated, with secondary interstitial keratitis, vascularisation and uveitis, during the latter part of the investigation. In this animal corneal pathology had modified arcus development with enhanced lipid deposition in the damaged region of cornea. There were numerous irregular to round extracellular spaces, particularly beneath the basal lamina of the anterior epithelium, larger acicular clefts were also present extracellularly, and fibroblasts contained large quantities of intracytoplasmic vacuoles and were of degenerate appearance.

The other three micrographs were from a dog which had developed arcus lipoides corneae unmodified by corneal damage. There were less numerous irregular spaces and acicular clefts, but the portions of fibroblasts which appear in all three micrographs are degenerate and some of the intracytoplasmic vacuoles therein would appear to have derived from damaged mitochondria. In one fibroblast a pyknotic nucleus adds to the general impression of dying cells. In considering the pattern of lipid deposition, the author postulated that in non-vascularised corneas lipid first accummulated in the fibroblasts, whereas in vascularised corneas there was the

possibility of direct deposition of lipid from blood vessels to corneal stroma. He makes no mention of the fibroblast as a source of extracellular lipid material except for the statement "Although it is possible that lipid was deposited in the stroma, entered the keratocytes and then returned to the stroma, such a sequence is unlikely when significantly smaller amounts of stromal lipid were formed in the non-vascularised corneas despite consistent hypercholesterolaemia". The possibility of corneal cells being a source of extracellular lipid in lipid keratopathy was clearly expressed in a paper by Cogan and Kuwabara (1958).

Ultrastructural studies of lipid keratopathy in man and the rabbit confirm the origin of granular lipid from degenerating fibroblasts filled with globular lipid. In the rabbit, Rodger (1971) showed that experimentally induced hypercholesterolaemia resulted in an arcus lipoides corneae which differed from that of man in having obvious fibroblast involvement, a situation which was dramatically enhanced if a limbotomy was performed when hypercholesterolaemia was present; in which case there was considerable fibroblast disintegration with marked stromal disorganisation. In man, Jack and Luse (1970) showed similar disintegration of fibroblasts in lipid keratopathy. They also illustrated the close association of blood vessels and nerves which was observed in the present study and their photographs illustrate the reduplication of the basal lamina which seems to be a characteristic of the new blood vessels which invade the stroma in lipid keratopathy. Macrophages were found to be common, especially near blood vessels. They did not comment on the numerous circular spaces, some with osmiophilic rims, which had accummulated between

the endothelial cell and its multilayered basal lamina. In the present study the possibility that these represent lipid infiltrating from blood vessel to corneal stroma has been suggested by the evidence obtained from fluorescein angiography, light and electron microscopy.

In the female patient described by Jack and Luse, triglyceride levels were raised and cholesterol levels were on the high side of normal, no serum electrophoresis was performed so the lipoprotein pattern was unknown.

The only other ultrastructural study of naturally occurring lipid keratopathy was reported in a neutered male cat by Carrington (1983). This animal had a raised level of serum cholesterol, cause unknown, and had originally developed a corneal ulcer as a result of a cat scratch. The lipid keratopathy had developed in association with the vascularisation which accompanied healing. Essentially the pathology was similar to that of the dog, rabbit and man in that there was a hypercellular zone at the periphery of the lipid-containing region, whereas free haemorrhage and necrosis were present in the less cellular area rich in lipid. In the cat, phagocytic cells, such as modified fibroblasts, macrophages and giant cells, would appear to mobilise rather faster than in the other animals examined; although this impression may also relate to the superficial nature of the lesion and the extensive vascularisation. Abnormal neurones were observed in some areas and neuronal elements were probably more abundant than in the normal feline cornea. A portion of unidentified cells contained membranous cytoplasmic bodies, which differed from similar structures in the dog by consisting of parallel, rather than whorled, membranous

lamellae.

In the dog the membranous lamellae of fibroblasts were apparently derived from microsomes in early lesions, and from both microsomes and lysosomes in later ones. Fibroblasts containing acid phosphatase-rich lysosomes also appeared to have phagocytic abilities, and their appearance was more that of the stem mesenchymal cell than the adult resting fibroblast. Despite these phagocytic actions the cells did not seem particularly effective in lipid catabolism and 'foam' cells filled with large poorly separated vacuoles were commonly observed. Whilst fibroblasts probably accounted for the majority of foam cells, a number of other cells, such as pericytes, smooth muscle cells, polymorphonuclear leukocytes, perineural cells and even tissue macrophages, were a possible source of foam cells; particularly in hyperlipoproteinaemic patients and those with considerable stromal damage.

Effective phagocytosis in central and paracentral lipid keratopathy lesions seemed dependent on adequate corneal capillarisation. Haematogenous macrophages and giant cells were common in established, vascularised, lesions. These cells differed from fibroblasts and fibroblast-like cells in being rounder and usually larger, rich in cell organelles and containing granules of variable size and electron density. In a proportion of material mononuclear and multinucleated phagocytic cells demonstrated subplasmalemmal linear densities, which consisted of a thin layer of electron-dense material immediately subjacent to the inner leaflet of the plasma membrane. The presence of subplasmalemmal densities has been previously reported in the macrophages and multinucleate giant cells of granulomata (Kawanami, Ferrans and Crystal, 1980).

Following uptake of lipid the macrophages contained numerous, clearly demarcated, membrane-bound vacuoles and, often, considerable quantities of osmiophilic material. The cells were generally rich in osmiophilic membranous lamellae, and it was noticeable that whereas fibroblast-like cells had a few, large, lysosomes, the macrophages had both large and small. Cells identified as macrophages ultrastructurally coincided with those of discrete staining ability and rich esterase activity under the light microscope. The lipid droplets these cells contained were usually anisotropic, comprising phospholipid and cholesterol ester of unsaturated type.

In summary, the results of ultrastructural study combined with earlier findings confirm that the free and esterified cholesterol which accumulates in the cornea in lipid keratopathy is largely, or wholly, derived from cell death; particularly that of the fibroblast. Whilst the fibroblast is capable of accumulating lipid, the presence of fibroblast-derived "foam" cells suggest that its capacities for lipid metabolism under these conditions are limited. The fibroblast's capacity for phospholipid synthesis and lysosome formation may be compensatory phenomena to reduce the deposition of hydrophobic lipids.

Haematogenous macrophages, and giant cells produced by macrophage circumfusion, seem to be key cells in the clearance of deposited lipid and the evidence obtained in this study suggests that phospholipid synthesis and conversion of saturated lipids to less saturated, or polyunsaturated, lipids may be of importance in achieving lipid clearance. Liposomes produced by phagocytic cells may be returned to the blood stream via the corneal vessels which

are a feature of lipid keratopathy.

6.0 FINAL DISCUSSION AND CONCLUSIONS

6.1 Introduction

Whereas lipid keratopathy is relatively uncommon in man (Davidson et al, 1951) and the dog (Crispin, 1982) predisposing factors, such as hyperlipoproteinaemia and anterior segment disease, are of frequent occurrence. In both species simultaneous hyperlipoproteinaemia and anterior segment disease does not necessarily result in lipid keratopathy, indicating that additional predisposing factors may be of importance. This section discusses the results obtained in this study in relation to some aspects of lipid metabolism and lipid interaction reported by other workers. In view of the limited information available concerning pathogenesis of lipid keratopathy in any species it was felt important to discuss the findings of the present study in a broader context in this Section.

6.2 Incidence

From the information available in the literature it would be impossible to arrive at accurate figures for the incidence of lipid keratopathy in the dog. An approximate idea of incidence can be assessed from cases seen by the author. Of some 8,000 dogs referred for ophthalmic consultation between 1972 and 1982; 48 animals were diagnosed as having lipid keratopathy, an incidence of 0.6%. It is relevant to note that 14 of the 48 had arcus lipoides corneae as well as lipid keratopathy when first presented. During the same period arcus lipoides corneae alone was seen in eight dogs (six Alsatians and two Rough Collies) as a bilateral, peripheral, lesion in the presence of hyperlipoproteinaemia involving raised cholesterol, triglyceride and phospholipid. This gives an

incidence for an established arcus lipoides corneae of 0.1%. In the same period two Labrador Retrievers with transient arcus lipoides corneae, in the presence of hypertriglyceridaemia as the only abnormality of serum lipids, were also seen. In both these animals the most marked feature was lipaemia and congestion of the conjunctival and episcleral blood vessels, with lipaemia of the aqueous in one dog. In both animals triglyceride levels returned to normal over several days and the congestion of the perilimbal vessels disappeared as did the lipaemia of the aqueous. The cloudy appearance of the peripheral cornea took about a week to clear.

Central/paracentral lipid dystrophy is recognised more frequently in dogs than lipid keratopathy and arcus lipoides corneae (Crispin, 1982; Crispin and Barnett, 1983) although it has proved difficult to establish inheritance, largely due to the difficulties of tracing the relatives of affected animals. If the incidence is computed for cases seen by the author over a ten year period as before, an incidence of just over 1% is reached for 82 dogs out of 8,000 with bilateral, non-inflammatory, evidence of central/paracentral lipid dystrophy. In none of these animals was arcus lipoides corneae or lipid keratopathy present, indicating that in these cases it might be rewarding to concentrate research efforts on lipid metabolism in fibroblasts, and also to investigate the possibility of rather subtle, possibly transient, changes in the pattern of serum lipids and lipoproteins.

With such small numbers of dogs affected by lipid keratopathy it would be rash to suggest a definite breed susceptibility; although further investigation of the Alsatian, Spaniel and Golden Retriever

might be indicated; the Golden Retriever is of special interest as the only breed in which the cases were all in bitches, with the exception of a single neutered male. In this breed the fibroblasts were frequently of bizarre shape and size early in the evolution of the disease. More information is also required for the Rough Collie and Old English Sheepdog.

The Alsatian and Rough Collie are the only breeds in which all three types of cholesterol-rich lesion (arcus lipoides corneae, central/paracentral lipid dystrophy and lipid keratopathy) seem to have been recorded, and future research might be concentrated on these breeds, particularly in relation to local ocular factors and detailed blood lipid chemistry. Further investigation may confirm that there are certain fundamental similarities of fibroblast response which can be modified by external influences. Offret, Payrau, Pouliquen, Faure and Bisson (1966) and Payrau, Pouliquen, Faure and Offret (1967) observed that the ultrastructural changes of a number of human corneal dystrophies were similar.

In man, arcus lipoides corneae is a common finding past middle age, whereas Schnyder's central crystalline dystrophy and lipid keratopathy are both uncommon, but without accurate figures of incidence being available. From the reports of human lipid keratopathy reviewed earlier it can be deduced that from 64 cases in which the sex of the patient was specified, 46 were in women and 18 in man; an incidence of 72% and 28% respectively. With the exception of the Golden Retriever, there was no apparent sex incidence of lipid keratopathy in the dog.

6.3 Serum Lipids and Lipoproteins

In the majority of animals which provided keratectomy specimens for microscopic examination the principal lipoprotein alteration was the enrichment of high density lipoprotein with cholesterol to produce HDL_C. In most patients the serum lipids and lipoproteins were restored to normal by appropriate dietary and exercise regimes, or hormonal therapy. The possible influence of normolipoproteinaemia alone could not be critically evaluated in this study because of other factors such as anterior segment disease, corneal neovascularisation and the effects of hormones. The local effects of lipoproteins on cells are discussed in Section 6.5 and 6.6 and the possible interactions of lipids and lipoproteins in connective tissues in Section 6.7.

The importance of examining lipoprotein patterns in individual cases has been emphasised by Lehmann and Lines (1972), for there are occasions when quantitative lipid results give no indication of underlying lipoprotein disorders. The results presented here for the dog underline the need for more detailed examinations of serum lipids and lipoproteins, particularly in conjunction with corneal analysis, in future work. The present study also draws attention to the paucity of information for man, where there has been little attempt to characterise the serum lipids and lipoproteins in most documented cases of lipid keratopathy (Klintworth, 1982).

A feature of human lipid keratopathy which has received little comment or study is the higher incidence in women. It is known that HDL-cholesterol levels are consistently higher in adult women than men, largely due to differences in HDL_2 concentration, (Nicoll, Miller & Lewis, 1980), and there may be some significance in the

size and composition of cholesterol-enriched HDL for the development of lipid keratopathy in dogs and humans.

6.4 Lipid Entry

In capillaries, lipid migrates right across the vessel wall (Courtice and Garlick, 1962; Parker α Odland, 1968) and lipid transport is more rapid in inflammed, or damaged vessels (Friedman and Byers, 1959; Walton, 1973) and in newly formed vessels (Friedman α Byers, 1962). Normal endothelium is permeable to plasma macromolecules even in healthy young animals (Bell, Adamson and Schwartz, 1974) and lipid deposition is likely to be enhanced with increased limbal blood flow, or when blood pressure is high as in hypertension.

The transfer of substances through the intact cornea is largely determined by phase solubility; epithelium and endothelium are most permeable to those substances possessing a fat soluble phase, and the stroma is permeable to those having a water soluble phase (Adler, 1965). The presence of soluble proteins has been reported in the corneal stroma by a number of workers. Holt and Kinoshita (1973) for example, reported on the presence of albumin, gamma globulin, transferrin, a lipoprotein and an unidentified protein of slow beta electrophoretic mobility in the bovine cornea. Their results indicate a direct linear relationship between the relative concentration of plasma derived constituents and molecular weight.

Maurice (1961) suggested that the largest molecules able to diffuse between the collagen fibrils of the normal stroma were between 60 Å and 185 Å in diameter and that the rate of diffusion slowed according to increasing size. The limiting size in the normal cornea corresponded to a molecular weight of approximately

500,000, although a swollen cornea allowed larger proteins to diffuse. As the apparent space between collagen fibrils was greater than the diameter of molecules capable of diffusion Maurice postulated that the corneal ground substance might also offer some resistance to diffusion. In a later publication, Maurice and Watson (1965) experimented with serum albumin (diameter, 70 A; molecular weight, 68,000) and showed that this protein apparently entered the cornea from the peripheral capillaries, diffused towards the centre and was slowly lost across the corneal surfaces.

There are important similarities between the situation proposed for the cornea and what is known concerning the intima of blood vessels. Smith (1977) has shown that most plasma proteins appear to be present in intima at concentrations that are a linear function of molecular weight and concentration in the plasma. Thus low density lipoprotein (molecular weight, 2×10^6) has the greatest retention relative to its plasma concentration, whereas the relative retention of albumin is only 15% of the relative retention of low density lipoprotein. The glycosaminoglycan gel of the intima appears to act as a molecular sieve, and an identical situation may be present in the cornea. Sheraidah, Winder α Fielder (1981) have indeed proposed that initial localisation of low density lipoprotein in human corneal arcus formation is probably a consequence of sieving by the stromal fibres at the limbus.

Negative staining of canine lipoproteins (Mahley et al, 1974) indicated a diameter of between 70 Å to 400 Å for cholesterol-rich high density lipoproteins, and their molecular weight would be approximately half the maximum value calculated by Maurice. It might therefore be possible for a proportion of intact high density

lipoproteins to gain access to the normal corneal stroma, whereas abnormal low density lipoprotein (170 to 360 Å) and very low density lipoprotein (240 - 550 Å) would almost certainly require some modification to size and structure before it could traverse the corneal stroma. The failure to detect intact high density lipoprotein, or the arginine rich apoprotein, using lipid and protein methods on parallel sections, has been referred to earlier in Section 5.0.

In addition to any deficiencies in the histochemical techniques employed there are a number of other factors to be considered; namely, the lipid enzyme, and other, activities of the blood vessel wall through which the cholesterol-rich plasma must pass to gain access to the cornea; the possible interactions plasma may undergo with a variety of corneal components once it has reached the cornea; the stability of HDL_c; and its soluble and insoluble constituents.

No enzyme degradative system for cholesterol exists in the blood vessel wall (Adams, 1978) whereas phospholipids and triglycerides can be influenced by phospholipases (Stein, Stein and Shapiro, 1963) and triglyceride lipases (Zemplényi, 1968) respectively. Thus lipoprotein or lipid could be modified by enzymes as it crosses the blood vessel wall and the enzymes may themselves be compromised when hyperlipoproteinaemia is present (Boyd, personal communication) or the blood vessel aged (Adams, 1973). Subtle changes in the surface properties of lipoproteins may have profound effects on their subsequent association with components of the subendothelial compartment, and, once plasma components cross the endothelial barrier, they reach an environment

with rheological properties quite different from those present in the vessel lumen (Camejo, 1982).

The lipid detected in the immediate vicinity of blood vessels in the present study, particularly that accummulating in the subendothelial space and between the multiple layers of the basal lamina in corneal neovascularisation, was found to be of a range of sizes, many of which were in excess of those reported for the largest lipoproteins, whereas the smaller ones could represent intact lipoprotein on the basis of size. The diameter increased, possibly because of coalescence, with increasing distance from the blood vessel. Similar ultrastructural evidence of accummulation has been illustrated in a variety of situations. For example, associated with corneal neovascularisation in naturally occurring human lipid keratopathy (Jack and Luse, 1970), in experimental hypercholesterolaemia in the dog aortic intima (Geer, 1965), and in rabbit dermal capillaries (Parker and Odland, 1968). These accummulations appear to correspond with the fine droplets of perivascular lipid seen by light microscopy and, in the present study, they were interpreted as being mainly, or exclusively, comprised of blood-derived lipid dissociated from its apoprotein vehicle. It is of interest that Sheraidah et al. (1981) demonstrated that human corneal arcus was not composed of unmodified low density lipoprotein, for the fatty acid pattern of the arcus deposit was not consistent with a recent origin from plasma lipoprotein. Apoprotein B detected in peripheral cornea probably reflected traces of freshly insudated lipoprotein, and it was present whether or not there was a macroscopic arcus lesion and formed no part of it. Plasma levels of high density lipoprotein do not apparently influence arcus

development, and it may be concluded that plasma low density lipoprotein may be concerned with the initiation of the arcus deposit, but does not form part of its composition.

Dissociation of soluble lipoprotein to give soluble and insoluble components may be a basic feature of plasma-derived lipid deposits in the cornea and could be a consequence of enzyme activity, the effects of filtration, or interactions between the lipoproteins and corneal components. There are some similarities with proposed mechanisms for atherogenesis. Page (1954) postulated that lipid-laden low density lipoprotein was unstable and became disrupted as it passed through the arterial wall. Soluble components passed on, whereas insoluble ones were deposited within the wall and themselves provoked tissue reaction. There is also considerable interest in possible interactions between lipoproteins and the arterial mesenchyme as factors in atherogenesis; a subject reviewed recently by Camejo (1982).

In the dog cholesterol-rich high density lipoprotein has an inherent instability. There is an increase in cholesterol, particularly esterified cholesterol, which occurs at the expense of other constituents such as protein, and this means that a minor conformational alteration will result in lipoprotein dissociation (Boyd, personal communication). Whilst protein is soluble and electrophoretically mobile, and phospholipid is soluble because of its amphipathic properties, cholesterol and cholesterol ester are insoluble and non-mobile. It would thus be quite possible for the insoluble components to accummulate by direct deposition in affected corneas, in addition to the more obvious derivation of extracellular lipidic debris from corneal cells. Examination of a proportion of

early, non-vascularised (Phase I) material in which extracellular, non-lamellated, cholesterol ester-rich perifibrous droplets were numerous, supports this hypothesis. In addition, there is the enhanced lipid deposition associated with hyperlipoproteinaemia, anterior segment inflammation, and localised corneal damage or neovascularisation. Excessive permeability to blood lipids is probably a property of all types of very young vascular tissue, regardless of the presence or absence of inflammation (Friedman and Byers, 1962) and can provide direct, perhaps unmodified, access to the corneal stroma for lipid-rich plasma in vascularised corneas. The results of the present study indicated that anterior segment disease and corneal neovascularisation could both enhance lipid deposition in affected animals. Vascularisation following lipid deposition produced a more plaque-like appearance of the corneal opacity.

Cholesterol-rich high density lipoprotein may have received less attention in man than in other mammals because low density lipoprotein is the principal cholesterol carrier in normal humans and, as such, has been extensively studied in atherogenesis. High density lipoprotein may actually exert a protective effect in atherosclerosis as reduced HDL levels are a characteristic of atherosclerosis prone species and human subjects at risk (Miller and Miller, 1975). However, cholesterol-rich human HDL is similar to HDL_C of other mammals in having higher levels of free and esterified cholesterol and lower levels of protein than normal HDL, or even normal human LDL. It is also inherently unstable because of these compositional changes (Boyd, personal communication) and these properties have renewed interest in its role in atherogenesis

(Smith, personal communication).

One other aspect of lipid entry relates to the ocular lesions which follow different types of diet. For example, in experimental hypercholesterolaemia of rabbits, Walton and Dunkerley (1974) showed that lipid deposition in the region of the deep scleral plexus occurred more quickly, and was more severe, with greater abundance of fat-filled cells, in animals fed a cholesterol diet, than those on a beef-fat diet. The authors commented that the LDL of rabbits fed cholesterol was "abnormal" in containing a much higher proportion of cholesterol than normal rabbit LDL, or LDL from rabbits given the beef-fat diet, which resembled normal LDL in composition. The results of the present study indicated that modifications to the diet of affected dogs may help to produce regression of lipid keratopathy in a proportion of cases, but that further work is required to establish the specific factors involved.

In summary, as regards lipid entry, the results of this study and those from other publications indicate that cholesterol enriched lipoprotein is common to a variety of apparently unrelated lipid-containing lesions. The possible significance of low density lipoprotein for peripheral corneal lesions and high density lipoprotein for more centrally located types has relevance for both arcus lipoides and lipid keratopathy.

6.5 The Fibroblast

Whilst in a proportion of early, Phase I, material it was possible to demonstrate a preponderance of extracellular cholesterol-ester-rich material with minimal fibroblast involvement, in most progressive lesions the fibroblast was an obvious and important source of lipidic debris and the major cell involved in Phase II.

In areas of necrosis and solid crystal formation the fibroblasts were degenerate, dying and dead, whereas at the periphery of necrotic areas there was often hypercellularity and indications of increased fibroblast activity.

The fibroblast is a cell of mesenchymal origin and diverse form whose properties vary more according to its site of origin than species of origin (Pollak, 1969). Its potential abilities will be reviewed in some detail in view of the central role it plays in lipid keratopathy.

The description of fibroblast is sometimes restricted to the metabolically active cell, whereas the less active or resting corneal cell is known as the fibrocyte, keratocyte, or corneal corpuscle. As there is considerable variation in metabolic activity many authorities designate all such cells as fibroblasts (Klintworth, 1969, 1977; Hogan, Alvarado and Weddell, 1971) and this is the procedure adopted in this study.

The corneal fibroblast possesses a number of properties; the most usual being synthesis of proteoglycan-rich ground substance and collagen precursors by the activated cell, of crucial importance in stromal wound healing in the adult cornea. Corneal injury, for example, results in cellular death in the immediate wound area, followed by a rounding-up of the fibroblasts and movement of activated cells towards the site of damage (Baum & Silbert, 1978). It is not always easy to ascertain whether the fibroblast-like cells found in damaged cornea derive entirely from in situ cells, or include extracorneal cells. Less readily identified fibroblast-like cells may originate from other mesenchymal sources; indeed Movat & Fernando (1962) in reviewing the fine structure of the

fibroblast admitted that they were "unable to separate the resting and apparently inactive fibroblast from the cell called a primitive (undifferentiated) mesenchymal cell" and that the picture was further complicated by "the proven ability of certain defined cells to become transformed into fibroblasts". In some of Phase II and Phase III material it was difficult to be sure of the origin of a proportion of phagocytic fibroblast-like cells, although an origin from fibroblasts seemed most likely.

After studying traumatic injury to the cornea, Walb (1875) suggested that corneal cells had phagocytic abilities, and, more recently, Klintworth (1969) investigated the phagocytic capability of the rabbit corneal fibroblast in vivo and in vitro. Klintworth concluded that the corneal fibroblast manifested a pronounced capability to ingest a wide variety of foreign particulate matter on the basis of experiments in which diverse substances were injected into the substantia propria of the central cornea in the living rabbit, as well as into in vitro tissue explants and confluent corneal fibroblast cultures. Following phagocytosis, the ingested material became sequestered in intracytoplasmic single membrane bound vacuoles. Some material, such as haemoglobin, was digested, while other indigestible foreign material, such as thorium, accumulated and eventually almost filled the cytoplasm. A proportion of canine fibroblasts in established lesions demonstrated phagocytic abilities in the present study and characteristically accumulated intracytoplasmic single membrane bound vacuoles similar to those described above.

Lipid metabolism in fibroblasts has been studied in vitro and, less commonly, in vivo. Much of the original interest in the cell

derived from its possible involvement in atherosclerosis, but as Pollak (1969) commented "the fibroblast, whether from mouse or man, is the cell least suited to the study of lipid metabolism", and he added that the role of the endothelial cell and smooth muscle cell with regard to lipid metabolism was of greater interest in the study of atherogenesis.

In relation to tissue culture work in which fibroblasts had been used, Pollak made the important observation that "the spontaneous appearance of fat droplets is very characteristic. This must be taken into account by those who study lipid metabolism of fibroblasts...". Other factors to be considered when studying the lipid histochemistry of fibroblasts include the methods used for lipid fixation and identification; a subject which was examined in some detail in Section 3.9.3. In some of the publications reviewed below it is apparent that the techniques have not always been specific, particularly with regard to the differentation of triglycerides and cholesterol esters.

Cogan & Kuwabara (1954 & 1955) used live rabbits and *in vitro* corneal explants from a number of species to demonstrate lipogenesis by corneal fibroblasts, corneal epithelial cells and corneal endothelial cells.

In the presence of rabbit serum and following injection of oleic acid or sodium oleate into the cornea a zone of necrosis containing birefringent crystals developed at the site of injection. The crystals stained a dense blue with Nile blue sulphate, were occasionally faintly Sudanophilic and were positive with Von Kossa's stain for calcium. They were identified as calcium soaps of fatty acids. At the periphery of the necrotic zone Sudanophilic

intracellular fat globules appeared within six hours of injection. The globules increased progressively in size for at least seven days and attained a diameter of several micra; they were a bright red colour with Sudan stains and pink with Nile blue sulphate.

The authors concluded that the globules represented neutral fat formed by an enzymatic process and that the fibroblasts contained synthetic, rather than hydrolytic lipases when engaged in lipogenesis. They were unable to demonstrate any catalytic lipase activity with the Gomori technique on normal corneas and corneal sections incubated with stearate and oleate esters. The limitations of the Gomori technique have been discussed previously.

A series of further experiments to define the factors involved in experimental aberrant lipogenesis were also undertaken. Cogan α Kuwabara (1957a) provided evidence that the serum factor involved in this phenomenon was protein-based and species non-specific and that it probably provided a vehicular system for transport of the oleate substrate across the cell wall. In defining the substrate factor, Kuwabara α Cogan, (1957a) concluded that experimental lipogenesis depended upon the presence of oleic acid, some oleate compound, or some lipid derivative of tissue which contained oleates. Lipogenesis did not occur with neutral fats unless they had been previously hydrolysed. Fatty acids other than oleic acid could not be substituted for oleic acid.

The tissue factor (Cogan α Kuwabara, 1957b) appeared to be inherent in all the native cells of the cornea, although stromal cells (fibroblasts) were more susceptible to the toxic effects of oleate than the surface epithelial and endothelial cells. No difference was found in the lipogenesis of central and peripheral

cornea. The fat globules developed exclusively in the cell cytoplasm and never in the nuclei. Attempts to correlate the site of fat formation with mitochondria were inconclusive.

Kuwabara α Cogan (1959) extended their studies to demonstrate that a non-Sudanophilic, birefringent, crystalline and acid haematein positive fat could be produced in corneal cells if stearate or palmitate were substituted for oleate. On the basis of some similarity of properties they postulated that the fat synthesised by the cells may have been a neutral fat such as tristearin or tripalmitin and phospholipid.

In rather similar experiments Moskovitz (1967) presented albumin-fatty acid complexes to L-strain mouse cells in culture. Unsaturated fatty acid such as oleate, linoleate and linolenate behaved similarly and the cytoplasm of cells exposed to these fatty acids contained Sudanophilic droplets which also stained bright pink with Nile blue sulphate, were positive with osmium tetroxide and negative with the Schultz reaction and Baker's acid haematein.

When saturated fatty acids such as palmitate or stearate were used the cell cytoplasm developed non-staining acicular inclusions which were negative with all the techniques employed, and which were also much more likely to lead to death of the cell than fat droplets, derived from unsaturated fatty acids, which the cells tolerated well. Another important difference was that the cells exposed to unsaturated fatty acids demonstrated lipase activity once fatty acid ester synthesis had stopped, with concomitant disappearance of lipid droplets, whereas no such activity could be demonstrated in cells with accumulations of saturated fats. The author concluded that the reduction in the number of fatty acid

ester particles (assumed to be triglyceride) was therefore most likely to be a consequence of hydrolytic lipase activity rather than any form of secretory mechanism whereby intact particles were extruded. The apparent failure of lipolytic response in the cells which had accumulated saturated fat was interpreted as a factor accounting for the observed irreversibility of the saturated lipid accumulation. This work also indicated that lipid synthesis probably occurred in intimate association with portions of the endoplasmic reticulum which is known as the site of many enzymes concerned with lipid synthesis (Oda, Yoshizawa, Nakamoto, Kubo α Okazaki, 1958; Norum, Berg, Helgerud α Drevon, 1983). This relationship was observed in the present study. Fatty acid esterification occurs in the endoplasmic reticulum of many cells, and while a few cells can pick up lipid droplets directly by pinocytosis, or micropinocytosis, most cells pick up the component parts of lipid which has been hydrolysed. Lipid is then resynthesised in the endoplasmic reticulum and, at some stage of the process, usually appears in the Golgi complex (Ghadially, 1982).

Using C_{14} labelled oleate Hill, Kinoshita α Kuwabara (1959) were able to confirm that the corneal cells were synthesising lipid from oleic acid, rather than simply storing it, as the lipid formed by the cell was a substance other than free oleate and presumably formed as a consequence of oleate esterification. Ciccarelli α Kuwabara (1959) chemically identified the fats produced in vitro by the rabbit cornea as esterified glycerol, esterified fatty acids, lipid phosphorus and cholesterol.

Broekhuyse and Daemen (1977) reviewed the rather limited information available concerning lipid metabolism in the intact

cornea. In bovine ocular tissues, for example, Andrews (1966) suggested that pathways for lipid metabolism similar to those of the liver were probably present and Broekhuyse (1968) found that the cornea, and other ocular tissues, incorporated [^{32}P]Pi into its phospholipid, while Thiele α Denden (1967) detected a seasonal influence on total lipids, with a high percentage of cholesterol esters in stroma and endothelium and mainly free cholesterol in the epithelium. Smidt-Martens and Hohorst (1972) disagreed with these findings in demonstrating a predominantly cholesterol ester-rich component in bovine corneal epithelium.

Culp, Cunningham, Tucker, Jeter α Determan (1970) investigated in vivo synthesis of lipids in various ocular tissues of the rabbit including the cornea. [1- ^{14}C] acetate was rapidly incorporated into fatty acids and to a lesser extent into cholesterol. Esterified fatty acids of neutral lipids showed a higher incorporation rate than those of phospholipids.

Klintworth α Hijmans (1970) demonstrated lipid accumulation and storage in explants of rabbit cornea. The epithelium, endothelium and fibroblasts all progressively accumulated osmiophilic and Sudanophilic intracytoplasmic lipid droplets in media containing homologous or heterologous normolipaemic serum. The extent of lipid storage was directly related to the quantity of serum in the medium and to the duration of growth within it. The lipid appeared as refractile bodies with phase contrast microscopy, was neither birefringent nor autofluorescent, and gave a negative Schultz reaction. The individual droplets often reached 15um diameter in older cultures, and their number also increased progressively with time.

The lipid droplets were digested with lipase and readily extracted by organic solvents such as ethanol and acetone. The lipid was not consistently associated with acid phosphatase activity.

The appearance of the intracytoplasmic lipid inclusions varied under the electron microscope according to the method of fixation, and the lipid was clearly not ingested in the form seen within cells. Similar lipid material was not observed extracellularly and there was no evidence of pinocytosis or phagocytosis.

In the least severely involved cells osmiophilic particles were not related to any particular organelles and it appeared that the cytoplasmic ground substance might be the site of synthesis. Older cultures contained cells with lysosomes and lysosomal-like particles with dense osmiophilic material within them; most of the cytoplasmic accumulates were not related to such structures.

The authors concluded that fatty acids, which are normally bound to serum albumin, entered the cell, possibly by simple diffusion, and became esterified into triglycerides within the cytoplasmic ground substance.

Tanaka α Kuwabara (1978) investigated the fine structure of lipogenetic cells of the cornea and postulated the formation of lipid within the ground substance. Tritiated oleate was injected into rabbit cornea, and immediately stromal cells in the vicinity of the injection became diffusely radioactive. The cytoplasm of the corneal stroma began to form small clear spaces of approximately 0.05um diameter in the matrix. Such spaces were never radioactive, were not related to any organelles or any radioactive concentrations of fat droplets and became membrane-bound oil droplets of some 0.05

to 1.0um diameter within five to six hours of injection. Their size and number increased over a few days, they produced no degenerative changes within the cell and were assumed from earlier studies to be triglycerides. Some longstanding fat droplets developed a few myelin figures and radioactive oleate was heavily incorporated into the lamellated membranes.

Lipid metabolism in non-ocular fibroblasts has attracted many workers but, as Pollak (1969) makes clear in his review of fibroblasts in tissue culture, the properties of non-ocular fibroblasts may be quite different from those of the eye and specifically the cornea. Furthermore, in vitro findings from cells in tissue culture should be extrapolated with caution to the in vivo situation.

However, there is important work concerning non-ocular fibroblasts which may be of relevance in the pathogenesis of lipid keratopathy. Goldstein α Brown (1974 α 1975) and Brown α Goldstein (1976) reported the presence of cell surface lipoprotein receptors in cultured human fibroblasts. Using LDL they demonstrated internalisation of the surface-bound lipoprotein by a process of endocytosis and lysosomal hydrolysis of its protein and cholesterol ester components. The free cholesterol which resulted was transferred to the cellular compartment where it suppressed 3-hydroxy-3-methylglutaryl-co-A reductase and activated acyl-co-A-cholesteryl acyl transferase, thus facilitating its own re-esterification. Normal fibroblasts grown in the absence of low density lipoprotein contained low levels of cholesterol esters. When LDL was added to the medium and allowed to bind to its receptor a ten-fold rise in cellular cholesterol ester content was observed.

The "LDL pathway" proposed by these workers has now been well documented in several cell lines in many laboratories (Norum, et al., 1983).

Bersot, Mahley, Brown α Goldstein (1976), using swine, demonstrated that HDL_c became bound to the LDL receptor in human fibroblasts, acting similarly to LDL and resulting in a net accumulation of cholesterol and cholesterol ester within the cell. They also showed that swine HDL_2 behaved in similar fashion to human HDL in that it did not react with the LDL receptor.

Mahley, Innerarity, Pitas, Weisgraber, Brown α Gross (1977) showed that treating LDL or HDL_c with cyclohexanedione (a reagent specific for the amino acid arginine) abolished the binding activity of both LDL and HDL_c. Mahley α Innerarity (1977) demonstrated that in the dog binding of HDL_1 or HDL_c to the LDL receptor was due to the content of arginine-rich apoprotein in the HDL_1 or HDL_c. They postulated that the determinant of the lipoprotein structure which allowed selective binding of specific lipoproteins may reside in the apoprotein content. Innerarity α Mahley (1978) succeeded in isolating dog HDL_c with arginine-rich protein as its only protein constituent and this fraction had markedly enhanced binding to fibroblasts compared with LDL.

The demonstration of a specific fibroblast receptor that binds plasma LDL or HDL_c is of particular interest in view of the proposed regulatory function of the receptor on sterol influx and stimulation of cholesterol esterification within the cell. In addition, there is evidence (Stein, Vanderhoek α Stein, 1976) that normal high density lipoproteins might influence sterol efflux, with high density apolipoprotein acting as a cholesterol acceptor.

Human skin fibroblasts enriched in cholesterol by previous incubation with low density lipoprotein lost their cholesterol in the presence of lipoprotein-depleted serum or a low density apolipoprotein/phospholipid mixture, and these findings led the authors to suggest that fibroblasts, in spite of their capacity to synthesise cholesterol, depend on exogenous cholesterol for the maintenance of normal levels. Receptor-mediated endocytosis of lipoprotein-associated cholesterol may be under negative-feedback control in a variety of cells including fibroblasts; it provides another way in which cells protect themselves against cholesterol overload. On a speculative basis, protective devices employed by cells could operate for lipid uptake, synthesis, metabolism, catabolism, or release, so the situation is understandably complex.

In addition, there are environmental effects, such as the type of lipoprotein, which may influence lipid accumulation and clearance. Henricksen, Evensen α Carlander (1979a) demonstrated that the concentration of LDL normally found in plasma could damage fibroblasts and endothelial cells in tissue culture, whereas HDL reduced the cell injuries produced by LDL (Henricksen, Evensen α Carlander, 1979b). HDL has also been shown to form liposomes over the surface of crystals of free cholesterol in vitro (Adams α Abdulla, 1978; Abdulla α Adams, 1978). The liposomes so formed varied in size from 30nm to 1,200nm and, in comparison with HDL, they contained more cholesterol, but less phospholipid. Liposome formation by corneal fibroblasts is discussed more fully in Section 6.8 and below.

Fibroblast involvement in dogs with lipid keratopathy was surprisingly complex, even when observations were limited to central

or paracentral lesions, as in this study. In fibroblasts apparently engaged in lipid synthesis, staining reactions combined with ultrastructural studies indicated that fatty acid esterification was taking place in the endoplasmic reticulum, which was often distended by cholesterol ester-rich lipid, and possibly also by glycosaminoglycans. Some lipid droplets appeared to form by budding off from the membrane of the endoplasmic reticulum to form single membrane bound lipid droplets within the cell cytoplasm. This phenomenon may be indicative of cholesterol overload in the cell membranes (Norum et al., 1983). No phagocytosis was observed in fibroblasts engaged in this type of lipid accummulation, but there were often numerous micropinocytotic vesicles along the cell border. It was not possible to determine the exact manner of lipid uptake by fibroblasts in this study, although the results obtained and the publications reviewed reveal a range of possibilities. Only the presence, or absence, of specific lipoprotein receptors still requires verification for the corneal fibroblast; their presence might help to explain the observed differences between the fibroblasts of clinical cases, particularly if such differences could also be related to the prevalent lipoprotein or lipid present in the cornea.

In areas of heavy lipid deposition fibroblasts exhibited a range of degenerate features and were a major source of extracellular lipid. In some dogs, most notably the Golden Retriever, the lipidic debris from progressive lesions was predominantly crystalline in nature, and this breed was also of note in occasionally having dystrophic calcification in long standing lesions (Crispin & Barnett, 1983). Investigation of fresh

squash and imprint preparations had suggested that there was some variation in melting point of mixtures consisting predominantly of cholesterol esters, and the simplest explanation of observed differences between breeds would relate to the degree of fatty acid saturation. However, there is a potential for extremely complex interaction between the substances involved in lipid keratopathy, including serum lipids and lipoproteins as well as corneal-derived elements, which will be discussed further in Section 6.7, and 6.8, particularly in relation to phospholipids.

Whilst it was possible to detect small quantities of phospholipid within a proportion of fibroblasts in early lipid keratopathy it became the prominent intracellular lipid in some of the fibroblasts and macrophages of later material. A characteristic feature of the fibroblast involved in lipid accumulation in progressive lesions and early regression was its association with various kinds of membranous lamellae or liposomes. Lipid histochemistry and aspects of their ultrastructural appearance indicated that the lamellae were phospholipid-rich.

The lamellae were usually associated with degenerate changes in the fibroblast, and were often particularly profuse in the cell processes, but with increased cell involvement they were more generalised, and a proportion derived from cell microsomes. The formation of matrical lipid debris, or liposomes, would seem an entirely logical method of <u>in situ</u> necrosis for effete corneal cells remote from the limbus. Its presence in occasional normal corneas has previously been noted and there is an analogous situation in articular cartilage, another avascular tissue. Ghadially (1982) in reviewing the evidence concerning

disintegrating cartilage cells, concluded, that while some free lipid originated from the cells, a proportion derived from their extruded cytoplasmic processes. He postulated that the formation and discharge of lipidic debris was a way in which the products of cell necrosis, and indirectly necrotic cells, were disposed of by an avascular tissue.

Lysosomes in fibroblasts and fibroblast-like cells were also associated with phospholipid-rich membranous lamellae. Ultrastructural histochemistry had indicated acid phosphatase within the primary lysosomes and at the periphery of secondary lysosomes. Whorled membranous lamellae of the second lysosomes became separated as the lysosomes enlarged, until only a peripheral osmiophilic rim surrounded a large electrolucent vacuole. The membranous lamellae of the fibroblasts, whether derived from the cell microsomes or lysosomes, had a thickness of approximately 30 A which varied slightly according to how closely the lamellae were packed.

There are interesting parallels between the observations concerning fibroblasts and those for lipid deposition in atherosclerosis. In early life the intimal membrane probably contains less than a full complement of free cholesterol and little, or no, cholesterol ester (Small α Shipley, 1974). If there is an increase of cholesterol in the membrane (or lamellar) fraction of the cell, then a compensatory synthesis of sphingomyelin-rich phospholipid is apparently instigated with a consequent increase in the amount of membrane lipid. Once cholesterol reaches maximum saturation in the cell membrane it appears to stimulate cholesterol ester formation. As cholesterol ester has little structural role in cell membranes, but rather serves as a means of cholesterol storage

and transport, the cell membrane rapidly becomes saturated with cholesterol ester as well as cholesterol and there is separation of cholesterol ester as a second lipid phase, either as oil droplets, or mesophase liquid crystals. Once the amount of free cholesterol increases above its solubility in either the membrane phase or the oily cholesterol ester phase, then it is possible for the precipitation of cholesterol crystals to produce a third, solid, phase. There are thus two compensatory processes which may help to maintain cholesterol in a non-crystalline state: one is the increased membrane production which may be caused primarily by the increased sphingomyelin-rich phospholipid and the other is the increased production of cholesterol ester.

