# STRUCTURE AND OPTICS OF THE ANTERIOR SEGMENT OF THE CETACEAN EYE

A Thesis presented in fulfilment of the requirements for the degree of
Master of Philosophy
at
Massey University

**LYNDA MELLOR**

**1999**

## ABSTRACT

The anterior segment of the mammalian eye is concerned with the function and maintenance of its optical components, the most important of these being the maintenance of transparency and stable intraocular pressure. The structures of the eye change throughout life. Continuous growth occurs in the lens, and a number of other changes associated with aging also occur, many of which reduce visual capacity. Many of these manifest in humans because of their long life span but have very little relevance in terms of survival. However, other long lived animals such as some birds, reptiles and whales, could be severely compromised by complete or partial blindness associated with aging. The aims of this study were to evaluate the importance of vision as a sense in whales by observation of the structure and optics of the anterior segment, and compare the findings with other species whose visual functions are well known. Pathological changes were recorded where appropriate.

The findings in this thesis are based on a three year survey of eyes from 45 whales in which i) differences between species in the size of the globe, lens, and cornea are described ii) the unique histological structure of the uveal tract is demonstrated and defined iii) evidence of emmetropia in both air and water from NMR images of two eyes is given iv) lens shape and capsular features which indicate that there could be a capacity for accommodation, are described, and v) lens pathology (four cataracts and one case of phacolysis) is described in five animals.

The largest whales (baleen and sperm) had the largest eyes, but this was mainly due to the thickness of sclera. Internal dimensions showed little variation with respect to body size, suggesting that there is an upper limit on internal size which is dependant on the focal length of the lens, a structure which enlarges only slightly with age. Corneal and lens sizes were especially large in the baleen whales, and particularly small in the sperm whale.

The uveal tract was found to be very vascular when compared to other species, and particularly well innervated with specialised nerve endings which are thought to be unique to cetaceans. Although the findings are not conclusive, evidence from this study suggests that the whales' unique uveal vasculature and aqueous drainage methods may be instrumental in modifying the dioptric strength of the eye. The abundance of specialised pressure-receptors in the ciliary body indirectly supports a proposed mechanism for this, whereby the engorged ciliary body raises intraocular pressure causing increased corneal curvature, and releases tension on the zonule to allow 'rounding up' of the lens.

Optically, the study showed that eyes from two long-finned pilot whales were

emmetropic by virtue of a cornea with only a very small amount of optical power in both air and water, and a very powerful lens (about 72D in water). Emmetropia was thus not affected unduly by transition from air to water as it is in most mammals, where the cornea is optically very significant in air but neutral in water. Lenses in both animals showed an unusual 'bump' on the central posterior surface, and the increased radius of curvature in this area was responsible for the very high dioptric strength of the lenses.

The prevalence of lens pathology, particularly cataracts in young animals, was high, but in all cases the cause was unknown.

**Acknowledgments**

This thesis represents the requirement for a Master of Philosophy degree, which was undertaken in the former Department of Veterinary Pathology and Public Health (now the Institute of Veterinary, Animal and Biomedical Sciences) at Massey University.

The study lasted for three years, and eyes from 45 dead stranded cetaceans were examined. The work would not have been possible without the cooperation of the Department of Conservation (DOC) and its staff who have helped to develop a network of communication which enables tissues to be collected and distributed as soon as possible *post mortem.*

I am indebted to the late Professor David Blackmore for so willingly giving his time in discussions for the planning of this study. His wisdom, knowledge, and breadth of vision were a source of inspiration; his enthusiasm a source of energy; and his advice, to 'follow your passion', has been invaluable.

I am most grateful to my supervisors Associate Professor Maurice Alley and Dr Per Madie for their flexibility of approach, their cheerful advice and support, and their willingness to embrace areas of study which were outside their own particular interests. Many hours of painstaking attention to detail by Associate Professor Alley have ensured that the material is presented as fluently and comprehensibly as possible, and I thank him for improving my writing skills.

Dr Craig Eccles supervised Chapter 7 and was instrumental in producing NMR images and processing the data in a ray drawing package which he developed specially for thick lenses to suit the purposes of this work. I am sincerely grateful to him.

I would like to thank Professor R.D. Jolly and Mr Mervyn Birtles for their invaluable advice on the interpretation of electron micrographs and light microscopy slides.

Professor N. Gregory, Dr A. Davies and Dr R. Crawford participated in useful discussions at various times. Dr G. Barnes provided help and support in some aspects of the optical investigations. Dr Ritesh Poudyal and Dr A. Mc Knight offered helpful advice on cataracts and aqueous drainage. I thank them all.

I am grateful to Dr Chris Clarke for his support in the use of NMR imaging. Histological sections were prepared by Mrs Pam Slack and Mrs Pat Davey, and Mrs Slack also prepared the tissues for electron microscopy. I thank them for their cheerful and willing cooperation. Mr D.H. Hopcroft and Mr R.J. Bennett of Hortresearch Palmerston North, helped with the electron microscopy and printed the

electron micrographs.

Eyes were collected from cadavers in the field and in the PM room by a variety of DoC staff, Dr Per Madie, Mr Alan Nutman, Dr Jane Hunter, Dr Padraig Duignan and Mr Gareth Jones. I am most grateful to them all.

I would like to thank Mr R. Palmer for providing an ophthalmoscope, so that if the opportunity to examine a live whale's eye arose, I would be equipped.

My husband David, after discussion and agreement, had no input into the scientific aspects of this project, but provided encouragement throughout to pursue this study as a means of both increasing current knowledge of whale biology, and more personally, as a means of acquiring research, writing, and life skills. I thank him for this, and for his role model in patiently and painstakingly striving to achieve excellence.

# ADDENDUM

PAGE 11.

A rete is a body of convoluted blood vessels, commonly found in mammals, connecting arteries to veins, arteries to arteries, and veins to veins. They are believed to store blood and regulate its flow.

PAGES 25, 56, 107, 109, 111, 114, 115.

There are significant limitations involved in measurement of material in these cases for the following reasons;

i] many of the specimens were suffering from some degree of autolysis. This may have affected subsequent dimensions. In addition, lens and cornea increase in thickness with *post mortem* imbibition of water.

ii] formalin fixation can also alter the dimensions of structures. Although this was investigated prior to the study and found not to be a significant factor for structures measured to the nearest millimetre, it may have been a significant factor for microscopic techniques with measurements in microns.
This has not been taken into account when presenting data.

iii] artefactual changes associated with tissue processing may have affected the sizes of structures in histological studies. Where these changes were obvious, the sites were avoided for measurement.

iv] some of the variations in size may have been attributable to the age and size of the animal. Lenses enlarge throughout life, and capsular thickness varies with age.

PAGE 54.

An unequivocal conclusion that whales lack pectinate ligaments could only be made using more sophisticated techniques, such as scanning electron microscopy or photographs taken with a dissecting microscope of the '*en face*' view of the ciliary cleft. This study merely reports that the pectinate ligament was not evident in any of the histological sections examined in these whales.

PAGE 56.

Dimensions of the ciliary body would have been better expressed as a percentage of overall ocular size.

PAGES 59, 62.

There are some limitations involved in the use of indian ink to trace aqueous outflow pathways using in vitro specimens; there is no possibility of active transport mechanisms operating; autolysis may damage the permeability of membranes and affect the resistance to flow of molecules; in addition, fixation will contract all membranes, valves and tissue spaces, and coagulate all proteinaceous substances within them. Better results would have been obtained using more sophisticated techniques, such as tracing the passage of latex microspheres or radioactively labelled substances in live animals.

PAGES 130-141

The study documents a small number of lesions in moderately autolysed specimens. Cataract was diagnosed on the basis of gross, rather than histological findings. However, strictly speaking, *post mortem* diagnosis of cataract should meet very specific histological criteria. The value of this chapter is to highlight the need to examine whales' eyes for cataracts with an ophthalmoscope at every possible opportunity, particularly at *post mortem* of by-catch or stranded animals.

**LIST OF FIGURES** **PAGE NO.**

# LIST OF CONTENTS

APPENDICES

# INTRODUCTION

The order cetacea demonstrates extreme diversity between its 78 species. Morphologically, size can vary from the small Hector's dolphin at 1.3m in length to the 30m blue whale (Dawson 1985). Habitats can range from polar to tropical seas, with a few freshwater species. Some species, such as some of the beaked whales, are seen rarely. This could be due to their pelagic habits and relatively short periods spent at the surface, or could indicate a genuinely small population. Other species, such as many dolphins, are seen frequently because they have a coastal habit and spend large periods of time near the surface.
Whales evolved in the late eocene period around 53 million years ago (Dawson 1985). Both mysticetes and odontocetes are believed to have evolved from land based artiodactyls (even toed ungulates).
The ancient Greek philosopher Aristotle (384-322 BC) described dolphins as having lungs, and as being like humans in their behaviour, growth, and reproduction, but still referred to them as fish (Dawson 1985). Similarly in biblical times, the whale was considered to be a fish (Jonah, Old Testament). However, by 1776AD Linnaeus had devised a theory for the classification of animals which is still in use to day, in which he states;

"I hereby separate the whales from the fish....
..... On account of their warm bilocular heart,
their lungs, their movable eyelids,
their hollow ears, penem intrantum
feminam mammis lactentem".

Even in 1851, the claim that the whale is a mammal and not a fish, was disputed. In Moby Dick (Herman Melville 1851) the description is given;

"to be short then, the whale is a spouting fish
with a horizontal tail".

Melville's problem with the mammal theory was probably that it was inconceivable that the inhospitable and alien environment of the deep ocean could be inhabited by anything other than fish. The very fact that this 'fish' had a horizontal tail, unlike any other fish, and a body motion in a corresponding plane, should have indicated its mammalian rather than piscine nature.
Earliest investigations of the cetacean eye were in the sixteenth century (Pierre Belon, 1517 - 1564, and Guillaume Ronderkelt 1507 - 1566, cited by Waller 1984). In 1719 Anton van Leewenhoek dissected a whale's eye that had been preserved in wine by the captain of a Greenland whaling boat, and noted the thick sclera.

Leewenhoek, who was famous for his invention of the light microscope, was able to put this instrument to good use in identifying and describing the ophthalmic rete mirabile, and the lamellar construction of the lens (Waller 1984).

Walls in 1942 and Prince in 1956 produced texts on comparative anatomy which were mainly descriptive in orientation. They included small amounts of information on cetacean eyes.

Since 1972, further innovative and original study of the cetacean eye has occurred, covering many aspects of anatomy, histology and optics.

## 1.1 ADAPTATIONS TO A MARINE ENVIRONMENT

### 1.1i Body Adaptations

Massive evolutionary adaptations have been necessary for whales to maintain themselves in an environment which is extremely hostile to a class that originally evolved for land living.

The ocean environment represents a challenge because it presents extremes of pressure, temperature and light intensity.

Pressure increases by one atmosphere for about every 10m increase in depth (Kooyman and Ponganis 1998). A shallow dive of this depth would therefore be equivalent to 2 atmospheres (202.6kPa or 30psi). During dives, the flexible ribs collapse to reduce lung volume and gas becomes compressed into the upper airways (Kooyman and Ponganis 1998). Heart rate slows, and blood is diverted to muscle and the central nervous system (CNS). Although cardiac output reduces, stroke volume and blood pressure are maintained (Kooyman 1989). Air filled compressible areas become filled with engorged rete vessels. In addition to mechanical effects on anatomical structures, pressure affects proteins at a molecular level, which could affect muscular contraction and nerve conduction (Schmidt-Nielson 1990) so special adaptations to alleviate this effect must exist.

Water temperature can be variable, but more importantly the thermal conductivity of water is so much higher than that of air that there is a significant need to maintain body temperature. Fur and feather insulation have been replaced by a thick layer of blubber.

Light intensity decreases with depth so that depending on the quality of the water, 90% of light may be absent at 9m and 99% at 35m (Walls 1942).

There is a constant potential for drowning, and the whale has developed mechanisms to reduce the threat of this in the form of specific diving adaptations, such as an extremely high oxygen carrying capacity by virtue of a high haematocrit during dives and high mean corpuscular haemoglobin content

(MCHC). The circulating and stored blood volumes are similarly increased, and much more oxygen is stored as myoglobin than in humans (Schmidt-Nielson 1990). The animal is always alert by virtue of the brain's arrangement that only one cerebral hemisphere shuts down at a time during sleep (Ridgway 1988).

### 1.1ii Ocular Adaptations

The cetacean eye has also adapted dramatically to meet the needs of wide variations in light intensity, temperature, pressure, osmotic effects and the loss of corneal power underwater. Hydrostatic pressure adaptations are complex. If an analogous plastic bag full of water is taken underwater to a pressure of 10 atmospheres, it will not suffer deformation. Compressive forces only exist where there is an air interface, such as around the thorax, sinuses and middle ear, and this is where the most profound adaptation occurs. There is a distinction between a compressive force, and hydrostatic pressure. It is not known what adaptations to increased hydrostatic pressure the eye has made, particularly with respect to the protection of nerve function. "High pressure nervous syndrome" has been described in several species (not cetaceans) as a direct mechanical effect of pressure on nerves, but the mechanism remains unexplained (Kooyman 1989).

The osmotic gradient imposed by seawater affects mainly the cornea. A thick layer of viscous jelly protects the corneal surface (Prince 1956). This layer is not thought to contribute to the dioptric strength of the eye (Dawson 1980). The jelly also provides protection from suspended particulate matter, since eyelashes are absent, as they are of little use in this medium.

The loss of dioptric power by the cornea when the eye is immersed in water occurs because the refractive indices of the cornea and water are similar (Walls 1942; Prince 1957; Dawson 1972). In land animals, the cornea is the main refractive device contributing about 2/3 of the dioptric strength of the eye while the lens contributes 1/3 (Spooner 1957) When the eye is submerged and the effect of the cornea is lost, the lens has sole responsibility for refraction. Land animals are therefore longsighted (hyperopic) underwater. Cetaceans have adopted the fish model of a powerful, spherical lens to combat this effect. However, unlike the fish whose vision is entirely aquatic, some whales, such as dolphins and orca (*Delphinidae sp.*) also need good aerial vision. Another problem arises when the whale eye with its powerful lens is taken out of water and into air. It is believed that if the cornea is reinstated as an important refractive element, when coupled with a powerful lens the system becomes hopelessly myopic. However, in reality we know that dolphins have good eyesight in air from their displays in dolphinaria. Visual acuity in *Delphinidae sp.* has been tested and found to be similar to that of a cat in daylight (Spong *et al.* 1971; White *et al* . 1971; Herman *et al.* 1975).

## 1.2 IMPORTANCE OF VISION AS A SENSE

Whales are "top feeders" (ie. they are at the top of the food chain consuming an energy rich, carnivorous diet of plankton, krill, fish or squid) and are therefore reliant on their intuitive and acquired skills as predators.

When the anatomy of the ungulate eye and the cetacean eye are compared it is obvious that the cetacean eye differs in a number of ways, suggesting that vision has become highly adapted and is therefore a sense of some significance.

If eyesight is important to cetaceans, it is possibly more important to some species than to others. In the Ganges River Dolphin, which is reputed to be sightless, the eye is small and the optic nerve is a mere thread (Tinker 1988; Waller 1983).

Madsen and Herman (1980) have suggested that vision has many important functions in cetacean life, including navigation, group movements, prey detection and capture, predator defence, and for identification and communication of behavioural state.

Visual problems may be a relevant consideration in the question of why whales 'actively' strand. It is noteworthy that strandings occur mainly in a few odontocete species and all are deep diving whales with non-coastal habits. In New Zealand, the commonest stranders are pilot whales, false killer whales and sperm whales, and these are responsible for 84% of individual and a high proportion of mass strandings (Brabyn 1992).

It is not known how migration in whales is accomplished, but magnetite has been found in the dura mater of *Delphinus delphis* which suggests that magnetic information may be used for orientation (Zoeger *et al.* 1981). In addition, a relationship between magnetic minima and mass strandings has been demonstrated (Klinowska 1985; Kirschvink 1986) and also between magnetic storms and strandings (Klinowska 1985).

Other methods of orientation or 'homing' such as chemotaxis, the ability to detect vibrations with a lateral line system or electric fields using electroreceptors, detection of polarised, infra red or ultraviolet light have not been demonstrated in cetaceans. Local orientation is achieved by echolocation in toothed whales. Spatial orientation is assumed to be achieved, like other mammals, by a series of proprioceptive and vestibular reflexes. These may be of more significance in aquatic environments because these animals may need to be able to orientate without the aid of fixed visual topographical cues such as a horizon, sky, or ground. The effect of gravity may be small, even zero, depending on the buoyancy of the body. Without light, reference points, and in neither positive nor negative buoyancy it can be possible to suffer from spatial disorientation until proprioceptive mechanisms stabilise. Although downwelling light may be absent as a navigational cue in the deep sea, sensitive vision is necessary to perceive light produced by marine organisms, either from photophores, or from

bioluminescent organisms in the gut.

When considering the cranial nerves, their comparative sizes are often used as indicators of their relative importance. The optic nerve is the third largest cranial nerve in whales although this is variable between species. This adds further support to the statement that vision is a significant sense in cetaceans. The largest cranial nerve in mysticetes is trigeminal five and in odontocetes is auditory eight. Odontocetes have well developed echolocation skills, hence the size and importance of this nerve. The second largest is auditory eight in mysticetes and trigeminal five (supplying the melon) in odontocetes (Tinker 1988).

The facial nerve is relatively large in both families. Other nerves associated with the eye such as oculomotor three, abducens six, and and trochlea four, vary according to the mobility of the eye. Hypoglossal, olefactory and glossopharyngeal nerves are small, reflecting the relative unimportance of these senses (Tinker 1988). This information indicates that the importance of vision has often been severely underrated as a special sense in whales.

The aims of the present study were to examine the anterior segments of several species of whale to determine their anatomical and histological features and correlate these with a range of possible functions, but particularly accommodative capacity; to determine the refractive state of one or more whales to establish whether there is a refractive error in air; and to record any pathological changes observed.

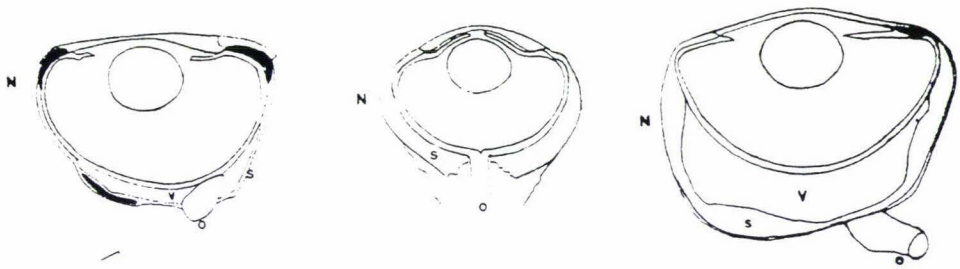
From these observations, some hypotheses can be formulated about the importance of vision as a sense, and how this, or its pathology, may contribute to the stranding phenomenon.

# LITERATURE REVIEW

## 2.1 GROSS ANATOMY AND BIOMETRICS

### 2.1.i Size and Shape of the Globe

The cetacean globe is an axially flattened sphere, horizontally ovoid in shape, and described as having reverted to fish type (Walls 1942). In order to retain a non-spherical shape the sclera has become very thick and rigid, not unlike the scleral ossicles of fish (Walls 1942). These comparative features have been illustrated by Waller (1984) in Figure 2-1.




Left, tuna *(Katsuwonus pelamis)*. Middle, dolphin *(D.delphis)*. Right, swordfish *(Istiophorous platypterus)*. The enlarged anterior segment and slit-like anterior chamber are anatomical features common to the three species. Rigid ossicles (black) strengthen the anterior face of the eye of the tuna and swordfish. The fibrous sclera (s) of the dolphin eye and the ossified sclera (s) of the swordfish eye are greatly thickened in comparison to that of the tuna eye (fibrocartilaginous sclera). The eyes of both tuna and dolphin have a peripherally thickened cornea. The vascular body (v) lies internal to the sclera in the eye of the tuna and swordfish. Horizontal sections (natural size), from author's dissections. o, optic nerve. N, nasal pole. The retina and choroid are shown as a single layer.

**Figure 2-1. Comparative ocular morphology of three marine vertebrates. From Waller 1984.**

The size of the eye in any species is limited by ophthalmic considerations such as the size of the lens and its supporting structures, the nature of the retina, and the nature of light itself, since diffraction would occur at the pupil if the aperture was too small (Spooner 1957). The relationship of eye size to body size is inversely proportional. There is a range of sizes for mammalian eyes from 3mm in diameter in the mouse to 33mm in the elephant (Spooner 1957). However, much of the large size of the cetacean eye is by virtue of the thick sclera.

Specific data on globe biometrics has been provided for the fin and minke whale (Pardue *et al.* 1993), blue, humpback and sperm whales (Anderson 1969) and fin, humpback and sperm (Dawson *et al.* 1972). Other studies have examined the cornea of the harbour porpoise (Kroger and Kirschfeld 1992) and fin and minke whales (Pardue *et al.* 1992). Specific analysis of the corneal data has revealed information about its dioptric strength ( see below; 2.3, and Chapter 7, Optics).

### 2.1ii Muscles

The extraocular muscles are very large compared to most mammals and encircle the globe like a sac. Their arrangement is typically mammalian with four rectus, two obliques and a large retractor bulbi. The presence of the retractor bulbi was disputed by Walls (1942). The rectus muscles insert around the equator of the eye, but a large proportion of the muscle continues on into the eyelid (Jansen and Jansen 1969 ). This suggests that there is good eyelid and globe mobility.

### 2.1iii Glands

The large ducts of numerous Meibomian glands are present around upper and lower conjunctival sacs. Of the three types of gland present in most mammals - lacrymal, Harderian and Meibomian, the former has disappeared along with the tarsal plate (Walls 1942). There is no associated tear draining mechanism or nictitating membrane.

### 2.1iv Lids

The lids appear to be 'like a button hole' (Johnson 1901) with 'little prospect of mobilility' although there are folds in mysticete lids which suggest that movement could be possible (Walls 1942). Eyelid closure associated with retraction of the globe has been described in dolphins (Dawson 1980).

## 2.2 THE UVEAL TRACT AND IRIDOCORNEAL ANGLE

### 2.2i General Structure and Function

The two primary components of the uveal tract are blood vessels and muscle. Internally there is a double layer of epithelium. The innermost layer is pigmented on the posterior surface of the iris and becomes unpigmented over the ciliary body. At the ora serrata this epithelium becomes non-neural retina, and eventually, neural retina. The second outer layer is non-pigmented in the iris, pigmented in the ciliary body, and forms the retinal pigment epithelium in the

posterior segment. Hogan *et al.* (1971) states that' because of its great vascularity, the choroid has been compared to an erectile tissue. The calibre of choroidal vessels depends partly on the intraocular pressure. They dilate when intraocular pressure is reduced, and assume their usual calibre when it returns to normal. The change in blood volume in these vessels probably plays some part in maintaining intraocular pressure.'

There are three separate functional areas within the uveal tract; anteriorly, the iris, the function of which is to modify the size and shape of the pupil and in some animals, the lens; posteriorly, the choroid, the function of which is mainly nutritive; and between these, the ciliary body which functions are i) to both produce and drain aqueous humour ii) to produce the vitreous body and iii) to accommodate, by altering lens curvature.

The iridocorneal angle is composed of the ciliary body and limbus. Its functional importance is in providing an outflow mechanism for aqueous humour.

The rete is important in providing arterial stem vessels for the uveal tract (Pilleri and Wandeler 1964) and possibly the retina (Dawson 1987).

In order to protect the rod-rich, highly sensitive retina, the iris must be rapidly mobile and capable of constricting to pinpoint size in bright light (Walls 1942). Cetaceans can experience a very wide range of rapidly changing light intensities when undertaking a rapid descent or ascent. The iris appears to have a well developed umbraculum (Walls 1942; West *et al.* 1991) in addition to substantial musculature. In the human, there are two main muscle blocks in the iris. One is circular, close to the pupil and controls constriction. The other is radial, sited peripherally with respect to the pupil and controls dilation (Hogan 1971). Similar musculature and numerous small arterioles have been described in the narwhal and beluga whale (West *et al.* 1991). A small amount of neural tissue is described in the beluga (*Delphinapterus leucas*) but not the narwhal (*Monodon monocerus*). The musculature of the iris is described as well developed by West *et al.* (1991) and iris vasculature is described as 'massive' by Wickham (1980) coupled with 'extreme excavation' of the iris stroma. In humans the ciliary body is primarily a muscular structure, but an anterior site, adjacent to the scleral spur, has an important drainage function (Hogan *et al.* 1971). In the whale, this ciliary muscle is rarely found (Table 2-1, compiled from Waller 1992; West *et al.* 1991), and when it does occur, the fibres have been described as 'sparse and small' (West *et al.* 1991) or as 'discreet, finely branching strands' which would be capable of producing an effect of only minimal amplitude (Waller 1992).

**TABLE 2-1 REPORTS OF CILIARY MUSCLE IN CETACEAN SPECIES**

| AUTHOR | DATE | SPECIES | CILIARY MUSCLE DEMONSTRATED |
|---|---|---|---|
| PUTTER | 1903 | | + |
| SLIJPER | 1962 | | + |
| WALLER | 1992 | LONG-FINNED PILOT | + |
| WEST ET AL | 1991 | NARWHAL | + |
| ROCHON DUVIGNEAUD | 1940 | | – |
| DRAL | 1975 | | – |
| DAWSON | 1980 | DOLPHIN | – |
| KASTELEIN | 1990 | | |
| WEST ET AL | 1991 | BELUGA | – |

The degree of development of muscle in the ciliary body is used as an indicator of the potential for lens deformation, and therefore accommodation. It is poorly developed in nocturnal species (Walls 1942) but in other species there is a wide variation in accommodative range : cat 3.5-8 dioptres (D), rat 0 D , river otter 40 - 60D, and cormorant 20- 40D (West *et al.* 1991; Walls 1942).
In humans refractive range varies according to age. It decreases from 20D at less than 1yr of age to 1D at middle age (Spooner 1957). A decrease in the malleability of the lens is thought to be responsible for this. Changes associated with age in the uveal tract itself include clubbing of the ciliary processes, and drusen (granular material) formation in Bruchs membrane (Hogan *et al.*1971). Studies of accommodation in live, captive dolphins have established that there were no accommodative movements under laboratory conditions (Dawson *et al.* 1972), but this does not confirm an inability to do so in a natural environment or exclude the possiblity that other species may do so.

### 2.2ii Limbus and Drainage Angle

Other important functions of the ciliary body are aqueous production and drainage. In man, the architecture of the trabecular meshwork with its corneal and uveal zones and of Schlemm's canal are well known (see Appendix 5-1). Similar areas have not been well documented in whales. Circular collagen (suggestive of corneoscleral zone) in the posterior ciliary body of narwhal and beluga (West *et al.* 1991), a "poorly developed irido-corneal spongium" of long -

finned pilot whales *Globiocephala melaena* (Waller 1992) large uveal and small corneoscleral areas in a spotted dolphin *Stenella attenuata* (Wickham 1980) have been described.

### 2.2iii Innervation of the Ciliary Body

One of the most interesting observations in the ciliary body in whales has been that of specialised sensory nerve endings, or encapsulated receptors (ERs). Sensory nerve endings can be interoceptive, proprioceptive, or exteroceptive (usually cutaneous). Interoceptive structures can be chemoreceptors, baroreceptors, or receptors to sense the distension of hollow viscera (mechanoreceptors) or proprioceptors, which monitor skeletal position, tension and movement (Burkitt *et al.* 1993).

Exteroceptors are usually cutaneous receptors and can be divided into simple and compound types. Simple types are free nerve endings which can be bare or Schwann cell wrapped. Compound types have special organisation of the neural and associated tissue to compliment their function (eg encapsulation, special senses). An overview of receptor structure and types is given in Appendix 6-1. The blowhole (nares) of the dolphin, has been reported to have receptors with a paciniform structure, particularly anteriorly (Bryden 1986). Similar receptors have also been described in the intraoral rete of the right whale (*Eubalaena glacialis*) where it is postulated that they sense either temperature or hydrodynamic flow (Ford and Kraus 1992) This rete has only recently been described and is thought to aid heat loss via the uninsulated oral cavity in order to protect the brain from hyperthermic blood. Other reports of ERs in the oral cavity of cetaceans have been made (Donaldson 1977).

In the dog and human, motor innervation of the anterior segment of the eye is achieved by parasympathetic fibres in oculomotor 3 and sympathetic fibres from the cervical ganglion to iris, ciliary muscles and blood vessels. These control the pupillary diameter and accommodative function of the ciliary body and also have a vasomotor function. Sensation from the cornea and iris is carried in trigeminal 5 (Peterson Jones 1989). In the human, unmyelinated fibres with a Schwann cell wrapping appear frequently in the ciliary body (Hogan *et al.* 1971).

Recently mechanoreceptors, similar to visceral mechanoreceptors, and thought to detect stretch in the sclera spur, have been described in the human limbus (Tamm *et al.* 1994).

The presence of specialised sensory nerve endings in whales suggests that the ciliary body may have a different function to that of other species. No other mammalian order is known to have ERs in the ciliary body (Wickham 1980). Encapsulated receptors in the iridocorneal angle of the cetacean eye have been described by Rochon Duvigneaud (1940), Pilleri and Wandeler (1964), Wickham (1980) and Vrabec (1972). The structures appear to be unique in mammals

(Wickham 1980) although similar structures have been described in the snow goose (Vrabec 1961). Wickham's study published in 1980 was the result of a field survey in which 42 eyes from nine species were collected over a number of years. A rigorous sampling regimen was undertaken (serial 2 micron sections for 300 microns in meridional section and serial 2 micron sections for 2mm in tangential section). Each set of serial sections was photographed. Encapsulated receptors were found in all but *Stenella longirostris*. Size and orientation of the ERs and their supplying nerve was recorded.

Sites in which ERs occurred varied between loose trabecular meshwork and dense scleral collagen. They were usually only found in one site in each species, not both; in trabecular meshwork of *Inia, Ziphidae* and *Delphinidae* and in sclera of *Kogia, Delphinapterus, and Stenella.*

Orientation varied, occurring circumferentially in all species, latitudinally in all but *Kogia* and beluga, and longitudinally in all but *Tursiops* and *Stenella.*

The ultrastructural features described varied between species according to size, the number of wrapping layers, and whether they were single or multiple within a common perineural capsule. Multiple association was commonest, with singles found exclusively in *Kogia* and beluga, and *Stenella.* The *Stenella sp.* could be distinguished by having a relatively small number of wrapping layers.

Ultrastructure was not described in detail in this study as follow up studies were planned.

Wickham's suggestions for the possible functions of ERs included: sensing the distortion of the vitreous; sensing pressure changes associated with filling and emptying of massive vascular system of the iris; lid closure causing a transient pressure increase; change in intraocular pressure with aqueous dynamics, and a thermosensory function.

Descriptions of the histology of the ciliary body in beluga, narwhals and pilot whales by West *et al.* (1991) and Waller (1992) are notable for their absence of any mention of ERs. However, Waller makes reference to 'numerous nerve bundles'.

### 2.2iv Choroid, Rete and Tapetum

Detailed choroid studies are not evident in the scientific literature, although its similarity to and close association with the much-researched rete tissue suggest such studies could be fruitful.

It is not known how the ophthalmic rete connects with the choroid and/or retinal vessels although the anatomy of vessels in this area in man and domestic animals is well known. Dawson (1980) has cited work which demonstrates that, in the fin whale, the rete supplies the long and short ciliary artery systems (Pilleri and Wandeler 1964). In man, the central retinal artery supplying the inner retina is a branch of the ophthalmic artery (Spooner 1957). An analogous vessel in the whale may either bypass the rete or become incorporated in it (Dawson 1987).

The tapetal layer within the choroid, however, has been studied in cetaceans. It is remarkable for its large number of layers (Young *et al.* 1988) and is typically ungulate-like, being a collagenous tapetum fibrosum. Its choroidal situation differentiates it from the tapetum cellulosum of carnivores, situated in the pigment epithelial layer (Young *et al.* 1988). In most whales it covers the whole fundus (Dawson 1972; Young *et al.* 1988), a phenomenon which has been attributed to the fact that their habitat is "homogeneous " and lacks a horizon (Young *et al.* 1988). Silver/blue and silver/green are commonly observed colourations (Dawson 1980), and this has been shown to correspond to the diameter and spacing of collagen fibrils, providing "increased spectral purity and efficiency"(Young *et al.* 1988).

## 2.3 THE ANTERIOR SEGMENT - ANATOMY OF DIOPTRIC ELEMENTS AND REFRACTIVE STATE

### 2.3i Anatomy of the Cornea

The cetacean cornea is markedly thinner centrally than peripherally (Dral 1975; Dawson 1980; Pardue *et al.* 1993). Such a phenomenon is not unusual in mammals, but in the whale it is pronounced. The human cornea has a central thickness of 0.6mm and peripheral thickness 0.9mm (Spooner 1957). In fish and many other aquatic vertebrates, the cornea has developed as a protective element for the lens and is optically neutral. Recently, research has been carried out to determine whether the role of the cornea in aquatic mammals is primarily for protection or if there is an significant optical function (Kroger and Kirschfeld 1992).
Johnson (1901) noted that the cornea is flattened in the horizontal plane.
Corneal astigmatism is recognised in whales (Dral 1975; Dawson 1987) although there is some disagreement as to whether it is mild and regular or occurs in local sites. Local astigmatism has been demonstrated as an 'emmetropic porthole' for vision in air is described by Dral (1975). This was not confirmed in a later study by Dawson (1987) but mild regular astigmatism was demonstrated. In humans, the curvature of the cornea is routinely measured using a keratoscope, an instrument which projects concentric circles of light of known size onto the cornea. If the pattern becomes distorted, this indicates astigmatism. By using quoted figures for refractive index and finding the anterior curvature of the cornea in this way, and assuming the posterior curvature lies parallel to it, focal length and thus dioptric strength of the cornea can be calculated. Since most dioptric errors lie either in the cornea or the axial length of the globe in humans, this information can be used by an optometrist to fit corrective lenses.
The corneal curvature in cetaceans has been measured using both corneal casts (Dawson 1972) and a keratoscope (Dawson 1987). Using corneal casts from

cadaver material, radii of curvature of 14.8mm anteriorly and of 13.4 mm posteriorly were measured. Using Matthiessen's figures for refractive index, (1893) the dioptric power was calculated to be 22.6D. Using a keratoscope, a dolphin was shown to have markedly elliptical concentric circles, showing astigmatism, with power variable according to site but ranging from 22-33D. The radius of curvature appeared to be around 20mm. Since no information is gained about the posterior refracting surface in this way, it is of limited value in cetaceans since in humans assumptions can be made about the curvature of the posterior surface and the refractive index (RI) of the cornea. Such assumptions cannot be made in cetaceans. Pardue *et al.* (1993) also noted central thinning of the cornea, and making rough calculations estimated the dioptric strength of two baleen whale corneas as 3D in air and -1D in water.

There is some evidence that the RI of the cornea in cetaceans is much higher at 1.53 (Kroger and Kirschfeld 1992) than 1.38 as was previously quoted by Matthiessen (1886), whose original technique using an Abbe refractometer only gave information about the surface of the cornea and is inferior to modern laser interferometry techniques which can assess the whole tissue. This figure (Matthiessen 1886) exceeds the RI of lens core at 1.51. The concept of softer corneal tissue having a greater optical density than a solid lens core is a novel one.

Observations on the corneal anatomy of two baleen whales and three seals demonstrated that the cornea is thicker in marine mammals than in man, due to a thicker stromal layer (Pardue *et al.* 1993). Other layers were relatively thinner, especially Descemet's membrane and the endothelium. These studies were conducted using light and transmission electron microscopy, with each structure measured five times from five different sections. Pinnipeds showed similar features, although the endothelium and Descemets membrane are not quite as thin as in cetaceans. At the limbus, the transparent cornea becomes opaque, forming the sclera. One of the most striking features of the cetacean eye is its enormously thick sclera. This was noted very early by John Hunter in 1787 (Waller 1984). Animals that are large with proportionately small eyes, such as whales, sharks and elephants, tend to have thick scleras. The thick cetacean sclera was believed to be necessary to withstand the pull of the large extraocular muscles (Walls 1942) but the eye was subsequently described as immobile due to the thick sheath of the optic nerve. Walls also failed also to describe the ophthalmic rete, and it is assumed that his observation of hard formalinised specimens led him to conclude this immobility. He goes on to state that cetacean ocular immobility would be of little consequence, since the eyes are a third of the way along the body with little prospect of forward or binocular vision.

## 2.3ii Lens

The cetacean lens has been described as small and hard with a high refractive index and short focal length (Walls 1942; Waller 1992) and therefore is very powerful in terms of dioptric strength.

Johnson (1901) recognised an asymmetry in the anterior and posterior surfaces and described a thick lens with a spherical anterior and parabolic posterior surface. Dral (1975) characterised the lens as a being aquatic and fish like. It is considered necessary to have a more powerful lens in water if the lens is the only refractive element functioning because the cornea has become optically neutral underwater, since a RI of 1.335 (Spooner 1957) is similar to that of the surrounding water (1.33).

The structure and function of the lens has been poorly researched in cetaceans. By using a fundus camera to photograph light reflected from the fundus through the lens, some convincing striations (zones of discontinuity) have been demonstrated (Dawson *et al.* 1992). Similar zones in humans appear at about one new zone every four years. Young cetaceans have few zones and older ones have many. They are thought to represent the refractive state of lens fibres as they reach a certain age and degree of compression. These zones were not demonstrable using conventional histology, but can be seen in excised, etched lenses using scanning electron microscopy. They can also be visualised in the living animal using a clinical slit lamp. Their presence may have some relevance in providing a method of estimating age and health history in cetaceans (Dawson *et al.* 1992).

Waller (1992) observed that the lens capsule has a uniform thickness apart from at the posterior pole where it is much thinner (22 microns), although this is still fairly thick by human standards, where 23 microns is the maximum width and 2-4 microns the minimum width of the capsule (Hogan *et al.*1971).

## 2.3iii Optics of the Cetacean Eye

The refractive state of an eye denotes whether the image of a distant object in a resting eye is brought to a focus in front of, upon or behind the retina (myopia, emmetropia, hyperopia). Refractive state has been rigorously assessed in man, and studied in many other species such as cephalopods (Sivak 1991), elephants (Murphy *et al.* 1992), otters (Murphy *et al.* 1990), and owls (Murphy *et al.* 1983). Refractive power is dependent upon the radii of curvature and refractive indices of each of the refracting elements of the eye and the distances between them. Refractive state is most often assessed in the live animal using a retinoscope, a device similar to an opthalmoscope in which a series of lenses are interposed between the eye and a camera.

Amphibious animals require a very wide refractive range and have adapted in a

variety of ways to meet the challenge of acute aerial and aquatic vision.
The otter has a novel mechanism whereby aqueous is shunted between anterior and posterior segments to cause lens displacement, at the same time as the powerful iris sphincter squeezes the lens into a cone shape (Murphy *et al.* 1992). The lens of the turtle is extremely soft (Walls 1942) and can similarly be moulded by the iris into a cone shape. The cormorant and turtle have similar iris mechanisms. Some diving ducks additionally have a transparent, refractile nictitating membrane which is brought over the eye. *Anablebs* (four eyed fish) have two pupils in each eye, one above and the other below the waterline, with two separate images formed on the retina (Walls 1942).
In most terrestrial animals, a wide accommodative range is unnecessary, and is achieved simply by lens flexure giving a range of 2-3D in middle aged humans (Spooner 1957) and 1-2D in the dog, cat and bovine (Prince 1957) . These species are irretrievably hyperopic underwater.
Although cetaceans are totally aquatic mammals, they spend a large proportion of their time at the surface in order to breathe. It is not known if aerial vision is important to these animals and there may be species variation in requirements. Some families, such as the *Delphinidae* (includes dolphins, *orca* and long-finned pilot whales) are well known for leaping, spy-hopping, and other surface activities which are likely to require good vision. Other species, notably the beaked whales, are rarely seen on the surface, and aerial vision may therefore be of little value.
How well an animal can see depends on the eye's refractive state and whether this is variable, its visual acuity and sensitivity, and its neural processing, in addition to how well the eye is adapted to a specific habitat. Behavioural studies in live dolphins and *orca* have shown their sight to be excellent in both air and water (Herman *et al.* 1975; Spong and White 1971; White *et al.* 1971).
More recent studies by Cronin *et al.* (1998) have found that live, unrestrained dolphins are emmetropic in water at distance, and myopic in air. This study also concluded that there may be some accommodative ability.
Ophthalmoscopic studies of the refractive state of the dolphin eye have shown aerial myopia (Dawson 1972; Dral 1975), so the challenge to find explanations for paradoxically good aerial vision has been pursued (Dawson 1972; Dral 1975, 1987; Herman 1975). Some of the explanations advanced to date are:-

a] The pinpoint pupil mechanism (Walls 1942, Dawson 1972) makes use of the physical principle that, at high F - stop values, depth of field is very wide.

b] The double slit pupil mechanism (Herman *et al.* 1975)

c] An emmetropic porthole/astigmatic cornea in air has been described by Dral (1975) but this finding was not supported in later work which describes 'mild, regular spoon shaped astigmatism (Dawson 1987). However, specimens in this study were difficult to assess as they had

a number of corneal lesions, such as scars and a lumpy gel layer. The emmetropic porthole (Dral 1975) was found to correspond with the position of the inner pupillary aperture in a fully constricted pupil, suggesting that there may be selective use of this site in a nasoventral direction in air. Later work by Dral (1987) demonstrated that a figure of 8-shaped visual streak exists, where it could be postulated that one area of resolution would be useful for vision laterally and the other for forward, binocular vision, further supporting the 'double image' theory.

d] The lens zone theory proposed by Rivamonte (1976) is based on the fact that spherical aberration is not encountered in living lenses because a refractive index gradient exists which is high at the core and low at the periphery. The theory suggests that the lens is overcorrected, causing rays striking the periphery to focus behind the retina, so that in air, when rays are likely to be restricted to the periphery through the crescent shaped pupil, reinstatement of the cornea as a refractive device will produce an emmetropic image. Rivamonte suggests that in lower light conditions underwater, the whole lens would be used, its central portion producing a focused image.

e] The ramp retina theory as described by Dawson (1980) proposes that the animal makes preferential use of areas of its retina, which has regional variations in distance from the lens.

More recently, a divergent cornea in the harbour porpoise has been demonstrated, and this principle could be extrapolated to provide an explanation for good aerial and aquatic vision. Model calculations on a porpoise eye using Matthiessen's values have suggested that it may be overpowerful even underwater (Kroger and Kirschfeld 1992) Originally, the cornea was thought to be insignificant underwater. However, Matthiessen's RI values (1886) have been disputed by Kroger and Kirschfeld (1992) whose work in the porpoise has redefined the cornea as a significant refractive element, by virtue of a significantly higher RI (1.53) than was previously thought and by its divergent shape. Calculation of focal lengths in water at a number of sites (using data from diagrams of an enucleated porpoise eye and RI values as cited by Matthiessen for the lens, and his own revised value for the cornea) he was able to demonstrate two optical models. In a model with a spherical approximation of the posterior corneal surface, there was central emmetropia and peripheral myopia and in a model with an elliptical approximation of this surface, most of the retina was emmetropic.

## 2.4 EXPERIMENTAL TECHNIQUES used in the ASSESSMENT of REFRACTIVE STATE

In the living eye, the refractive state is routinely measured using a retinoscope. Studies of refractive states in the enucleated eye are problematic because:

**a)** a retinoscope cannot be used as the cornea collapses *post mortem* and the lens and cornea quickly lose their transparency (Spooner 1957).

**b)** if the eye is enucleated and incised its normal architecture is destroyed, so the original sizes, shapes and distances between the refractive elements are lost.

**c)** calculations for geometrical optics require refractive index values.

Values in living tissues such as lens and cornea are hard to obtain because they are not uniform in distribution. Studies on human, bovine (Bettelheim and Wang 1974; Pierscionek *et al.* 1988) and squid (Sivak 1991) lenses have revealed that a gradient exists.

Refractive index values quoted by Matthiessen in 1886 have not been reviewed until recently (Dawson 1980; Kroger and Kirschfeld 1992). Dawson (1980) used ultrasonography to obtain measurements between intraocular structures and compared these values with those obtained by direct measurement after dissection. They were found to be very different. Dawson's explanation was that the ultrasound machine had been calibrated using human plasma, of similar value to Matthiessen's value for intraocular media in cetaceans, which is the same as the density of human intraocular media. If the accuracy of Matthiessen's values for intraocular media are questionable, then those for cornea and lens could also be questioned. However, the technique used by Dawson of imaging a section through the cornea may have meant that a significant proportion of the beam traversed lens material rather than media, and since lens is considerably more dense than the media, his methods and results may also be questionable.

Recent work by Kroger and Kirschfeld (1992) on the shape and refractive index of the cornea reveals a refractive index value of 1.53, exceeding Matthiessen's 1886 value of 1.38. The former value of 1.53 is likely to be more accurate than readings made at the surface with an Abbe refractometer, because the laser interferometry technique used takes account of the existence of a RI gradient within living corneal tissue where surface layers are less refractile and deeper layers more refractile (Kroger and Kirschfeld 1992).

### 2.4i Recent Techniques for the Measurement of Refractive Index in Lenses.

Sophisticated techniques to measure RI gradient in bovine and human lenses have now been described. However, there is considerable difficulty in obtaining the refractive index at a given point in an intact lens. Pierscionek *et al.*(1988)

described methods that have been used in previous attempts to obtain values, and all of these involve dissection of the excised lens. They are:

i) Refractometry - Abbe Refractometer / Pulfrich Refractometer
Thin sections of lens are placed on a prism and a light beam is shone through the prism and sample. The resulting deflection of the beam can be registered on a scale. To be effective the whole prism should be covered, and the surfaces must be flat and smooth. This is easy with fluids but almost impossible to achieve with lenses. However, the technique was used with some success by Bettelheim and Wang (1974), who commented that the values obtained were slightly low due to incomplete coverage of the prism.

ii) Protein densitometry
If the protein concentration of a sample of lens is measured, then the Gladstone - Dale formula which relates protein concentration to the refractive index, can be applied.

iii) Interferometry
Using an immersion and interferometry technique, Bettelheim and Wang (1974) removed the capsule from a lens, submerged it in fluids of variable RI, and looked for minimum scattering of light at various points. This technique relies on the fact that when the lens is immersed in a medium with a RI identical to that of the lens, it becomes invisible. A series of solutions with varying RI's were set up and the lens immersed sequentially until a match was achieved. The medium must be non-reactive. Bettelheim and Wang (1974) were able to devise an experimental regimen using butylphthalate and hexamethyldisiloxane mixed in varying proportions. Sucrose solutions of varying osmolarity will give results but the technique has to be rapid to avoid the lens fibres being affected by a non-isotonic solution. Detection of a similar RI was accomplished by directing a laser beam onto the lens at a known locus, and the pattern of scatter that it produced was photographed. When scattering was minimal, the RI of the immersion medium matched that of the lens. A map of surface RI could then be constructed. The topographic distribution of RI obtained by this technique was closely matched by direct readings taken on isolated samples of cortex using the Abbe refractometer. The only non-destructive method in an isolated lens reported to date was described by Pierscionek *et al.* (1988), who was able to use a variation of the immersion and refraction technique. The paths of a split laser beam through a bovine lens suspended in an agar gel of RI and isotonicity identical to aqueous were recorded. One ray was fixed and central, therefore not refracted and the other was moved in three planes and was variably refracted. A locus was defined by identifying its position on x, y, z axes, and by measuring the emergent angle of the refracted ray a mathematical model could be used (as defined by Chan *et al.* 1987) to give the RI

at the given locus. Refractive index could be plotted as a function of radial distance from the centre on a three dimensional map.
Their results indicated that the adult bovine lens has a continuous gradient towards the centre, whereas human lenses exhibit a plateau to a radius of about 3mm with gradual reduction in RI from 3-5mm. The youngest human lens was 16yrs old, and the oldest bovine lens 2.8yrs. A possible explanation for the difference would be that there was not sufficient time for the bovine to develop a plateau since the lens is constantly growing and developing.
None of these techniques has yet been applied to a cetacean lens.

## 2.5 PATHOLOGY OF THE CETACEAN EYE

There is a paucity of literature concerning eye disease in cetaceans. There are a few anecdotal reports of ophthalmic problems in live animals such as conjunctivitis in captive dolphins and lenticular opacities in a seal (Lilley 1997). Dawson notes that in captive dolphins, lesions occur frequently on the cornea (Dawson *et al.* 1987).
In cetaceans it is thought that corneal trauma and subsequent inflammation (keratitis and conjunctivitis) are the lesions most commonly seen, with the ability to retract the globe providing some protection against such injury (Sweeney and Ridgeway 1975). In a survey of seals (Stoskopf *et al.* 1985) 4.6% were found to have eye lesions of which corneal scars were commonest, followed by prominent lens sutures and cataracts. In another seal survey, 46% of seals exhibited lens lesions, which is surprising given that only three previous reports of such lesions were published in the literature (Schoon and Schoon 1992).

Cataracts were reported in one case. In this case, completely
opaque, bilateral cataracts were reported in a mature adult (19.3m) fin whale (Panilov 1975). The animal was described as behaving normally and in good body condition with a full stomach. However, there were no investigations to ascertain the aetiology or age of the cataracts to support the author's statements that good vision is superfluous for survival.
In contrast, there is a surfeit of literature concerning cataracts in humans. Congenital cataracts appear to be frequently accompanied by other physical and mental abnormalities (Zimmer *et al.* 1993; Gripp *et al.* 1996). Inherited cataracts have been described in sheep (Brooks 1981) but also occur in many other species. Other causes of cataract in humans are systemic diseases, old age and environmental causes.
The aetiology of cataract is poorly understood (see Chapter 8). The presence of

toxic polychlorinated biphenyls (PCB's) in marine mammals has been described extensively in the literature and is thought to be of considerable concern (Kannan *et al.* 1989). The aromatic benzene compound, napthalene, is recognised as a cause of cataract in humans (Hogan 1962) and it is not inconceivable that some of the other aromatic benzene compounds such as PCB's could also be implicated as causal agents for cataract (Schoon and Schoon 1992).

Recent research has identified and quantified several risk factors for the development of cataracts in humans:

i] Latitude.

The prevalence of cataract increases with decreasing latitude, an effect which is thought to be at least partly due to to the dose of UV received by the lens (Javitt and Taylor 1995; Merriam 1996). In the US the rate of cataract surgery increases by 3% per degree south (Javitt and Taylor 1995).

ii] Diet.

A number of studies have shown that certain foodstuffs can increase or decrease the rate of cataract occurrence. It is believed that fruit and vegetables, calcium, folic acid, vitamin E (Tavani 1996) and other antioxidants (West and Valmadrid 1995) are protective against cataracts, while fat (except olive oil) and salt (Tavani *et al.* 1996) and alcohol (West and Valmadrid 1995) increase the risk of cataractogenesis. The Body Mass Index (BMI) appears to be a successful method for assessment of risk which is independent of other risk variables such as age, smoking, diabetes (Glynn *et al.* 1995).

iii] Chronic Steroid Use

Systemic and topical steroid use appear to be associated with an increased incidence of cataract formation (Hodge *et al.* 1995).

iv] Gender

In one case controlled study of 1, 940 people, the incidence of cataracts in older people was higher in females than in males (Harding *et al.* 1993).

v] Effects of oestrogen

A protective effect on the lens by oestrogen has been reported after a study found that women receiving postmenopausal oestrogen, and women who were younger than average at menarche or older than average at menopause, developed less severe cataracts.

In addition to opacity of the lens, discolouration can also be encountered. Yellow filters in the cornea have been described in diurnal, terrestrial species (geckos, squirrels, snakes) and yellow lenses in some squirrels and prairie dogs (Walls 1942). A yellow filter absorbs short wavelength light. The value of this would be that contrast in the blue/green area of the spectrum is improved, and glare is eliminated, since most scattered light is of short wavelength. Chromatic aberration is also reduced. In humans, yellow lenses occur normally with advancing age due to deposition of urochrome pigment (Hogan *et al.* 1971), which affects the perception of colour, so that the lens of a child absorbs only 10% of

blue light while that of an 72 year old absorbs 85% (Walls 1942). In whales, yellow lenses were noted as an incidental finding by Dawson (1980) in four specimens of *Inia* (freshwater dolphins). A possible advantage to this species would be the reduction of glare in relatively shallow water. However, for marine species in deep water, the advantages of a yellow filter are unclear. In deep water, long and short wavelength light is absorbed, so that at 250m only 520 nm wavelength light remains (Beebe 1934). Fish rhodopsins have a peak sensitivity at this wavelength (Walls 1942), but pigment extracted from a dolphin had a maximum spectral sensitivity at 486nm (Madsen and Herman 1980). Paradoxically, when sensitivity was tested behaviourally, 495nm light was perceived best. A similar situation is described in porpoises (Lavigne and Ronald 1972; Wartzok 1979). The two hypotheses for visual object detection in water are i) the sensitivity hypothesis (Munz 1965) and ii) the contrast hypothesis (Lythgoe 1968). The sensitivity hypothesis requires that the absorption spectrum of visual pigments should coincide with the wavelengths of light that best penetrate water at depth. The contrast theory requires the absorption spectrum of pigments to coincide with the wavelengths of light reflected from prey. Dolphins and porpoises have pigments which, when extracted, support the sensitivity theory, but behaviourally they support the contrast theory. Possible explanations for this are that there are multiple pigment systems allowing good visual function over a wide range, or that not all penetrating light reaches the retina because of absorption within the eye (Watkins and Wartzok 1985). The presence of a yellow filter in the lens would support the latter explanation.