It would seem that there is an increase of sphingomyelin-rich phospholipid associated with ageing in the normal central cornea (Broekhuyse, 1976) and that the sphingomyelin-rich phospholipid could derive from synthesis in the fibroblast. If such a mechanism exists in the healthy cornea then it would almost certainly be deployed as a protective device in disease as suggested by the present study. Similarly, the accummulation of intracellular cholesterol esters was the commonest indication of fibroblast involvement in lipid keratopathy and the cornea is probably capable of the compensatory processes proposed for other biological systems in avascular, or relatively avascular, situations.

Once the lipid-filled cells degenerate, then the potential for direct deposition of insoluble cholesterol and its esters is high. There is a vicious circle of cell degeneration and phagocytosis with a connective tissue response which seems to vary according to the type of lipid present. In the dog, dense collections of

crystalline cholesterol and crystalline or liquid cholesterol ester were present in early Phase II lesions in which there was little histochemical or ultrastructural evidence of phospholipid. With the ability to detect spingomyelin-rich phospholipid in corneal cells there was considerable increase in the number of anisotropic droplets, and it is suggested that these lamellated 'liposomes' represent a means whereby cholesterol ester and cholesterol can be dispersed within the hydrophobic parts of the bimolecular leaflets of phospholipid. The detergent effect of the phospholipid means that such droplets are not deposited like crystalline substances but have a certain mobility, indeed they tend to accumulate beneath the basal lamina in a variety of stromal lipid disorders. The importance of lipid-lipid interactions for the physical state of lipid deposits in lipid keratopathy is discussed in Section 6.7.

6.6 The Macrophage and Giant Cell

Efficient phagocytosis, characteristic of the regressive, Phase III, lesion was associated with extensive capillarisation and the presence of macrophages of apparently haematogenous origin. However, the phagocytic ability of corneal fibroblasts has also been mentioned and the mesenchymal origin of both kinds of cell meant that the distinction of fibroblasts and macrophages was not always easy.

As a class macrophages are poorly endowed with granular endoplasmic reticulum and profiles of endoplasmic reticulum are poorly populated by ribosomes. This is due to relatively short polyribosome chains (4 or 5 ribosomes) as compared with much larger chains (up to 30 ribosomes) in fibroblasts and is a useful point of distinction (Leibovich α Ross, 1975). Carr's (1970) description of

the macrophage possessing a "prominent granular endoplasmic reticulum" is misleading and not corroborated by the illustrations which accompany his monograph.

Stimulated macrophages actively engaged in phagocytosis certainly contain greater than normal quantities of granular endoplasmic reticulum, presumably necessary for the synthesis of hydrolytic enzymes and primary lysosomes, utilising the well-known pathway through the granular endoplasmic reticulum and Golgi complex. Such cells also possess well-developed cell processes with which to enwrap material for ingestion (Ghadially, 1982).

A large variety of enzymes have been demonstrated within macrophages; there are some species differences and considerable variation in the proportion of individual enzymes at differing sites under various conditions. These differences reflect considerable quantitative heterogeneity among macrophages which may indicate adaptation to local environmental conditions rather than the existence of biochemically different populations of cells (Vernon-Roberts, 1972).

Macrophages with a highly developed capacity for endocytosis usually contain abundant lysosomes richly endowed with acid hydrolases, of which acid phosphatase has been the commonest marker of lysosomal activity. Other enzymes of relevance to fat digestion are present such as esterases and lipases, better termed fatty acid hydrolases in view of their lack of specificity for particular fatty acid chain lengths (Lake, 1972).

The lysosomes of macrophages may also contain non-enzymatic material. Novikoff (1961) demonstrated a positive staining reaction for phospholipid with acid haematein and, occasionally, PAS positive

diastase-resistant material can be demonstrated (Metcalf, 1966).

The formation of macrophage lysosomes is largely determined by the environmental factors to which the cell is exposed, a subject which has been reviewed by Cohn (1968). The primary lysosome of the macrophage is a tiny smooth-surfaced Golgi vesicle, which is a package of enzyme not yet in contact with substrate. The secondary lysosome contains both substrate and enzyme and is composed of constituents of the extracellular environment in addition to the endogenously synthesised hydrolytic enzymes. Lysosomes can vary in size and shape. Secondary lysosomes may be large and their fusion may induce the formation of complex residual bodies. Granules of lipofuscin and the closely related, or identical, pigments ceroid and haemofuscin for example, represent residual bodies left behind in the cell after lysosomal activity. Lipofuscin represents undigested residues derived from lysosomal hydrolysis and modification of sequestrated material prior to and following digestion.

The ability of lysosomal hydrolases to degrade lipids varies between cell types. Gaton and Wolman (1977) demonstrated that haematogenous macrophages in the experimentally induced atheroma of rabbits were rich in acid esterase activity and the macrophages contained small quantities of finely-emulsified lipid, whereas smooth muscle cells (the other major cell type involved) were poor in acid esterase activity and contained bulky, poorly emulsified lipid. Deficient lysosomal lipolytic activity leading to the overloading of smooth muscle lysosomes with formation of lipid-laden foam cells was described previously by Peters, Takano and de Duve (1973) and these workers had compared lipid accumulation in the atheromatous blood vessel wall with genetic storage diseases in

which there is deficiency, or absence, of a lysosomal hydrolase.

In addition to lysosomal (acid) esterases, which are hydrolytic, the macrophage may also possess microsomal (alkaline) esterases which are synthetic, so that both synthesis and hydrolysis of cholesterol ester are possible (Day, 1960). Using alpha naphthyl acetate esterase at pH 5.8 and 7.5, with and without the inhibitor E600, Bayliss (1975) showed that the E600-resistant esterase activity of rabbit macrophages and giant cells resided in the lysosomes, whereas E600-sensitive esterases were distributed diffusely within the cytoplasm. Similar results were obtained in this study within the macrophages and giant cells of resolving lesions.

The role of the macrophage in various aspects of lipid metabolism has been expertly reviewed by Day (1964). The findings of Day and his colleagues, together with other available evidence, serve to demonstrate the impressive range of abilities which these cells possess. Lipid particles of all sizes, including chylomicrons, can be taken up by macrophages (Casley-Smith & Day, 1966). Free diffusion, micropinocytosis and phagocytosis would appear to be the mechanisms employed. As in fibroblasts there is receptor-mediated endocytosis of lipoprotein-associated cholesterol, although human tissue macrophages lack the negative-feedback control present in human fibroblasts (Norum et al., 1983).

Lipoprotein may enter the macrophage by endocytosis in pinocytotic vesicles, both intact, and as lipid dissociated from its apoprotein vehicle. Macrophages may also have the ability to form soluble lipoprotein complexes following esterification of cholesterol as suggested by Tompkins (1946). Day & Gould-Hurst

(1961) demonstrated that ^{14}C-labelled cholesterol suspensions taken up by macrophages were returned to the media in a soluble form which might be a lipoprotein and Bierman, Stein & Stein (1974) produced some evidence for regurgitation of partially changed lipoproteins.

Free fatty acids probably enter the cell by a process of non-enzymatic diffusion. ^{14}C labelling has been used to study the *in vitro* uptake of acetate precursors and fatty acids into macrophages and their subsequent incorporation into phospholipid, triglyceride and, to a lesser extent, into cholesterol ester and mono- and di-glyceride (Day, 1964).

Triglycerides appear to be readily phagocytosed and, like free fatty acids, oxidised, according to *in vitro* studies (French & Morris, 1960).

Cholesterol esters were shown to be taken up directly by macrophages *in vivo* (Adams, Abdulla & Bayliss, 1971) possibly by pinocytosis rather than trans-membrane entry into the cell. By this route direct uptake into the macrophage occurred without obligatory hydrolysis to free sterol and re-esterification within the cell. Earlier work by Rothblat, Hartzell, Miahle & Kritchevsky (1967), using cells in tissue culture, had suggested that cells could only absorb and secrete cholesterol in its free form.

The free cholesterol which results from hydrolysis of cholesterol ester may be excreted into the medium, probably in soluble form. *In vitro* studies by Werb & Cohn (1971) showed that cholesterol linoleate was hydrolysed much more readily than cholesterol palmitate. Day & French (1961) administered cholesterol and cholesterol esters intraperitoneally to rats and demonstrated

that the suspensions were readily taken up by the reticulo-endothelial cells of the sternal lymph nodes. Sudanophilia at the storage site diminished some two months after cholesterol injection but persisted beyond 18 months after injection of cholesterol oleate. Interestingly, triglyceride emulsions administered under the same circumstances had disappeared within a few days (French & Morris, 1960).

Cholesterol in colloidal form is readily engulfed by macrophages, whereas in crystalline form a giant cell reaction may ensue (Bayliss, 1976). The ability of macrophages to esterify cholesterol has been demonstrated by chemical means and using ^{14}C-labelled cholesterol. Whilst cholesterol uptake is accompanied by an increase in phospholipid synthesis, phospholipid apparently decreases the esterification of free cholesterol, although it also promotes the hydrolysis of lipoprotein cholesterol (Day, 1964).

Werb & Cohn (1971) studied cholesterol metabolism of the macrophage in vitro. They demonstrated that there was an exchange of free cholesterol molecules of the macrophage membranes with those of serum lipoproteins, that exchangeable cholesterol occurred in a rapidly exchanging plasma membrane compartment and a slowly exchanging intracellular compartment, and that an intralysosomal cholesterol compartment was formed following phagocytosis of cholesterol-containing particles. Cholesterol was excreted from the macrophage at a single exponential rate which depended on the concentration of acceptor lipoproteins in the medium. Bates & Rothblat (1974) found that HDL was more efficient than LDL in promoting the efflux of sterols from cells in culture.

Giant cell formation is apparently a consequence of circumfusion by macrophages in order to incorporate cholesterol crystals too large for ingestion by individual macrophages (Spector α Lykke, 1966; Mariano α Spector, 1974; Bayliss, 1976). The mutlinucleate cells which form in this way esterify the crystalline cholesterol before dissociating into individual lipid-filled macrophages. Giant cell formation was characteristic of regressing lipid keratopathy lesions in which cholesterol crystals were present.

Phospholipids such as lecithin, sphingomyelin and cerebrosides are readily taken up by macrophages (Day, 1964). The synthesis of phospholipid by macrophages has been suggested as a way by which it accumulates in the arterial wall in atherosclerosis and differences between plaque and plasma phospholipid suggest that the endogenesis synthesis of sphingomyelin-rich phospholipid is a major source of phospholipid in atheromatous arteries (Adams, 1963).

Dunnigan (1964) demonstrated phospholipid within the macrophages of human atheromatous plaques with Sudan IV, Nile blue sulphate, acid haematein and OTAN. He noted that whereas some macrophages contained only one type of lipid, others had both hydrophobic lipid and phospholipid in the same cell. Around the atherosclerotic plaque there was also some variation in cell morphology, ranging from rather elongated cells of possible smooth muscle origin to classical foam cells.

Endogenous synthesis of phospholipid by the macrophage has been suggested as a local defence mechanism against sterol deposition in atheroma. The ability of corneal cells to engage in phospholipid synthesis in canine lipid keratopathy has likewise been interpreted

as a compensatory device to mitigate the effects of sterol deposition. As both fibroblasts and macrophages are cells of mesenchymal origin their similar response is predictable. Whilst a proportion of corneal fibroblasts or fibroblast-like cells demonstrated phagocytic abilities in established lesions (Phase II and Phase III), they were apparently ineffective in lipid catabolism and this was the major point of distinction between such cells and haematogenous macrophages.

The lipoprotein receptors described for haematogenous macrophages, tissue macrophages, and fibroblasts differ. Tissue culture studies indicate that tissue macrophages have receptors for LDL which has been modified in same way, by acetylation for example, whereas there are very few receptors for unmodified, or native, LDL. Non-macrophage cells such as fibroblasts and smooth muscle cells have receptors for native LDL, but not for modified LDL. The receptor-mediated uptake of native LDL in non-macrophages and of modified LDL in tissue macrophages is strikingly different, in that the latter appear incapable of "downregulating" receptor activity for modified LDL uptake with increasing accumulation of esterified cholesterol within the cell. It has been suggested that tissue macrophages may be a source of foam cells because of this lack of negative feedback control (Norum et al., 1983). However, it should be noted that hypercholesterolaemia may overload transport systems in normal cells and the role of LDL and HDL_c is of relevance in terms of fibroblast-derived cholesterol deposition.

Macrophages derived from human monocytes have receptors for both native LDL and modified LDL (Schecter, Fogelman, Haberland, Seager, Hokom α Edwards, 1981) and, if this finding is applicable to

the dog, it may help to explain their obvious versatility as scavenger cells.

In summary; the progressive type of lipid keratopathy lesion in which lipid accumulated within fibroblasts and extra corneal cells to produce foam cells, with cell death in the absence of effective phagocytic cells, bore some resemblance to atheromatous lesions in which lipid accumulates in modified smooth muscle cells and "myogenic" foam cells. As Adams, Bayliss & Turner (1974) commented, "the atheromatous artery seems to be ineffectively populated by macrophages or possibly partly populated by ineffective macrophages".

In lipid keratopathy, regression of the lesion and effective phagocytosis was associated with cells rich in phospholipid containing finely emulsified, less saturated, esterified cholesterol, than that detected in fibroblasts and fibroblast-like cells. These cells were usually rich in both microsomal and lysosomal esterases and their properties were consistent with those described by others for the haematogenous macrophage. Of these properties, one of the most interesting is the apparent ability of the macrophage to donate polyunsaturated fatty acid from the phospholipid it synthesises, so that polyunsaturated cholesterol ester is produced from cholesterol (Adams & Abdulla, 1972). The regressing lipid keratopathy lesion rich in macrophages was the only lesion in which unsaturated lipids could be detected with osmium tetroxide, and parallel sections demonstrated that the lipids were located within phospholipid-rich macrophages. This finding is relevant to the known differences between polyunsaturated esters and monounsaturated or unsaturated esters in body tissues.

6.7 The Responses of Connective Tissue to Lipid Deposition

This section is concerned with the corneal response to lipid deposition, rather than the factors which initate such deposition. The results of the present study are compared with those of other workers and the emphasis is on the physico-chemical state of the lipids involved.

As early as 1929 Schoenheimer & Yuasa implanted cholesterol crystals subcutaneously in the dog and evoked a giant cell reaction. They also demonstrated by chemical means that the increased Sudanophilia and spherocrystals of macrophages which had taken up cholesterol were a consequence of its partial esterification.

Abdulla, Adams & Morgan (1967) were the first to investigate connective tissue reactions to a range of chemically pure lipids. They implanted sterol, sterol esters, phospholipids, glycerides and free fatty acids subcutaneously in rats and were able to show that, with certain exceptions, the rate of resorption of the lipid implants was approximately proportional to their sclerogenic activity. The results indicated that phospholipids provoked little or no sclerosis in connective tissues and that glycerides were only feebly sclerogenic. Polyunsaturated free fatty acids provoked a fairly intense acute inflammatory and fibrotic reaction. Free cholesterol was intensely sclerogenic. Cholesterol esters of saturated, monosaturated and trans-polyunsaturated fatty acids were slowly resorbed and strongly sclerogenic. Some of the monounsaturated cholesterol esters, such as cholesterol oleate, were actually more damaging to the tissues than cholesterol. Cis-polyunsaturated cholesterol esters, the commonest polyunsaturated esters of animal tissues, were mobilised rapidly and were relatively

non-sclerogenic. It was inferred from these results that the synthesis of cis-polyunsaturated sterol esters is an important method of mobilising cholesterol from tissues and that phospholipid may play a role in this esterification system by donating polyunsaturated fatty acid to cholesterol.

Waters (1959 and 1965) used the cornea as a "model" of the arterial intima and studied the stromal reactions in the dog and rabbit cornea following direct injection of lipoprotein-containing serum and other lipids such as chylomicrons. Direct injection of homologous hyperlipoproteinaemic serum into the central corneal stroma resulted in the formation of lipid-containing fibrocytic, foam-cellular, reactions, thought by the author to reproduce many of the morphological features of small atherosclerotic plaques in man. Prior delipidisation of the serum virtually eliminated the corneal tissue reaction. It was concluded that there was direct evidence for the dependence of such lesions on the lipoprotein lipid contained in the injected serum and, in rabbits, the severity of corneal reaction increased as the level of total cholesterol in the injected serum was raised. It would seem logical to extend investigations of this type to the injection of chemically pure lipids and specific lipoproteins; but it should be remembered that the injection itself induces some stromal disruption and that this route of lipid entry would not be utilised in naturally occurring disease.

From the results obtained in the present study of naturally occurring lipid keratopathy, it was apparent that the corneal response did vary, at least in part, according to the predominant type of lipid. Crystalline lipids produced the most marked stromal

reaction, particularly lesions rich in high melting point, esterified cholesterol, followed by those in which free cholesterol predominated. Non-crystalline esterified cholesterol did not produce quite such a damaging reaction. Large quantities of phospholipid were associated with a diminution in the amount of crystalline lipid present and hence a reduction in stromal reaction. The quantities of free fatty acid and triglyceride detected were never sufficient to allow their effects to be gauged, although their very scarcity would suggest that they were not of any great individual significance.

It is much more difficult to assess the possible interactions of the various lipids and lipoproteins. They may interact with other lipids, plasma constituents, cellular enzymes, components of the blood vessel wall and corneal matrix, in their short journey from the blood vessel lumen to the corneal stroma. The probability that insoluble and crystalline components accumulate, whereas soluble and liquid components are more likely to escape, further complicates the issue. In this context it should also be remembered that the presence of predominantly crystalline, high melting point, lipids, does not necessarily indicate their exclusive production, but rather, the more rapid removal of non-crystalline, lower melting point, lipids.

Against this rather complicated background, the predominance of esterified and free cholesterol together with the apparently beneficial effect of phospholipid is not a situation unique to lipid keratopathy; indeed the fact that similar lipids accumulate in other biological systems strengthens the arguments propounded in this thesis.

Small and Shipley (1974) felt that interactions other than lipid-lipid interactions might be of qualitative, rather than quantitative importance, in determining the physical state of lipid in atherosclerotic lesions. They therefore constructed phase diagrams in which the components phospholipid, cholesterol, cholesterol ester and water, were at a fixed temperature of 37°C and a fixed pressure of one atmosphere, in order to study lipid-lipid interactions. As a result of these experimental models they were able to predict the physical state of lipids within the arterial intima. For example, at a water concentration of 70% in a lecithin-cholesterol ester-cholesterol-water system, phase equilibrium studies indicated that four major zones existed. Zone I consisted of a membrane or lamellar liquid-crystalline phase formed by phospholipids, with the ability to incorporate up to one molecule of cholesterol per molecule of phospholipid, plus a very small amount of cholesterol ester. The amount of cholesterol esters incorporated did not exceed more than two to three percent by weight of the total lipid. Zone II consisted of an oily cholesterol ester phase containing up to 8% by weight of free cholesterol, but no phospholipid. In Zone III two phases co-existed; the oily cholesterol ester phase and the lamellar liquid-crystalline phase. Zone IV contained three phases of invariant composition; the oily cholesterol ester phase saturated with free cholesterol, the lamellar liquid-crystalline phase saturated with both cholesterol and cholesterol ester, and crystals of cholesterol monohydrate. Small and Shipley went on to show that the three physical states (lamellar phase, oily phase and crystalline cholesterol monohydrate) of lipids, predicted from the quaternary phase diagram of a model

system of the component lipids, have been identified in aortic intimal tissue, which contains approximately 70% of water. The results of the present study suggest that similar physical states exist for the lipids prominent in lipid keratopathy. The cornea probably contains between 72 and 82% water and the sclera rather less (Hogan, Alvarado & Weddell, 1971) values which are reasonably close to the water content of arterial intima.

The cornea differs from intima in having a temperature below that of the rest of the body. Corneal temperature is primarily influenced by environmental sources (external environment, tears, aqueous humour and lids) and the result of a decrease in environmental temperature is to produce a linear drop in corneal temperature. A decrease of temperature will influence the solubility characteristics of the various lipid phases. For example, cholesterol becomes less soluble in the oily cholesterol ester phase as the temperature is lowered (Small, 1970), and to judge from the melting point of lipids such as cholesterol esters in a proportion of the squash and imprint preparations, the temperature may have had some influence on the physical state of these lipids in vivo.

It is known that the functional properties of biological membranes are influenced by membrane fluidity, and that the fluidity of a bilayer at a given temperature is determined by its composition. An artificial bilayer of a single type of phospholipid has a sharp and characteristic phase-transition, or melting point, at which it changes from a rigid gel phase to a liquid crystalline state (Chapman, 1973). Above the transition temperature there is a greater mobility of the fatty acids in the phospholipids and, as

each phospholipid molecule with its fatty acids covers a larger surface, the membrane permeability is increased. The phase-transition temperature increases and the permeability of the membrane decreases with chain length and saturation.

Cholesterol is an important component of lipoproteins and cellular membranes; when introduced into a bilayer it condenses the packing of phospholipid molecules and reduces membrane permeability; the phase-transition temperature is also raised.

Membrane bilayers are normally in a liquid crystalline state as their phase-transition temperature is below mammalian body temperature. The temperature gradient which has been demonstrated for the canine cornea may be an important influence on the activities of temperature-sensitive enzymes, lipid deposits and cellular membrane bilayers. The latter are discussed in more detail below.

6.8 Phospholipids and Membranous Lamellae

Phospholipids appear to play a crucial role in the regression of lipid keratopathy. Squash and imprint preparations of the cornea demonstrated, in conjunction with other microscopic examination, that phospholipids were surface acting dispersing agents. The droplets so formed were mainly anisotropic and did not coalesce when they came into contact, suggesting that their surfaces were stabilised and they could be considered as emulsion droplets. Extracellular droplets of this type were mainly derived from fibroblasts and such droplets tended to accumulate beneath the basal lamina of the corneal epithelium, which acted as an effective barrier to further progress when it was intact. Whether other substances, such as those of the corneal intercellular matrix,

interacted with droplets of this type, or with any of the lipids which were present in the cornea, was an aspect of lipid keratopathy which received little investigation in the present study. It could be, that possible interactions between lipoproteins, lipids and extracellular elements in normal cornea are amplified in diseased tissue, because of modifications to such intercellular matrix components as collagen, glycosaminoglycans and proteoglycans. In addition, unusual components, such as fibronectin and oxytalan, might further exacerbate the problem.

The fibroblast in lipid keratopathy actually demonstrated a variety of responses which were interpreted as compensatory devices to keep cholesterol, and possibly esterified cholesterol, in the membrane, or lamellar phase. The commonest histological manifestation was the production of large quantities of membrane-like lamellae and intracellular cholesterol ester droplets.

In fibroblast-like cells and in well developed phagocytic cells such as haematogenous macrophages and giant cells, phospholipid-rich membranous material was associated with lysosomes. In lysosomes it appeared to be a method of incorporating cholesterol and cholesterol ester so as to produce anisotropic, finely emulsified, cholesterol ester-rich inclusions. Weller (1966) suggested that the molecular arrangement of such lamellated structures might facilitate enzyme activity and the possibility that the phospholipid therein might donate polyunsaturated fatty acid to cholesterol for the formation of polyunsaturated cholesterol esters has been previously discussed. Hata et al. (1974) regarded the cholesterol ester molecule as the primary unit of structure in these inclusions and their estimations of the thickness of a lamella one ester molecule thick produce a

maximum of about 40 Å with the molecules fully extended, and a minimum of about 24 Å with the molecules fully folded. These figures are remarkably close to the 30 Å thick electron-dense lamellae observed in the present study.

From what is known of membranous lamellae it seems likely that the dark bands represent an aqueous phase with ionisable groups of phospholipid embedded in it. If protein is present then it would also be part of the aqueous phase. Cholesterol and cholesterol esters would be dispersed among the long hydrocarbon chains of the phospholipids in the unstained, light, bands of the lamellated structures (Engström & Finean, 1958).

In summary, lamellated structures are ubiquitous in biological systems, as the diversity of possible names would suggest (membranous lamellae, myelin figures, myelinoid figures, micelles and liposomes for example). In lipid keratopathy, membranous lamellae provided a means for dispersing dead cells such as fibroblasts and also hydrophobic sterol compounds. Additionally, membranous lamellae were intimately involved in the regression of lipid keratopathy lesions and there was some evidence to suggest that liposomes formed in haematogenous macrophages were returned, via the corneal blood vessels, to the general circulation - an intriguing possibility in view of the putative similarity of liposomes to lipoproteins.

6.9 Conclusions

In relation to lipid keratopathy the results of this study indicate that the normal canine cornea does not accumulate macroscopically visible quantities of lipid as a regular feature of ageing and that lipid detected histochemically in the stroma is more

likely to correlate with the pattern of serum lipids and lipoproteins. In the age range encountered in the clinical cases there were no regular alterations of the serum lipids and lipoproteins according to age, sex or breed. However, there was some unexplained individual variation which should be examined in more detail as it may accord with the propensity of certain breeds to develop lipid keratopathy. The effect of diet on serum lipids and lipoproteins was not critically evaluated in this thesis, but the information obtained from preliminary investigations has been of sufficient interest to encourage further study.

There was usually a history of anterior segment disease in dogs which developed lipid keratopathy. In addition to direct corneal insults like ulceration, there was a high incidence of perilimbal inflammations such as episcleritis, scleritis and uveitis and the type of ocular disease, particularly in relation to corneal involvement, influenced the development and appearance of the lipid keratopathy. In a high proportion of cases there were also demonstrable abnormalities of serum lipids and lipoproteins, some of which were secondary to disease, whereas others may have been indicative of long term overindulgence, individual variation, or breed variation. Serum enzymes should also be examined in such animals. In some dogs no abnormalities of serum lipids or lipoproteins were demonstrated, despite examining a series of sequential samples; such cases might be investigated further on fasting and non-fasting samples over longer periods.

The possibility that some cases did have primary abnormalities of fibroblast metabolism must also be considered, and could be investigated further in tissue culture, particularly with regard to

fibroblast enzymes. In all the animals which form the subject of this study there were some areas of cornea which were of normal appearance, suggesting that corneal factors per se would be insufficient to account for the condition.

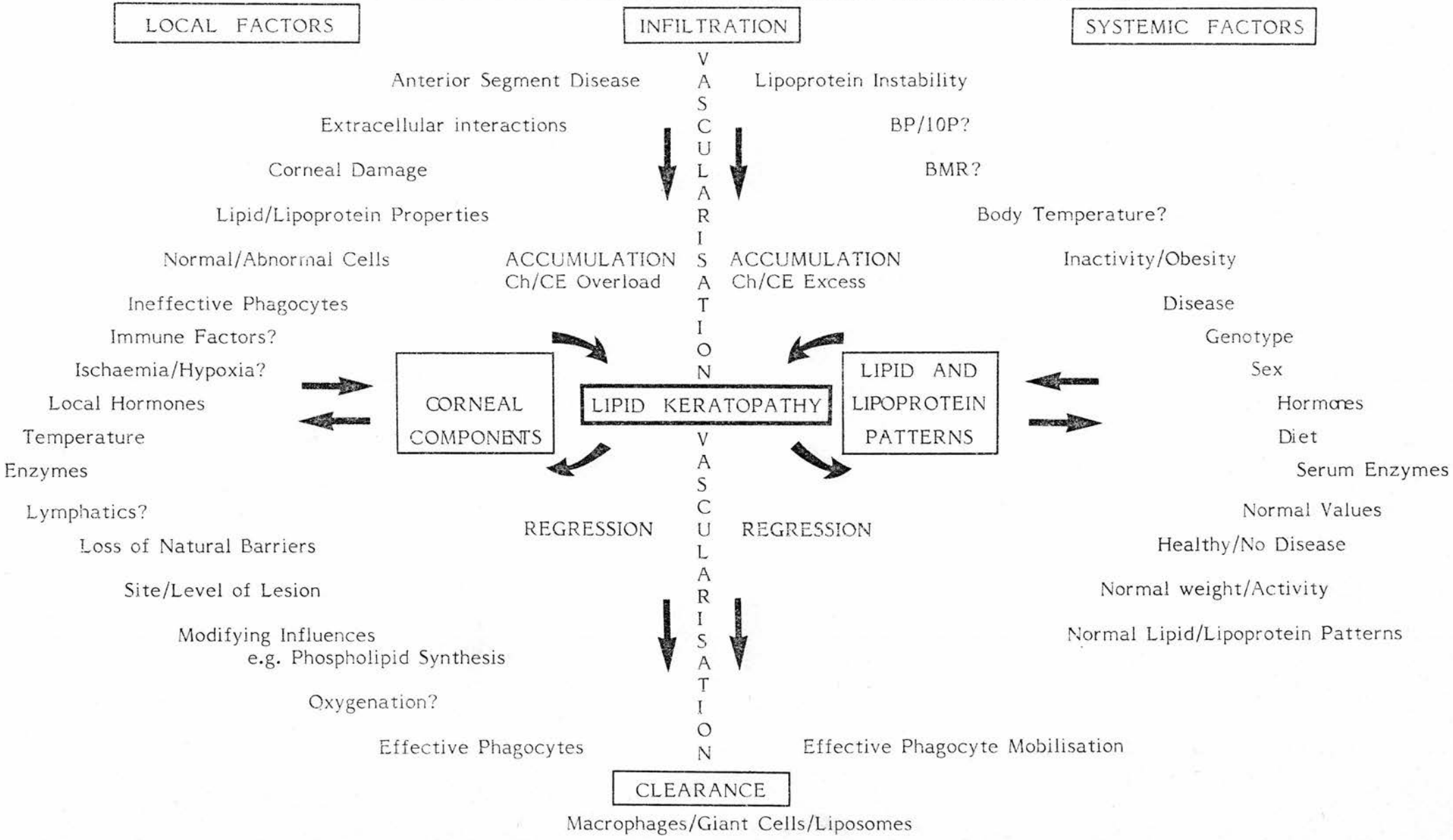
Phagocytosis of corneal lipid deposits appeared to be associated with corneal neovascularisation. Normolipoproteinaemia was also necessary if phagocytosis was to effect lipid clearance. In addition to providing direct access for haematogenous macrophages corneal neovascularisation would produce local changes in the corneal environment, such as an increase of temperature and tissue oxygen tension. The temperature may be of importance in determining cellular enzyme activity and whether lipids are present in liquid or solid state. Tissue oxygen tension may also influence enzyme activity and it is known that hypoxia can depress lipid phagocytosis and transport (Robertson, 1968). Dixon (1982) has reviewed the effects of intimal hypoxia in promoting atherosclerosis, and, in the cornea, Baum and Silbert (1978) observed that vascularisation assisted wound healing and that central lesions healed more slowly than peripheral ones.

The influence of various hormones on lipid keratopathy and other diseases of canine lipid metabolism appears a likely field for future investigation. In the present study hormones could be implicated in quite a high proportion of the clinical cases. Whilst the widespread effects of a chronic deficiency of thyroid hormone can be readily detected and corrected, it is not so easy to assess the possible effects of fluctuating levels of such hormones as oestrogen, particularly as cases are frequently presented some time after the initiating event. There are also numerous, poorly

understood actions of hormones, including "local" hormones such as prostaglandins or even topical corticosteroids, at cellular level, which may be relevant to the pathogenesis of lipid keratopathy.

There is little doubt that lipid keratopathy is the visible expression of a most complex disorder, and a speculative diagram of some possible inter-relationships is presented in Fig. 6.9/1.

Figure 6.9/1 Some Local and Systemic Factors which may be Inter-related in Canine Lipid Keratopathy



Abdulla, Y. H., Adams, C. W. M., α Morgan, R. S. (1967) Connective tissue reactions to implantation of purified sterol, sterol esters, phosphoglycerides, glycerides and free fatty acids. J. Path. Bact., 94, 63-71.

Abdulla, Y. H. α Adams, C. W. M. (1978) The action of human high density lipoprotein on cholesterol crystals. Part 2. Biochemical observations. Atherosclerosis. 31, 473-480.

Abe, M., Kramer, S. P. α Seligman, A. M. (1964) The histochemical demonstration of pancreatic-like lipase and comparison with distribution of esterase. J. Histochem. Cytochem., 12, 364-383.

Adams, C. W. M. α Sloper, J. C. (1955) Technique for demonstrating neurosecretory material in the human hypothalamus. Lancet, 1, 651-652.

Adams, C. W. M. (1957) A p-dimethylaminobenzaldehyde-nitrite method for the histochemical demonstration of tryptophan and related compounds. J. Clin. Path., 10, 56-62.

Adams, C. W. M. (1959) A histochemical method for the simultaneous demonstration of normal and degenerating myelin. J. Path. Bact., 77, 648-650.

Adams, C. W. M. (1960) A histochemical method for tryptophan applicable to formalin-fixed tissues. J. Path. Bact., 80, 442-445.

Adams, C. W. M. (1961) The perchloric acid-naphthoquinone method for the histochemical localisation of cholesterol. Nature, 192, 331-332.

Adams, C. W. M. (1963) Multiple factors in the pathogenesis of atherosclerosis. Guy's Hospital Reports, 112, 222-253.

Adams, C. W. M. α Bayliss, O. B. (1963) Histochemical observations on the localisation and origin of sphingomyelin, cerebroside and cholesterol in normal and atherosclerotic human artery. J. Path. Bact., 85, 113-119.

Adams, C. W. M. (1965) Lipid histochemistry. In Neurohistochemistry, Ed. C. W. M. Adams, Elsevier, Amsterdam, pp 6-66.

Adams, C. W. M., Abdulla, Y. H., Bayliss, O. B. α Weller, R. O. (1966) Histochemical detection of triglyceride esters with specific lipases and calcium lead sulphide technique. J. Histochem. Cytochem., 14, 385-395.

Adams, C. W. M., Abdulla, Y. H. α Bayliss, O. B. (1967) Osmium tetroxide as a histochemical and histological reagent. Histochemie, 9, 68-77.

Adams, C. W. M., Abdulla, Y. H., Bayliss, O. B. α Morgan, R. S. (1967) Modification of aortic atheroma and fatty liver in cholesterol-fed rabbits by intravenous injection of saturated and polyunsaturated lecithins. J. Path. Bact., 94, 77-87.

Adams, C. W. M. α Bayliss, O. B. (1968) Reappraisal of Osmium tetroxide and OTAN histochemical reactions. Histochemie, 16, 162-166.

Adams, C. W. M. (1969a) Lipid histochemistry, Adv. Lipid Res., 7, 1-62.

Adams, C. W. M. (1969b) Enzyme histochemistry: applications and pitfalls. In Handbook of Neurochemistry. ed. C. W. M. Adams, Elsevier, Amsterdam, pp 525-538.

Adams, C. W. M., Abdulla, Y. H. α Bayliss, O. B. (1971) Entry of esterified cholesterol into foam cells. Atherosclerosis, 13, 111-119.

Adams, C. W. M. (1972) Lipoproteins and the reversibility of atherosclerosis. Lancet, 1, 635-636.

Adams, C. W. M. α Abdulla, Y. H. (1972) Polyunsaturated phospholipid and experimental atherosclerosis. In Phospholipide in Biochemie, Experiment und Klinik, Georg Thieme Verlag, Stuttgart, pp 44-49.

Adams, C. W. M. (1973) Tissue changes and lipid entry in developing atheroma. In Atherogenesis: Initiating factors, Ciba Foundation Symposium 12, eds. R. Porter α J. Knight. Elsevier, Amsterdam, 5-37.

Adams, C. W. M. α Bayliss, O. B. (1973) Acid mucosubstances underlying lipid deposits in ageing tendons and atherosclerotic arteries. Atherosclerosis, 18, 191-195.

Adams, C. W. M., Morgan, R. S. α Bayliss, O. B. (1973) No regression of atheroma over one year in rabbits previously fed a cholesterol-enriched diet. Atherosclerosis, 18, 429-444.

Adams, C. W. M. α Bayliss, O. B. (1974) Lipid histochemistry. In Techniques of Biochemical and Biophysical Morphology, eds. D. Glick α R. M. Rosenbaum. Volume 2, Wiley-Interscience, New York, pp 99-156.

Adams, C. W. M., Bayliss, O. B. α Turner, D. R. (1974) Phagocytes, lipid removal and regression of atheroma. J. Path., 116, 225-238.

Adams, C. W. M. α Bayliss, O. B. (1975) Crystals in atherosclerotic lesions: real or artifactπ Atherosclerosis, 22, 629-636.

Adams, C. W. M., Knox, J. α Morgan, R. S. (1975a) The resorption rate of atheroma lipids in situ and implanted subcutaneously. Atherosclerosis, 22, 79-90.

Adams, C. W. M., Knox, J. α Morgan, R. S. (1975b) Exchange of plasma radioactive cholesterol with atheroma lipids in situ and implanted subcutaneously. Atherosclerosis, 22, 229-240.

Adams, C. W. M. α Morgan, R. S. (1977) Regression of atheroma in the rabbit. Atherosclerosis, 28, 399-404.

Adams, C. W. M. (1978) Some cellular and metabolic aspects of regression of atherosclerosis, and the compensatory effect of senile arterial dilatation. Rheinisch-Westfalische Akademie der Wissenschaften. International Symposium on State of Prevention and Therapy in Human Arteriosclerosis and in Animal Models, Westdeutscher Verlag, Germany, pp 119-126.

Adams, C. W. M. α Abdulla, Y. H. (1978) The action of human high density lipoprotein on cholesterol crystals. Part I. Light-microscopic observations. Atherosclerosis, 31, 465-471.

Adams, C. W. M. α Bayliss-High, O. B. (1980) Preliminary oxidation in histochemical staining methods for cholesterol. J. Microscopy. 119, 427-430.

Adams, C. W. M., Miller, N. E., Morgan, R. S. α Rao, S. N. (1982) Lipoprotein levels and tissue lipids in fatty-fibrous atherosclerosis induced in rabbits by two years' cholesterol feeding at a low level. Atherosclerosis, 44, 1-8.

Adler, F. H. (1965) Physiology of the eye, 4th Edition. CV Mosby Co., St. Louis.

Anderson, A. C. (1970) The eye-gross and subgross anatomy. In The Beagle as an Experimental Dog, ed. A. C. Anderson. Iowa State University Press, Iowa, pp 374-385.

Andrews, J. S. (1966) Corneal lipids I. Sterol and fatty acid synthesis in the intact calf cornea. Invest. Ophthalmol., 5, 367-370.

Anitschkow, N. (1913) Über die Veränderungen der Kaninchenaorta bei experimenteller Cholesterinsteatose. Beitr. path. Anat., 56, 379-404.

Anitschkow, N. α Chalatow, S. (1913) Ueber experimentelle cholesterinsteatose und ihre Bedeutung für die Enstehung einiger pathologischen Prozesse. Zbl. allg. Path. path. Anat., 24, 1-9.

Anitschkow, N. (1933) In Arteriosclerosis, ed E. V. Cowdry. Macmillan, New York, pp 271-322.

Arbeiter, K. von α Lorin, D. (1981) Organ- und Blutbefunde bei hormonal bedingten Gynakopathien der Hündin. Wein. tierarztl. Mschr., 68, 191-196.

Archibald, R. W. R. α Orton, C. C. (1970) Specific identification of free and esterified fatty acids in tissue sections. Histochem. J., 2, 411-417.

Arslan, S. H. (1980) Diagnosis of canine hypothyroidism: some clinical and laboratory methods. PhD. Thesis, University of Edinburgh.

Ashton, N. (1959) Histochemical studies on the normal and swollen cornea. Am. J. Ophthalmol., 47, 229-232.

Ausprunk, D. H., Boudreau, C. L. α Nelson, D. A. (1981) Proteoglycans in the microvasculature I. Histochemical localisation in microvessels of the rabbit eye. Am. J. Path., 103, 353-366.

Axenfeld, T. (1930) Dystrophien des Kornea-parenchyms. Klin Monatsbl. Augenh., 85, 493-507.

Babel, J. (1950) Les surcharges graisseuses de la cornee. Arch. d'opht., 10, 5-21.

Bachstez, E. (1921) Über Verfettung in der Hornhaut. Arch. f. Ophth., 105, 997-1036.

Baker, J. R. (1945) The structure and chemical composition of the Golgi element. Quart. J. micr. Sci., 85, 1-71.

Baker, J. R. (1946a) The histochemical recognition of lipine. Quart. J. micr. Sci., 87, 441-470.

Baker, J. R. (1946b) Acid haematein method for choline-containing phospholipids. Quart. J. micr. Sci., 87, 441-445.

Baker, J. R. (1947) The histochemical recognition of certain guanidine derivatives. Quart. J. micr. Sci., 88, 115-121.

Baker, J. R. (1956) The histochemical recognition of phenols, especially tyrosine, Quart. J. micr. Sci., 97, 161-164.

Baker, J. R. (1958) Fixation in cytochemistry and electron microscopy. J. Histochem. Cytochem., 6, 303-308.

Barka, T. (1960) A simple azo dye method for histochemical demonstration of acid phosphatase. Nature, 187, 248-249.

Barka, T. α Anderson, P. J. (1962) Histochemical methods for acid phosphatase using hexazonium pararosaniline as coupler. J. Histochem. Cytochem., 10, 743-753.

Bates, S. R. α Rothblat, G. H. (1974) Regulation of cellular sterol flux and synthesis by human serum lipoproteins. Biochim. Biophys. Acta., 360, 38-55.

Baum, J. L. (1969) Cholesterol keratopathy. Am. J. Ophthalmol., 67, 372-375.

Baum, J. L. α Silbert, A. M. (1978) Aspects of corneal wound healing in health and disease. Trans. ophthal. Soc. U.K., 98, 348-351.

Baumgarten, P. (1876) Ophthalmologisch - histologische Mitteilungen : I Ein Fall von sclerosirender Keratitis. Albrecht v. Graefes Arch. Ophthal., 22, 185-203.

Bayer, J. (1914) Augenheilkunde, 3rd Edition, Braumueller, Vienna.

Bayliss, High, O. B. (1972) Lipid histochemistry: Validity of techniques, M. Phil. Thesis, University of London.

Bayliss, O. B. α Adams, C. W. M. (1972) Bromine-Sudan Black: a general stain for lipids including free cholesterol. Histochem. J., 4, 505-515.

Bayliss, High, O. B. (1975) The cellular response to cholesterol crystals. Ph.D. Thesis, University of London.

Bayliss, O. B. (1976) The giant cell in cholesterol resorption. Br. J. exp. Path., 57, 610-618.

Bayliss, High, O. B. (1981) The histochemical versatility of Sudan Black B. Acta Histochemica. Suppl.-Band XXIV, S. 247-255.

Bayliss, High, O. B. (1982) Lipids. In Theory and Practice of Histological Techniques, eds. J. D. Bancroft α A. Stevens, 2nd Edition. Churchill Livingstone, Edinburgh, pp 217-241.

Bell, F. P., Adamson, I. L. α Schwartz, C. J. (1974) Aortic endothelial permeability to albumin: focal and regional patterns of uptake and transmural distribution of 131^{I} albumin in the young pig. Exp. Mol. Pathol., 20, 57-68.

Bellhorn, R. W. α Henkind, P. (1967) Ocular nodular fascitis in a dog. J. Am. Vet. Med. Ass., 150, 212-213.

Berman, E. R. (1982) Lysosomal storage diseases. In Pathobiology of Ocular Disease Part B, eds. A. Garner α G. K. Klintworth. Marcel Dekker, Switzerland, p 849.

Bersot, T. P., Mahley, R. W., Brown, M. S. α Goldstein, J. L. (1976) Interaction of swine lipoproteins with the low density lipoprotein receptor in human fibroblasts. J. Biol. Chem., 251, 2395-2398.

Bevans, M., Davidson, J. D. α Abell, L. L. (1951) Early lesions of canine arteriosclerosis. Arch. Path., 51, 278-287.

Bierman, E. L., Stein, O. α Stein, Y. (1974) Lipoprotein uptake and metabolism by rat aortic smooth muscle cells in tissue culture. Circ. Res., 35, 136-150.

Bonnet, P., Paufique, L. α Bonamour, G. (1934) Cristaux de cholesterine au centre de la cornée avec gerontoxon. Bull. Soc. d'opht de Paris, 46, 225-228.

Bortz, W. M. (1968) Reversibility of atherosclerosis in cholesterol-fed rabbits. Circ. Res., 22, 135-139.

Boyd, G. S. α Oliver, M. F. (1958) Physiology of circulating cholesterol lipoproteins. In Cholesterol, ed. R. P. Cook, Academic Press, New York. pp 181-208.

Brockerhoff, H. (1970) Substrate specificity of pancreatic lipase. Biochim. Biophys. Acta., 212, 92-101.

Broekhuyse, R. M. (1968) Phospholipids in tissues of the eye I. Isolation, characterisation and quantitative analysis by two dimensional thin-layer chromatography of diacyl and vinyl-ether phospholipids. Biochim. Biophys. Acta., 152, 307-315.

Broekhuyse, R. M. (1975) The lipid composition of ageing sclera and cornea. Ophthalmologica, 171, 82-85.

Broekhuyse, R. M. (1976) Lipids in tissues of the eye XIV. Corneoscleral lipids during ageing and in arcus senilis. Doc. Ophthalmol., 7, 313-321.

Broekhuyse, R. M. & Daemen, F. J. M. (1977) The Eye. In Lipid Metabolism in Mammals, Vol II, ed. F. Snyder pp 145-188.

Bron, A. J. & Easty, D. L. (1970) Fluorescein angiography of the globe and anterior segment. Trans. ophthal. Soc. U.K., 90, 339-367.

Bron, A. J. (1973) A simple scheme for documenting corneal disease. Brit. J. Ophthal., 57, 629-634.

Brown, E. V. L. & Katz, D. (1932) Dystrophia adiposa corneae. Tr. Am. Ophth. Soc., 30, 173-211.

Brown, M. S. & Goldstein, J. L. (1976) Receptor mediated control of cholesterol metabolism. Science, 191, 150-154.

Brunn-Jensen, J. (1969) Fluorescein angiography of the anterior segment. Am. J. Ophthalmol., 67, 842-845.

Burstone, M. S. (1958) Histochemical demonstration of acid phosphatase with naphthol AS-phosphates. J. Natl. Cancer Inst., 21, 523-539.

Burstone, M. S. (1962) Enzyme Histochemistry. Academic Press, New York.

Busacca, A. (1952) Biomikroskopie und Histopathologie des Auges. Volume 1. Schweizer Druck-und Verlagshaus, Zurich.

Cain, A. J. (1947) The use of Nile Blue in the examination of lipoids. Quart. J. micr. Sci., 88, 383-391.

Cain, A. J. (1948) A further note on Nile Blue. Quart. J. micr. Sci., 89, 429.

Camejo, G. (1982) The interaction of lipids and lipoproteins with the intercellular matrix of arterial tissue: Its possible role in atherogenesis. Adv. Lipid Res., 19, 1-53.

Carr, I. (1973) The Macrophage. Academic Press, London.

Carrington, S. D. (1983) Lipid keratopathy in a cat. J. sm. Anim. Pract., 24, 495-505.

Carson, F. L., Martin, J. H. & Lynn, J. A. (1973) Formalin fixation for electron microscopy: a re-evaluation. Am. J. Clin. Path., 59, 365-373.

Casley-Smith, J. R. & Day, A. J. (1966) The uptake of lipid and lipoprotein by macrophages in vitro: an electron microscope study. Quart. J. exp. Physiol., 51, 1-10.

Cavara, V. (1937) L'adiposi primaria della cornea (contributo alla studio della eosidetta degerazione grassa della cornea). Boll. d'ocul., 16, 1-38.

Chapman, D. (1957) Liquid crystalline nature of phospholipids. In Ordered Fluids and Liquid Crystals. Advances in Chemistry Series 63, ed. R. F. Gould, American Chemical Society, Washington, D.C., p 157.

Chapman, D. (1973) Some recent studies of lipids, lipid-cholesterol and membrane systems. In Biological Membranes, eds. D. Chapman & D. F. Wallach, Academic Press, New York, Volume 2, pp 91-144.

Choromokos, E. A. (1982) A simplified technique for anterior segment fluorescein angiography. In Ophthalmic Photography, ed. J. Justice Jr., Little, Brown & Co., Boston, pp 193-197.

Choudhury, S. R. (1972) The nature of non specific esterases: A subunit concept. J. Histochem. Cytochem., 20, 507-517.

Chuma, M. (1923) Ueber Organveranderungen nach Lanolinfutterung beim Kaninchen. Virchow's Arch. path. Anat., 242, 275-309.

Ciccarelli, E. C. α Kuwabara, T. (1959) Experimental aberrant lipogenesis VI. Biochemical characterisation of the sudanophilic material produced. Arch. Ophthal., 62, 125-129.

Clausen, W. (1923) See Verderame (1922).

Cogan, D. G. (1951) Recurrent bilateral scleritis of many years duration with unusual corneal infiltration. Arch. Ophthal., 46, 341-342.

Cogan, D. G. α Kuwabara, T. (1954) Lipogenesis by cells of the cornea. Science, 120, 321-322.

Cogan, D. G. α Kuwabara, T. (1955a) Fat deposition in the cornea and lipogenesis. Acta. XVII Conc. Ophthalmol., 523-535.

Cogan, D. G. α Kuwabara, T. (1955b) Lipogenesis by cells of the cornea. Arch. Path., 59, 453-456.

Cogan, D. G. α Kuwabara, T. (1955c) Fat formation and foam cells following injection of brain tissue into the cornea. J. Neuropath. exp. Neurol., 14, 95-107.

Cogan, D. G. α Kuwabara, T. (1957a) Experimental aberrant lipogenesis I. Serum factor, Arch., Path., 63, 381-386.

Cogan, D. G. α Kuwabara, T. (1957b) Experimental aberrant lipogenesis III. Tissue factor. Arch. Path., 64, 23-33.

Cogan, D. G. α Kuwabara, T. (1958a) Lipid keratopathy and atheroma. Circulation. 18, 519-525.

Cogan, D. G. α Kuwabara, T. (1958b) Lipid keratopathy and atheroma. Trans. Am. Ophth., Soc., 56, 109-122.

Cogan, D. G. α Kuwabara, T. (1959) Ocular changes in experimental hypercholesterolaemia in rabbits. Arch. Ophthal., 61, 219-225.

Cogan, D. G. (1960) Some aspects of fat in degeneration. In The Transparency of the Cornea, eds. S. Duke Elder & E. S. Perkins, Blackwell Scientific Publications, Oxford, pp. 149-163.

Cohn, Z. A. (1968) The structure and function of monocytes and macrophages. Adv. Immunol., 9, 163-214.

Constantinides, P. (1965) Experimental Atherosclerosis. Elsevier, Amsterdam.

Conway, J. & Loewenstein, A. (1943) A third entity of primary symmetrical fatty corneal dystrophy. Brit. J. Ophthal., 27, 49-54.

Courtice, F. C. & Garlick, D. G. (1962) The permeability of the capillary wall to the different plasma lipoproteins of the hypercholesterolaemic rabbit in relation to their size. Quart. J. exp. Physiol., 47, 221-227.

Cramp, D. G. (1972) Lipid methodology. J. Clin. Path., 26, Supplement, 5, 17-21.

Crispin, S. M. & Barnett, K. C. (1978) Arcus lipoides corneae secondary to hypothyroidism in the Alsatian. J. sm. Anim. Pract., 19, 127-142.

Crispin, S. M. (1980) Anaesthesia for ophthalmic surgery. Proc. Ass. Vet. An. G.B. & Ireland, 9, 170-183.

Crispin, S. M. & Langslow, D. R. (1980) Diabetes mellitus in the dog. In Physiological Basis of Small Animal Medicine, eds. A. T. Yoxall and J. F. R. Hird, Blackwell Scientific Publications, Oxford, pp 3-25.

Crispin, S. M. (1982) Corneal dystrophies in small animals. In the Veterinary Annual, 22nd Issue, eds. C. S. Grunsell & F. W. G. Hill, John Wright, Bristol, pp 298-310.

Crispin, S. M. α Barnett, K. C. (1983) Dystrophy, degeneration and infiltration of the canine cornea. J. sm. Anim. Pract., 24, 63-83.

Crouse, J. R., Grundy, S. M. α Ahrens, E. H. Jr. (1972) Cholesterol distribution in the bulk tissues of man: variation with age. J. Clin. Invest., 51, 1292-1296.

Cuignet (1880) Keratites parenchymateuses graisseuses. Recueil d' opthalmologie p 665.

Culp, T. W., Cunningham, R. D., Tucker, P. W., Jeter, J. α Determan, Jr., L. H. (1970) In vivo synthesis of lipids in rabbit iris, cornea, and lens tissues. Exp. Eye Res., 9, 98-105.

Davidson, A. (1947) Primary lipid dystrophy of the cornea. Arch. Ophthal., 37, 433-443.

Davidson, B., Pilz, C. G. α Zeller, R. W. (1951) Generalised xanthomatosis with corneal involvement. Am. J. Ophthalmol., 34, 233-236.

Davidson, J. D. α Kendall, F. E. (1951) Regression of lesions in canine arteriosclerosis. Arch. Path., 51, 288-292.

Davis, B. J. α Ornstein, L. (1959) High resolution enzyme localisation with a new diazo reagent hexazonium pararosanaline. J. Histochem. Cytochem. 7, 297-298.

Day, A. J. (1960) Cholesterol esterase activity of rabbit macrophages. Quart. J. exp. Physiol., 45, 55-59.

Day, A. J. α French, J. E. (1961) Further observations on the storage of cholesterol and cholesterol esters in lymph glands in the rat. J. Path. Bact., 81, 247-248.

Day, A. J. α Gould-Hurst, P. R. S. (1961) Esterification of ^{14}C-labelled cholesterol by reticulo-endothelial cells. Quart. J. exp. Physiol., 46, 376-382.

Day, A. J. (1964) The macrophage system, lipid metabolism and atherosclerosis. J. Atheroscler. Res., 4, 117-130.

Denti, A. V. (1926) Primary bilateral fatty degeneration of the cornea. Boll. Ocul., 5, 284.

Dice, P. F. (1974) Corneal dystrophy in the Airedale. Proc. Am. Coll. Vet. Ophthal., Texas. 5th Annual Science Programme, pp 80-86.

Dixon, K. C. (1982) Cellular Defects in Disease. Blackwell Scientific Publications, Oxford, pp 383-385.

Donaldson, D. D. (1980) Corneal degenerations. In Atlas of External Diseases of the Eye: Volume III Cornea and Sclera, 2nd Edition, C. V. Mosby, St. Louis.

Donovan, R. H., Carpenter, R. L., Schepens, C. L. α Tolentino, F. I. (1974) Histology of the normal Collie eye: I Topography, cornea, sclera and filtration angle. Ann. Ophthalmol., 6, 257-266.

Dreyfuss, M. (1930) Symmetrische zentrale Hornhautverfettung beim Hund. Albrecht von Grafes Arch. Ophthal., 125, 67-85.

Duke-Elder, S. α Smith, R. J. H. (1962) The examination of the eye. In The Foundations of Ophthalmology Volume VII, Heredity, Pathology, Diagnosis and Therapeutics, ed. S. Duke-Elder, Henry Kimpton. London, pp 233-365.

Duke-Elder, S. α Leigh, A. G. (1965) Corneal degenerations, dystrophies and pigmentations. In System of Opthalmology Volume VIII, Part 2, Diseases of the Outer Eye, eds. S. Duke-Elder, Henry Kimpton, London, pp 873-879.

Dunnigan, M. G. (1964) The distribution of phospholipid within macrophages in human atheromatous plaques. J. Atheroscler. Res., 4, 144-150.

Dunnigan, M. G. (1968) The use of Nile Blue Sulphate in the histochemical identification of phospholipids. Stain Technol., 43, 249-256.

Dunphy, E. B. (1949) Ocular conditions associated with idiopathic hyperlipaemia. Tr. Am. Ophth. Soc., 47, 197-211.

Easty, D. L. α Bron, A. J. (1971) Fluorescein angiography of the anterior segment: its value in corneal disease. Brit. J. Ophthal., 55, 671-682.

Eckersall, P. D. α Williams, M. E. (1983) Thyroid function tests in dogs using radioimmunoassay kits. J. sm. Anim. Pract., 24, 525-532.

Ehlers, N. (1970) Some comparative studies on the mammalian corneal epithelium. Acta. Ophthalmol. 48, 821-828.

Ekins, M. B., Waring, G. O., Roth, A. M., Spangler, W. L., Sodhi, H. S. α Gupta, L. M. (1979) Oval corneal opacities in hypercholesterolaemic Beagles. Invest. Ophthalmol. Vis. Sc., 18, (Supplement) 142.

Ekins, M. B., Waring, G. O. α Harris, R. R. (1980) Oval lipid corneal opacities in Beagles: II Natural history over four years and the study of tear function. J. Am. Anim. Hosp. Ass., 16, 601-605.

Elleder, M. α Lojda, Z. (1971) Studies in lipid histochemistry VI. Problems of extraction with acetone in lipid histochemistry. Histochemie, 28, 68-87.

Elleder, M. α Lojda, Z. (1972) Studies in lipid histochemistry IX. The specificity of Holczinger's reaction for fatty acids. Histochemie, 32, 301-305.

Elschnig, H. H. (1923) Ueber einen merkwürdigen Fall von Fettdystrophie der Peripherie beider Hornhäute. Klin. Monatsbl. Augenh., 71, 720-723.

Engström, A. α Finean, J. B. (1958) Biological Ultrastructure, Academic Press, New York, p 223.

Etherton, J. E. α Botham, C. M. (1970) Factors affecting lead capture methods for the fine localisation of rat lung acid phosphatase. Histochem. J., 2, 507-519.

Feigin, I. (1956) A method for the histochemical differentiation of cholesterol and its esters. J. Biophys. Biochem. Cytol., 2, 213-214.

Fine, B. S., Townsend, W. M., Zimmerman, L. E. α Lashkari, M. H. (1974) Primary lipoidal degeneration of the cornea. Am. J. Ophthalmol., 78, 12-23.

Fisher, T. M. (1979) Ocular manifestations of canine lipaemias. Mod. Vet. Pract., 60, 48-50.

Forni, S. (1951) Diagnostic et symptomatologie de la degenerascence cristalline hereditaire de la cornée. Arch. Ophtal., 11, 541-563.

Forsius, H. (1954) Arcus senilis corneae. Its clinical development and relationship to serum lipids, proteins and lipoproteins. Acta Ophthalmol., 42, 1-78.

Forsius, H. (1961) Lipid keratopathy: a clinical and serum lipid study of sixteen cases. Acta Ophthalmol., 39, 272-283.

Francis, J. α Victoria-Troncoso, V. (1978) Lysosomes in corneal pathology. Bull. Soc. belge. Ophtal., 182, 13-24.

Francois, J. α Neetens, A. (1964) The ocular manifestations of experimental hypercholesterolaemia in rabbits. Ophthalmologica. 148, 57-79.

French, J. E. α Morris, B. (1960) The uptake and storage of lipid particles in lymph glands in the rat. J. Path. Bact., 79, 11-19.

Friedlander, M. H., Cavanagh, H. D., Sullivan, W. R., Gallagher, M. J. α Dickersin, G. R. (1977) Bilateral central lipid infiltrates of the cornea. Am. J. Ophthalmol., 84, 781-787.

Friedman, M. α Byers, S. O. (1959) Some local factors affecting iridic lipid infiltration in hypercholesterolaemic rabbits. Am. J. Physiol., 197, 842-849.

Friedman, M. α Byers, S. O. (1962) Excess lipid leakage: a property of very young vascular endothelium. Brit. J. exp. Path., 43, 363-372.

Fuchs, E. (1901) Ueber ringformige und scheibenformige Keratitis (keratitis annularis et disciformis). Klin. Monatsbl. Augenh., 39, 513-523.

Garner, A. (1976) Histochemistry in the study of the ocular manifestations of inherited metabolic disorders. In Birth Defects: original article series, 12, 53-64.

Gaton, E. α Wolman, M. (1977) The role of smooth muscle cells and haematogenous macrophages in atheroma. J. Path., 123, 123-128.

Geer, J. C. (1965) Fine structure of canine experimental atherosclerosis. Am. J. Path., 47, 241-269.

Ghadially, F. N. (1975) Ultrastructural Pathology of the Cell. Butterworths, London.

Ghadially, F. N. (1982) Ultrastructural Pathology of the Cell and Matrix. 2nd Edition, Butterworths, London.

Gilbert, W. von (1929) Uber Xanthomatose der Hornhaut und Lederhaut. Arch. f. Augenh., 100-101, 329-333.

Glauert, A. M. (1965a) Section staining, cytology, autoradiography and immunochemistry of biological specimens. In Techniques for Electron Microscopy, ed. D. Kay, 2nd Edition, Blackwell Scientific Publications, Oxford.

Glauert, A. M. (1965b) The fixation and embedding of biological specimens. In Techniques for Electron Microscopy, ed. D. Kay, 2nd Edition, Blackwell Scientific Publications, Oxford.

Goldstein, J. L. α Brown, M. S. (1974) Binding and degradation of LDL by cultured human fibroblasts. Comparison of cells from a normal subject and from a patient with homozygous familial hypercholesterolaemia. J. Biol. Chem., 249, 5153-5162.

Goldstein, J. L. α Brown, M. S. (1975) Lipoprotein receptors, cholesterol metabolism, and atherosclerosis. Arch. Path., 99, 181-184.

Gomori, G. (1941) Distribution of acid phosphatase in the tissues under normal and under pathologic conditions. Arch. Path., 32, 189-199.

Gomori, G. (1950) An improved histochemical technique for acid phosphatase. Stain Technol., 25, 81-85.

Gomori, G. (1952) Histochemistry of esterases. International Review of Cytology, 1, 323-335.

Grande, F. α Schultz, A. (1965) Effect of coconut oil on serum lipids of normal and of thyroidectomised dogs. Proc. Soc. Exp. Biol. Med., 45, 161-174.

Gresham, G. A. α Howard, A. N. (1962) Atherosclerosis produced by semisynthetic diet with no added cholesterol. Arch. Path., 74, 1-5.

Guidry, M. A., Geer, J. C. α Robertson, W. B. (1964) Lipid pattern in experimental canine atherosclerosis. Circ. Res., 14, 61-72.

Gwin, R. M. α Gelatt, K. N., (1977) Bilateral ocular lipidosis in a cotton tail rabbit fed an all milk diet. J. Am. Vet. Med. Ass., 171, 887-889.

Hale, C. W. (1946) Histochemical demonstration of acid polysaccharides in animal tissues. Nature, 157, 802.

Halliday, N. (1949) The effect of formalin on liver lipids. J. Biol. Chem., 129, 65-69.

Harrington, G. A. α Kelly, D. (1980) Corneal lipoidosis in a dog with bilateral thyroid carcinoma. Vet. Pathol., 17, 490-517.

Hashimoto, S. α Dayton, S. (1974) Cholesterol-esterifying activity of aortas from atherosclerosis-resistant and atherosclerosis-susceptible species. Proc. Soc. Exp. Biol. Med., 145, 89-92.

Hata, Y., Hower, J. α Insull, W. (1974) Cholesteryl ester-rich inclusions from human aortic fatty streak and fibrous plaque lesions of atherosclerosis. Am. J. Path., 75, 423-453.

Hayat, M. A. α Giaquinta, R. (1970) Rapid fixation and embedding for electron microscopy. Tissue and Cell, 2, 191-195.

Hayat, M. A. (1973) Principles and Techniques of Electron Microscopy: Biological Applications. Volumes 1 to 4. Van Nostrand Reinhold Company, New York.

Hayat, M. A. (1981) Fixation for Electron Microscopy, Academic Press, New York.

Heath, P. (1932) Ocular lipid histiocytosis and allied storage phenomena. Trans. Am. Acad. Ophthalmol., 37, 121-141.

Heath, P. (1935) Lipin interstitial keratitis. Arch. Ophth., 13, 614-619.

Heath, I. D. (1961) Staining of sulphated mucopolysaccharides. Nature, 191, 1370-1371.

Henriksen, T. S., Evensen, A. α Carlander, B. (1979a) Injury to human endothelial cells in culture induced by low density lipoproteins. Scand. J. Clin. Lab. Invest., 39, 361-368.

Henriksen, T. S., Evensen, A. α Carlander, B. (1979b) Injury to cultured endothelial cells induced by low density lipoproteins: protection by high density lipoproteins. Scand. J. Clin. Lab. Invest., 39, 369-375.

Highman, B. (1946) Improved methods for demonstrating amyloid in paraffin sections. Arch. Path., 41, 559- 562.

Hill, K., Kinoshita, J. H. α Kuwabara, T. (1959) Experimental aberrant lipogenesis V. Biochemical evidence of oleate-induced lipid formation in the cornea. Arch. Ophthal., 61, 361-365.

Hoe, C. M. α Harvey, D. G. (1961) An investigation into liver function tests in dogs. J. sm. Anim. Pract., 2, 109-127.

Hogan, M. J., Alvarado, J. A. α Weddell, J. E. (1971) Histology of the Human Eye. W. B. Saunders Co., Philadelphia.

Holczinger, L. (1959) Histochemischer Nachweis freier Fettsäuren. Acta. Histochem., 8, 167-175.

Holmes, R. S. α Masters, C. J. (1968) A comparative study of the multiplicity of mammalian esterases. Biochim. Biophys. Acta., 151, 147-158.

Holt, W. S. α Kinoshita, J. H. (1973) The soluble proteins of the bovine cornea. Invest. Ophthalmol., 12, 114-126.

Innerarity, T. L. α Mahley, R. W. (1978) Enhanced binding by cultured human fibroblasts of Apo-E-containing lipoproteins as compared with low density lipoprotein. Biochemistry, 17, 1440-1447.

Iverius, P-H. (1972) The interaction between human plasma lipoproteins and connective tissue glycosaminoglycans. J. Biol. Chem., 247, 2607-2613.

Jack, R. L. α Luse, A. A. (1970) Lipid keratopathy: An electron microscope study. Arch. Ophthal., 83, 678-691.

Jacob, H. (1920) Tierärztliche Augenheilkunde. R. Schoetz, Berlin.

Jaensch, P. A. (1928a) Die Rolle der Histiozyten bei der degenerativen Verfettung des Bulbus. Ber. u. d. Versamml. d. Deutsch. ophth. Gesellsch., 47 251-257.

Jaensch, P. A. (1928b) Die degenerative Verfettung am Auge I. Das Fettvorkommen bei Erkrankungen der Hornhaut unter besonderer Berücksichtigung der Verhältnisse bei der Keratomalazie. Arch. f. Ophth., 119, 198-226.

Jaensch, P. A. (1933) Verfettung des Auges. Zentralbl. ges Opth., 30, 145-161.

Jaensch, P. A. (1935) Die degenerative Verfettung am Auge II. Pathologic lipoidosis of the cornea and sclera; anatomic findings in scleritis. Arch. f. Ophth., 133, 361-377.

Janes, R. G. (1964) Changes in the rabbit's eye caused by cholesterol feeding. Am. J. Ophthalmol., 58, 819-828.

Jensen, H. E. (1974) Histological changes in the canine eye related to ageing. Proc. Am. Coll. Vet. Ophthal., Texas. 5th Annual Science Programme. (Cited by D. H. Slatter, 1975).

Jones, D. α Gresham, G. A. (1966) Reaction of formaldehyde with unsaturated fatty acids during histological fixation. Nature. 210, 1386-1388.

Kamocki, V. (1893) Ein Fall von Fettentartung der Hornhaut mit intermittirenden Reizerscheinungen. Arch. f. Ophth., 39, 209-223.

Kaneko, J. J. (1980) Thyroid function. In Clinical Biochemistry of Domestic Animals, 3rd Edition. ed. J. J. Kaneko, Academic Press Inc., New York, pp 491-512.

Katz, D. α Delaney, P. A. (1933) Dystrophia adiposa corneae. Arch. Ophthal., 9, 78-101.

Kawanami, O., Ferrans, V. J. α Crystal, R. G. (1980) Sub-plasmalemmal linear densities in cells of the mononuclear phagocytic system in lung. Am. J. Path., 100, 131-150.

Keilig, I. (1944) Über spezifitatsbreite und Grundlagen der Markscheidenfärbungen. Virchow Arch. path. Anat., 312, 404-420.

Kern, T. J. α Riis, R. C. (1980) Ocular manifestations of secondary hyperlipidaemia associated with hypothyroidism and uveitis in a dog. J. Am. Anim. Hosp. Ass., 16, 907-914.

Kiernan, J. A. (1981) Histological and Histochemical methods: Theory and Practice, Pergammon Press, Oxford.

Klintworth, G. K. (1969) Experimental studies on the phagocytic capability of the corneal fibroblast. Am. J. Path., 55, 283-294.

Klintworth, G. K. α Hijmans, J. C. (1970) The induction by serum of lipid storage in cells of the cornea grown in culture. Am. J. Path., 58, 403-418.

Klintworth, G. K. (1977) The cornea-structure and macromolecules in health and disease. Am. J. Path., 89, 718-808.

Klintworth, G. K. (1982) Degenerations, depositions and miscellaneous reactions of the cornea, conjunctiva and sclera. In Pathobiology of Ocular Disease Part B, eds. A. Garner and G. K. Klintworth, Marcel Dekker, Switzerland. pp 1445-1447.

Kolen, A. A. (1927) Uber die Ablagerung von Lipoiden im Auge und ihre Beziehung zum Alter, sowie zu der Lipoidablagerung in den Bindesubstanzen anderer Korperteile. Virchow Arch. Path. Anat. Physiologie., 263, 46-48.

Kossa, J. von (1901) Ueber die im Organismus kunstlich erzeugbaren Verkalkungen. Bietre. path. Anat., 29, 163-202.

Kronfeld, D. S., Johnson, K. α Dunlap, H. (1979) Inherited predisposition of dogs to diet-induced hypercholesterolaemia. J. Nutr., 109, 1715-1719.

Kusama (1921) Ein Beitrag zur primären fettigen Degeneration der Kornea. Klin. Monatsbl. Augenh., 66, 111-114.

Kuwabara, T. α Cogan, D. G. (1957) Experimental aberrant lipogenesis II. Substrate factor. Arch. Path., 63, 496-501.

Kuwabara, T. α Cogan, D. G. (1959) Experimental aberrant lipogenesis IV. Production of non-sudanophilic fat. Arch. Path., 67, 34-38.

Lake, B. D. α Patrick, A. D. (1970) Wolman's disease: deficiency of E600-resistant acid esterase activity with storage of lipids in lysosomes. J. Pediat., 76, 262-266.

Lake, B. D. (1971) Histochemical detection of the enzyme deficiency in blood films in Wolman's disease. J. Clin. Path., 24, 617-620.

Lake, B. D. (1972) Is the histochemical demonstration of lipase activity possible? Histochem. J., 4, 71-73.

Lake, B. D. (1973) Acid esterase in human leukocytes. J. Histochem. Cytochem., 21, 759.

Lake, B. D. (1976) In discussion of 'Histochemistry in the study of the ocular manifestations of inherited metabolic disorders'. In Birth Defects original article series, 12, 66-67.

Landmann, O. (1925) A case of tubercular iridocyclitis with fatty degeneration and infiltration of the anterior segment of the eye. Arch. f. Ophth., 54, 261-264.

Lang, P. D. α Insull, W. (1970) Lipid droplets in atherosclerotic fatty streaks of human aorta. J. Clin. Invest., 49, 1479-1488.

Lavelle, R. B. α Yoxall, A. T. (1980) The adrenal glands. In Physiological Basis of Small Animal Medicine, eds. A. T. Yoxall α J. F. R. Hird, Blackwell Scientific Publications, Oxford, pp 39-68.

Lavignette, A. M. α Carlton, W. W. (1974) A case of ocular nodular fasciitis in a dog. J. Am. Anim. Hosp. Ass., 10, 503-506.

Leary, T. (1941) The genesis of atherosclerosis. Arch. Path., 32, 507-555.

LeBaron, F. N. α Folch, J. (1956) The isolation from brain tissue of a trypsin-resistant protein fraction containing combined inositol, and its relation to neurokeratin. J. Neurochem., 1, 101-108.

Lehmann, H. α Lines, J. G. (1972) Hyperlipoproteinaemia classification: the optimum routine electrophoretic system and its relevance to treatment. Lancet, 1, 557-560.

Leibovich, S. J. α Ross, R. (1975) The role of the macrophage in wound repair. Am. J. Path., 78, 71-100.

Lendrum, A. C., Fraser, D. S., Slidders, W. α Henderson, R. (1962) Studies on the character and staining of fibrin. J. Clin. Path., 15, 401-413.

Lewis, B. (1976) The Hyperlipidaemias, Blackwell Scientific Publications, Oxford, pp 383-420.

Lewis, L. A., Green, A. A. α Page, I. H. (1952) Ultracentrifuge lipoprotein pattern of serum of normal, hypertensive and hypothyroid animals. Am. J. Physiol., 171, 391-400.

Lillie, R. D. α Ashburn, L. L. (1943) Supersaturated solutions of fat stains in dilute isopropanol for demonstration of acute fatty degenerations not shown by Herxheimer technique. Arch. Path., 36, 432-435.

Lillie, R. D. (1954) In Histopathologic Technique and Practical Histochemistry, 2nd Edition, ed. R. D. Lillie, Blakiston, New York. p 312.

Lindall, A. W., Grande, F. α Schultz, A. (1971) The effect of dietary fats on the serum lipoproteins of normal dogs. Proc. Soc. Exp. Biol. Med., 136, 1032-1037.

Lison, L. α Dagnelie, J. (1935) Methodes nouvelles de colouration de la myeline. Bull. d'Histol. Appliq. a la physiol., 12, 85-91.

Loewenstein, A. (1942) Some contributions to the lipoidal pathology of the ocular tissue. Trans. ophth. Soc. U.K., 62, 159-167.

Lorenz, M. D. α Stiff, M. E. (1980) Serum thyroxine content before and after thyrotropin stimulation in dogs with suspected hypothyroidism. J. Am. Vet. Med. Ass., 177, 78-81.

McManus, J. F. A. (1946) Histological demonstration of mucin after periodic acid. Nature, 158, 202.

MacMillan, A. D., Waring, G. O., Spangler, W. L. α Roth, A. M. (1979) Crystalline corneal opacities in the Siberian Husky. J. Am. Vet. Med. Ass., 175, 829-832.

Mahley, R. W. α Weisgraber, K. H. (1974) Canine lipoproteins and atherosclerosis: I. Isolation and characterisation of lipids from control dogs. Circ. Res., 35, 713-721.

Mahley, R. W., Weisgraber, K. H. α Innerarity, T. L. (1974) Canine lipoproteins and atherosclerosis: II. Characterisation of plasma lipoproteins associated with atherogenic and non atherogenic hyperlipidaemia. Circ. Res., 35, 722-733.

Mahley, R. W. α Innerarity, T. L. (1977) Interaction of canine and swine lipoproteins with the LDL receptor of fibroblasts as correlated with heparin/manganese precipitability. J. Biol. Chem., 252, 3980-3986.

Mahley, R. W., Innerarity, T. L., Pitas, R. E., Weisgraber, K. H., Brown, J. H. α Gross, E. (1977) Inhibition of lipoprotein binding to cell surface receptors of fibroblasts following selective modification of Arginyl residues in Arginine-rich and B apoproteins. J. Biol. Chem., 252, 7279-7287.

Mallory, F. B. (1938) Pathological Technique, W. B. Saunders, London.

Mann, I. & Pullinger, B. D. (1942) The pathology of cholestrin and fat deposition in mustard gas injuries of the cornea. Brit. J. Ophthal., 26, 503-507.

Mann, G. V. & Stare, F. J. (1954) Nutrition and atherosclerosis. In Symposium on Atherosclerosis, Washington D. C. National Academy of Sciences, National Research Council. pp 169-180.

Manning, P. J., Corwin, L. A. & Middleton, C. C. (1973) Familial hyperlipoproteinaemia and thyroid dysfunction of Beagles. Exp. Mol. Pathol., 19, 378-388.

Mapstone, R. (1964) Determinants of corneal temperature. Brit. J. Ophthal., 52, 729-741.

Mariano, M. & Spector, W. G. (1974) The formation and properties of macrophage polykaryons (inflammatory giant cells). J. Path., 113, 1-19.

Martin, C. L. (1969) Slit lamp examination of normal canine anterior ocular segment. J. sm. Anim. Pract., 10, 143-149.
10, 151-162.
10, 169-184.

Martin, C. L. & Anderson, B. G. (1981) Ocular Anatomy. In Textbook of Veterinary Ophthalmology, ed. K. N. Gelatt, Lea & Febiger, Philadelphia. pp 12-121.

Martonyi, C. L. (1982) Fluorescein angiography of the conjunctiva. In Ophthalmic Photography, ed. J. Justice, Jr., Little, Brown & Co., Boston, pp 187-192.

Masson, P. (1929) Some histological methods: trichrome stainings and their preliminary technique. J. Tech. Meth. and Bull. Inst. Assn. Med. Mus., 12, 75-90.

Matsui, M. α Justice, J. Jr. (1982) Anterior segment fluorescein angiography. In Ophthalmic Photography, ed. J. Justice Jr., Little, Brown α Co., Boston, pp 179-185.

Maumenee, A. E. (1962) Histopathology of corneal degenerations and dystrophies. Acta XIX Conc. Ophthal., 1, 509-522.

Maurice, D. M. (1961) The use of permeability studies in the investigation of submicroscopic structure. In The Structure of the Eye, ed. G. Smelser, Academic Press, New York, pp 381-391.

Maurice, D. M. α Watson, P. (1965) The distribution and movement of serum albumin in the cornea. Exp. Eye Res., 4, 355-363.

McManus, J. F. A. (1946) Histological demonstration of mucin after periodic acid. Nature, 158, 202.

Meesman, A. (1924) Anatomischer Befund eines Auges mit massenhafter Ablagerung von Cholesterinkristallen im vorderen Kammer und sekundärer Atheromatose der Hornhaut. Arch. f. Augenh., 94, 56-72.

Meesman, A. (1927) Die Mikroskopie des lebenden Auges Gullstrandschen Spaltlampe mit Atlas typischer Befunde. Wein, Urban α Schwarzenburg, Berlin, p 66.

Metcalf, D. (1966) The Thymus, Springer-Verlag, Berlin.

Meyer, H. (1928) Beitrag zum Krankheitsbild der dystrophia adiposa corneae (Primäre xanthomatosis corneae). Klin. Monatsbl. Augenh., 81, 786-793.

Miller, G. J. α Miller, N. E. (1975) Plasma high density lipoprotein concentration and development of ischaemic heart disease. Lancet, 1, 16-19.

Mitsui, Y., Matsubara, M. α Kanagawa, M. (1969) Fluorescence irido-corneal photography. Brit. J. Ophthal., 53, 505-512.

Monroe, C. W. α Frommer, J. (1967) Neutral Red-Fast Green FCF, a single solution stain for mammalian tissues. Stain Technol., 42, 262-264.

Morrin, L. A., Waring, G. O. α Spangler, W. (1982) Oval lipid corneal opacities in Beagles: Ultrastructure of normal Beagle cornea. Am. J. Vet. Res., 43, 443-453.

Moskovitz, M. S. (1967) Fatty acid-induced steatosis in monolayer cell cultures. In Lipid Metabolism in Tissue Culture Cells, eds. G. H. Rothblat α D. Kritchevsky, Wistar Symp. Monogr. No. 6, Wistar Inst. Press, Philadelphia, pp 49-62.

Movat, H. Z. α Fernando, N. V. P. (1962) The fine structure of connective tissue I. The fibroblast. Exp. Molec. Path., 1, 509-534.

Mowry, R. W. (1956) Alcian blue techniques for the histochemical study of acidic carbohydrates. J. Histochem. Cytochem., 6, 82-83.

Nicoll, A., Miller, N. E. α Lewis, B. (1980) High-density lipoprotein metabolism. Adv. Lipid Res., 17, 53-97.

Norum, K. R., Berg, T., Helgerud, P. α Drevon, C. A. (1983) Transport of cholesterol. Phys. Rev., 63, 1343-1419.

Novikoff, A. B. (1961) Lysosomes and related particles. In The Cell, Volume 2, eds. J. Brachet α A. E. Minsky, Academic Press, New York, pp 423-488.

Oda, T., Yoshizawa, K., Nakamoto, T., Kubo, Y. α Okazaki, H. (1958) An electron microscopic study on lipogenesis. Acta Med., Okayama, 12, 29-41.

Offret, G., Payrau, P., Pouliquen, Y., Faure, J-P. α Bisson, J. (1966) La structure fine de certaines dystrophies cornéenes. Arch. d' Ophtal., 26, 171-181.

Olin, D. D., Rogers, W. A. α MacMillan, A. D. (1976) Lipid-laden aqueous humour associated with anterior uveitis and concurrent hyperlipaemia in two dogs. J. Am. Vet. Med. Ass., 168, 861-864.

Orzalesi, F. (1947) Degenerazione eredo-familiare della cornea con reperto istologico atipico. Rass. ital. Ottal., 16, 393-406.

Page, I. H. (1954) Atherosclerosis: an introduction. Circulation, 10, 1-27.

Palich-Szánto, O. (1960) Das Infiltrat der Hornhaut im Gebiete des Gerontoxons. Ophthalmologica, 140, 161-167.

Parker, F. α Odland, G. F. (1966) A correlative histochemical, biochemical and electron microscopic study of experimental atherosclerosis in the rabbit aorta with special reference to the myointimal cell. Am. J. Path., 48, 197-239.

Parker, F. α Odland, G. F. (1968) Experimental xanthoma: A correlative biochemical, histologic, histochemical and electron microscopic study. Am. J. Path., 53, 537-566.

Payrau, P., Pouliquen, Y., Faure, J-P α Offret, G. (1967) La Transparence de la Cornée. Masson α Cie, Paris, pp 508-523.

Pearse, A. G. E. (1960) Histochemistry: Theoretical and Applied. 2nd Edition, Churchill, London.

Pearse, A. G. E. (1968 α 1972) Histochemistry, Theoretical and Applied. 3rd Edition, 2 volumes, Churchill-Livingstone, Edinburgh.

Pease, D. C. (1964) Histochemical Techniques for Electron Microscopy. Academic Press, London.

Pedigree Petfoods (1976) Breeder and Veterinary Service. Product Data Sheets.

Perls, M. (1867) Nachweis von Eisenoxyd in gewissen Pigmenten. Virchow's Arch., 39, 42-48.

Perera, R. N. (1969) Basal cells of the corneal epithelium in man and monkey. Brit. J. Ophthal., 53, 592-605.