# MATERIALS AND METHODS

## 3.1i Sources of Material

Eyes were obtained from 45 whales from five families, two of the suborder mysticeti and three odontoceti (see Appendix 4-1) which had either stranded and died around the coast of New Zealand or had been accidentally caught in fishing nets. The eyes were removed, often by DOC staff and placed in 10% buffered formalin as soon as possible after death. They were then transported by courier to the Department of Veterinary Pathology and Public Health at Massey University. Whenever possible a broad incision across the central cornea was made to enable fixative to penetrate inside the globe. Sections of the globe were made at intervals after fixation which varied from three days to several weeks. For long term storage, some specimens were transferred to 50% alcohol.

## 3.1ii Preparation of Material

All eyes were were bisected longitudinally and examined in an identical manner. Standard terms are used to describe positions (Figure 3-1).

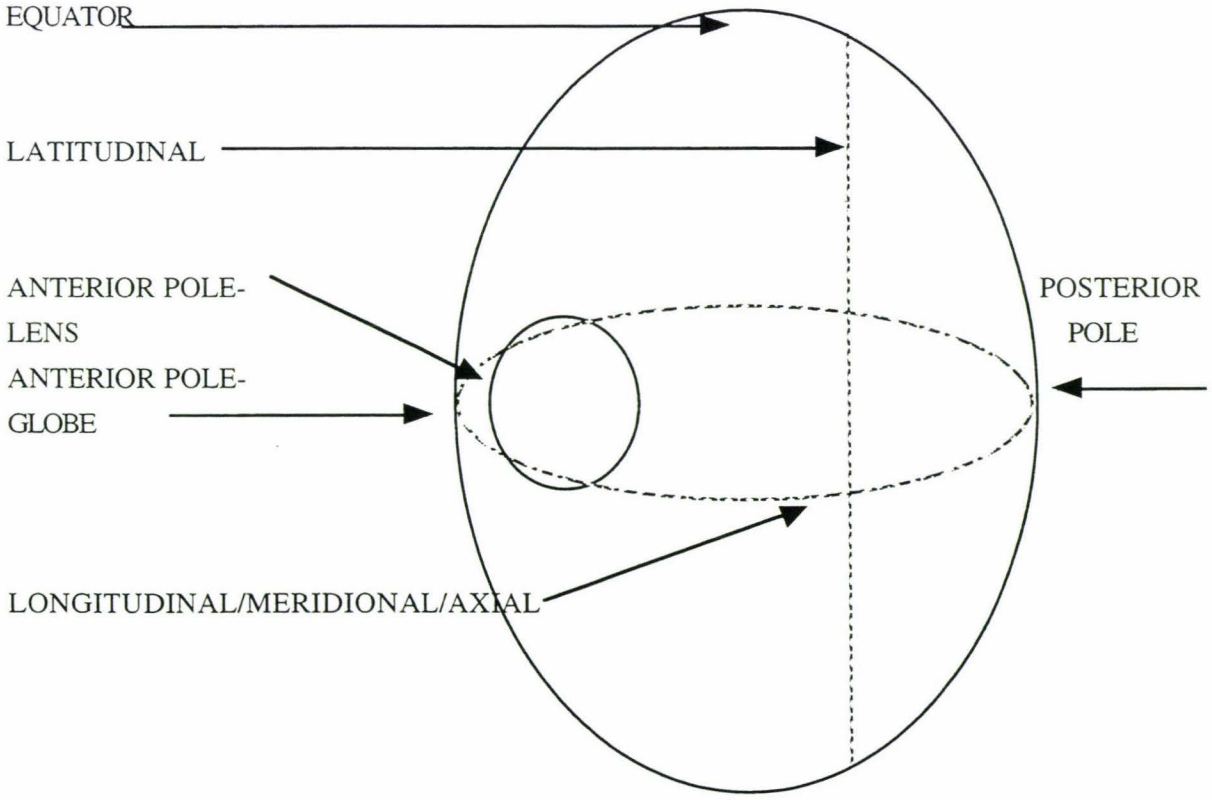



**Figure 3-1. Standard terms for positions on the globe.**

In 38 whales, the globe, cornea, lens, sclera and optic nerve were measured for a biometrical survey (Chapter 4). Twenty eyes were fresh enough to be used in histological surveys (Chapters 5 and 6). One fresh eye (DOC no.38-98) and one fixed eye from long finned pilot whales were used in a nuclear-magnetic resonance (NMR) study (Chapter 7). Two fixed eyes from long-finned pilot whales had Indian ink injected into the anterior chamber in order to study percolation through the drainage angle (Chapter 5). Five whales were involved in the ocular pathology survey (Chapter 8).

Samples were collected from the midline following a standard regimen of six areas:

1 Anterior segment A-B fig. 3-1
2 Posterior segment C-D fig. 3-1
3 Lids (if available)
4 Lens (if available)
5 Rete (if available)
6 Retina ( if detached from posterior segment )

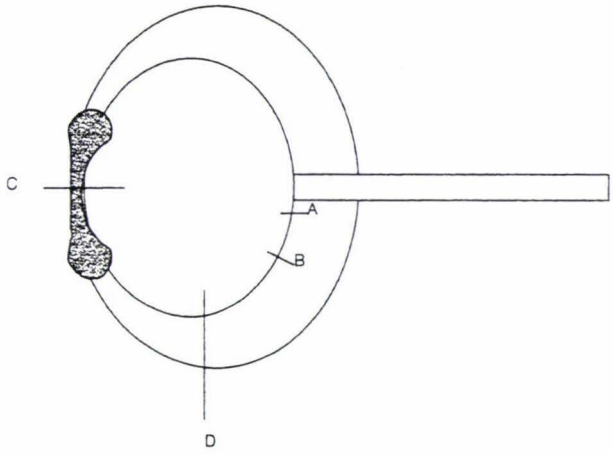



Figure 3-2. **Longitudinal, midline section of cetacean eye, showing areas sampled.**

### 3.1iii Preparation for Histology

All sections were processed by routine histological methods and embedded in paraffin wax. Sections were cut 3-4 microns in thickness and stained with haematoxylin and eosin. Those eyes showing least evidence of autolysis were selected for more detailed study and some were further stained with Van Gieson, Masson's Trichrome, Luxol fast blue, periodic acid schiff (PAS) and Holmes' silver (Figure 5-2). In six whales samples from the ciliary body were cut equatorially (perpendicular to the standard direction) in an attempt to demonstrate the presence of circular muscle fibres.

Each chapter deals with materials and methods specifically.

# BIOMETRICS AND GROSS ANATOMY

## 4.1 ABSTRACT

AIMS: To investigate relationships between the shapes and sizes of eye features in different species of whale.
METHOD: Measurements from the eyes of 38 whales in six families were recorded in a spreadsheet.
RESULTS: There were clear differences in the sizes of anatomical features between mysticetes and odontocetes, and sperm whales.
CONCLUSION: Toothed whales had eye sizes which were proportional to their body sizes. Their scleral thickness, corneal and lens sizes increased proportionately with eye size. Baleen whales had proportionally larger eyes and larger lenses with respect to body size, but disproportionately thicker scleras and larger corneas than odontocetes. The sperm whale was exceptional in having a cornea and lens which were proportionately smaller than the other toothed whales.

## 4.2 INTRODUCTION

The relative sizes, shapes and positions, or gross anatomy, of many anatomical structures are related to the way in which they function. The eyes of different species can be compared to predict whether the eye is useful in light, dark or both (diurnal, nocturnal or arrythmic) and whether there is likely to be a capacity to alter the refractive state. If the optical elements of the eye are examined, they can be used to assess the eye's refractive state. The eye is an optical instrument which is independent of body size (Spooner 1957). The relatively largest eyes do not necessarily belong to the largest animals, but to animals requiring good vision in dim light because they are either hunters (eg. seals) or hunted (eg. deer).
Animals which have a requirement for good vision have the largest eyes that their skulls can comfortably accommodate. Walls (1942) explains that in the case of nocturnal animals, a large eye is required to accommodate a large pupil which allows in more light, so a large lens is required to fill the pupil. A large lens may be flat or spherical but in nocturnal animals it tends to be spherical, to allow a smaller brighter image to be formed at the retina. This effect is enhanced if the cornea is also strongly curved. The diurnal eye also needs to be large, in order to have a large image for good acuity. In this case the lens and cornea flatten to increase focal length and provide a larger, but dimmer, image than in the nocturnal eye. In the arrythmic eye, balance is achieved between these extremes, and the photon catch is enhanced by the presence of a tapetum. In all cases, there are appropriate retinal adaptations.
Accommodative ability is less predictable from eye shape and size, but generally, a

deeper anterior chamber indicates that space is required for 'rounding up' of the lens (Walls 1942) and this is associated with the diurnal eye, where accommodation and acuity are of prime importance.

In the case of cetaceans, the eye is large and flattened axially (Walls 1942; Prince 1957; Dawson 1980). The lens is near spherical (Dral 1975; Dawson 1980; Waller 1992) which indicates that a small, bright image will be formed with a wide angle of vision, and a tapetum is present (Walls 1942, Dawson 1980), all factors which indicate that the eye is adapted for use in dim light. It has been suggested that baleen whales have better acuity than odontocetes due to their larger eyes (Waller 1980) but this neglects the fact that the sclera is much thicker in mysticetes (Walls 1942, Prince 1956, Dawson 1980) and the internal dimensions may actually be very similar to odontocetes. In addition, it is the lens size, not eye size, which dictates image size.

The aim of the present study was to make a biometrical analysis of eye dimensions of 38 whales in five different families in order to increase knowledge in this area, since to date little information has been published. The data obtained could then be used to:

1] ascertain the variations of eye size and anatomy between species, and

2] compare eye size with respect to corneal and lens sizes.

## 4.3 MATERIALS AND METHODS

Eyes from 38 dead, stranded cetaceans were obtained and fixed in 10% buffered formalin. For the purposes of this study these were subdivided into six groups (Table 6-1). Measurements of the horizontal and vertical diameters of the globe and cornea were made before the eye was bisected along a vertical axial plane. The internal axial length (cornea to optic nerve head) was measured and recorded. Thicknesses of central and peripheral cornea and optic nerve were recorded. The vertical diameter and axial length of the lens was recorded. All measurements were made to the nearest millimetre with a clear plastic ruler or a micrometer screw gauge. Data was processed using Clarisworks (spreadsheet) and Cricket graph to produce a series of charts and graphs to illustrate relationships between parameters.

## 4 - 4 RESULTS

After removal, the globe often retained its extraocular musculature, and this was very extensive, with four rectus, two obliques and a large retractor bulbus muscle, surrounding a substantial ophthalmic rete. The limbus was often 1-2 mm wide, and the cornea was thinner centrally than peripherally. In most cases peripheral

measurements of 2mm and central measurements of 1mm were made. (Appendix A4-1 and Chapter 7).

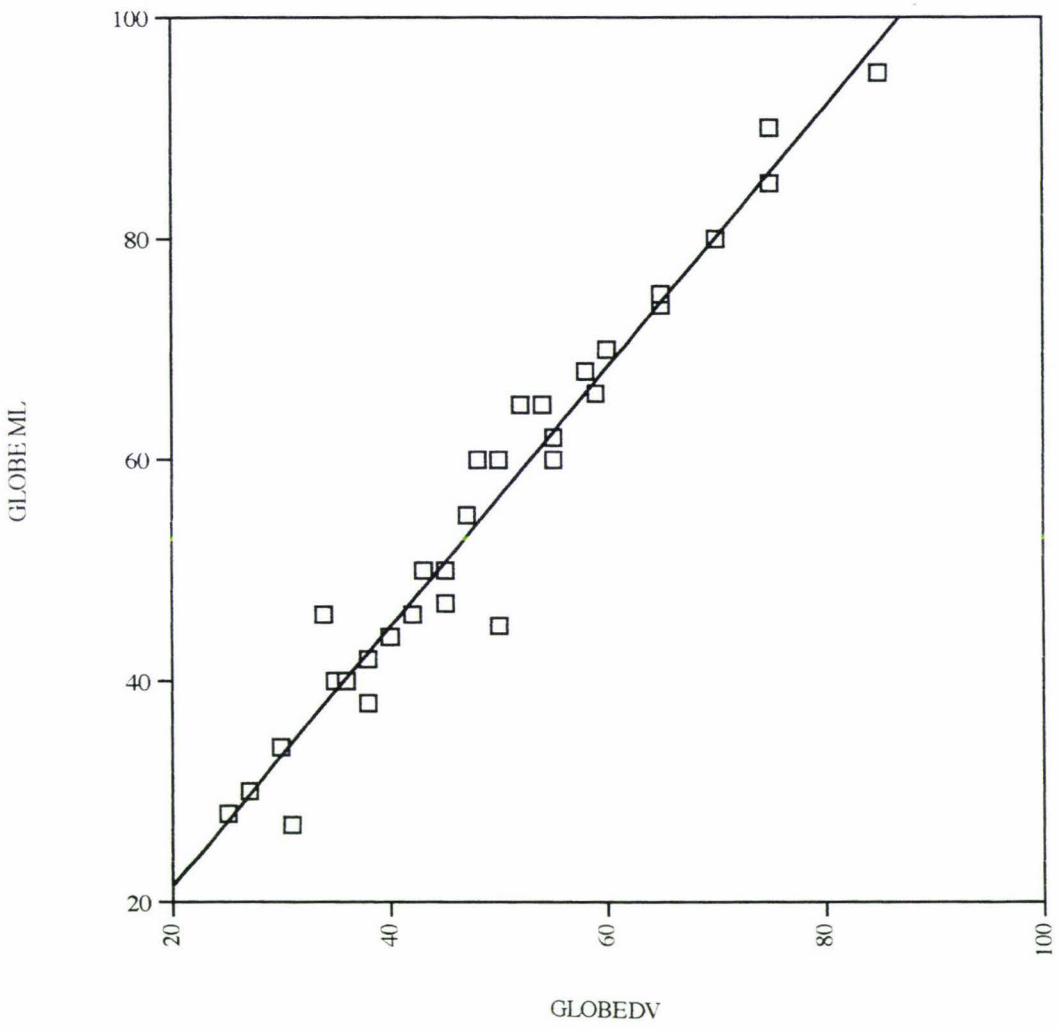
Charts to illustrate comparative size relationships between species were formulated (Figures 4-1 to 4-7).

**TABLE 4.1. WHALES INVOLVED IN BIOMETRICAL ANALYSIS**

| BIOMETRICS REFERENCE | FAMILY | WHALE |
|---|---|---|
| 1 | B | Pygmy right (adult) |
| 2 | A | Brydes |
| 3 | L | Minke |
| 4 | E | Minke |
| 5 | E | Minke |
| 6 | N | Dwarf minke |
| | | |
| 7 | S | Sperm |
| 8 | P | Sperm |
| 9 | E | Sperm |
| 10 | R | Sperm |
| | M | |
| | | |
| 11 | P S | Pygmy Sperm |
| 12 | Y P | Pygmy Sperm |
| 13 | G E | Pygmy Sperm |
| 14 | M R | Pygmy sperm |
| 15 | Y M | Pygmy sperm |
| | | |
| 16 | B | Shepherds |
| 17 | E | Straptoothed |
| 18 | A | Grays |
| 19 | K | Grays |
| 20 | E | Grays |
| 21 | D | Southern right bottlenosed |
| 22 | | Cuviers |
| 23 | | Cuviers |
| 24 | | Splaytoothed |
| | | |
| 25 | L | Long finned pilot |
| 26 | O | Long finned pilot |
| 27 | N | Long finned pilot |
| 28 | G | Long finned pilot |
| 29 | FINNED | Long finned pilot |
| | P | Long finned pilot |
| | I | Long finned pilot |
| | L | Long finned pilot |
| | O | Long finned pilot |
| | T | Long finned pilot |
| | | |
| 30 | | Orca |
| | | |
| | D | Spectacled porpoise |
| 31 | O | Bottlenosed dolphin |
| 32 | L | Dolphin |
| 33 | P | Dolphin |
| 34 | H | Dolphin |
| | I | Dolphin |
| 35 | N | Dolphin |
| | S | |
| 36 | | Juvenile pygmy right |
| 37 | | Juvenile long finned pilot |
| 38 | | Foetal pyg. sperm |

## 4.4i Ratio of Dorsoventral to Mediolateral Diameters

The ratio of dorsoventral:mediolateral globe measurements was a constant one across the species, with horizontal size always exceeding vertical size, confirming that the globe is mildly elliptical (Figure 4-1). In larger species of whales, the eyes were larger, with baleen whales having the largest eyes, and beaked and sperm whales the second largest. This is illustrated in Figure 4-2 where the whales are grouped according to species.




DV = dorsoventral diameter
ML = mediolateral diameter

**Figure 4-1. The relationship between dorsoventral and mediolateral globe diameters.**

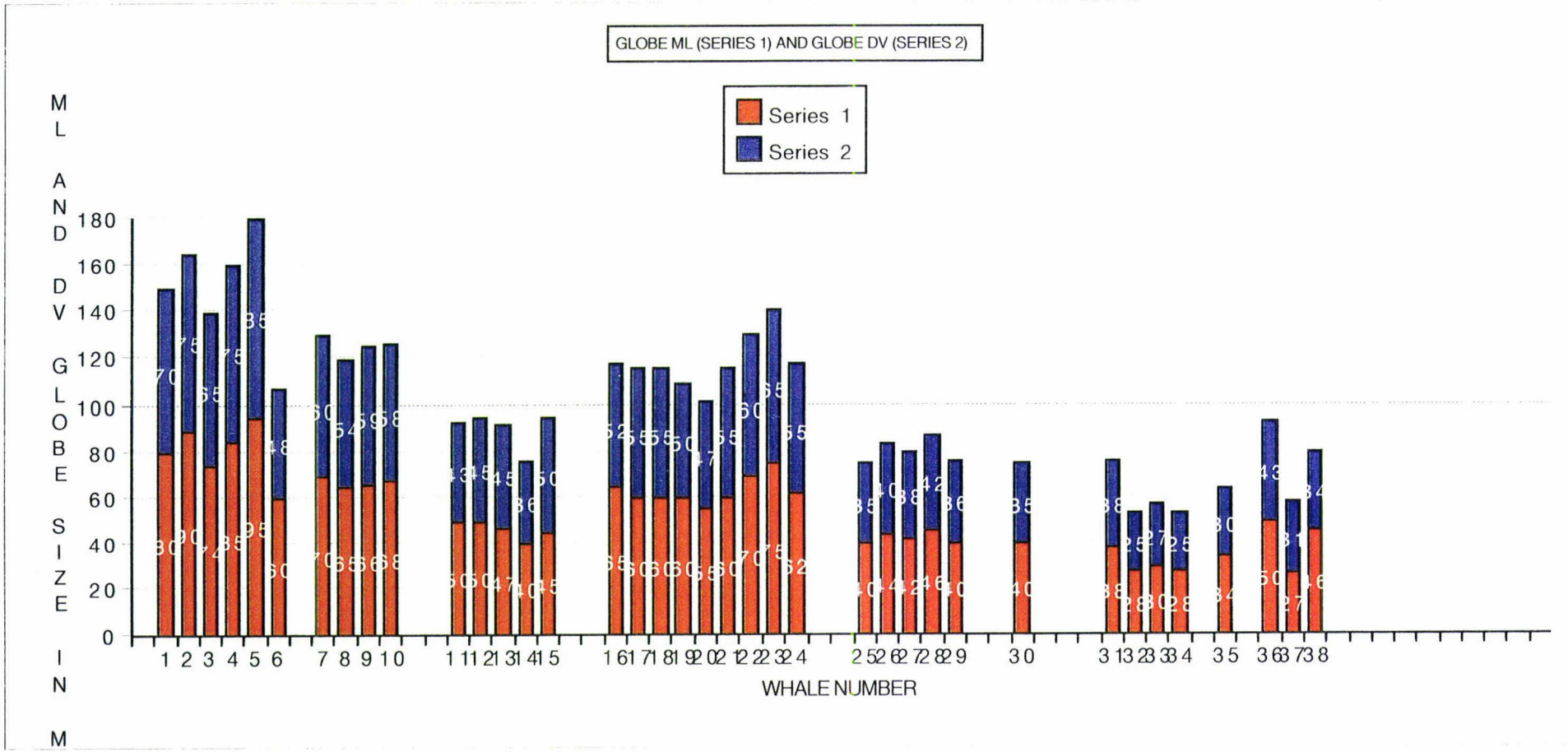



**Figure 4-2. The relative sizes of the mediolateral and dorsoventral globe (see table 4-1 for details).**

### 4.4ii Corneal and Globe Size Relationships.

The relationship between corneal size and globe size showed a moderately variable distribution. It was noted that baleen whales, beaked whales and pygmy sperm whales had the largest corneas with respect to globe size (Figure 4-3) and that the cornea of the sperm whale was relatively small with respect to globe size (Figure 4-3). Although most whales were clustered within a broad range of values, nine whales (four baleen, four beaked and two pygmy sperm) had corneal values which plateaued around 30 mm regardless of globe size (Figure 4-3). The pygmy right whale had a cornea which was noticeably smaller than those of many other whales.

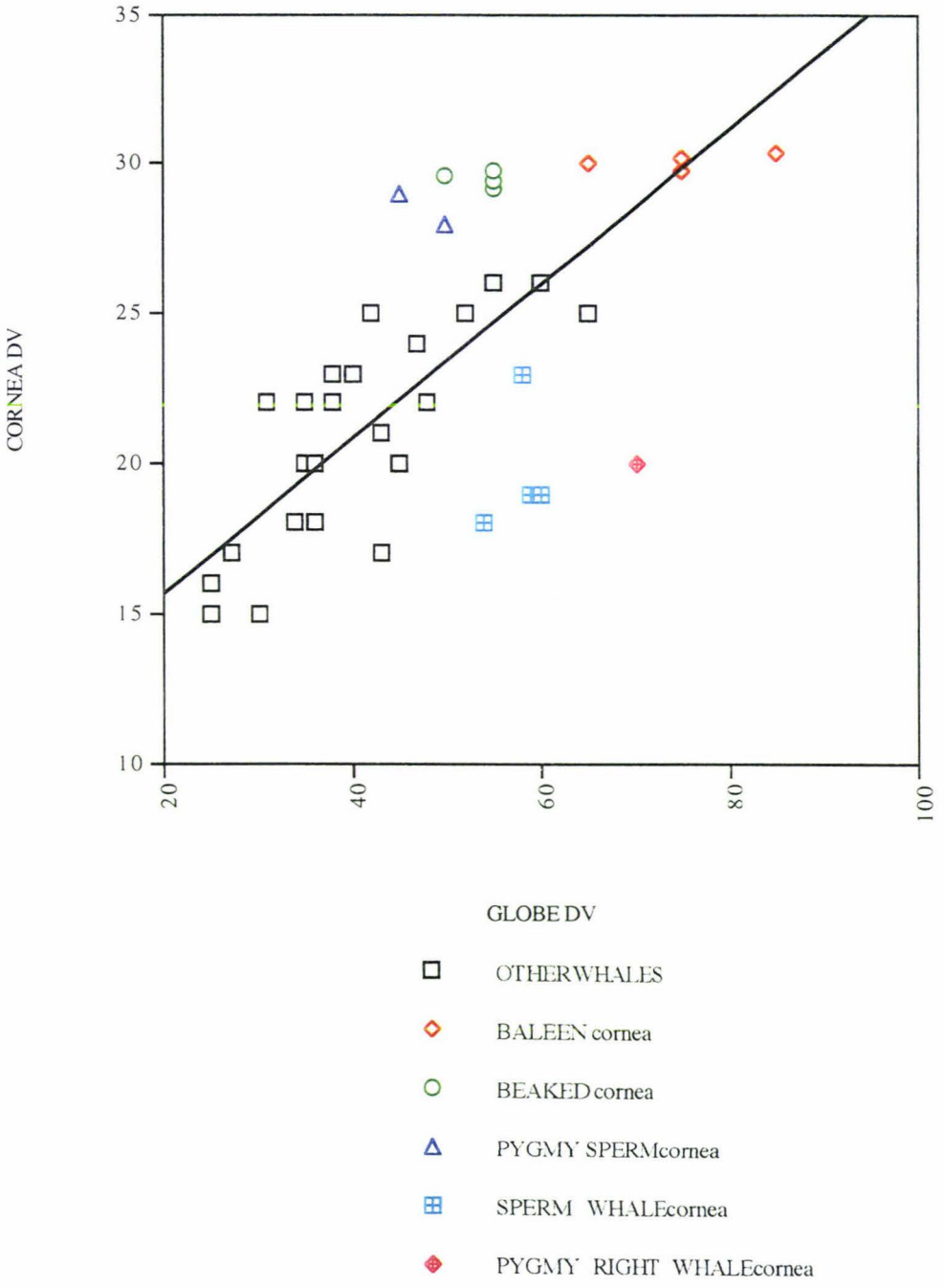



**Figure 4-3. Relationship between globe size and corneal size in 38 whales.**

### 4.4iii The relationship between Scleral Thickness and Globe Size, and Internal Globe Size compared to External Globe Size.

In most whales, the sclera increased in width proportionately with increasing globe size(Figure 4-4). However, the baleen and sperm whales showed a disproportionate increase in scleral thickness with increasing globe size. This was not accompanied by the proportionate decrease in internal axial length as would be anticipated. Internal axial lengths in these animals were closely aligned to the curve drawn for the other whales, suggesting that internal axial measurements increase proportionately with external dorsoventral measurements. The disproportionately increased thickness of sclera may have affected those parameters which have not been measured, ie. external axial globe or internal dorsoventral globe (Figure 4-4).

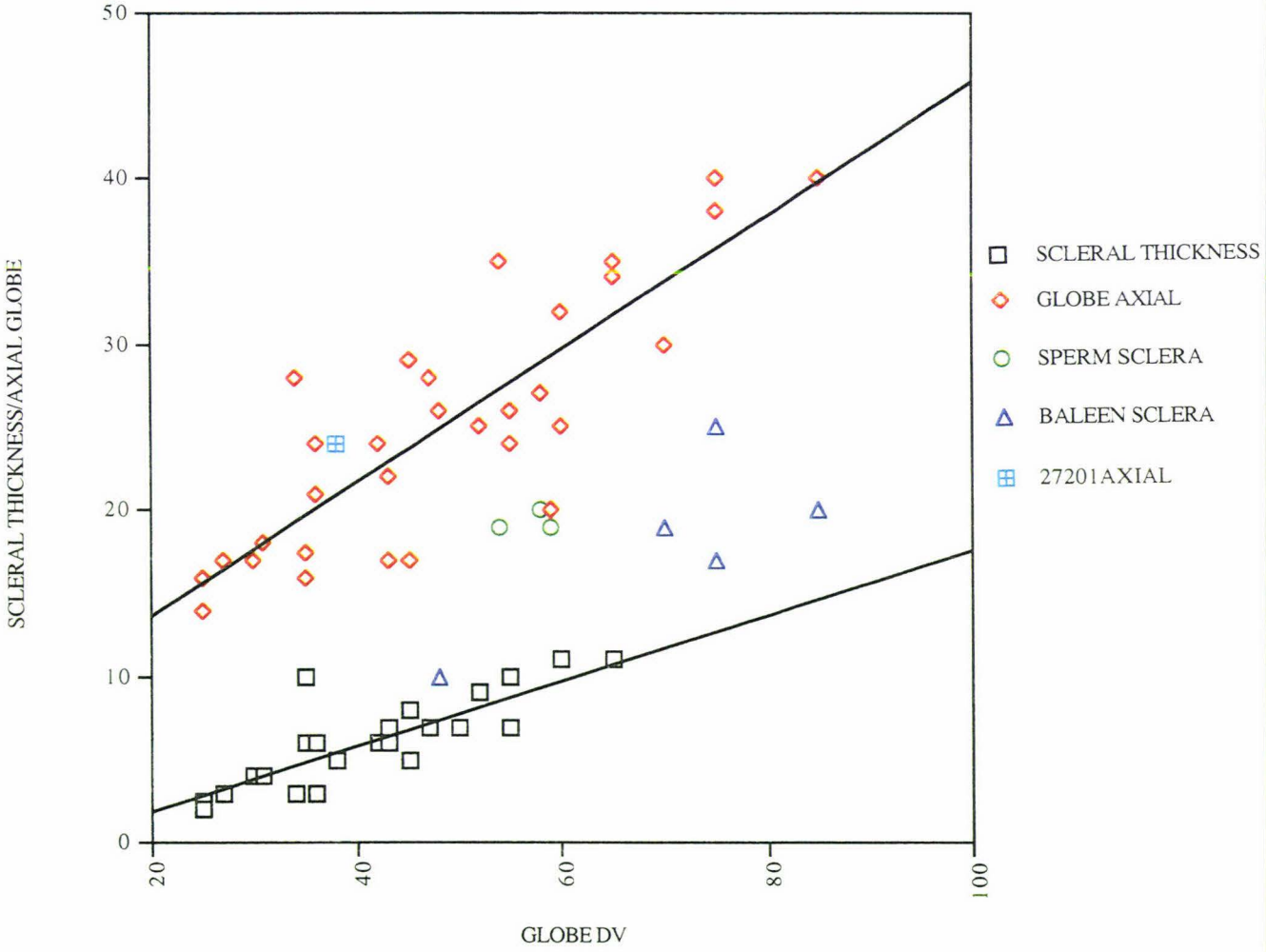



Figure 4-4. The relationships between globe size, scleral thickness, and internal axial diameter.

### 4.4iv Relative Sizes and Shapes of Lenses.

Lens size varied from 6-20mm. One exceptionally small lens, in a Shepherd's beaked whale, was considered an artefactual error. The largest lenses were found in baleen whales, and lenses in other whales appeared to fall within the relatively narrow range of 7-15mm. In most cases the vertical length exceeded the axial length indicating that the lens was elliptical. In some cases (whale no.13, a pygmy sperm whale and whale no.30, an orca) the measurements were identical, indicating that the lens was spherical (Figure 4-5).

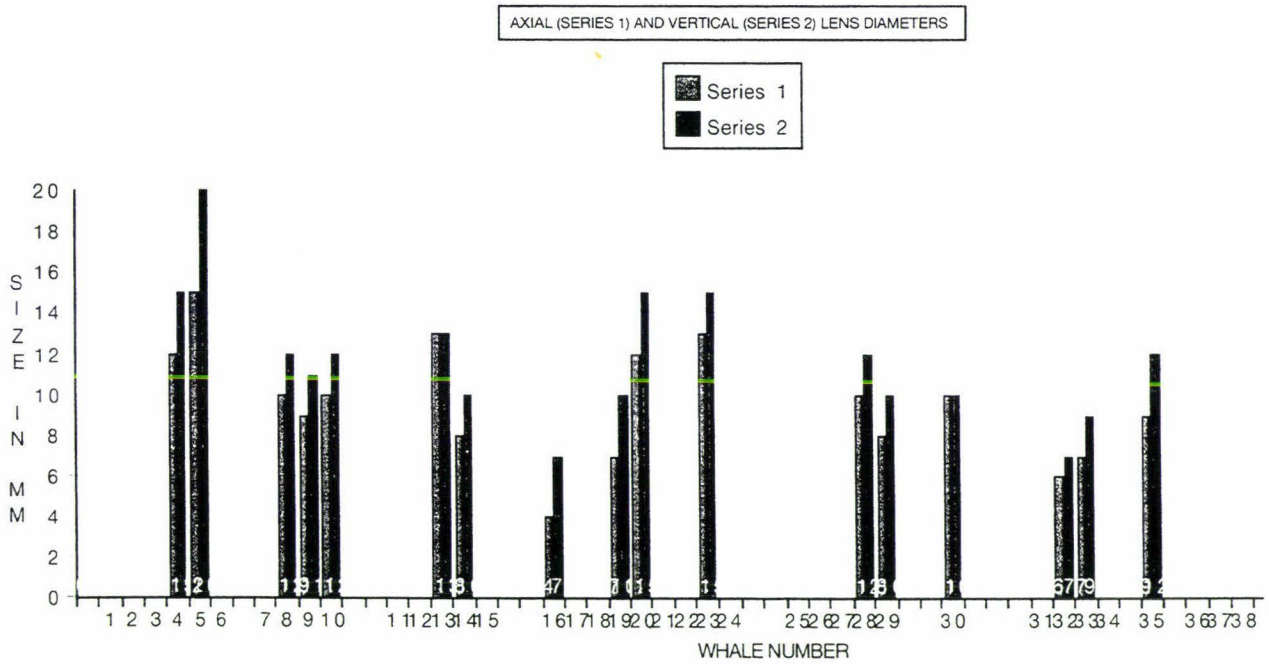



**Figure 4-5.** **Axial and vertical lens values for 17 whales. (Refer Table 4-1).**

### 4.4v Internal Axial Globe Length Related to Size of Lens.

Since lens size and curvature are related to its focal length (which is equivalent to the internal axial length of the globe) the relationship between this and lens dimensions are of interest. The largest lenses, found in baleen whales, were found to have the longest axial lengths, as would be expected. There are insufficient data for meaningful analysis, but in most cases the internal axial (focal) length was about twice the vertical diameter of the lens (Figure 4-6). One lens was very much smaller than the others (whale no.16, Shepherd's beaked), but this was probably due to the loss of its cortex during autolysis.

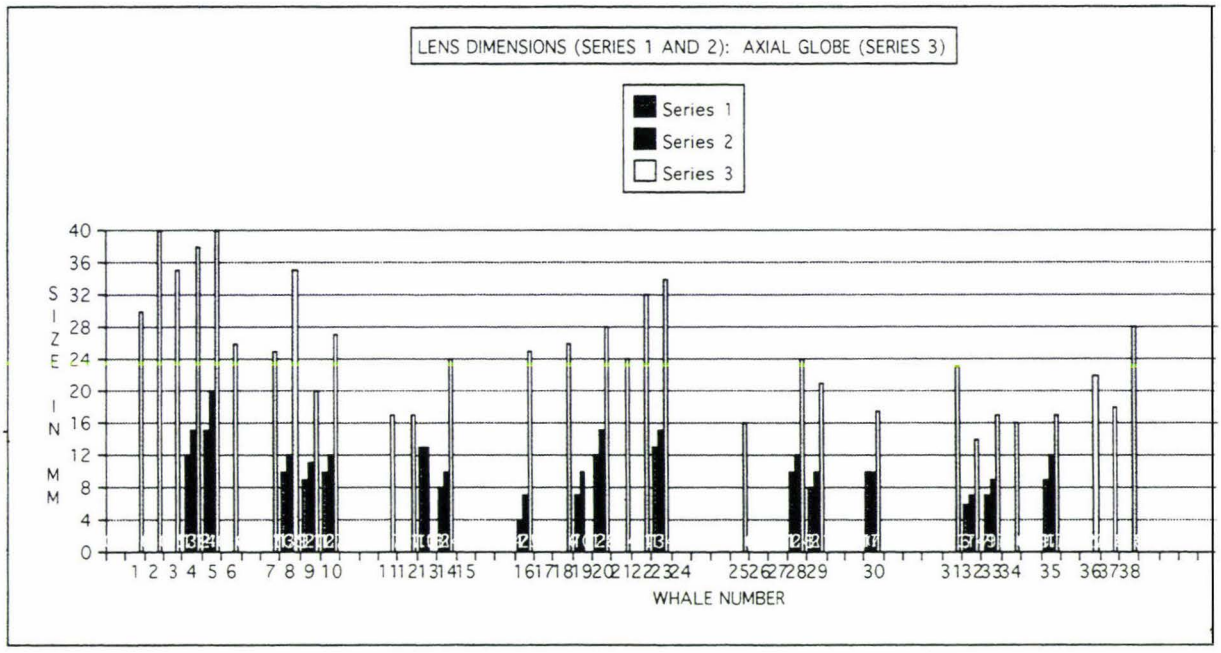



**Figure 4-6.** **Axial globe, axial and vertical lens dimensions in 15 whales.**

### 4.4vi Corneal Diameter Relative to Lens Diameter.

Corneal diameter was assessed as an indicator of potential pupillary dilation and was often nearly double the diameter of the lens (Figure 4-7) .

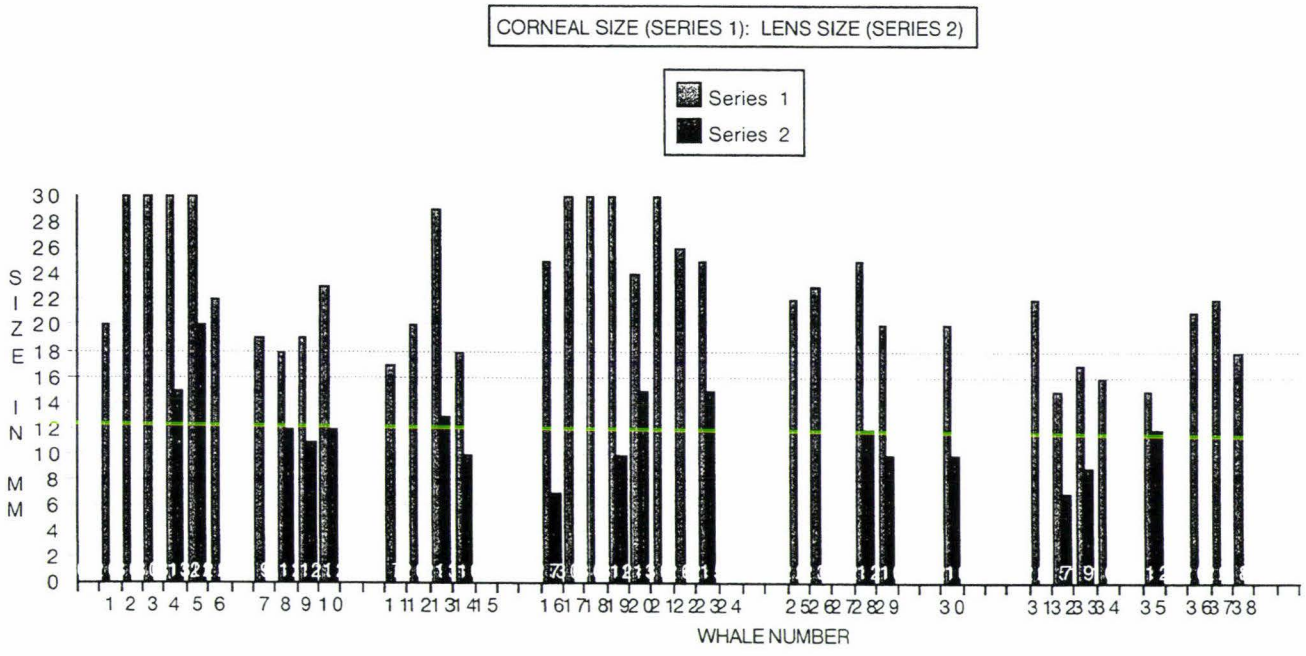



**Figure 4-7. Corneal size with respect to lens size in 17 whales.**

## 4.5 DISCUSSION

The data obtained in this study confirmed the general belief that baleen whales have larger eyes than toothed whales, and this was accompanied by a disproportionate increase in scleral thickness. Eye shape in all cetaceans was found to be horizontally elliptical. In toothed whales, the sclera increased proportionately with eye size, apart from the sperm whale, where the increase was disproportionate. The reasons for variable scleral thickness are unclear, but possibilities include maintenance of an elliptical shape during extensive mobility of the eye, including use of eyelids, or maintenance of shape during periods of raised intraocular pressure. It is unlikely that raised hydrostatic pressure with deep diving is a causative agent, since baleen whales tend to remain in shallow waters while sperm whales dive to considerable depths

Adult body sizes obtained from the literature were used to classify whales from largest to smallest in order to make comparisons of eye parameters to body size, as in Figure 4-2 (Table 4-2).

**TABLE 4-2. ADULT BODY LENGTHS OF FAMILIES OF CETAÇEANS** (Compiled from ^Harrison and Kooyman 1971, *Dawson 1985)

| SPECIES | BODY LENGTH | TYPICAL DIVE DEPTH |
|---|---|---|
| **Baleen** | | |
| Fin whale | *22 m | ^500 m (harpooned) |
| Minke | *8 m | |
| Brydes | *12-14 m | |
| **Sperm** | *12-20 m | ^1,134 m (caught in cables) |
| **Pygmy sperm** | *3-4 m | |
| **Beaked** | | |
| Cuvier's | *6.4 m | |
| Straptoothed | *3.6-5.5 m | |
| **Long finned pilot** | *4.8-6 m | ^366 m (feeding habits) |
| **Dolphins** | | |
| Bottlenose | *3 m | |
| Common | *2 m | *up to 42 m |

Corneal size also increased with globe size, and three separate categories occurred (Figure 4-3). In one category (some beaked, baleen and one pygmy sperm whale) the cornea appeared to have reached a maximum size plateau which was

independant of body size. In the other category, containing odontocetes, there was a proportionate increase in corneal size with eye (and body) size. In three of the four sperm whales, and in the pygmy right whale (adult) corneal size was proportionately smaller than in the majority of odontocetes.

Lens size also increased with eye size, with the largest lenses (>15mm vertical diameter) being found in baleen whales and some beaked whales. The sperm whale lens was disproportionately small compared to its eye size. Since larger lenses produce larger images than small lenses, baleen whales are likely to have larger, dimmer images than odontocetes. It has been suggested that the consequences of this would be to limit their visual capacity with increasing depth (Waller 1984). This is consistent with the behaviour of baleen whales which are known to inhabit mainly shallow water.

Because of their small lenses sperm whales will have small bright images, better suited to dim light conditions.

Other odontocetes are likely to have reached a compromise position, somewhere between these two extremes.

Lens diameter varied from 10mm to 20mm (excluding the Shepherd's beaked rogue value, which appeared to be low due to loss of the cortex following autolysis) and internal axial measurements varied from 14-40mm. A rough calculation of Matthiessen's ratio (focal length of lens/radius of lens) was made, using the average axial length (adjusted for depth of anterior chamber and thickness of lens) and average lens radius, giving a value of 3. Matthiessen's average value for fish is 2.55 (Matthiessen 1886). Since most fish have spherical lenses and the whale has a near spherical lens, this indicates that the fish lens is more refractile than the cetacean lens and this must be due to refractive index. Values for the cichlid are 1.56 centrally and 1.38 at the surface (Fernald and Wright 1983). This compares with the harbor porpoise values of 1.51 centrally and 1.38 at the surface (Kroger and Kirschfeld 1992).

Lens size may also indicate the presence of an aphakic area (peripheral area devoid of lens) in certain circumstances. The average corneal diameter in the present study of 23.8mm exceeded the average lens diameter of 11.8mm by 12mm. This is in contrast to humans where the lens is around 8mm and the cornea 11mm giving only 3mm difference (Spooner 1957). Assuming that the pupil dilates to a diameter slightly smaller than the corneal diameter, the lenses in this study were significantly smaller than the corneas, and by inference, the pupillary diameter. This situation has been documented in other vertebrates (Sivak 1980). If an aphakic area is present with the pupil fully dilated, the consequences may be that more of the retina is illuminated, although the light is not focused peripherally and crisp, bright images would not be formed. However, with high sensitivity and contrast perception and exceptional movement perception, as is suggested by retinal anatomy (Harrison 1977; Watkins and Wartok 1985; Murayama *et al.* 1995) it seems likely that this would be an ideal adaptation for vision and predation in deep

ocean conditions which are devoid of light apart from that emitted by bioluminescent organisms, some fish, and squid.

# THE UVEAL TRACT , IRIDOCORNEAL ANGLE and RETE

## 5.1 ABSTRACT

AIM: To compare the microscopic structure of the uveal tract, iridocorneal angle and rete in five different families of cetaceans with that of terrestrial mammals and to demonstrate possible routes of aqueous drainage in whales' eyes.

METHOD: Histological observations were recorded, photomicrographic montages compiled and diagrams prepared from sections of the uveal tract of 20 whales of five different families. Indian ink was injected into the anterior chamber of the eyes of two whales, and its percolation into drainage channels was examined.

RESULTS: Ciliary muscle was not present in any of the whales examined, but large numbers of venous blood vessels, aqueous drainage vessels and specialised nerve endings were present. In all whales examined, one large sinus was present in the ciliary body which was confluent with a plexus of vessels, extending from iridal veins to choroidal veins. In some cases, this large sinus was separated from the trabecular spaces by just one endothelial cell wall thickness. The Indian ink percolation study demonstrated percolation of ink into trabecular spaces but not into the large sinus.

CONCLUSION: The possible consequences of such an anatomical arrangement suggest that if the uveal tract becomes filled with blood to its maximum capacity, the cornea could increase its radius of curvature, the lens may be moved anteriorly or posteriorly, and tension on the zonule could be released leading to 'rounding up' of the lens in a similar way to muscular release of tension on the zonule.

## 5.2 INTRODUCTION

In terrestrial mammals the two primary components of the uveal tract are blood vessels and muscle. There are three separate functional areas within the tract; anteriorly, the iris, whose function is to modify the size and shape of the pupil and in some animals, the lens; posteriorly, the choroid, whose function is mainly nutritive; and between these, the ciliary body whose functions are i) to both produce and drain aqueous humour ii) to produce the vitreous body and iii) to accommodate, by altering lens curvature.

The tract is lined by a double layer of epithelium whose innermost layer is pigmented at the iris, becomes unpigmented over the ciliary body, then at the ora

serrata becomes non-neural retina and eventually, neural retina. The second layer is non-pigmented in the iris, pigmented in the ciliary body, and forms the retinal pigment epithilium in the region of the posterior segment.

The iridocorneal angle is comprised of the ciliary body and limbus. Its functional importance is in providing an outflow mechanism for aqueous.

The rete is important in providing arterial stem vessels for the uveal tract (Pilleri and Wandeler 1964) and possibly the retina (Dawson *et al.* 1987).

Despite the presence of unusual features in the cetacean eye, little is known of its precise mechanism of accommodation. There is evidence of ciliary muscle in certain species of whales viewed in certain planes (Waller 1992; Putter 1903; Slijper 1962; West *et al.* 1991) but in the majority of whales it is believed to be absent, or if fibres are present they are scanty and not functional (Dawson 1980; Rochon-Duvigneaud 1940; Dral 1975; Kastelein 1990; West *et al.* 1991). Some mammals have evolved systems which achieve a clear focus without lens flexure or movement, such as small lenses which have a wide depth of field, an increase in length of the receptor cells, a ramp retina, or a stenopaeic pupil all of which have been described by Walls (1942). Fishes rely on lens movement by muscular force, and recent work has shown that the otter uses a unique mechanism to achieve lens movement whereby the fluid volume (and therefore pressure) of the anterior segment is varied with respect to the posterior segment, resulting in movement of the lens. A special adaptation of the ciliary muscle can empty or fill a large sinus, situated in the posterior part of pars plana (Murphy *et al.* 1992).

The drainage apparatus of cetaceans is not well documented. Dawson's evidence of wide fluctuations of intraocular pressure (IOP) in dolphins (1992) suggests that these mammals do not maintain a constant IOP as other mammals do simply by regulation of aqueous outflow. In humans, aqueous is believed to drain by two direct routes i) via the canal of Schlemm and episcleral veins, ii) via the aqueous veins (Ascher 1942, 1953) and also by one indirect route via perineural and perivascular lymphatics. In some species of monkey, the suprachoroid space is also a significant drainage route, and this is true to a lesser extent in cats (Bill *et al.* 1965, 1966). This route has not been assessed in humans (Hogan *et al.* 1971). It has been demonstrated that there is no communication between the uveoscleral and canal of Schlemm drainage routes (Jocson and Grant 1965).

The literature suggests that cetacean pupillary function is similar to other mammals, but it is exceptional in dolphins (and possibly other cetaceans) in that a double slit is formed (Dawson *et al.* 1972). The iris is noted to have vessels which are detatched from the stroma (West *et al.* 1991) and this could affect the composition of aqueous, as occurs in humans (Hogan *et al.* 1971).

This investigation provided an opportunity to compare the anatomy of the uveal tract region in cetaceans of five different families with that of other orders. Bovine and human eyes were chosen as suitable terrestrial mammals for comparison because there is an evolutionary link with the former, and a wealth of

literature available for the latter. The features to be compared were;

i] size and shape of trabecular zones

ii] placement of encapsulated receptors

iii] size, type and position of blood vessels

iv] musculature

This study has also examined the aqueous drainage of the cetacean eye using Indian ink percolation in order to assess the potential significance of the canal of Schlemm compared with the suprachoroid route of drainage.

## 5.3 MATERIALS AND METHODS

### 5.3i Histological Survey

Twenty whales were surveyed (see Table 5-1). All were between four and 72 hours *post mortem* following stranding.

**TABLE 5-1. WHALES AND DOLPHINS SURVEYED HISTOLOGICALLY.**

| GROUP | REF.NO. | SPECIES | AGE | SOURCE |
|---|---|---|---|---|
| GROUP 1 BALEEN | E428/95 | PYGMYRIGHT | JUVENILE | STRANDED |
| | 28609-97 | MINKE | | |
| | 28831-98 | MINKE | | |
| GROUP2 SPERM | E90/97 | SPERM | ADULT | STRANDED |
| | E430/95 | SPERM | ADULT | STRANDED |
| GROUP 3 PYGMY SPERM | E413/95 | PYGMY SPERM | JUVENILE | STRANDED |
| | 27961/97 | PYGMY SPERM | ADULT | STRANDED |
| GROUP4 BEAKED | E15/97 | CUVIERS BEAKED | ADULT | STRANDED |
| | 28543/98 | CUVIERS BEAKED | | |
| | E433/95 | GRAYS BEAKED | ADULT | STRANDED |
| GROUP 5 LONG FINNED PILOT | E425/95 | LONG FINNED PILOT | ADULT | STRANDED |
| | E432/95 | LONG FINNED PILOT | ADULT | STRANDED |
| | E189/98 | LONG FINNED PILOT | ADULT | STRANDED |
| | E195/98 | LONG FINNED PILOT | ADULT | STRANDED |
| | E198/98 | LONG FINNED PILOT | ADULT | STRANDED |
| | E199/98 | LONG FINNED PILOT | ADULT | STRANDED |
| | E200/98 | LONG FINNED PILOT | ADULT | STRANDED |
| GROUP 6 DOLPHINS | 27796/98 | DOLPHIN | | |
| | 27865/98 | DOLPHIN | | BYCATCH |
| | 28112/97 | DOLPHIN | | |

Eyes were fixed in 10% buffered formalin as soon after death as practical. When possible, a small incision was made at the limbus to assist the penetration of fixative. This was found to be a more reliable method than injection of fixative through the sclera.

The globe was bisected longitudinally and 4-5mm blocks cut from the midline. In

some cases, 2-3mm latitudinal sections were taken along the limbus/ciliary body. Choroid sections were taken from a midline site adjacent to the optic nerve and included retina and 1mm of sclera. Samples of the rete were taken 1-2 cm from the optic papilla. The sections were processed routinely for histology, embedded in paraffin and cut at 2-3 microns. All blocks were stained initially with haematoxylin and eosin (H&E). In some cases, additional staining techniques were used (see Table 5-2.)

**TABLE 5-2. PLANES AND STAINS USED IN UVEAL TRACT SECTIONS.**

| GROUP | REF.NO. | SPECIES | PLANES | STAINS |
|---|---|---|---|---|
| GROUP1 BALEEN | E428/95 | PYGMY RIGHT | LONG | H&E, VG, MT |
| | 28609/97 | MINKE | LONG | H&E, H&E &AB |
| | 28831/98 | MINKE | LONG AND LAT | H&E |
| GROUP2 SPERM | E90/97 | SPERM | LONG AND LAT | H&E, H&E&AB, |
| | E430/95 | SPERM | LONG | H&E, MT |
| GROUP 3 PYGMY SPERM | E413/95 | PYGMY SPERM | LONG AND LAT | H&E, VG, MT, |
| | 27961/97 | PYGMY SPERM | LONG AND LAT | H&E, H&E&AB, |
| GROUP4 BEAKED | E15/97 | CUVIERS BEAKED | LONG AND LAT | H&E, H&E &AB, MT, VG |
| | 28543/98 | CUVIERS BEAKED | LONG | H"E, H&E&AB |
| | E433/95 | GRAYS BEAKED | LONG | H&E, VG, MT |
| GROUP 5 LONG FINNED PILOT | E425/95 | LONG FINNED PILOT | LONG | H&E, VG, MT |
| | E432/95 | LONG FINNED PILOT | LONG | H&E, VG,PAS |
| | E189/98 | LONG FINNED PILOT | LONG AND LAT | H&E |
| | E195/98 | LONG FINNED PILOT | LONG | H&E |
| | E198/98 | LONG FINNED PILOT | LONG | H&E |
| | E199/98 | LONG FINNED PILOT | LONG | H&E |
| | E200/98 | LONG FINNED PILOT | LONG | H&E |
| GROUP 6 DOLPHINS | 27796/98 | DOLPHIN | LONG | H&E |
| | 27865/98 | DOLPHIN | LONG | H&E |
| | 28112/97 | DOLPHIN | LONG | H&E |

Key:-H&E, haematoxylin and eosin; VG, Van Gieson; AB, alcian blue: MT, Masson's trichrome.

Different stains enhanced the appearance of particular tissue types, as shown in Table 5-3. This facilitated differentiation of tissues which, when stained with H&E, may have looked similar, such as smooth muscle and peripheral nerve.