Peters, T. J., Takano, T. α de Duve, C. (1973) Subcellular fractionation studies on the cells isolated from normal and atherosclerotic aorta. In Atherogenesis: Initiating factors. Ciba Foundation Symposium 12, eds. R. Porter and J. Knight, Elsevier, Amsterdam, 197-222.

Pollak, O. J. (1969) Monographs on Atherosclerosis I. Tissue Cultures. S. Karger, New York.

Prince, J. H., Diesem, C. D., Eglitis, I. α Ruskell, G. L. (1960) The Anatomy and Histology of the Eye and Orbit in Domestic Animals. Charles C. Thomas, Springfield, Illinois.

Prince, J. H. (1964) Comparative Anatomy of the Eye. Charles C. Thomas, Springfield, Illinois.

Reynolds, E. S. (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol., 17, 208-212.

Roberts, S. R., Dellaporta, A. α Winter, F. C. (1966) The Collie ectasia syndrome, pathology of eyes of young and adult dogs. Am. J. Ophthalmol., 62, 728-758.

Robertson, A. L. (1968) Oxygen requirements of the human arterial intima in atherogenesis. Progr. Biochem. Pharmacol., 4, 305-316.

Robin, V. α Charton, A. (1939) La lipoidose cornéene du chien. Rec. Med. Vet., 115, 321-331.

Robinson, G. (1982) Electron microscopy 2: Transmission. In Theory and Practice of Histological Techniques, 2nd Edition, eds. J. D. Bancroft α A. Stevens, Churchill-Livingstone, Edinburgh, pp 482-518.

Robinson, G. α Terras, S. (1982) Electron microscopy 3: Specialised techniques. In Theory and Practice of Histological Techniques, 2nd Edition, eds. J. D. Bancroft α A. Stevens, Churchill-Livingstone, Edinburgh. pp 519-534.

Rodger, F. C. (1971) A study of the ultrastructure and cytochemistry of lipid accumulation and clearance in cholesterol-fed rabbit cornea. Exp. Eye Res., 14, 88-93.

Rodgers, W. A., Donovan, E. F. α Kociba, G. J. (1975a) Idiopathic hyperlipoproteinaemia in dogs. J. Am. Vet. Med. Ass., 166, 1087-1091.

Rodgers, W. A., Donovan, E. F. α Kociba, G. J. (1957b) Lipids and lipoproteins in normal dogs and in dogs with secondary hyperlipoproteinaemia. J. Am. Vet. Med. Ass., 166, 1092-1100.

Rohrschneider, W. (1925a) Beitrag zur Kenntnis der experimentellen Hypercholesterinamie des Kaninchens. Virchow's Arch. f. path. Anat., 256, 139-149.

Rohrschneider, W. (1925b) Ueber die Bildung intracellulärer anisotroper Tröpfchen nach örtlicher Einspritzung von Cholesterin in die Cornea und Vorderkammer des Kaninchen-auges. Virchow's Arch. f. path. Anat., 256, 150-157.

Rohrschneider, W. (1927a) Zur Entstehung der pathologischen Hornhautverfettung. Bericht über die XLVI Zusammenkunft der deutschen Opth. Geselsch. in Heidelberg, 46, 269-275.

Rohrschneider, W. (1927b) Experimentelle Untersuchungen über die infiltrative Verfettung der Cornea beim Kaninchen. A. v. Graefes Ophthal., 115, 535-561.

Roozemond, R. C. (1967) Thin layer chromatographic study of lipid extraction from cryostat sections of rat hypothalamus by some fixatives. J. Histochem. Cytochem., 15, 526-529.

Roscoe, H. G. α Vogel, A. W. (1968) Lipid changes in the eye concomitant with the development of atherosclerosis in the aorta of the rabbit. Circulat. Res., 23, 633-643.

Roscoe, H. G. α Riccardi, B. A. (1969) Phospholipid changes in the eye and aorta of cholesterol-fed rabbits. J. Atheroscler. Res., 10, 123-130.

Ross, R. J. M. α Valori, R. M. (1982) Measurement of thyroid hormones in Labradors. Vet. Rec., 111, 329.

Roth, A. M., Ekins, M. B., Waring, G. O., Gupta, L. M. α Rosenblatt, L. S. (1981) Oval corneal opacities in Beagles: III. Histochemical demonstration of stromal lipids without hyperlipidaemia. Invest. Ophthalmol., Vis. Sci., 21, 95-106.

Rothblat, G. H., Hartzell, R., Mialhe, H. α Kritchevsky, D. (1967) Cholesterol metabolism in tissue culture cells. In Lipid Metabolism in Tissue Culture Cells, eds. G. H. Rothblat α D. Kritchevsky, Wistar Symp. Monogr. No. 6. Wistar Inst. Press, Philadelphia, pp 129-149.

Saunders, L. Z. α Rubin, L. F. (1975) Ophthalmic Pathology of Animals, S. Karger, Basel, pp 14-15.

Schecter, J. A., Fogelman, M., Haberland, M. E., Seager, J., Hokom, M. α Edwards, P. (1981) The metabolism of native and malondialdehyde-altered low density lipoprotein by human monocyte - macrophages. J. Lipid Res., 22, 63-71.

Schiller, I., Berglund, N. E., Terry, J. R., Reichlin, R., Trueheart, R. E. α Cox, G. E. (1964) Hypercholesterolaemia in pet dogs. Arch. Path., 77, 389-392.

Schnabel, R. (1964) Eine topochemische Methode zur Differenzierung des freien und veresterten Cholesterins. Acta histochemica, 18, 161-167.

Schock, K. (1910) Eine noch nicht beschriebene Hornhautaffektion beim Hund. Arch. vergl. Ophthal., 1, 313-316.

Schoenheimer, R. α Yuasa, D. (1929) Über die Pflanzensteriine für den tierischen Organismus; verfügt der tierische Organismus über Pflanzensterinesterasen? Ztschr. f. physiol. Chem., 180, 19-23.

Schultz, A. (1924) Eine Methode des mikrochemischen Cholesterinnachweises am Gewebeschnitt. Zbl. Allg. Path. Pathol. Anat., 35, 314-316.

Schwartz, B. (1964) The effect of lid closure upon the ocular temperature gradient. Invest. Ophthalmol., 3, 100-106.

Schwartz, B. (1965) Environmental temperature and the ocular temperature gradient. Arch. Ophthal., 74, 237-243.

Shapiro, L. A. α Farkas, T. G. (1977) Lipid keratopathy following corneal hydrops. Arch. Ophthal., 95, 456-458.

Sheraidah, G. A. K., Winder, A. F. α Fielder, A. R. (1981) Lipid-protein constituents of human corneal arcus. Atherosclerosis, 40, 91-98.

Shively, J. N. α Epling, G. P. (1970a) Fine structure of the canine eye: cornea. Am. J. Vet. Res., 31, 712-722.

Shively, J. N. α Epling, G. P. (1970b) The eye-fine structure. In the Beagle as an Experimental Dog, ed. A. C. Anderson. Iowa State University Press, Iowa, pp 386-411.

Shull, K. H., Mann, G. V., Andrus, S. B. α Stare, F. J. (1954) Response of dogs to cholesterol feeding. Am. J. Physiol., 176, 475-482.

Sigler, R. L. (1977) Ocular manifestations of hyperlipaemia in a dog and cat: case reports. J. Am. Anim. Hosp. Ass., 9, 288-291.

Slatter, D. H. (1975) Effects of hyperlipoproteinaemia and ageing on canine eyes. PhD Thesis. Colorado State University, Fort Collins, Colorado.

Slatter, D. H. (1980) Effects of extreme hypercholesterolaemia on the canine eye. Fellowship Thesis, Royal College of Veterinary Surgeons, London.

Small, D. S. (1970) The physical state of lipids of biological importance: cholesteryl esters, cholesterol, triglyceride. In Surface Chemistry of Biological Systems. Advances in Experimental Medicine and Biology Volume 7, ed. M. Blank, Plenum Press, New York, pp 55-83.

Small, D. M. α Shipley, G. G. (1974) Physical-chemical basis of lipid deposition in atherosclerosis. Science, 185, 222-229.

Smidt-Martens, F. W. α Hohurst, H. J. (1972) Free fatty acids in bovine cornea and corneal epithelium. Ophthalmic. Res., 3, 126-128.

Smith, E. B. α Slater, R. S. (1973) Relationship between plasma lipids and arterial tissue lipids. Nutr. Metab., 15, 17-26.

Smith, E. B. (1977) Molecular interactions in human atherosclerotic plaques. Am. J. Path., 86, 665-674.

Smith, J. S., Bistner, S. α Riis, R. (1976) Infiltrative corneal lesions resembling fibrous histiocytoma: clinical and pathologic findings in six dogs and one cat. J. Am. Vet. Med. Ass., 169, 722-726.

Spanlang, H. (1927) Beiträge zur Klinik und Pathologie seltener Hornhauterkrankungen. Z. Augenheilk., 62, 21-41.

Spangler, W. L., Waring, G. O. α Morrin, L. A. (1982) Oval lipid corneal opacities in Beagles. Vet. Pathol., 19, 150-159.

Spector, W. G. α Lykke, A. W. (1966) The cellular evolution of inflammatory granulomata. J. Path. Bact., 92, 163-167.

Spreull, J. S. A. (1966) Anatomy and physiology of the cornea of the dog. J. sm. Anim. Pract., 7, 253-256.

Steedman, H. F. (1950) Alcian blue 8GS: a new stain for mucin. Quart. J. micr. Sci., 91, 477-479.

Stein, Y., Stein, O. α Shapiro, B. (1963) Enzymic pathways of glyceride and phospholipid synthesis in aortic homogenates. Biochim. Biophys. Acta. 70, 33-42.

Stein, O., Vanderhoek, J. α Stein, Y. (1976) Cholesterol content and sterol synthesis in human skin fibroblasts and rat aortic smooth muscle cells exposed to lipoprotein-depleted serum and high density apolipoprotein/phospholipid mixtures. Biochim. Biophys. Acta., 431, 347-358.

Steiner, A. α Domanski, B. (1941) Dietary hypercholesterolaemia. Am. J. Med. Sci., 201, 820-830.

Steiner, A. α Kendall, F. E. (1946) Atherosclerosis and arteriosclerosis in dogs following ingestion of cholesterol and thiouracil. Arch. Path., 42, 433-444.

Stevens, A. (1982) Pigments and minerals. In Theory and Practice of Histological Techniques, 2nd Edition, eds. J. D. Bancroft α A. Stevens, Churchill-Livingstone, Edinburgh. pp 242-266.

Stewart, G. T. (1961) Mesomorphic forms of lipid in the structure of normal and atheromatous tissues. J. Path. Bact., 81, 385-393.

Suzuki, M. (1972) Experimental cerebral atherosclerosis in the dog. Am. J. Path., 67, 387-394.

Szasz, A. (1937) Ein eigenartiger Fall von xanthomatosis corneae. Arch. f. Augenh., 110, 373-381.

Szily, A. von (1923) Ueber orangegelbe Verfärbung des vorderen Bulbusabschnittes infolge von Verfettung im Bereiche der vorderen augenkammer und der iris (xanothomatosis bulbi) mit histologischen befunden. Klin. Monatsbl. Augenh., 71, 30-44.

Takayasu, M. (1912) Über eine primäre Fettdegeneration der Kornea. Arch. f. Ophth., 82, 475-488.

Tanaka, M. α Kuwabara, T. (1978) Fine structure of lipogenetic cells of the cornea. Invest. Ophthalmol., Vis. Sci., 17 (Supplement) 200.

Tertsch, R. (1911) Ein Fall von primärer fettiger Degeneration beider Hornhäute. Klin. Monatsbl. Augenh., 49, 1-13.

Thiele, O. W. α Denden, A. (1967) Über die Verteilung der Lipide in der Cornea des Rindes. Z. Physiol. Chem., 348, 1097-1101.

Thomas, C. I. (1955) Dystrophies of the cornea. In The Cornea, C.C. Thomas, Springfield, Illinois, 276-374.

Tompkins, E. H. (1946) Reaction of reticulo-endothelial cells to subcutaneous injections of cholesterol. Arch. Path., 42, 299-319.

Toth, Z. (1933) Ein Fall von primärer annulärer cholesterinamischer Infiltration der Kornea. Klin. Monatsbl. Augenh., 91, 502-507.

Treacher Collins, E. (1907) Corneal changes in a case of myxoedema. Trans. ophthal. Soc. U.K., 27, 47-48.

Tremblay, M. α Dubé, I. (1975) La keratopathie lipidique. L'union Medicale der Canada. 104, 1383-1385.

Troncoso, M. U. α Castroviejo, R. (1936) Microanatomy of the eye with the slit lamp microscope. Am. J. Ophthalmol., 19, 481-492.

Tschetter, R. T. (1976) Lipid analysis of the human cornea with and without arcus senilis. Arch. Ophthal., 76, 403-405.

Uberreiter, O. (1970) Biomikroskopische Befunde der Netzhaut und der Papille bei Hund und Katze. Wien Tierartze Monatsschr., 57, 269-274.

Vainisi, S. J. α Goldberg, M. F. (1974) Animal models of inherited human eye disease. In Genetic and Metabolic Eye Disease, ed. M. F. Goldberg. Little Brown α Co., Boston, pp 215-236.

Van Buskirk, E. M. (1979) The canine eye: the vessels of aqueous drainage. Invest. Ophthalmol. Vis. Sci., 18, 223-230.

Van Canneyt, J. (1948) Xanthoma de la cornée. Bull et Mem. Soc. Fr. D'Ophtal., 61, 169-176.

Van Gieson, I. (1899) Laboratory notes of technical methods for the nervous system. New York Medical Journal, 50, 57.

Van Herwaarden, A. (1937) Arcus lipoides corneae bij experimenteele hypercholesterinaemie van het konijn. Portielje, Amsterdam.

Vannas, M. (1931) A case of white fatty dystrophy of the corneal periphery. Klin. Monatsbl. Augenh. 87, 289.

Veenendaal, H. (1928) Twee nog weinig bekende hoornvliesaandoeringen bij den hond. T. Diergeneeskde, 55, 853-869.

Veenendaal, H. (1937) Dystrophia corneae adiposa bij den hond. T. Diergeneeskde, 64, 913-921.

Verderame (1922) Cited by W. Clausen (1923). Anatomical and histochemical investigation of a case of symmetrical, bilateral, fatty degeneration of the cornea and arcus senilis. Zentrabl. f. d. ges. ophth., 10, 56.

Vernon-Roberts, V. (1973) The Macrophage. Cambridge University Press, Cambridge.

Versé, M. (1916) Über die Blut- und Augenveränderungen bei experimenteller Cholesterinamie. Munch. Med. Woschr., 2, 1074-1076.

Versé, M. α Rohrschneider, W. (1924) Über die Enstehung des arcus lipoides corneae in Tierexperimenten und beim Menschen. Klin Wschr., 32, 1528-1529.

Versé, M. (1924) Uber die Augenveränderungen (lipoidosis oculi) bei der experimentellan lipocholesterinaemie des kaninchen. Virchow Arch. Path. Anat., 250, 252-272.

Versé, M. (1940) Zur Frage der scheibenförmigen und ringförmigen fortschreitenden Hornhautverfettungen. Klin. Monatsbl. Augenh., 104, 553-571.

Wada, M., Minamisono, T., Erhart, L. A., Naito, H. K. & Mise, J. (1977) Familial hyperlipoproteinaemia in Beagles. Life Sci., 20, 999-1008.

Walb, H. von. (1875) Ueber die traumatische Hornhautentzundung. Virchow Arch., 64, 113-135.

Walker, A. (1983) Feeding of dogs and cats. In the Henston Veterinary Vade Mecum, ed. J. M. Evans. Adlard & Son, Dorking, pp 252-269.

Walton, K. W. & Williamson, N. (1968) Histological and immunofluorescent studies on the evolution of the human atheromatous plaque. J. Atheroscler. Res., 8, 599-624.

Walton, K. W., Williamson, N. & Johnson, A. G. (1970) The pathogenesis of atherosclerosis of the mitral and aortic valves. J. Path., 101, 205-220.

Walton, K. W. (1973a) Studies on the pathogenesis of corneal arcus formation I. The human corneal arcus and its relation to atherosclerosis as studied by immunofluorescence, J. Path., 111, 263-273.

Walton, K. W. (1973b) The role of altered vascular permeability in the induction of experimental xanthomata. Nutr. Metab., 15, 59-67.

Walton, K. W., Thomas, C. & Dunkerley, D. J. (1973) The pathogenesis of xanthomata. J. Path., 109, 271-289.

Walton, K. W. α Dunkerley, D. J. (1974) Studies on the pathogenesis of corneal arcus formation II. Immunofluorescent studies on lipid deposition in the eye of the lipid-fed rabbit. J. Path., 114, 217-229.

Waring, G. O., Muggli, F. M. α MacMillan, A. (1977) Oval corneal opacities in Beagles. J. Am. Anim. Hosp. Ass., 13, 204-208.

Waring, G. O., Rodrigues, M. M. α Laibson, P. R. (1978) Corneal dystrophies of the epithelium, Bowman's layer and stroma. Surv. Ophth., 23, 71-122.

Waring, G. O., MacMillan, A., Spangler, W. L. α Roth, A. M. (1978) Crystalline corneal opacities in Siberian Huskies. Invest. Opthalmol. Vis. Sc., 17, (Supplement). 298.

Waring, G. O., MacMillan, A. D., Roth, A. M., Spangler, W. L. α Ekins, M. B. (1979) Lipid corneal opacities in Beagles and Siberian Huskies. Proc. Am. Coll. Vet. Ophthalmol., 10, 1-27.

Waters, L. L. (1959) Studies on the pathogenesis of vascular disease. Yale J. Biol. Med., 31, 213-230.

Waters, L. L. (1965) Corneal stromal reactions in rabbits following injection of hyperlipaemic and of delipidized homologous serum. Am. J. Path., 47, 51-59.

Watson, P. α Holt-Wilson, A. D. (1974) Corneal involvement in episcleritis and scleritis. Trans. ophthal. Soc. U.K., 94, 46-51.

Weller, R. O. (1966) The ultrastructure of intracellular lipid accumulations in atheroma. J. Atheroscler. Res., 6, 184-189.

Werb, Z. α Cohn, Z. A. (1971) Cholesterol metabolism in the macrophage. J. Exp. Med., 134, 21-44.

Winder, A. F., Sheraidah, G. α Fielder, A. R. (1978) Plasma low density lipoprotein and the composition of human corneal arcus. Biochem. Soc. Trans., 6, 1392-1393.

Winder, A. F. (1983) Relationship between corneal arcus and hyperlipidaemia is clarified by studies in familial hypercholesterolaemia. Brit. J. Ophthal., 67, (12) 89-94.

Wolman, M. (1974) Acid esterase as a factor in atherosclerosis. Atherosclerosis. 20, 217-223.

Wright, R. E. (1936) Degeneration of the cornea, calcareous and fatty. Arch. Ophthal., 15, 803-810.

Wyman, M. α McKissick, G. E. (1973) Lipaemia retinalis in a dog and cat: case reports. J. Am. Anim. Hosp. Ass., 9, 288-291.

Zeeman, W. P. C. α Groen, J. (1947) Lipidosis corneae. Geneeskundige bladen uit kliniek en laboratorium voor de praktijk, 41, 375-411.

Zemplényi, T. (1968) Enzyme Biochemistry of the Arterial Wall. Lloyd-Luke, London.

APPENDIX I

Case Histories and Synopsis of Findings.

Further details of the clinical cases are summarised in Fig. 5.1/1 and Tables 5.1/1, 5.7/1 and 5.7/7.

Alsatian 27 The owners had originally noticed a white spot in the animal's right eye when he was almost four years of age; it was situated paracentrally and inferiorly. It was diagnosed as an ulcer by the owner's veterinary surgeon and treated symptomatically. The opacity had gradually extended circumferentially to form a complete broad annulus, separated from the limbus by a relatively clear zone and extensively vascularised. The eye was noted to be reddened on occasions. Approximately two months after the opacity in the right eye was noticed a white spot appeared paracentrally and inferiorly in the left cornea.

The dog was referred for second opinion approximately five months after the opacity in the right eye had first been noticed. No observations concerning the animal's general health were made by the referring veterinary surgeon other than the fact that the dog suffered occasional bouts of diarrhoea. Examination indicated a placid and rather overweight dog with bilateral vascularised corneal opacities (Fig. 5.1/1).

Slit lamp examination revealed a dense white annular opacity in the right eye which extended throughout the full thickness of the stroma and was particularly dense in the region of the epithelial basement membrane and Descemet's membrane, possibly including the anterior portion of the latter. The stromal opacity consisted of numerous dot-like opacities and aggregates of scintillating

crystals; it was richly vascularised at all levels. The sclera was reddened and the conjunctival and episcleral vessels were injected.

The left eye contained an almost circular paracentral lesion into which ran a single superficial blood vessel. The lesion was composed of numerous dots and crystalline aggregates and located in the anterior and middle stroma. The eye was also reddened.

The results of laboratory examination were normal except for a slight elevation of serum lipids manifest on lipoprotein electrophoresis as an increased level of HDL_1 (as HDL_c). The free thyroxine level was 7.8pmol/l and total thyroxine was undetectable. Blood glucose was 4.1 mmol/l.

Keratectomy was performed and blood samples were repeated one month later. On this occasion cholesterol was 7.17mmol/l and free thyroxine 6.9pmol/l. It was felt that the animal's appearance and the results of laboratory examination indicated mild hypothyroidism and the dog was treated with L-thyroxine sodium (Eltroxin, Glaxo Labs. Ltd.) as set out by Crispin & Barnett (1978).

Approximately six months after surgery the owner reported that the dog's eyes appeared normal and that his health was much improved. Nothing further was heard for two years and then the dog was referred again for a second opinion and with a new owner. The new owner had not realised that the dog required continuous thyroid replacement therapy so none had been given for the nine months that the dog had been in her possession. The owner had sought veterinary advice because the dog had had occasional epileptiform fits and had apparently been blind for some three days following the last one two weeks previously. On examination the dog was grossly overweight (71kg) and bilateral corneal opacities were present. In addition to

a central/paracentral lesion which involved most of the cornea there was a peripheral arcus lesion with lipaemia and other abnormalities of the perilimbal vessels (Figs. 5.1/2; 5.1/3; 5.3/2). The changes in the perilimbal vessels were suggestive of atherosclerosis, which would conform with a history of epileptiform fits as a consequence of atheromatous emboli.

The results of laboratory examination showed cholesterol 18.06mmol/l, triglyceride 3.21mmol/l and phospholipid 9.25mmol/l. Agarose gel electrophoresis indicated an increase of HDL_c, LDL and VLDL.

Blood glucose was 2.96mmol/l and a normochromic, normocytic, anaemia was present. Total thyroxine was less than 5nmol/l and free thyroxine was less than 2pmol/l.

The dog was treated with L-thyroxine prior to keratectomy of both corneas and appeared in much better general health at a maintenance level of 0.2mg twice daily, although serum lipids and lipoproteins remained slightly abnormal, in that HDL_c persisted.

Alsatian 36 In this animal the owners had noticed recurrent bouts of redness in both eyes followed by the appearance of a rapidly developing opacity in the right eye and then, almost three months later, in the left eye.

On examination the dog appeared in good general health although slightly overweight. The right eye was more involved than the left and in both eyes there was extensive superficial vascularisation with some free haemorrhage and pigmentation. There was bilateral episcleritis. In the right eye an arcus was also present, as a complete peripheral annulus continuous with the limbus. The stromal

opacity in the right eye involved about half the stromal thickness, that of the left eye involved the anterior third (Fig. 5.1/1 α 5.1/4).

An initial blood sample revealed no lipoprotein abnormality other than increased HDL_1. A keratectomy was performed three months later, despite the eyes being somewhat inflammed; a blood sample taken at this time indicated cholesterol, 8.68mmol/l; triglyceride, 1.01mmol/l; phospholipid, 3.99mmol/l; and free thyroxine was estimated as 6.48pmol/l. Thyroid replacement therapy was instituted on the basis of these results and the dog was in good general health, without recurrrence of the more central corneal opacities and some clearance of the peripheral arcus, some two years after keratectomy.

Alsatian 153 The history was of an animal in good general health which developed bilateral corneal opacities within a week of each other and with no apparent ocular abnormality other than perilimbal reddening. Slit lamp examination indicated a vascularised dense white crystalline opacity which was almost symmetrical and involved the anterior third of the stroma. There was a clear zone between the opacity and the limbus and the sclera in close proximity to the opacity was reddened and inflamed (Fig. 5.1/1).

Laboratory examination revealed no abnormalities and samples repeated two months later were also normal (cholesterol, 5.41mmol/l; triglyceride, 0.29mmol/l; phospholipid, 4.01mmol/l). By this time there was also epithelial involvement in the left eye and a number of ulcerated areas stained positively with fluorescein. There did,

however, seem to be some reduction in the density of the opacity in the ulcerated areas and, as this was accompanied by only minimal ocular discomfort, the dog was not treated but was kept under observation.

Over a period of six months there was a reduction in the density of the opacity in both eyes accompanied by increased vascularisation; the new blood vessels arising at conjunctival, episcleral and scleral levels. Punctate erosions occurred in both corneas but were more numerous in the left eye. The animal was lost to follow up approximately eight months after being seen for the first time.

Alsatian 160 The history was of the sudden appearance of bilateral corneal opacities within one week of each other in this bitch of almost four years of age. She was overweight but otherwise in good general health.

The corneas of both eyes contained crescentic paracentral opacities of white, plaque-like, appearance (Fig. 5.1/1). Slit lamp examination demonstrated subepithelial, scintillating, acicular crystals and a more diffuse, non-crystalline, haze, the latter being more readily observed at the periphery of the main opacity. Blood vessels entered the lesions at the level of the opacities. Keratectomy was performed in the right eye a week after referral.

No abnormalities were detected on laboratory examination other than a mild increase in serum cholesterol identified as HDL_c on serum electrophoresis.

The owners were advised to give the dog more exercise and to feed only proprietary dog foods. On this regime the dog lost 2kg in

weight and there was a reported decrease in the intensity of the opacity in the left eye four months after the regime was introduced.

Alsatian X 150 The owners had noticed a grey haze which developed first in the right eye, and then in the left, over a period of six months when the dog was only one year old. There were no signs of pain and the dog appeared in otherwise good health. Their veterinary surgeon tentatively diagnosed chronic superficial keratitis and the dog was put onto betamethasone topically (Betsolan drops, Glaxovet) and by mouth (Betsolan tablets, Glaxovet). This treatment produced little change in the clinical appearance and a second opinion was asked for. All medication was stopped for four weeks before the dog was seen and examination took place eight months after the lesion had first been noted in the right eye (Fig. 5.1/1).

The bitch was in good general health although slightly overweight. She had been neutered at the age of six months prior to her first oestrus. Serum cholesterol and phospholipid were slightly raised. Free thyroxine was 12.5pmol/l and this rose to 30.2pmol/l 12 hours after the intramuscular injection of 10 units (0.4 Units/kg) of bovine T.S.H. (Lorenz α Stiff, 1980).

Slit lamp examination indicated extensive superficial greyish opacities in both eyes, pigmentation and vascularisation. Stromal involvement was apparently limited to the anterior third and the lesion was composed of a myriad of fine grey dots and a more diffuse grey haze beyond the resolution of the slit lamp. In the limbal region immediately underlying the nictitating membrane there was a very fine arcus which merged imperceptibly with the limbus. In

opaque regions other than those formed by the arcus occasional collections of scintillating crystals were apparent within the anterior stroma. Pigmentation appeared to be limited to the posterior layers of cells in the epithelium.

Intravascularly injected sodium fluorescein appeared in the cornea approximately nine seconds after injection; it leaked into the opaque areas from the extensive new blood vessels and a small amount of leakage from the tips of some superficial vessels into the tear film was also observed.

Keratectomy was performed, the animal returned home on a low fat diet and the owners were instructed to give the dog more exercise. Thyroid function was certainly below average in this dog and will be reassessed at the six-monthly check up, or before, should any overt signs of hypothyroidism develop.

Golden Retriever 1 The owners of this dog had noticed bilateral opacities of somewhat crystalline appearance when the dog was about two years of age; the opacity of the left eye had preceded that of the right. The dog was said to be in good general health and no ocular discomfort had accompanied the development of the lesions.

Examination confirmed the dog's excellent general condition and the results of laboratory examination were normal. There were bilateral, but asymmetrical, subepithelial and anterior stromal opacities in both corneas and these had a dense white crystalline appearance with the slit lamp. No anterior segment inflammation or pain was present, and the perilimbal vessels appeared normal (Fig. 5.3/1). Fine superficial corneal neovascularisation was apparent in

the infero-temporal quadrants of both eyes (Fig. 5.1/1). Both lesions were completely removed by keratectomy at the time of referral, there had been no recurrence two years post-operatively.

Golden Retriever 4 This dog was the mother of the previous Golden Retriever (1) and, in her case, the opacity had developed in the left eye almost 12 months before. Within two weeks there was also involvement of the other eye (Fig. 5.1/1).

The owners had noticed some intermittent ocular discomfort and the eyes were reddened during these episodes; they thought that some of these periodic attacks had preceded the appearance of visible corneal opacities.

Examination indicated a slightly overweight animal in good general health. Laboratory examination demonstrated no abnormalities other than a mild hypercholesterolaemia with an increase of high density alpha$_2$-lipoproteins (HDL_c).

Slit lamp examination revealed two extensive opacities of "candy floss" appearance in the right eye with areas of punctate epithelial erosion which were fluorescein positive. Some vascularisation was present, mainly derived from conjunctival vessels but also from the deeper perilimbal vascular plexus. The crystalline opacity was most marked as a well defined band approximately half way through the stroma with clearer regions of stroma anteriorly and posteriorly. There was also a less clearly defined, more diffuse, haze which was most obvious immediately beneath the corneal epithelium and at the borders of the crystalline lesion (Fig. 5.1/6). Findings in the left eye were substantially similar except that one opacity was present, there was less

epithelial erosion, and the crystalline opacity was more readily defined as an irregular lattice of white spicules and larger more amorphous masses with some clear spaces (Fig. 5.1/7).

In both eyes there was irregularity of corneal thickness and mild injection of superficial and deeper vessels in the immediate vicinity of the corneal lesions. The ocular discomfort may have been a consequence of the epithelial erosions. Keratectomy was performed at the time of referral, and no specific therapy other than a low fat diet was advised. There was mild recrudescence in both eyes a few months after surgery, but without any epithelial erosions or discomfort; at this time HDL_C was the only obvious lipoprotein abnormality. The owners reported that the appearance of the eyes had changed little one year later and that the dog was in good general health.

Golden Retriever 6 This dog had intermittent reddening of the right eye over a period of two months; during this time the owner's veterinary surgeon had treated the eye with topical corticosteroids. At some time in the first month of anterior segment inflammation two opacities appeared. The left eye appeared normal throughout.

The dog was referred for a second opinion approximately four months after the red eye had first been noticed. At this stage the eye was quiet, there were no signs of systemic disease, laboratory examinations were normal and the dog was of normal weight and appearance. The iris of the affected eye was darker than that of the unaffected eye, suggesting a previous iritis.

Slit lamp examination indicated that the two opacities of the right eye involved the epithelium and were located mainly in the

anterior stroma. The opacity of the supero-nasal quadrant was slightly less dense and more heavily vascularised than that of the infero-temporal quadrant. Both lesions were composed of scintillating golden-yellow crystals and white dense amorphous masses with occasional clearer spaces. As in other cases the opacities were bordered by diffuse grey dots rather than crystalline material (Figs. 5.1/1 α 5.1/8). There was some variation in epithelial and subepithelial thickness in the region of the opacities, whereas intervening areas were of normal appearance.

Keratectomy was performed a few weeks after the dog had been presented for a second opinion, she made an uneventful recovery and repeated laboratory examinations, during the time of observation and the year following surgery, were uneventful.

Golden Retriever 9 The owners reported that the dog had suffered from recurrent bouts of ocular redness over the previous five months; they were also aware that she was gaining weight despite feeding a reduced amount of carbohydrate. They thought that the white opacities had appeared, first in the right eye and then, a month later, in the left eye, about one month after the reddening of the eyes had first been noticed and that the opacities enlarged slightly each time the eyes became reddened.

The dog had shown irregular oestrus cycles of late and was reluctant to exercise; she was also intolerant of cold.

Examination revealed a placid overweight animal with a subnormal temperature (37.9°C) and bilateral white plaque-like corneal opacities (Fig. 5.1/1).

Slit lamp examination indicated that the opacities were

situated within the anterior stroma with prominence of diffuse haze anterior to more deeply situated scintillating crystals. The epithelium was intact but appeared thicker in some places than others, and there was also subepithelial infiltration (Fig. 5.1/9). Conjunctival and episcleral vessels appeared congested and tortuous and the sclera next to the opacities was diffusely reddened. There was mild ocular discomfort and the opacities were vascularised.

Laboratory examination indicated that all lipoprotein fractions other than alpha lipoproteins and chylomicrons were increased, and that serum lipids were raised. Blood glucose was low at 2.5mmol/l and free thyroxine was 5.7pmol/l. The dog was put on to L-thyroxine sodium and serum lipids and lipoproteins returned to normal within two months.

She was kept under observation for one year, during this time becoming livelier and losing weight; the density of the corneal opacities also reduced, so that clear zones of almost normal cornea were present next to dense crystalline aggregates (Fig. 5.1/5). The owner was kind enough to permit keratectomy of what was judged to be a resolving lesion in a normolipoproteinaemic animal at this stage. After surgery, the dog remained in good general health, and there was no recurrence of anterior segment inflammation, or lipid deposition, during the three years that she was followed up.

Golden Retriever 14 This dog was apparently in normal health until the owners noticed that she was blinking more frequently than usual; they observed that the left eye was a diffuse, salmon-pink colour and that over a period of days rather cloudy crescentic opacities developed in a paralimbal position. During

the next six weeks the opacities became a dense chalky-white and the eye was less inflamed and free of pain (Fig. 5.1/1).

Examination confirmed the presence of a unilateral lesion composed of two separate vascularised plaque-like opacities; the eye was free of pain and redness. Dense crystalline and amorphous material was located as a fairly well defined band at almost the middle of the stromal thickness with a finer less dense haze of numerous small grey-white dots anterior to the band and around its entire circumference. Both opacities were of the same distribution and appearance and the intervening cornea appeared normal. Partial keratectomy of the temporal lesion was performed, the opacity was too deep for complete removal using this procedure.

Laboratory examination indicated an increase of high density alpha$_2$-lipoproteins (HDL_c) as the only abnormality and the dog was put onto a low fat diet of commercial dog food. The owners reported that the opacities were less dense approximately nine months later and that the opacity in the area of incomplete removal had almost completely disappeared.

Golden Retriever 79 This animal was training as a guide dog for the blind. He had apparently been perfectly fit and working well when bilateral corneal opacities developed over a matter of days. These were treated with antibiotic ointment and the dog was thought to be improving a few days later, but then there was another period when the eyes appeared reddened and inflamed and the opacities enlarged and became denser. Subconjunctival methylprednisolone (Depomedrome, Upjohn) was given and the eyes remained quiet for almost six months. There was then another flare

up diagnosed as superficial keratitis and treated with topical framycetin (Soframycin, Roussel) and betamethasone (Betnesol, Glaxo) with no effect. The dog was referred for a second opinion approximately seven months after the original eye trouble (Fig. 5.1/1).

Examination indicated a reasonably fit castrated male dog with no obvious abnormalities on physical examination except his eyes. Slit lamp examination indicated three discrete round opacities of differing density in the right eye and a single paralimbal crescentic opacity in the left eye (Figs. 5.1/10 α 5.1/11). There was some tortuosity and injection of the conjunctival and, to a lesser extent, episcleral vessels, and superficial vessels invaded all the opacities. Both irises were dark and a low grade anterior uveitis was present.

The opacities of the right eye consisted of dense white crystalline aggregates for one of the lesions and much more diffuse grey amorphous masses for the other two. The crescentic opacity of the left eye closely resembled the single crystalline opacity of the right. Fine, superficial blood vessels supplied the almost circular crystalline and amorphous aggregates in the right eye; a further crystalline opacity developed quite precipitately in a previously non-vascularised area of amorphous corneal opacity, coincident with neovascularisation.

No reason for the keratitis could be found on clinical and ophthalmoscopic examination, which included corneal and conjunctival smears and culture and Schirmer tear testing, in addition to the other tests routinely employed; a diagnosis of sclerosing keratitis was made because of the progressive nature of the condition, and

also the close association of perilimbal inflammation and corneal changes. The dog was treated with topical oxyphenbutazone ointment (Tanderil, Geigy) and the eyes quietened leaving clearly demarcated white lesions.

Examination of venous blood samples consistently revealed an increased level of serum cholesterol and high density alpha$_2$-lipoprotein (HDL_c) as the only abnormality; the dog was put onto a low fat diet of commercial dog food and retired as a potential guide dog in view of the guarded prognosis. Insufficient material was available for comparison to assess any effect castration may have had on the serum lipoprotein pattern.

The dog was lost to follow up when a new home was found for him, but there had been no change in the appearance of the sclerosing keratitis in the 14 weeks of follow up prior to this.

Golden Retriever 151 This rather overweight bitch was thought to have a skin problem by the owners as her coat had thinned over the past few months. They sought veterinary advice when they noticed dull golden opacities in both corneas. The dog was lethargic and placid, she had bilateral and similar central and paracentral corneal opacities which took the form of multiple golden needle-like opacities when viewed with the slit lamp. There was no vascularisation and the opacities were situated throughout the stroma although there was no apparent involvement of Descemet's membrane and no diffuse opacification was present in the subepithelial zone (Fig. 5.1/1). The dog had normal vision and no pain although the owners had noticed occasional bouts of circumcorneal redness over the past weeks. Keratectomy was

performed in the right eye at the time of first examination.

Laboratory examination was normal except for raised serum lipids and an increase of high density alpha$_2$-lipoproteins (as HDL_c). The free thyroxine level was 8.3pmol/l on the first occasion. Two months later it was 7.6pmol/l and the free thyroxine level rose to only 12.4pmol/l after TSH stimulation. It was decided that her clinical appearance and the results of laboratory tests warranted a diagnosis of hypothyroidism and she was treated with liothyronine sodium (Tertroxin; Glaxo) at a maintenance dose of 20mcg four times daily. According to the owners, she progressed well on treatment and was generally livelier. No recurrence of the lesion in the right eye was reported, the left eye was said to look the same as before.

Golden Retriever 154 This dog had developed eye trouble in the left eye when she was just over three years of age. There were no other details available.

Examination indicated a slightly underweight bitch in good general health; the owners reported that she had a huge appetite. The corneal opacities were in the form of round and crescentic subepithelial crystalline aggregates. Superficial vessels ran into the crescentic opacity whereas the round opacity was not vascularised (Fig. 5.1/1).

Venous blood samples were normal except for a slightly high blood glucose level (5.8mmol/l) which was repeated two months later and found to be slightly lower (5.3mmol/l). The owners were advised to return the dog for further investigation if there were any other changes in eating and drinking habits.

As the left eye was quiet and free of pain no treatment was prescribed. Unfortunately the dog was lost to follow up about 14 months after the eye trouble had first been noticed, although the owners reported that the appearance of the eye had changed little over a 12 month period.

Golden Retriever 155 The owners had noticed recurrent episodes of pain and redness in this dog's eyes when she was just over three years of age. Approximately one month after these signs had first been noticed bilateral cloudy crescentic corneal opacities were noticed close to a very reddened region of sclera. No treatment of any kind had been given and the dog was referred immediately for a second opinion when the corneal opacities were noticed (Fig. 5.1/1).

Examination indicated a dog in good general health with signs of active scleritis and bilateral and similar crescentic corneal opacities. There was also a low grade anterior uveitis.

With the slit lamp the opacities were seen to consist of masses of closely packed needle-like crystals situated in the middle and anterior corneal stroma. Blood vessels entered the cornea at superficial and deeper levels; ramifying freely within the lesion at superficial level and pursuing a much straighter course when of deeper origin. The sclera adjacent to the crescentic opacities was of a dark salmon-pink colour and episcleral vessels in this region were darkened and tortuous.

Venous blood samples indicated a mild increase of high density alpha$_2$-lipoprotein as the only abnormality. The owners were given advice on feeding a diet with less fat and increasing the dog's exercise. The eyes were treated with oxyphenbutazone cream

applied topically and the eyes became less reddened within two days of starting this treatment, there had been no recurrence of the redness some six weeks later and the lipoprotein pattern was within normal range (cholesterol, 5.2mmol/l; triglyceride, 0.41mmol/l; phospholipid, 4.8mmol/l). The corneal opacities were less dense approximately one year later.

Springer Spaniel 65 This animal developed a whitish-grey vascularised opacity in the right eye following an anterior uveitis of unknown cause. Laboratory examinations were essentially normal, there was a mild increase in HDL_c.

The lesion extended quite deeply within the stroma and was composed of numerous opaque dots of various sizes and aggregations of scintillating crystals. The epithelium appeared normal. Vascularisation was entirely at stromal level and corneal melanosis was absent (Fig. 5.1/1).

Keratectomy was performed and the animal was put onto a low fat diet of commercial dog food only. The owners reported that the dog was in good health and with no recurrence of eye trouble approximately three months after keratectomy.

Springer Spaniel 66 This dog was noticed to have grey-white rather granular lesions in both corneas which developed without any apparent inflammatory antecedent. In the left eye the opacity was more discrete with a crescentic outline and oedematous appearance. Crystalline deposits could be seen within the stroma and there were some very fine blood vessels present. In the right eye crystalline deposits were also apparent but of a much more diffuse disposition

and without vascularisation (Fig. 5.1/1).