**TABLE 5-3. STAIN PREDILECTION SITES (Compiled from Burkitt *et al.* 1993, Culling 1985).**

| STAIN | COLOURS | TARGET SITES | STRUCTURES STAINED |
|---|---|---|---|
| H&E | Blue(basic dye) | acid proteins | nucleus, ribosomes, rough ER |
| | Pink(acid dye) | basic proteins | cytoplasm |
| MASSONS TRICHROME | Blue/black | | nuclei |
| | Blue | | collagen/mucin |
| | Red | cytoplasm | muscle,erythrocytes |
| VAN GIESON | Black | | nuclei |
| | Red | collagen | connective tissue |
| | Yellow | cytoplasm | muscle, erythrocytes |
| PAS | Magenta | complex | mucin, basement membranes,collagen, glycogen |
| HOLMES' SILVER | Black | neurofilaments neurotubules | nerve |

### 5.3ii Comparative Study of Iris

An H&E stained iris section was obtained from a sperm whale (E430-95). Photomicrographs were taken at magnification x 80 in preparation for assembly of a photomontage (Figure 5-7b).

### 5.3iii Comparative Study of Ciliary Bodies

Longitudinal blocks of ciliary body were selected from the eyes of whales as listed in Table 5-1. Sections from these blocks were prepared as described in Chapter 4. Similar blocks of the ciliary body from a 2-year-old steer were selected and prepared in a similar manner.

Photomicrographic prints were assembled to produce photomontages which were photocopied to produce images at a magnification x80, or in the case of the larger ciliary bodies of the sperm whale and steer, magnification x20 (See Appendix 5-1). Diagrams were made from the full sized photomontages, and reduced in size on the photocopier to produce images magnification x32, or in the case of the sperm whale and steer, magnification x 12 (Figures 5-14).
Colour coding of structures and zones (KEY, Figures 5-17) allowed ease of comparison.

Sizes of ciliary bodies were obtained by measurement directly from the slide, using an eyepiece graticule. The end of Descemet's membrane and the end of the pars plicata were used as reference points. The pars plana was not included due to the variability of its length within the ora serrata (Table 5- 3).
In the Indian ink studies, one fresh frozen and one fixed eye from two long-finned pilot whales were used. Indian ink and 10% buffered formalin were injected into the fresh eye (2.5ml) and ink was injected into the fixed eye (2.5ml). Both eyes were stored in 10% buffered formalin for 4-5 days, and then sectioned for histological examination as described above.

## 5.4 RESULTS

The bisected eye varied between species with respect to the position of the optic nerve (central or slightly offset dorsally, possibly ventrally in some species), the thickness of the sclera, and the size of the lens (see Chapters 4 and 7). It was found that lenses do 'round up' when they are excised from unfixed eyes (autolysis of the cortex may accentuate this tendency), and a range of shapes from spherical to flattened sphere has been observed in fixed specimens (see Chapter 8).
The position of the ciliary body and limbus within the eye is illustrated in Figures 5-1a and b. The overall microscopic appearance of the iridocorneal area is illustrated in Figures 5-2a and b.

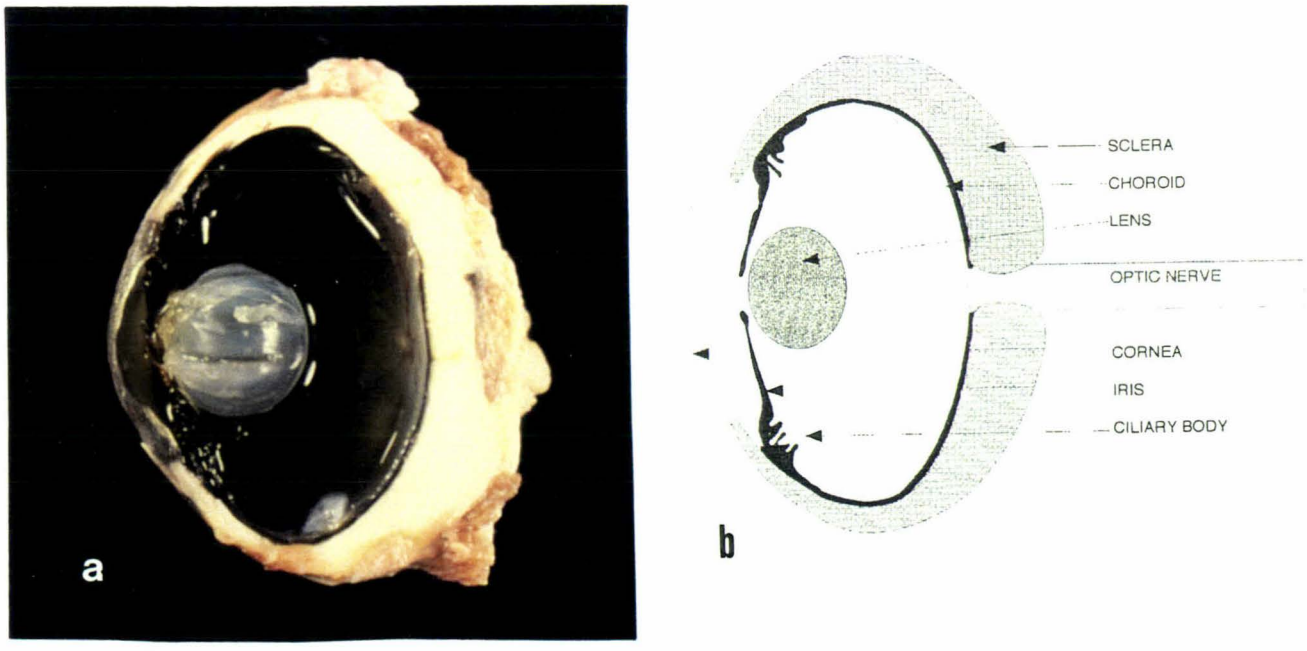
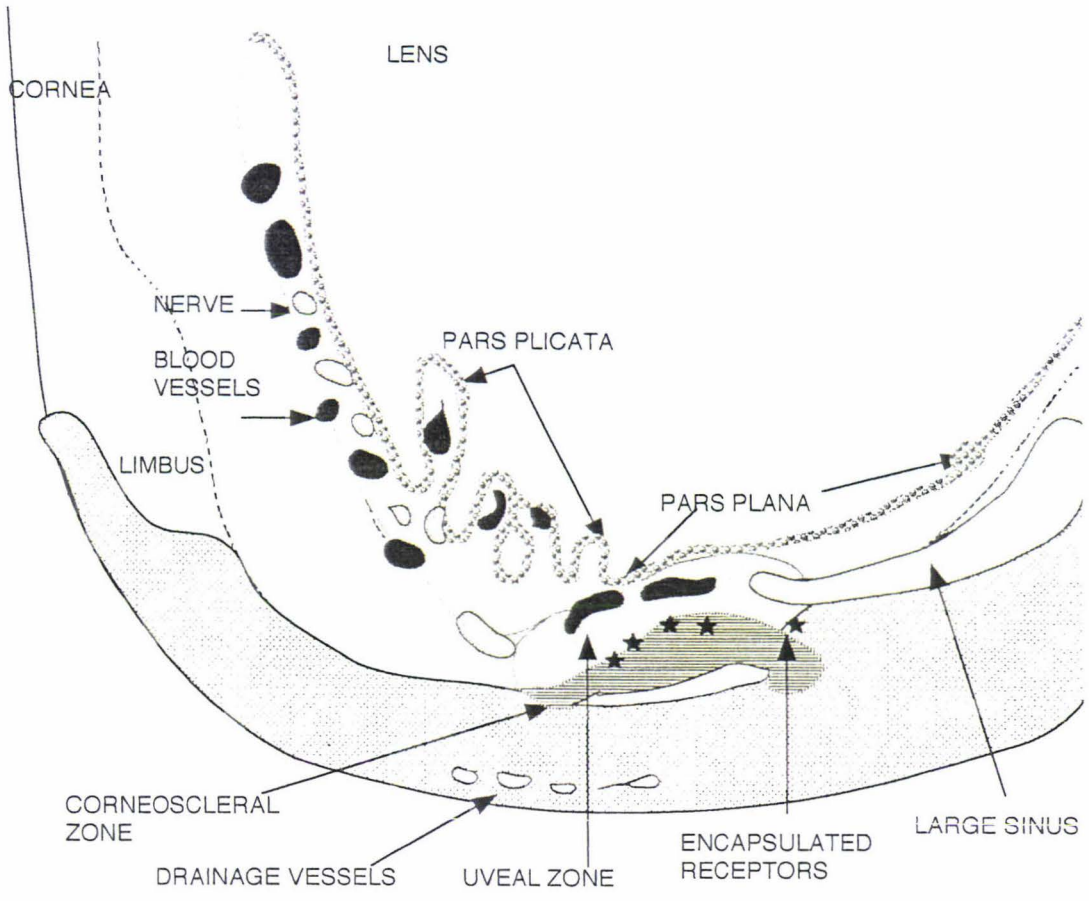
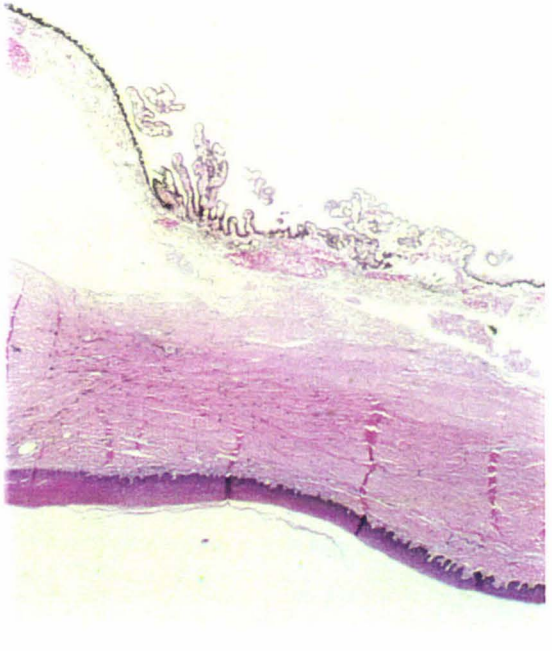



Figure 5.1a and b. **Photograph and diagram of the eye of a pygmy sperm whale x 1.25.**

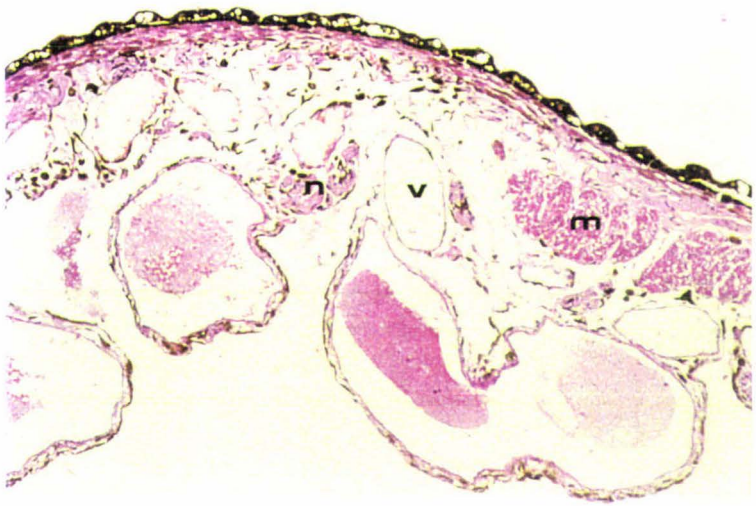


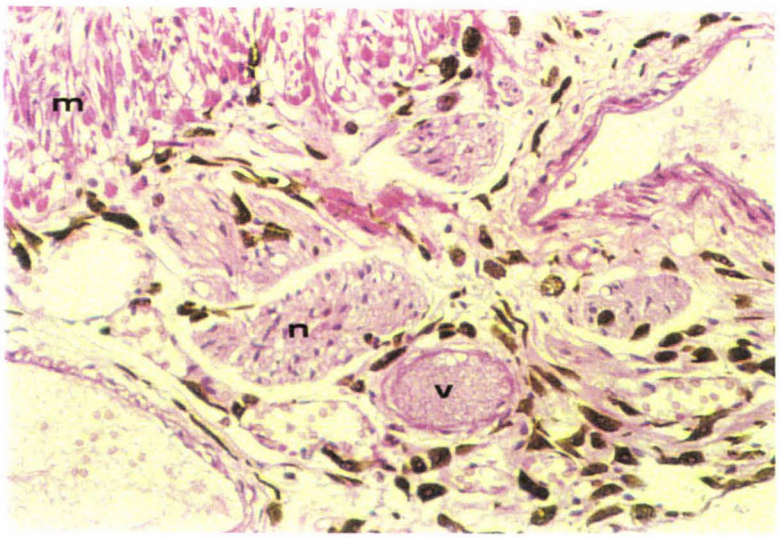
**Figures 5-2a and b.** a) Diagram and b) photomicrograph of the ciliary body area of a pygmy sperm whale H&E x14

### 5.4i Comparative Study of Iris

A double layer of epithelium lined the posterior border of the iris, of which the external layer was heavily pigmented. A sparse stroma, consisting of fibroblasts and collagen fibres, supported an abundance of nerve tissue, blood vessels and muscle. Melanin granules also occurred within the stroma in melanocytes and other cells such as histiocytes (Figure 5-3a and b).




a




b

Figure 5-3a and b. Iris of Cuvier's beaked whale E15-97 a) H&Ex56 b) H&Ex140. n, nerve; m, muscle; g, melanin granules; v, vessels.

There were very large numbers of blood vessels distributed evenly along the anterior border. These usually possessed thin walls and large lumina, and very little smooth muscle within the walls. These were interpreted as venous sinusoids (Figure 5-4) In some cases, these were detatched from the stroma. A large nerve at the root of the iris was a commonly observed feature (Figure 5-5).

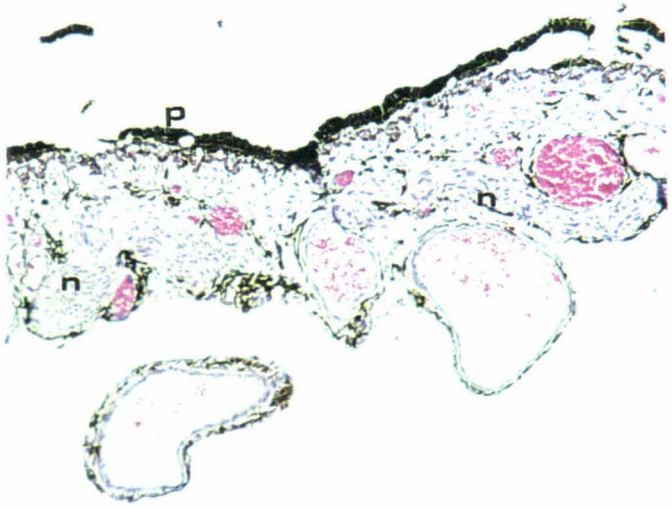



Figure 5-4. Iris of long finned pilot whale. H&E x54 n, nerve; p, posterior surface of iris. H&E x80.

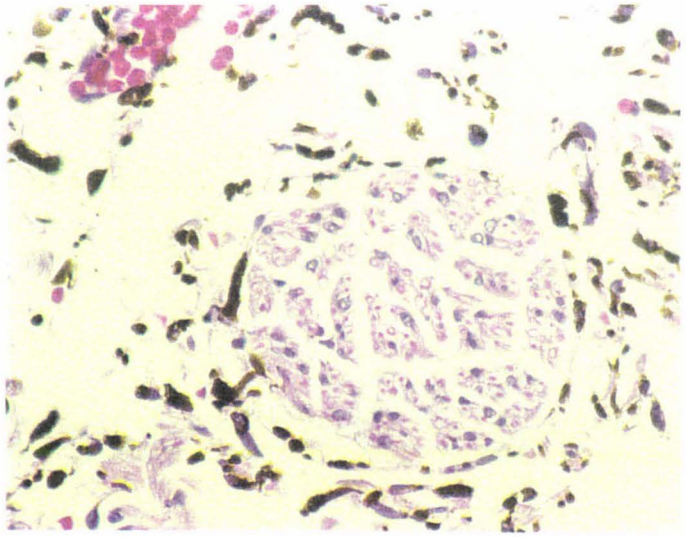


Figure 5-5. Cross section of a nerve at the base of the iris. H&E x400.

In all whales there were two types of muscle present in the iris. Close to the pupillary margin, circular sphincter muscle fibres were seen in cross section. The sphincter muscle took the form of a dense block of tissue, extending half the length of the iris. The dilator muscle fibres formed a more diffuse structure adjacent to the posterior epithelium and were at times indistinguishable from it due to the heavy pigmentation of this layer. The fibres were sparse close to the pupil but more abundant peripherally (Figure 5-6).

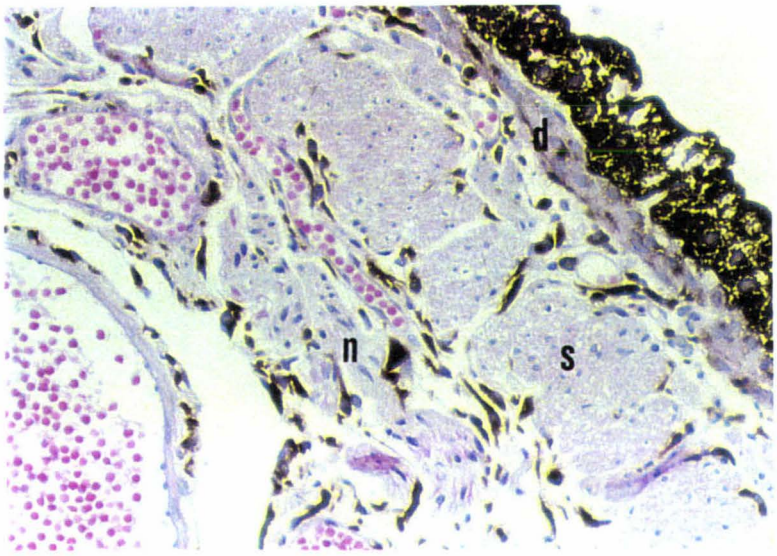



**Figure 5-6. Iris of a long-finned pilot whale. The nerve tissue has similar tinctorial properties to smooth muscle. s, sphincter; d, dilator, n, nerve. H&E x133**

There was an abundance of nerve tissue in all cetacean irises. Van Gieson and Masson's trichrome stains were used to demonstrate that these structures were nerve, since with H&E staining Schwann cells were similar to fine bundles of smooth muscle (Figure 5-7).

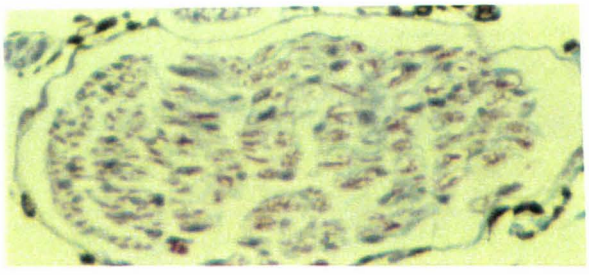
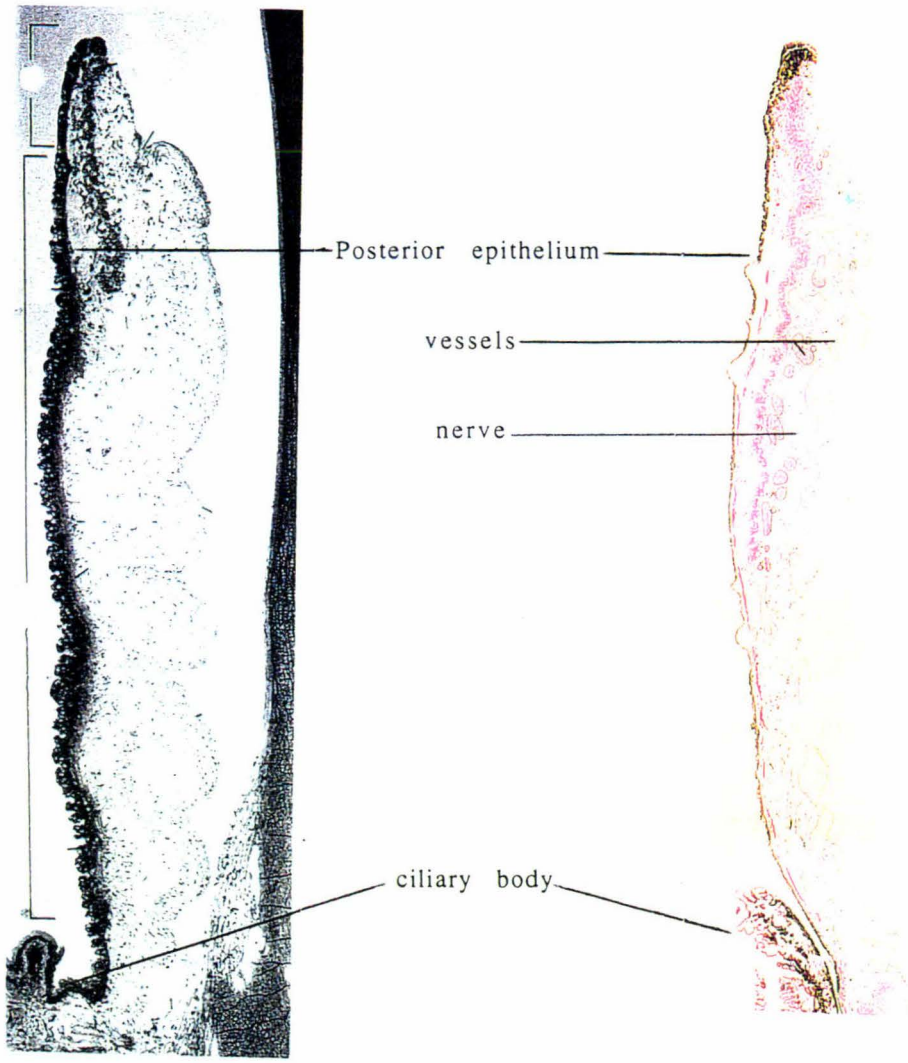


**Figure 5-7. Cross section of nerve in the iris, stained with Masson's trichrome. x266**

By comparison, the bovine iris was less vascular, and similar in appearance to that of the human as seen in the photomontage of a human iris which was obtained from Hogan *et al.* (1971) for comparison with cetacean material. There was a much more substantial stroma, and far fewer nerves and vessels than occur in cetaceans, but the quantity of muscle was comparable (Figures 5-8a and b).




**Figures 5-8a and b.** **a) Photomontage of a human iris from Hogan *et al.* (1971) at magnification x40 compared with b) H&E&AB stained section of the iris of a sperm whale x12.5.**

## 5.4ii Comparative Study of a Cetacean Ciliary Body

The ciliary body showed the general mammalian pattern of two parts - a pars plicata (pleated part) anteriorly, and a pars plana (flat part) posteriorly. There was a double layer of epithelium covering both parts, the inner pigmented layer being a continuation of pigment epithelium from the retina. There was a junctional zone where ciliary body pigment epithelium transferred from being the inner layer of a double epithelium to the outer layer lining the posterior surface of the iris.

The ciliary processes were long in some animals and shorter in others. In all species they were richly vascular, with vessels being mainly venous sinusoids (Figures 5-9 and 10).

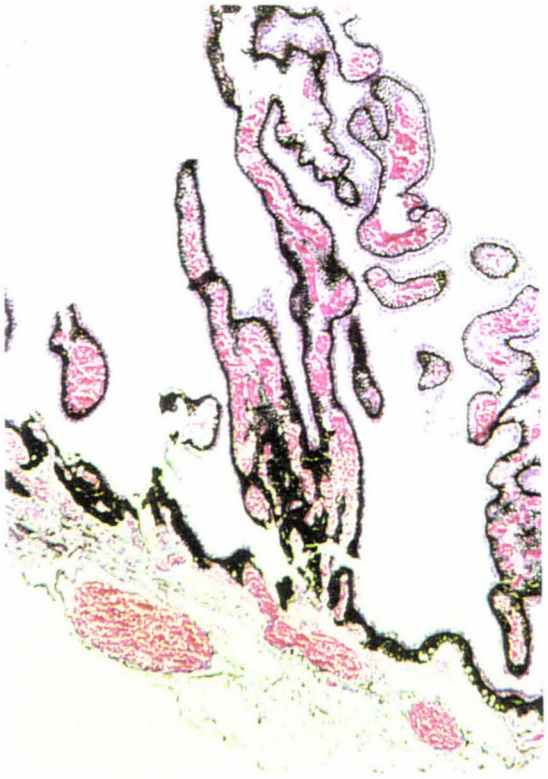


**Figure 5-9. Ciliary processes of a long -finned pilot whale stained H&E x54.**

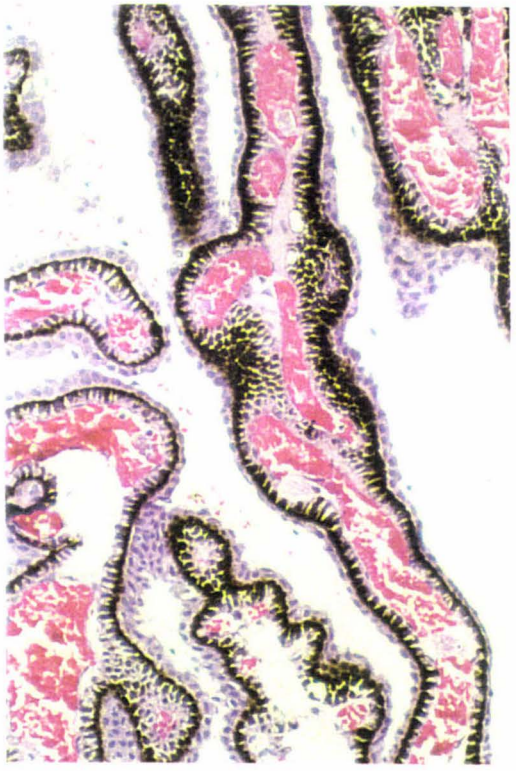


**Figure 5-10. High power view of processes H&E x133**

Plexus of Veins

The ciliary body was relatively narrow from inner to outer edge but long from anterior to posterior edge. Many thin walled, large lumened blood vessels, identified as venous sinusoids were observed in the ciliary body adjacent to the processes. In many instances, these were observed as being confluent with process vessels, forming a complex, interconnected plexus of veins within the ciliary body (Figure 5-11). In minke whale 28609/97, it was evident that one large vessel drained the iris and ran posteriorly toward the choroid (Figure 5-12). Arterioles were only observed in small numbers, and were of small size, running anteriorly near the root of the iris.

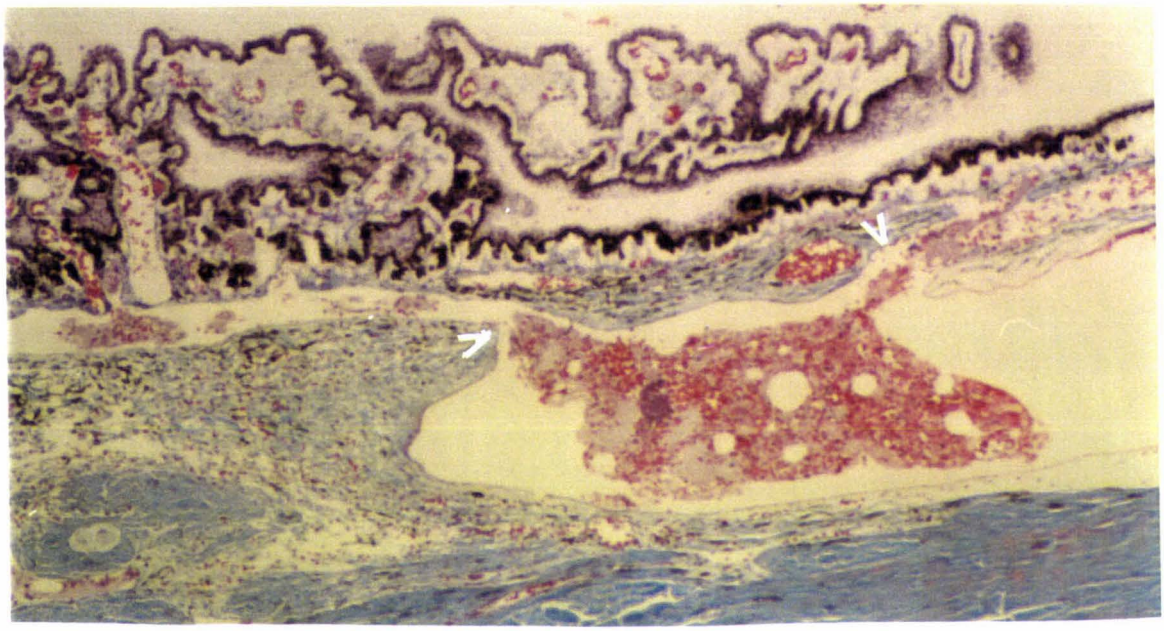


Figure 5-11. Ciliary body of pygmy sperm whale E413-95 showing a large venous sinusoid confluent with vessels coursing anteriorly towards the iris and posteriorly towards the choroid (arrows). Masson's trichrome x80.

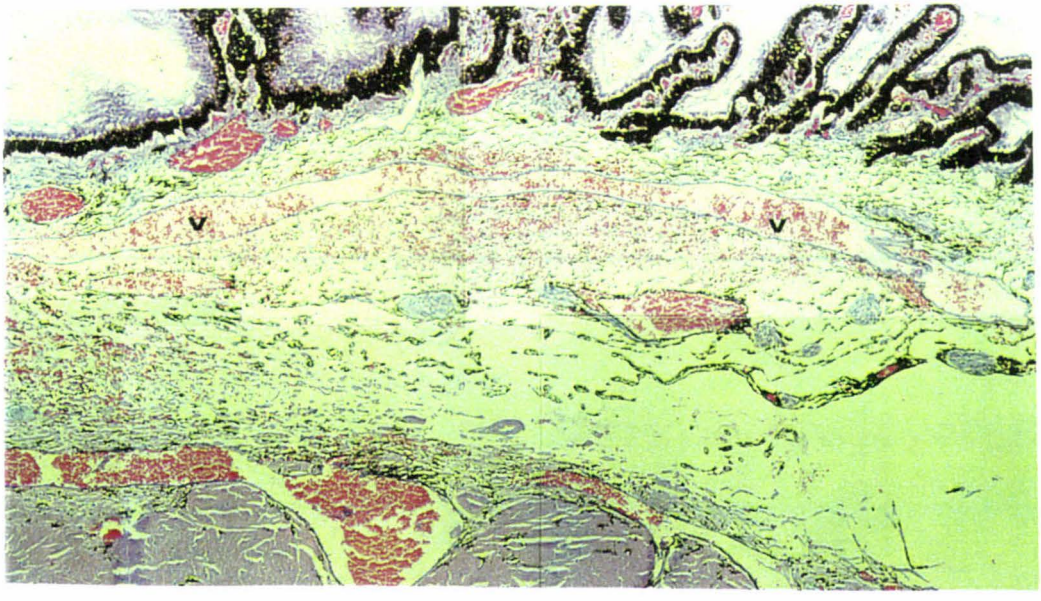



Figure 5-12. Iris of minke whale 28609-97. A vein, v, sectioned longitudinally extends from the iris to the choroid. H&E x80.

Venous Sinusoids in Layers

A constant feature in most whales was the presence of a particularly large sinus in the pars plana which extended posteriorly to the suprachoroid. The sinus was often fluid filled but was occasionally blood and fluid filled (Figures 5-13a and b). In many whales, vessels appeared to be arranged in two layers; an inner layer of large lumened, thin walled vessels which were usually blood filled; and an outer layer of larger thin walled vessels which frequently appeared empty (Figures 5-14a, b, c) or contained a few blood cells (Figures 14 d and e) .

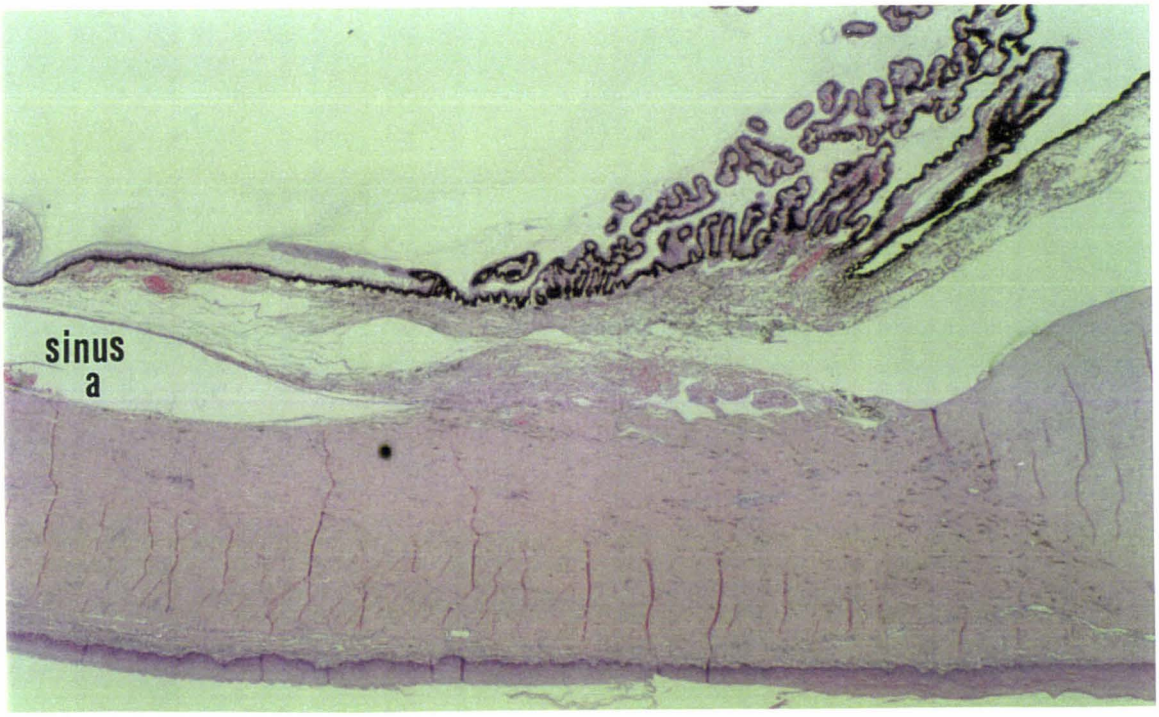



Figure 5-13a. Photomicrograph from pygmy sperm whale 27961-97 showing large fluid filled sinus and associated venous plexus. a, lumen of large sinus. H&E x20.

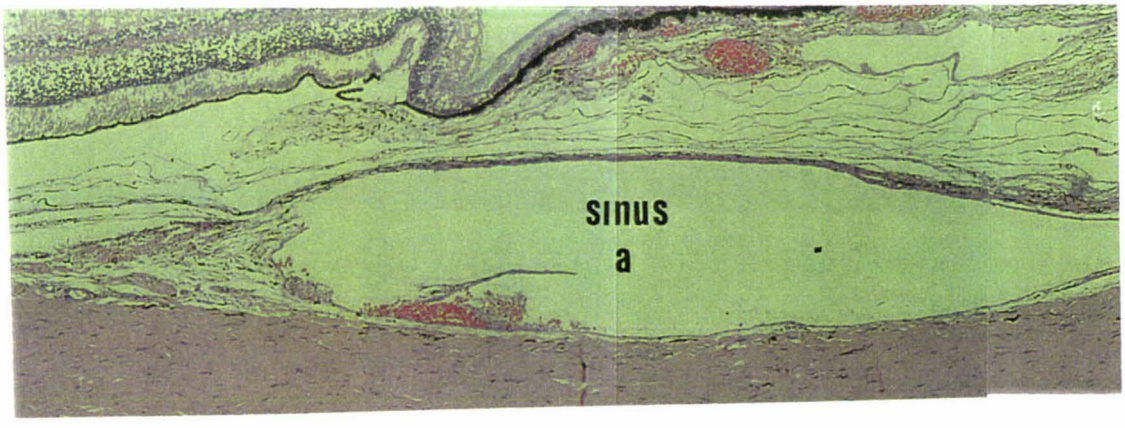
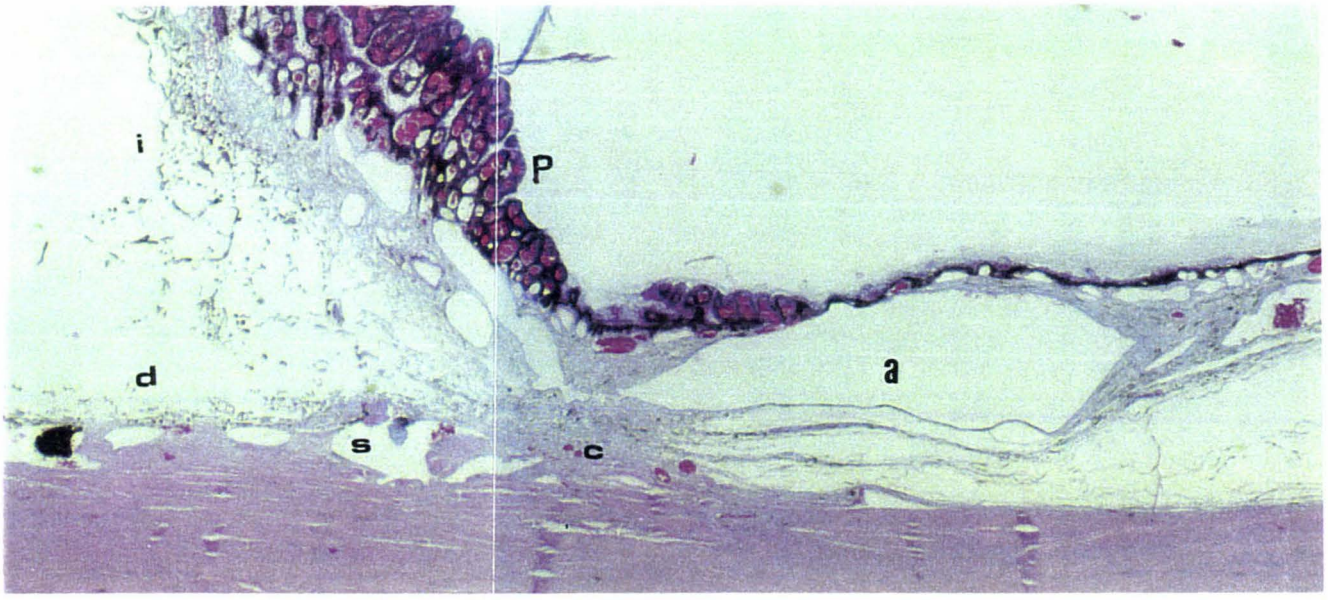
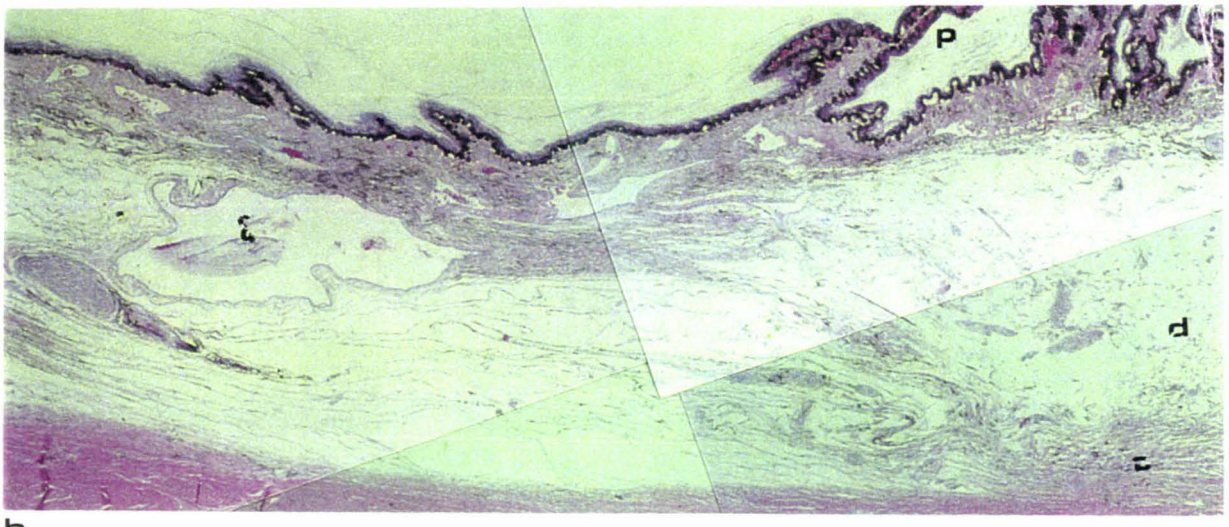


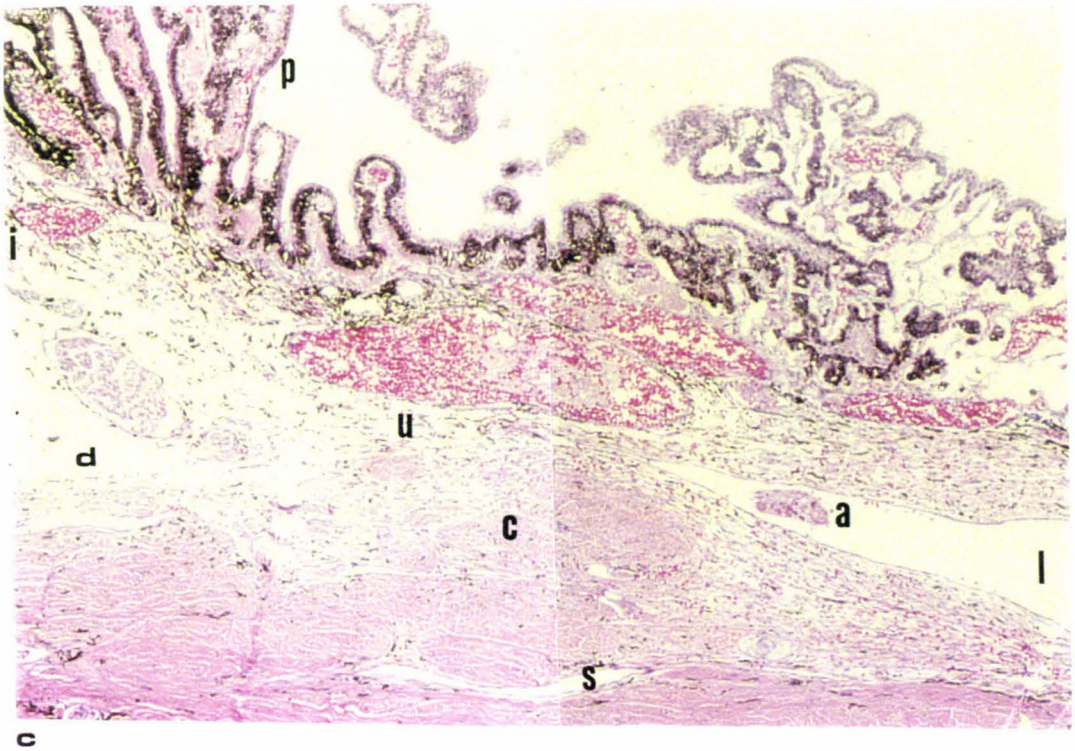

Figure 5-13b. Photomontage of large sinus at higher power, a, sinus lumen. H&E x66




a

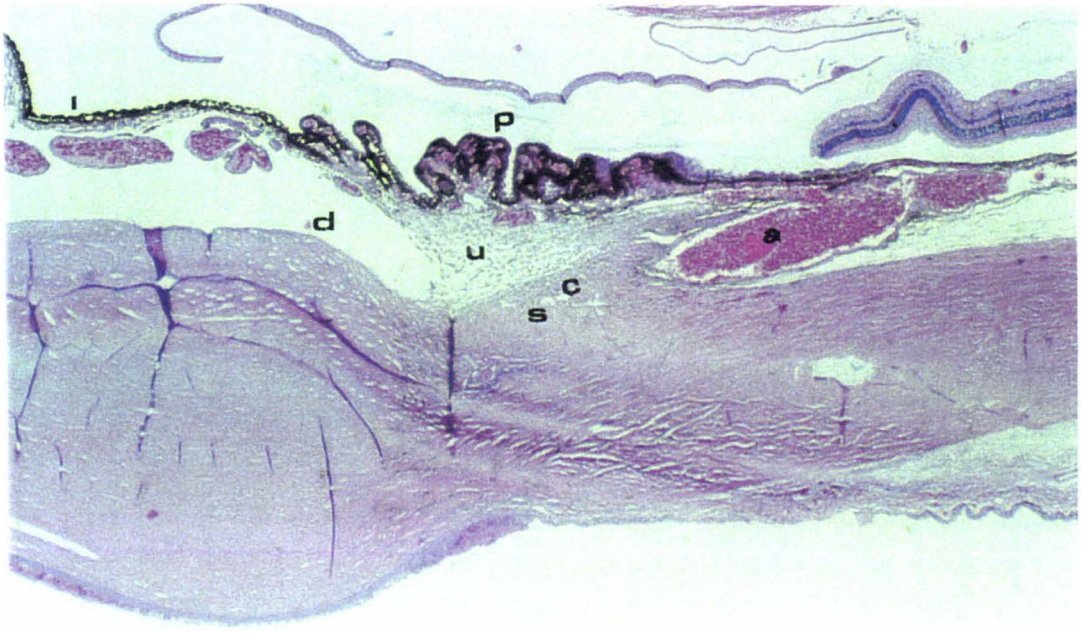



b




c

Figures 5-14a, b and c (facing page). Empty sinuses in outer layer. a) Cuvier's beaked whale 28543-97 H&E x20. b) Minke whale 28831 H&E x56 c) pygmy sperm whale E413-95 H&E x56. a, lumen of large sinusoid; c, corneoscleral meshwork; d, drainage angle; i, iris; p, ciliary processes; s, Schlemm's canal equivalent; u, uveal meshwork



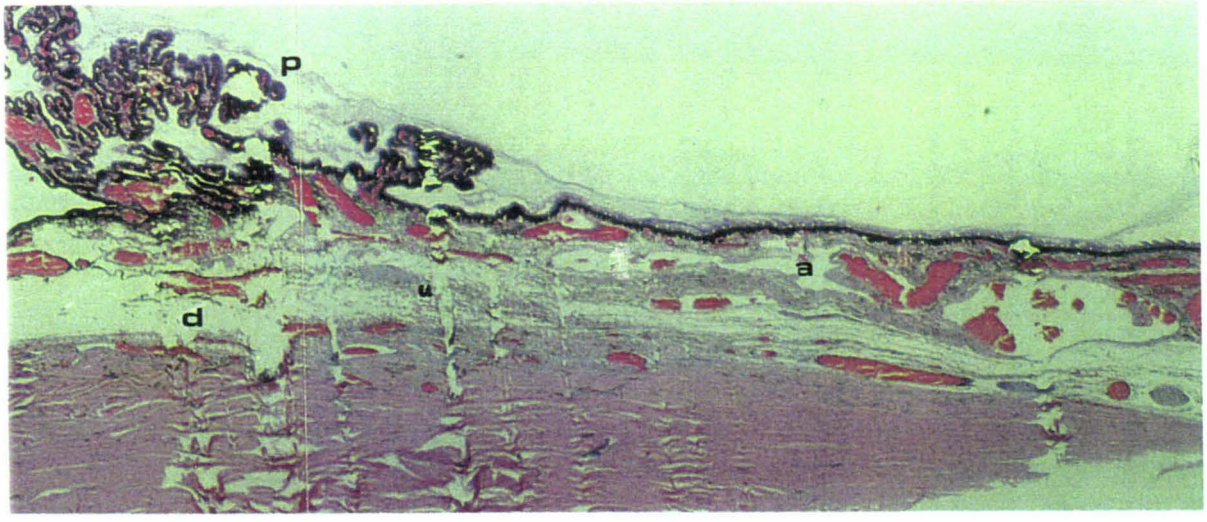




Figure 5-14 d and e. Blood filled ciliary sinusoids in a] Dolphin 27796-97 H&E x28 and b] Minke whale 28609-97 H&E x16. a, lumen of large sinusoid; c, corneoscleral meshwork; d, drainage angle; i, iris; p, ciliary processes; s, Schlemm's canal equivalent; u, uveal meshwork

Zones of Meshwork

In all of the whales in this study, the stroma of the ciliary body appeared to have analogous areas to those described in man as the trabecular meshwork of the drainage angle. Trabecular zone tissues are characterised by a collagen core which is surrounded by endothelial cells (Hogan *et al.* 1971). A loosely packed uveal zone (Figure 5-15) was found to surround a more compact corneoscleral zone. These zones were loosely identified by their light microscopic appearance based on criteria described by Hogan *et al.* (1971). In the corneoscleral zone, the collagen was densely packed, with fibres oriented latitudinally. In the uveal zone, fibres were more randomly oriented anteriorly, but had a longitudinal orientation in the pars plana, with wide intertrabecular spaces giving an open appearance (Figures 5-16 a and b)

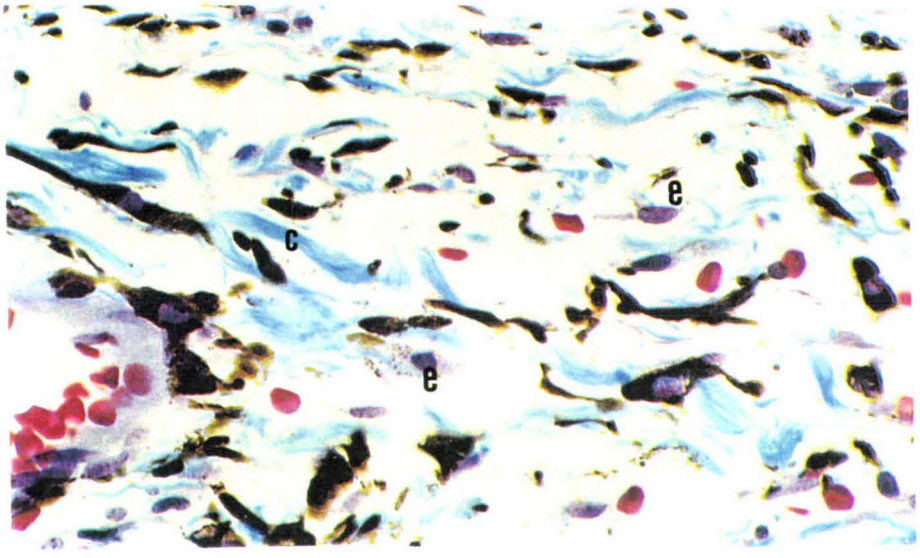



Figure 5-15. Uveal trabecular meshwork of pygmy sperm whale E413-95 showing random collagen cores surrounded by endothelial cells. e, endothelial cell, c, collagen Masson's trichrome x660

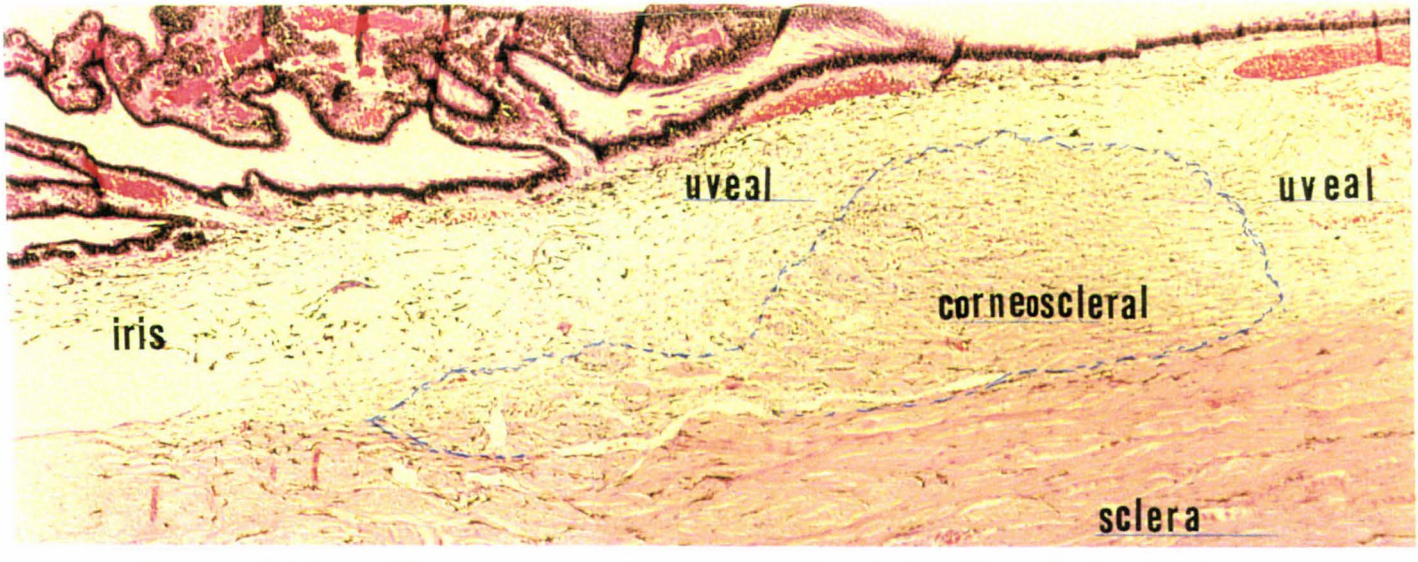



Figure 5-16a. Photomontage of pygmy right whale ciliary body showing zones of meshwork and sites of ERs (arrows). H&E x26.

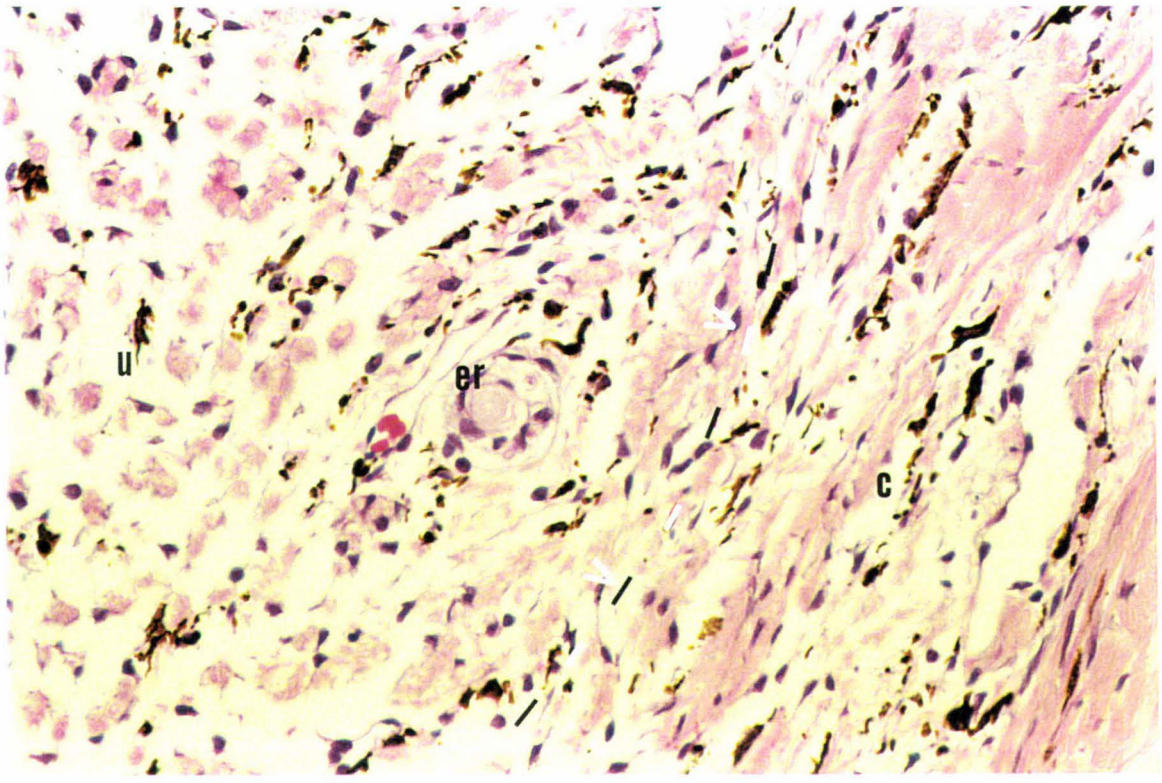



Figure 5-16b. **High power view of a single encapsulated receptor situated at the junction of uveal and corneoscleral zones (white arrows). u, uveal zone; er, encapsulated receptor; c, corneoscleral zone. H&E x 400.**

Aqueous drainage vessels were present within the limbus. These occurred at two sites; the scleral/trabecular junction and adjacent to the scleral epithelium. Ocassionally there were a few blood cells within the lumen of these vessels. In 18 of the 20 whales and dolphins surveyed, encapsulated receptors were present in transverse and/or longitudinal section in the trabecular meshwork and/or sclera. These have been described in detail elsewhere (Chapter 6).

### 5.4iii Comparative Study of Cetacean, Bovine and Human Ciliary Bodies and Limbi.

The ciliary body photomontages from cetaceans and a steer are shown in Appendix 5-1.

Diagrams traced from these photomontages were used to analyse and compare the zones in the drainage angle, the vasculature and innervation of this region Figures 5-17 a to e. The analysis of these diagrams revealed the following features:-

## 1 Zones of Trabecular Meshwork

Three histological zones were broadly evident within the limbus/drainage angle area. These were the sclera, the corneoscleral trabecular meshwork and the uveal trabecular meshwork. In humans these areas are relatively small and well defined and one large aqueous collecting vessel, the canal of Schlemm, is evident. In the steer, the zones were more diffuse and ill defined owing to the more extensive nature of the vessels which are canal of Schlemm equivalents. In whales, the areas were also comparatively less well defined.

## 2 Vasculature

A venous plexus appeared to be present in the cetacean ciliary body, but few arteries were evident. This contrasted with human and bovine eyes which possessed a good arterial supply to the iris and ciliary processes, but far fewer venous sinusoids.

The corneoscleral zone (CSZ) contained very few vessels in whales eyes. Those present were concentrated mainly at its peripheral areas where the CSZ blended with uvea internally and sclera externally.

## 3 Innervation and Encapsulated Receptors

Encapsulated receptors were not seen in the bovine ciliary body but they were seen in all whales except the dolphins, in which only one specimen showed them. The sites in which they occurred were;

i) Within the body of the trabecular meshwork (Long finned pilot, pygmy right, pygmy sperm and sperm whales) particularly at the junctional areas.

ii) In the dense scleral collagen of the limbus (pygmy sperm whale, sperm whale). In this site the orientation was mainly meridional (see later - Chapter six, Topographical Study).

## 4 Muscle

Muscle tissue was only found in the bovine ciliary body. There was no evidence of muscle in the whale eye in longitudinal or latitudinal sections.

## 5 Other features

There was no pectinate ligament in whales as described in the cow.

Figures 5-17 to a to f. Diagrams taken from photomontages of cetacean ciliary bodies, with large numbers of blood/aqueous drainage vessels and nerve tissue evident.
Figures 5-17e and 5-17f illustrate bovine and human ciliary bodies, in which the presence of muscle was a dominant feature.

Key:

Muscle

Nerve tissue

Vasculature/
drainage vessels

a, muscle b, sclera, c, ciliary processes, d, iris e, canal of Schlemm or its equivalent
g, trabecular meshwork.


Figure 5-17a. Long-finned pilot whale. All receptors are located within the corneoscleral zone or at its junction. x26


Figure 5-17b. Pygmy right whale. All receptors are located within the corneoscleral zone. x26

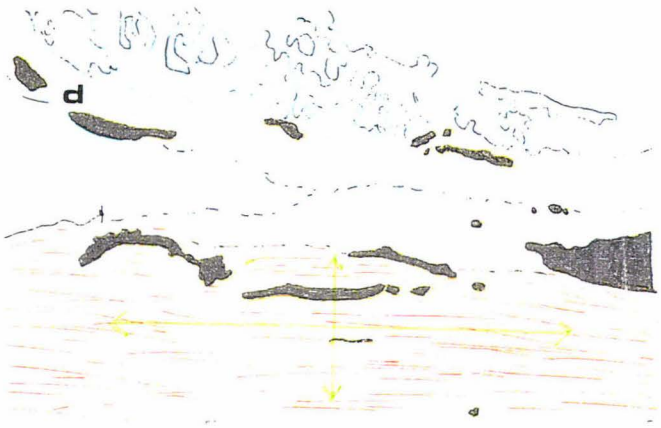



Figure 5-17c. Pygmy sperm whale. No receptors are visible in this plane, but many where visible on latitudinal section located within the sclera longitudinally at a position along the green axis x26.

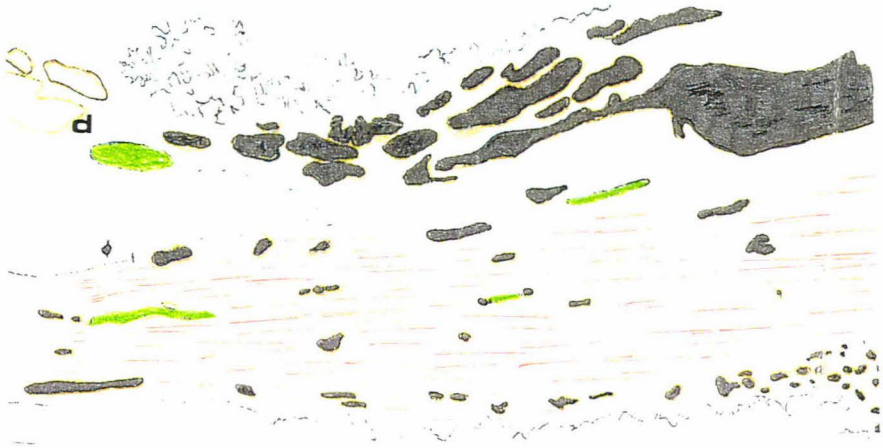



Figure 5-17d. Sperm whale. Large receptors are present in all areas shown x12.

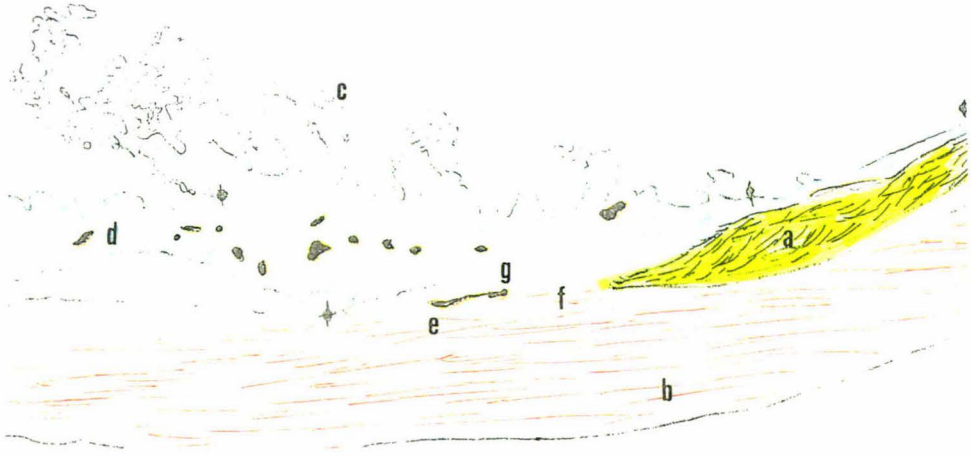



Figure 5-17e. Bovine ciliary body. x12.

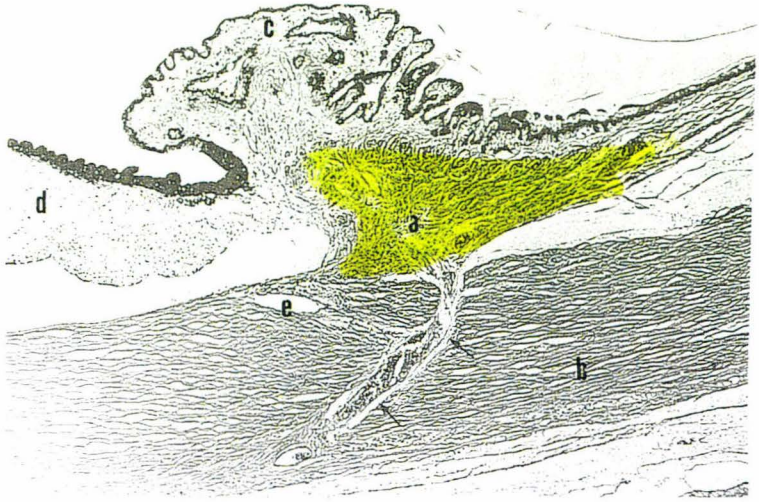



Figure 5-17f. Diagram of a human ciliary body taken from Hogan *et al.* (1971). This section was specially selected by the authors for the rare appearance of an episcleral vessel (arrow).

6 Size of Ciliary Bodies

The human ciliary body is about 6mm in length; 2mm of pars plicata and 4mm of pars plana (from Hogan *et al.* 1971 ). The bovine ciliary body is 6-10mm in length (Prince 1956). There was a wide variation observed in the lengths of the pars plicata area of ciliary bodies in the whale eyes examined. It ranged in length from 1-11.25mm when measured from the end of Descemet's membrane to the start of pars plana.

**TABLE 5- 4. RELATIVE LENGTHS OF PARS PLICATA AREA OF CILIARY BODIES IN 20 WHALES.**

| SPECIES | LENGTH OF CILIARY BODY - MICRONS |
|---|---|
| BALEEN | |
| PYGMY RIGHT - JUVENILE - E428/97 | 1000.. |
| MINKE 28609/97 | 5000.. |
| MINKE 28831/97 | 10000.. |
| SPERM | |
| E90/97 | 3,250 - 7,500 |
| E430/95 | 5000.. |
| PYGMY SPERM | |
| JUVENILE - E413/95 | 4,250.. |
| 29761/97 | 2000.. |
| BEAKED | |
| CUVIER'S BEAKED E15/95 | 11,250.. |
| CUVIER'S BEAKED 28543/97 | 3,500.. |
| GRAY'S BEAKED E433/95 | 5,250.. |
| LONG-FINNED PILOT | |
| E425/95 | 2,250.. |
| E432/95 | 2,250.. |
| E189/97 | 2,750.. |
| E195/97 | 3,250.. |
| E198/97 | |
| E199/97 | |
| E200/97 | |
| DOLPHINS | |
| 27796/97 | 1500.. |
| 27865/97 | |
| 28112/97 | |

### 5.4iv Choroid

Choroidal layers observed in cetaceans were ; i) pigment epithelium, containing pigment in non-tapetalised areas and devoid of pigment in tapetalised areas; ii) a dense capillary layer, the choriocapillaris; iii) a dense layer of collagen fibrils, the tapetum, traversed by capillaries iv) the large vessels of the choroid, a thick vascular layer and v) the suprachoroid, a layer of loose collagen stroma (Figure 5-18a). Its total thickness varied between specimens, but was 370 microns in pygmy sperm whale E413-95. A thick tapetum demands a thick choroid (Prince 1956). In humans the thickness of the choroid is 30 microns. Bovine choroid was observed to be much thinner than cetacean choroid due to the relative thicknesses of tapetum and suprachoroid, and had less extensive choroidal vasculature (Figure 5-18b). A thickness of 100-150 microns for the thickness of the bovine choroid is cited by Prince (1960) . In humans, blood enters the choriocapillaris from ciliary arteries and drains through the stroma into choroidal veins, which leave the eye as either episcleral or vortex veins. A similar arrangement is probably present in the pygmy sperm whale E413-95 (Figures 5-19a and b).

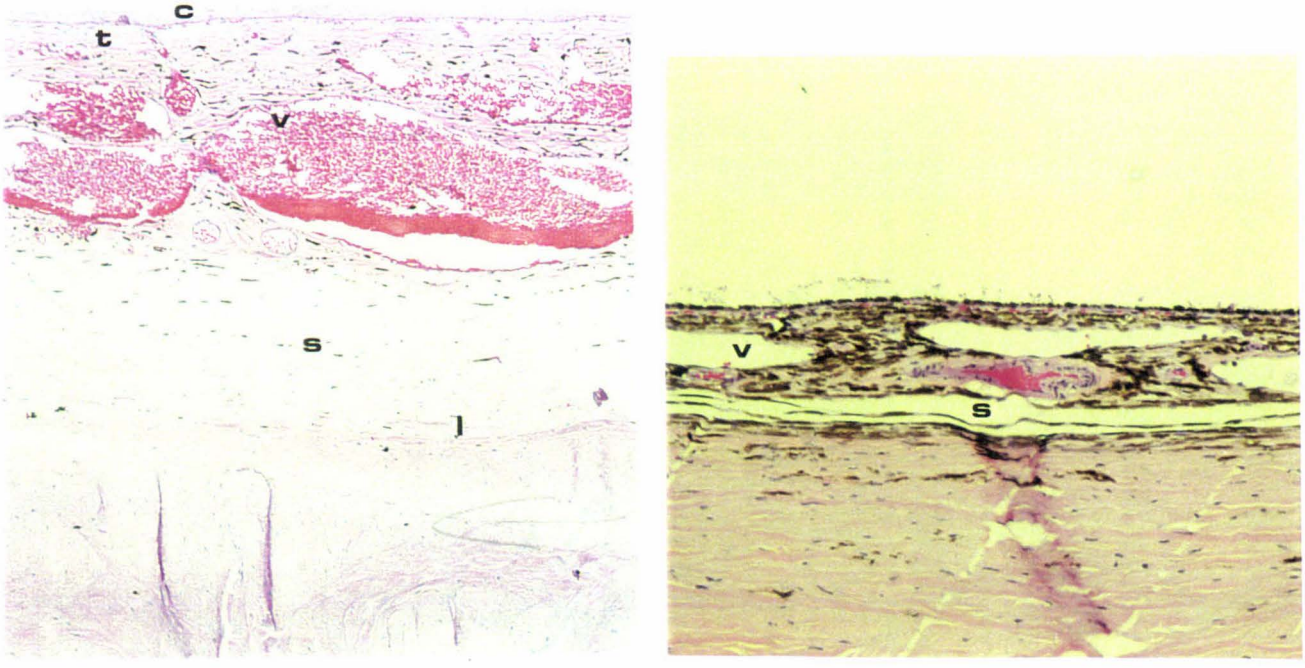
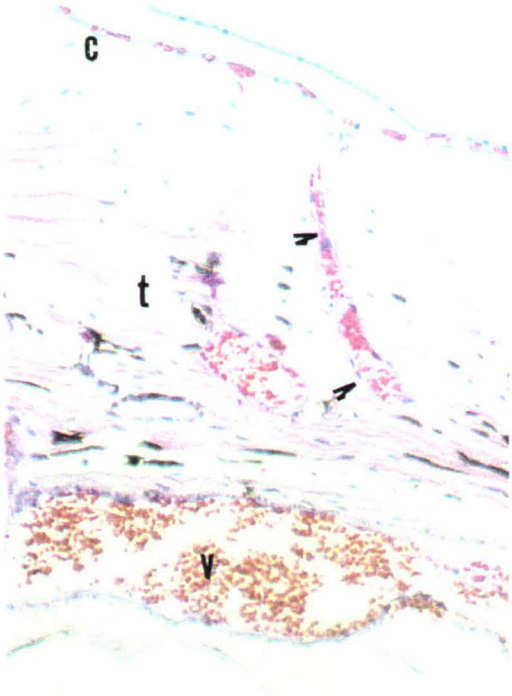
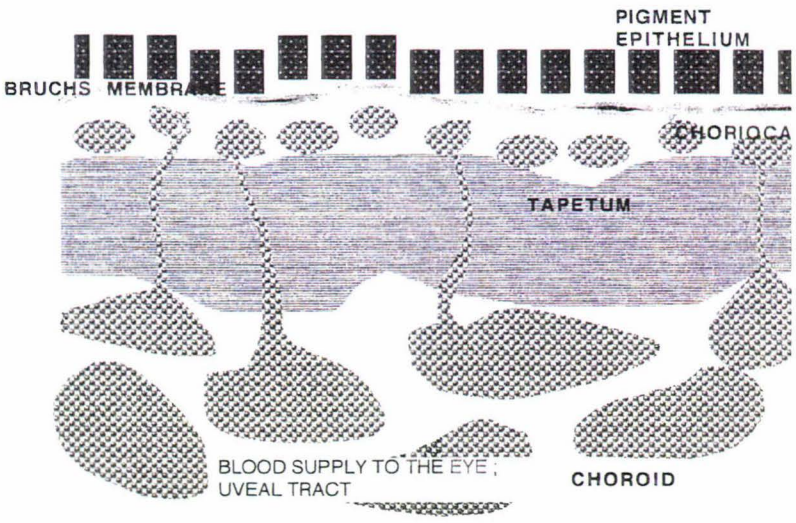



Figure 5-18 a and b. a) Area of tapetalised choroid in a pygmy sperm whale E413-95 stained with H&E x54. b) Area of non-tapetalised choroid in a steer. H&E x80 c, choriocapillaris; t, tapetum; v, choroidal vessels; s, suprachoroid; l, lamina fusca;




Figures 5-19 a and b. a) blood supply to the choroid of pygmy sperm E413-95 H&E x266. c, choriocapillaris; t, tapetum; v, choroidal vessels.
b) diagram of blood supply.

## 5.4v Rete

Rete vessels in all the cetacean species examined had thick walls containing smooth muscle and were therefore interpreted as arterioles. A few vessels were smaller, with thin walls, and these were interpreted as venules (Figures 5-20, 21 and 22).

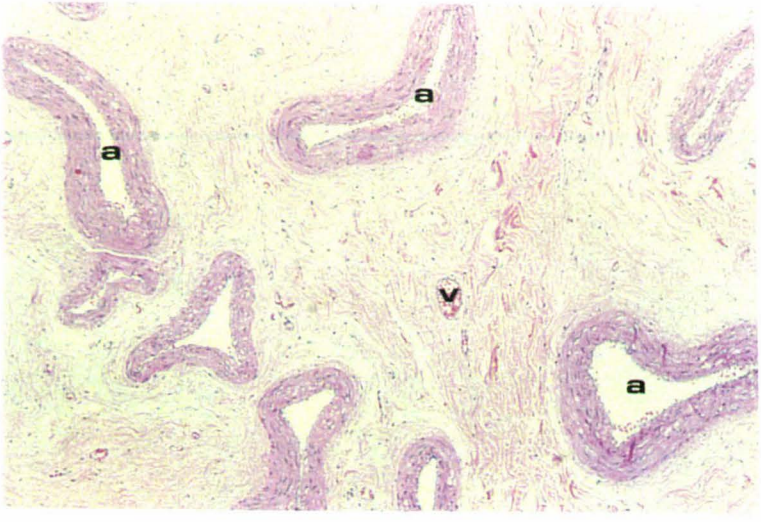



Figure 5-20. Pygmy sperm whale rete. a, arteries v, venules H&E x133

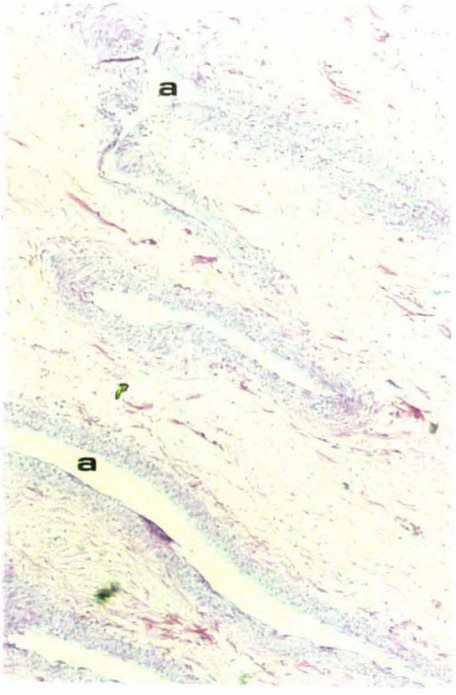



Figure 5-21. Long-finned pilot rete. a, arteries ; v, venules H&E x133

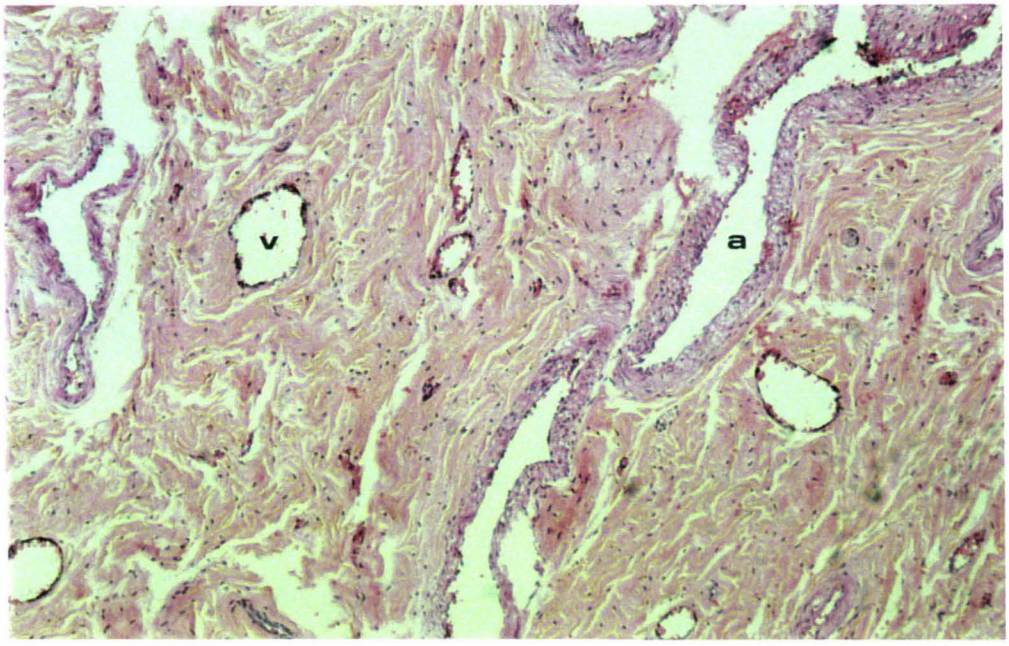



Figure 5-22. Rete of a Southern right bottlenosed whale. a, arterioles; v, venules. H&E x 200

## 5.4 vi Drainage of the Anterior Chamber

The ink injected into the anterior chamber was found to accumulate in the drainage angle, and penetrate for some distance into the trabecular spaces (Figure 5-23). However, the large sinus did not show any evidence of Indian ink particles.

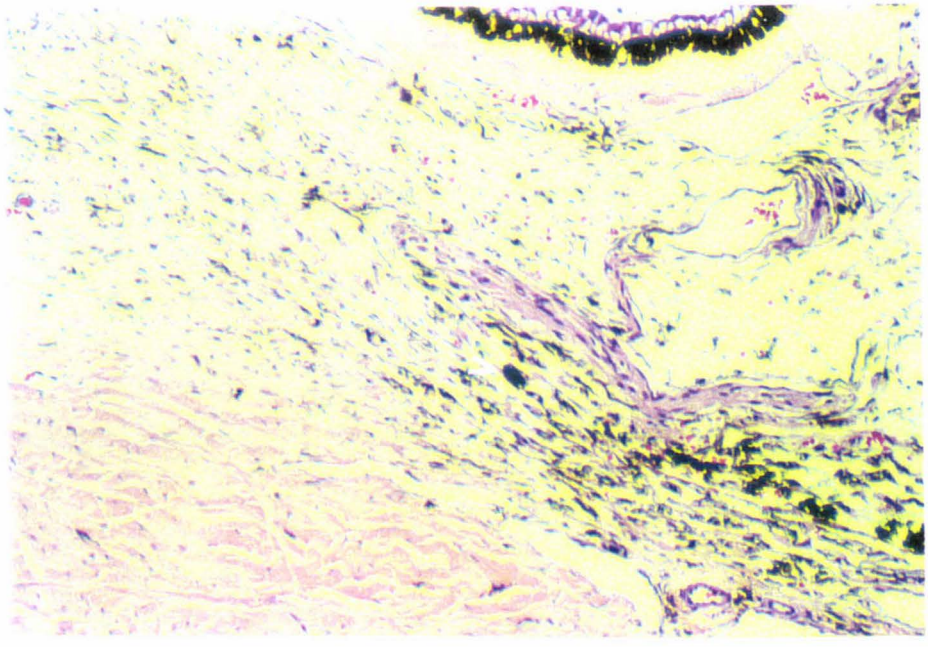


Figure 5-23. Drainage angle of long-finned pilot whale showing limits of Indian ink percolation (white arrows). H&Ex 200

## 5.5 DISCUSSION

The cetacean iris is similar to bovine and human irises in having circular and radial muscle fibres for pupillary adjustment. In the dolphin the pupillary reflex is partially consensual, while its eye movements are dysjunctive (Dawson 1980). It is assumed that the iris responds by using a similar mechanism to other animals, namely constriction of the sphincter muscle under parasympathetic control of oculomotor three, and dilation by sympathetic control of vagosympathetic trunk (Peterson Jones 1989). In whales, the collagenous stroma is very sparse in comparison to humans and cattle. It has been described by Wickham (1980) as "somewhat ephemeral" and its vasculature, which is mainly venous, is far more extensive than in most other mammals. The loose, open appearance of the iris reflects that seen in the ciliary body. This raises the question of whether the cetacean iris has a greater role to play in aqueous drainage than it does in humans, where it is responsible for about 5% of drainage and its role is thought to be mainly involved in modification of the composition of aqueous (Hogan *et al.* 1971).

In most species, the anterior and posterior uveal tracts have a distinctly different histological appearance and each major area (iris, ciliary body, and choroid) has a distinctly different function. The anterior uvea is mainly fibromuscular, and is responsible for dynamic events such as pupillary control and lens flexure, while the choroid is a vascular structure responsible for nutrition of the retina. It is not clear whether cetaceans are capable of altering the dioptric power of the eye, or whether their mechanism to achieve sharp focus is by alternative methods such as a stenopaeic pupil, ramp retina, or long receptor cells (see Chapter 2). Evidence supporting both active accommodation and alternative methods is equivocal. Some experiments on live dolphins have not required cycloplegia to paralyse accommodative movements (Dawson 1972, 1980, 1987), and the dioptric power appears to remain stable. In other studies, weak lenses were required in the ophthalmoscope because the dioptric power was variable, indicating some accommodation (Dral 1975). The most recent study in this area used videoretinoscopy to show that there may be some evidence of varying refractive ability, either actively or by an alternative method (Cronin et al. 1998). Strong aerial myopia has been observed in dolphins by Dawson (1972) Dral (in certain directions of gaze, 1972) and Cronin et al. *et al.* (1998). Good visual acuity, suggestive of either active accommodation or an alternative method of focusing, has been demonstrated in dolphins (Herman *et al.* 1975; Spong and White 1971) and the killer whale (White *et al.* 1971).

An important observation in this study was that differences in structural features between different areas of the uveal tract are well defined in the bovine and human ciliary body, whereas in the whale eye these differences are less obvious, and the anterior uvea is very similar in appearance to the posterior uvea. This is due partly to the absence of ciliary muscle tissue in the cetacean eye and finding this confirms the work of Dral (1975) and Dawson (1980), and also West *et al.* (1991), who found muscle in the narwhal but not in the beluga. The most recent study in this area (Waller 1992) describes some ciliary muscle in long-finned pilot whales, but this was only visible in the latitudinal plane. However, observations made in a similar plane in the present study did not support this. Finding this lack of ciliary muscle imparted a very loose, open trabecular appearance on the cetacean ciliary body. The wide, endothelium lined spaces, suggest that the capacity of this tissue for aqueous drainage (or storage) is very large. The poor junction between uvea and sclera, and lack of pectinate ligament suggests that the ciliary body is not subjected to much tension, either from the zonule or by muscular force. This implies that there is little resting tension in the zonule (as in the relaxed eye of the human or bovine) or no muscular forces during accommodation.

The current study has found that lenses do 'round up' when they are excised from unfixed eyes (autolysis of the cortex may accentuate this tendency), and a range of shapes from spherical to a flattened sphere have been observed in fixed specimens (Chapter 7).

A second major finding was the presence of a large sinus in the pars plana region of the uvea of cetacean eyes. This is similar to that described in the sea otter, where it has been suggested that if the sinus is dilated by muscular action, it could fill with aqueous, leading to anterior movement of the lens (Murphy *et al.* 1990). The presence of a large sinus in this site suggests that it will be aqueous filled, and on most sections the clarity of the lumen supports this possibility. In some sections, small numbers of blood cells are present, suggesting that there could be a mixture of blood and aqueous, as in the aqueous veins of Ascher in humans . However in other sections, there were clearly connections between the large sinus and smaller venous sinusoids (Figure 5-11) and the sinus was blood filled (Figure 15d and e).

It has been observed in whales (Dral 1975) that wide spaces of the choroid consist of delicate strands of connective tissue supporting a number of melanocytes and that the contents of the ciliary body are continuations of the suprachoroid lamellae, with the spaces between them empty, attributable to processing artefact. In the present study, the ciliary body was found to consist of endothelial cells surrounding collagen in a trabecular pattern (Figure 5-16), and also collagenous strands as described by Dral (1975). The establishment of a link between the ciliary body and the large sinus as described, was not conclusive. Efforts to trace an anatomical link using Indian ink in a limited number of eyes (two cetacean

eyes, one 'fresh' and one fixed, and one bovine eye) demonstrated passage of ink into the trabecular spaces, but not the sinus, of one cetacean eye. This does not exclude the possiblity that a link may have existed between the spaces, the large sinus and the suprachoroid. Agitation or massage of the globe to 'pump' the ink through the 1-2mm distance of anterior chamber to the sinus, the use of a much fresher specimen, and use of a dye with a smaller particle size, may have assisted more extensive penetration.

A third finding in this study is that the anterior uvea had large numbers of venous sinusoids, and these were arranged in two layers, with a congested inner layer and a fluid filled outer layer. The significance of this is unclear, but it could indicate that the outer layer fills only intermittently as a reservoir, or that a substantial amount of aqueous is present in this region.

These observations have led to the development of the following hypothesis.

## THE VASCULAR ENGORGEMENT CONCEPT

The dense vascularity of the uvea gives the structure an appearance similar to that of erectile tissue. Vascular engorgement of this tissue could lead to raised intraocular pressure, and the hypothetical possibility of intraocular events such as increased corneal curvature, movement or remodelling of the lens, and movement of the retina.

In humans the calibre of choroidal vessels is partly dependent on the intraocular pressure. They dilate when intraocular pressure (IOP) is reduced, and assume their usual calibre when it returns to normal. The change in blood volume in these vessels probably plays some part in maintaining intraocular pressure (Hogan *et al.* 1971). In humans, IOP is largely derived from capillary hydrostatic pressure and aqueous osmotic pressure (Spooner 1957) and is maintained at a constant level of around 15-20mm Hg (Hogan *et al.* 1971). In order for arterial blood pressure to have an effect on IOP the rise needs to be massive (Dawson *et al.* 1991), but a small rise in venous pressure can affect IOP (Smythe 1958). Venous stasis would arise if IOP exceeded venous pressure, and the difference between these is very small (about 2mm Hg in humans). Small transient rises are normal and occur frequently with eyelid closure and other movements, but if IOP remains high for long periods, pathological changes in the retina result. This suggests that humans maintain a stable, constant IOP primarily by the constant, regular flow rates of aqueous production and drainage, with vascular adjustments to compensate for raised or reduced IOP as a result of aqueous dynamics. Recent work has shown that mechanoreceptors, similar to stretch receptors, are present in the limbus of the human eye, and probably monitor the degree of stretch in the collagen, and hence, IOP (Tamm and Lutjen-Drecoll 1996).

Observations made by Dawson (1987) have shown that dolphins in experimental

conditions are unusual in having variable intraocular pressures of up to 75mm Hg. The mechanism of this variation is not known, but since aqueous production rate is probably limited by the physiology of membrane transfer, it seems most likely that the variation is of vascular origin, since membrane transfer dynamics would limit the rate of aqueous production to a fairly low level.

If this hypothesis is correct, variation in IOP in dolphins is primarily vascular, not aqueous, in origin.

The present study, with its three major findings, raises the question of the overall effect of total engorgement of the uveal tract. The large capacity of the sinusoids compared to the diminutive arterial capacity suggests that they may be used as a reservoir, similar to cavernous erectile tissue (eg. corpus cavernosum penis). The ratio of venous : arterial tissue within the eye is reversed when the rete is considered, since its main capacity is arterial with a diminutive venous capacity. It can therefore be hypothesised that a rise in venous pressure would result in raised intraocular pressure. This is supported by the observation that intraocular pressure may vary between 20-75mm Hg in dolphins over a 40 minute period (Dawson *et al.* 1992).

Intraocular pressures increase in a normal eye until the elastic limit of that eye is reached and then, because fluids are incompressible, a plateau is reached as the eye contains its maximum volume of fluid at maximum pressure. In whales, the elastic limit of the eye is very small due to the thick, rigid sclera. Further pressure increases can only be made by a piston effect. This effect is analogous to the arterial pressure in the ophthalmic artery. In a whale, when the volume plateau has been reached, arterial pressure, as long as it exceeds IOP and venous pressure in the uveal tract, would exert a piston effect. Venous pressure would be high due to passive congestion, by virtue of the constriction of venous outflow .

Possibilities for constriction within the cetacean eye are either a) veins are constricted at their point of exit from the sclera or b) arteries within the rete compress veins. The arterial capacity of the rete is very large, and their unusually large lumina suggest that they may also function as a reservoir. If this occurs, thin walled veins which pass through the rete could be occluded. The arterial ophthalmic rete probably fills as part of the dive response, in a similar way to those found in the thorax and around the spinal cord. In these sites, the functions of the rete are uncertain, but are believed to be an adaptation to diving. Current hypotheses suggest that their function as a volume reservoir is very small, and it is their role as a pressure reservoir that is of major importance. Because an arterial rete consists of numerous small vessels arranged collaterally, it has very low resistance and little effect on mean pressure, but avoids the pulse pressure problems that would be associated with a single, large,muscular tube (Vogl and Fisher 1982). Hence, the entire pre-CNS arterial vascular bed acts as a 'windkessel' or shock absorber, so that during dives blood is directed towards them, and their outflow is directed through a few vessels which penetrate the dura

to supply the CNS (Vogl and Fisher 1982).

The overall effect of an engorged uveal tract and subsequent rise in IOP could cause the following sequence of events:-

i] incompressible structures within the eye such as lens, vitreous body, and aqueous, would be forced to remodel as extra blood fills vessels in the anterior segment. The cornea would stretch to its elastic limit, and become more curved, increasing its dioptric power.

ii] the volume of blood entering would displace an equal volume of aqueous posteriorly into the pars plana sinus. Since this sinus lies in the posterior segment, equal pressures would be maintained, without movement of any other structures, such as the lens. If equal pressures are not maintained, lens movement may occur to compensate.

iii] the engorged ciliary body and dilated sinus would substantially increase in size. This could reduce tension on the zonule, and allow the lens to 'round up' similar to the more usual muscular action. Thus the dioptric power of the lens would be increased.

By this vascular engorgement mechanism, it would be possible for the whale eye, which has been demonstrated as emmetropic (ie. the relaxed lens allows distant objects to appear in focus) in water (Dawson *et al.* 1972; Dral 1972; Kroger 1992; Cronin et al. *et al.* 1998) to accommodate during dives, thus enabling it to view close objects as well as distant ones, using a mechanism that is related to the dive response. The other effects of an engorged uvea during dives may be to maintain warmth of the eye in its uninsulated site, and maintain the supply of oxygen and nutrients, as described for arterial rete in other sites (Vogl and Fisher 1982). Further work in the present study (Chapter 7) provides evidence that the relaxed eye may also be emmetropic in air. Accommodation would therefore be required for near vision, and although the vascular engorgement concept may operate, this seems unlikely if it is linked to the dive response. This hypothesis is further supported by the observations of Herman *et al.* (1975) that aerial vision in dolphins is better at distance (suggesting a relaxed eye), whereas aquatic vision is better for near objects (suggesting active accommodation of an emmetropic eye).

# ENCAPSULATED RECEPTORS

## 6.1 ABSTRACT

AIM: To describe the occurrence, sites, orientation and morphology of encapsulated receptors (ERs) in the ciliary body, limbus, and sclera of the cetacean eye.

METHOD: A detailed topographical study of the size, shape and position of five individual longitudinal encapsulated receptors in the sclera of a pygmy sperm whale (*Kogia breviceps*) was made using histological and photomicrographic techniques. Histological examinations were made of eyes from 20 whales to record the site, orientation and types of encapsulated receptor present, and electron microscopy was used to show the ultrastructure in two of these cases.

RESULTS: The topographical survey demonstrated that the structures examined were nerve endings, with connection to an axon, although this did not always occur at the end of the structure. Encapsulated receptors were found in the majority of whales. They occurred in specific sites, varied histologically between species and demonstrated particular orientations.

CONCLUSION: Receptors seemed to conform mainly to a loosely paciniform structure with three broad variations recognisable - a large, loose type, a small, dense type, and an intermediate type. The sperm and pygmy sperm whales' ERs showed some individual differences in histology, site and orientation. One structure resembling a Ruffini corpuscle was evident in a long-finned pilot whale.

## 6.2 INTRODUCTION

Encapsulated receptors (ERs) are nerve endings that are associated with a specialised arrangement of non-neural tissue to augment their function (Burkitt *et al.* 1993). They occur widely in cutaneous and deep sites, such as pancreas, conjunctiva, mesentry and joint capsules. The ultrastructure of ERs has been described in the joint capsules of the pig, cat (Tachibana *et al.* 1988) and pigeon (Halata and Munger 1980; Meyer and Neurand 1981) the blowhole of the dolphin (Bryden and Molyneux 1982), and the pharynx of a right whale (Ford and Kraus 1992). They have been described in the cetacean ciliary body (Rochon Duvigneaud 1940; Pilleri and Wandeler 1964; Vrabec 1972; Wickham 1980) as well as in the snowgoose (Vrabec 1961). In human eyes they have been described in the limbus as mechanoreceptors, similar in structure to muscle spindles, and are thought to monitor the tensile strength in limbal collagen fibres (Tamm *et al.* 1996). Encapsulated receptors up to 50 microns in diameter and 350 microns in length have also been described in the iridocorneal angle of cetaceans (Wickham

1980). Data for Wickham's study was obtained by photographing a large number (150 longitudinal and 1000 tangential) of serial sections in 42 individuals.

Comprehensive, detailed data on the ultrastructure of ERs in cetacean ciliary bodies is not available. Wickham's study in 1980 describes their orientation and size for nine cetacean species but detailed ultrastructure is lacking. No further studies to broaden the range of species have since been undertaken. Wickham postulated that ERs have four main functions; to sense changes in intraocular pressure associated with accommodative movements, with filling of the iris vascular system, with lid closure, and with aqueous movement.
It is assumed that the function of mechanoreceptors can be postulated on the basis of their structure (Iggo 1976). Historically, most receptors have had their functions revealed after electrophysiological studies. However, the mechanoreceptor function of the encapsulated receptor types described in this chapter is not proven, merely postulated. It is known that in humans, specific axon types relay specific sensations, although certain types are polymodal, such as free nerve endings for pain and touch (Munger and Ide 1988). Since many cutaneous receptors are identical to interoreceptors, the assumption that the function of ERs found in the present study can be predicted from their structure can be made with some confidence.

The present study was performed in order to improve our understanding of the structure and distribution of ERs in whales. It aimed to find whether they are of similar morphology and orientation in each species, and to hypothesise further about their possible functions as mechanoreceptors.

## 6.3 MATERIALS AND METHODS

### 6.3i Topographical Study

A *Kogia breviceps* (pygmy sperm whale 27961-97 ) was chosen as the most suitable specimen for this study because of the high density of longitudinal ERs present in latitudinal section. The site studied was in the sclera, adjacent to the conjunctiva (Figure 6-1).

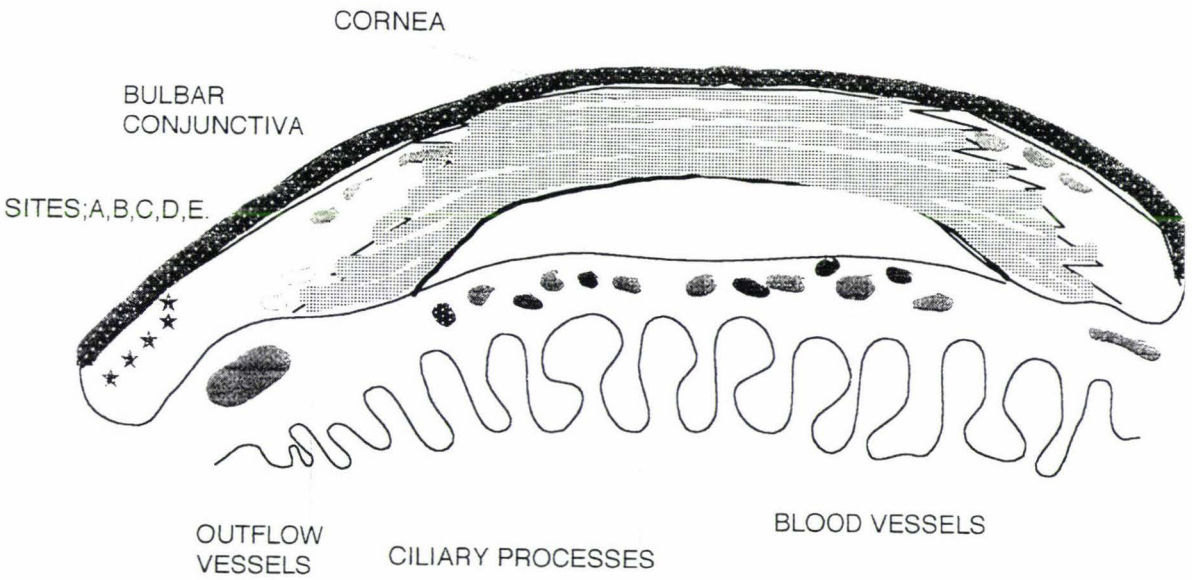



**Figure 6-1. Diagram of a latitudinal section of the ciliary body in pygmy sperm whale 27961-97 showing study sites.**

A formalin fixed eye was sectioned and processed routinely for histology as described previously in Chapter 5.

One hundred and fifty latitudinal sections, four microns thick were examined. The area chosen for study had four easily recognised ERs and a nerve trunk which were within an area of 2-3 high power (x 20) fields. Each ER was identified by a label a,b,c,d, and e (Figure 6.1) and each was traced through up to 150 serial sections. Diagrams were drawn at each stage because of the slightly different appearance of consecutive slides. The lengths and positions of receptors were

recorded graphically (Figure 6-2). One of these (e) was chosen for detailed study because of its ease of recognition. A series of photomicrographs was compiled to illustrate its progress (Figures 6-4a to g).

### 6.3ii Histology and Ultrastructure

The iridocorneal areas of 20 whales representing five different families were examined using an Olympus BH-2 light microscope. The histological techniques used have been described previously in Chapter 5. Each tissue was stained with haematoxylin and eosin and selected blocks were also stained with PAS, Van Gieson and Holmes silver (see Appendix 6-1 and Table 6-1).
Samples for electron microscopy were fixed in buffered 10% formalin. Blocks of one cubic millimetre were cut, embedded in epoxy resin and initially thick (0.5um) sections were cut and stained with toluidine blue. These thick, toluidine blue-stained sections from six whales were examined with a light microscope, ERs were located in each, and the block was trimmed selectively for the sites having ERs. Thin (60nm) sections were then cut and stained with uranyl acetate and lead citrate for examination under a Philips EM 201c electron microscope.

The following features of ERs were recorded ;

1] The association with vessels in three whales; pygmy sperm, sperm and long finned pilot.

2] The size, site, orientation and histology of ERs in a series of twenty whales (Table 6-1)

TABLE 6-1. SECTION PLANES AND STAINS IN THE EYES OF TWENTY WHALES.

| GROUP | REF.NO. | SPECIES | PLANES | STAINS |
|---|---|---|---|---|
| GROUP1 | E428/95 | PYGMY RIGHT | LONG | H&E, VG, MT |
| BALEEN | 28609/97 | MINKE | LONG | H&E, H&E &AB |
| | 28831/98 | MINKE | LONG AND LAT | H&E |
| GROUP2 | E90/97 | SPERM | LONG AND LAT | H&E, H&E&AB, AG |
| SPERM | E430/95 | SPERM | LONG | H&E, MT |
| GROUP 3 | E413/95 | PYGMY SPERM | LONG AND LAT | H&E, VG, MT, AG |
| PYGMY SPERM | 27961/97 | PYGMY SPERM | LONG AND LAT | H&E, H&E&AB, AG |
| GROUP4 | E15/97 | CUVIERS BEAKED | LONG AND LAT | H&E, H&E &AB, MT, VG |
| BEAKED | 28543/98 | CUVIERS BEAKED | LONG | H*E, H&E&AB |
| | E433/95 | GRAYS BEAKED | LONG | H&E, VG, MT |
| GROUP 5 | E425/95 | LONG FINNED PILOT | LONG | H&E, VG, MT |
| LONG-FINNED PILOT | E432/95 | LONG FINNED PILOT | LONG | H&E, VG,PAS, AG |
| | E189/98 | LONG FINNED PILOT | LONG AND LAT | H&E |
| | E195/98 | LONG FINNED PILOT | LONG | H&E |
| | E198/98 | LONG FINNED PILOT | LONG | H&E |
| | E199/98 | LONG FINNED PILOT | LONG | H&E |
| | E200/98 | LONG FINNED PILOT | LONG | H&E |
| GROUP 6 | 27796/98 | DOLPHIN | LONG | H&E |
| DOLPHINS | 27865/98 | DOLPHIN | LONG | H&E |
| | 28112/97 | DOLPHIN | LONG | H&E |

Abbreviations: H&E, haematoxylin and eosin; VG, Van Gieson; MT, Masson's trichrome; Ag, Holmes' silver.

## 6.4 RESULTS

### 6.4i Topographical Study

Five ER-like structures found in the sclera of a pygmy sperm whale were chosen for detailed study over 150 serial sections. These were identified as structures a to e. One of these was subsequently identified as a nerve. The length and axon connection of these structures is shown in Table 6-2.

**TABLE 6-2. LENGTHS AND CONNECTIONS OF FIVE RECEPTORS IN THE SCLERA OF A PYGMY SPERM WHALE**

| RECEPTOR/ NERVE | START Section no. | FINISH Section no. | AXON APPEARED | TOTAL LENGTH in microns |
|---|---|---|---|---|
| a | 29 | 51 | 29(FROM NERVE B) | 120 |
| NERVE b | 1 | - | | - |
| c | 1 | 21 | 21 | 84 |
| d | 21 | 62 | 42-45 | 164 |
| e | 96 | 129 | | 132 |

The range in receptor length was from 84-164 microns. Receptors c and a were connected to an axon at one end, while d and e connected at a midpoint (see Table 6-2).

The sizes and shapes of these receptors is represented graphically in Figure 6-2 and receptors a to d are illustrated in photomontage Figure 6-3.

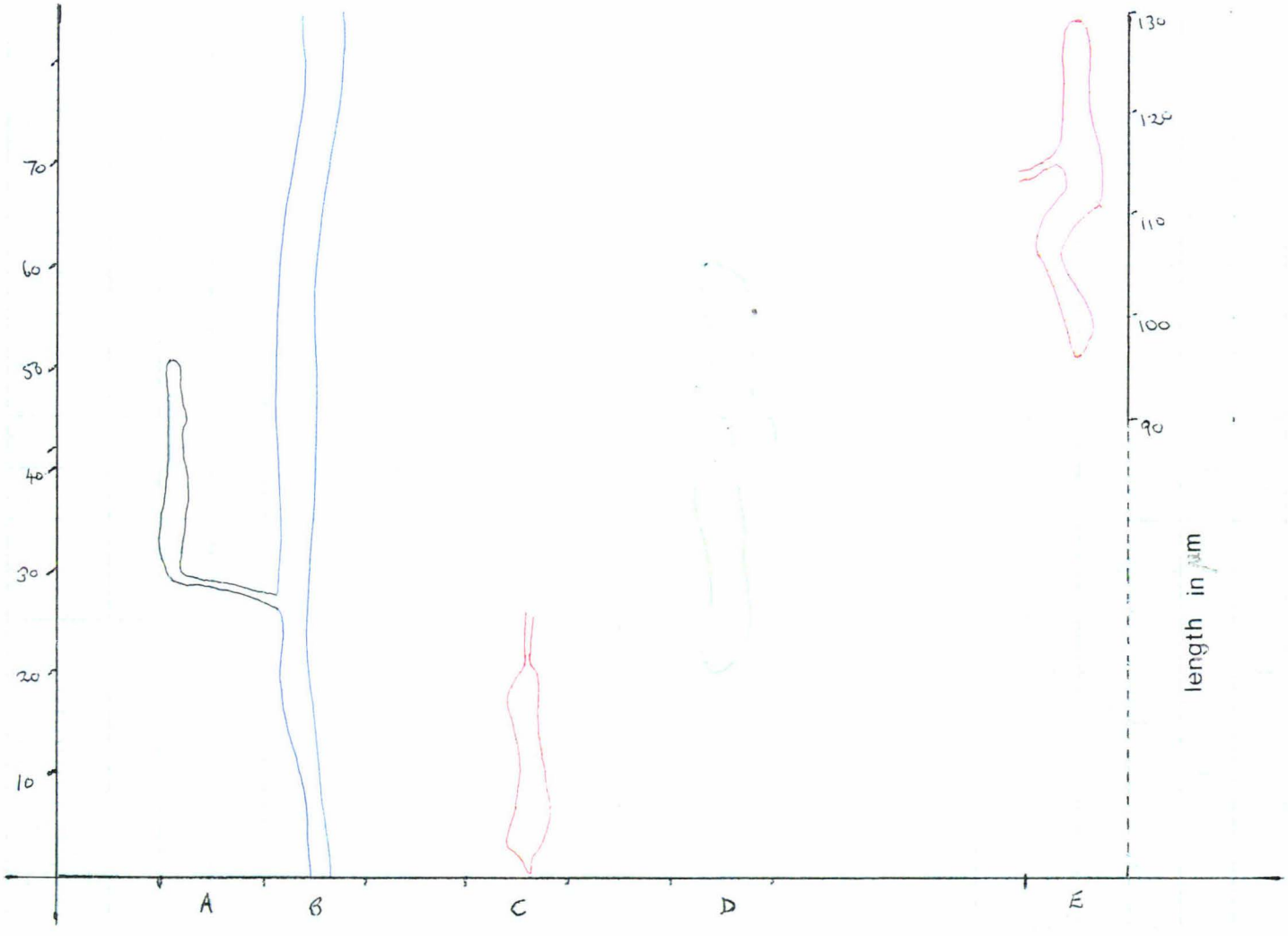



Figure 6-2. The relative shapes, lengths and distribution of four ERs a, b, c, d and e in pygmy sperm whale 29761-97.