Serum lipids were raised and there was an increase in both high density alpha$_2$- and low density lipoproteins. Free thyroxine was not estimated, total thyroxine was 36nmol/l. Keratectomy was performed at the time of first examination and the dog made an uneventful recovery, only to develop lymphosarcoma two months later. She was destroyed three months after the keratectomy and was not available for post mortem.

A litter sister also developed crystalline opacities in both corneas and she had a cholesterol level of 8.76mmol; triglyceride, of 1.26mmol/l; and phospholipid, 6.04mmol/l. Another litter sister had normal serum lipids (cholesterol, 5.14mmol/l; triglyceride, 0.51mmol/l) and no ocular involvement; whereas her son, aged seven months, had a cholesterol level of 7.39mmol/l and triglyceride of 0.92mmol/l, but without ocular involvement.

The mother of the three bitches (aged 4 years 9 months) had a cholesterol level of 8.79mmol/l, and triglycerides of 1.56mmol/l with no detectable total thyroxine, whereas her mother aged 7 years 5 months had normal serum lipids (cholesterol, 4.54mmol/l and triglyceride, 0.70mmol/l). Neither of these dogs had any signs of ocular involvement.

Unfortunately further investigation of the family was not possible after the original patient (66) had died. The possibility of hypothyroidism in the patient and her relatives must remain conjectural.

Springer Spaniel 131 A cat had scratched this dog's right eye about four months before. The ulcerated superficial lesion had been

treated symptomatically and had healed satisfactorily with vascularisation from the limbus. Approximately three months after the initial injury the cornea became cloudy, rather suddenly, at the site of the original scratch.

The dog was in good general health and serum lipids were on the high side of normal. There was a vascularised peripheral opacity which was of a crystalline and granular appearance centrally, fringed by a more diffuse finely granular haze peripherally (Fig. 5.1/1).

Vascularisation was entirely superficial and the opacity was located in the anterior stroma, the epithelium was intact and slightly raised in comparison with epithelium adjoining the opaque area.

Repeated serum lipid estimations and other laboratory tests revealed no abnormality other than a mild increase of high density $alpha_2$-lipoproteins and a concomitant decrease in $alpha_1$-lipoproteins. The lipoprotein pattern returned to a more normal high $alpha_2$-lipoprotein distribution after approximately three months of a low fat, high fibre, diet. Eventually the site of the original ulcer was marked by a dull grey-brown opacity and numerous ghost vessels which were only visible with the slit lamp biomicroscope.

English Springer Spaniel 142 In this animal the history was of a cat scratch to the left eye followed by the rapid development of a corneal opacity. The animal was overweight and rather lethargic and the owners reported that oestrus had not occurred at regular intervals for about two years. Serum lipids and

lipoproteins were abnormal with an increase of high density alpha$_2$-lipoprotein, low density lipoprotein and very low density lipoprotein.

Slit lamp examination indicated extensive ocular involvement of the left eye. The conjunctival and episcleral blood vessels were tortuous and dilated and lipaemia was evident. Two main areas of corneal opacity containing granular and crystalline material were present, in addition to a peripheral arcus of more diffuse, whiter, and pigmented composition. The two discrete areas of opacity were richly supplied by arborescent superficial blood vessels (Fig. 5.1/1).

Thyroid function tests were abnormal. Free thyroxine was less than 2pmol/l and remained at less than 2pmol/l following TSH stimulation.

The animal was treated with L-thyroxine sodium and maintained at a level of 0.15mg twice daily. Serum lipids and lipoproteins returned to normal on this regime and she became more active. According to the owners there was some clearance of the corneal lesion over a period of two years.

Welsh Springer Spaniel 42 The owners reported that the dog had suffered from intermittent bouts of ocular pain and a watery discharge for approximately eight months. Their veterinary surgeon had treated the eyes with a topical corticosteroid-antibiotic preparation (Betsolan, Glaxovet) but the owners thought that this may have exacerbated the problem as they noticed white spots in both corneas within two days of the treatment and some seven months after the trouble first started. The dog was referred for a second

opinion. She was in good general health although slightly overweight, and had large white, subepithelial, corneal opacities of finely granular appearance in both eyes (Fig. 5.1/1). There was a fine vessel running into the opacity of the left eye and it terminated in a less dense area. No vascularisation was present in the right eye. The eyes were not inflamed at this time but the owners were requested to bring her back as soon as there was any recrudescence. They returned six days later and episcleritis and scleritis were present on this occasion accompanied by mild ocular discomfort and a serous ocular discharge. Topical oxyphenbutazone was prescribed and a low fat diet suggested. The bitch returned for keratectomy of the right corneal opacity approximately one month after it was first noticed. The lesion extended throughout the superficial stroma without involvement of the epithelium, no crystals were apparent and the opacity comprised a diffuse grey-white film composed of small discrete dots and a more homogeneous haze.

Almost two months after successful surgery to the right eye (Fig. 5.1/12), the dog returned for keratectomy to the left eye. In all, her lipids and lipoprotein patterns were examined five times during the period of investigation up to keratectomy, and results were similar to those given in Table 5.7/1 in that a mild increase of high density alpha$_2$-lipoprotein as HDL$_c$, was the major abnormality, in conjunction with rather low triglyceride levels. The last sample of this series was taken approximately two months after instituting a low fat diet and this indicated cholesterol, 6.03mmol/l; triglyceride, 0.28mmol/l and phospholipid, 5.47mmol/l. Following surgery the bitch remained in good general health and free

of eye problems for more than a year. There was then an exacerbation of the earlier ocular signs with recurrent episodes of ocular redness and, after the recrudescence, opacities reappeared in both corneas, but were less extensive than originally (Fig. 5.1/13). The owners admitted that they had allowed the low fat diet to lapse and a blood sample taken at this time indicated cholesterol, 7.38mmol/l; triglyceride, 0.12mmol/l and phospholipid, 6.96mmol/l. Fluorescein angiography of the anterior segment indicated considerable leakage of fluorescein within the stroma of both corneas at a time when the eyes were reddened and perilimbal vessels prominent (Fig. 5.4/4).

Partial keratectomy of the right eye (R2) was undertaken when the eyes were quiet following topical corticosteroid therapy. The lesions had a dense, white, plaque-like appearance at this time (Fig. 5.1/14). The corticosteroid may have modified the morphology of the lesion because of its inhibitory effects on neovascularisation.

Repeated episodes of anterior segment inflammation with marked pain, redness and photophobia, resulted in the deposition of further quantities of lipid in both corneas, but to a much lesser extent than previously. The opacities enlarged with each flare-up of inflammation. There were also signs of a low grade anterior uveitis which culminated in posterior synechiae formation and darkening of both irises. Lipids and lipoproteins remained abnormal during the time of episodic anterior segment inflammation; cholesterol was 8.04mmol/l and triglyceride 0.18mmol/l in one sample taken during this period.

The dog was treated symptomatically with a topical parasympatholytic (atropine) and a non-steroidal anti-inflammatory agent

(oxyphenbutazone). In addition, anti-prostaglandins (alternate use of aspirin and phenylbutazone) were given orally. Diet was modified so as to include a higher proportion of fibre and the amount of daily exercise was increased. It would be difficult to assess the efficacy of any single aspect of this regime, but it was of interest that serum lipids and lipoproteins returned to a more normal distribution over a period of months and there were no further bouts of anterior segment inflammation; perhaps the abnormal blood lipids provoked the anterior segment inflammation. Furthermore, the lesions started to regress spontaneously. Within a year of regression commencing, the bitch was admitted for removal of a small skin tumour in the flank region and the owners kindly allowed removal of the resolving opacity in the right cornea at the same time (R3).

The dog has remained in good health for the past 18 months, the right eye has remained free of opacity and the left eye has almost cleared spontaneously (Fig. 5.1/15). There has been no recrudescence of the ocular reddening during this time and the last blood sample taken indicated cholesterol, 5.8mmol/l; triglyceride, 0.22mmol/l; phospholipid 6.1, mmol/l.

Cocker Spaniel 24 The owners reported that the dog had developed hazy eyes rather suddenly almost three months previously with the right eye developing slightly in advance of the left. The eyes appeared red on occasions and the dog was seen to rub them frequently.

On examination the dog was found to be slightly overweight and the eyes appeared dry and irritating. Slit lamp examination

indicated a bilateral stromal opacity which extended from superficial stroma to at least mid-stroma. The epithelium was slightly uneven but not ulcerated. The conjunctival and episcleral vessels were congested and there was a mild mucoid discharge. The Schirmer tear test was marginally below normal at 9mm/minute (normal rate in the dog is at least 10 to 25mm/minute). The stromal opacity was of a mainly milky-white colour in both eyes and in, the right eye, there were occasional aggregations of fine scintillating golden crystals with sparse vascularisation at approximately episcleral level, vascularisation was not seen in the left eye.

Laboratory examination indicated a blood glucose of 2.6mmol/l and raised serum lipids with an increase of alpha$_2$- high density lipoproteins particularly, but also an increase of low density lipoproteins. Free thyroxine was 8.6pmol/l. Lipid analysis was repeated one month later and yielded cholesterol, 11.86mmol/l; triglyceride, 1.02mmol/l and phospholipid, 5.63mmol/l. The free thyroxine level was 6.4pmol/l and increased to only 9.7pmol/l after TSH injection. The dog was put onto L-thyroxine sodium and one month after reaching a maintenance dose of 0.1mg twice daily the serum lipids and lipoprotein patterns appeared normal. Keratectomy was performed at the time of first referral and the eyes remained free of lipid following this procedure. The owners were given a false tear preparation to apply to the dog's eyes as necessary.

Cocker Spaniel 83 This animal had had a skin problem of unknown cause for most of his life. The skin was dry and scurfy, the coat was dull and lifeless and he had been treated with a variety of saturated fats to improve his appearance. In addition he

had been given a five week course of corticosteroids by mouth, the type and amount was not specified. Recently he had developed a recurrent redness of both eyes but more marked in the infero-temporal region of the left eye. A grey haze had been noticed within the cornea adjacent to the inflammed region and separated from the limbus by a clear zone (Fig. 5.1/1). There was also a much less clearly defined but almost symmetrical grey zone in the right eye.

Examination indicated that the dog was slightly overweight and rather lethargic. The owners volunteered the information that he was never very energetic; whereas two other dogs kept were much livelier. His skin was dry and cool to the touch.

Both scleras were diffusely reddened and a nodular scleritis was present in the left eye. The crescentic grey opacities consisted of a multitude of fine dots and a grey haze beyond the resolution of the slit lamp; no vascularisation was present. The main opacity was at the level of anterior Descemet's membrane and the posterior stroma, with a very faint fine haze beneath the anterior epithelium as a minor component of the lesion (Fig. 5.1/16).

Serum lipids were all increased and both high density alpha$_2$- and low density lipoproteins stained with greater intensity; alpha$_1$- staining was reduced. Total thyroxine was estimated at 12.0nmol/l, free thyroxine at 15.2pmol/l, and mild hypothyroidism was suspected.

The dog was put on to a low fat diet consisting of commercial tinned dog food as 30% of his diet and dry biscuits as 70%. The recurrent scleritis responded to topical corticosteroid (Synalar Lotion; I.C.I.).

The dog was seen three months later and there was no change in his

general condition. The scleritis had not recurred but the grey opacities persisted in the corneas.

Laboratory examinations were repeated approximately two months later as the patient was actually gaining weight despite the low fat diet. At this stage apathy was marked, he weighed 17.5kg and there was a discoidal opacity in the left eye in close proximity to the original arcus and invaded by a single fine superficial blood vessel from the limbus. (Fig. 5.1/17). Slit lamp examination confirmed that the opacity consisted largely of glistening crystals in a largely subepithelial location.

Cholesterol levels were 10.6mmol/l; triglyceride, 1.62mmol/l and phospholipid, 8.20mmol/l. Free thyroxine was estimated as 8pmol/l; total thyroxine was unrecordable. A normocytic, normochromic, anaemia was present (red cells 4.72 x 10^{12}/l; PCV, 0.33l/l; haemoglobin, 10.9g/dl; white blood cells 6.1 x 10^{9}/l).

Despite the fact that the only clinical signs were apathy and increased body weight all the other investigations indicated hypothyroidism and the dog was placed on oral treatment with L-triiodothyronine sodium (Tetroxin; Glaxo Ltd.). The initial level was 40mcg in divided doses arriving at a maintenance level of 120mcg in divided doses.

On this regime there was a swift improvement in his appearance, the apathy and lethargy disappeared to reveal a friendly lively dog. The vascularised corneal opacity started to clear from the centre within one week of thyroid replacement therapy commencing, the arcus lipoides corneae remained unchanged (Fig. 5.1/18).

Serum lipids were restored to near normal within one month of treatment, and the dog's weight went down to 15.4kg over a period of

six months.

Cocker Spaniel 133 This dog was referred because of a striking proptosis of the left eye of fairly acute origin and no obvious cause (Fig. 5.1/1).

The animal was slightly subdued with obvious ocular pain in the left eye and mild pyrexia (102.8°F). In other respects she was in good health. Examination indicated that the eye was proptosed, the nictitating membrane reasonably prominent and the cornea diffusely opaque and slightly oedematous. When the mouth was opened, a procedure resented by the patient, a slight swelling could be seen in the roof of the mouth, caudal to the last molar tooth and underlying the orbit of the left eye. A diagnosis of retrobulbar abscess was made and this was drained via the mouth under general anaesthesia. A five day course of penicillin (Mylipen, Glaxovet) was also given and the dog made an uneventful recovery. Whilst the appearance of the eye generally appeared normal after treatment, the corneal opacity gradually assumed a less diffuse and more granular appearance and there were occasional aggregations of scintillating crystals. The cornea became vascularised some six months after drainage of the abscess and gradually the opacity cleared.

The dog was next seen some two years after the original surgery and small amounts of opacity remained within the vicinity of the blood vessels. It was of interest that, whilst originally there had been a marginal increase in HDL_1 only, and no other abnormalities detected; two years later the cholesterol level was 8.43mmol/l; triglyceride, 0.84mmol/l; and phospholipid, 6.02mmol/l. Free thyroxine was 18.4pmol/l and the dog was now overweight. A low

fat diet was advised and, as the owners owned a public house, the importance of enforcing this was emphasized. The cornea continued to clear on this regime and the dog was reported to be more active for a time. The owners later reported that her weight was increasing and that she had become very lazy; they were asked to bring her back for further investigation but have not yet done so.

Rough Collie 73 The client's veterinary surgeon noticed what appeared to be a fluid-filled lesion in the dog's left eye when she was neutered at approximately two and a half years of age. There was also some form of opacity in the right eye. When questioned the client thought that there had been something in both eyes for about six months and that they were first noticed when the bitch was on heat. The animal was referred to a second veterinary surgeon for another opinion and keratectomy was performed on the left eye only; no lipid was found. A blood sample taken at the time of surgery indicated 4.52mmol/l of cholesterol.

The lesion of the left eye recurred, or possibly was incompletely removed, and the dog was referred for a third opinion nine months later (Fig. 5.1/1). The owners reported that the dog was in good health, that there was no pain associated with the opacities and the animal's vision was unaffected.

Examination indicated a crystalline paracentral opacity of the right eye and a larger, more paralimbal, opacity of the left eye (Fig. 5.1/19). No vascularisation was present in the right eye and the opacity was limited to the anterior stroma. In the left eye there was epithelial involvement with slight superficial ulceration, vascularisation and pigmentation. The opacity in the left eye

comprised crystalline aggregates and a diffuse grey haze with areas of clear cornea of more normal appearance in between. Some of the clear areas coincided with active vascularisation; and the impression was of vascularisation aiding regression of the opacity in such regions.

Fluorescein angiography revealed the rich vascular pattern of the opacity in the left eye, whereas no fluorescein was located within the opacity of the right eye. The fluorescein appeared in the opacity of the left eye ten seconds after intravenous injection; at this time it was located entirely within the superficial blood vessels, but by 20 seconds there was a rather more diffuse spread within the opacity.

The lesions were completely excised by superficial keratectomy and the results of laboratory examination at the time of surgery were normal. The appearance of the left eye was partly due to the presence of ocular nodular fasciitis, as judged from its histological appearance (Bellhorn & Henkind, 1967; Lavignette & Carlton, 1974; Smith, Bistner & Riis, 1976).

Rough Collie 134 This dog was referred for a second opinion because of hyphaema in the right eye. On examination there was resolving intraocular haemorrhage in the right eye and severe Collie eye anomaly in the left eye. The haemorrhage in the right eye was almost certainly a consequence of Collie eye anomaly. In addition to the intraocular abnormalities the dog also had an opacity of very crystalline appearance paracentrally in the right eye with an extensive supply of ghost blood vessels. There was a lesion resembling nodular fasciitis in the region of the temporal limbus

in the left eye, which encroached slightly on the cornea, and a crescentic opacity of finely granular material bordered it (Fig. 5.1/1).

No abnormality of serum lipids other than a slight increase in alpha$_2$ high density lipoproteins was demonstrated. Other laboratory investigations were normal.

The nodular fasciitis was treated with topical corticosteroids and the owners advised as to a suitable low fat diet and increased exercise for the dog. The nodular fasciitis resolved after six weeks treatment with a gradual tailing off of corticosteroid therapy after four weeks of treatment. The intraocular haemorrhage of the right eye also disappeared during this time and the presence of severe collie eye anomaly in this eye was confirmed. Pre-retinal vascular loops were also observed. The appearance of the crystalline lesion in the right eye showed little change, whereas the cornea of the left eye cleared except for multiple crystalline aggregates of diffuse distribution which were so small that they could only be observed with a slit lamp. The significance of these aggregates in the left eye is unclear. Approximately one year after the original referral the dog was presented again for hyphaema in the left eye; at this time cholesterol was 7.53mmol/l; triglyceride, 0.36mmol/l; and phospholipid, 6.08mmol/l. The owners were given further advice about feeding a low fat, low cholesterol, diet of commercial dog food. The hyphaema was probably a consequence of intraocular haemorrhage from abnormal choroidal or retinal blood vessels associated with the Collie eye eye anomaly; it appeared to be undergoing slow resorption. Two years after the original referral, the owners wrote to say that the dog had been destroyed

because of a possible brain tumour; no post-mortem was performed.

Rough Collie 149 The owners had noticed that the right eye was very prominent and their veterinary surgeon had diagnosed an anterior lens luxation and performed a lendectomy. Some six months after this the owners had noticed that the dog appeared in pain from time to time and that these episodes were accompanied by anorexia, diarrhoea and occasional vomiting. The right eye became white and opaque during this period; the left eye appeared normal. There was also weight loss, from being slightly overweight the dog became thin and hyperactive; she also shed her coat more readily. Investigation included tests of pancreatic function and a tentative diagnosis of subacute pancreatitis was made and this was treated symptomatically. A blood sample taken at this time was sent for lipid and lipoprotein analysis and the results are given in Table 5.7/1. The results of lipoprotein electrophoresis revealed a prominent beta-lipoprotein band and a mild increase in alpha$_2$-lipoprotein.

The owner's veterinary surgeon referred the case for second opinion. Examination indicated a thin but alert dog with no abnormalities detectable on physical examination other than ocular ones. The left eye had severe Collie eye anomaly with a large scleral ectasia, the right eye was phthitic and blind with considerable granular and crystalline opacification and an arcus of milky appearance. The opacity involved most of the cornea with a clearer zone between the peripheral arcus and the major, more central, lesion and it extended through the cornea up to, and including, Descemet's membrane. Some vascularisation was

present (Fig. 5.1/1). The difference between the milky-white arcus which adjoined the limbus and the more crystalline central and paracentral lipid keratopathy was quite striking, possibly indicating that different lipoproteins or lipids might be concerned with their origin and formation (Fig. 1.0/4).

Serum amylase was estimated at this time and when she next had a bout of abdominal pain, and the diagnosis of acute pancreatitis confirmed by levels in excess of 6,000 u/l immediately following an acute attack. Lipids and lipoproteins were also analysed again and the most notable findings were a hypertriglyceridaemia which produced a visibly lipaemic serum sample. A prominent beta lipoprotein band was present on serum lipoprotein electrophoresis. Flare-ups were treated symptomatically and the owners reported that the dog appeared quite normal some six months later. She was seen again approximately eleven months after this report as the owners had noticed a reduction in visual acuity. Examination revealed retinal detachment, with partial disinsertion, in the left eye, sufficient to account for the signs noted by the owners. The already blind and phthitic right eye was remarkably free of major opacity, rather patchy crystalline areas remained with numerous intervening clear zones; some vascularisation was present. A blood sample taken at this time indicated a normal lipoprotein pattern and serum lipids were: cholesterol, 4.23mmol/l; triglyceride, 0.58mmol/l; and phospholipid, 3.02mmol/l.

Old English Sheepdog 13 According to the owners this dog had had eye trouble for the nine months that they had owned him. The most obvious features were recurrent redness and mild ocular discomfort,

although two months ago they had noticed a white opacity first in the left eye and then in both eyes. On examination the dog was in good general health with obvious plaque-like vascularised opacities in both eyes (Fig. 5.1/1). In the right eye two lesions were present, one of which was vascularised the other not; they occupied a paracentral position. In the left eye a central vascularised opacity was present. Both scleras were reddened and the conjunctival and episcleral vessels were rather tortuous and slightly engorged. Neovascularisation of the cornea was mainly from vessels at episcleral level. The corneal stroma was involved throughout most of its depth in the left eye and in the anterior third of the right eye. The epithelium was irregularly thickened but no erosions were present.

Laboratory examination revealed no abnormality other than raised high density lipoprotein as HDL_C. Similar results were obtained at the time of keratectomy four months after the opacity had first been noticed. The dog was given topical corticosteroids post-operatively because of the history of recurrent redness and he made an uneventful recovery.

The opacity was completely excised from the right eye, only partially so from the left. A low fat diet was prescribed post-operatively and on this regime the right eye remained clear and the left eye showed partial clearance of the deeper opacification. The dog was lost to follow up nine months after surgery.

Old English Sheepdog 38 This animal was overweight and lethargic and the owners had noticed marked reddening of the eyes on a number of occasions. They had also observed a grey-white

opacity develop in the left eye about twelve months ago and in the right eye two months later. Their veterinary surgeon diagnosed episcleritis of unknown cause and referred the dog for a second opinion. Ocular examination indicated extensive vascularised central and paracentral opacities of both eyes surrounded by a clear periphery (Fig. 5/1/1). The epithelium was thickened in places and the opacity extended throughout the anterior stroma with greater involvement of the left eye. With the slit lamp, multiple areas of crystal aggregates were evident, as was the extensive nature of corneal vascularisation at all levels of the stromal opacity. There was some engorgement and congestion of the perilimbal vessels (Fig. 5.1/20).

The results of laboratory examination indicated raised serum lipids, particularly an increase of HDL_C. Free thyroxine was 7.20pmol/l, other values were within normal range.

The dog was put onto thyroid replacement therapy reaching a maintenance dose of 120mcg of L- triidothyronine sodium daily. Keratectomy was performed two months after this regime was started and when his serum lipid and lipoproteins were of more normal distribution.

Despite the fact that keratectomy, particularly of the left eye, was incomplete, the eventual result was excellent with complete clearance of both opacities according to the owners. The owners further reported that the dog was much livelier and enjoying walks and this state persisted up to the time of the last check-up two years post-surgery.

Great Dane 74 About one year previously the owner had noticed

grey opacities in the dog's left eye, followed by similar opacities in the right eye four months later. In other respects the dog appeared well.

On examination two central opacities situated immediately beneath the epithelium to a depth of approximately one third of the stroma were present in both eyes. They were not vascularised and comprised scintillating crystals set against a more diffuse background of small grey dots (Fig. 5.1/1). The eyes were reddened and the dog had an active iritis. Iris cysts were present in both eyes but were more numerous in the right eye, and distichiasis was apparent on both upper lids (Fig. 5.1/22).

Laboratory examination yielded substantially normal results. The extra eye lashes were removed. The iritis, of unknown cause, was treated with atropine and corticosteroids for approximately two months. Keratectomy was performed once the eye became quiet, and within one month of treatment starting.

The dog remained in good health and the eyes appeared normal for approximately 18 months and then the owners indicated that the corneal opacities had suddenly reappeared and were more extensive than before. On examination the corneas were quite normal but bilateral cortical cataracts were present, both irises were extremely dark, the number of iris cysts had increased and the eyes were reddened (Fig. 5.1/23). The owners admitted that they had observed recurrent redness of the eyes over the past few months without thinking it was of any significance.

The dog had also become overweight and a blood sample indicated cholesterol, 7.02; triglyceride, 0.73; and phospholipid, 5.69mmol/l. Other laboratory investigations were normal.

A unilateral intracapsular cataract extraction was performed and the dog made an uneventful recovery. He remains in good health and has lost a little weight on a low fat diet and increased amounts of exercise. The owners keep a hotel and apparently find it difficult to stop the guests feeding the dog potato crisps.

Great Dane 130 This dog was noticed to have central opacities of both corneas approximately seven to eight months previously. Their appearance had not been associated with any other ocular signs and the dog appeared fit and well, although the owners reported occasional digestive upsets and a tentative diagnosis of chronic pancreatitis had been made by the referring Veterinary Surgeon.

The lesion in the right eye enlarged centrifugally over a period of months and consisted of almost discrete circles of scintillating crystalline aggregates situated in a more diffuse, slightly oedematous stroma. Superficial vascularisation developed at the infero-temporal border of the lesion and the appearance was of chronic granulation tissue with a small area of superficial ulceration. The lesion in the left eye changed little in appearance and was neither vascularised nor ulcerated. Both opacities were situated sub-epithelially in the anterior one third of the stroma and were readily removed by superficial keratectomy at the time of referral (Fig. 5.1/1).

Serum lipids and lipoproteins were normal save for raised triglycerides and a prominent beta band; other laboratory tests were within normal range. The dog was in excellent general health and lean, the owners have reported no change in his condition since surgery. The corneas remain clear.

Bearded Collie 26 This dog was noticed to develop, over a period of several days, a crescentic opacity situated temporally and paracentrally in the left eye. The eye was reddened and some ocular discomfort was present.

The dog was referred for second opinion approximately four months after the opacity had first been noticed. Examination indicated a dog of normal weight and in good general health. The right eye was of normal appearance whereas the left eye was reddened and painful with a striking plaque-like white opacity situated as already described (Fig. 5.1/1). The iris of the left eye was darker than that of the right and responded poorly to light. The opacity was situated subepithelially and extended throughout the entire stromal thickness; there was no epithelial involvement and vascularisation was present in the stroma at all levels.

A diagnosis of uveitis was made and the dog was treated with topical atropine and corticosteroids. The eye became quieter and when re-examined several days later aqueous flare had disappeared although the iris remained darker than that of the right eye. No posterior segment abnormalities were apparent. Over the course of several months there were occasional flare-ups of anterior uveitis in the left eye, with reddening and soreness. These recrudescences were treated symptomatically when they occurred. The crescentic opacity of the left eye extended circumferentially so as to involve a greater area of cornea, the shape was originally half-moon whereas later it was more like a horse-shoe (Fig. 5.1/21). The density of the lesion increased, and there was some epithelial involvement. With the slit lamp the presence of dense aggregates of crystalline

and more amorphous material was apparent.

Laboratory examination yielded normal results and repeated examinations over a three year period were normal. The owners requested euthanasia for the dog because of undesirable changes of temperament some three years after the opacity had first been noticed. At the time of euthanasia the eye was quiet, few blood vessels were present and the opacity had become less dense with clear zones between aggregates of crystalline and amorphous material. The epithelium was intact. The eyes were made available for microscopic examination but a post-mortem was not performed.

Jack Russell Terrier 132 This dog was referred because of apparent chronic conjunctivitis, first in the right eye over a period of 18 months and then in both eyes for the last five days (Fig. 5.1/1). The dog was rather subdued and was eating less than usual.

On examination of the right eye a long-standing posterior lens luxation with a total retinal detachment were the most obvious findings. The intraocular pressure was slightly higher than normal and there were some chronic changes within the central cornea and some fine fracture lines in Descemet's membrane. The eye was without sight.

The left eye was glaucomatous with a posterior lens luxation and a widely dilated pupil. Vision was limited to light-dark appreciation. In the supero-temporal quadrant of the left cornea an extensively vascularised opacity was present (Fig. 1.0/5) and the owners reported that this had gradually developed at the site of a previous cat scratch which had been received about nine months

previously. Blood samples at this time revealed a slight rise in lipid levels as the only abnormality; other laboratory investigations were normal.

Lendectomy was performed on the left eye and a small piece of superficial cornea containing crystalline aggregates and more homogeneous plaque-like masses was removed at the same time.

The eye healed uneventfully following intraocular surgery and the dog was bright and pain-free when seen for suture removal from the canthotomy incision ten days later.

The owners sent regular reports as to the dog's progress over the next months with the animal on no treatment other than commercial dog food only. They observed a gradual diminution in the size of the white-gold opacity coupled with an increase in the amount of black pigment. The dog was seen again three years after surgery and the owners' observations confirmed. No lipid was detectable within the cornea, save for two small dense plaques within the anterior stroma, there was obvious melanosis (Fig. 1.0/6). Ghost blood vessels were apparent on slit lamp examination, but none of the vessels carried blood. The animal was in excellent general health and surprisingly mobile considering that there was now a total retinal detachment in both eyes and very little appreciation of light in the left eye. The right eye had become phthitic. Intraocular pressure of the right eye was lower than normal (10mm Hg) and within normal range in the left eye (19mm Hg).

German Short Haired Pointer 75 This dog belonged to a veterinary surgeon who had noticed recurrent attacks of anterior segment inflammation over the past few months which affected the right eye

more than the left. Similar anterior segment inflammation had also occurred a year or so earlier when she had suffered from a systemic infection with intermittent jaundice, diagnosed as some form of viral hepatitis. Following recovery from the infection she regained condition but a harsh dry cough persisted and there were times when she suffered from ill-defined malaise.

Ophthalmic examination indicated occasional focal inactive chorioretinitis lesions in both fundi, extensive iris atrophy and posterior synechiae, corneal opacities and reddening of the sclera (Fig. 5.1/1). The corneal opacities were similar between the two eyes except that that of the right eye was more extensive and contained more crystalline material. There was a relatively inactive vascularised pannus forming a crescent-shaped superior corneal infiltration and containing many ghost vessels. A relatively clearer zone existed between the infiltrating edge of the grey pannus and subepithelial aggregations of crystalline appearance (Fig. 5.4/1). The scleras were reddened and visible blood vessels were tortuous and slightly injected.

Venous blood samples indicated raised triglycerides and slight prominence of the beta lipoprotein band as the only obvious abnormality at this stage, although an earlier blood sample taken soon after recovery from the acute hepatitis had apparently shown an increase of all serum lipids, particularly cholesterol and phospholipid. Further laboratory examination indicated a free thyroxine level of 26.3pmol/l and a range of liver function tests was performed. The relevant results were: alanine aminotransferase, 85.0mU/ml (normal 6-24mU/ml); alkaline phosphatase, 295mU/ml (normal 0-170mU/ml); aspartate aminotransferase, less than 5mU/ml (normal

6-20mU/ml) and total bilirubin, 2.9umol/l (normal 0.29-7.55umol/l). In the light of these findings, and other negative results, a diagnosis of chronic intrahepatic cholestasis was made.

Fluorescein angiography of the anterior segment demonstrated positive fluorescence within the area of pannus within 15 seconds of injection, whereas the area containing crystalline aggregates was fluorescein negative (Figs. 5.4/2 α 5.4/3).

Basset Hound 77 This dog was referred for second opinion because of crescentic paracentral opacities of both corneas which had been present for some time, in association with ocular irritation.

Examination indicated an overweight animal with a pendulous abdomen and symmetrical hair loss. The owners had noticed an increase in eating and drinking which started some time after treatment with prednisolone was given over a long period for contact dermatitis. Polyuria was also present according to the history.

Ophthalmic examination indicated ocular discomfort and epiphora in both eyes, distichiasis was also present and was more severe in the right eye than the left. The corneal opacities were immediately subepithelial and comprised fine grey dots and a diffuse haze, no vascularisation was present and the epithelium was intact (Figs. 5.1/1 α 5.1/24). The extra eye lashes were removed by epilation under local analgesia and the corneas were almost clear of opacities when the owners collected the dog three hours later (Fig. 5.1/25).

Venous blood samples indicated a reduction in alpha$_1$-lipoprotein and a prominent beta and pre-beta lipoprotein band. Other results of relevance were a mild eosinopaenia; an alkaline phosphatase of 230mU/ml (normal 0-170mU/ml); alanine

aminotransferase of 56mU/ml (normal 6-24mU/ml); and normal levels of free thyroxine (22.4pmol/l), blood glucose (5.0mmol/l) and blood urea (6.2mmol/l). Hepatomegaly was also present. The clinical signs and laboratory tests were suggestive of iatrogenic Cushing's syndrome when taken in conjunction with the history and examination. Gradual withdrawal of prednisolone over a period of days was recommended without further investigation of adreno-cortical function. Whether this dog is truly representative of an early lipid keratopathy is not easy to determine as the corneal opacities were more typical of arcus lipoides corneae save for their more central distribution. However, the chronic low grade trauma from the extra eyelashes might be considered a contributory factor in lipid deposition and this case is therefore arbitrarily classified as a potential lipid keratopathy.

The diminution in opacity within a few hours of the irritating lashes being removed may indicate resolution of a mild corneal opacity rather than actual lipid resorption. The case is of interest in bearing some resemblance to that in a young boy with an ectopic cilium reviewed earlier (Heath, 1949).

APPENDIX II

The Assessment and Treatment of Dogs with Lipid Keratopathy

1. Establish serum lipid levels and lipoprotein pattern.
2. Diagnose and treat any underlying systemic disease which is producing secondary hyperlipoproteinaemia - common in dogs.
3. Diagnose and treat primary hyperlipoproteinaemia - rare in dogs.
4. Overindulgence hyperlipoproteinaemia should be treated by a low fat, complete, commercial dog food such as "Chappie" (Pedigree Petfoods).
5. The amount of daily exercise should be increased.
6. For refractory cases of overindulgence hyperlipoproteinaemia, the diet can be further changed by increasing the amount of fibre, or feeding a commercial obesity diet (Pedigree Petfoods).
7. Diagnose and treat any existing ocular disease.
8. In early, progressive, lesions, anti-inflammatory drugs are indicated.
9. In later, regressive, lesions, with corneal neovascularisation as the only sign of anterior segment inflammation in normolipoproteinaemic patients, anti-inflammatory drugs should not be used. Corticosteroid preparations, for example, effectively reduce corneal neovascularisation, thus preventing access by haematogenous macrophages, in addition there may be other local effects of importance.
10. Keratectomy, or keratoplasty, is of value in cases where the ocular lesions are painful, extensive and progressive, such that vision is threatened. It is important that surgery is only undertaken in normolipoproteinaemic patients where pre-existing anterior segment inflammation has been brought under control.

Post-operative treatment with anti-inflammatory drugs may be required.

11. Prospective use of intravenous phospholipids might be considered in refractory, vascularised, lesions, in view of the results of this investigation and their known beneficial effects in experimental rabbits. Adams, Abdulla, Bayliss α Morgan (1967), used polyunsaturated phosphatidyl choline (Lipostabil) to modify aortic atheromas and fatty livers in cholesterol-fed rabbits.

APPENDIX III

Author's Published Papers on Canine Corneal Lipidoses.

J. small Anim. Pract. (1983) **24**, 63–83.

Dystrophy, degeneration and infiltration of the canine cornea

S. M. CRISPIN* AND K. C. BARNETT

Unit of Comparative Ophthalmology, Animal Health Trust Small Animals Centre, Lanwades Park, Kennett, Newmarket, Suffolk CB8 7PN

ABSTRACT

Three conditions of the cornea in the dog are described; a form of corneal dystrophy (central/paracentral lipid dystrophy, lipidosis, lipoidosis, cholesterolosis), degeneration (fatty and calcareous degeneration) and infiltration (arcus lipoides corneae, anterior embryotoxon, pre-senile arcus). The clinical appearance, together with histopathological and ultrastructural details, are recorded. The age, sex, breed incidence and possible hereditary factors are also included. Reference is made to previous reports in the veterinary literature and the three conditions are compared with similar conditions in man.

INTRODUCTION

The term corneal dystrophy should be reserved for primary, inherited, bilateral affectations of the cornea which occur unaccompanied by systemic disease (Bron & Tripathi, 1970). Duke-Elder (1965) states that they usually develop without obvious cause in an apparently normal eye and in the absence of inflammation and vascularization, although secondary inflammatory changes may occur. Having reached a certain stage they are usually static, occasionally they regress, but they may progress to produce considerable visual disability.

Corneal degeneration implies an essentially pathological change within the tissue, usually fatty, hyaline or calcareous in nature. More than one type of degenerative change may be present and inflammation, vascularization and melanosis may accompany, or even precede, the changes. Degenerative corneal

* Present Address: Department of Anatomy, Royal (Dick) School of Veterinary Studies, Summerhall, Edinburgh EH9 1QH.

0022-4510/83/0200-0063 $02.00

conditions are not necessarily inherited, although, as with dystrophies and infiltrations, there may be a breed prevalence.

Corneal infiltration by substances such as lipids may occur in an otherwise normal cornea or in one in which disease already exists. Some of the factors which have been implicated in the deposition of lipids in the cornea have been discussed in a previous paper (Crispin & Barnett, 1978).

The distinction between dystrophies, degenerations and infiltrations is somewhat arbitrary, particularly as knowledge of these conditions in the dog is, at present, limited. The conditions which are described in this paper all occur bilaterally in corneas which previously appeared normal and are unassociated with any form of concurrent ocular disease. They should be distinguished from the various forms of secondary fatty change, such as lipid keratopathy, which will be discussed in a subsequent paper. Secondary fatty change is often a feature of pathological alterations within tissues; in the cornea it may be associated with disease of the cornea, of the nearby ocular tissues, or even of the eye as a whole and it is not necessarily bilateral. The significance of secondary fatty change in the context of this paper is that it may occur as a sequel to any of the three conditions which are described as they themselves are producing corneal abnormality. The early history and clinical appearance are thus critical in the diagnosis of these forms of corneal disease as the ultrastructural appearance may be considerably modified by secondary degenerative changes.

The canine cornea is derived embryologically from ectoderm and mesoderm. In the adult, blood vessels are absent except for a fine capillary arcade in the limbal region. The peripheral cornea is thus supplied by the terminal branches of conjunctival, episcleral and scleral vessels. Other sources of corneal nutrition are the aqueous humour and the tear film. Lymphatic vessels are not present in the normal cornea. Sensory innervation is mainly derived from the ophthalmic division of the fifth cranial nerve and is richest in the superficial cornea.

Structurally, four division can be recognized (Fig. 1):

(1) A stratified, non-keratinized, epithelium covered anteriorly by the tear film and bounded posteriorly by a basement membrane on which lies a single layer of basal columnar cells connected to the basement membrane by hemidesmosome attachments (Fig. 2). Above the basal epithelial cells are two to four layers of polyhedral, or wing, cells and anterior to these some four to ten layers of squamous cells. The basement membrane on which the basal epithelial cell layer rests contains fine argyrophilic fibrils embedded in a mucoprotein matrix which stains characteristically with P.A.S.

(2) The connective tissue stroma, or substantia propria, of the cornea consists of collagen fibrils which are organized into bundles, or lamellae, embedded in an abundant ground substance (Fig. 3). In the anterior stroma the lamellae are arranged obliquely whilst there is a more regular orientation in the deeper layers of the stroma. The lamellae, and the collagen fibrils which compose them, appear to extend across the entire corneal diameter. Stellate keratocytes, the corneal

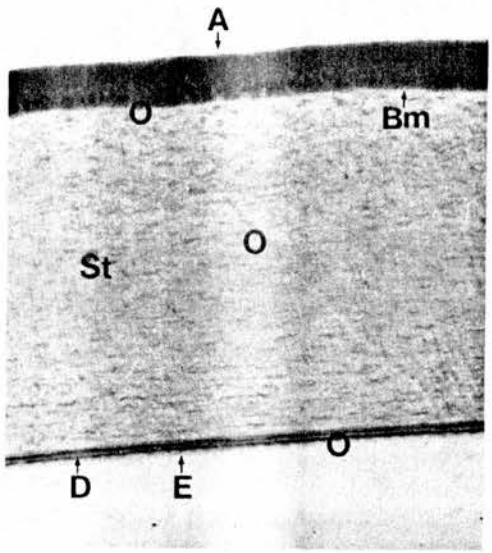


FIG. 1. Light micrograph of normal central cornea in a cross-bred collie. Ringed areas shown at higher magnification in Figs 2, 3 and 4. A—Anterior epithelium; Bm—Basement membrane; St—Stroma; D—Descemet's membrane; E—Endothelium (mesothelium).

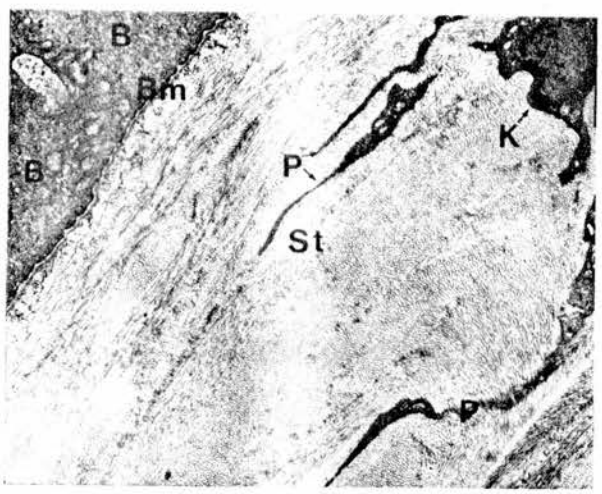


FIG. 2. Electron micrograph of basal anterior epithelium showing part of two basal columnar cells (B), the basement membrane (Bm) and the anterior stroma (St) with a keratocyte (K) and keratocyte processes (P) between the collagen fibrils.