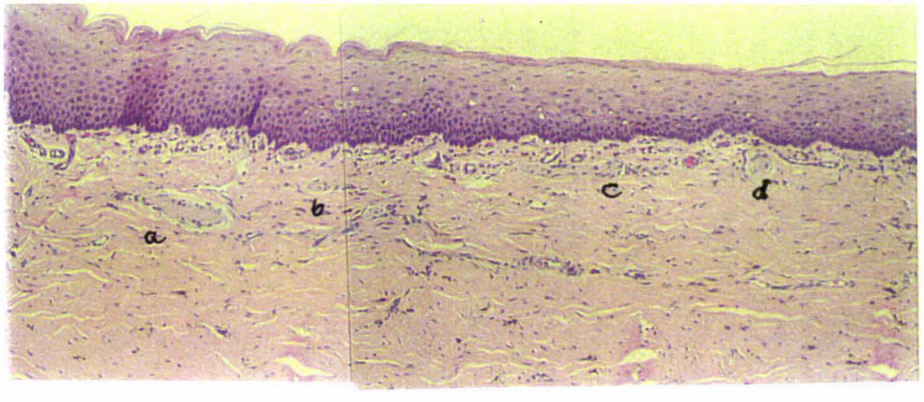



Figure 6-3. Photomontage of ERs a, b, c, d in sclera of pygmy sperm whale 27961-97. H&E x40.

Receptor 'e' was 132 microns long with a small axon joining at 116 microns. Throughout its length it was situated subconjunctivally, with a distinctive outer capsule and inner core. It had a diameter which varied from 25 microns to 75 microns. The structure of receptor 'e' is illustrated in a series of photomicrographs (Figures 6-5a to g) from sections stained with H&E at x400.

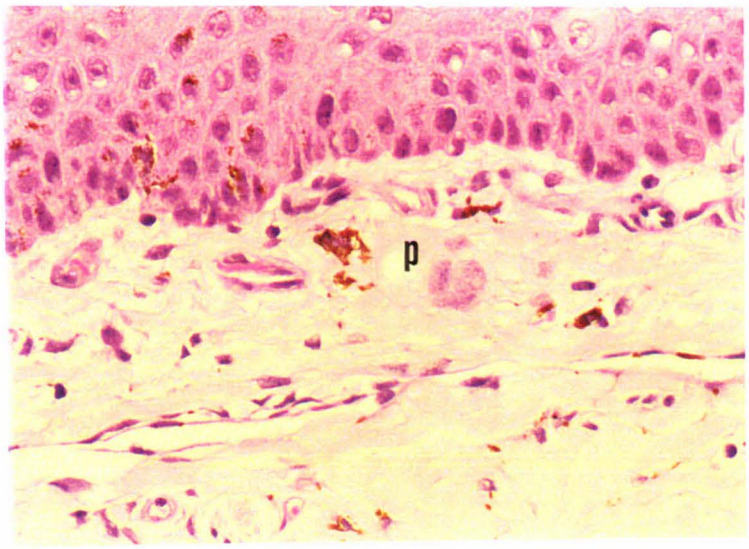



Figure 6-4a. First evidence of ER 'e' appears as a small group of perineural cells, flat view. p, perineural capsular cells.

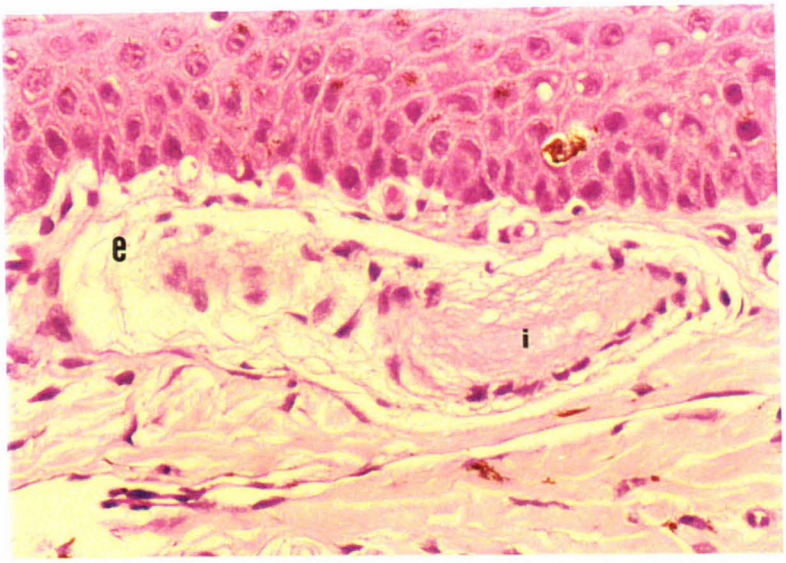



Figure 6-4b. At 40 microns ER 'e' is seen in oblique section. e, ER 'e'; i, inner core.

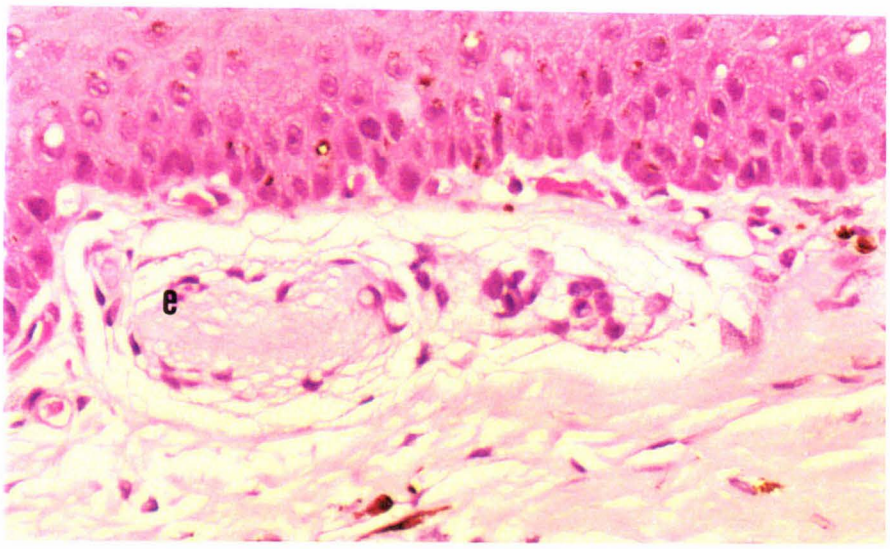



Figure 6-4c. At 60 microns, the section of ER 'e' is still oblique.

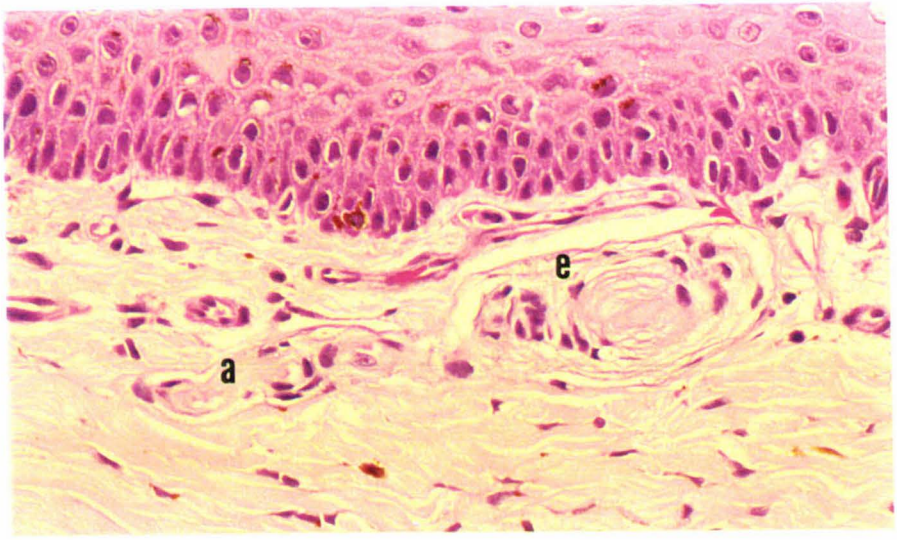



Figure 6-4d. At 76 microns, a cross sectional view of ER 'e' with a small axon joining from the left. a, axon; e, ER 'e'.

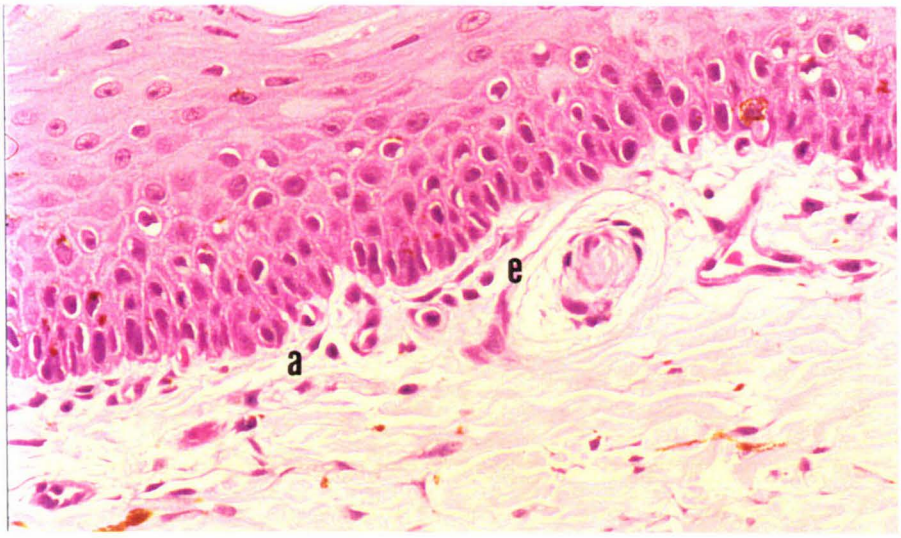



Figure 6-4e. At 116 microns, a cross sectional view of ER 'e' with small remnants of an axon still visible. a, axon; e, ER 'e'.

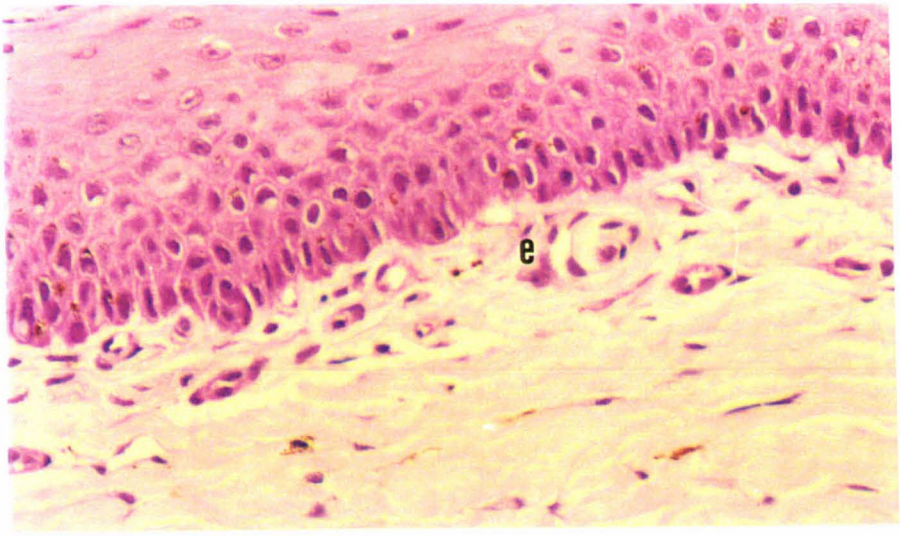



Figure 6-4f. At 124 microns, a cross sectional view of ER 'e', now much reduced in diameter.

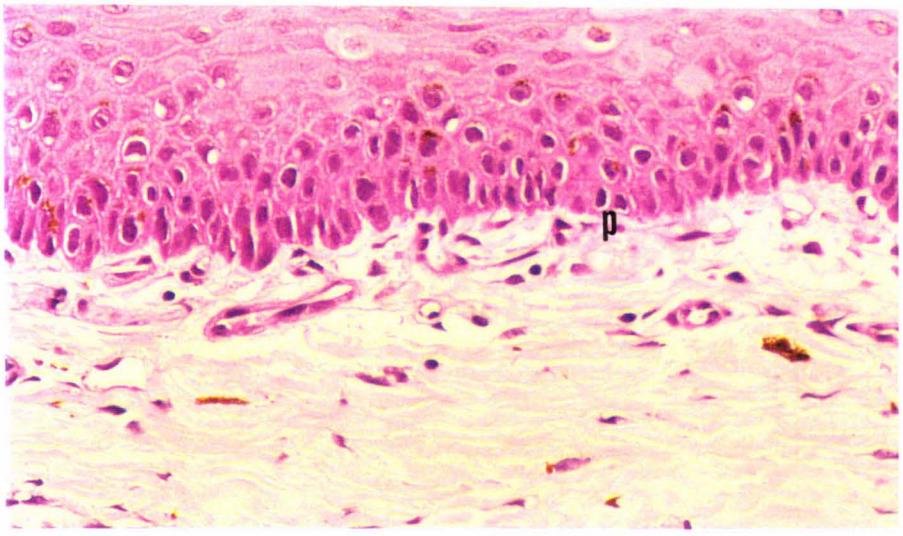



Figure 6-4g. At 132 microns, the last evidence of ER 'e'. p, perineural capsular cells.

### 6.4ii Association with Vessels

Subjectively, there did not appear to be a close relationship between vessels and ERs. This is illustrated in Figures 6-5, 6-6, 6-7 where the presence of an ER is denoted by the symbol ✦ .

One sperm whale (E90-97) and two pygmy sperm whales (27961-97, E413-95) were randomly selected and photomontages of their ciliary body areas produced. In each case ERs occurred mainly in dense scleral connective tissue. Of the 21 receptors captured 16 were in dense scleral connective tissue and five were in trabecular connective tissue. Only six appeared to be in close proximity to either blood or aqueous drainage vessels.

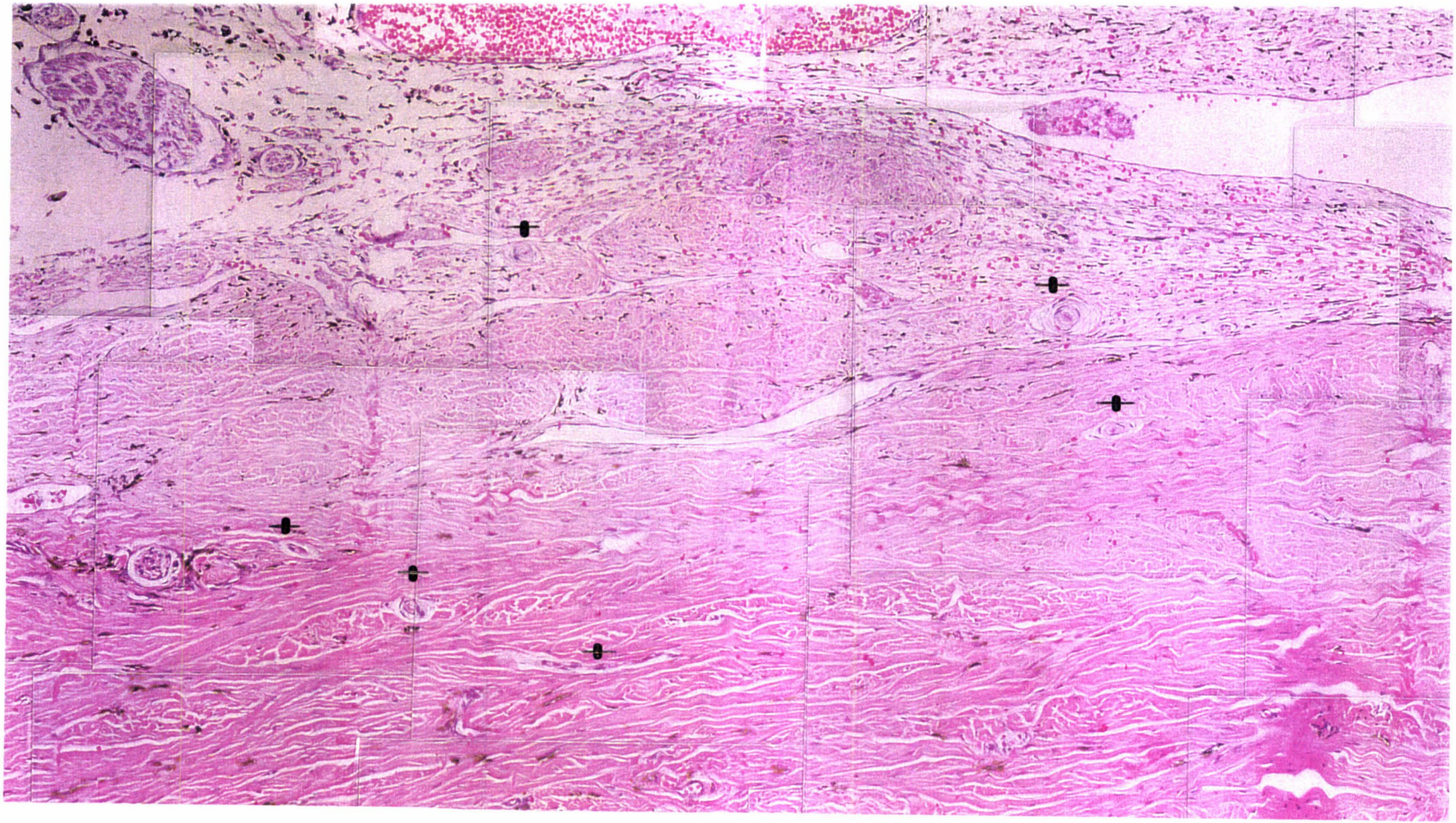


Figure 6-5. Photomontage of the ciliary body of a pygmy sperm whale (E413-95) showing positions of ERs ( ⊸ ) with respect to vessels. H&Ex140

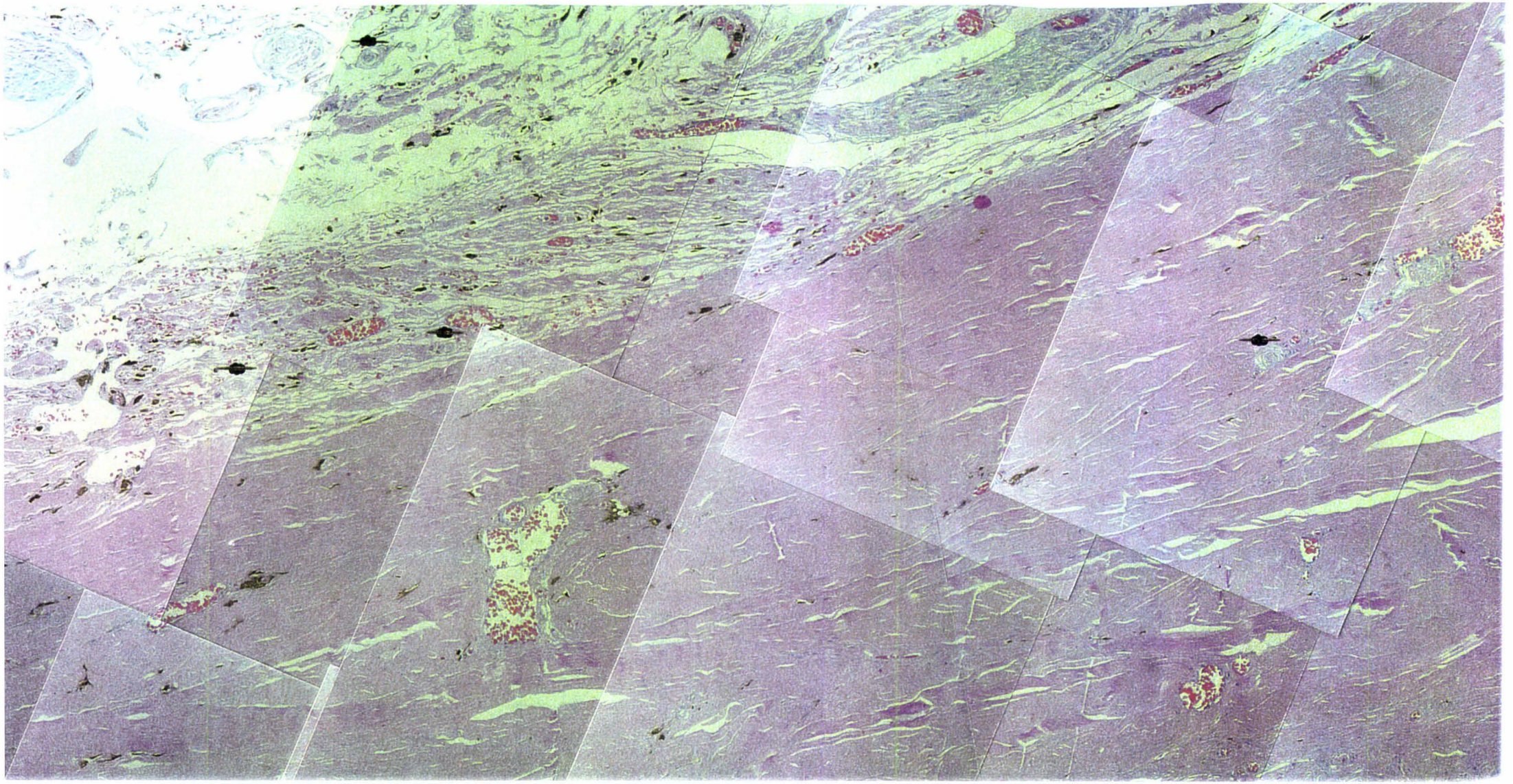


Figure 6-6. Photomontage of the ciliary body of a sperm whale (E90-97) showing positions of ERs ( ) with respect to vessels. H&Ex56

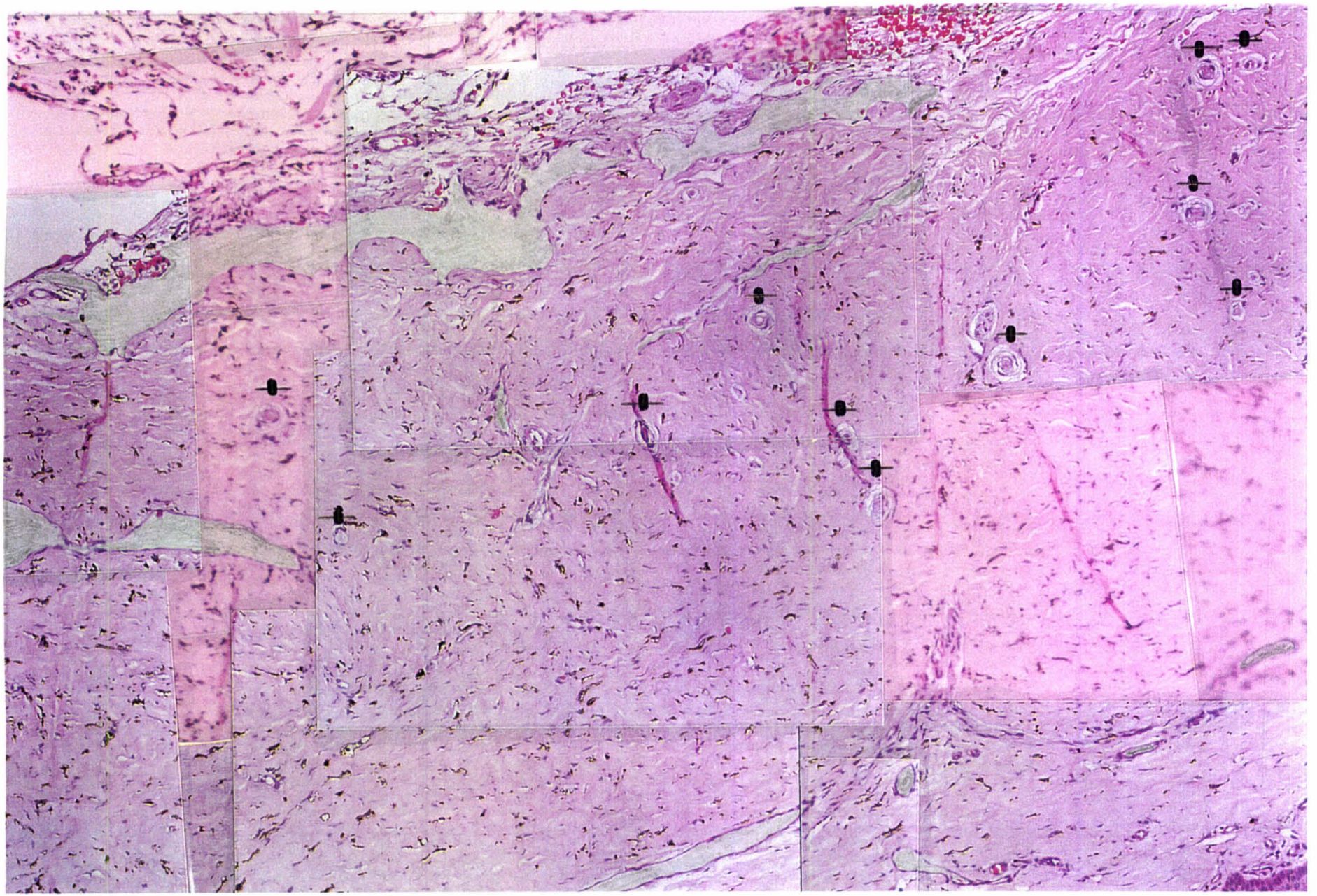


Figure 6-7. Photomontage of the ciliary body of a long finned pilot whale (27961-97) showing positions of ERs ( ⊣ ) with respect to vessels. H&Ex56

### 6.4iii Size, Site and Orientation of Encapsulated Receptors in the Eyes of Twenty Whales

Most whales had receptors in the 25-80 micron diameter range. In these, the capsule and core were of roughly equal widths. In minke whales, larger receptors of more than 80 microns diameter were seen, in which the capsule was relatively wider than the core. In dolphins, receptors were only present in one of the three animals examined, and these were very much smaller than those encountered in other whales at 5-25 microns.

Receptors were loosely classified into four types on the basis of their size :-

Type 1: Large, loose type usually occurring singly.

Type 2: Intermediate type, medium-sized and occurring either singly or as groups.

Type 3: Small, dense type occurring in groups.

Type 4: Atypical type, occurring in sperm whales.

Other variations were in orientation (running latitudinally, longitudinally, or radially) and site (within uveal or corneoscleral trabecular meshwork, or sclera). The latitudinal orientation was found consistently in every whale except pygmy sperm whale 27961-97, and only sparsely in the sperm whale E90-97. Longitudinal receptors were found mainly in the sclera and occurred only in sperm and pygmy sperm whales, with the exception of one example in the sclera of a Cuvier's beaked whale. Encapsulated receptors occurred either as one or several discrete, single structures (all whales) or in groups, within the same perineurium (sperm, Cuvier's beaked, and dolphins) (Table 6-3 ).

TABLE 6-3. COMPARATIVE ASPECTS OF ENCAPSULATED RECEPTOR SIZE, SITE AND ORIENTATION.

| GROUP | ABSENT | DIAMETER <25μm | DIAMETER 25-80μm | DIAMETER >80μm | ORIENTATION | SITE | SINGLE OR CLUSTERED |
|---|---|---|---|---|---|---|---|
| TYPE 1-LARGE | | | | | | | |
| Minke | | | 28609/97<br>28831/97 | 28609/97<br>28831/97 | LONG AND LAT | TRABECULAR MESHWORK | |
| Sperm | | | E430/95<br>E90/97 | | MAINLY LONG. | SCLERA | CLUSTERED |
| TYPE 2-MEDIUM | | | | | | | |
| Pygmy sperm | | | E413/95<br>27961/97 | | LONG AND LAT | SCLERA | |
| Beaked | | | E15/97<br>28543/97 | | LONG AND LAT | TRABECULAR MESHWORK | CLUSTERED |
| Long finned pilot | | | E432/95 E425/95<br>E 189, 195, 198, 199, 200/97 | | LAT | TRABECULAR MESHWORK | |
| Pygmy right | | | E428/95 | | LAT | TRABECULAR MESHWORK | |
| TYPE 3-VERY SMALL | | | | | | | |
| Dolphins | | 27865/97 | | | LAT | TRABECULAR MESHWORK | CLUSTERED |
| ABSENT | | | | | | | |
| Dolphins | 27796/97<br>28112/97 | | | | | | |
| | | | | | | | |

## 6.4iv Histology of Encapsulated Receptors in a Series of Twenty Whales

Encapsulated receptors had a typically paciniform appearance (Figure 6-12) in all whales except sperm whale E90-97. Receptors were round or ovoid in cross or oblique section and cylindrical in longitudinal section with two distinct parts; an outer capsule and an inner core. The outer capsule was often loosely lamellated with wispy, myxomatous material, staining basophilically with H&E and strongly blue with alcian blue, suggestive of a proteoglycan component and in appearance reminiscent of the structure of Renaut bodies (Summers 1995). In some cases, small dense particles could be seen in the matrix of the outer core. With Van Gieson stain the outer capsule stained red and with Masson's trichrome it stained blue, indicating the presence of collagen. Cell nuclei were often present within the outer core. By contrast, in types 1-3 the inner core was devoid of nuclei though its periphery was delineated by several nuclei. The inner core often showed eosinophilic staining characteristics with H&E, with few layers, each of which was relatively thick and more densely stained than the basophilic outer core. With Van Gieson stain, the core stained yellow, typical of cytoplasm, suggesting these were lamellae of modified Schwann cells or fibroblasts. With Masson's trichrome, the inner core stained pale blue, indicating that some collagen is also present in the inner core.

### Type 1 Receptors

These receptors were only found in the minke whales and were greater than 80 microns in diameter, with a distinct inner core and outer capsule. The outer capsule was of greater width than the inner core, and was typically basophilic with an open, wispy, myxomatous appearance. The inner core was devoid of nuclei with quite dense, eosinophilic staining properties with H&E.
In one minke whale (28831-98) they occurred in large numbers and had exceptionally wide diameters of up to 100 microns (Figure 6-8).

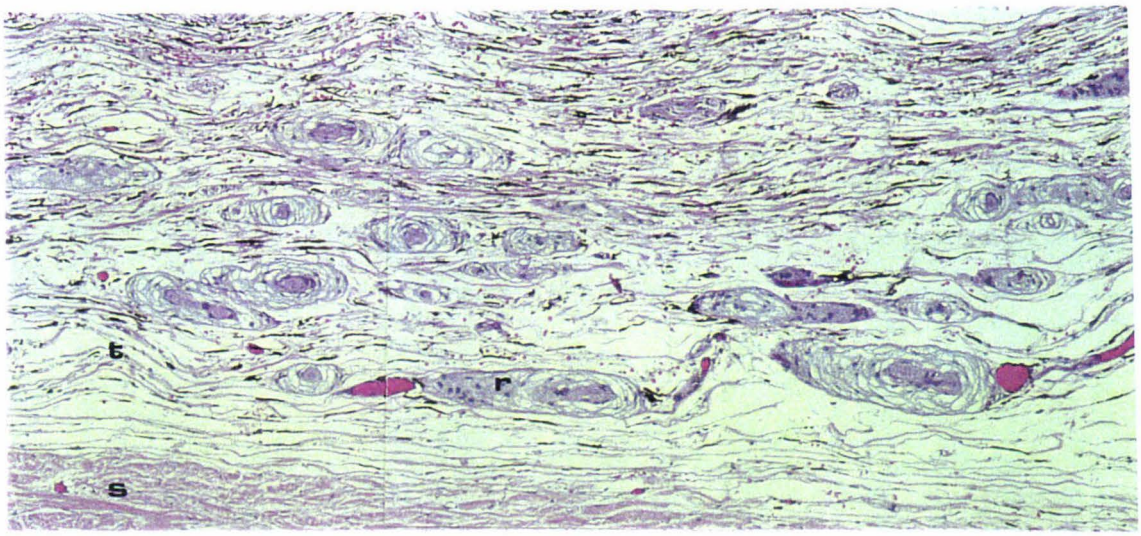



Figure 6-8. Type one receptors in latitudinal section of ciliary body of minke whale 28831-98. Some receptors are in slightly oblique section but most are in cross section. s, sclera; t, trabecular meshwork; r, receptors. H&E and alcian blue x 280

## Type 2 receptors

These were classified as having a diameter between 25 and 80 microns. Inner core and outer capsule were evident, but the outer capsule was frequently much thinner than the inner core. These occurred in all whales except dolphins. In sperm and Cuvier's beaked whales, they frequently occurred as groups, within a common perineurium and often accompanied by small axons (Figures 6- 9, 6-10, 6-11) within the same perineurium.

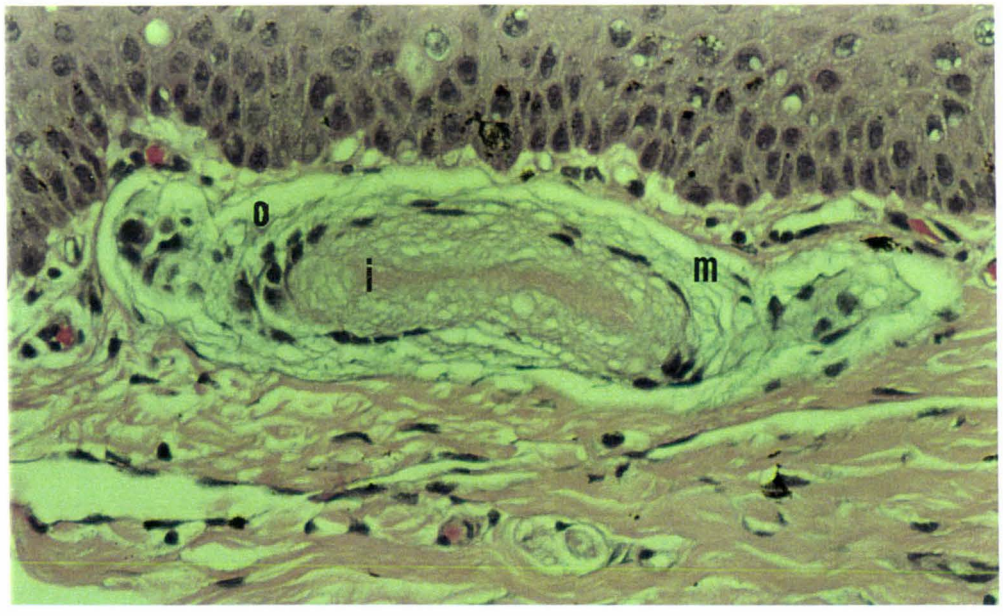



Figure 6-9. Oblique section of an encapsulated receptor from pygmy sperm whale 27961/97 stained with H&E x400 i, inner core; o, outer capsule; m, myxomatous material.

Figures 6-10 and 6-11 show an ER with characteristic core details. By contrast, a small nerve to the right showed a number of medium sized axons and well developed perineurium.

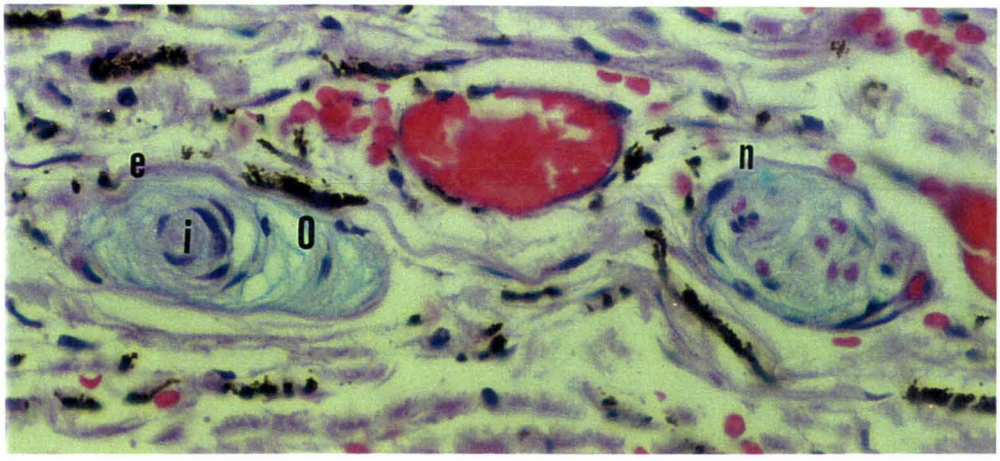



**Figure 6-10.** Section of trabecular meshwork in a minke whale (28609-97) e, encapsulated receptor i, inner core n, nerve o, outer capsule H&E x400.

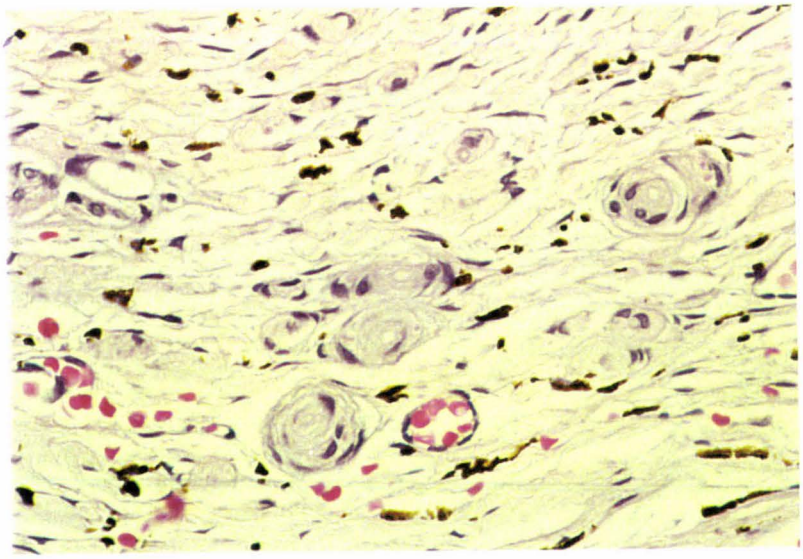


**Figure 6-11.** Section of trabecular meshwork of long-finned pilot whale (E425-95) H&E x400.

Figures 6-12 a,b,c,and d show staining characteristics of an ER in a pygmy sperm whale (E413-95) with H&E, Van Gieson, Masson's trichrome and Holmes' silver stains. The silver stain clearly demonstrates that an axon is present centrally.

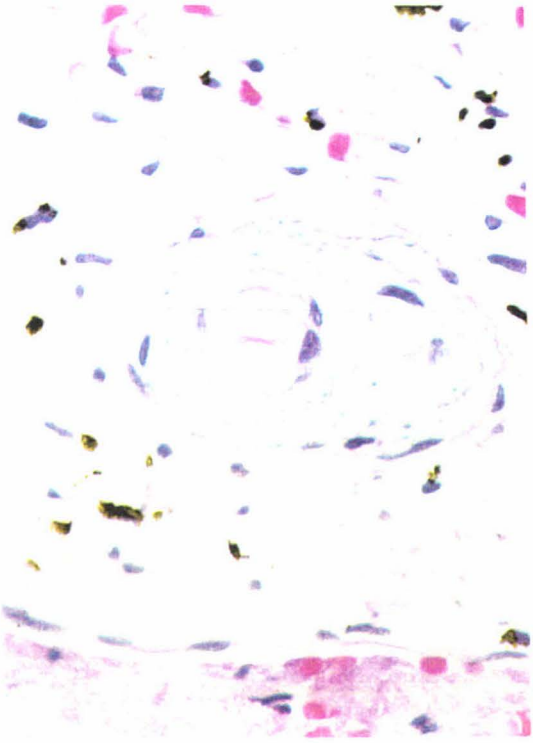


Figure 6-12a. Single ER with characteristic eosinophilic core and basophilic capsule H&E x400.

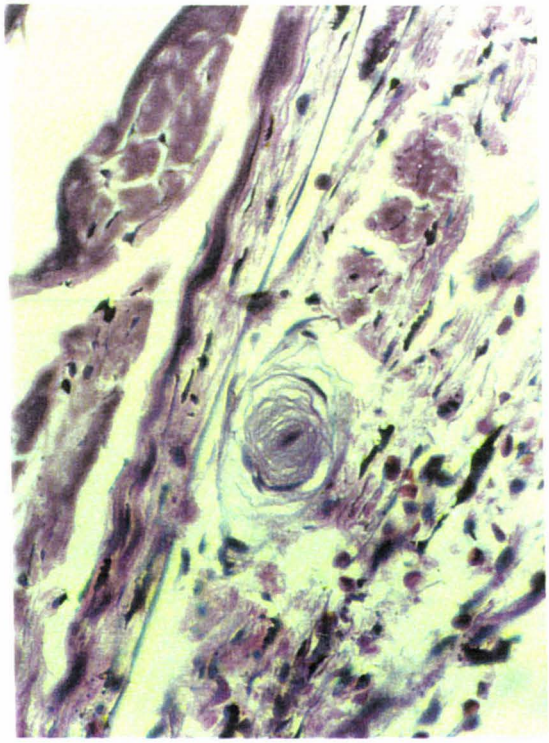


Figure 6-12b. Holmes' silver x280.

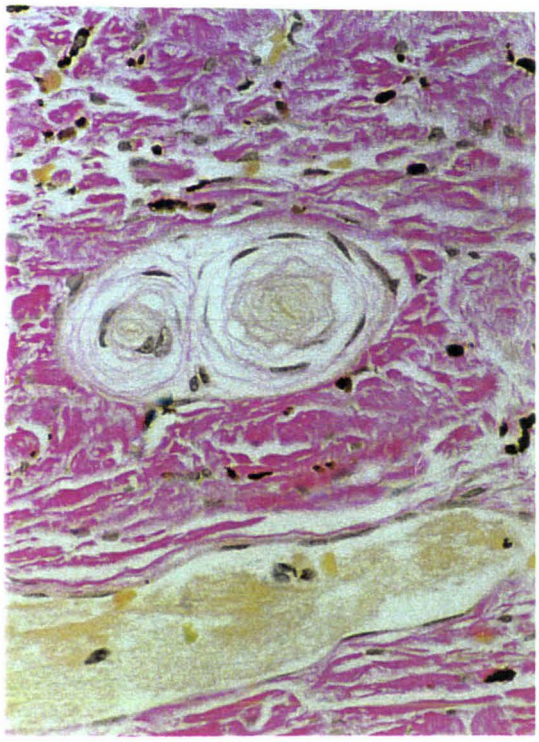


Figure 6-12c. Van Gieson stain x400.

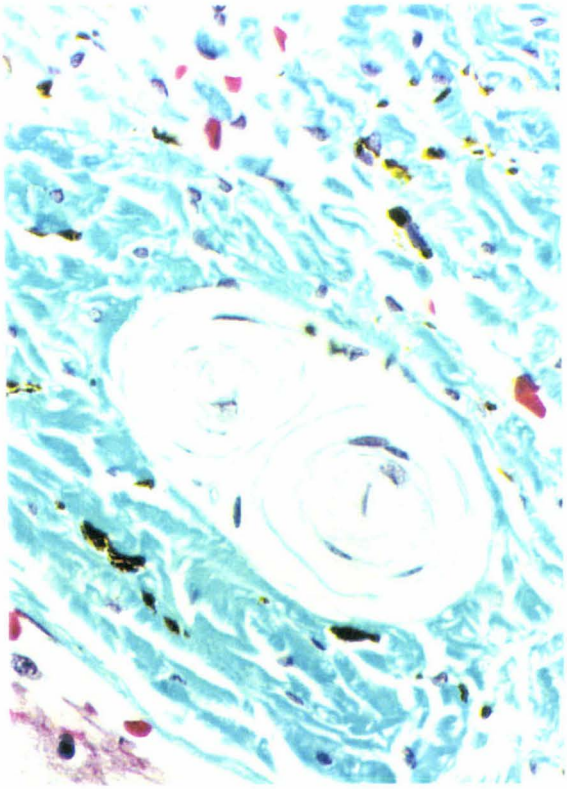


Figure 6-12d. Masson's trichrome x666.

In some instances, longitudinal sections of considerable length were fortuitously obtained (Figures 6-13 and 6-14). The ERs which were arranged in groups within a common perineurium resembled myelinated nerve, but ERs were of larger overall diameter than myelinated axons due to multiple concentric cytoplasmic lamellae around each axon. The larger numbers of nuclei associated with the core also differentiated these from myelinated axons. Differences in appearance between groups of lamellated receptors and myelinated nerve in longitudinal and cross sections were demonstrated using Holmes' silver stained (Figures 6-15 a, 6-15b, 6-16a, 6-16b) sections from the ciliary body of a Cuvier's beaked whale (E15-97) x280.

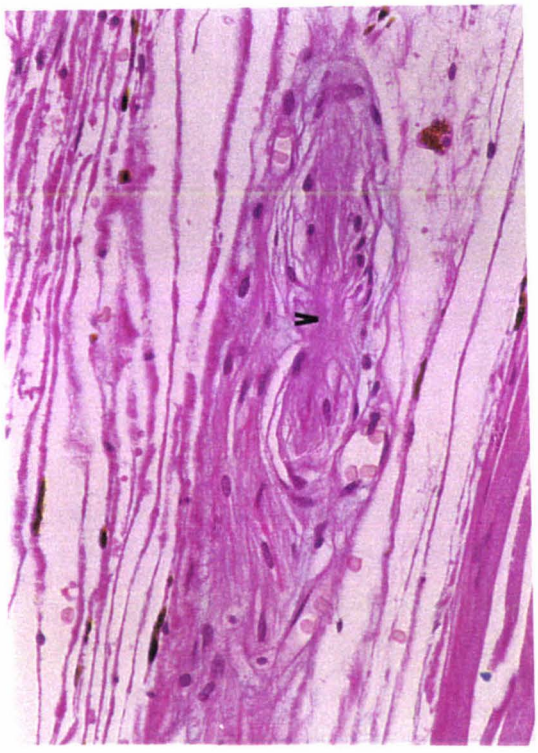


Figure 6-13. Longitudinal receptor from a Cuvier's beaked whale (E15-97). The outer capsule remains intact centrally (arrow), giving a tunnel-like appearance. H&Ex280

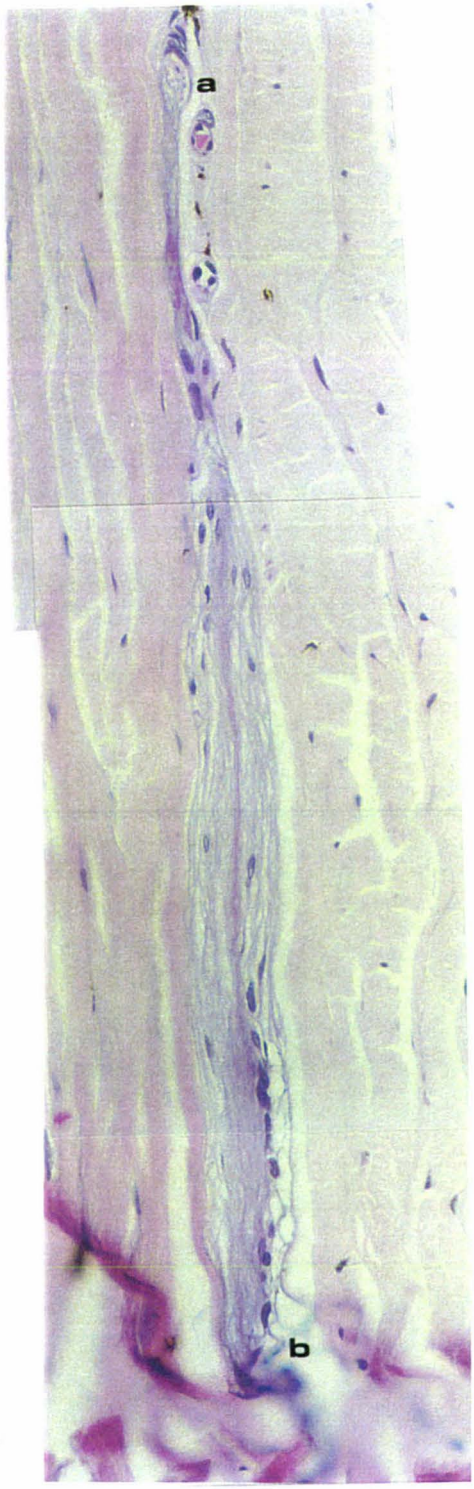



Figure 6-14. Longitudinal section of an ER from a pygmy sperm whale (E413-95). The receptor measures 800 microns from a to b. H&E x200.

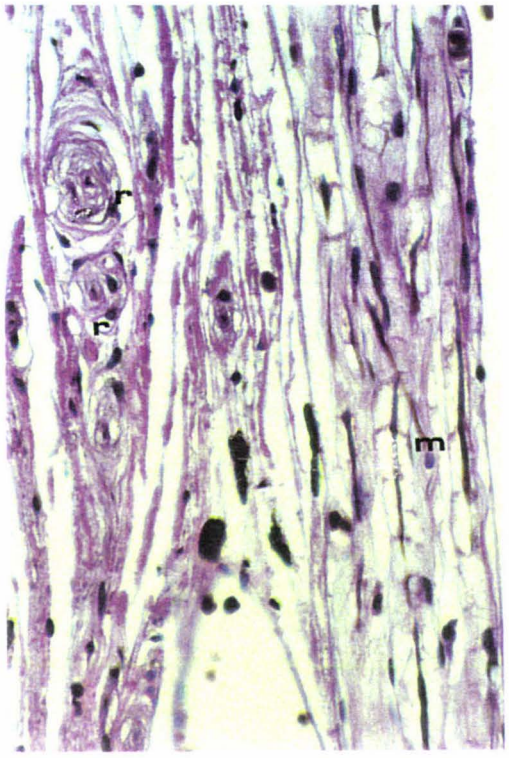



Figure 6-15a. Myelinated nerve in longitudinal section. r, receptor m, myelinated nerve. Silver x280

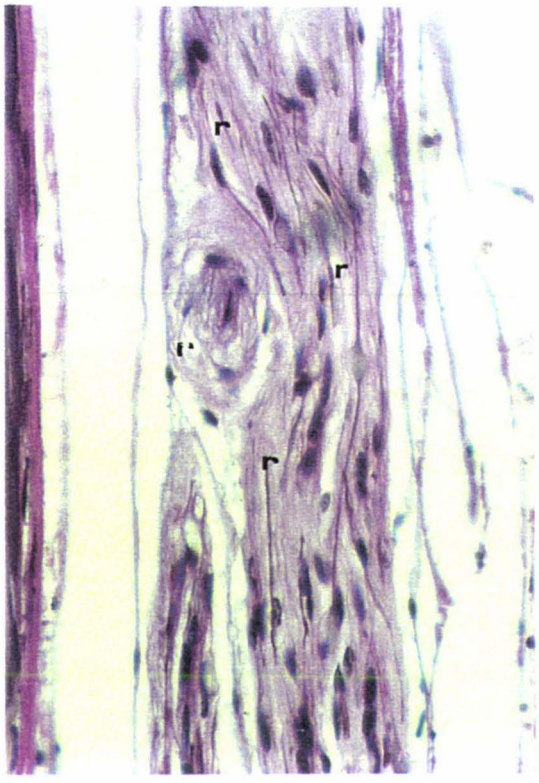



Figure 6-16a. Group of receptors in longitudinal section. Silver x280

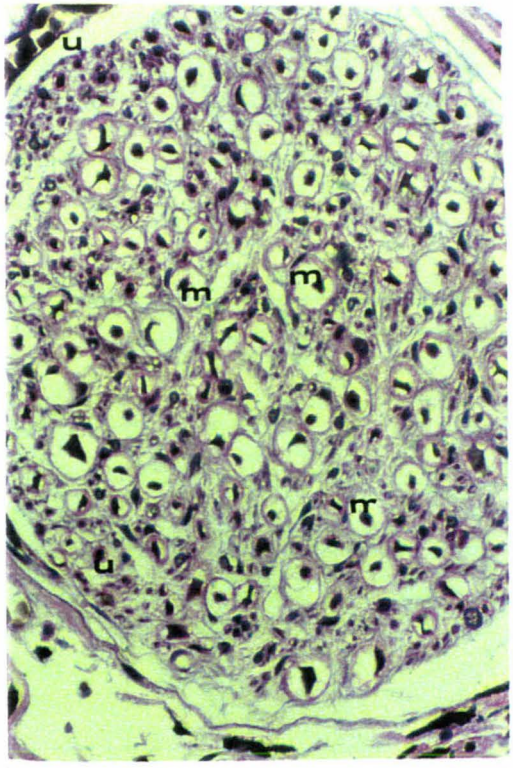



Figure 6-15b. Cross section of mixed, mainly myelinated nerve.
m, myelinated axons;
u, unmyelinated axons. Silver x280

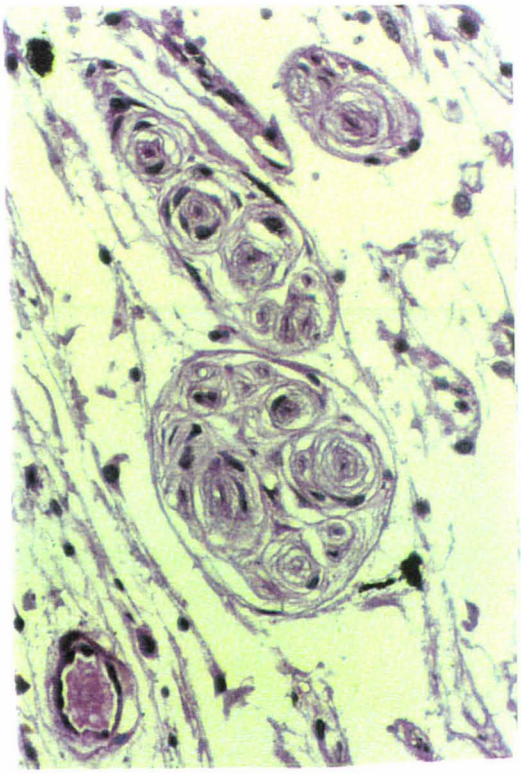


Figure 6-16b. Cross section of a group of encapsulated receptors. Silver x280

## Type 3 Receptors

These were less than 25 microns in diameter with a dense appearance. Inner and outer cores were not clearly distinguishable. This type only occurred in dolphins and was only present in one of the three dolphins examined (27865-97). They consisted of thick, densely packed, eosinophilic lamellae, resembling an inner core, but with little evidence of an outer capsule (Figures 6-17a and 6-17b).

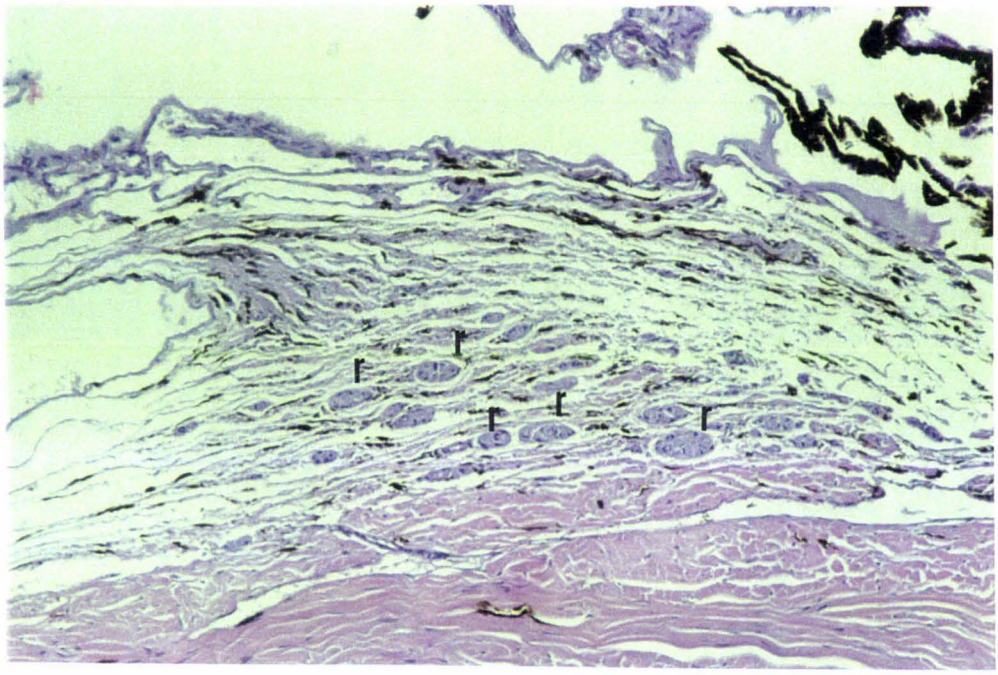



Figure 6-17a. Type 3 receptors in ciliary body of dolphin 27865-97. A large number of groups of receptors can be seen r, receptors. H&E and alcian blue x120

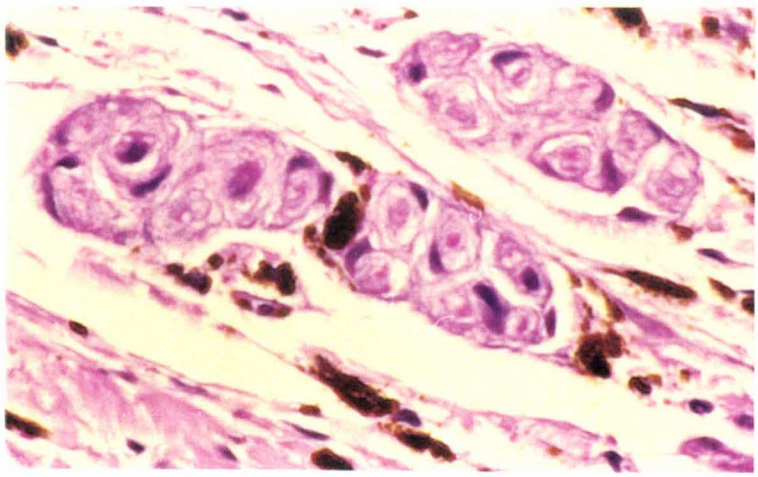


Figure 6-17b. High magnification of a group of receptors from Figure 6-17a H&E x800

## Type 4 Receptors - Sperm Whale

The sperm whales examined had ERs which appeared to be atypical in appearance, due to i] grouping within large, nerve like structures (Figures 6-18 and 6-19) ii] extensive amounts of myxomatous material between individual ERs iii] an inner core which often contained several nuclei and iv] an outer capsule which was diffuse.

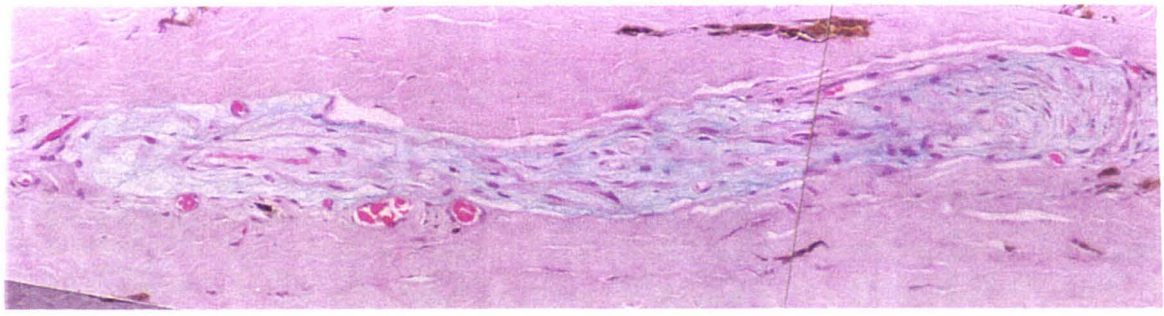


Figure 6-18. Group of encapsulated receptors from sperm whale E90-97 in dense sclera. H&E and alcian blue x40

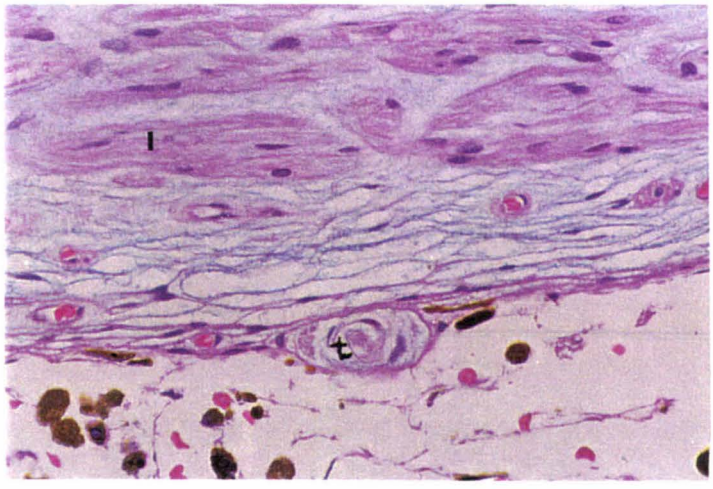



Figure 6-19. Group of lamellated receptors from sperm whale E90-97 l, longitudinal section t, transverse section H&E and alcian blue x280

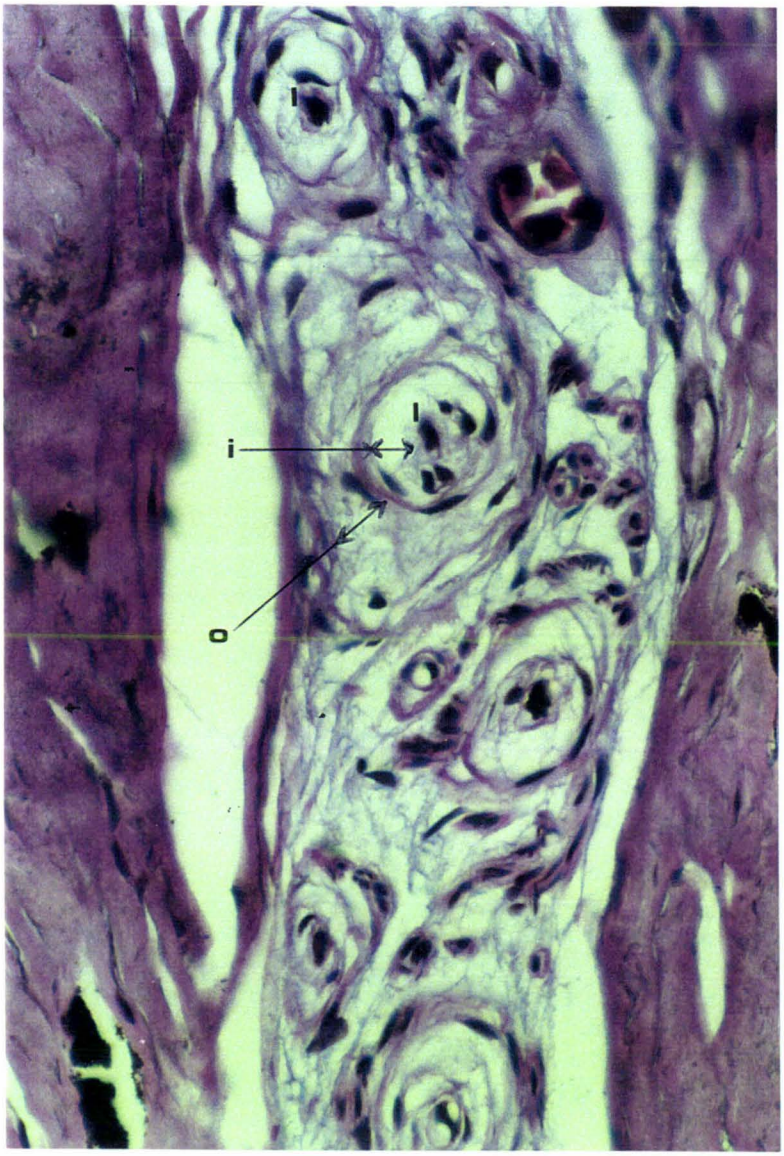
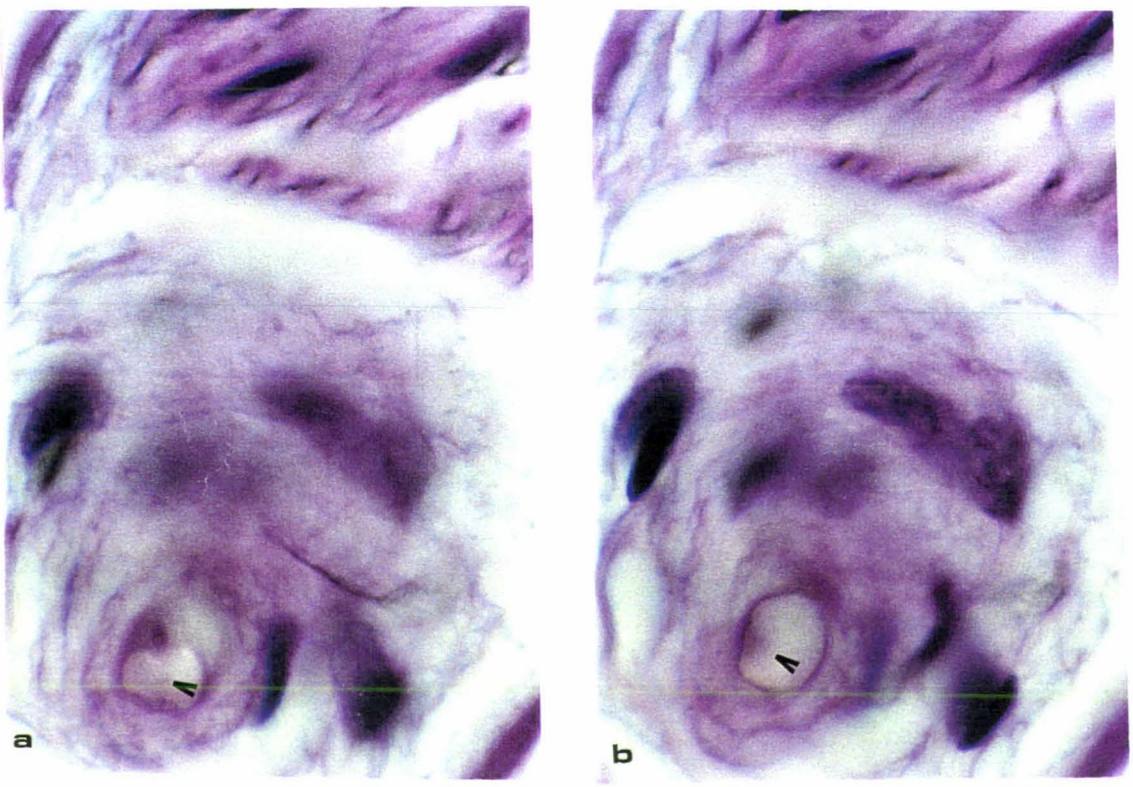


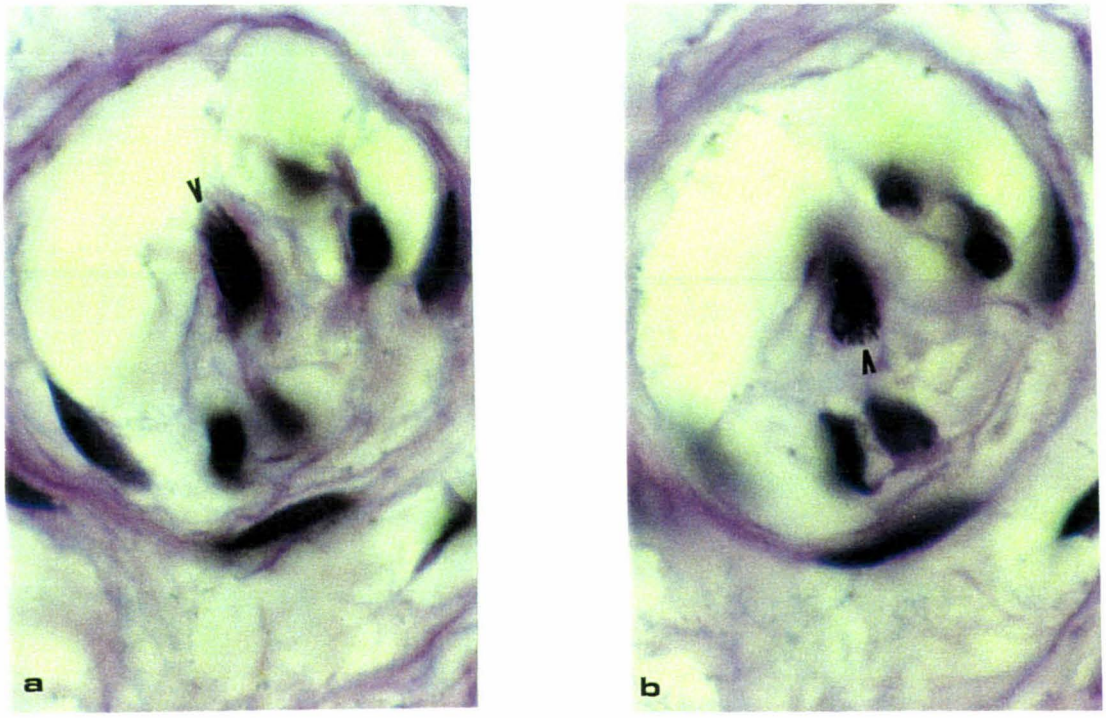

Figure 6-20. Type 4 ERs in the ciliary body of sperm whale E90-97.
u, unmyelinated axons;
l, large axon; e, encapsulated receptor;
o, outer capsule; i, inner core
Holmes'silver x400


Figure 6-21. High power view of group of receptors the in the ciliary body of a sperm whale showing a wide range of axon sizes
u, small, unmyelinated axon
l, large axon. Holmes' silver with oil immersion x1400

Centrally, an ovoid or circular area, devoid of stain, was often seen. This was interpreted as an artefactual hole produced by the loss of the axon from the area (Figures 6-22a and b). In some sections, an eosinophilic structure, interpreted as an axon, occupied the central area. Holmes' silver stain was used to confirm the argentophilic nature of this structure (Figures 6-23a and b).




Figures 6-22a and b. A sperm whale ER, focused at different planes to illustrate the 'hole' or 'tunnel' ( ➤ ) that occurs in some cases. This is probably an artefact due to an axon vacating the space. Oil immersion x1400




Figures 6-23 a and b. Sperm whale ER stained with Holmes' silver and focused in deep and superficial planes. The neurofilaments have taken up the stain and show tasselled edges at the cut surface of the axon ( ➤ ). Oil immersion x1400

### 6.4v Encapsulated Receptors in Other Sites

In whales where eyelids were sampled, ERs were evident. The histology of ERs in this site was similar to those seen in the ciliary body (Figure 6-24).

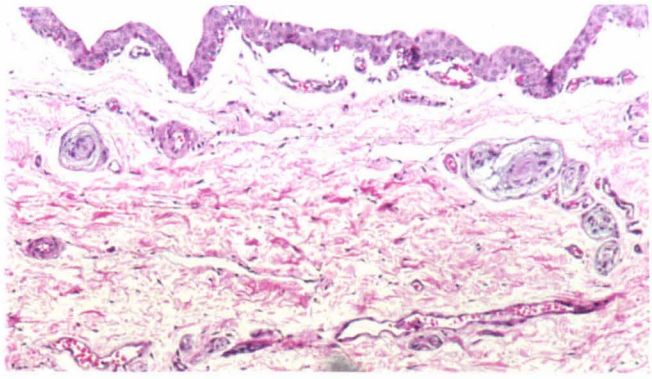


Figure 6-24. Conjunctival surface of eyelid with dermal ERs. H&E x80

In long-finned pilot whales E432-95, E425-95, receptors similar to those seen in the ciliary body were seen in the dermis adjacent to the blowhole, closely associated with epidermal pegs (Figures 6-25 and 6-26).

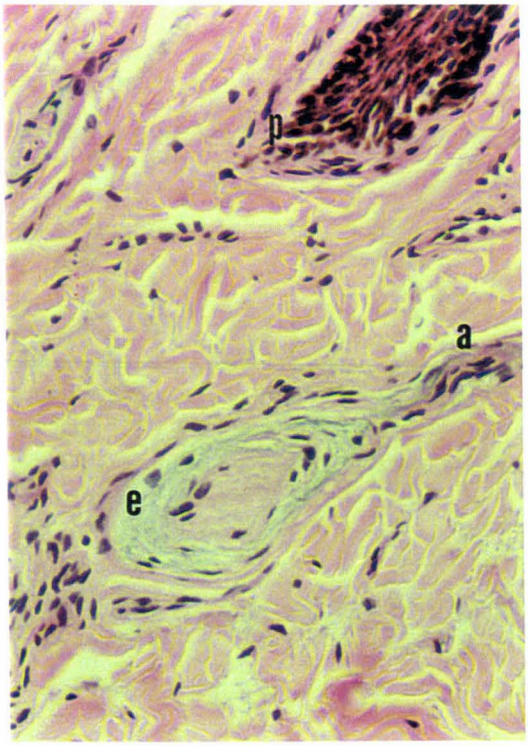



Figure 6-25. Encapsulated receptor in dermis of anterior blowhole skin of a long-finned pilot whale. An axon can be seen supplying this receptor. a, axon e, encapsulated receptor H&E x400

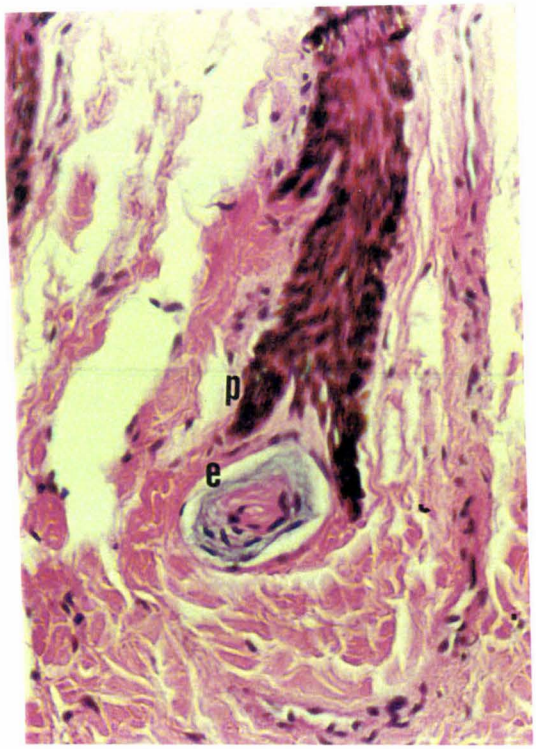



Figure 6-26. Small ER at the tip of an epidermal peg in a long-finned pilot whale. a, axon p, peg H&E x400

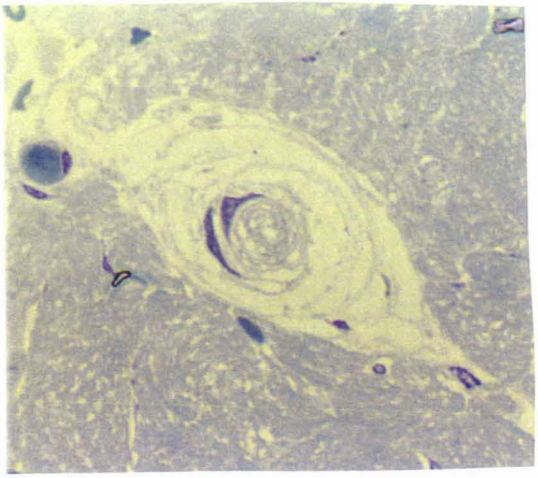
## 6.5 ULTRASTRUCTURE OF ENCAPSULATED RECEPTORS

Of the six whales' eyes embedded for electron microscopy, only two whales had ERs which were sufficiently well preserved to allow detailed ultrastructural examination. These were; sperm whale E90-97 in which four encapsulated receptors were observed and long-finned pilot whale E432-95 in which one structure that consisted of a mixed group of lamellated axons and encapsulated corpuscles was studied.

The general structure of ERs consisted of concentric lamellae dervived from fibroblasts and Schwann cells. There were fine collagen fibrils between the lamellae, which were either sparsely or densely packed. Axons were not always distinguishable, but may have been pulled out during processing, as appeared to have occurred in some specimens prepared for light microscopy. Artefactual holes were commonly seen between the lamellae. Five receptors were examined in detail.

*Receptor 1 - Sperm Whale E90-97*

Examination of thick (0.5 micron) epoxy resin embedded sections stained with Toluidine blue (Figure 6-27) revealed a large encapsulated receptor with distinct inner and outer cores embedded in the dense connective tissue of the sclera. This receptor consisted of loose lamellar structures peripherally, which were devoid of material between the lamellae, and more densely packed lamellae centrally (Figure 6-27a). The elongated nucleus of an attenuated spindle cell resembling a fibroblast was also visible to one side of the central core. There appeared to be large numbers of collagen fibrils between the lamellae, however large numbers of artefactual spaces were also visible, presumably the result of autolysis.



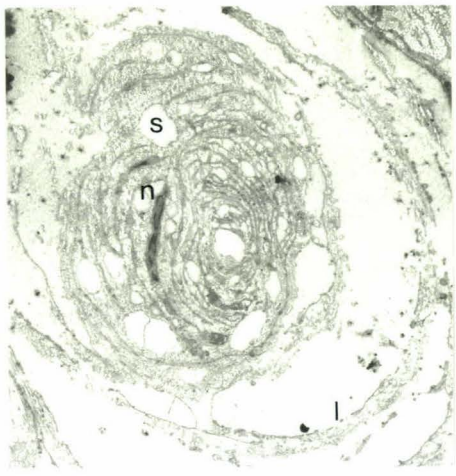



**Figure 6-27a.** Light micrograph of a single ER in sperm whale E90-97. The inner core appears considerably more dense than the outer capsule. Epoxy resin embedded Tol. blue x800.

**Figure 6-27b.** Electron micrograph of the same ER. s, artefactual spaces; n, fibroblast nucleus; l, lamellae of outer capsule. TEM x 2,300 .

At higher magnification, the vacuous nature of the interlamellar spaces was evident (Figure 6-28). Centrally, a structure which has been interpreted as an axon was visible within an artefactual space. Closely packed, concentric lamellae surrounded the axon, giving the appearance of a symmetrical encapsulated receptor.

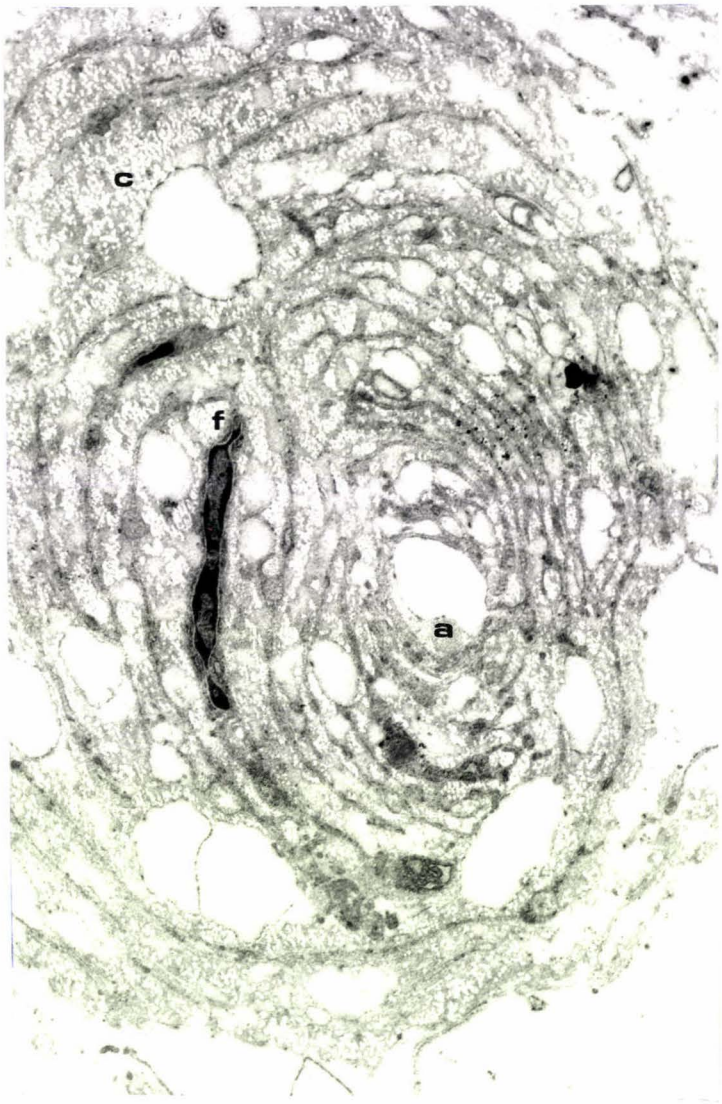



**Figure 6-28. Inner core of an ER in a sperm whale. Collagen fibrils are visible as small bright white spots between the lamellae, more easily seen against areas of darker background. Many artefactual holes are present. Lamellae are artefactually broken and distorted. a, axon; c, collagen fibrils; f, fibroblast nucleus. TEM x 6,800 .**

*Receptor 2 - sperm whale E90-97*

This consisted of extremely thin lamellae, which were little more than plasmalemma and a thin layer of cytoplasm, devoid of organelles, forming concentric arcs. Dense collagen fibrils could be seen externally, with small numbers of fine fibrils also visible between the lamellae (Figure 6-29).

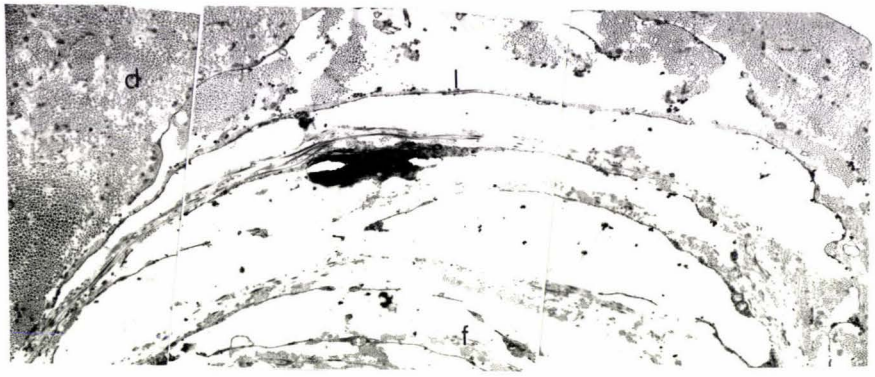



**Figure 6-29. Outer capsule area of an ER from sperm whale E90-97. l, lamella; d, dense collagen of sclera; f, fine sparse interlamellar collagen. TEM x800**

*Receptor 2- sperm whale E90-97*

This receptor contained two closely lamellated axons. An accompanying nerve with three myelinated axons possibly supplied the structure. The receptor axons within the ERs contained neurofilaments, neurotubules, and several mitochondria. Nuclei surrounding the structure were round or ovoid in form, and resembled Schwann cell nuclei. Interlamellar areas contained sparse collagen fibrils (Figure 6-30). There did not appear to be an expanded outer capsule in this specimen .

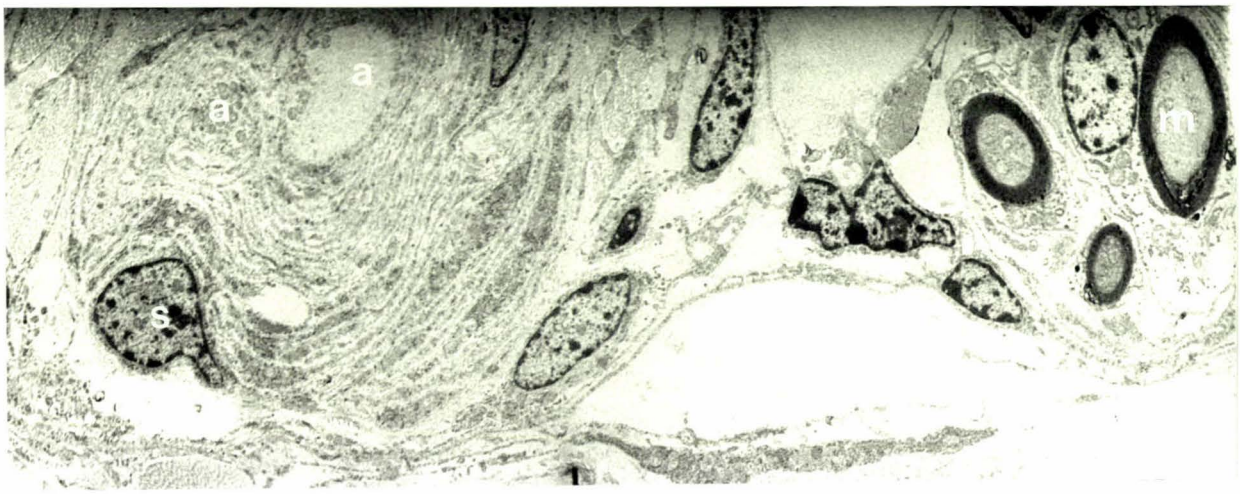



**Figure 6-30. Mixed nerve from sperm whale E90-97 showing ERs 'e' within its structure. a, axon; m, myelinated axons; s, Schwann cell nuclei. TEM x 6,800**

At higher magnification, mitochondria and neurofilaments/neurotubules were seen (Figure 6-31).

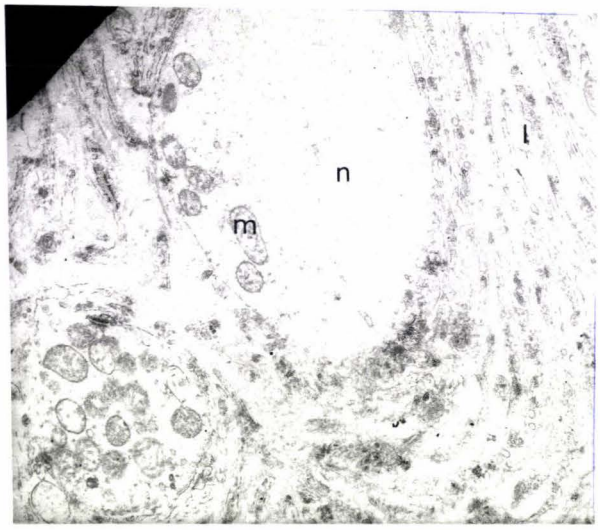



**Figure 6-31. Encapsulated receptor from sperm whale E90-97 m, mitochondria; n, neurotubules/filaments; l, lamellae. TEM x10,500**

*Receptor 3 - sperm whale E90-97*

In this specimen a large melanocyte was situated at the periphery of the outer lamellae. Two nuclei were present within the lamellar area. The nuclei resembled those of a fibroblast, because of their elongated shape and large, dark areas of euchromatin. There was an abundance of fibrillar collagen between the lamellae. There were three large interlamellar holes, which may have been artefactual or may have accommodated axons. A myelinated axon, and a capillary were also visible (Figures 6-32 and 6-33).

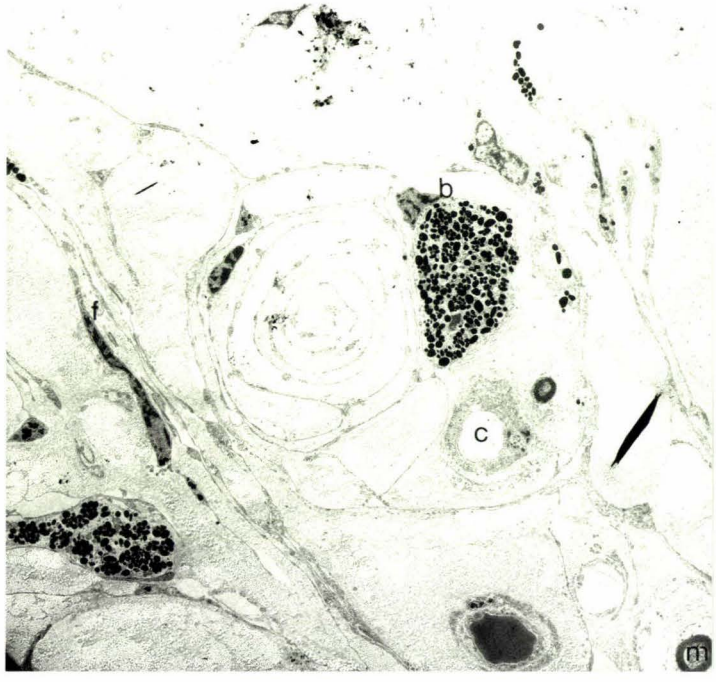



**Figure 6-32. Encapsulated receptor from sperm whale E90-97. b, melanocyte; m, myelinated axon; f, fibroblast nucleus; c, capillary. TEM x 2,300**

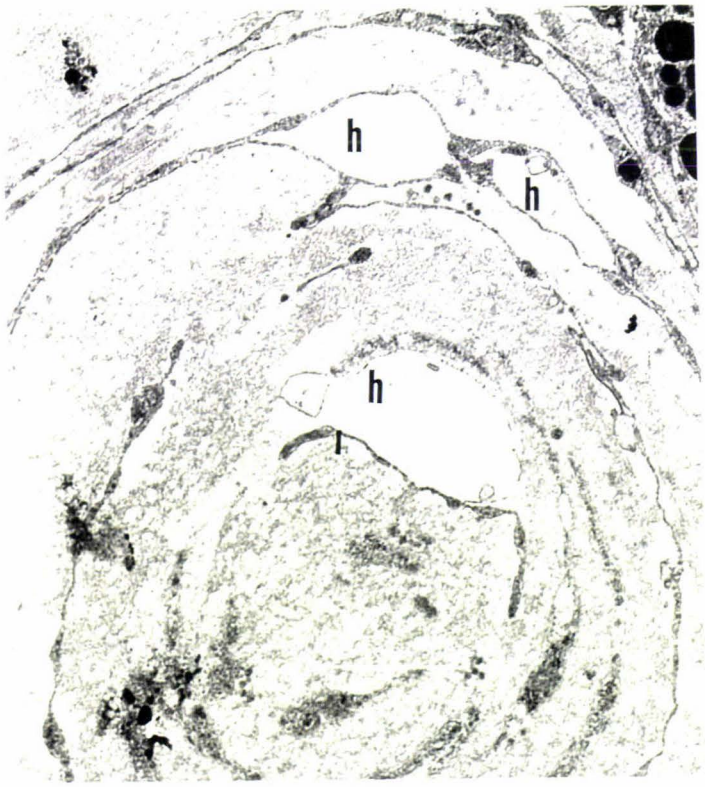



**Figure 6-33. Higher magnification of ER in Figure 6-32. Holes containing no structures between the lamellae have been interpreted as artefacts. h, hole; l, lamella. TEM x 10,500**

*Receptor 4 - sperm whale E90-97*

Lamellae did not have a concentric circular form in this receptor. Four nuclei within the lamellar area resembled fibroblast nuclei. Two structures resembling axons were visible and there was an abundance of collagen fibrils. The non-symmetrical and poorly lamellated appearance of this receptor suggested that it may have been a Ruffini-type structure (Figures 6-34 and 6-35).

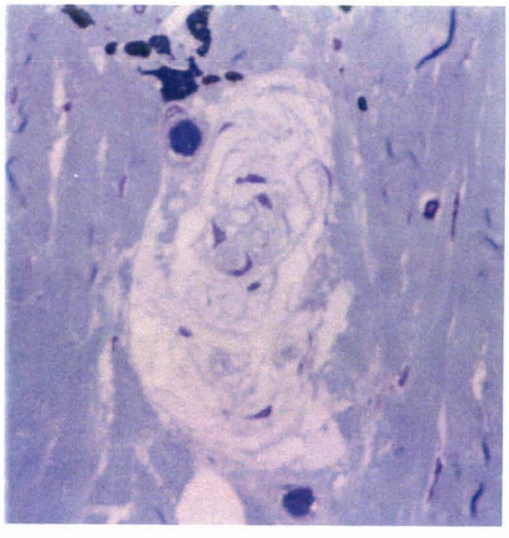


**Figure 6-34. Receptor from sperm whale E90-97 showing non-symmetrical form. Thick epoxy section Toluidine blue x400**

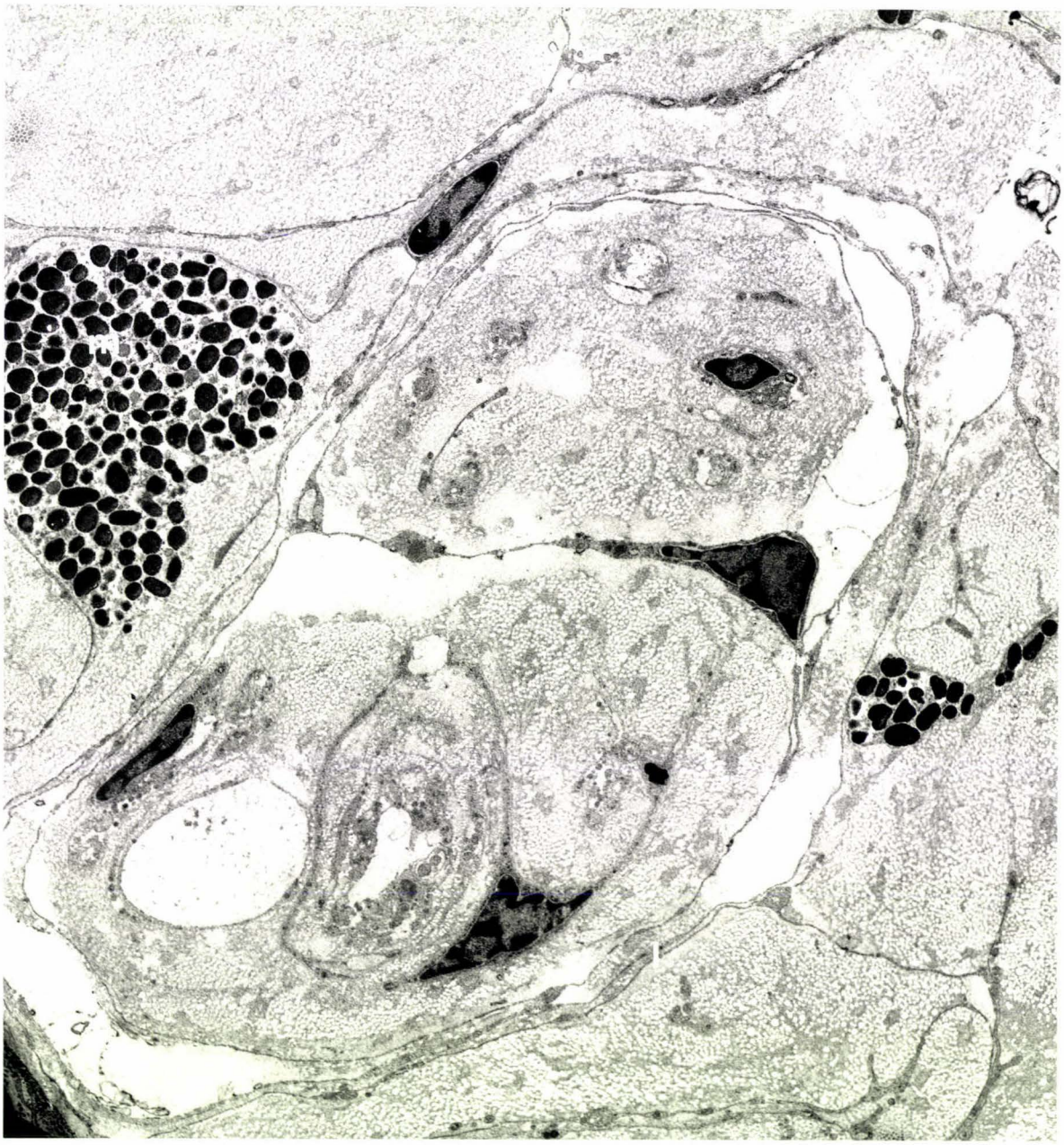


Figure 6-35. Higher magnification of a non-symmetrical, Ruffini type receptor. A few non-concentric, folding lamellae can be seen. a, axon l, lamellae m, melanocyte. TEM x 6,800

*Receptor 5 - long-finned pilot whale E432-95*

This structure occurred in a long-finned pilot whale. It consisted of a mixed nerve containing a large number of nuclei of two types, three myelinated axons, and a non-concentric lamellar pattern with an abundance of interlamellar collagen fibrils (Figure 6-36).

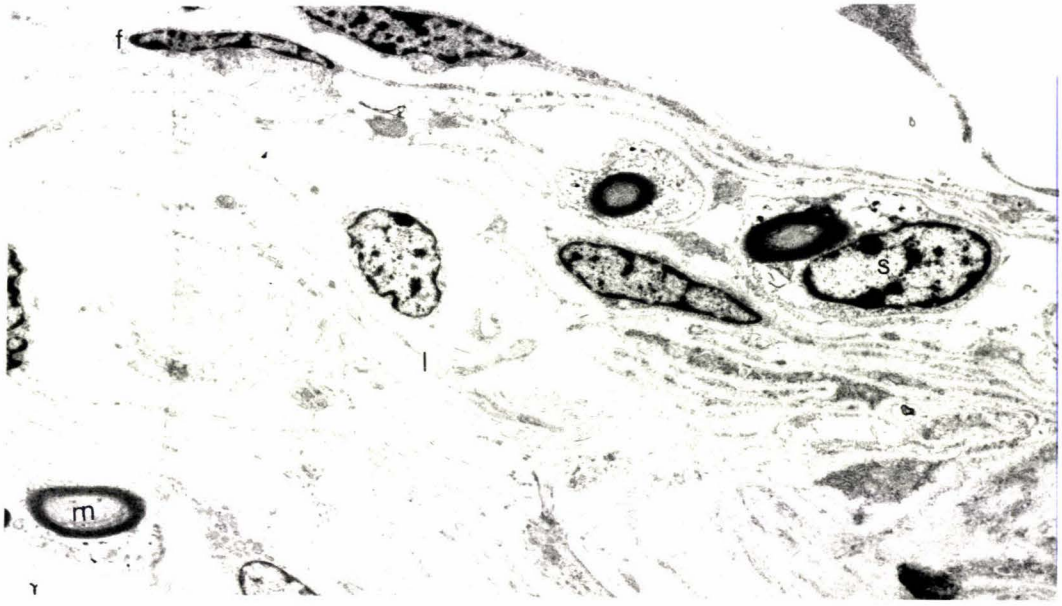



Figure 6-36. **Mixed nerve containing lamellated receptors. f, fibroblast nucleus l, lamellae m, myelinated axon s, Schwann cell nucleus. TEM x4,600**

## 6.6 DISCUSSION

The histological and ultrastructural study found encapsulated receptors in the ciliary bodies of 18 out of 20 whales examined, with dolphins being the only specimens in which observations failed to reveal any ERs. This confirms the findings of Wickham (1980), who found that ERs were present in all but Spinner dolphins. In the present study, Cuvier's beaked whales, pygmy sperm whales, and common dolphins were examined, in common with Wickham's study (1980), which also examined Amazon river dolphins, atlantic bottlenosed dolphins, spinner dolphins and spotted dolphins, a beluga whale and Sowerby's beaked whale. The current study also examined baleen whales, sperm whales, Gray's beaked whales and long-finned pilot whales.

The presence of ERs in eyelids has not been described previously in whales, but similar encapsulated receptors (Krause end bulbs) are well known in this site in humans (Burkitt et al. 1993; Munger 1988; Chouchkov 1986). The current finding of ERs around the blowhole in a long-finned pilot whale complements similar findings in a dolphin and false killer whale (Bryden 1982). Similar ERs have been described in the nasal diverticula of dolphins (Khomenko 1970) and in skin (Palmer and Weddel 1964). A very small number of skin samples from the body in the present investigation in three whales failed to reveal any ERs.

### Topographical Study

The main aim of the topographical study was to demonstrate that the structures studied were nerve endings, and not merely neurons with a Schwann cell wrapping 'en route' to other structures in the anterior segment of the eye. The material available from these whales was less than optimal, in most cases it was more than 24 hrs old before fixation occurred. Autolysis that was not visible with the light microscope became evident with the electron microscope, and only material from two whales was of suitable quality for detailed ultrastructural study. The topographical study revealed that the receptors in this *kogia* (pygmy sperm whale) were slightly shorter at 84-164 microns than longitudinal and radial receptors found in *kogia* in Wickham's 1980 study (175 microns). However, receptor diameters were much greater in the current study (up to 100 microns in one minke whale) than Wickham's findings, where 50 microns was the greatest diameter, found in a pygmy sperm whale.

### Sites of Occurrence

The corneoscleral zone (CSZ) and uveal zone areas were both of substantial size in all cetaceans apart from the dolphins, and CSZ was important as a site for ER occurrence. This is in contrast to Wickham's study (1980), which described the CSZ as small compared to the large uveal zone, and this was supported with an

electronmicrograph of the iridocorneal angle in Stenella *attenuata*. Wickham's "posterior collagen pad" as described in the *Delphinus* and *Tursiops* may, in our study, have been classified as CSZ. There may have been other differences in classification of zones which led to this discrepancy (see Chapter 5 for trabecular zone classification).

Wickham's description of the close association of ERs to aqueous outflow channels was not confirmed in this study by subjective observation but in some cases there was an association with blood vessels.

Siting is likely to be significant with respect to function, for detection of compressive or stretching forces, and mechanical deformity. Scleral and limbal sites are more likely to be stimulated by stretching or deformity from external forces such as extraocular muscles and eyelids, or distortions of the cornea if intraocular pressure rises. Recently, small mechanoreceptors similar to visceral stretch receptors have been discovered in the human limbus (Tamm et al. 1994). It has been suggested that they monitor tensile strength in the collagen and thus possibly intraocular pressure. Usually, Ruffini type corpuscles are responsible for this type of monitoring and can be found in dense collagen sites such as joint capsules (Halata 1976; Halata and Munger 1980). Pacinian corpuscles have also been found in joint capsules but are situated in the less dense collagen of the stratum synoviale/stratum fibrosum junction (Halata 1976). Trabecular sites are more likely to be useful for monitoring compression from intraocular sources.

Orientation of Receptors

Orientations were found to be site-dependent. In the trabecular meshwork, ERs could be longitudinal or latitudinal, but in the sclera, they tended to be longitudinal. This confirms Wickham's previous findings (1980).

All animals in both studies had latitudinal (circumferential) receptors, but longitudinal (meridional) receptors were not evident in *Tursiops* or Stenella (Wickham 1982) or *Delphinus*, *Caparea*, and *Globiocephala* in the present study. The third orientation described by Wickham was equatorial, or perpendicular to the axis. This orientation occurred in most species. However, the present study rarely detected this orientation because a tangential plane of sectioning was unfortunately not included in the protocol.

Histology of Receptors

Light microscopy enabled the receptors to be divided into four broad groups, based mainly on size but also, to a lesser extent on histological structure. The only species which could not be classified in this way was the sperm whale, which was considered 'atypical'.

In each receptor, an outer capsule was present, with basophilic staining properties, and large spaces between the layers. This suggests that proteoglycan material or DNA-rich structures such as rough endoplasmic reticulum and free

ribosomes are present. This is not suggestive of a typical Pacinian corpuscle, in which the outer capsule, has elongated perineural cells which stain eosinophilically, and has very narrow spaces of 300-800nm between the layers (Chouchkov 1978). Nuclei were frequently present in the outer core, which is typical of Pacinian corpuscles.

Internal to the spacious outer capsule, a nuclear layer was typically present in receptors. This has been interpreted as the endoneurial compartment as described by Chouchkov (1978), containing fibroblasts and macrophages. Macrophages are believed to protect the neuron by collecting foreign material from its cytoplasm in endocytic vesicles. There is a cleft in the inner core which permits their contact (Munger and Ide 1988).

The inner core stained eosinophilically and was devoid of nuclei centrally, but they were present peripherally. This is typical of the inner core of Pacinian corpuscles, where Schwann cell nuclei are seen peripherally, giving rise to concentric circular lamellae. An axon was frequently present centrally, though not always.

Light microscopy suggested that most of these receptors were a symmetrical type, such as paciniform corpuscles but with an expanded outer capsule in the larger Types 1 and 2, possibly no capsule at all in Type 3, and considerable amounts of myxomatous tissue in Type 4 (atypical).

Although the structure of Pacinian corpuscles has been recognised for many years, recent studies have elucidated ultrastructural details (Munger et al. 1988; Ide et al. 1988). The outer core consists of thin cells that concentrically encircle the inner core, and each cell layer is separated by a connective tissue compartment which contains scattered collagen fibrils and ground substance. Inner layers of the outer capsule are coupled with tight junctions (Ide and Hayashi 1988). Tight junctions function to establish barriers in epithelial systems and would thus prevent ionic flow from inner to outer cores. The outer core is merely an expanded perineural compartment and the inner core is an expanded endoneurial compartment. The capsule is clearly continuous with the perineurium of the nerve trunk innervating the corpuscle (Shanthaveerappa and Bourne 1963).

By contrast, the inner core is, according to Munger and Ide (1978), remarkable in having numerous gap junctions between the lamellae which are limited to each half. Since gap junctions are the cellular basis for low resistance between cells (Loewenstein et al. 1978), they concluded that the halves are separately coupled. The close proximity of a nerve in Cuvier's and sperm whales to aggregates of ERs may indicate that the ERs start within the nerve and branch off in groups, which may eventually separate into single ERs.

Ultrastructure of Receptors

Electron microscopy in the present study revealed that at least two types of ER exist; symmetrically and non-symmetrically lamellated, containing either one or several axons. In addition to the paciniform receptors described above from light micrographs, the non-symmetrical encapsulated receptors have been interpreted as Ruffini corpuscles.

Ultrastructural studies of ERs in cetaceans by Bryden (1982) and Wickham (1980) have confirmed the presence of some of the characteristic ultrastructural features of Pacinian corpuscles; an unmyelinated receptor axon linked by desmosomes to the surrounding Schwann cell lamellae, an inner core of 5-7 layers and an outer capsule of up to 6 layers Bryden (1986). The inner core and outer capsule did not appear to exceed 50 microns in total diameter (Wickham 1980).

Possible Functions of Encapsulated Receptors

The exclusive presence of encapsulated receptors in the ciliary bodies of the order cetacea, raises questions about their functional significance and how their site, orientation and histology might affect this function. Suggested functions have been to monitor intraocular pressure and external forces on the sclera/cornea (Wickham 1980). How IOP might vary has been already postulated within discussion of the vascular engorgement theory (Chapter 5). Why IOP might vary is postulated as:- i) part of an accommodative mechanism (Chapter 5) ii) for pooling of blood in the uveal tract to act as a venous rete, for temperature regulation and oxygen supply iii) as an external pressure monitor, or iv) as a means of triggering the dive reflex.

The eye is uniquely vascularised to enable it to maintain arterial and venous blood pressures above intraocular pressure, which is higher than normal tissue pressure, in order to avoid venous stasis. It must therefore have an independent means of monitoring blood pressure and intraocular pressure, so an additional role of ERs as a pressure gauge for external pressure is a hypothetical possibility. This information could give the animal an indication of its depth underwater.

The dive reflex is of special importance to aquatic mammals. The primary stimulus in humans and ducks for activation of the dive reflex is wetting of the facial or bill skin (Gooden 1994). Face wetting (trigeminal stimulation) and lung volume, with cessation of breathing, are thought to lead to the bradycardia associated with diving (Dykes 1974). In addition, the oculocardiac reflex (eye pressure leading to bradycardia) may be of some relevance in cetaceans, though in humans external pressure on the eye does not appear to be proportional to the degree of bradycardia (Risavi *et al.* 1983).