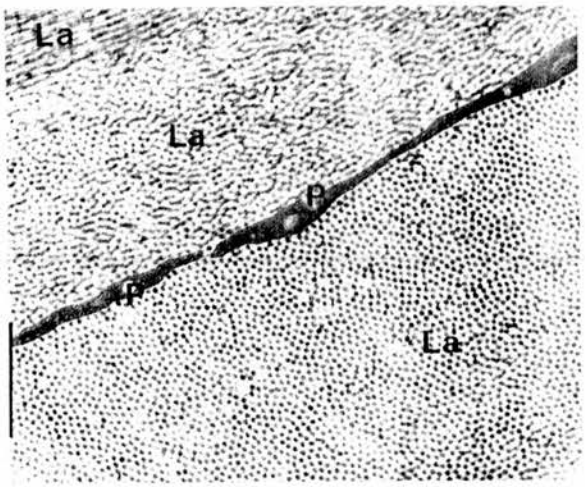


FIG. 3. Electron micrograph of mid-stromal region showing the regular orientation of the collagen lamellae (La) and attenuated keratocyte processes (P).

equivalent of fibroblasts, are distributed throughout the stroma and these cells produce presumptive ground substance and collagen fibrils. Occasional lymphocytes and macrophages may be seen and, rarely, perhaps abnormally, polymorphonuclear leucocytes. Bowman's layer, a concentration of randomly oriented collagen fibrils beneath the basement membrane, is poorly defined and not designated as a separate layer in the dog, but rather as a cell-free zone.

(3) Descemet's membrane is a well-formed, acellular, homogeneous, eosinophilic, P.A.S. positive and mainly collagenous structure, largely produced by the underlying endothelial, or more correctly, mesothelial cells (Fig. 4).

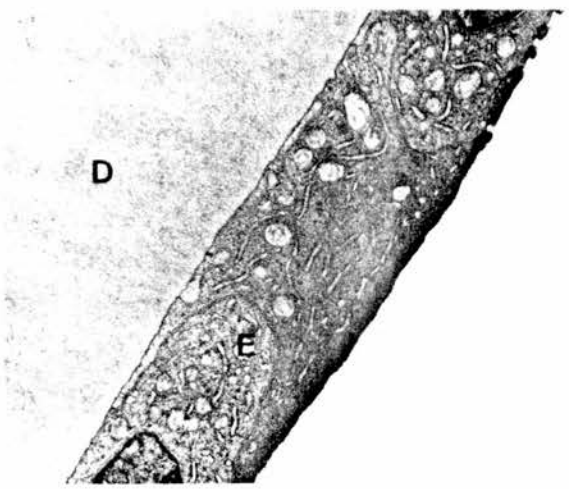


FIG. 4. Electron micrograph of part of Descemet's membrane (D) and the corneal endothelium (E).

(4) The mesothelial layer is a single layer of polygonal cells whose anterior cell membranes are in contact with Descemet's membrane and whose posterior cell membranes are bathed by the aqueous humour of the anterior chamber.

MATERIALS AND METHODS

A detailed history was taken, including information on the patient's relatives. A routine clinical examination and detailed ophthalmological examination were carried out in all cases.

Ocular examination was by observation, aided by a pen torch and a magnifying loupe, by direct ophthalmoscopy, by slit-lamp biomicroscopy and using photography.

Blood samples from the cephalic vein of a number of fasting patients were submitted for routine haematological examination and for the estimation of serum lipoproteins, blood glucose, blood urea, calcium, magnesium and inorganic phosphate. More specific investigations were performed as and when they were required.

Routine urinalysis was carried out on a sample obtained at around the time of blood collection.

In some patients the corneal lesions were removed or sampled by superficial keratectomy. Whenever possible the lesion was removed completely along with a proportion of the normal corneal tissue surrounding the opacity. During fixation and processing the tissues were handled so as to preserve the normal corneal architecture as far as possible for light and transmission electron microscopy.

Light Microscopy

(a) Frozen sections. Pieces of tissue were placed directly into 10 per cent buffered formol saline or 10 per cent formalin with 1 per cent calcium acetate. Smaller pieces of tissue (less than 1 cm^2) were placed directly into a freezing mixture of liquid nitrogen with Freon, sometimes supported by 20 per cent gelatin or a 7 per cent gelatin in Azide mixture before immersion. 10–15 micron (μ) sections were cut on a freezing cryostat and stained in most instances as described by Pearse (1968) with oil-red-0, Nile blue, Sudan black and Sudan III and IV and the modified Schultz method for cholesterol and its esters.

(b) Paraffin-wax embedded sections. These portions of tissues were placed in 10 per cent buffered formol saline and 5–15 μ sections cut after routine histological processing and paraffin-wax embedding or Peterfi's double embedding technique. The specimens were stained with haematoxylin and eosin, Masson's and Mallory's trichrome methods, the periodic acid-Schiff reaction Alcian blue, Von Kossa's stain for calcium and the colloidal iron method for acid mucopolysaccharides counterstained with Van Gieson's solution. Suitable controls were employed where necessary.

Transmission Electron Microscopy

Samples were placed in (a) 4 per cent gluteraldehyde with phosphate buffer at pH 7·4 or (b) 3 per cent gluteraldehyde with cacodylate buffer at pH 7·2. Approximately half the specimens were post-fixed in buffered osmium tetroxide, the remainder were not osmicated and were stored in buffer during the time of osmication.

(a) Samples originally taken into 4 per cent gluteraldehyde with phosphate buffer were dehydrated with graded alcohols following post-fixation in 1 per cent buffered osmium tetroxide or storage in phosphate buffer for two hours. Transitional stages in propylene oxide were followed by embedding in Araldite. Thick (μ) sections were cut and stained with 1 per cent alkaline toluidine blue or 1 per cent methylene blue and examined with a light microscope. Thin sections were stained with uranyl acetate and lead citrate and examined at between 50 and 120 Kv in either an Hitachi HS8 or a Philips 400 electron microscope.

(b) Samples taken into 3 per cent gluteraldehyde with cacodylate buffer were dehydrated with graded acetones following a period of osmication with 1 per cent buffered osmium tetroxide or storage in cacodylate buffer which lasted for 30 minutes. The processed samples were embedded in Epon or Araldite and sectioned, stained and examined as described above.

I CORNEAL DYSTROPHY

Clinical Appearance

This form of corneal dystrophy in the dog appears clinically as a grey-white or silver, almost metallic, opacity situated in the central corneal region usually just below and temporal to the exact centre. It is essentially a bilateral and often symmetrical lesion, although it may appear on one side before the other, and the lesion in the two eyes may not be exactly similar in either size or density. The area involved varies considerably in size (see Fig. 5–10) but is usually small and never

---

FIG. 5. Typical appearance in the Rough Collie with an oval area of opacity slightly temporal and inferior to the centre of the cornea.

FIG. 6. Higher magnification (slit-lamp microscope) showing sub-epithelial diffuse deposit (Rough Collie bitch).

FIG. 7. Same dog as in Fig. 6 showing similar, but not identical, lesion in the other eye.

FIG. 8. Slip-lamp photograph of Cavalier King Charles Spaniel, larger and more circular area with dense periphery.

FIG. 9. Slit-lamp photograph of another Cavalier King Charles Spaniel, large diffuse and faint area of opacity.

FIG. 10. Slit-lamp photograph of Rough Collie, oval with linear form.

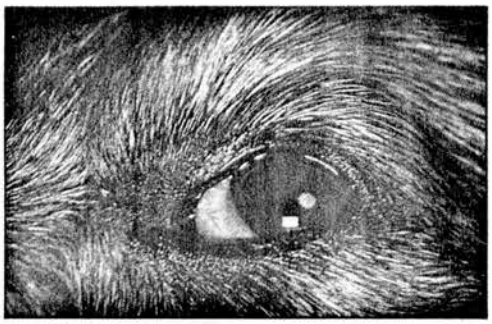


FIG. 5

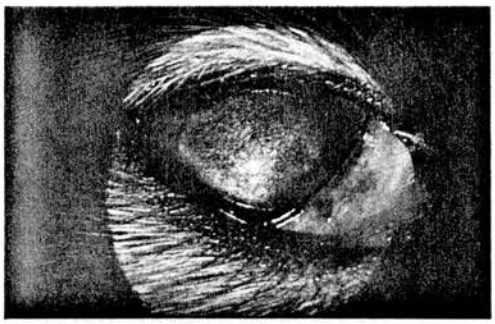


FIG. 6

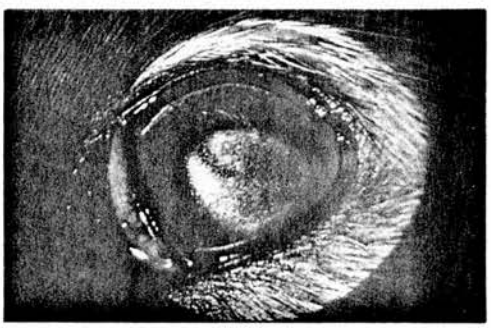


FIG. 7

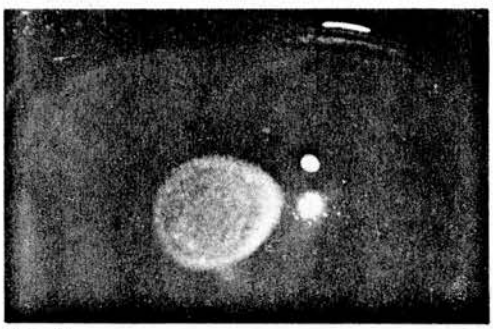


FIG. 8

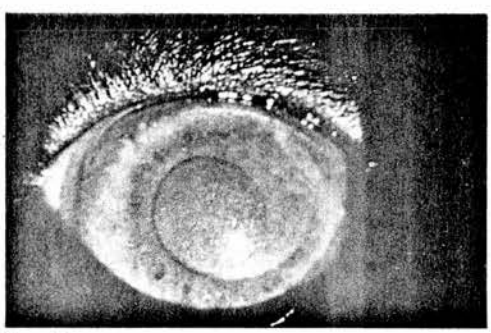


FIG. 9

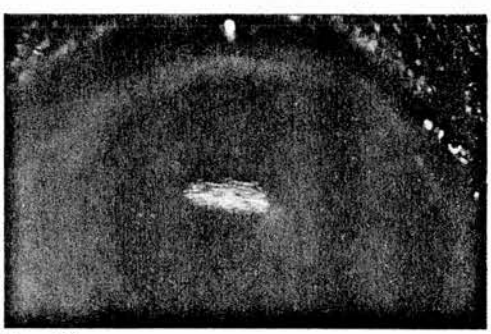


FIG. 10

(*Facing p.* 68)

involves the whole cornea, there always being a broad and clear region inside the limbus. The density of the opacity also varies from the completely opaque (often small) spot to a faint region (often larger) through which details of the anterior segment of the eye can easily be discerned. Commonly, the peripheral border is denser than the centre. These variations in size and density may well be stages in progression of the same lesion. Typically, the shape is oval, sometimes more circular in appearance and the edge of the lesion is usually well demarcated from the surrounding clear cornea. The lesion is sub-epithelial (anterior stromal) with an intact epithelium. A perfect corneal reflection to a light source can easily be demonstrated over the actual opaque area. This procedure, together with the fact that the lesion does not take up intravital stains, e.g. fluorescein, is useful in diagnosis and differentiates it from a corneal ulcer. There is never any associated inflammation, vascularization or pigmentation.

The different forms of the lesion may relate to progression and regression of the condition. There may be a myriad of fine, small particles dispersed throughout the cornea and only visible on slit-lamp examination. In many cases there are discrete round or oval focal areas of uniform opacity whereas other animals may present with round or oval ring-like opacities and a clear central zone. The dystrophy is usually slow to appear and possibly forms as a result of aggregation of the fine particles seen with slit-lamp biomicroscopy. Alternatively, it may start as a small dense lesion becoming larger and less dense with time. The lesions may remain stationary as discrete opacities, or as ring-like opacities, or they may regress by centrifugal dispersion of the visible particles. The ring-like opacities with a clear central zone perhaps represent a stage in the aggregation or dispersion of the fine particles.

Corneal dystrophy of this type, although frequently of concern to the owner, causes no irritation to the dog, mainly because of the absence of epithelial involvement and, whatever the size or density of the opacity, their effect on vision is minimal. There is no associated ocular or systemic disease although an increase in the animal's serum lipoprotein levels may modify the evolution of the dystrophy so as to make it more obvious and of greater area. Corneal dystrophies of this type are not particularly uncommon and a breed, age and sex prevalence can be demonstrated.

Pathology

The epithelium is intact and of normal thickness although focal changes in thickness of the basement membrane may develop if the lesion progresses. Extracellular crystals and lipid droplets predominate.

In the stroma, particularly the anterior third and often no deeper than this, there are both extracellular and intracellular vacuoles. In frozen sections small globules become impregnated with fat stains, such as oil-red-0 and the Sudan series. In electron micrographs these round spaces are empty or contain dark staining remnants. Studies to date indicate that they originally contained hydrophobic

lipids. There are also needle-shaped and rectangular areas in the superficial corneal lamellae in extracellular situations and occasionally intracellularly within the cytoplasm of the keratocytes of paraffin wax embedded material for light microscopy and preparations for transmission electron microscopy. Frozen unfixed sections examined with a polarized light source indicate birefringence in these regions and further confirmation of the presence of cholesterol is afforded by the modified Schultz reaction.

The normal lamellar architecture of the anterior stroma, and a proportion of the keratocytes, are disrupted by the lipid inclusions. Within the corneal stroma there are also poorly defined areas of granulo-fibrillar material which may represent unaggregated collagen filaments and are probably a non-specific feature of this and other corneal lesions (Garner & Tripathi, 1972).

Rarely, the abnormalities observed in the superficial stroma extend more deeply than the anterior third and there is minimal cellular reaction in affected areas (Figs 11–12). Descemet's membrane and the mesothelium are unaffected by the dystrophy.

Discussion

Corneal lipidosis, as has been stated, is not rare in the dog, and is being recognized with increasing frequency. During the period 1963 to 1980 inclusive, 144 cases were referred to the Comparative Ophthalmology Unit at the University of Cambridge Veterinary School, the Animal Health Trust Small Animals Centre and the University of Edinburgh Small Animal Clinic. Referral was mainly on account of lack of response to treatment and the fear of eventual blindness or, at least, a visual defect.

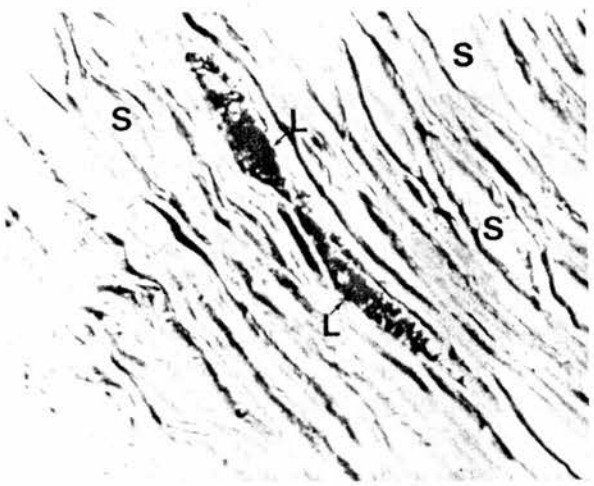


FIG. 11. Light micrograph of anterior stroma. Corneal lipidosis in a Rough Collie. Frozen section obtained by superficial keratectomy and stained with Sudan black showing splitting of the collagen lamellae (S) and the presence of globular lipids (L).

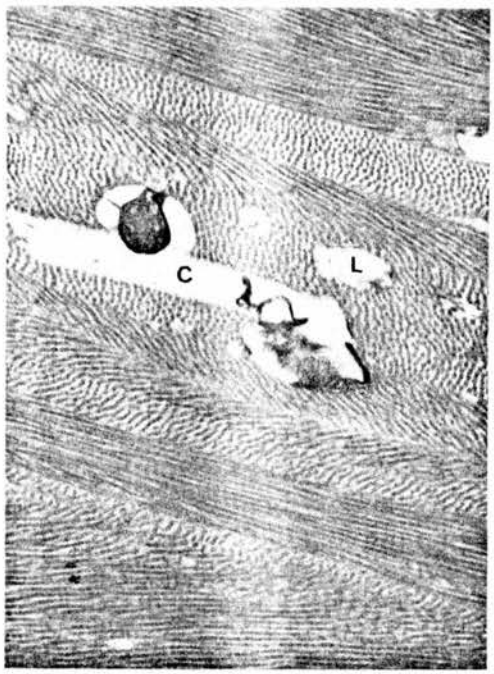


FIG. 12. Electron micrograph of anterior stroma in lipidosis from a Cavalier King Charles Spaniel. Showing characteristic outline of a dissolved cholesterol crystal (C) and a lipid droplet (L).

Perusal of the breed, age and sex incidence of the cases examined reveals some interesting facts. Four breeds, namely the Rough Collie, Shetland Sheepdog, Cavalier King Charles Spaniel and Alsatian (German Shepherd Dog), account for over two thirds of the total cases seen. This high breed incidence indicates some genetic factor and, referred to before, the term corneal dystrophy in man is reserved for hereditary conditions of the cornea. Corneal dystrophy, sometimes referred to by other terms, has been recognized in the dog, particularly in certain breeds, for some time (Schock, 1910; Veenedaal, 1928, 1937, 1953; Dreyfuss, 1930; Robin & Charton, 1939; Jubb & Kennedy, 1963; Startup, 1969; Magrane, 1971; Wyman & Donovan, 1971; Saunders & Rubin, 1975). It was once considered as part of the collie eye anomaly (Roberts & Dellaporte, 1965) but is now accepted as a quite separate condition; although it occurs in dogs with collie eye anomaly, it also occurs in collies showing no ophthalmoscopic evidence of this other hereditary condition. The incidence of corneal lipidosis in the collie breeds in Europe, in groups of dogs examined for collie eye anomaly, has already been mentioned in the Shetland Sheepdog (Barnett & Stades, 1979) and the Rough Collie (Stades & Barnett, 1981). An incidence in other breeds has been reported in the Afghan Hound (Vainisi & Goldberg, 1974) Dachshund, (variety not specified) (Vainisi &

Goldberg, 1974), Beagle (Waring, Muggli & MacMillan, 1977; Ekins, Waring & Harris, 1980; Roth *et al.*, 1981). The lipid dystrophies described by Dice (1974 and 1977) in the Airedale and MacMillan, Waring, Spangler & Roth (1979) in the Siberian Husky differ in depth and distribution from the central/paracentral lipid dystrophy under discussion. Crispin (1982) has proposed the topographical description of 'diffuse' lipid dystrophy for the type reported as inherited in related Airedales and 'annular' lipid dystrophy for the type reported in Siberian Huskies. This communication records central lipid dystrophy as a familial condition in the following breeds: Rough Collie, Shetland Sheepdog, Cavalier King Charles Spaniel and Alsatian (German Shepherd Dog), although no exact mode of inheritance has been demonstrated. In the Rough Collie and Cavalier King Charles Spaniel this type of corneal dystrophy has been seen through three generations. Superficial corneal dystrophy in three generations in the Collie and the Afghan has been recorded by Vainisi & Goldberg (1974). Cases have also been seen in the United Kingdom in the following breeds which, with further investigation of related animals, may also prove to be familial: Samoyed, American Cocker Spaniel, Afghan Hound and Cairn Terrier (cases occurring under 12 months of age).

Corneal dystrophy in closely related stump-tailed Manx cats has been recorded in America by Bistner *et al.* (1976) and takes the form of a progressive stromal oedema with secondary epithelial involvement; it is apparently inherited as a single autosomal recessive. In this country a form of corneal dystrophy has been observed in a white cat (Barnett, unpublished observation) of identical clinical appearance to the central/paracentral lipid dystrophy described in this paper. In addition a form of endothelial dystrophy, involving degeneration of the corneal endothelium and leading to corneal oedema and sometimes keratoconus has been described in closely inbred short-haired domestic cats (Crispin, 1982).

An endothelial dystrophy of similar pathology to that in the cat occurs in the Boston Terrier and Chihuahua in both America (Dice, 1981) and the United Kingdom (Barnett, unpublished observations). In both the cat and dog this dystrophy shows many similarities to Fuch's hereditary endothelial dystrophy in man.

A further type of corneal dystrophy, similar to human dystrophic recurrent-erosion, may well be the underlying cause of the indolent ulcer which is seen particularly in the Boxer (Roberts, 1965) and the Pembroke Corgi.

Typically, corneal lipidosis or central lipid dystrophy is a disease of the young adult animal and it is interesting that the definition by Duke-Elder (1965) refers to the dystrophy usually appearing during the first decades in the human. In the present series the age incidence ranged from two months to eight years but 25 per cent (108 cases) occurred in animals in the two to four year age groups. This is particularly evident in the Rough Collie in which 25 out of 30 cases occurred between one and four years of age.

The sex incidence is perhaps the most interesting feature of all, particularly as, to date, no sex-linked or sex-limited hereditary eye conditions have been proved in any

animal, although many such examples are known in man. In this series of 144 cases more than twice as many occurred in the female than in the male (100:44). In the Rough Collie there were 24:6 female to male. It is also interesting that in the group of Huskies reported by MacMillan *et al.* (1979) the sex distribution was 58 females to 20 males, although this discrepancy was not commented on by the authors. However, a sex-linked recessive inheritance (i.e. occurring in the male) has been suggested in the Airedale (Dice, 1980). Other authors have not remarked upon the sex incidence in their cases in the dog. Not uncommonly the history from the owner includes reference to the appearance of the corneal opacity in relation to pregnancy and/or the oestral cycle of the bitch. Two interesting cases were a five-year-old bitch, spayed at nine months of age but on stilboestrol therapy for two years for incontinence and a male on similar treatment for anal adenomata.

The fasting blood cholesterol values have been investigated in a number of cases but have been found to be within normal limits. This fact has also been reported by other authors although the typical appearance of cholesterol crystals and clefts can be demonstrated both on slit-lamp microscopy and in histological preparations of biopsy material.

Corneal dystrophy does not respond to topical medical treatment, in particular to antibiotic and/or corticosteroid eye ointments. The lesion can quite easily be removed surgically by superficial keratectomy and in a few cases in which this procedure has been undertaken there has been no tendency to recurrence up to five years post-operatively. However, this procedure is usually unnecessary as the condition rarely, if ever, interferes with vision and, because of its subepithelial position and non-inflammatory nature, causes no irritation to the dog. Corneal dystrophy is not always permanent and in some cases which have been followed over several years complete regression of the lesion has occurred. In many cases progression of the lesion does not continue beyond a certain point at which neither a visual defect nor ocular inflammation are present. However, progression to visual impairment in a few cases has been recorded for the annular lipid dystrophy of the Husky in the United States.

This form of lipid or cholesterol dystrophy is of interest as it bears some resemblance to Schnyder's central crystalline dystrophy (degeneratio corneae cristallinea hereditaria) in man (Van Went & Wibaut, 1924; Schnyder, 1929, 1939) which is characterized by the deposition of fats and cholesterol in an axial position in both corneas in early life and which has an autosomal and dominant mode of inheritance. Dellamen & Winkleman (1968) described five morphological phenotypes of Schnyder's crystalline dystrophy in man and in dogs too there appear to be different morphological phenotypes. It has been suggested above, with regard to this dystrophy, that some variation may be expected in the clinical appearance according to the progression, regression or stationary nature of the lesion. In man Schnyder's dystrophy is believed to be due to a localized defect of lipid metabolism (Bron, Williams & Carruthers, 1972) and the same is probably true of the dog (Crispin, unpublished observations).

II CORNEAL DEGENERATION

Clinical Appearance

Corneal degeneration of the type described here appears clinically as dense grey or white lesions in both eyes but not necessarily symmetrical and with a clearly defined smooth edge. This corneal degeneration appears as one or two irregular, curved, sausage-shaped bands of opacity situated in a paraxial or peripheral position but well inside the limbus and with clear cornea between it and the lesion and in the centre. Occasionally the opacity may be more central in position and appear as a medium to large irregularly circular area. Unlike the previous type, epithelial involvement occurs with vascularization at a relatively early stage. The degeneration is progressive leading to epithelial disruption and erosion, the surface appearing rough and with loss of the clear corneal reflection. The epithelial involvement leads to irritation; mild blepharospasm and slight lacrimation may be other ocular signs present. (Figs 13–14.)

Fatty and calcareous degeneration is a rare condition in the dog and with slit-lamp biomicroscopy the degenerative corneal changes are seen to involve the epithelium and stroma to varying depths. Generally there is infiltration of the subepithelial region by granular material, although small vacuoles and needle-like forms may also be observed. Similar depositions can often be seen in the deeper parts of the stroma.

Pathology

The epithelium is of variable thickness with excessive numbers of cell layers in some regions and erosion of the entire thickness in others. The basement membrane shows some focal thickening but there is also disruption which is complete in the areas of extensive epithelial erosion.

In the stroma of some cases there is a distinctive sub-epithelial band of fine granular appearance as well as very small vacuoles and needle-shaped areas. With Von Kossa's stain for calcium (using 0·1 per cent citrate buffer on parallel sections to remove calcium and provide controls) there is black staining of the fine granules

---

FIG. 13. Corneal degeneration in a Golden Retriever with two opaque areas inside the limbus.

FIG. 14. Corneal degeneration in a Golden Retriever. Slit-lamp photograph.

FIG. 17. Corneal arcus in the Alsatian. Complete narrow ring of opacity.

FIG. 18. Corneal arcus in the Alsatian. Complete broad ring of opacity.

FIG. 19. Corneal arcus in the Alsatian. Incomplete ring of opacity with clear outer zone immediately inside the limbus.

FIG. 20. Corneal arcus. Crystalline appearance of lesion on slit-lamp microscopy.

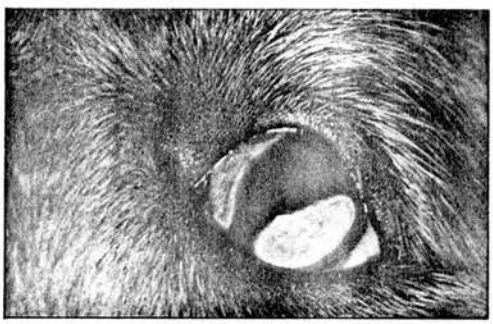


FIG. 13

FIG. 14

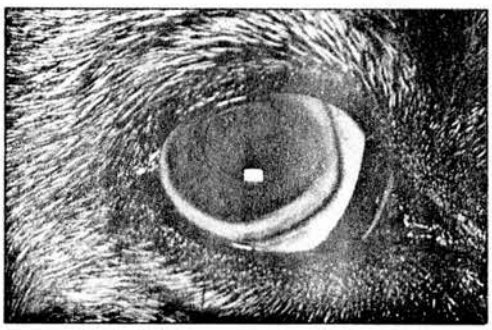


FIG. 17

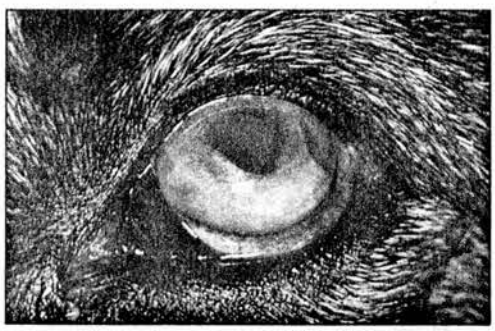


FIG. 18

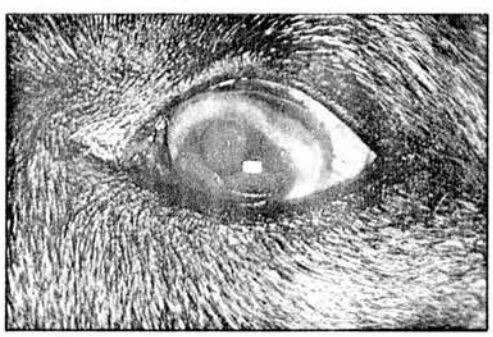


FIG. 19

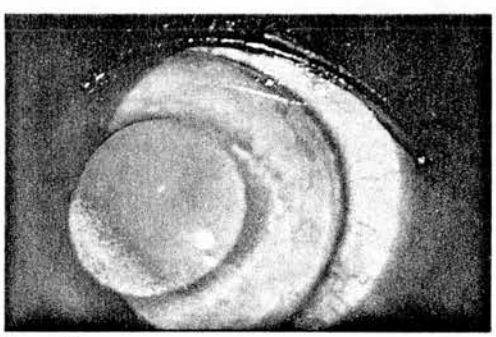


FIG. 20

(*Facing p.* 74)

representing the deposition of calcium salts. The small vacuoles are sudanophilic in frozen and fixed cryostat sections stained with the Sudan dyes, whereas the needle-shaped areas contain cholesterol.

In some patients there is often a region of normal stroma beneath the sub-epithelial deposition, more deeply within the anterior stroma fat droplets and crystals of cholesterol are found again. In the deeper stroma, to about half the total stromal thickness, there are frequently large fat globules present and these may be contiguous and confluent, forming chains of extracellular fat between the lamellae.

The abnormal deposition of fat and cholesterol is found in intracellular sites within the cytoplasm of keratocytes and occasional macrophages as well as in the extracellular sites described above.

The collagen fibres appear normal despite the infiltrative nature of the lesion. There is increased cellularity of the affected stroma and vascularization is a prominent feature of established degeneration. (Figs 15–16.)

Discussion

Fatty and calcareous degeneration may be primary or secondary in type, the former occurring in apparently normal eyes, the latter in diseased eyes. In man the primary types are very rare and consist of calcification of a previously apparently normal cornea. Axenfeld (1917) reported a bilateral case in a young boy which he termed a primary calcareous degeneration. Band-shaped keratopathy, first

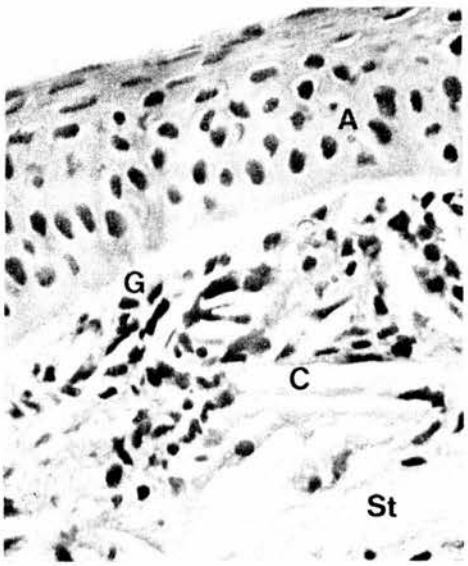


FIG. 15. Light micrograph of keratectomy specimen of anterior cornea from a Golden Retriever with corneal degeneration. Granular deposit (G) beneath the basement membrane of the epithelium (A), numerous cholesterol clefts (C) and increased cellularity of the stroma (St).

FIG. 16. Electron micrograph of anterior stroma from another Golden Retriever showing calcium deposits (Ca) and numerous cholesterol clefts (C) of different sizes.

described by Dixon (1848) is another form of primary or secondary calcareous degeneration and whether of primary or secondary origin, the condition develops as a grey band which moves axially from the limbus in the interpalpebral area of the cornea. There is occasionally an hereditary basis to the human types of band-shaped keratopathy. Spanlang described an unusual fatty and calcareous degeneration in man in 1927.

Calcareous degenerations secondary to systemic disease or secondary to some other corneal or ocular problem, are relatively common human conditions. Thus hypercalcaemia, hypophosphataemia, uraemia, hypervitaminosis D and Norrie's disease may be accompanied by secondary corneal calcification. Many diseases of the eye produce secondary calcareous degeneration. Fatty degeneration and hyaline degeneration may accompany, or precede, calcareous degeneration and it is not uncommon to find calcium in old scars, following uveitis, or in phthisical eyes.

In the dog fatty and calcareous degeneration has been seen as a bilateral condition without obvious antecedent eye disease. The Golden Retriever has been the breed most commonly affected and only bitches have been involved. Only in the case of a mother and daughter were the animals closely related. The mean age distribution is around four years. It is possible that this form of degeneration could be classed as a type of corneal dystrophy. Cases seem to occur in a cornea which was originally normal, and therefore of primary corneal origin, and the prevalence in the Golden Retriever indicates some genetic factor.

It is difficult to know whether the type of degeneration described here originates as a calcareous degeneration with secondary fatty degeneration, as a fatty

degeneration with secondary calcareous degeneration, or as a combined fatty and calcareous degeneration. Inflammatory changes are an early feature of this condition and such changes further obscure its true origin. The history and clinical appearance would suggest a combined degeneration or one in which fatty degeneration is the first abnormality to appear. Further investigation depends upon the early referral of animals with this condition if more pertinent observations are to be made.

Affected dogs, with one exception, have been clinically normal in other respects. The exception was an overweight Golden Retriever bitch with a fasting cholesterol level of 11·2 mmol/l and extensive degenerative involvement of both corneas. Dietary restrictions reduced the serum cholesterol to a more normal level of 6·2 mmol/l.

The majority of cases have required surgical treatment as the degeneration is progressive and the lesion has caused discomfort to the patient. The lesions are excised, completely if possible, by superficial keratectomy. Surgery may also be combined with the topical application of the chelating agent disodium ethylene diamine tetra-acetate (E.D.T.A.) at a concentration of 0·4–1·38 per cent in neutral solution. This regime has proved of value where calcium has been deposited within the stroma. In none of the cases described has the lesion been deep enough to require keratoplasty in addition to keratectomy.

Generally, the degenerative changes have not recurred if removal has been complete; sometimes, however, complete removal is technically difficult becuase of the area of cornea affected and the considerable corneal vascularization. In such cases keratectomy may have to be repeated.

III CORNEAL INFILTRATION

Clinical Appearance

Corneal infiltration may appear as a corneal arcus, a ring of corneal opacity of variable width just inside the limbus in the peripheral region. The central area is clear but is encroached upon in advanced cases. The lesions are bilateral, similar but not symmetrical and of a silvery blue-grey colour. The arcus may be narrow or broader, the width varying between dogs and, occasionally, between eyes in the same dog, although usually the two eyes are similar. The opaque ring may be adjacent to the limbus or just inside the limbus with a narrow area of clear cornea between it and the limbus itself (lucid interval). The inside border may show some evidence of spreading centrally, the edge being rather diffuse although the outer border is usually more definite. Usually the arcus is complete, occasionally there is a small break in the annulus. The opacity is made up of many closely packed dots seen with the slit-lamp and is sub-epithelial in position. There is, therefore, a complete corneal reflection to light with no irritation and no intravital staining. Inflammatory changes may develop in some cases and minor vascularization be part of this reaction. When inflammation does occur, secondary degenerative

changes will follow and the lesion will become more extensive and yellow in appearance.

Slit-lamp biomicroscopy reveals the opacity to be primarily in the region of Descemet's membrane and also under the basement membrane of the epithelium. Stromal involvement is initially not very marked but becomes more considerable as opacification becomes more obvious. The scintillating crystalline appearance so characteristic of cholesterol is not an early feature of arcus lipoides corneae unless the opacity is particularly dense, or in the event of secondary degenerative changes. (Figs 17–20.)

Pathology

In early cases the paraffin-wax embedded sections showed no abnormality. With fat stains the epithelium is normal and there is a granular sudanophilia consisting of extracellular fat in the corneal stroma and a hyaline sudanophilia of the region immediately beneath the anterior epithelium and the anterior part of Descemet's membrane. It is readily demonstrated on frozen sections with dyes such as oil-red-0, the Sudan series and Nile blue. The granular sudanophilia consists of numerous fat globules with these stains whereas the hyaline sudanophilia is of homogeneous appearance, particularly striking with Nile blue and oil-red-0.

As the corneal opacification becomes more marked there is an increase in the number and size of the fat globules, particularly in the deeper regions of the substantia propria. Extracellular and intracellular fat globules within the cytoplasm of the keratocytes can be discerned at this stage. Cholesterol becomes a feature of the developing opacity as demonstrated by the modified Schultz reaction, characteristic cholesterol clefts may be observed in paraffin-wax embedded material and by transmission electron microscopy and numerous vacuoles may also be observed in electron microscope preparations in the extracellular and intracytoplasmic sites described previously.

In addition to the infiltration of cholesterol, fatty acid esters and phospholipids there is a sterile inflammatory reaction so that there is an increase of corneal cellularity. In advanced lesions the heavy cholesterol infiltration may disrupt the basement membrane of the anterior epithelium and there may be some increase of thickness of the overlying epithelial cells but without epithelial erosion.

Fine fibrillar material occurs free in the stroma of more advanced lesions and in the deeper layers of the stroma large globules of eosinophilic material may be demonstrated on paraffin-wax embedded sections stained with haematoxylin and eosin (Figs 21–22).

Although neovascularization of the cornea may complicate advanced lesions, the majority of fat gains access to the cornea from the limbal vasculature and it is of interest that the types of lipids responsible for advanced arcus lipoides corneae, namely cholesterol, fatty acid esters and phospholipids, approximate to the types of lipids producing the systemic hyperlipoproteinaemia.

Unlike the other conditions described in this paper the presence of lipid is not

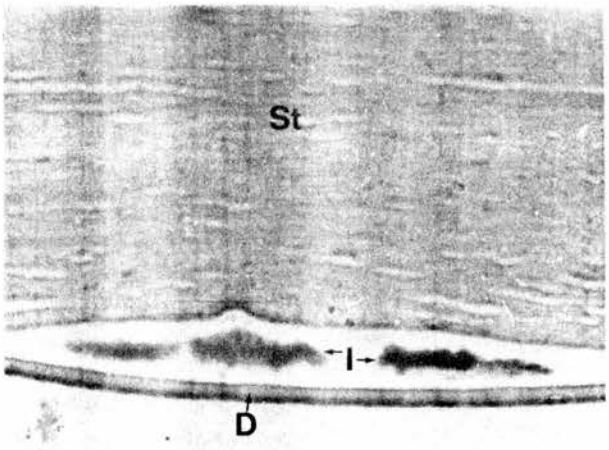


FIG. 21. Light micrograph of posterior cornea from an Alsatian with arcus lipoides corneae, showing amorphous infiltration (I) between posterior stroma (St) and Descemet's membrane (D).

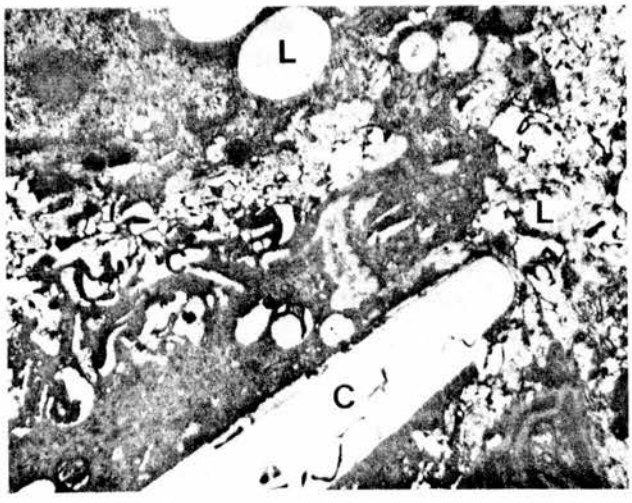


FIG. 22. Electron micrograph from a biopsy specimen of the anterior cornea from an Alsatian with arcus lipoides corneae showing numerous intracellular and extracellular vacuoles (L) typical of lipid droplets and clefts (C) typical of cholesterol crystals.

restricted to the cornea in these animals but may also be demonstrated post mortem within the anterior uvea and sclera.

Discussion

Corneal infiltration (arcus lipoides corneae) is rare and in most cases seen to date has been associated with primary acquired hypothyroidism and abnormally

high serum triglyceride and cholesterol levels. We have previously described five cases (Crispin & Barnett, 1978), all in the Alsatian (German Shepherd Dog). The fact that these naturally occurring cases were all in the same breed points to a possible familial incidence but, unfortunately, it was not possible to study the heredity as few pedigrees were available. The corneal infiltration is certainly a secondary change but primary hypothyroidism may be inherited.

In man, inherited hypothyroidism is well recognized and may be classified into several groups, each representing an abnormality of thyroid function at different points (de Groot & Stanbury, 1975). In the dog familial hypothyroidism and hyperlipoproteinaemia has been recorded in the Beagle (Manning, 1979). Blake Lapinski (1980) reviewed two thousand T4 results in different breeds and found that certain breeds had a high percentage of low T4 readings, in particular the Beagle, Labrador and Sled dogs, whereas other breeds (Dachshund and Schnauzer) showed considerably fewer cases. They concluded that certain breeds exhibit more hypothyroidism than others and that this is possibly hereditary. In addition, hypothyroidism, diagnosed on serum T3 and T4 values, was studied in 108 dogs by Nesbitt *et al.* (1980). Certain breeds showed some predilection and the larger breeds generally were more commonly affected and at an earlier age. However, there has been no suggestion, to date, that the German Shepherd Dog is particularly prone to hypothyroidism. It is interesting that secondary hypothyroidism does occur in association with pituitary dwarfism (Bush, personal communication) and other pituitary defects (panhypopituitarism). Pituitary dwarfism has been shown to be inherited in the German Shepherd Dog, and due to an autosomal recessive gene, in Australia (Nicholas, 1978) Denmark (Andresen & Willeberg, 1976) and the United Kingdom (Willis, 1981). However, it must be stated that there was no evidence of dwarfism in the German Shepherd Dogs in our series and in these dogs the hypothyroidism was considered to be primary.

Since the previous paper, one further case has been seen, in an adult male Shetland Sheepdog. The corneal arcus was identical to that described in the Alsatian and, although samples were not available for laboratory investigation, the dog clinically was a typical hypothyroid case with a poor and dry coat, obese and dull and with small, soft testicles. In the Alsatians investigated in detail affected with primary acquired hypothyroidism, all responded to treatment with oral L-thyroxine tablets (Eltroxin—Glaxo Laboratories Ltd.) in that clinical signs of hypothyroidism disappeared and the serum lipoprotein levels returned to normal. The corneal lesions became slightly less dense in some cases but the corneal appearance never returned to normal and this was confirmed at ultrastructural level on necropsy material from two cases which had both been successfully treated for several years. Further details of treatment were given in the previous paper.

Arcus lipoides corneae is a ring-shaped infiltration of the peripheral cornea. In the dog it has only been observed as a pre-senile change, almost certainly related to systemic hyperlipoproteinaemia and an apparent increase in vascular permeability of the limbal emissary vessels. Hyperlipoproteinaemia is not uncommon in the dog;

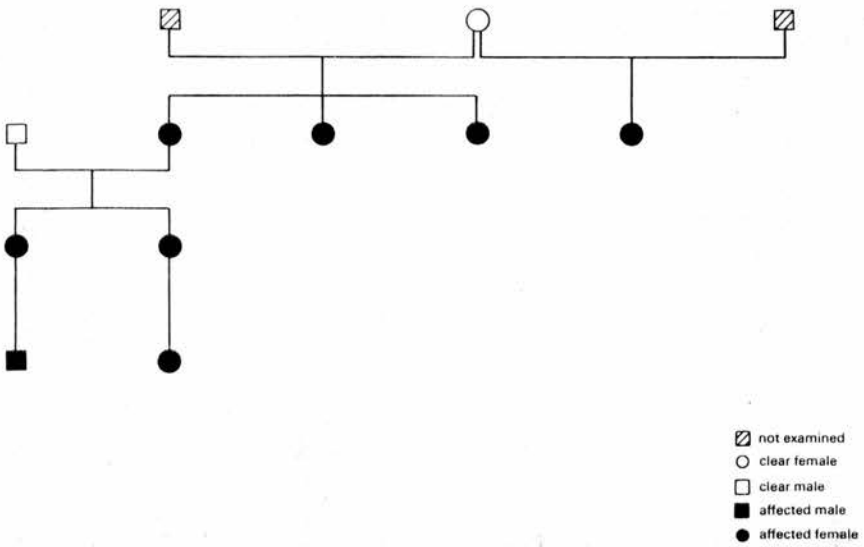


FIG. 23. Pedigree of cases of corneal lipidosis in the Rough Collie. (These cases were all young adult collies from different homes.)

it is rarely a primary condition but may occur secondary to dietary indiscretion, pregnancy, pancreatic disorders, hepatic disease, nephrosis, various endocrine dysfunctions and some infections. In view of the frequency with which hyperlipoproteinaemia can occur, it is perhaps surprising that arcus lipoides corneae is such a rare condition.

It is interesting that the cornea of the Alsatian may show several quite different conditions. As well as corneal dystrophy and corneal infiltration described here, the typical pannus or chronic superficial vascular keratitis and corneal dermoid are commonly seen in the Alsatian, the former perhaps exclusively.

ACKNOWLEDGMENT

This work was partly supported (S.M.C.) by the Clinical Studies Trust Fund of the British Small Animal Veterinary Association.

REFERENCES

ANDRESEN, E. & WILLEBERG, P. (1976) Pituitary dwarfism in German Shepherd dogs: Additional evidence of simple autosomal, recessive inheritance. *Nordisk Vet.* **28,** 481.

AXENFELD, T. (1917) Ueber doppelsetige primare progressive parenchymatose Verkalkung (Dystrophia calcera) der Cornea. *Klin. Monatsbel. f. Augenh.* **58,** 58.

BARNETT, K.C. & STADES, F.C. (1979) Collie eye anomaly in the Shetland Sheepdog in the Netherlands. *J. small. Anim. Pract.* **20,** 321.

BISTNER, S.I., AGUIRRE, G.D. & SHIVELEY, N. (1976). Corneal dystrophy in the Manx Cat. *Invest. Ophthalmol.* **15,** 15.

BLAKE, S. & LAPINSKI, A. (1980) Hypothyroidism in different breeds. *Canine Practice*, **7,** 48.

BRON, A.J. & TRIPATHI, T.C. (1970) Corneal disorders. In: *Genetic and Metabolic Eye Diseases* (Ed. M. F. Goldberg), pp. 281–323. Little Brown and Co. Boston.

BRON, A.J., WILLIAMS, H.P. & CARRUTHERS, M.E. (1972) Hereditary crystalline stromal dystrophy of Schnyder, I Clinical features of a family with hyperlipoproteinaemia. *Brit. J. Ophthalmol.* **56,** 383.

BUSH, B.M. (1981) Personal communication.

CRISPIN, S.M. & BARNETT, K.C. (1978) Arcus lipoides corneae secondary to hypothyroidism in the Alsatian. *J. small Anim. Pract.* **19,** 127.

CRISPIN, S.M. (1982). Corneal dystrophies in small animals. In: *The Veterinary Annual* (Eds C. S. Grunsell and F. W. G. Hill), pp. 298–310. John Wright, Bristol.

DE GROOT, L.J. & STANBURY, J.B. (1975) *The Thyroid and its Diseases*. John Wiley and Sons, New York.

DELLAMEN, J.W. & WINKLEMAN, J.E. (1968) Degeneratio corneae cristallinea hereditaria: A clinical, genetical and histological study. *Ophthalmologica*, **155,** 409.

DICE, P.F. (1974) Corneal dystrophy in the Airedale. *Proc. Am. Coll. Vet. Ophthalmol. 5th. Annual Scientific Programme* pp. 80–86.

DICE, P.F. (1977) Corneal dystrophy in the Airedale. In: *Current Veterinary Therapy*, Vol. VI (Ed. R. W. Kirk), p. 617. W. B. Saunders Co. Philadelphia.

DICE, P.F. (1980) Primary Corneal Disease in the dog and cat. In: *Vet. Clin. North America*, Vol. 10 (Ed. R. L. Peiffer), p. 339. W. B. Saunders Co.

DICE, P.F. (1981) The Canine Cornea. In: *A Textbook of Veterinary Ophthalmology* (Ed. K. N. Gelatt), p. 366. Lea and Febiger, Philadelphia.

DIXON, J. (1855) *Diseases of the Eye*, pp. 91–93. John Churchill, London.

DREYFUSS, M. (1930) Symmetrische zentrale Hornhautverfettung beim Hund. *Albrecht. v. Graefes Arch. Ophthal.* **125,** 67.

DUKE-ELDER, S. & LEIGH, A. G. (1965) Corneal degenerations, dystrophies and pigmentations. In: *System of Ophthalmology*, Vol. VIII, Part 2. Henry Kimpton, London.

EKINS, M.B., WARING, G.O. & HARRIS, R.R. (1980) Oval lipid corneal opacities in Beagles, Part II. Natural history over four years and study of tear function. *J. Am. Anim. Hosp. Ass.* **16,** 601.

JUBB, K.V.F. & KENNEDY, P.C. (1963) *Pathology of Domestic Animals*, Vol. 2. Academic Press, New York and London.

MACMILLAN, A.D., WARING, G.O., SPANGLER, W.L. & ROTH, A.M. (1979) Crystalline corneal opacities in the Siberian Husky. *J. Am. vet. med. Ass.* **175,** 829.

MAGRANE, W.G. (1977) *Canine Ophthalmology*, 3rd edn. Lea and Febiger, Philadelphia.

MANNING, P.J. (1979) Thyroid gland and arterial lesions of Beagles with familial hypothyroidism and hyperlipoproteinemia. *Am. J. vet. Res.* **40,** 820.

NESBITT, G.H., IZZO, J., PETERSON, L. & WILKINS, R.J. (1980) Canine hypothyroidism: A retrospective study of 108 cases. *J. Am. vet. med. Ass.* **177,** 1117.

NICHOLAS, F. (1978) Pituitary dwarfism in German Shepherd Dogs: A genetic analysis of some Australian data. *J. small Anim. Pract.* **19,** 167.

PEARSE, A.G.E. (1968) *Histochemistry, Theoretical and Applied*, 3rd edn. Little Brown, Boston.

ROBERTS, S.R. (1965) Superficial indolent ulcer of the cornea in Boxer dogs. *J. small Anim. Pract.* **6,** 111.

ROBERTS, S.R. & DELLAPORTE, A. (1965) Congenital posterior ectasia of the sclera in Collie dogs. *Am. J. Ophthalmology*, **59,** 180.

ROBIN, V. & CHARTON, A. (1939) La Lipoidose cornéene du chien. *Rec. Med. Vet.* **115,** 321.

ROTH, A.M., EKINS, M.B., WARING, G.O., GUPTA, L.M. & ROSENBLATT, L.S. (1981) Oval corneal opacities in Beagles. III. Histochemical demonstration of stromal lipids without hyperlipidaemia. *Invest. Ophthalmol. Vis. Sci.* **21,** 95.

SAUNDERS, L.Z. & RUBIN, L.F. (1975) *Ophthalmic Pathology of Animals* (*An Atlas and Reference Book*) p. 14. S. Karger.

SCHOCK, K. (1910) Eine noch nicht beschriebene Hornhautaffektion beim Hund *Arch. vergl. Ophthal.* **1,** 313.

SCHNYDER, W.F. (1929) Mitteilung ueber einen neuen Typus von familiarer Hornhauterkrankung. *Schweiz. Med. Wschr.* **59,** 559. (Schnyder's original observations presented at a meeting of Swiss Ophthalmological Society on June 18th, 1927. These observations published in January 1929).

SCHNYDER, W.F. (1939) Scheibenformige Kristaleinlagerungen in der Hornhautemitte als Erbleiden. *Klin. Mbl. Augenheilk.* **103,** 494.

SPANLANG, H. (1927) Beitrage zur klinik und Pathologie seltener Hornhauterkrankungen. *Zeitschrift fur Augenheilkunde*, **62,** 21.

STADES, F.C. & BARNETT, K.C. (1981) Collie eye anomaly in Collies in the Netherlands. *The Veterinary Quarterly*, **3,** 66.

STARTUP, F.G. (1969) *Diseases of the Canine Eye.* Bailliere Tindall and Cassell, London.

VAINISI, S.J. & GOLDBERG, M.F. (1974) Animal models of inherited human eye diseases. In: *Genetic and Metabolic Eye Disease* (Ed. M. F. Goldberg). Little Brown and Co., Boston.

VAN WENT, J. & WIBAUT, F. (1924) Een zeldzame erfelijke hoornvliesaandoening. *Ned. T. Geneesk.* **68,** 2996.

VEENENDAAL, H. (1928) Twee nog weinig bekende hoornvliesaandoeningen bij den hond. *Tijdschr. Diergeneeskde*, **55,** 853.

VEENENDAAL, H. (1937) Dystrophia corneae adiposa bij de hond. *Tijdschr. Diergeneeskde*, **64,** 913.

VEENENDAAL, H. (1953) Mededelingen mit de oogheelkundige afdeling van de Kliniek voor kleine huisdieren van de rijksuniversiteit te Utrecht. *Tijdschr. Diergeneeskde*, **78,** 662.

WARING, G.O., MUGGLI, F.M. & MACMILLAN, A. (1977) Oval corneal opacities in Beagles. *J. Am. Anim. Hosp. Ass.* **13,** 204.

WILLIS, M.B. (1981) Personal communication.

WYMAN, M. & DONOVAN, E.F. (1971) *Current Veterinary Therapy*, Vol. IV (Ed. R. W. Kirk). W. B. Saunders Co., Philadelphia.

J. small Anim. Pract. (1978) **19**, 127–142.

Arcus lipoides corneae secondary to hypothyroidism in the Alsatian

SHEILA M. CRISPIN* AND K. C. BARNETT†

* Department of Veterinary Surgery, Royal (Dick) School of Veterinary Studies, Summerhall, Edinburgh and † Animal Health Trust Small Animals Centre, Lanwades Park, Kennett, Newmarket, Suffolk.

ABSTRACT

Five cases of arcus lipoides corneae in the Alsatian are described. The affected animals were investigated and found to be hyperlipoproteinaemic and hypothyroid. Appropriate treatment was instigated and the results of treatment are presented. The laboratory findings for the five cases are compared with those for ten normal Alsatians of similar age range.

INTRODUCTION

Arcus lipoides corneae in man is an annular infiltration of the peripheral cornea and perilimbal zone of the sclera by lipid material (Duke-Elder, 1965). There is usually a distinct clear zone, or lucid interval, at the limbus and an arcus may appear as two concentric bands, sometimes incomplete and occasionally encroaching upon the centre of the cornea.

Hyperlipoproteinaemia is not always accompanied by arcus formation (Garn & Gertler, 1950; Lindholm, 1960; Harcourt, 1969) and this is particularly true of the secondary types of hyperlipoproteinaemia where arcus lipoides is rare. Raised cholesterol and triglyceride levels are usually implicated in the arcus lipoides corneae of hyperlipoproteinaemia (Bron, 1976).

This report describes five cases of arcus lipoides corneae in the Alsatian breed, associated with the secondary hyperlipoproteinaemia of hypothyroidism. This is believed to be the first record of association in the dog.

METHODS OF INVESTIGATION

Diagnosis was based upon the history, general clinical examination, detailed ocular examination and laboratory findings. Skin and corneal biopsies were

0020–4510/78/0200–0127 $02.00

taken from Cases1 and 2, and Cases 1 and 3 were submitted for post-mortem examination (histopathology only for Case 1) approximately three years after the original referral.

Ocular examination was by observation, direct opthalmoscopy, slit-lamp biomicroscopy and photography.

Skin biopsies were taken under general anaesthesia from the parascrotal/-inguinal region, fixed in 10% neutral buffered formalin, embedded in paraffin-wax and stained by haemotoxylin and eosin (H and E), per-iodic acid Schiff (PAS) and Masson's trichrome methods.

Corneal biopsies, obtained at the same time as the skin biopsies, were taken into 10% neutral buffered formalin for light microscopy and buffered gluteraldehyde for electron microscopy. Sections for light microscopy were cut with a freezing microtome after fixation only and conventionally after paraffin-wax embedding. Frozen sections were stained for lipids by the oil-red-O, Sudan black, and Sudan III and IV methods and also examined unstained with a polarized light source. Paraffin-wax embedded sections were stained with H. and E., Masson's trichrome, Van Gieson's, PAS and Von Kossa's stain for calcium. All material for electron microscopy was post-fixed in 1% buffered osmium tetroxide for two hours, dehydrated with graded alcohols and embedded in araldite following transitional stages in propylene oxide. Thick 1μm (micron) sections were cut and stained with alkaline toluidine blue. Thin sections were stained with uranyl acetate and lead citrate and examined at 50 kV in an Hitachi HS 8 electron microscope. The full results of microscopic examination are to be published at a future date.

Resting venous blood samples were taken from the cephalic vein for laboratory examination after the animals had undergone a standard 12–16 hours fast. Blood samples were taken for routine haematology, blood glucose, blood urea nitrogen, plasma cholesterol and triglyceride estimations, lipoprotein electrophoresis, serum sodium and potassium and serum thyroxine (T_4) assay. The biochemical investigations were later extended to include serum protein electrophoresis, serum triiodothyronine (T_3) estimations and ultra-centrifugational analysis of selected samples. Detailed results of biochemical analysis form the basis of a future communication: a synopsis only is presented in this paper.

Serum cholesterol and triglyceride levels were measured simultaneously on a dual channel autoanalyser using the N-24a and N-78a methodologies. Lipoprotein electrophoresis was carried out according to Lehmann & Lines (1972). T_4 and T_3 estimations were by radioimmunoassay using methods described by Ratcliffe, Challand & Ratcliffe (1974).

Routine urinalysis was also undertaken and, as far as possible, blood and urine samples were taken from affected and normal animals at 09.00 hours, immediately after the rectal temperature had been recorded.

Statistical analysis of the laboratory findings for the five cases and ten normal unrelated Alsatians (six males, four females) was made using Student's t test.

Comparisons were only made when the variance ratio (F) was found to be not significant and when the number of combined samples (n) was ten or more. Values of P less than 0·01 were considered to be of statistical significance.

CASE REPORTS

Case 1. Alsatian, male 4 years

Presented with a history of eye trouble which had started in the right eye almost eight months previously. Recent involvement of the left eye had caused the owner to seek veterinary advice.

The ocular lesion in the right eye (Fig. 1) appeared as a broad, complete annulus extending from the limbus towards the centre of the cornea, particularly in the inferior nasal quadrant; there was also slight distortion of the corneal epithelium and very fine superficial vascularisation. In the left eye (Fig. 2) the annulus was narrow and complete, encroaching on the centre of the cornea from the limbus. In this animal, as in the others of the series, the arcus was of a silvery blue–grey colour and located in the subepithelial and stromal layers of the cornea. In optical section the appearance was of a myriad of tiny bright dots and darker lattice-like lines. There was no pain, photophobia, blepharospasm or lachrymation and the right eye of Case 1 was the only eye in which corneal vascularization was observed. Vision in all cases was unaffected by the arcus lipoides.

On general examination the dog was both dull and lethargic and the owner observed that the dog had been like this for at least twelve months. Reduced

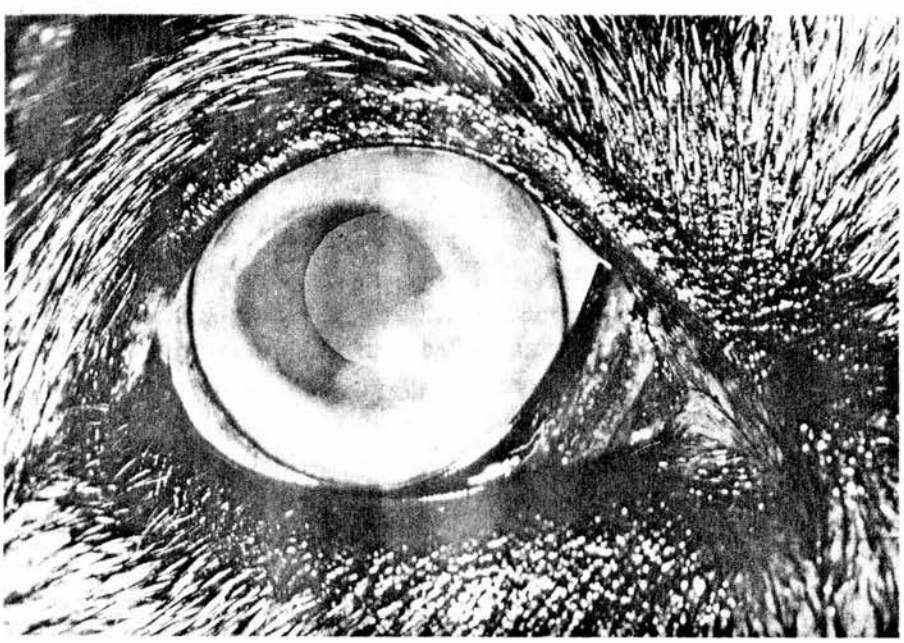


FIG. 1. Case 1. Right eye. Broad perilimbal corneal arcus.

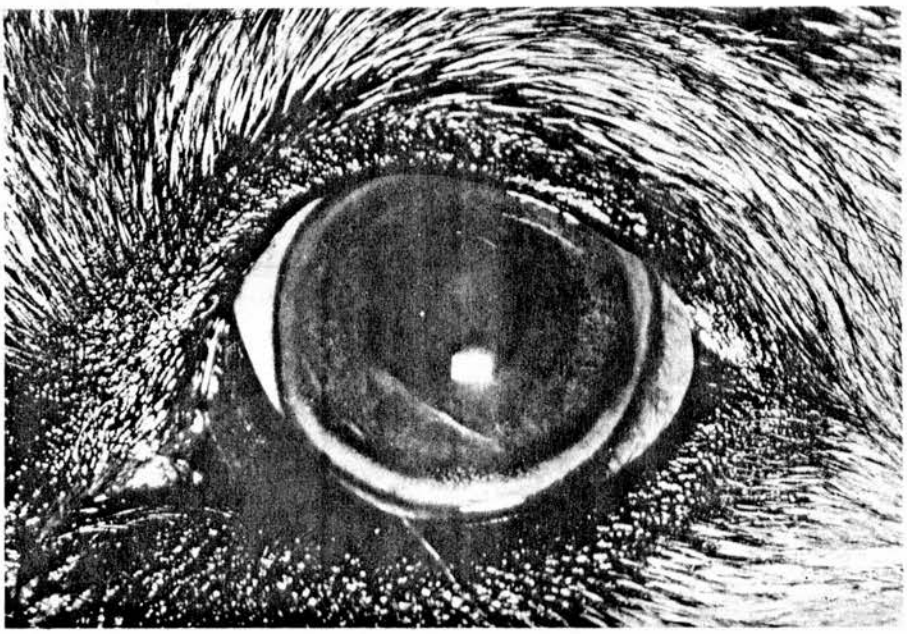


FIG. 2. Case 1. Left eye. Narrow perilimbal corneal arcus.

exercise tolerance had been ascribed to hip dysplasia, which was present, and to a gain in weight. The coat shed readily and the hair was dry, brittle and coarse. The skin was thickened and slightly scaly, with mild hyper-pigmentation in the axilla and groin; in these regions the skin was also noticeably cool to the touch. Both testicles were smaller than normal and the dog lacked libido. Appetite was capricious and there were occasional bouts of diarrhoea. The resting pulse rate taken from the femoral artery was between 96 and 100 beats per minute and the pulse was of normal volume; a standard bipolar limb lead electrocardiogram recorded in the conscious, unsedated animal showed no apparent abnormality.

Laboratory findings (Tables 1 and 2) confirmed the clinical diagnosis of hypothroidism. The mild anaemia was of a normochromic, normocytic type and serum from the animal was visibly lipaemic on standing (Fig. 3). Histological examination of the skin biopsy specimen showed a thin epidermis with marked atrophy of adnexal structures; hair follicles in particular were degenerate or absent. Dermal collagen was of increased quantity but relative accellularity and the collagen was separated by large amounts of ground substance of a mucopolysaccharide nature.

In the corneal biopsy specimens neutral fats and cholesterol (or characteristic cholesterol clefts) were found extracellularly within the corneal stroma and underlying the corneal epithelium. Some lipid material was present within the keratocytes.

Treatment with oral L-thyroxine sodium (Eltroxin, Glaxo Laboratories Ltd) was started at a dose rate of 0·05 mg twice daily. At the end of two weeks the dog

TABLE 1.

Values	Normals (n=10) Mean ± SEM	Abnormals (n=5) *(n=4) Mean ± SEM	Case 1	Case 2	Case 3	Case 4	Case 5	† Probability values
Rectal temperature °C	38·6 ± 0·09	37·8 ± 0·18	37·5	37·9	37·3	37·8	38·3	‡
Urine S.G.	1·036 ± 0·004	*1·035 ± 0·010	1·042	1·034	1·026	—	1·038	n.s.
Rbc × 10^{12}/l	6·4 ± 0·14	5·36 ± 0·25	4·8	5·1	5·4	5·2	6·3	‡
Hb g/dl	14·7 ± 0·32	14·2 ± 0·58	12·2	15·1	13·6	15·3	14·9	n.s.
PCV l/l	0·46 ± 0·099	0·39 ± 0·017	0·33	0·40	0·38	0·42	0·42	n.s.
Wbc × 10^{9}/l	9·67 ± 0·316	8·09 ± 0·315	7·58	8·35	9·20	7·50	7·80	n.s.
Total serum protein g/l	67·0 ± 1·32	*66·5 ± 4·46	57	68	63	—	78	n.s.
A/G ratio	0·96 (n=8)	1·08 (n=3)	—	1·02	1·28	—	0·96	—
Na mmol/l	142 ± 1·0	139 ± 1·1	142	136	138	140	141	n.s.
K mmol/l	4·5 ± 0·12	4·36 ± 0·07	4·6	4·3	4·4	4·3	4·2	n.s.
Blood glucose mmol/l	4·10 ± 0·155	*2·78 ± 0·144	3·06	2·88	2·38	—	2·78	§
Blood urea nitrogen mmol/l	2·45 ± 0·109	2·50 ± 0·170	2·5	2·7	3·0	2·3	2·0	n.s.

† Probability values: n.s. not significant; ‡ = < 0·01 > 0·001; § = < 0·001

TABLE 2.

Values	Normals (n = 10) *(n = 5) Mean ± SEM	Abnormals (n = 5) Mean ± SEM	Case 1	Case 2	Case 3	Case 4	Case 5	† Probability value
Total cholesterol mmol/l	4·84 ± 0·273	15·6 ± 0·692	17·8	16·3	15·0	13·4	15·6	‡
Range	3·62 – 6·33	13·4 – 17·8						
Total triglyceride mmol/l	0·7 ± 0·047	2·43 ± 0·1405	2·82	2·60	2·48	2·03	2·20	‡
Range	0·51 – 1·02	2·03 – 2·82						
Thyroxine (T_4) nmol/l	*89·2 ± 1·71	13·76 ± 1·12	13·3	14·3	14·8	16·7	10·0	‡
Triiodothyronine (T_3) nmol/l	*1·4 ± 0·05	0·95 (n = 2)	—	—	0·7	—	1·2	—
Serum lipoprotein electrophoresis	normal	Excess pre-beta, beta and alpha$_2$-lipoprotein						

† Probability values: n.s. = not significant; ** = < 0·01 > 0·001; ‡ = < 0·001

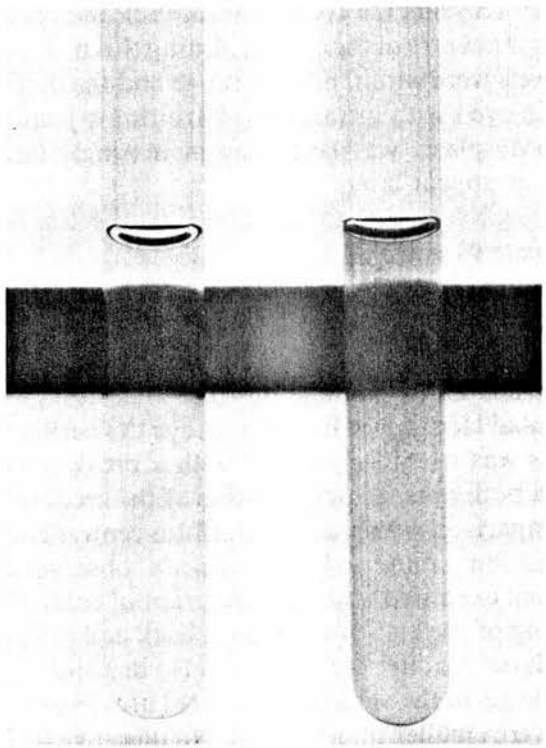


FIG. 3. Case 1. Serum sample RHS. Control LHS.

was more active and the dose rate was gradually increased over a period of two months to a maintenance level of 0·15 mg twice daily. There was some clinical improvement within a month but serum triglyceride and cholesterol levels remained higher than normal. In addition to L-thyroxin sodium the dog therefore received 500–1000 mg of Clofibrate (Atromid-S, Imperial Chemical Industries Ltd) by mouth each day. Dosage in excess of 500 mg caused digestive upsets and obvious discomfort and treatment with Clofibrate was discontinued when it was realized that the hormone replacement therapy being administered at this stage (0·1 mg L-thyroxine sodium twice daily) was inadequate. Within three months of diagnosis the rectal temperature had risen to a normal value of 38·5, presumably reflecting an increased basal metabolic rate. The coat and skin had assumed a much healthier appearance, although the patchy alopoecia took about six months to disappear. Throughout treatment the red cell count remained on the low side of normal ($5{\cdot}7 \times 10^{12}$/l) although the PCV improved (0·44 l/l) and blood glucose levels increased slightly (3·0 mmol/l).

The owners were warned as to the cumulative nature of L-thyroxine sodium and asked to report any side effects or signs of overdose such as restlessness, tachycardia and diarrhoea. The effectiveness of treatment was monitored by the animal's clinical appearance and serum cholesterol and triglyceride estimations.

Over a period of two years the corneal arcus in both eyes cleared slowly from the limbus towards the centre of the cornea; during this time the serum cholesterol and triglyceride levels were within normal range and the dog was fed a fairly low fat diet. It was destroyed with intravenous barbiturate just over two years after referral as the hip dysplasia was becoming increasingly incapacitating. Histopathological findings appear later.

Case 2. Alsatian, male 9½ years

Presented because of eye trouble in the preceeding six months. The owner had also noticed that the dog was apathetic and overweight but had attributed this to senility.

In the left eye the arcus lipoides cornea was complete with a sharply demarcated perilimbal clear zone. In the right eye the perilimbal region was also clear and the arcus was irregularly broad with a break in continuity in the 12 o'clock position. In both eyes the demarcation of the arcus in the perilimbal area was striking in comparison with the more diffuse central corneal infiltration.

General examination confirmed the owner's observations, the dog also fatigued readily when exercised and was intolerant of cold. There was bilaterally symmetrical thinning of the hair of the flanks, back and tail and the hair was dry and brittle and epilated readily; the skin was also dry and cool to the touch and there was some oedema in the ventral abdominal area. The animal lacked libido and both testicles were smaller than normal. No digestive disturbances had been noted and appetite was apparently unaffected.

The resting pulse rate was 56–60 beats per minute and of poor volume. Peaked *T* waves were recorded with a standard bipolar limb lead ECG which appeared normal in all other respects. Peaked *T* waves may themselves be a normal ECG feature but can be indicative of myocardial ischaemia, posterior wall infarcation and hyperkalaemia. Potassium levels were normal (Table 1) and, as the dog was not submitted for post-mortem examination, the possibility of underlying cardiac

TABLE 3.

Case 2	Before treatment	After 1 months treatment	After 6 months treatment
Total cholesterol mmol/l	16·28	4·34	4·15
Total triglycerides mmol/l	2·60	0·68	0·67
Case 3	**Before treatment**	**After 2 months treatment**	**After 18 months treatment**
Total cholesterol mmol/l	14·99	10·07	6·33
Total triglycerides mmol/l	2·48	1·13	0·62

pathology such as atherosclerosis, with consequent ischaemia or infarction, is conjectural. The histopathology of skin and corneal biopsies were as for Case 1.

Treatment with L-thyroxine sodium was started as for Case 1, a maintenance dose of 0·1 mg twice daily was reached after three weeks. Response to treatment was gratifying, the dog was brighter and livelier within two weeks and the skin and coat were normal within 6 months. In this dog the serum cholesterol and triglyceride levels were used as indices of adequate treatment (Table III), as described for Case 1. There was slight and incomplete clearing of the corneal arcus from the limbus in the left eye and no clearing in the right eye; the dog remained free of signs of hypothyroidism until his death when approaching the age of 13 years. The body was not available for post-mortem examination and the cause of death is unknown.

Case 3. Alsatian, female, 6 years 9 months

This bitch had been in poor health for eighteen months and had developed corneal opacities in both eyes within the last six weeks. In the right eye (Fig. 4) a broad band extended from the limbus towards the centre of the cornea. It was not of equal width all the way round and there was a clear zone between 10 o'clock and 1 o'clock. In the left eye the arcus was less broad and there was a perilimbal clear zone and a break in the corneal arcus between 11 o'clock and 2 o'clock. The animal also had a retinopathy.

Presenting clinical signs were as for Cases 1 and 2; mental and physical dullness, cold intolerance, obesity, coarse, dry and brittle hair and a puffy

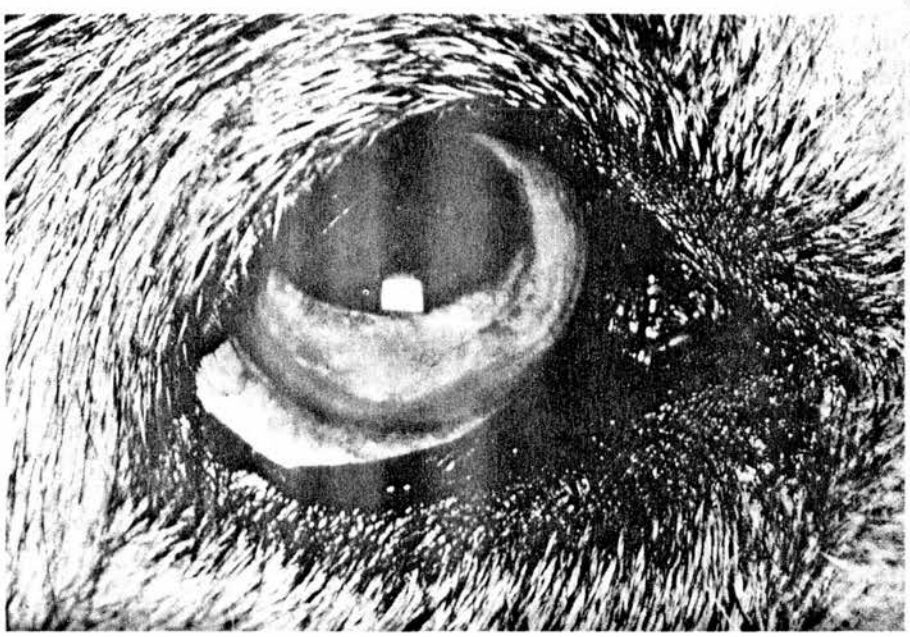


FIG. 4. Case 3. Right eye. Broad dense opaque corneal arcus.

FIG. 5. Case 3. Typical appearance of hypothyroid animal.

appearance (Fig. 5). She had whelped normally about three years before presentation. Her oestrus cycle had been irregular afterwards and was now absent, the owner had been unable to breed from her.

Investigations were performed as previously described, although no biopsy specimens were taken. The laboratory results are shown in Tables 1 and 2.

Treatment with 0·05 mg L-thyroxine sodium twice daily resulted in a clinical improvement within three weeks and a maintenance dose of 0·2 mg twice daily was found to be satisfactory after five weeks treatment. Serum tryglyceride and cholesterol returned to near normal levels, increasing the level of thyroxine administration did not reduce the lipoprotein levels any further. In the latter part of the animal's life the levels were always on the high side of normal, a possible reason for this is mentioned later. There was some clearance of the arcus lipoides corneae (Fig. 6).

The bitch continued in good health until almost ten when she gradually became dull and reluctant to eat. On presentation almost two months after the onset of this illness she was anorexic, cachexic and listless. A large mass was palpable in the anterior abdomen and ascites was present. The visible mucous membranes were pale, but not jaundiced. Clinical, radiological and laboratory examination indicated a liver tumour. Euthanasia with intravenous barbiturate was performed at the owner's request and post-mortem findings appear later.

Case 4. Alsatian, male, 6 years

This Alsatian was referred and described as ageing prematurely, with alopecia

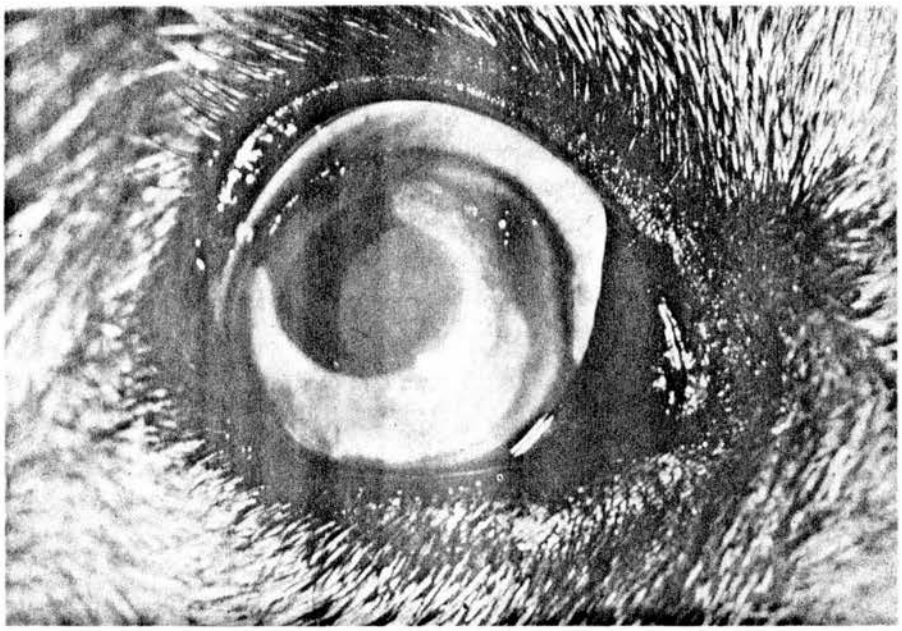


FIG. 6. Case 3. Fifteen months after treatment started showing reduction in density of corneal arcus. Note incomplete arcus at top.

in the tail region, a dry, scurfy coat and underdeveloped testicles, as well as corneal abnormalities.

In this dog the corneal arcus in the right eye was narrow and comprised two bands of differing density, whereas in the left eye the arcus was broad with a perilimbal clear zone.

The animal was lethargic, intolerant of cold and in all respects like the other dogs of the series. The resting pulse rate was between 87 and 95 beats per minute and of normal volume. Laboratory findings are given in Tables 1 and 2.

Treatment was with L-thyroxine sodium as described previously and the dog is at present maintained on 0·2 mg twice daily on which it appears euthyroid.

Case 5. Alsatian, male, 6 years

Another six-year old male Alsatian was presented because of a bilateral eye problem and with a history of decreased exercise tolerance. The corneal arcus in the right eye was a narrow but complete annulus with some variation in width and extending from the limbus. The arcus in the left eye was also complete and about twice as broad as that of the right eye, extending diffusely towards the centre of the cornea in the superior temporal quadrant.

Typical signs of hypothyroidism were present; the coat was sparse and dry, particularly in the flank region; the skin was cool and thickened with ridges and excessive pigmentation. The dog was overweight and placid and the owners felt that he had been unwell for some time, despite a normal appetite. The resting

pulse rate was between 82 and 86 beats per minute and pulse volume was normal. Visible mucous membranes were less pallid than those of the other cases. The testicles were small and soft. In this animal, and in all the others of the series, it was not possible to recognise any thyroid abnormality by palpation.

The treatment regime was as described for other cases and the dog is at present in good health on a maintenance dose of 0·2 mg L-thyroxine sodium twice daily. Treatment started in July 1976 and the owners have reported no change in the appearance of the corneal arcus, although serum cholesterol and triglyceride levels have been within the normal range since September 1976.

POST-MORTEM FINDINGS

Case 1

Formalin-fixed tissues only were available for post-mortem examination. The thyroid gland showed diffuse lymphoid infiltration and marked associated parenchymatous atrophy. Any follicles which remained contained little colloid. The parafollicular cells were hypertrophied.

In the liver and spleen there was some passive venous congestion and haemosiderin deposition. The pancreas and pituitary gland were normal, as was the central nervous system (CNS).

Case 3

The thyroid gland appeared small and atrophic. Histological examination revealed that the acini were smaller than normal and relatively few in number. There was a paucity of epithelial cells and the follicles were frequently devoid of colloid. There was no evidence of inflammation. The central nervous system, pituitary gland, adrenal glands and pancreas showed neither macroscopic nor microscopic abnormality.

In the liver there was a poorly differentiated bile duct adenocarcinoma. Biliary obstruction can itself lead to secondary hyperlipoproteinaemia and might account for the fact that serum cholesterol and triglycerides remained on the high side of normal, despite variations in the treatment regime.

DISCUSSION

The deposition of lipid within the cornea may be influenced by many factors, either acting together or independently. For instance, there may be local changes in corneal structure and function, in the corneal vascular supply and in the lipid content of nearby ocular tissues such as the aqueous humour, as well as the more generalized disturbances of lipid metabolism which characterise the primary and secondary hyperlipoproteinaemias.

Arcus formation is encouraged by increased vascularity, as from a tumour (Fig. 7) or chronic inflammation (Fig. 8) (Cogan & Kuwabara, 1959), and the

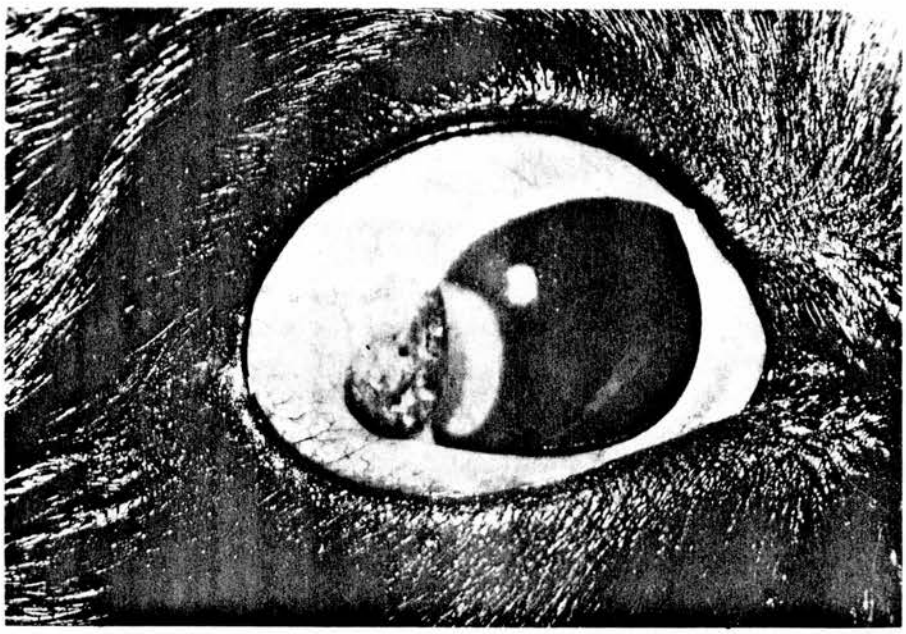


FIG. 7. Corneal arcus in region of a tumour.