There may also be a thermosensitive trigger for the dive reflex in ducks, whose encapsulated receptors, known as Herbst corpuscles are thought to have a thermosensitive function (Necker 1974). Since the ERs around the blowhole, in

the eyelid and at the iridocorneal angle of cetaceans appear to have similar histology to these, perhaps they have a similar function (ie. associated with the diving reflex and subsequent autonomic responses). The intraoral rete (Ford 1992) seems most likely to have ERs with a thermosensitive function, since the purpose of this rete appears to be to present venous blood for cooling at an uninsulated peripheral site, in a similar way to fin function. The concept of parallel processing (Munger and Ide 1988) in which the same structure is multiply innervated by several different types of axon terminals) and the concept of polymodality (one axon senses several modalities) suggests that within one site, ERs could have thermosensitive and/or pressure sensitive roles.

One of the most striking features of many of the ERs examined, in particular in the sperm and minke whales, was the amount of myxomatous material present between the lamellae, unlike paciniform receptors in other species. This appearance was reminiscent of Renaut bodies (Summers *et al.* 1995). Renaut bodies occur in response to nerve compression as a means of 'cushioning'. In addition, some demyelinating diseases produce an 'onion bulb' response, where concentric arrays of Schwann cell processes arrange around a central axon with endoneurial collagenous fibrillogenesis and perhaps to intrafascicular accumulations of Renaut-body-like mucoid tissue as well (Summers *et al.* 1995). Myxomatous material therefore appears to have a key role in mechanical, and maybe other forms of protection for vulnerable nerves. In humans, who free dive or SCUBA dive, and experimentally in other species, the neurological risks encountered in diving are associated with nitrogen narcosis, oxygen toxicity, high pressure nervous syndrome, decompression sickness (DCS), arterial gas embolism (AGE), and brain anoxia. Adaptive mechanisms including compressible air spaces, change in blood flow to tissues, increased blood volume with reservoirs in spleen and venous circulation, bradycardia to reduce myocardial workload, increased packed cell volume and mean corpuscular haemoglobin content and myoglobin capacity, exceptionally good buffering capacity at low pH, improved oxygen dissociation for unloading at tissues, decreased metabolic rate, resistance to hypercapnia (Harrison and Kooyman 1971; Schmidt-Nielson 1990; Kooyman 1989) have evolved to avoid these effects in natural divers. However, it is thought that DCS may occur, particularly in species which perform serial shallow dives, where the aetiology is time as well as depth dependant (Kooyman 1989). The pathogenesis of DCS involves bubble formation within tissues causing pain and itching, and within vessels where they may act as space occupying lesions and cause infarction, particularly in the central nervous system, leading to pelvic girdle pain, ataxia and unconsciousness (Greer and Massey 1996). Often the degree of pathological change is not reflected in clinical signs (Palmer 1987). The pathogenesis of high pressure nervous syndrome (HPNS) is presently unknown, but is thought to involve dysfunction of neurotransmitters and is clinically similar to 'serotonin syndrome' (Jain 1994). It is not known if this occurs in

naturally diving mammals. However, since neurological tissue is most at risk from the adverse effects of diving, it seems likely that protective mechanisms in addition to those adaptations already mentioned will be present, and that myxomatous tissue around axons may fulfil this role, either mechanically, or as a non-foaming tissue sink for gases (particularly nitrogen). Further study in this area could be fruitful.

## CRITIQUE

In order to confidently extrapolate the findings of the topographical study to every nerve tissue in this site with a similar appearance, more specimens, and more exhaustive studies would need to be undertaken.

In particular, the ERs in the subconjunctival site chosen may not be typical of ERs in the trabecular meshwork.

One of the difficulties experienced in this study was effective recording of the variable appearance of consecutive sections. Although the serial diagram technique (this study) and serial photography technique (Wickham 1980) appear clumsy and time consuming, alternative methods such as computer imaging, were not considered hugely advantageous.

For the histological studies, all sections were taken in the vicinity of the midline, in a longitudinally bisected eye. If every quadrant had been examined, differences between the type and distribution of receptors may have emerged. However, in the comprehensive survey undertaken by Wickham (1980) where this was done, no differences were found between the quadrants.

Accompanying nerves were rarely evident and only seen in longitudinal section in sperm, Cuvier's beaked and minke whales, running longitudinally in the posterior part of the ciliary body. This could indicate that they were a chance finding in these whales but would be equally as evident in other whales if more longitudinal sections were taken. These other whales might then equally show evidence of ERs in groups, where they are close to the nerve.

In retrospect, this study might be enhanced by

1] further comparisons eg. with other marine mammals such as seals and otters

2] more serial sections in three planes and quantitative analysis

# OPTICS AND THE ANTERIOR SEGMENT

## 7.1 ABSTRACT

AIMS: To obtain data about the geometric optics and the histological details of optical elements from cetacean eyes.
METHODS: Histological sections of corneas from seven whales were examined with a light microscope. The best specimen was drawn at 2x life size and compared to human and porpoise corneas, as described in the literature. Lenses from two whales were examined. Both were photographed and one was subjected to nuclear magnetic resonance (NMR) imaging. The images from both techniques were analysed in order to be able to describe their size, curvatures and shape. In addition, lenses were examined histologically. Entire globes from two whales were obtained and subjected to NMR imaging. Data relevant to the optical elements were then analysed using a computer ray drawing package.
RESULTS: The cornea was optically almost neutral in air and water. The lens was pliable, had asymmetrical anterior and posterior surfaces, and a capsule which could potentially produce changes in lens profile.
CONCLUSION: It is hypothesised that accommodative changes would be unnecessary for movements between air and water, and minor changes in lens curvature would be sufficient to produce a major effect on dioptric power.

## 7.2 INTRODUCTION

There is interest and speculation regarding how well whales can see in the context of a number of issues. These range from the accurate acrobatic displays of dolphins in shows, to the unfortunate capture of dolphins in fishing nets, the stranding phenomenon, and the frequent collisions of whales with marine vessels. Despite this interest, very little work on the acuity of cetacean vision has been published. Visual acuity is defined as the ability of the eye to differentiate two point sources of light, and is expressed as the angle subtended at the eye by those sources. In humans it is around 26 seconds (Guyton 1986). It seems likely that some unpublished work on acuity would exist within US Naval centres, where highly trained dolphins are used in underwater missions, and it has been shown that the killer whale and dolphin have a visual acuity in water of five to six minutes, which is equivalent to a cat in air, and that vision is likely to play a significant role in their sensory awareness (Spong and White 1971; White *et al.* 1971).
It is not known how acute cetacean aerial vision is, although work on dolphins has shown that in water close objects are viewed optimally and in air distant objects are

viewed optimally (Herman *et al.* 1975). This is explained in terms of the optical properties of a double slit pupil, where two images are produced either side of the focal point and these completely overlap at the focal point, the location of which would vary according to whether the eye was in air or water.
Factors determining how well an animal can see are both visual acuity and refractive state. Acuity is related to receptor and ganglion cell density, and to the processing of the image at the retina. The quality of that image is determined by the ability of the refractive elements of the eye to produce a good quality, focused image.
The best method to measure visual acuity is by direct measurement, in behavioural experiments in the live animal. However, if only cadaver material is available, it is possible to use cell density methods which hypothesise values for visual acuity (Murayama 1995, see Chapter 2). Refractive error is similarly most accurately measured in the live animal by use of a retinoscope, but can also be obtained from an enucleated eye by use of the basic principles of physics.

The aims of this study were to obtain data on the optical elements of the cetacean eye by using cadaver material. It also sought to obtain information on the refractive state of the cetacean eye by determining whether it is emmetropic in water or air and finding the range of accommodation required to achieve emmetropia in both these media.

This study explores the use of NMR techniques, as a non-invasive, non-destructive method to obtain an image of the refractive elements of the eye. The clinical use of NMR is well known, and includes its use in diagnosing ocular pathology (Sassani *et al.* 1984). Biological material, because of its high water content, is "protonaceous" and therefore an ideal subject for proton spin NMR imaging, which maps the spatial distribution and mobility of protonaceous species (Clarke *et al.* 1996). Slices along any plane can be selected, while the signal intensity is recorded as a gray scale. The brightest areas in this experiment (most signal) were given by fat and vitreous, darkest (least signal) by the lens, and moderate signal by nerve.

## 7.3 MATERIALS AND METHODS

Studies were conducted in three separate areas:

1] the cornea.

2] the lens.

3] the globe.

NMR imaging was used as a technique, mainly in the globe studies, because it provided an opportunity to produce an image with all the optical elements *in situ* which could then be used for drawing ray diagrams. A custom built 200MHz spectrometer connected to a 4.7 Tesla, horizontal superconducting magnet, was used (Eccles *et al.* 1998).

### 7-3i Corneal Study

Formalin fixed globes from seven dead, stranded cetaceans were sectioned and routinely processed and stained with haematoxylin and eosin.(See Appendix Tables 4-1 for details of whales).

The thickness of each corneal layer was measured taking, where possible, four peripheral measurements and four central measurements using a light microscope at x400 and an eyepiece graticule (Table 7-1, graph 7-1).

Diagrammatic representation was made of one of these corneas (a juvenile pygmy sperm whale E413-95) using an anterior radius of curvature value taken from another, adult whale of the same species (27821-97). An assumption was made that, despite the differences in body and corneal size between these two whales, radius of curvature would remain relatively constant. In humans the eye volume doubles as the neonate grows to adulthood. However, throughout this development, the corneal curvature only increases from 7mm to 7.8mm (Spooner 1957).

Diagrams of the juvenile pygmy sperm whale (E413-95), a porpoise (using data from Kroger and Kirschfeld 1992), a human (using data from Spooner 1957), and a long-finned pilot whale (using data from NMR image of 5.1, Table 7-5) were compared to illustrate the relative overall sizes and curvatures of corneas (Figures 7-3 to 7-6).

### 7-3ii Lens Study

Lenses were obtained from three whales.

1] Lenses from a fixed dolphin eye (28782-98) and a long-finned pilot whale eye were photographed.

2] Lenses were obtained from a long-finned pilot whale and a steer. Each lens

was 4 days *post mortem*, so some autolysis was present. They were subjected to NMR imaging.

3] Capsular width was measured in histological sections of lenses prepared from whales in the histological survey described previously in Chapter 5.

### 7-3iii Globe Study

One fresh globe and one formalin-fixed globe were obtained from two long-finned pilot whales (numbers 38-98 and 44-98). The fresh globe was chilled, and the anterior chamber inflated to a smooth curve using 1ml of ovine aqueous humour and 1ml of air. It was then 'set' into 10% agar gel, to maintain the curve and provide better contrast for imaging.

Both globes were imaged using NMR in 2 planes; vertical and horizontal axial. Images were then analysed to obtain focal lengths using a ray drawing package, 'Ray Trace', developed specially for thick lenses (Craig Eccles 1998, personal communication).

In all of the ray diagrams, the refractive indices which were used for the lens and cornea were derived from the literature (Kroger and Kirschfeld 1992); cornea - 1.53; lens - 1.51.

# 7.4 RESULTS

## 7.4i Corneal study

Separate measurements for each corneal layer are recorded in Appendix 7-1. Total corneal thickness was about double peripherally compared to centrally (Table 7-1, Figure 7-1). The average values for each individual layer were:-
Epithelium; 177 microns. Bowmans capsule; 17.9 microns. Stroma; 1476 microns Descemets membrane; 1.9 microns. Endothelium; 3.6 microns.
There was insufficient data for statistical analysis, but some variation between individuals was apparent. Epithelial thickness varied widely between species, from 345 in the pygmy sperm whale to 73 microns in the straptoothed whale. Similarly, Bowman's membrane width varied from 28.6 in the long-finned pilot whale to 5 microns in the pygmy sperm whale. There was less species difference in stromal values, although these were often twice as wide peripherally compared to the central measurement, thus accounting for the overall cross sectional profile of the cornea. Compared to human and bovine eyes, these values were comparatively thicker in all layers except Descemet's membrane and the endothelium.

**TABLE 7-1. CENTRAL AND PERIPHERAL CORNEAL THICKNESS IN 7 WHALES.**

| | WHALE | AVERAGE CENTRAL μM | AVERAGE PERIPHERAL μM |
|---|---|---|---|
| 1 | LONG FINNED PILOT E432-95 | 1000 | 1875 |
| 2 | JUVENILE LONG FINNED PILOT 27471 | 1030 | 1805 |
| 3 | STRAPTOOTHED E429-95 | 2083 | 2320 |
| 4 | PYGMY SPERM E413-95 | 856 | 1718 |
| 5 | CUVIERS BEAKED E15-97 | 758 | 2008 |
| 6 | MINKE E18-97 | 1233 | 1843 |
| 7 | GRAYS BEAKED E433-95 | 1412 | 2806 |

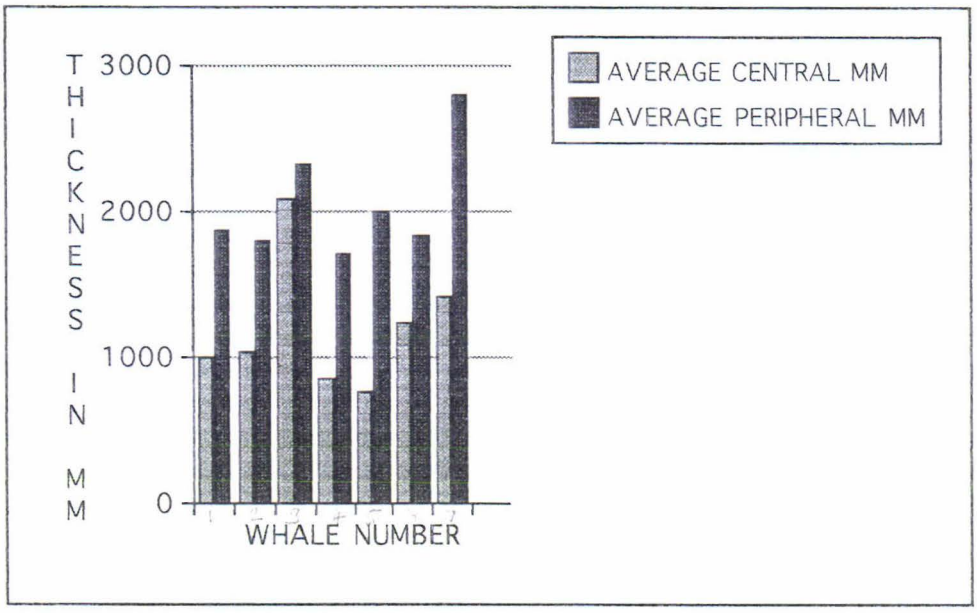



**Figure 7-1. Central and peripheral corneal thicknesses in 7 whales.** Histologically, a typically mammalian arrangement of 5 layers was observed; epithelium, basement membrane, stroma, Descemet's membrane, and endothelium. In this particular specimen, small capillaries of just one corpuscle width were observed within the epithelial layer. This observation was not made in other specimens (Figure 7-2).

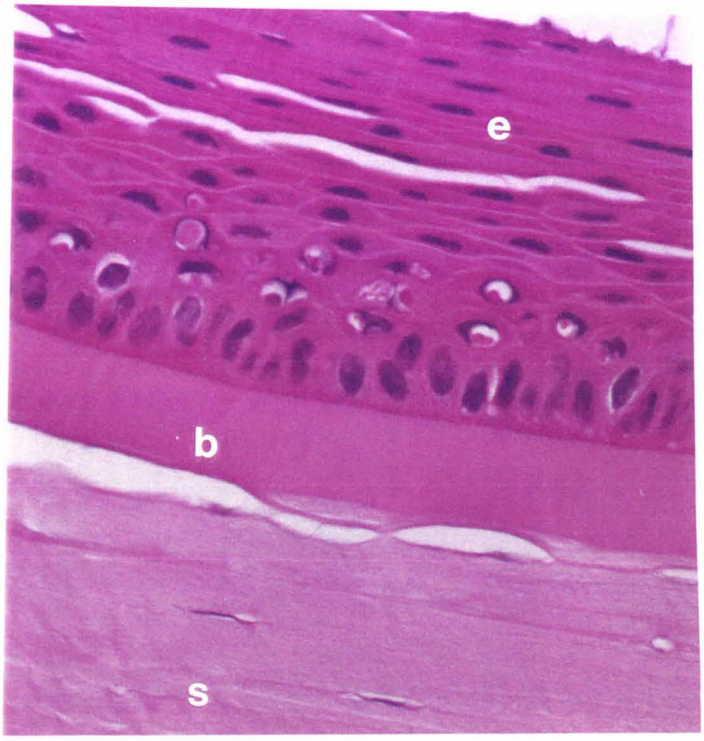



**Figure 7-2. Cornea of dolphin showing typical mammalian structure and small epithelial capilleries. e, epithelium b, basement membrane s, stroma H&E x 400**

Using the data obtained from long-finned pilot whale 38-98 and pygmy sperm whale E413-95, diagrams of the corneal size and shape at 2x life size were drawn (Figures 7-3 and 7-4) and compared with similar diagrams drawn using data from the literature (Spooner 1957; Kroger and Kirschfeld 1992)(Table 7-2). The results confirm the concave shape of the cetacean cornea and illustrate its size in relation to the globe (Figures 7-4 and 7-5).

## TABLE 7-2. CORNEAL DATA FOR THREE CETACEANS AND A HUMAN EYE.

| | |
|---|---|
| LONG FINNED PILOT WHALE | |
| RADIUS OF CURVATURE-ANT. | 32 |
| RADIUS OF CURVATURE-POST. | 18.2 |
| CENTRAL THICKNESS | 1.3 |
| LENGTH | 32 |
| PYGMY SPERM WHALE E413-95 | |
| RADIUS OF CURVATURE-ANT. | 17.8 |
| RADIUS OF CURVATURE-POST | 8.9 |
| CENTRAL THICKNESS | 0.9 |
| PERIPHERAL THICKNESS | 1.2 |
| LENGTH | 17 |
| PORPOISE | |
| RADIUS OF CURVATURE-ANT. | 13.5 |
| RADIUS OF CURVATURE-POST. | 3.9 |
| CENTRAL THICKNESS | 0.6 |
| LENGTH | 16 |
| HUMAN | |
| RADIUS OF CURVATURE-ANT. | 7.8 |
| RADIUS OF CURVATURE-POST. | 6.7 |
| CENTRAL THICKNESS | 0.6 |
| LENGTH | 11 |

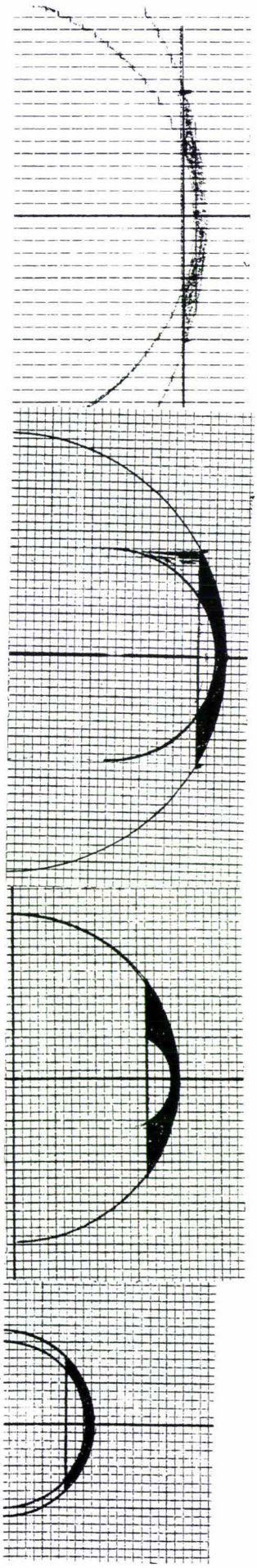


Figure 7-3. Diagram of a long-finned pilot whale cornea at 2x life size.

Figure 7-4. Diagram of a pygmy sperm whale cornea at 2x life size.

Figure 7-5. Diagram of a harbour porpoise cornea at 2x life size.

Figure 7-6. Diagram of a human cornea at 2x life size.

### 7.4ii Lens Study

The vertical diameter of lenses studied was approximately 10mm in both the dolphin and long-finned pilot whale species, which is a similar size to that of the human but only half the size of bovine lenses (Prince 1957).
Fresh lenses were of a moderately soft and pliable consistency, which visibly altered in shape from asymmetrical (Figure 7- 7) to spherically symmetrical when removed from the zonule, suggesting that in life this capacity may be used in accommodation ( in man, a similar phenomenon is observed when the lens is removed from the zonule (Prince 1957). The macroscopic appearance of entire lenses was similar to that of other species. When bisected sagitally, there was a marked lamellar appearance (Figure 7- 8). At about four days *post mortem*, fresh lenses became sufficiently autolysed for the cortex to liquefy within the intact capsule, with the harder nucleus palpable within. Bovine lenses were found to degenerate in a similar manner. The NMR images depict concentric zones of differing proton density, which was interpreted as tissue density (Figures 7-9a and b).
In one specimen, it was possible to observe which area of the lens was utilised during moderate pupillary constriction, although not with sufficient accuracy to state peripheral or cortical (Figure 7-10).

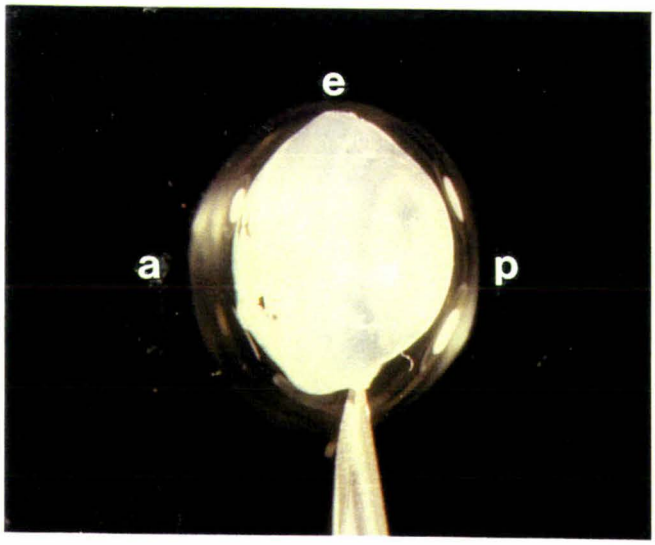



Figure 7-7a. Dolphin lens a, anterior p, posterior e, equator

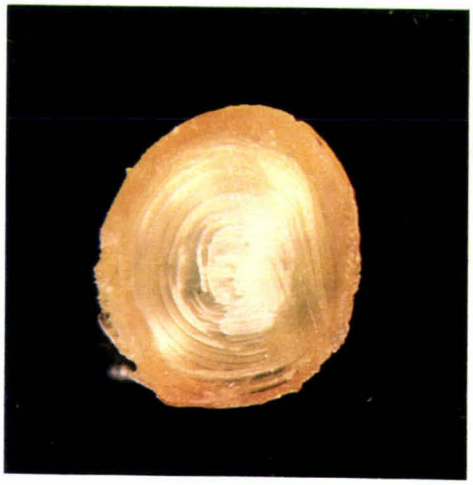


Figure 7-7b. Bisected dolphin lens showing concentric lamellations

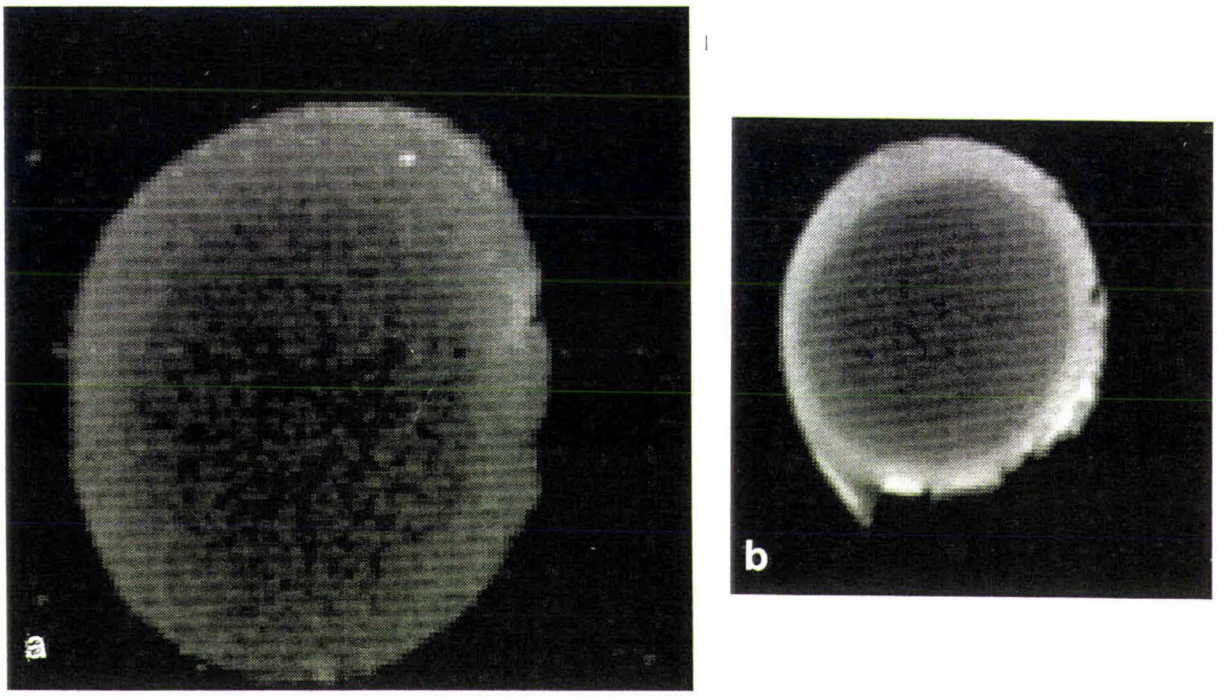



Figure 7-8 a] NMR image of bovine lens and b] NMR image of pilot whale lens.

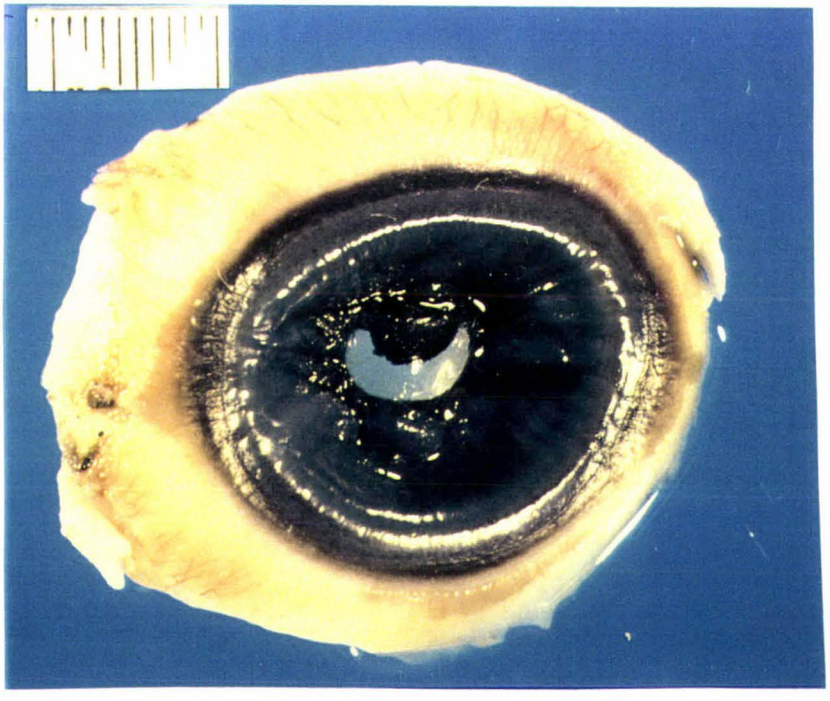
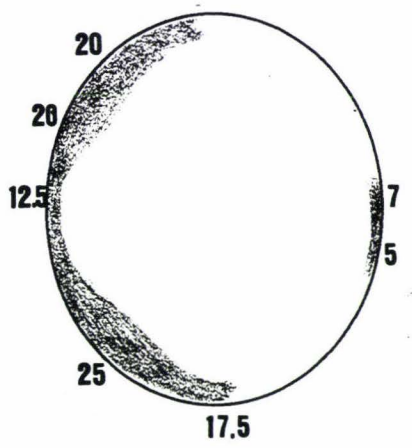


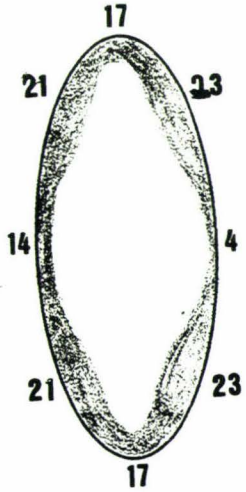
Figure 7-9. View of anterior segment with moderately constricted pupil.

In many histological sections, lens capsule was absent due to the difficulty of processing the lens tissue. When it was present, capsular width was measured using an eyepiece graticule (Table 7-3) and a clockface convention was used to identify sites (Figure 7-10). (Shaded areas represent capsule).




ANTERIOR.....POSTERIOR

**Figure 7-10. Diagram of a vertical axial section of a cetacean lens to identify sites of capsule measurements.**




ANTERIOR.....POSTERIOR

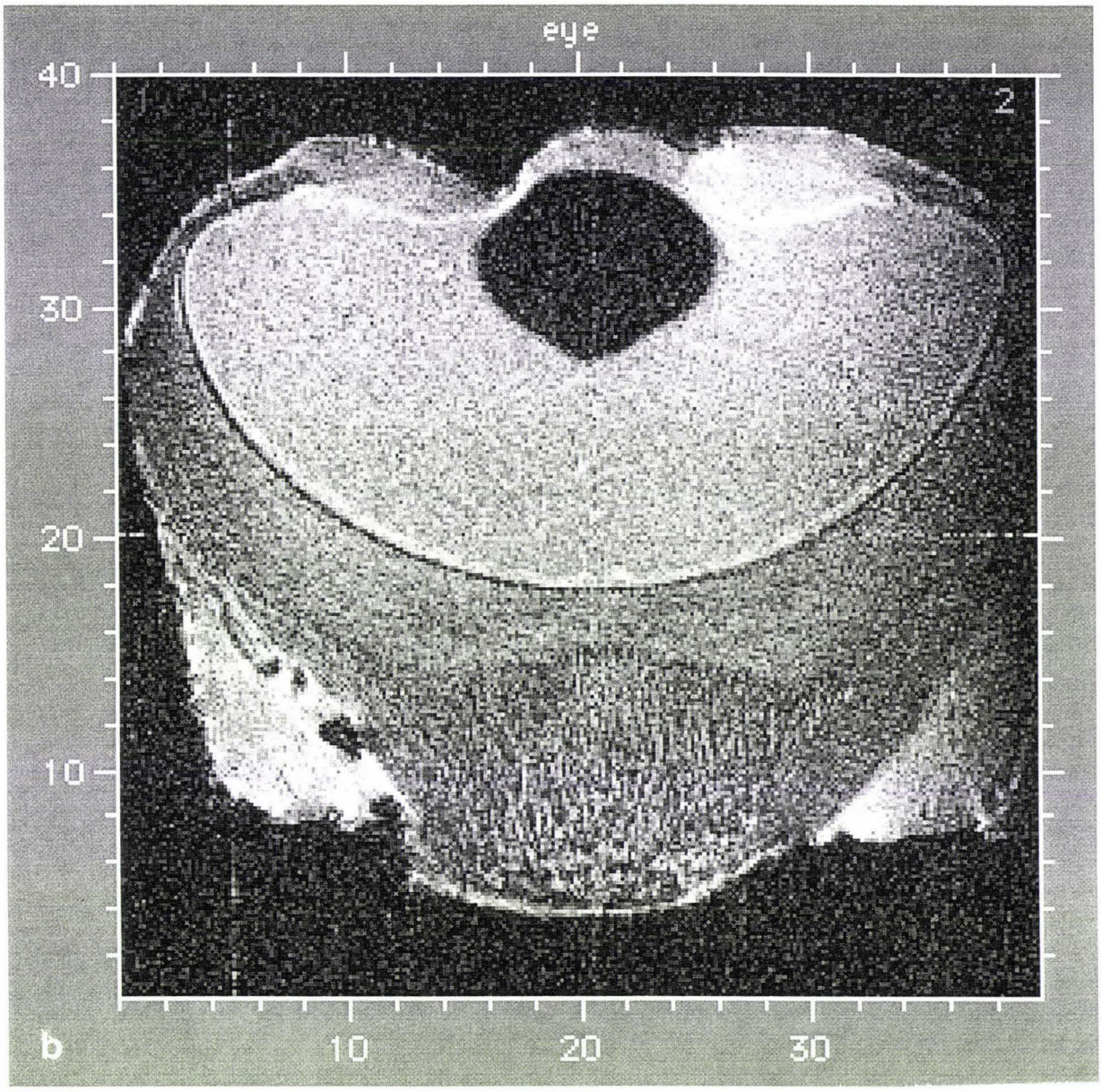
**Figure 7-11. Diagram of a vertical axial section of a human lens to identify sites of capsule measurements.**

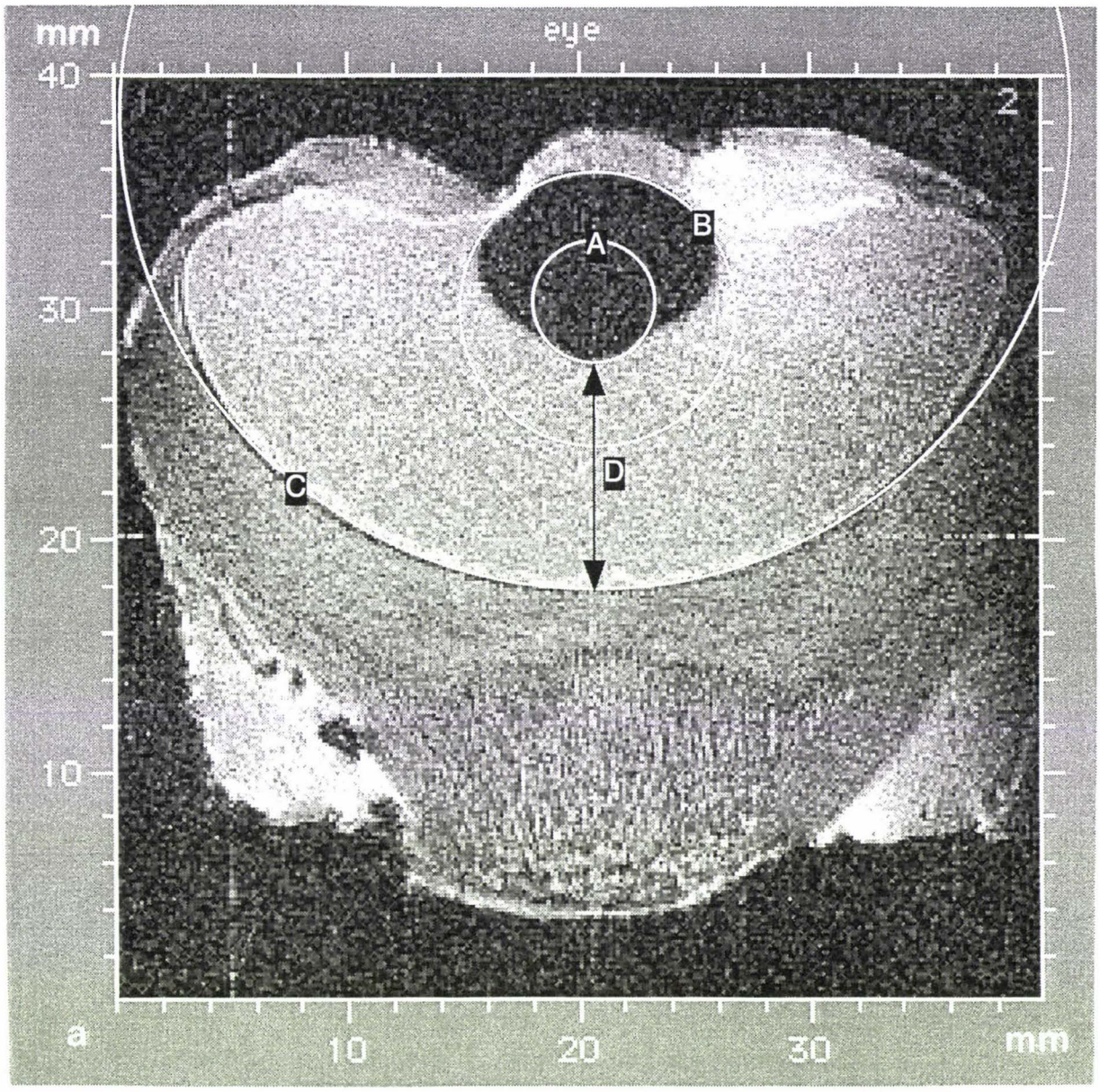
**TABLE 7-3. CAPSULAR THICKNESS IN CETACEAN, BOVINE AND HUMAN LENSES.**

| WHALE | AREA | WIDTH IN MICRONS |
|---|---|---|
| CUVIER'S | Anterior | 15 |
| BEAKED | Posterior | 8 |
| PYGMY RIGHT | Posterior | 5 |
| LONG-FINNED | 12-00 BOW | NOT AVAILABLE |
| PILOT E268-98 | 1-00 | NA |
| | 2-00 | NA |
| | 3-00 POSTERIOR | 7 |
| | 4-00 | 5 |
| | 5-00 | NA |
| | 6-00 BOW | 17.5 |
| | 7-00 | 25 |
| | 8-00 | NA |
| | 9-00 ANTERIOR | 12.5 |
| | 10-00 | 20 |
| | 11-00 | 20 |
| BOVINE | Anterior | 30 |
| | At bow | 7.5 |
| HUMAN (from | *12-00 | 17 |
| Hogan 1971) | 1-00 POSTERIOR | 23 |
| | 3-00 POSTERIOR | 4 |
| | 5-00 POSTERIOR | 23 |
| | *6-00 BOW | 17 |
| | 7-00 ANTERIOR | 21 |
| | 9-00 ANTERIOR | 14 |
| | 11-00 ANTERIOR | 21 |

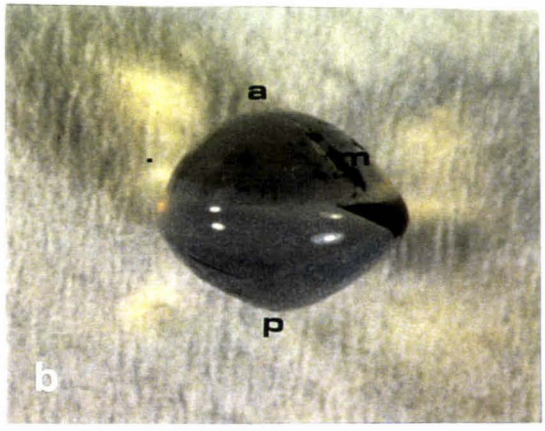
### 7.4.iii Globe study

Nuclear magnetic resonance images of satisfactory quality in horizontal and vertical planes were obtained from both whales (long-finned pilot 38-98 and long-finned pilot 44-98) (Figures 7-12a and 7- 13). The shape of the lens in serial sections in both planes revealed a 'protruberance' on the posterior surface, which was also visible to the naked eye in whale no. 44-98 (Figure 7- 12b). Horizontal plane images were chosen for analysis because they were clearest in terms of resolution, and showed the protruberance best. Subsequent analysis by the computer ray drawing package revealed that both whales were near emmetropic in both air and water. Although no.44-98 (the fixed eye, 2.1) appeared slightly myopic in air, and no.38-98 (the fresh eye, 5.1) appeared slightly hyperopic in both air and water, this could easily have been due to inaccuracies in the techniques. In particular, in 2.1 the plane of imaging was slightly offset from the midline, producing slightly smaller overall dimensions for the lens than would have been present at midline and therefore a shorter apparent focal length. In addition, data for the cornea in whale 44-98 (image 2.1) was not available and was 'borrowed' from image 5.1.


eye
40
30
20
10
2
b
10
20
30

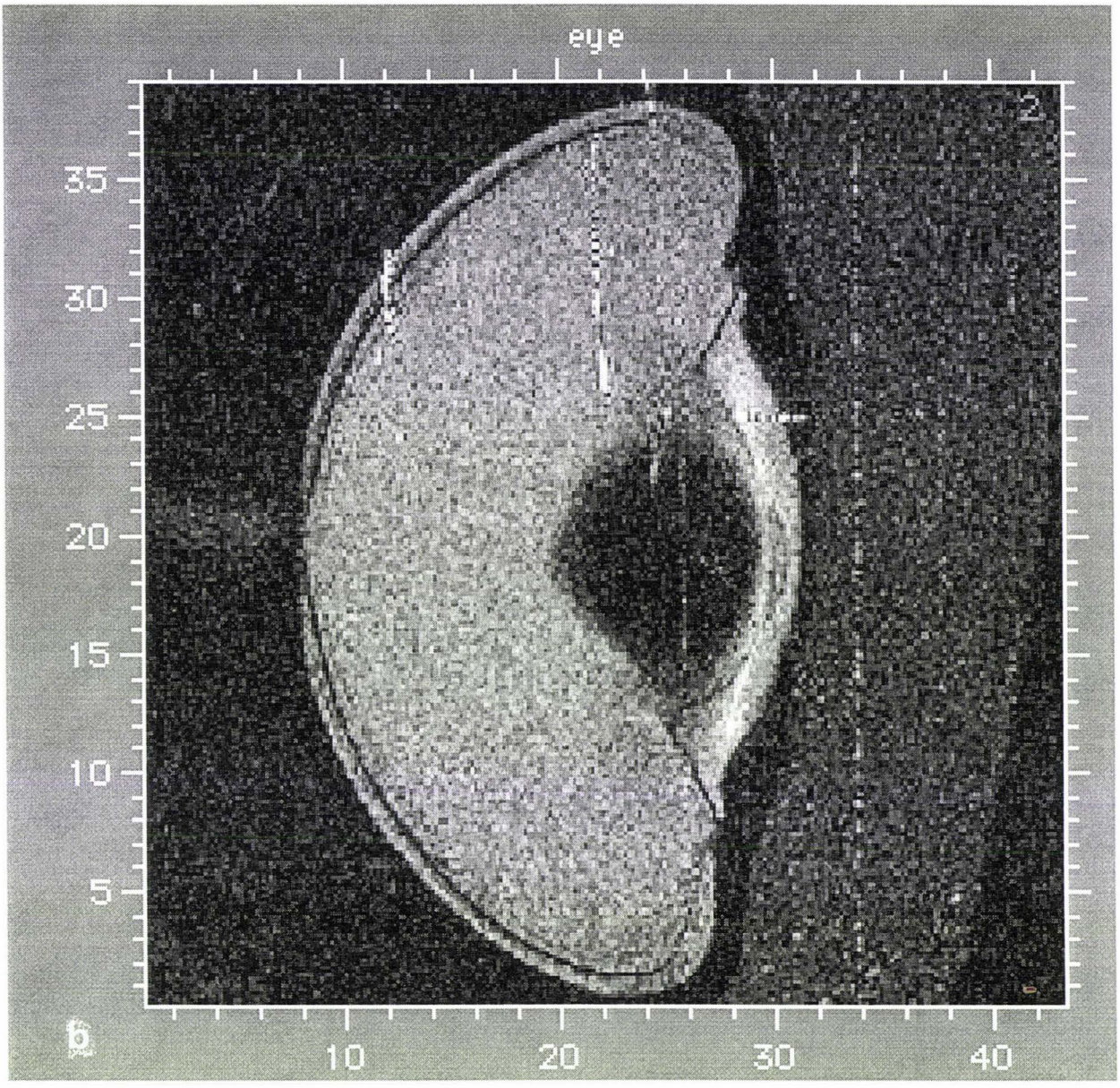



Figures 7-12 a] NMR image 2.1 of long finned pilot whale 44-98, scale in mm. b] photograph of the lens from this specimen. a, anterior m, melanin pigment from iris p, protruberance c] (facing page) NMR image as above, without markers.




## TABLE 7-4 DATA OBTAINED FROM NMR IMAGE 2.1

| | | |
|---|---|---|
| A | Post. lens radius of curvature | 2.6mm |
| B | Ant. lens radius of curvature | 5.8mm |
| C | Retina radius of curvature | 20.5mm |
| D | Retina -lens distance | 9.5mm |


eye
35
30
25
20
15
10
5
10
20
30
40

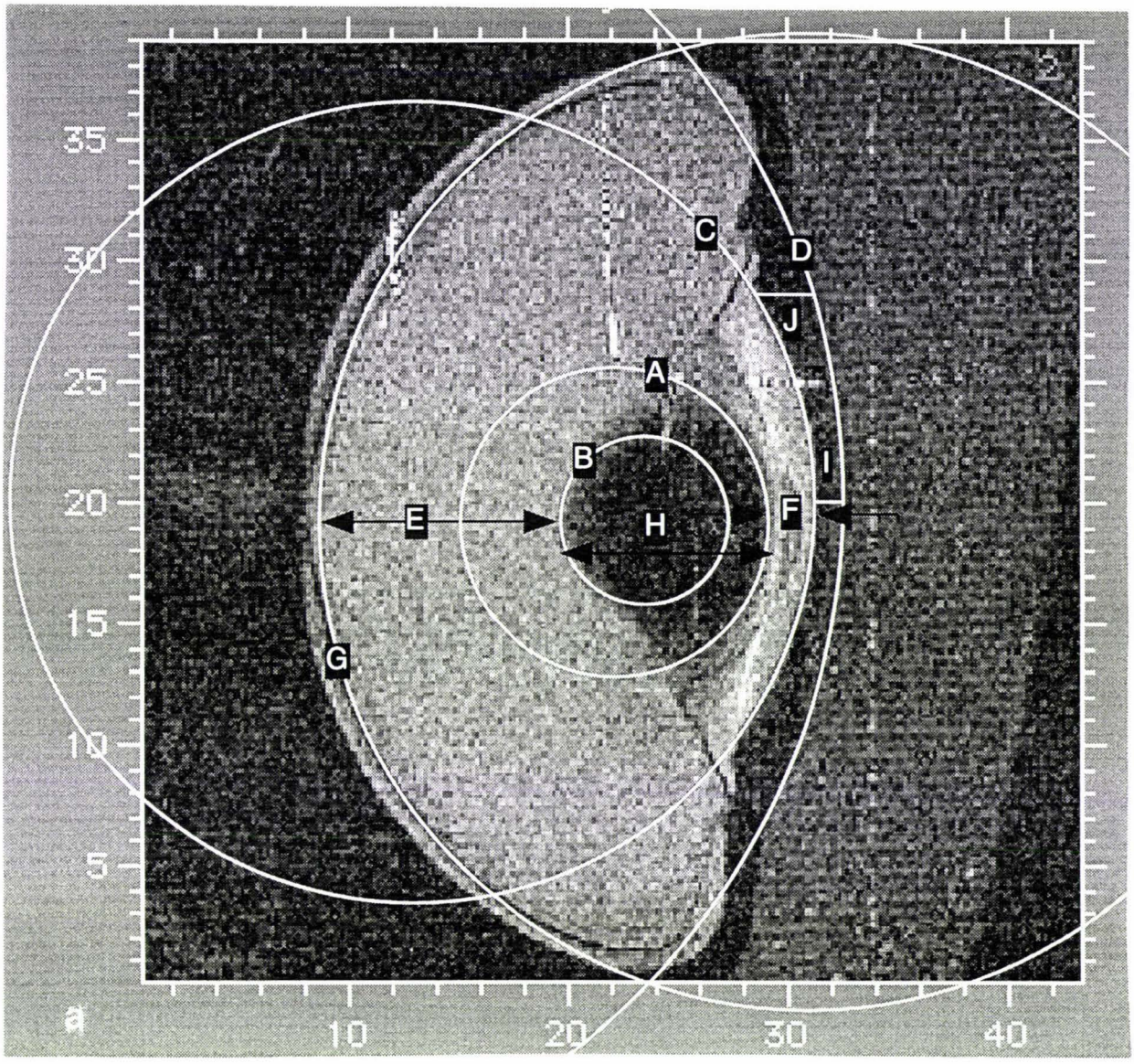


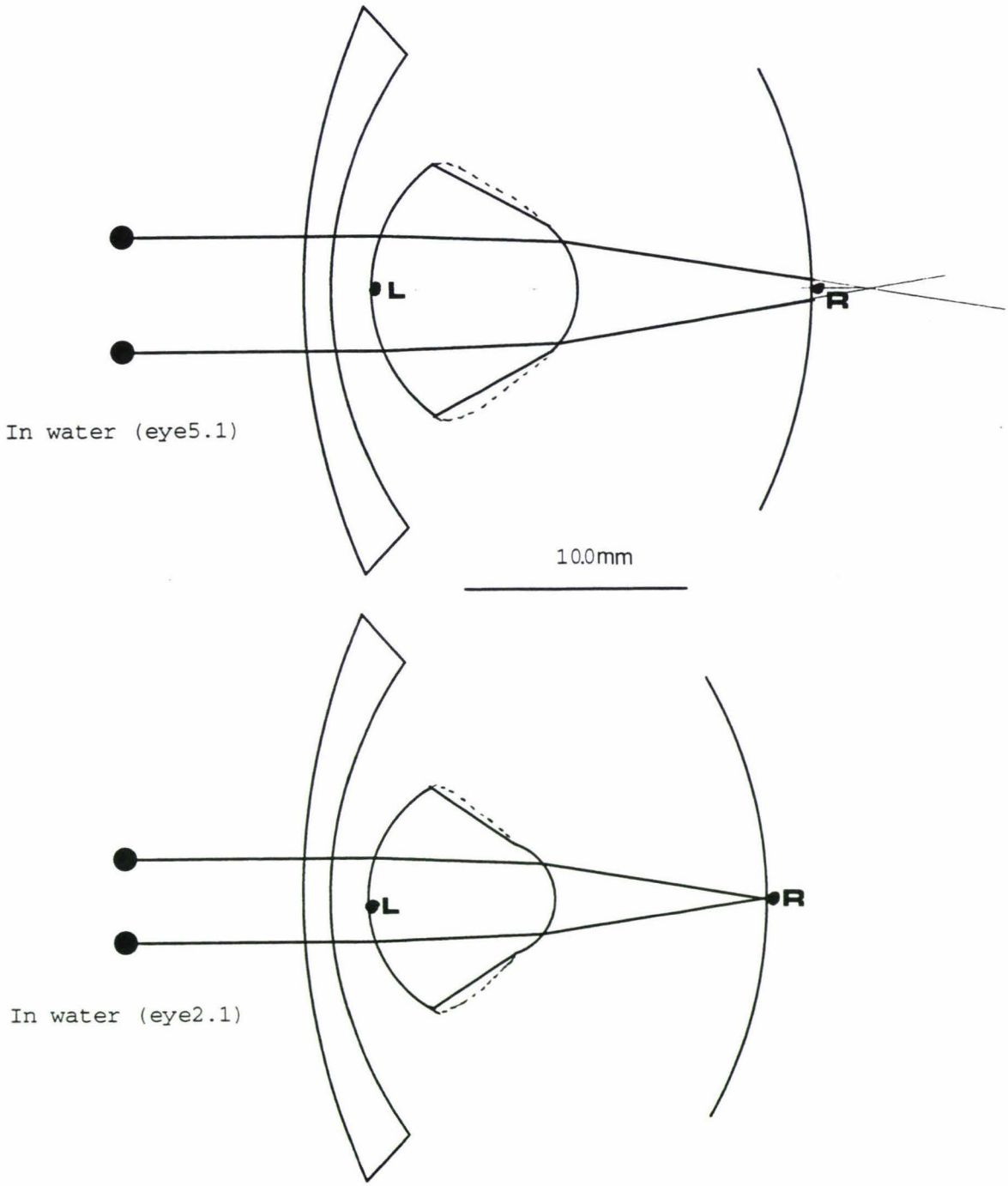

Figure 7-13a] NMR image 5.1 of fresh eye of long finned pilot whale 38-98 (scale in mm). b] (facing page) NMR image as above but without markers.

**TABLE 7-5. DATA OBTAINED FROM NMR IMAGE 5.1.**

| | | |
|---|---|---|
| A | Lens radius of curvature ant. | 7.5mm |
| B | post | 3.8mm |
| C | Corneal radius of curvature ant. | 32mm |
| D | post. | 18.2mm |
| E | Lens-retina | 10.7mm |
| F | Cornea-lens | 2mm |
| G | Retina R of C | 22.2mm |
| H | Lens thickness | 9.7mm |
| I | Central Corneal thickness | 1.3mm |
| J | Peripheral corneal thickness | 2.6mm |
| | Lens height | 11.4mm(circle A) |
| | | 5.7mm(circle H) |

**TABLE 7-6. DATA FROM RAY TRACING DIAGRAMS DERIVED FROM IMAGES 5.1 AND 2.1 (Figures 7-14a and b).**

| WHALE NUMBER | CORNEA R OF C | LENS RADIUS OF CURVATURE | LENS DIMENSIONS IN MM | FL IN AIR | FL IN WATER | DISTANCE -LENS to RETINA |
|---|---|---|---|---|---|---|
| Long finned pilot 5.1 | 32mm-ANT. 18.2mm POST | 7.05mm-ant 3.8mm-post | 10X8 | 21mm | 22mm | 19.7mm |
| Long finned pilot 2.1 | 32mm ANT. 18.2mm POST | 5.8- ANT. 2.6-POST. | 10X8 | 17mm | 17.6mm | 17.9mm |




Figures 7-14 a] and b] facing page. Ray trace diagrams obtained from NMR images 2.1 and 5.1 of long-finned pilot whale eyes. a] in air and b] in water. Solid lines (computer model interpretation). Dotted lines (histologist's interpretation). L, anterior surface of lens deemed 'nodal point'. R, retina. Scale 3.4:1.