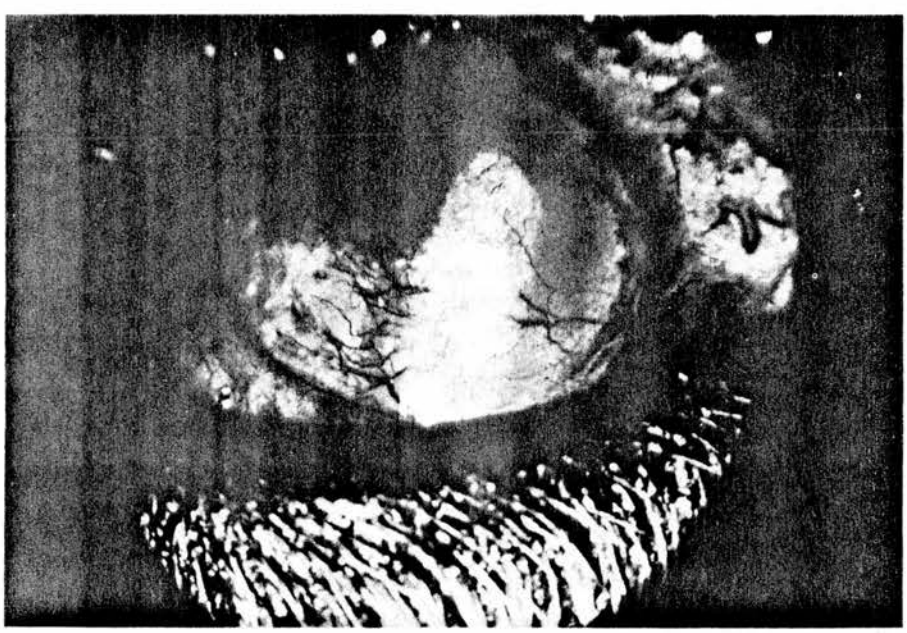


FIG. 8. Corneal arcus in area of chronic superficial keratitis (lipid keratopathy).

deposition of lipid at the donor–recipient interface, the most frequent cause of loss of transparency in a lamellar corneal graft, has been connected with the presence of deep blood vessels (Maumenee, 1964). Lipid is most commonly deposited by blood stream spread and both Andrews (1962) and Walton (1973) have suggested that increased blood vessel permeability allows lipids to enter the cornea. The passage of lipids into the corneal stroma by other routes, for instance from the aqueous via the endothial layers, is unlikely to be an important portal of entry in the normal eye but may assume significance in the presence of corneal inflammation or oedema. The entry and diffusion of lipid in the cornea is probably affected by the type of lipid, the composition of the cornea, particularly the ground substance of the corneal stroma, as well as by the degree of vascularization, inflammation and oedema.

In the cases under discussion the arcus lipoides showed little tendency to regress (although in some cases it certainly became less dense) even though serum lipoprotein levels had returned to normal and this is in accordance with such infiltrations in man (Forsius, 1954).

Human hyperlipoproteinaemias are divided into primary and secondary types. For primary hyperlipoproteinaemia five genetically determined phenotypes were distinguished by Frederickson, Levy & Lees (1967) and the scheme has been slightly modified in the World Health Organisation classification (1970) to include the overindulgence hyperlipoproteinaemia so commonly encountered in civilized countries.

Secondary hyperlipoproteinaemia may be a consequence of the diet; natural phenomena such as late pregnancy; various infections; pancreatic disease such as diabetes mellitus; hepatic disease, particularly where biliary obstruction is present; endocrine dysfunction, which may be hypothalamic, hypopituitary, hypothyroid or hyper-adrenal in origin; or even iatrogenic as after prolonged steroid or oestrogen therapy and, in man, hyperlipoproteinaemia may also occur as part of the nephrotic syndrome, a complication as yet unreported in the dog.

In the five cases presented hyperlipoproteinaemia was associated with an increase in pre-beta, principally the very low-density lipoprotein (VLDL) which is mainly triglyceride, an increase in the beta, low-density lipoprotein (LDL) which is mainly cholesterol and an increase in the alpha$_2$-lipoproteins which are of high density (HDL) and mainly phospholipid. Prominent beta, pre-beta and alpha$_2$-lipoproteins on serum electrophoresis have been reported previously by Manning, Corwin & Middleton (1973) in hypothyroid Beagles and Rogers, Donovan & Kociba (1975) in a number of different breeds of hypothyroid dog.

In the Alsatians described in this report no clinical signs of CNS or pituitary dysfunction were observed and no CNS or pituitary abnormality was found in the two animals which came to post-mortem. Increased serum lipoprotein levels correlated with a reduction in thyroid activity and with signs suggestive of acquired primary hypothyroidism, which is the commonest type in dogs (Rijnberk, 1974). The inverse relationship between thyroid activity and the level

of serum cholesterol (Abell, Mosbach & Kendall, 1956) in the uncomplicated case of acquired primary hypothyroidism provided a useful means of estimating the adequacy of hormone replacement therapy.

Lipaemia retinalis, associated with systemic lipoproteinaemia, has been reported in a dog and cat (Wyman & McKissick, 1973) and in another cat (Rubin, 1974). A further case occurred in an eight-year-old cross-bred Dachshund bitch. The owner had noticed sudden blindness and ophthalmoscopic examination revealed lipaemia retinalis. The resting total serum cholesterol level was 22·1 mmol/l (Crispin, unpublished observations). Transient opacity of the aqueous humour due to hyperlipoproteinaemia has also been recorded in a dog with serum cholesterol levels of 8·32 mmol/l and serum triglyceride levels of >50 mmol/l. (Schmidt, personal communication).

Familial hyperlipoproteinaemia was associated with hypothyroidism in the Beagles described by Manning *et al.* (1973). In the only animal of this series whose relatives could be traced (Case 3) the bitch's mother and daughter were hyperlipoproteinaemic and the daughter showed clinical signs of hypothyroidism, although neither of these dogs had a corneal arcus. The presence of hyperlipoproteinaemia in three generations and the identical breed incidence of all five cases, indicates that the hyperlipoproteinaemia, and the hypothyroidism with which it is associated, may have an hereditary basis in the Alsatian breed.

ACKNOWLEDGEMENTS

The authors are most grateful to the Veterinary Surgeons who referred these cases to us and also the owners of the animals. They are greatly indebted to Dr J. G. Lines of the Department of Biochemistry, Addenbrooke's Hospital, Cambridge for all the enthusiasm, advice and help he has given.

One of us (SMC) was in receipt of the PDSA Small Animal Scholarship at the time these investigations were in progress.

REFERENCES

ABELL, L.L., MOSBACH, E.H. AND KENDALL, F.E. (1956) Cholesterol metabolism in the dog. *J. Biol. Chem.* **220,** 527.

ANDREWS, J.S. (1962) The lipids of arcus senilis. *Arch. Ophthal.* (Chicago) **68,** 264.

BRON, A.J. (1976) Dyslipoproteinaemias and their ocular manifestations. *Birth Defects: Original Article Series,* Volume XII, 3, 257.

Bulletin of the World Health Organisation (1970) **43,** 891.

COGAN, D.G. & KUWABARA, T. (1959) Arcus senilis, its pathology and histochemistry. *Arch. Ophthal.* **61,** 553.

DUKE-ELDER, S. (1965) *System of Ophthalmology VIII,* Part 2, p. 873. Henry Kimpton, London.

FORSIUS, H. (1954) Arcus senilis corneae. Its clinical development and relationship to serum lipids, proteins and lipoproteins. *Acta. ophthal. (Copenhagen) Suppl.* **42,** 78.

FREDRICKSON, D.S., LEVY, R.I. AND LEES, R.S. (1967). Fat transport in lipoproteins – an integrated approach to mechanisms and disorders. *New Eng. J. Med.* **276,** 34, 94, 148, 215, 273.

GARN, S.M. & GERTLER, M.M. (1950) Arcus senilis and serum cholesterol levels in Aleut. *New Eng. J. Med.* **242,** 283.

HARCOURT, R.B. (1969) Ocular manifestations of inborn errors of metabolism. *Br. J. Hosp. Med.* **2,** 831.

LEHMANN, H. & LINES, J.G. (1972) Hyperlipoproteinaemia classification: the optimum routine electrophoretic system and its relevance to treatment. *Lancet,* **i,** 557.

LINDHOLM, H. (1960) Arcus lipoides corneae and arteriosclerosis. *Acta Med. scand.* **168,** 45.

MANNING, P.J., CORWIN, L.A. & MIDDLETON, C.C. (1973) Familial hyperlipoproteinaemia and thyroid dysfunction of Beagles. *Exp. Mol. Path.* **19,** 378.

MAUMENEE, A.E. (1965) The histopathology of corneal grafts. In: *The Cornea* (Eds King & McTigue) p. 707. Butterworths, London.

RATCLIFFE, W.A., CHALLAND, G.S. & RATCLIFFE, J.G. (1974) A critical evaluation of separation methods in radioimmunoassay for total T_3 and T_4 in unextracted human serum. *Ann. Clin. Biochem.* **11,** (6) 224.

RIJNBERK, A. (1974) Hypothyroidism. In: *Current Veterinary Therapy. V Small Animal Practice* (Ed. Kirk, R.W.) p. 791. W.B. Saunders Company, Philadelphia.

ROGERS, W.A., DONOVAN, E.F. & KOCIBA, G.J. (1975) Lipids and lipoproteins in normal dogs and in dogs with secondary hyperlipoproteinaemia *Am. Vet. med. Ass.* **166,** 11, 1092.

RUBIN, L.F. (1974) *Atlas of Veterinary Ophthalmoscopy,* p. 256. Lea and Febiger, Philadelphia.

WALTON, K.W. (1973) Studies on the pathogenesis of arcus formation. I. The human corneal arcus and its relation to atherosclerosis as studied by immunofluorescence. *J. Path.* **111,** 263.

WYMAN, M. & MCKISSICK, G.E. (1973) Lipaemia retinalis in a dog and cat: case reports. *J. Am. Anim. Hosp. Ass.* **9,** 288.

SHEILA CRISPIN

Corneal dystrophies in small animals

THE TERM 'CORNEAL DYSTROPHY' implies defective or faulty nutrition of the cornea. The description should be reserved for functional and morphological changes occurring bilaterally, but not necessarily symmetrically, in a previously normal cornea and not caused by disease elsewhere in the eye or elsewhere in the body. The reasons for such alterations are unknown but genetic predisposition is implicated. Typically, they make their appearance in early adult life, although the age of onset and symptomatology are variable.

Diagnosis of corneal dystrophies is based on the history, age, breed and sex of the affected animal. The usual methods of ophthalmoscopic examination are employed with slit lamp biomicroscopy as a basic requirement for detailed study. Wherever practicable, microscopic examination of corneal biopsy specimens should form part of the investigation and as much detail as possible of the patient's relatives should be obtained.

Classification of corneal dystrophies in animals has not been attempted although it would seem logical to adopt a topographical classification based on the site of origin of the early corneal changes—which is the approach used in this article—as proposed for similar conditions in man (Franceschetti and Forni, 1950; Francois, 1961; Duke Elder and Leigh, 1965; Bron and Tripathi, 1974).

There is some confusion associated with the correct recognition of corneal dystrophies in both man and animals. This is partly because the primary dystrophy may be modified in its evolution and appearance by secondary degeneration, or by infiltration of the cornea, or by coexisting systemic abnormalities such as hormonal or mineral imbalance. There is also little doubt that some types of corneal dystrophy progress to corneal degeneration by virtue of the substances they contain: calcium and cholesterol for instance can both promote a cellular response, whereas other types of corneal dystrophy produce no inflammatory changes, remain static once formed and may regress or even disappear completely. The time scale of dystrophic corneal changes is one which may occupy months or even years.

THE CAT

EPITHELIAL AND STROMAL DYSTROPHY IN THE MANX CAT

A progressive form of corneal dystrophy which is apparently inherited as a simple autosomal recessive has been reported in a group of closely inbred

Stump-tailed Manx cats (Bistner et al., 1974). The dystrophy was first noticed at about 4 months of age when clouding of the corneas became evident. The most obvious microscopic feature was oedema of the anterior corneal stroma with swelling and disintegration of the collagen fibres. Changes also occurred in the corneal epithelium and basement membrane but these were thought to be secondary to the stromal oedema. Extensive vesicle formation was observed in the epithelium with coalescence of the small vesicles to form large vacuoles so that epithelial oedema was particularly marked in the central, or axial, region of the cornea.

Over a period of months the whole of the anterior cornea became oedematous, and in some cases bullous keratopathy with subsequent ulceration of epithelium and stroma resulted. In view of the progressive and severe nature of this dystrophy it has been suggested that only palliative treatment is possible (Dice, 1980). The cause of the dystrophy is unknown.

ENDOTHELIAL DYSTROPHY IN THE CAT

A progressive, bilateral and severe endothelial dystrophy has recently been encountered in a group of inbred Short-haired Domestic Cats; both sexes have been involved.

In affected animals stromal oedema may be detected as early as three to four weeks of age, beginning in a central position and spreading towards the limbus, but with perilimbal sparing. Keratoconus and keratoglobus are frequent complications.

The earliest microscopic change is probably in the central corneal endothelium. The cytoplasm of the endothelial cells becomes vacuolated and the regular orientation of the cells and their nuclei is altered. Towards the limbus the endothelial cells are of normal appearance. There is stromal oedema and increased stromal thickness. The corneal epithelium shows no abnormality early on in the disease but later becomes thinner than normal because of a reduced number of layers; a change of corneal profile is clinically evident at this stage.

As with the other corneal conditions in this chapter, inheritance should be demonstrated to justify the description 'dystrophy' and investigations of the mode of inheritance are currently in progress. Treatment, if attempted, would involve penetrating keratoplasty.

THE DOG

A number of dystrophies have been described; some fulfill the criteria suggested in the introduction, others may do so when more information is available and a few have been wrongly ascribed at the outset. Incorrect designation particularly applies to secondary corneal disease, ocular disease which may produce corneal changes and systemic disease with corneal involvement.

EPITHELIAL DYSTROPHIES

Recurrent epithelial erosion

This is a condition in which there is defective adhesion between the corneal

epithelium and anterior stroma. It is seen particularly in Boxers, Corgis and Boston Terriers from early middle age onwards.

There may be a history of trauma and it is important to consider such predisposing or causal agents as extra eyelashes, foreign bodies, scratches and infections, particularly with unilateral lesions. In a small percentage of cases no cause of the erosion (or ulcer) can be established and a corneal dystrophy may be present. Whether such dystrophic changes are of primary origin or occur secondary to other abnormalities such as hormonal deficiency, vitamin imbalance and fifth cranial nerve involvement is uncertain.

The eyes are not necessarily affected simultaneously by erosions of unknown cause although bilateral corneal abnormalities may be apparent on examination. The animal usually shows signs of superficial ocular pain with blepharospasm, excessive lacrimination and eyeball retraction.

Ophthalmoscopic examination reveals single or multiple superficial erosions which extend the full thickness of the corneal epithelium to the surface of the anterior stroma. The epithelium at the edge of the lesion has a characteristic 'banked up' appearance with a more peripheral zone in which epithelium covers but does not adhere to, the underlying stroma. There is typically an absence of inflammation or neovascularization, indicative of the ulcer's indolence or unwillingness to heal (*Fig.* 1).

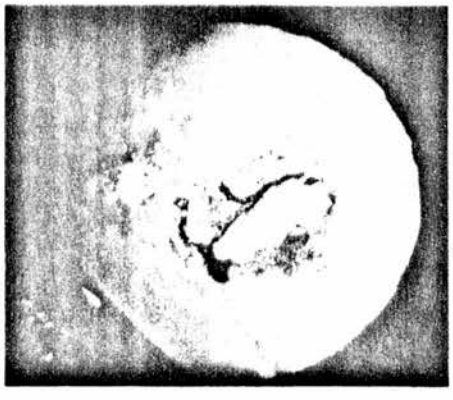


Fig. 1

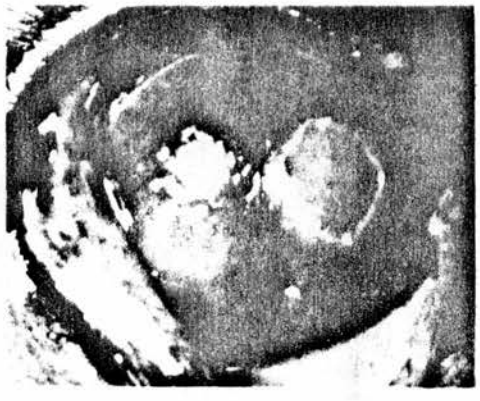


Fig. 2

Fig. 1. Large area of epithelial erosion in a Corgi observed by retro-illumination. Note numerous defects of the basal epithelium. (Unilateral.)

Fig. 2. Large area of epithelial erosion and several smaller areas of abnormality in a Boxer. On the large area note the apparent extent and the actual extent of abnormal epithelial adhesion. (Bilateral, eyes dissimilar.)

Slit lamp biomicroscopy may detect punctate and linear opacities in the basement membrane region of the epithelium of both eyes whether or not erosions are present.

Treatment of recurrent epithelial erosion involves removal of loose corneal epithelium from the apparent margin of the ulcer to the region of normal epithelial adhesion (*Fig.* 2), a process readily achieved with a chemical cauterizing agent such as pure liquid phenol. Occasionally the support of a nictitating membrane flap may be required if cautery proves ineffective.

Hormone replacement therapy as an adjunct to treatment has been used in males with testicular atrophy and in neutered females or females with irregular oestrus cycles, following Roberts' (1965) observation that all the cases of superficial indolent ulceration which he recorded in Boxer bitches were in neutered animals. Vitamins have been similarly employed as part of treatment; the Vitamin B complex, particularly riboflavin, Vitamin A and Vitamin C may be worthy of further investigation in the treatment of this interesting and poorly understood condition.

Subepithelial basophilic deposits

Saunders and Rubin (1975) described a self-limiting type of dystrophy of young dogs. Although sometimes present as a primary central corneal condition it may also be found in association with congenital ocular anomalies. It consists of a collection of basophilic PAS-positive deposits in the basement membrane of the epithelium which disappear as the animal matures. No treatment is necessary and no mode of inheritance has been suggested.

Verticillate corneal dystrophy

In man, Fleischer's vortex dystrophy (Grüber's cornea verticillata, verticillate corneal dystrophy) has long been considered a type of corneal dystrophy, although it is more likely to be one of the clinical manifestations of Fabry's disease, a glycolipid disorder transmitted as a sex-linked recessive. The rather unusual whorl patterns which form superficially in the cornea may be the only manifestation of this systemic disease in female carriers.

A common mechanism may operate to produce whorl patterns within the cornea, and when pigmented opacities are present it has been suggested that a centripetal slide phenomenon from epithelial pigment cells at the limbus is responsible for the gross appearance (Cowan, 1964). Striate melanokeratosis produced in this fashion is not uncommon and may occur in association with inflammatory conditions such as keratitis and uveitis; it probably accounts for the possible verticillate corneal dystrophy described in a poodle by Barnett and Grimes (1971) in which they noted that there was no evidence of inheritance.

STROMAL DYSTROPHIES

Corneal lesions containing fats of various kinds are not uncommon in the dog. The following conditions may be excluded from further discussion as they do not fulfill the criteria outlined earlier for corneal dystrophies.

Fatty change occurring secondary to corneal disease. This is commonly the result of some form of previous insult to the cornea such as a scratch, ulcer or surgical incision. The lesion is commonly unilateral. Should the fatty change

occur following corneal vascularization (*Fig.* 3), it is termed a lipid keratopathy (Cogan, 1960).

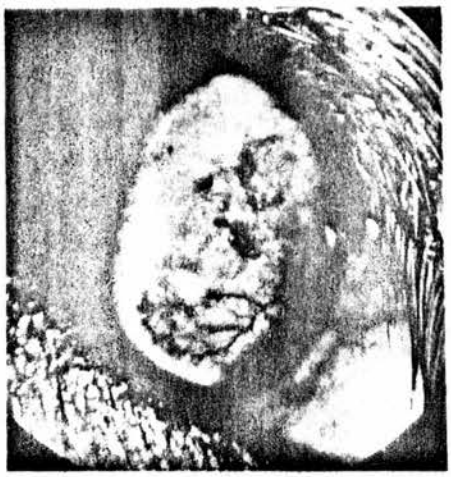


Fig. 3

Fig. 4

Fig. 3. Lipid keratopathy in a Jack Russell Terrier which followed damage due to a cat-scratch. (Unilateral.)

Fig. 4. Fatty change secondary to episcleritis in a Springer Spaniel. (Bilateral and similar.)

Fig. 5

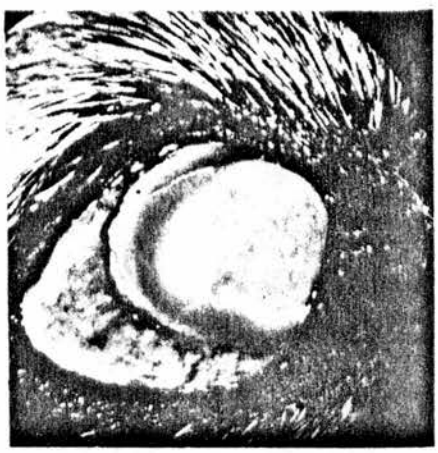


Fig. 6

Fig. 5. Arcus lipoides corneae secondary to hypothyroidism in an Alsatian. (Bilateral and similar.)

Fig. 6. Lipid keratopathy and arcus lipoides corneae in a Rough Collie. Lipid keratopathy followed surgical section of the cornea for lendectomy and primary hyperlipoproteinaemia also present. (Unilateral.)

Fatty change secondary to ocular disease elsewhere in the eye. Diffuse involvement of both corneas, frequently with perilimbal sparing, may occur in conjunction with such conditions as uveitis, scleritis or episcleritis (*Fig.* 4).

Fatty infiltration due to primary and secondary systemic dyslipoproteinaemias. For instance, bilateral arcus lipoides corneae (*Fig.* 5) may occur in conjunction with raised serum lipoprotein levels in the dog (Crispin and Barnett, 1978). The relationship between high serum lipoprotein levels and the development of arcus lipoides corneae is equivocal, and it is of interest that some degree of lipid infiltration (arcus senilis) is regarded as a normal feature of ageing in man but that no such change occurs as part of ageing in the dog.

Fatty change and fatty infiltration may occur together (*Fig.* 6), and the modifying influence of the various degenerative and infiltrative conditions on primary corneal dystrophies has been mentioned previously.

LIPID DYSTROPHIES OF THE CORNEAL STROMA

Three possible types of stromal lipid dystrophy are described. They are arbitrarily separated into (1) a dystrophy which commences centrally or paracentrally and affects the superficial stroma, (2) a dystrophy which commences with more diffuse involvement of all but the perilimbal region of the cornea and extends more deeply into the stroma and (3) a dystrophy which commences as an incomplete or complete annulus of peripheral opacity with clear cornea centrally and perilimbally and stromal involvement which varies from partial to complete in depth.

'Central/paracentral' corneal dystrophy

The commonest lipid corneal dystrophy in the dog is a bilateral and reasonably symmetrical central or paracentral opacity situated subepithelially in the anterior stroma to approximately one-third of its depth. One eye may be affected in advance of the other. This type of opacity has been seen in a number of breeds of dog, although the Rough Collie, Cavalier King Charles Spaniel, Shetland Sheepdog, Afghan Hound and Alsatian are most commonly affected in the UK. The dystrophy has been followed through three generations of Rough Collie (Crispin and Barnett, 1981). Although the dystrophy has been seen as early as a few weeks of age in the Rough Collie and Shetland Sheepdog, it occurs most frequently in the young adult. In some breeds, there is predilection for females and the dystrophy may first be observed following oestrus, or, after pregnancy, during lactation.

This dystrophy is very similar to human central crystalline dystrophy (Schnyder's corneal dystrophy) for which Delleman and Winkelman (1968) demonstrated five morphological phenotypes; there appears to be a similarly wide range of phenotypical expression in the dog.

The clinical appearance ranges from central or paracentral opacities of varying densities occurring in a number of shapes, of which oval (*Fig.* 7) and circular (*Fig.* 8) are commonest, to more diffuse corneal involvement (*Fig.* 9). The composition of the dystrophy also varies; cholesterol crystals have a scintillating

Fig. 7. 'Central/paracentral lipidosis' in Afghan hound. (Bilateral and symmetrical.)

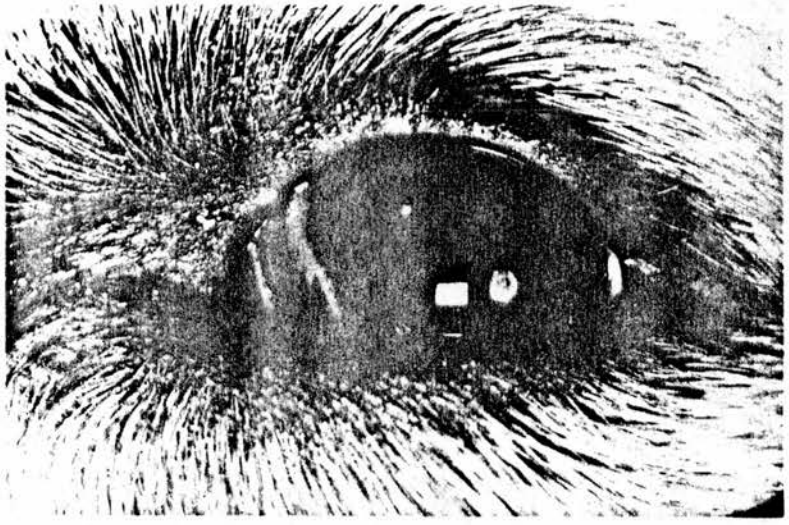


Fig. 8. 'Central/paracentral lipidosis' in Shetland Sheepdog. (Bilateral and similar.)

Fig. 9. 'Central/paracentral lipidosis' in Rough Collie observed with a slit lamp biomicroscope. (Bilateral and symmetrical.)

golden/silver appearance and may be aggregated into needle-like forms, whereas a more homogeneous greyish opacity indicates non-crystalline lipids such as cholesterol esters, triglycerides and phospholipids. Usually both crystalline and non-crystalline lipids are present together although their proportions differ. From the three generations of Rough Collies examined in some detail it seems that the range of expression may, in part, be related to the evolution and regression of the dystrophy. There is no inflammation or vascularization, the epithelium is intact and undisturbed and only rarely are the opacities extensive enough to cause any effect on vision, in which case they may be removed by superficial keratectomy; otherwise no treatment is required.

The speed and completeness of their development is variable; frequently they remain stationary once formed, but they may disperse and ultimately disappear over a period of months or years.

This type of corneal dystrophy probably corresponds to that described by Schock (1910), Veenendaal (1928), Dreyfuss (1930), Robin and Charton (1939), Roberts et al. (1966) and Waring et al. (1977).

'Diffuse' lipid dystrophy in the Airedale

Dice (1974) described a progressive bilateral and symmetrical lipid dystrophy in three closely related male Airedale Terriers which were presented at between 9 and 11 months of age because of a milky opalescence of both corneas. The opacities involved the whole cornea except for a clear perilimbal zone of 4–6 mm. This zone remained lucid throughout the development of the opacity, which became dense enough to produce visual disturbance by the time the animals were 3–4 years of age.

The corneal stroma was affected most densely in the subepithelial region and less densely to a depth of one-half to three-quarters of the corneal thickness. Inflammation, vascularization and oedema were absent and serum lipoprotein levels were normal. Histochemical studies of the cornea demonstrated phospholipids and triglycerides.

Treatment by superficial keratectomy, lamellar keratoplasty and penetrating keratoplasty were all tried, but opacification returned at between 4 months and 2 years depending on whether partial or full-thickness removal of corneal tissue was performed. A sex-linked recessive pattern of inheritance was proposed by the author.

'Annular' lipid dystrophy in the Siberian Husky

A lipid dystrophy of Siberian Huskies between 7 months and 12 years of age, has been reported by MacMillan et al. (1979). The animals had bilateral, usually symmetrical, round or horizontally oval opacities with a clear centre, occupying a peripheral corneal position with a lucid interval in the perilimbal region. No. inflammation or vascularization was present but the opacity was progressive, becoming deeper and denser as the animal grew older until only the perilimbal area remained clear.

The lipids comprising the opacity were identified as cholesterol, neutral fats and phospholipids; the proportions and distribution varied with the age of the lesion.

No pattern of inheritance was established although several of the affected dogs were closely related. No other relevant ocular or systemic abnormalities were detected in the affected animals and no mention is made of any attempts at treatment.

Fatty and calcareous degeneration of the anterior cornea in the Golden Retriever

Calcareous degeneration of the cornea secondary to corneal disease, or disease elsewhere in the eye, may accompany fatty change or, less commonly, occur on its own. In man and animals calcium deposition in sites which include the cornea may arise following systemic diseases which create mineral imbalances and in primary conditions of this type (such as hypercalcaemia or hypophosphataemia). In the dog deposition of calcium on the surface of the cornea may follow parotid duct transposition.

Inherited primary calcareous degenerations of the cornea are rare in man and unproven in animals, although Mittle et al. (1970) described corneal calcification in certain strains of spontaneously diabetic laboratory mice.

Because deposition of calcium tends to occur most obviously in the interpalpebral area of the cornea, the observable lesion is usually referred to as a band-shaped keratopathy whether of primary or secondary origin.

In the dog a progressive and bilateral form of fatty and calcareous degeneration has been seen in four Golden Retriever bitches (Crispin and Barnett, 1981). The lesions developed during early adult life in the absence of any other ocular disease. No systemic abnormality was present in three of the animals but

the other was grossly overweight with a resting serum cholesterol of approximately 11 mmol/l. The high cholesterol levels may have exacerbated progression of the corneal changes which were particularly marked in this case.

Although both corneas are affected the lesions may be asymmetrical commencing at the periphery of the cornea (*Fig.* 10) or with more diffuse central involvement (*Fig.* 11). Initially, the lesions are limited to the subepithelium and anterior stroma but inflammation and vascularization are relatively early features and epithelial erosion follows.

Once erosion has occurred the condition causes pain, blepharospasm, excessive lacrimation and apparent enophthalmos.

Microscopic study of biopsy specimens indicates calcium salts immediately beneath the basement membrane with fatty change (crystalline and non-crystalline lipids) in the anterior stroma. As the condition progresses the deeper stroma becomes involved and the epithelium and basement membrane are disrupted. There is marked cellular reaction in the regions where cholesterol and calcium occur.

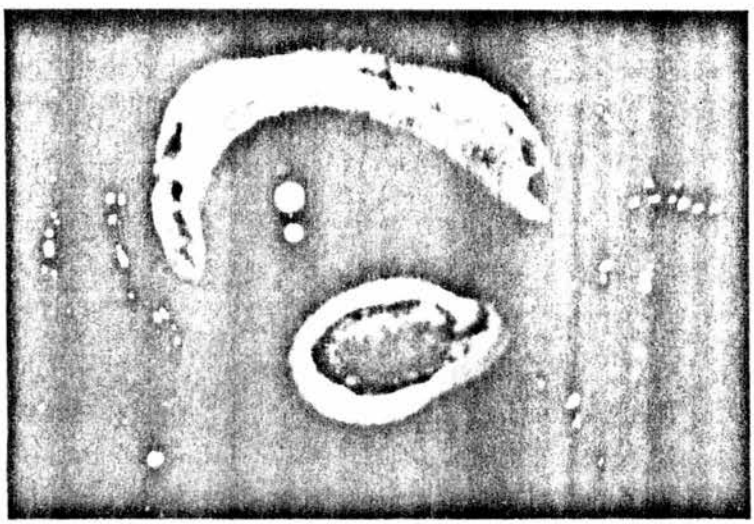


Fig. 10. Peripheral fatty and calcareous degeneration in a Golden Retriever. Note epithelial erosion of oval lesion. (Bilateral, eyes dissimilar.)

Treatment of fatty and calcareous degeneration is usually requested once epithelial erosion or visual impairment develop, but the earlier superficial keratectomy is performed the better the prognosis. The chelating agent EDTA has also been applied to the cornea at the time of surgery to aid removal of calcium.

It is not possible to determine whether or not this is a form of corneal dystrophy, or even whether calcareous degeneration does actually precede fatty change or vice versa, on the limited material so far studied.

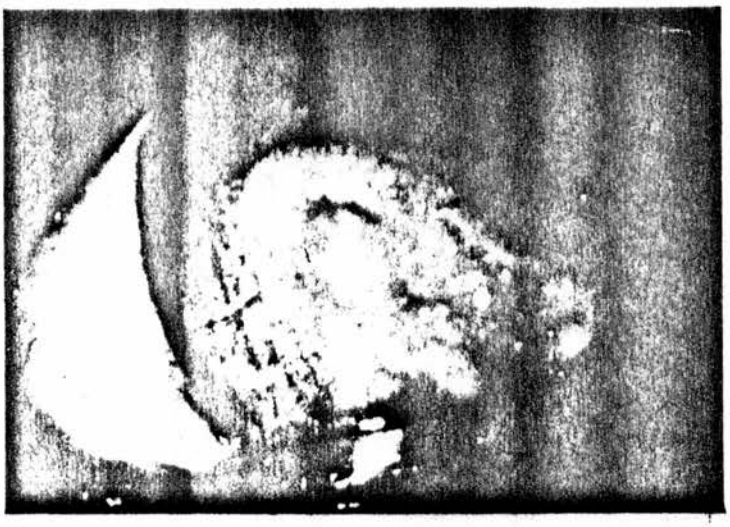


Fig. 11. More central fatty and calcareous degeneration in a Golden Retriever. Note superficial vascularization at temporal (lateral) canthus. (Bilateral, eyes similar.)

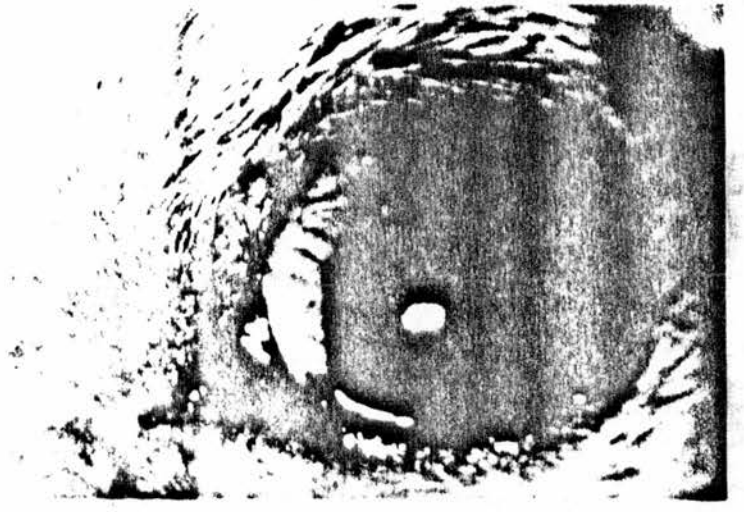


Fig. 12. Endothelial-epithelial dystrophy in a Chihuahua. (Bilateral and symmetrical.) (Photograph courtesy of Dr K. C. Barnett.)

ENDOTHELIAL DYSTROPHIES

Endothelial-epithelial corneal dystrophy

A progressive and permanent corneal oedema as a consequence of abnormalities of the endothelial (mesothelial) layer of the cornea has been described in the Boston Terrier, Chihuahua and Dachshund (Dice and Martin, 1976; Dice,

1980). They reported that bitches are most commonly affected and present at about 5 years of age with one or both eyes affected by corneal oedema, initially most marked at the temporal corneal limbus. The entire cornea of both eyes is eventually involved over a period of 2–3 years (*Fig.* 12). Prior to total involvement no discomfort or inflammation is present. A bullous keratopathy develops and rupture of epithelial bullae and subsequent erosions cause pain, blepharospasm and excessive lacrimation. Keratoconus of the central cornea may result.

Microscopic examination of the dystrophy in a 7-year-old Boston Terrier (Dice, 1980) indicated a primary degeneration of the endothelial cell layer in places. In the central cornea endothelial cells were sometimes absent or abnormal and flat excrescences occasionally appeared in Descemet's membrane. Epithelial and stromal oedema with disruption of parts of the basement membrane were considered to be secondary to the endothelial abnormalities. Capillary ingrowth had occurred in the superficial stroma and there was an overall increase in cellularity.

No mode of inheritance was postulated with regard to this dystrophy and treatment is unlikely to be more than palliative, although penetrating keratoplasty is of possible value (Dice, 1981).

CONCLUSIONS

Various corneal conditions of small animals reported as corneal dystrophies have been reviewed and discussed in relation to the site of origin of the early corneal changes and the criteria which should be met if the description is to be applied with accuracy.

The majority of corneal dystrophies reported in the dog and cat are characterized by their breed specificity and possible inheritance, their chronic nature and the lack of corneal reaction to their development, whether or not corneal degeneration is also present. A number of corneal dystrophies do progress to produce considerable visual impairment and a minority provoke inflammatory responses such as cellular infiltration and neovascularization.

Prognosis and treatment are largely determined by the rate of development, the area and depth of corneal involvement and the local effects of the dystrophy. More knowledge of existing small animal corneal dystrophies is required and many more conditions of this type await discovery.

REFERENCES

Barnett K. C. and Grimes T. D. (1971) Verticillate corneal dystrophy in a dog. *J. Small Anim. Pract.* **12**, 297.

Bistner S. I., Aguirre G. and Shiveley N. (1974) Hereditary corneal dystrophy in the Manx cat. *Proc. Am. Coll. Vet. Ophthalmol., Fifth Annual Scientific Program*, p. 87.

Bron A. J. and Tripathi R. C. (1974) Corneal disorders. In: Goldberg M. F. (ed.), *Genetic and Metabolic Eye Disease*. Boston, Little Brown, p. 281.

Cogan D. G. (1960). In: Duke Elder S. and Perkins E. S. (eds.), *The Transparency of the Cornea*, Oxford, Blackwell, p. 151.

Cowan T. H. (1964) Striate melanokeratosis in negroes. *Am. J. Ophthalmol.* **57**, 443.
Crispin S. M. and Barnett K. C. (1978) Arcus lipoides corneae secondary to hypothyroidism in the Alsatian. *J. Small Anim. Pract.* **19**, 127.
Crispin S. M. and Barnett K. C. (1981) Unpublished observations.
Delleman J. W. and Winkelman J. E. (1968) Degeneratio corneae cristallinea hereditaria: a clinical, genetical and histological study. *Ophthalmologica* **155**, 409.
Dice P. F. (1974) Corneal dystrophy in the Airedale. *Proc. Am. Coll. Vet. Ophthalmol., Fifth Annual Scientific Program*, p. 80.
Dice P. F. (1980) Primary corneal disease in the dog and cat. In: Peiffer R. L. (ed.), *Vet. Clin. North Am.* **10**. Philadelphia, Saunders, p. 339.
Dice, P. F. (1981). The canine cornea. In: Gelatt K. (ed.), *Veterinary Ophthalmology*. Philadelphia, Lea & Febiger, p. 366.
Dice P. F. and Martin C. (1976) Corneal endothelial-epithelial dystrophy in the dog *Proc. Am. Coll. Vet. Ophthalmol., Seventh Annual Scientific Program*, p. 36.
Dreyfuss M. (1930) Symmetrische zentrale Hornhautfettung beim Hund. *Albrecht von Graefe's Arch. Klin. Ophthalmol.* 125, 67.
Duke Elder S. and Leigh A. G. (1965) Corneal degenerations, dystrophies and pigmentations. In: Duke Elder S. (ed.), *System of Ophthalmology*, Vol. VIII, Part 2. London, Henry Kimpton, p. 921.
Franceschetti A. and Forni S. (1950) The heredo-familial degenerations of the cornea. A. Clinical Aspects. In: *XVI Concilium Ophthalmologicum, Brittania* (*Acta*, Vol. 1). London, British Medical Association, p. 193.
Francois J. (1961) *Heredity in Ophthalmology*. St Louis, Mosby.
MacMillan A. D., Waring G. O., Spangler W. L. et al. (1979) Crystalline corneal opacities in the Siberian Husky. *J. Am. Vet. Med. Assoc.* **175**, 829.
Mittle R., Galin M. A., Opperman W. et al. (1970) Corneal calcification in spontaneously diabetic mice. *Invest. Ophthalmol.* 9, 137.
Roberts S. R. (1965) Superficial indolent ulcer of the cornea in Boxer dogs. *J. Small Anim. Pract.* **6**, 111.
Roberts S. R., Dellaporta A. and Winter F. C. (1966) The Collie ectasia syndrome, pathology of eyes of young and adult dogs. *Am. J. Ophthalmol.* **62**, 728.
Robin V. and Charton A. (1939) La lipoidose cornéene du chien. *Rec. Méd. Vét.* **115**, 321.
Saunders L. Z. and Rubin L. F. (1975), *Ophthalmic Pathology of Animals*. Basel, Karger, p. 14.
Schock K. (1910) Eine noch nicht beschriebene Hornhautaffektion beim Hund. *Arch. Vergl. Opthalmol.* **1**, 313.
Veenendaal H. (1928) Twee nog weinig bekende hoornvliesaandven igen bij den hond. *Tijdschr. Diergeneeskd.* **55**, 853.
Waring G. O., Muggli F. M. and MacMillan A. (1977) Oval corneal opacities in Beagles. *J. Am. Anim. Hosp. Assoc.* **13**, 204.