**7.4iv Simulations of Hypothetical Situations a] isolated corneal focal length b] isolated lens focal length c] remodelling of posterior lens surface to achieve emmetropia.**

a] Isolated cornea focal length

The data from the fresh cornea in image 5.1 was used in the computer Ray Trace package to obtain information about its focal lengths in air and water. The generalised lensmakers formula was also applied to obtain focal lengths/dioptric powers in both media (Appendix 7-1 and Table 7-8).

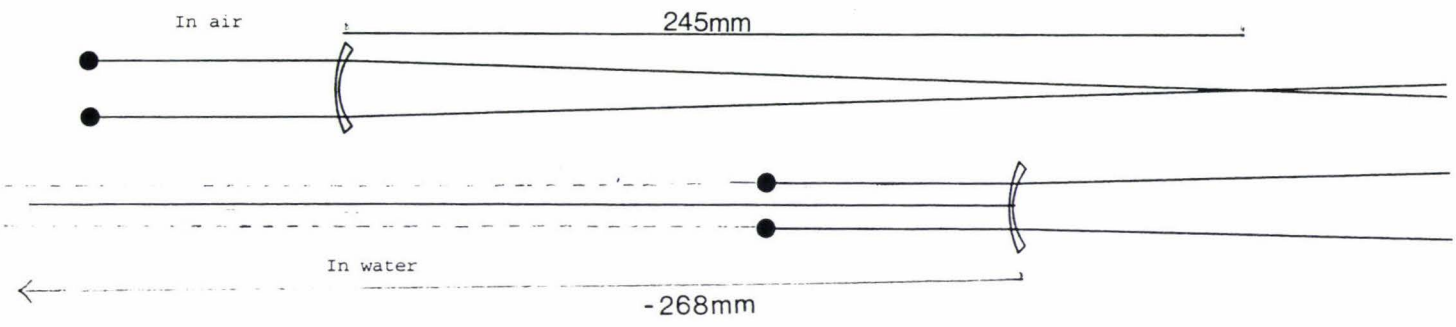



Figure 7-15. **'Ray Trace' diagram of simulation of corneal focal length in the isolated cornea from image 5.1 in air to water and water to water.**

**TABLE 7-7. DATA DERIVED FROM FIGURE 7-15 COMPARED TO THAT DERIVED BY CALCULATION USING THE LENSMAKERS FORMULA (APPENDIX 7-2)**

| | RAY TRACE | FORMULA |
|---|---|---|
| FOCAL LENGTH IN AIR | 245MM<br>4.1D | 238MM<br>4.2D |
| FOCAL LENGTH IN WATER | -268MM<br>-3.7D | -280MM<br>-3.6D |

b] Isolated lens focal lengths - symmetrical and asymmetrical surfaces.

A hypothetical analysis of a symmetrical lens shape with a posterior radius of curvature equal to that of the anterior surface was made for comparison with the non-symmetrical shape observed in image 2.1 in order to reveal the magnitude of change that the protruberance causes (Figure 7-16). A focal length of 17.8mm was observed in the non symmetrical lens, compared with around 24.1mm in the non symmetrical lens, a difference of 6.3mm. The generalised lensmakers formula was also applied to obtain focal lengths/dioptric powers (Table 7-9 and Appendix 7-2).

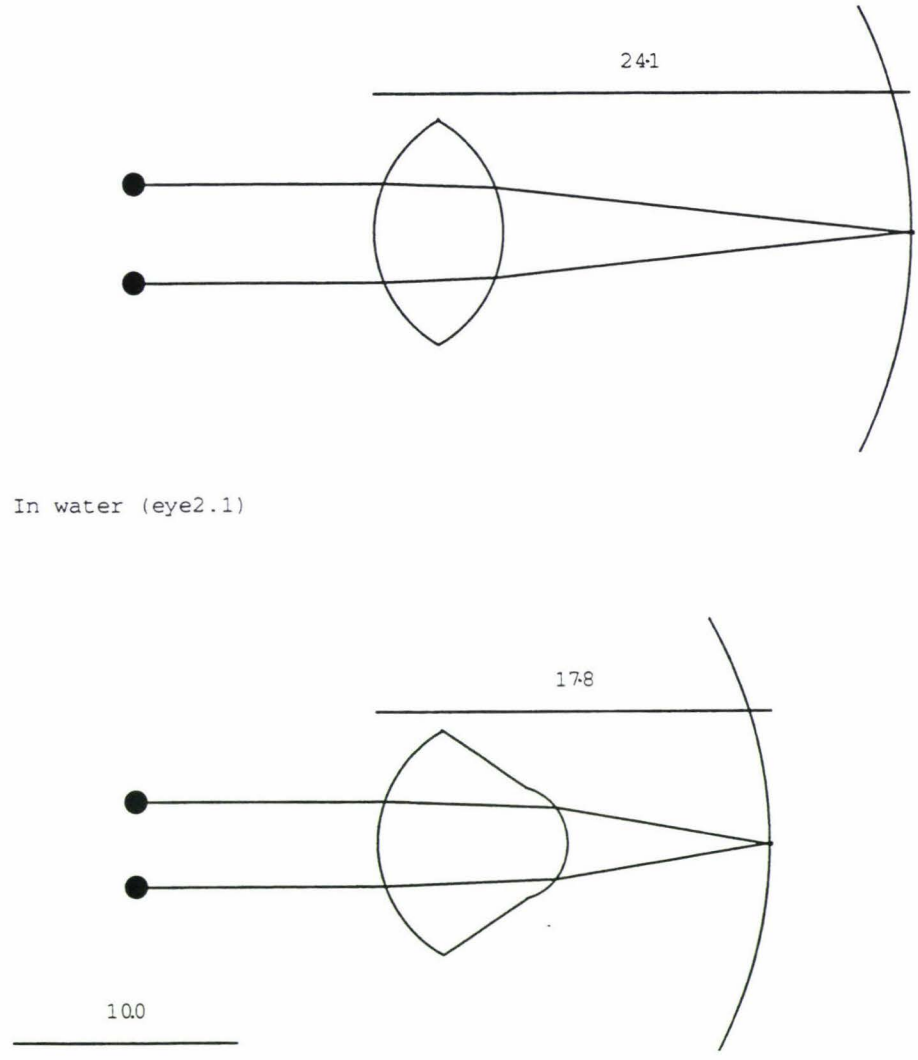



**Figure 7-16. Focal lengths in symmetrical and asymmetrical lenses.**

**TABLE 7-8. DATA DERIVED FROM FIGURE 7-16; FOCAL LENGTHS IN SYMMETRICAL AND ASYMMETRICAL LENSES.**

| | RAY TRACE | FORMULA |
|---|---|---|
| SYMMETRICAL LENS<br>RofC 5.8/5.8 | 24.1MM<br>45.6D | 21.4MM<br>46.7D |
| ASYMMETRICAL LENS<br>RofC5.8/2.6 | 17.8MM<br>71.4D | 13.3MM<br>75.4D |

c] Accommodated lens focal lengths

The radius of curvature of the posterior lens surface in image 5.1 was varied from 3.8mm to approximately 3.3mm in order to produce emmetropic images in water and air. The radius of curvature of the posterior lens surface in image 2.1 was varied from 2.6 mm to 2.7mm and 2.8mm in order to produce emmetropic images in water and air (negative accommodation) (Figure 7-17).
The radius of curvature (R of C) of the posterior lens surface in image 5.1 was changed from 3.8mm to 3.3mm (air and water) to correct the hypermetropia (Figure 7-17) existing in the natural state (Figures 7-14a and b).
The R of C was similarly changed in image 2.1 from 2.6mm to 2.8mm (air) 2.7mm (water) to correct the slight myopia existing in the natural state.

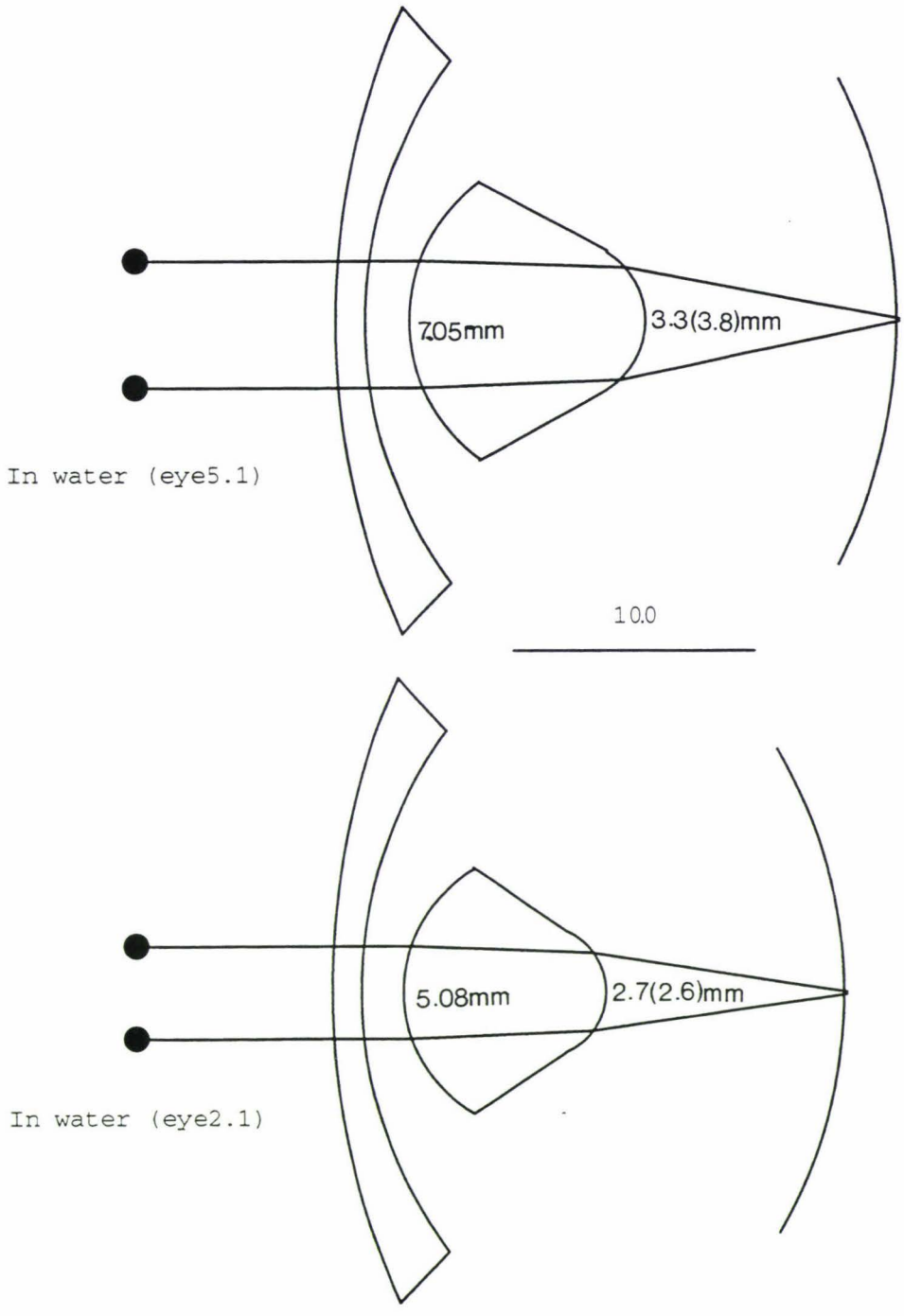


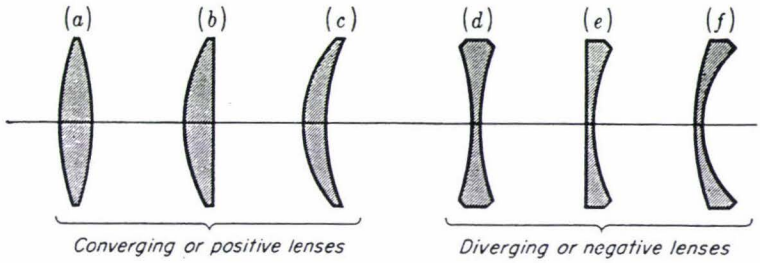

**Figure 7-17.** **Ray trace diagrams to simulate the effects on focal length of modifying radius of curvature of posterior lens surfaces in images 2.1 and 5.1. Original radius of curvature in brackets.**

# 7.5 DISCUSSION

## 7.5i Corneal Study

The corneal study data demonstrate that in the whales examined, the cornea was around half the thickness centrally compared to the periphery (mean 960 microns centrally, 1800 peripherally). The cornea is usually considered to be converging because the anterior and posterior surfaces in many mammals are concentric. However, lenses of refractive index greater than 1 which are thinner centrally than peripherally are divergent in air (Jenkins and White 1957 p.28).

The three *diverging*, or *negative, lenses,*




which are thinner at the center, are (*d*) *equiconcave*, (*e*) *plano-concave*, and (*f*) *negative meniscus.*

**Figure 7-18. Cross sections of common types of thin lenses (from Jenkins and White 1957).**

Previous data (Pardue *et al.* 1993) state corneal thickness values of 1474 microns (fin whale) and 1204 microns (Minke whale) but do not specify whether these are peripheral or central. Pardue's rough assessment of corneal power was +3D in air and -1D in water. Kroger and Kirschfeld (1992) defined the cornea as a "weak diverging" lens without specifying values. Kroger's values for refractive index (1992) were used in the present study to reveal that the cornea was weakly diverging (-3.6D) with the eye in water, and weakly converging (+4.2D) with the eye in air, thus supporting Pardue *et al.* (1993) and Kroger and Kirschfeld (1992) but refuting Dawson (1972) who calculated 22.8D in air from a fixed specimen, later repeated by keratoscopy in a live animal to reveal a power of 26.8D in air (1987) with optical neutrality in water.

The apparent difference in results between Kroger and Kirschfeld (1992) and this

study, and Dawson's study occurred because Dawson used RI values from Matthiessen (1894) which are much lower than those used by Kroger and Kirschfeld (1992). In addition Dawson used a keratoscope to obtain the radius of curvature of the cornea but this would only give information about the anterior surface. In humans, for whom this instrument was designed, the posterior surface approximates the anterior surface so closely that they are considered parallel. Its use in cetaceans is therefore inappropriate.

Differences between the total focal lengths of the globe in air and water is small (1mm in 5.1; and 0.6mm in 2.1)(Table 7.7) indicating that the cornea has little effect on the dioptric power of the eye.

### 7.5ii Lens Study

Lenses in the two small species of whale examined (dolphin and long-finned pilot) were smaller than bovine lenses (19mm quoted by Prince *et al.* 1960) and around the same size as a human lens (9mm cited by Spooner 1957). Results in this study suggest that the lens is not a symmetrical sphere, because clear differences exist in lenses from the dolphin and long-finned pilot whale illustrated (Figures 7-7 and 7-12) and this is confirmed in the NMR study, where in the latter whale the posterior lens surface had a smaller radius of curvature than the anterior surface. It is possible that this finding was an artefact, produced by a moulding effect of the hyaloid canal on the soft lens cortex. Further studies would confirm or refute this hypothesis.

The moderately soft, pliable consistency of the lens appeared to be restricted to the cortex, unlike the nucleus, which was solid. Previous studies (Walls 1942, Dawson *et al.* 1980, Waller 1992) have merely described the lens as hard.

In the present study, capsular width was examined, since this is an indicator of the potential for the lens to deform. Width was found to be slightly thinner than in the cow and human, but variable in different areas of the lens. However, the small sample size (3 whales, 2-7 measurements each) does not allow an unequivocal statement that this is a general trend. Other studies (Waller 1992) have described the capsule as much thicker than in humans (a claim which is clearly refuted in Figure 7-11a and b) but very thin posteriorly.

Further 'evidence' to support a potential for lens flexure is found in the capsular structure. In man, rounding up of the lens is achieved when tension on the zonule is released, allowing the lens to bulge into thinner anterior and posterior parts of the capsule (Hogan *et al.* 1971) The radius of curvature is reduced from 10mm to 5.3 anteriorly, and 6mm to 5.3mm posteriorly during accommodation (i.e. the lens becomes a symmetrical, near spherical shape). In this study, long-finned pilot whale lenses were found to have an anterior radius of curvature of 5.8mm and a posterior radius of curvature of 2.6mm (Figures 7-11 and 7-12) and it is not known how this shape may alter. However, the refractive index of the human lens at 1.39

(Hogan 1971) is considerably less than has been described in whales at 1.51 for the core, 1.37 peripherally (Kroger and Kirschfeld 1992) and is more closely allied to that described in fish at 1.56 centrally and 1.38 peripherally (Fernald and Wright 1983). The consequences of this would be that very minor alterations in curvature of the cetacean lens would produce a profound variation in its dioptric power. An increase in radius of curvature of the posterior surface in image 2.1 from 2.6mm to 5.8mm produced a large increase in focal length from 13.2mm (75.7D) to 21.4mm (46.7D) (Figure 7-16).
Similarly, if the radius of curvature on the posterior surface of the lenses featured in NMR studies is altered to achieve emmetropia, only minor changes in radius of curvature on the posterior surface of 0.1-0.5mm are required (Figure 7-17).

### 7-5 iii Globe Study

In the globe study it was found that the fresh eye (image 5.1) was slightly hyperopic in air and water and the fixed eye (image 2.1) was slightly myopic in air and water. A possible artefactual cause for the myopic result has already been given, but additional confounding factors in this experiment include:-
Uncertainty with respect to the exact position of the nodal point, the poor contrast of the NMR imaging machine, assumptions made regarding data for refractive indices, artificial inflation of the anterior chamber, and possible *postmortem* changes affecting the shape of the cornea and lens. In particular, the apparent 'bulge' in the posterior surface of the lens may be the result of the the vitreous humour gel solidifying after death, thereby causing the retrolental fossa to become more pronounced and allowing it to mould the lens surface. In order to make definitive statements about the focal length of long-finned pilot whale eyes, more data from more, very fresh eyes, or the eyes of live whales, is mandatory.

When the cornea and lens were considered as separate elements in computer simulations, the following situation occurred:- In both air and water, the cornea appeared to be optically almost neutral with just 3.6 dioptres of negative power in water and 4.2 dioptres of positive power in air/water (Figure 7-15, Ray Trace). Most of the dioptric power of eye 2.1, long finned pilot whale 44-98 was attributable to the lens, whose focal point at 17.8mm (Figure 7-16, Ray Trace) very closely approached the actual distance from lens to retina of 17.9mm. When the corneal power is added to the lens power, the total power of the eye would produce further slight myopia in air (17.2 mm), but emmetropia or mild hyperopia in water (18.1mm).
Assuming that the hypothesis proposed in this study is correct (i.e. emmetropia in air and water without a need for significant accommodation), further ramifications should be considered. The implications of a relatively small posterior bulge for the whale are that it is the central lens area that provides an emmetropic image. This

occurs in a relaxed eye with an object at distance in both air and water, but accommodation would be necessary for close objects in both media. In order to utilise the central area exclusively, pupillary shape may be of some importance. The whale is remarkable for its large, muscular umbraculum. In the cow and horse, the umbraculum is formed from nodules arising from the pigment layers of the iris, and functions to shade the sensitive retina from downwelling light, as well as reducing pupillary size (Prince 1956). In whales the umbraculum is a substantial muscular structure and in addition to the above functions, would be capable of producing extensive pupillary dilatation, as would be necessary in low-light level, deep sea conditions. It is uncertain which area of the lens the light would be restricted to during pupillary constriction - central or peripheral. More studies are required to ascertain this. In order to obtain an emmetropic image it may be necessary to adjust the direction of gaze/eye position such that light passes through the central area of the lens for emmetropic vision. Such a behavioural modification has been noted in dolphins (Dral 1975) but the explanation made was that an 'emmetropic porthole' existed in the dorsoventral area of the cornea. The porthole was not definitively demonstrated with a keratoscope, and appeared to reveal emmetropia in water also, contrary to expectations of hyperopia. A subsequent observation (Dawson *et al.* 1987) failed to confirm the finding. It may be possible that Dral's observation was not in the use of a specific area of the cornea, but of the lens. Rivamonte's theory (1976) proposed that the passage of light selectively through the peripheral lens (of lower refractive index) would result in a longer focal length, which when combined with corneal power in air, could result in an emmetropic image. In water, with dimmer light conditions and pupillary dilation, the whole lens would be utilised, with its central area of higher refractive index producing an emmetropic image.

Reports on the refractive state of the eye are ambiguous, but generally describe aerial myopia. Aerial myopia with emmetropia in water has been described by Walls (1942), Dawson (1980, 1987) Dral (1975) and Cronin *et al.* (1998). Myopia in air and hyperopia in water has been described by Dawson (1972). Shifting accommodation has been described by Dral (1975) and Cronin *et al.* (1998). Aerial and aquatic emmetropia, as described in this study, has been described in a restricted capacity (an 'emmetropic porthole'), by Dral (1975) and Pack and Herman (1995). Good visual acuity in water and air has been described by Herman *et al.* (1975), Spong and White (1971) and White *et al.* (1971). Dolphins have been shown to have aerial vision which improves with increasing distance and aquatic vision which improves with decreasing distance (Herman *et al.* 1975). This is contrary to expectations, since most reports describe aerial myopia. The apparent anomoly is explained in terms of the physical properties of a double slit pupil, whereby a clear image is produced when there is a 100% overlap of the two images at the focal point, which occurs at an image distance of just one metre in air. Resolution deteriorates at greater distances, in contrast to the situation in air,

where acuity improves with increased viewing distance because complete separation of the two images, although blurred, allows better resolution than when incomplete overlap occurs.

The presence of a near emmetropic focus in air and water as described in the present study would reduce the range of accommodation required for both media from tens of dioptres to just a few dioptres. This does not preclude the use of other devices which make accommodation unnecessary (as described in Chapter 2), variations of which have been suggested in cetaceans, such as the stenopaeic pupil (as in ungulates and cats), effective length of receptor cells increased (some bats), a ramp retina (as in the horse), or a bifocal lens (as in the kingfisher, and *Anaplebs*) (Walls 1942).

Rivamonte's hypothesis (1976) for the avoidance of aerial myopia by the selective use of different lens zones gains some support from the present study, but a central lens area with an increased radius of curvature is described in addition to the refractive index gradient. The present study describes the use of the central lens for emmetropic vision in both water and air. In contrast to this, Rivamonte hypothesises that emmetropia in air would be achieved by exclusive use of the peripheral lens because use of the central lens would result in myopia. It is unclear whether the constricted, crescent-shaped pupil restricts the passage of light to central or peripheral lens areas. A simple technique using a marker pen to delineate the edges of the aperture on the lens could be used to investigate this. The main tenets of the hypothesis described in this study for emmetropia in air and water were i] that the cornea was optically almost neutral in air and water (Figure 7-15) thereby endowing the lens with the majority of the refractive power ii] the posterior lens surface displayed a central area with a small radius of curvature, thereby providing the lens with a shorter focal length.

# EYE PATHOLOGY

## 8.1 ABSTRACT

AIM: To record the prevalence and type of pathological changes observed in a field survey of 50 cetacean eyes from 16 different species.
METHOD: Enucleated eyes from stranded cetaceans were examined grossly and histologically.
RESULT: Six whales had pathological eye changes; three had cataracts, all occurring in young animals; two had excessive yellow colouration of the lens and in one of these a small cataract was present histologically; and one had a resolved case of phacolysis, arising probably from a penetration injury and subsequent cataract or from penetration and traumatic phacolysis. There were incidental findings of oedema in the suprachoroid in two other animals, presumably as a result of an agonal death.
CONCLUSION: The findings in this study are the first report of phacolysis in a dolphin, and third report of cataracts in cetaceans. The rate of occurrence of juvenile cataracts in humans is 0.4% (Ruttum *et al.* 1987). In this study, the rate of occurrence was higher at 6%.

## 8.2 INTRODUCTION

There are few reports of eye disease in cetaceans, and only one report of cataract. One case of completely opaque, bilateral cataracts has been reported in a mature adult (19.3m) fin whale (Panilov 1975). The animal was described as behaving normally and in good body condition with a full stomach. However, there were no investigations to ascertain the aetiology or age of the cataracts to support the author's statements that good vision is superfluous for survival.

There are several anecdotal reports of extraocular eye disease in live animals such as conjunctivitis in captive dolphins and lenticular opacities in a seal (Lilley 1997, personal communication). Dawson notes that in captive dolphins, lesions occur frequently on the cornea (Dawson *et al.* 1987) and are thought to be due to the effects of estuarine habitats. cohort studies in seals have found 22% prevalence of corneal lesions (Greenwood 1985). It is thought that corneal trauma and subsequent inflammation (keratitis and conjunctivitis) are the lesions most commonly seen, with the ability to retract the globe providing some protection against more serious injury (Sweeney and Ridgway 1975).
More generally in marine mammals, there are reports of lens and anterior

segment diseases in seals (Stoskopf *et al.* 1985). Bilateral lens defects have been reported as an incidental finding in 48% of a population of seals during an outbreak of Phocine Distemper Virus (PDV)( Schoon and Schoon 1992). The aetiology of the lesions was uncertain, but PDV involvement was suspected. Some of the animals also had high tissue levels of mercury and PCBs, and these could also have been causative agents, particularly in young animals whose enzyme systems and blood-aqueous barriers would be immature.
Yellow lenses occur normally in some mammals (squirrel, prairie dog Walls 1942) and as a result of old age in humans (Hogan and Zimmerman 1962). Yellow lenses have been documented in four eyes from *Inia* (Dawson 1980)

## 8.3 MATERIALS AND METHODS

Between 1995 and 1998 the eyes of 50 cetaceans (details of 45 of these whales are given in Appendix 4-1) from 16 different species were examined. The carcasses of most animals were also subjected to a thorough necropsy. Eyes were fixed in 10% buffered formalin. Generally the globe was dissected free of most of the surrounding muscle and a 10mm incision was made in the limbus to enable fixative to penetrate more easily. After fixing, the eye was bisected along a vertical meridian, and two sections for histology were taken in this plane; an anterior segment section, taking cornea, ciliary body, and iris; a posterior segment section, taking an arc of the fundus between ciliary body and the optic nerve. The lenses were removed separately. The samples were embedded in paraffin wax, sectioned at 2-3 microns, and routinely processed for staining with haematoxylin and eosin. Lenses were prepared in a similar way.

## 8.4 RESULTS

Whales with eye pathology are listed in Table 8-1.

**TABLE 8-1. SPECIES OF WHALE AND EYE PATHOLOGY**

| SPECIES | REF. | AGE | DEATH | EYE PATHOLOGY |
|---|---|---|---|---|
| MINKE | 25485-95 | Juvenile | Stranded | Cataract-unilateral |
| DWARF MINKE | 27926-97 | Young | Stranded | Cataract |
| GRAY'S BEAKED | E411-95 | Unknown | Stranded | Yellow lens with cataract |
| PYGMY SPERM WHALE | 27685-96 | Foetal | Dam caught in net | Cataract-bilateral |
| BOTTLE NOSE DOLPHIN | 27201-97 | Adult-2.5m | Stranded | Phacolysis |
| BRYDE'S WHALE | E16-97 | Adult-6m | Stranded | 'Oedema' of suprachoroid |
| LONG-FINNED PILOT WHALE | E193-97 | unknown | Stranded | 'Oedema' of suprachoroid |

In many of the eyes examined, signs of an "agonal death" were evident such as extreme congestion of blood vessels with a high density of red cells, extravasation of blood (petechiae) and in two animals (Bryde's whale and long finned pilot whale) exudation of a densely eosinophilic, proteinaceous fluid into the suprachoroid (Figures 8-1 and 8-2).

Specimens which had undergone some *post mortem* change before fixation showed signs of cellular breakdown and often had gram negative rods in large numbers within blood vessels without concurrent signs of inflammation such as leucocytosis.

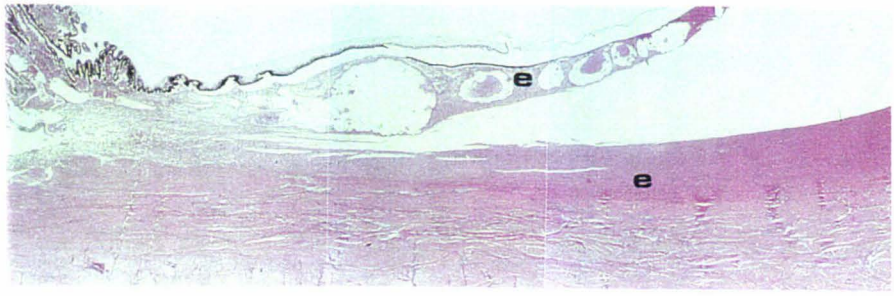



Figure 8-1. Photomontage of ciliary body area, choroid, suprachoroid and sclera of Bryde's whale with proteinaceous exudate. e, exudate. H&Ex10.

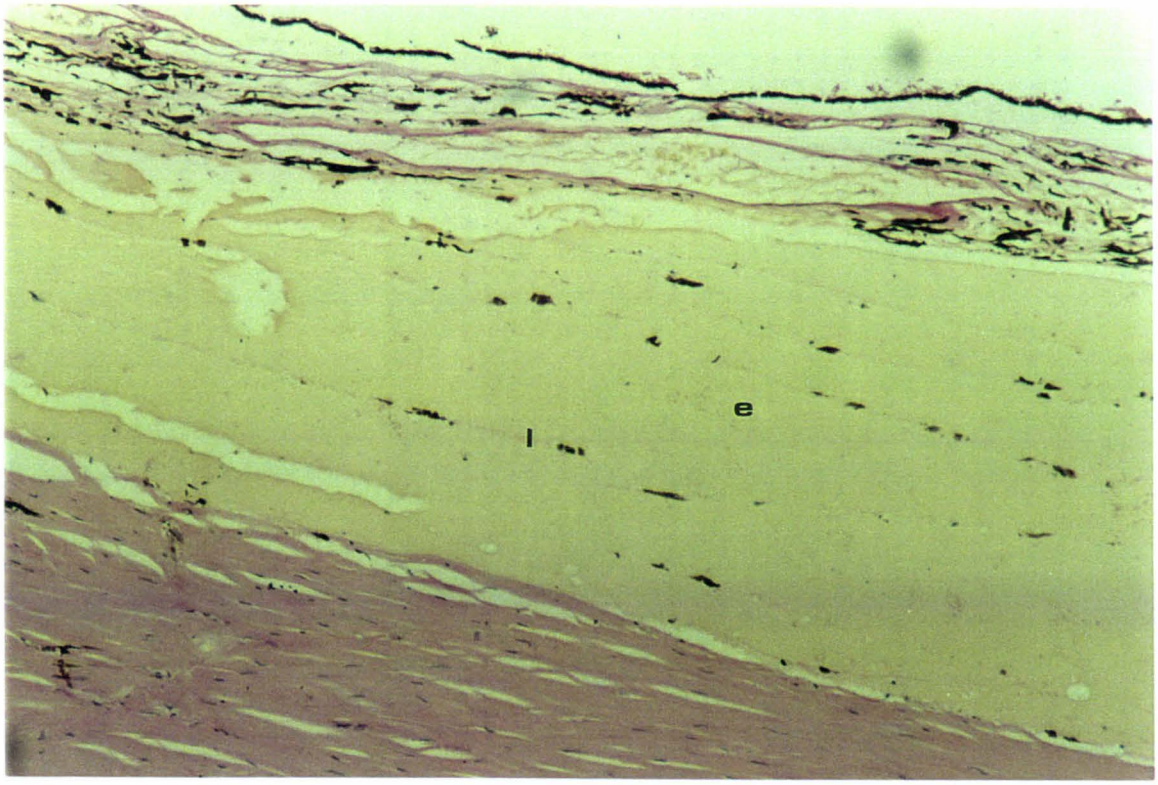



Figure 8-2. Choroid, suprachoroid and sclera of long finned pilot whale E193-97 with marked lamellar separation where exudate occurs. l, lamella, e, exudate. H&Ex80.

## Case Studies

### 8.4i Minke Whale 25485-95 with Cataract

A young (about 2 wks old) female minke had one normal lens, while the other was grossly abnormal. It was shrunken, deformed, densely opaque throughout its nucleus and cortex, and white/pink in colour. Histologically, some fibre walls were still visible, but most were broken and the cells appeared swollen. Within the fibres eosinophilic material with a granular appearance was observed. Large clefts containing similar material were present between the fibres (Figure 8-3).

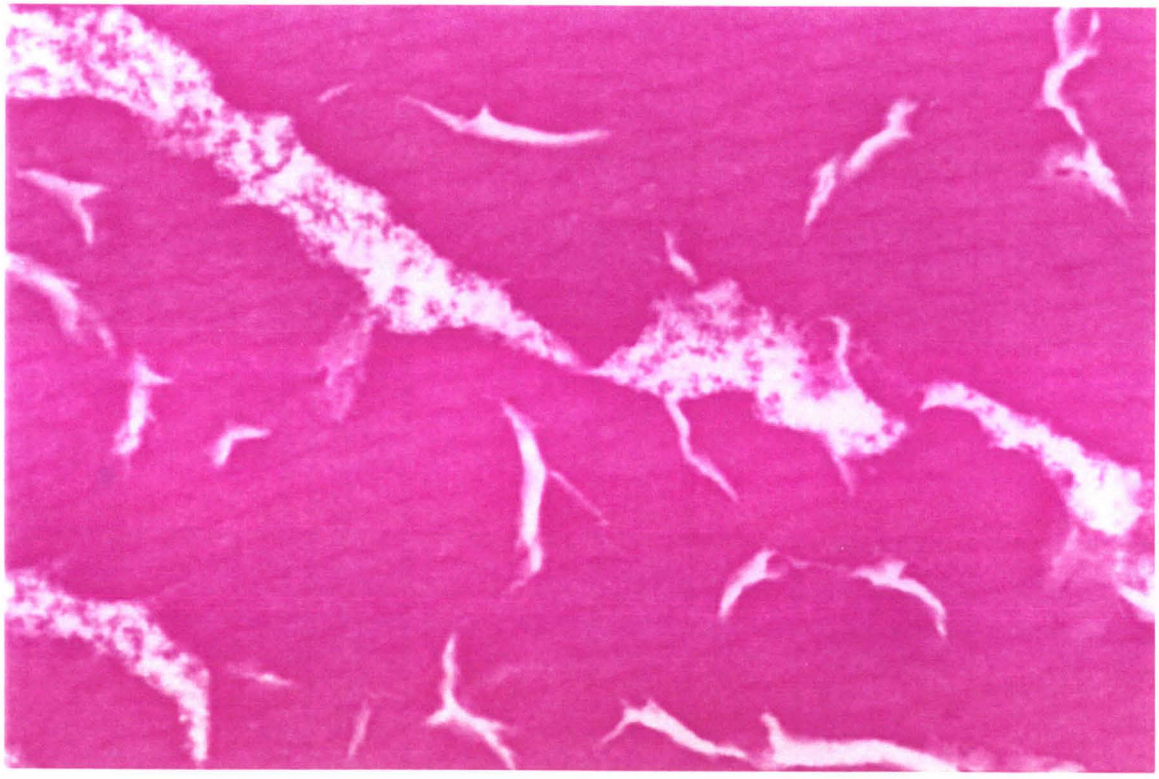


Figure 8-3. Minke lens (25485-95) in an area where lens fibre cell walls are still present. H&E stain x800

### 8.4ii Dwarf Minke Whale 27926-97 with Cataract

Only one eye was examined in this animal. This had an abnormal lens which was shrunken, deformed, and had a moderately opaque, quartz-like appearance (Figure 8- 4). Histologically, the anterior capsule and subcapsular areas showed discreet, localised lesions (Figure 8-5). Normal fibre architecture was lost in these lesions, and was replaced by aggregates of granular eosinophilic material, surrounded by strands of basophilic material. Additional staining techniques of Von Kossa, PAS, and alcian blue were used to identify the basophilic material, and these revealed that it was neither calcium or mucin.

Figure 8-4. Lens of dwarf minke 27926-97 with cataract (left) compared with the normal lens of a dolphin (right).

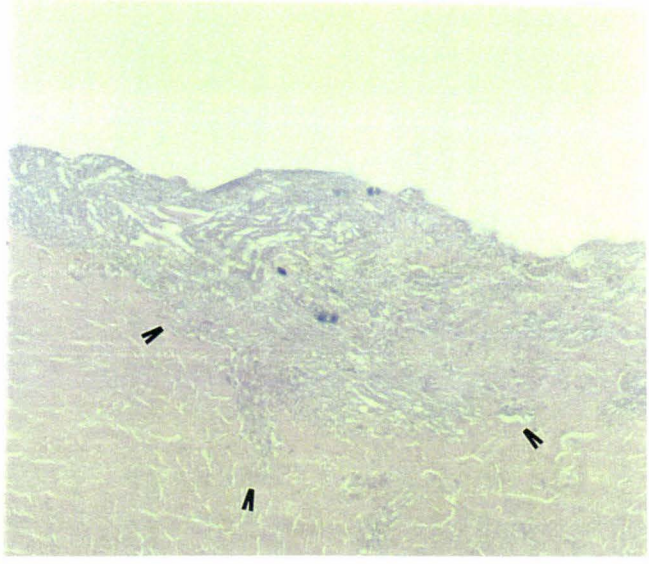


Figure 8-5. Anterior Capsular/subcapsular area dwarf minke whale lens, showing large lesion (arrows). H&Ex80

### 8.4iii Pygmy Sperm Whale 27685-97 with Bilateral Cataracts

A near-term foetal pygmy sperm whale, had bilateral cataracts. Both lenses were of normal size and shape, but were densely opaque. (Figure 8-6). When sectioned, the spherical shape was lost giving the lens a collapsed appearance. Histologically, epithelium completely surrounded the lens. This may have been an unusual plane of section, or migration of the anterior epithelium to a posterior site. The epithelium was stratified in parts and the nuclei had a bilobar appearance. There was no evidence of lens fibre cell walls. A mass of homogeneous, densely eosinophilically stained material replaced the cortex and nucleus. The rest of the eye appeared to be normal.

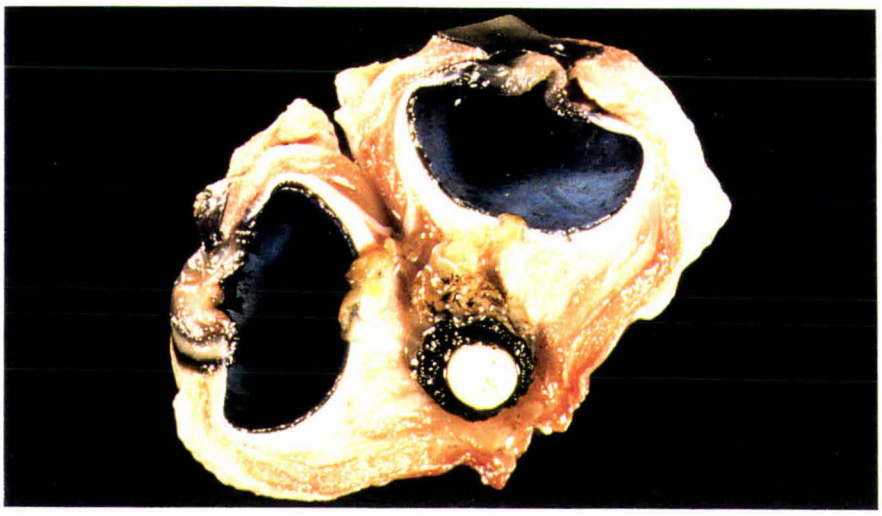


Figure 8-6. Bisected eye of foetal pygmy sperm whale with cataractous lens dissected out and displayed on iris and retrobulbar structures.

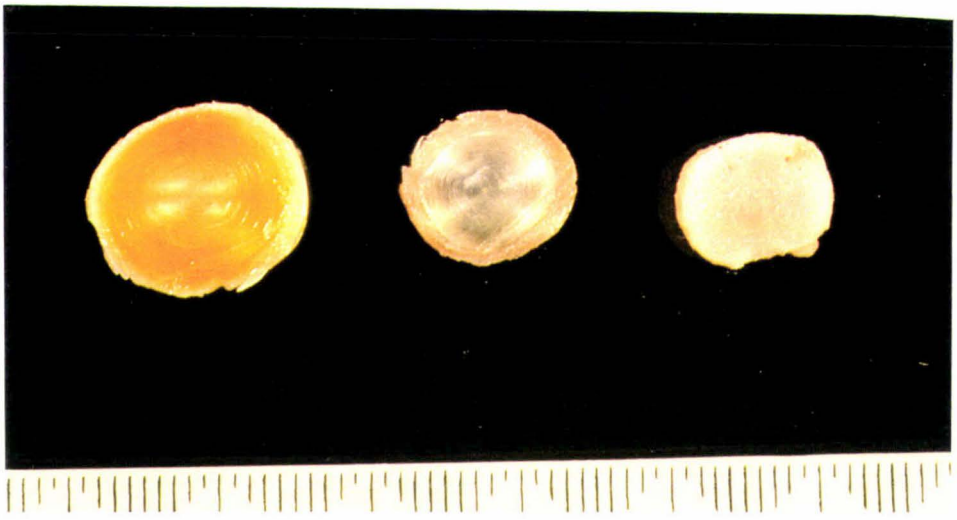


Figure 8-7. Comparison of lens sizes and shapes. a, adult Gray's beaked whale; b, dolphin; c, minke 27926-97.

### 8-4 iv Bottlenose Dolphin 28112-97 with Phacoclastic Uveitis

An adult male bottlenose dolphin had a fibrous lump located ventrally in the posterior segment of the left eye where the lens was absent, indicating that there had been lens rupture resulting in phacoclastic uveitis. Grossly, there was a mildly scarred area on the cornea. Histologically, there was large, thickened plaque of fibrous tissue attached to the interior choroid and ciliary apparatus on one side of the eye. The plaque was composed of bundles of mature collagen which was loose in central areas and more dense peripherally. It was covered in one area by the remnants of a lens capsule.

The other findings elsewhere in this animal were extensive recent skin erosions

and contusions on the left side of head, left flank and flipper. Histologically, the lung showed generalised oedema with a slight excess in numbers of alveolar macrophages many of which contain Gram negative organisms. Colonies of faintly Gram positive short rods were also present in some alveolar capillaries. The kidney contained numerous colonies of similar organisms within glomerular tuft capillaries. The stomach showed occasional foci of lymphoid cells within the lamina propria of the superficial mucosa. In the testis there was moderate, diffuse, testicular degeneration with very few viable sperm present in tubules. The bladder, lymph node, spleen intestine muscle and flipper showed no significant changes. The liver was too autolysed for critical examination. Bacteria were recovered in pure growth from the blood. The diagnosis that was made for this animal was phacoclastic uveitis with recent trauma to left side and terminal bacteraemia (Erysipelas), although there is no histological evidence of an overwhelming infection and it seems likely that bacterial invasion was a terminal event.

### 8.4v Yellow Lens Colouration in Beaked Whales

In two specimens of beaked whales, a straptoothed (E429-96) and a Gray's beaked whale (E411-95), lenses appeared to be excessively yellow, although not opaque when examined grossly (Figures 8-8 and 8-9). Histologically, the lens from the straptoothed whale was normal. However, the Gray's beaked lens showed cataractous changes in a posterior subcapsular site, occupying one quarter of the circumference of the lens. The fibres were swollen, of differing shapes and sizes, and more globular in cross sectional appearance than the usual hexagonal form. The fibre contents were granular. Some clefts appeared between the fibres, often with granular contents. In some subcapsular sites, homogeneous eosinophilic material was present (Figures 8-10a and b).

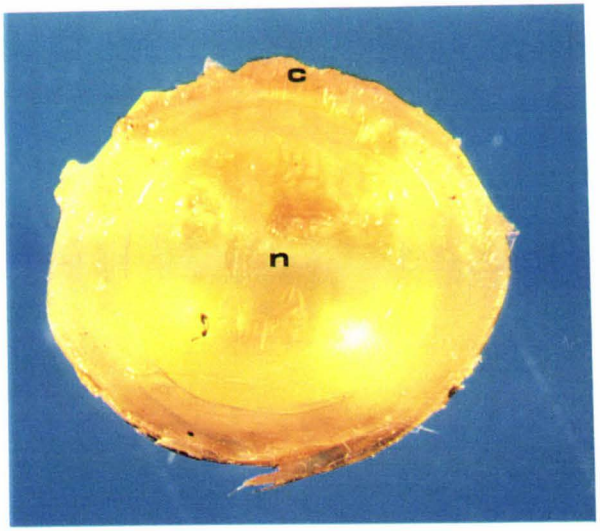



**Figure 8-8.** **Lens of straptoothed whale. c, capsule n, nucleus.**

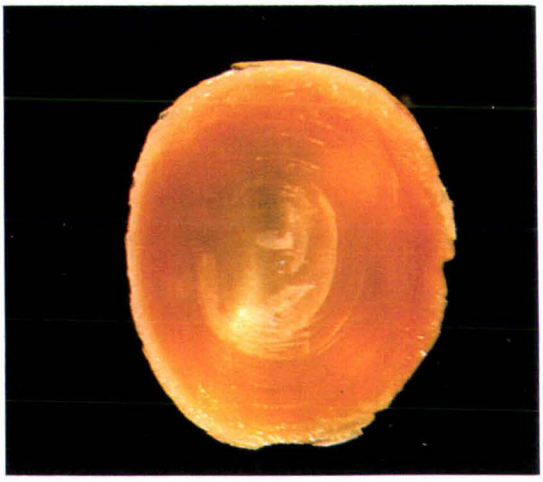
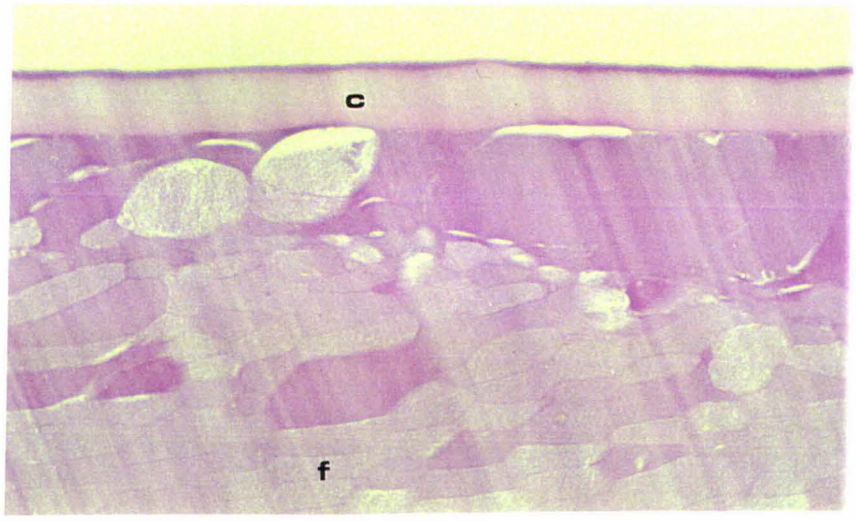
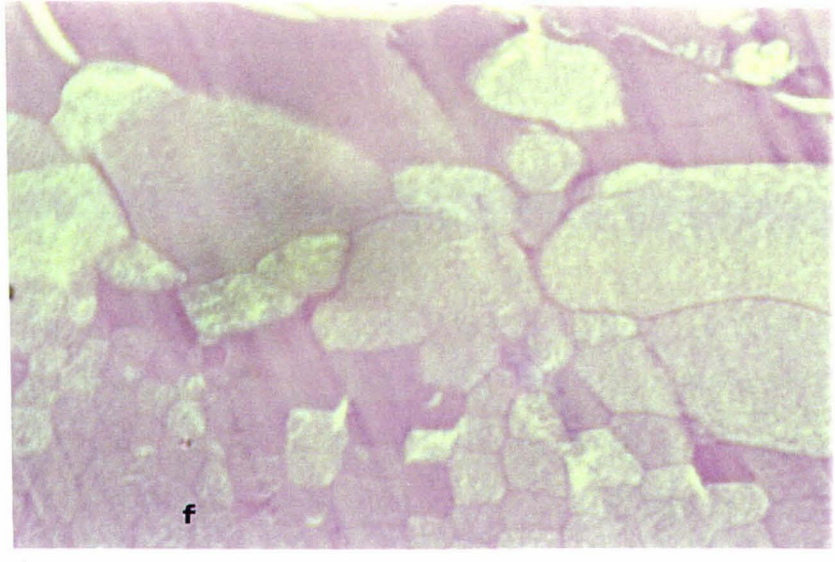


Figure 8-9 Lens of Gray's beaked whale (E411-95).




a

Figures 8-10 a and b. Photomicrograph of Gray's beaked lens in subcapsular site showing degenerative swelling and lysis of fibres. a) H&Ex400 b) H&E with oil immersion x800




b

## 8.5 DISCUSSION

The major finding in the current study was the high prevalence of cataracts, all of which occurred in young animals. The normal overall incidence of cataracts in humans is very variable according to age, disease status and genetic factors, and also many environmental factors such as geographical latitude, diet, drug use, gender and oestrogen levels. Juvenile cataracts have been predicted to occur at a rate of 0.4% in humans (Ruttum *et al.*, 1987).

An incidental finding in this study was that of yellow lenses in two whales. In one of these, a Gray's beaked whale, a small cataract was present. Yellow lenses in whales may be a natural phenomenon, a pathological feature or may, as in humans, have occurred as a result of old age. Urochrome pigment (a metabolic breakdown product of proteins) occurs in human lenses at all ages, but in increasing quantities with age (Hogan and Zimmerman 1962). The effect of a yellow lens is to filter short wavelength (blue) light (Walls 1942). In some animals, this filter effect is deliberately used to enhance contrast in its environment. However, contrast may also be diminished, depending on which colours are being observed. Supposedly natural yellow lenses have also been observed in *Inia* (river dolphins) at *post mortem* (Dawson 1980). In shallow water the absorption of short wavelength scatter within both water and within the eye itself, may be useful. However, in deeper water, as inhabited by some marine species, the effect is unknown, but may be to counter camouflage colouration of animals swimming above (Walls 1942; Tansley 1965; Lythgoe 1979).

### 8.5 i Development of cataracts

A cataract is any discreet or generalised opacity in the lens (Newell and Ernest 1974). Local involvement is usually as a result of the deposition of opaque material, such as cholesterol, whereas generalised changes occur as a result of disturbed metabolism within the lens and subsequent coagulation of lens protein (Paterson 1979). Histopathological changes that occur are hyperplasia and metaplasia of the epithelium, hydropic changes in the fibres leading to necrosis and sometimes, the deposition of calcium and cholesterol crystals (Schoon and Schoon 1992). The aetiology of the cataract affects its pathogenesis, as described by Hogan and Zimmerman (1962). Cataracts can arise as a result of: 1] Aging 2] Environmental causes 3] Systemic disease (naturally occurring or iatrogenic) 4] Inheritance.

1] Nuclear cataracts which form as a result of old age are the result of excessive nuclear sclerosis, a process which occurs normally in the aging lens. Small amounts of urochrome pigment are also deposited in the lens throughout life, and if this occurs excessively, a brown ('brunescent') cataract results (Hogan and Zimmerman 1962). Senile cataracts may also be cortical, where slower lens

metabolism leads to acidification, causing the lens fibres to shrink and lose fluid which collects in clefts or vacuoles. When lens fibre proteins coagulate, an opacity occurs (Hogan and Zimmerman 1962).

2] Cataracts which form as a result of environmental factors can be cortical and/or nuclear. Epidemiological studies have implicated geographical latitude, excessive salt and fat in the diet, alcohol, smoking, use of steroids, selenate, hair dye, oestrogens, gender, and high body mass index as contributing factors. Positive effects have been correlated with other dietary items such as meat, cheese, many fruits, and the ingestion of calcium, folic acid and vitamin E.

3] Cataracts which develop secondarily to systemic disease can be unilateral (local causes) or bilateral (systemic causes) and are capsular, epithelial, cortical and/or nuclear. Their aetiology can be diabetes, galactosaemia, inflammation of the anterior segment, electric shock, hypocalcaemic tetany, and foetal infections such as rubella, or foetal compromise such as maternal parathyroid deficit. The pathogenesis of the cataract depends on the disease which causes it. Damage to the epithelium from inflammatory, neoplastic or traumatic events in the anterior segment can lead to opacity of adjacent fibres and newly formed fibres in the 'bow' region. In disorders of carbohydrate metabolism, such as diabetes and galactosaemia, high blood-sugar levels lead to high sugar levels in aqueous, with a subsequent osmotic crisis in the lens fibres.

Experimental or iatrogenic pathological causes of cataract include a] high levels of galactose, xylose, or fructose. The lens can only utilise glucose, and these substances are believed to produce cataracts either because of an osmotic effect, or due to interference with glutathione synthesis b] amino acid deficiency c] riboflavin deficiency d] a low calcium:phosphate ratio, as a result of parathyroidectomy e] ingestion of poisons such as dinitrophenol, napthalene, thallium, or ergot.

4] Cataracts can form because of an inherited tendency, and in humans may be associated with other systemic defects such as mongolism and cretinism. More recently, a syndrome of myopathy, cardiomyopathy and cataract has also been described (Cruysberg *et al.* 1986). Inherited cataracts have also been recorded in mice, rats, cattle, dogs and sheep (cited by Brooks, 1981) and cats (Kramer *et al.* 1977). The histopathological changes of inherited bilateral cataracts have been documented in Romney sheep by Brooks (1981). In the early stage of formation at about two months of age, there was fibre swelling in the anterior cortex, with some breakdown of fibres releasing a mass of proteinaceous debris. In adjacent epithelial areas, vacuolation was seen. The posterior cortex also became subsequently involved. The equator appeared resistant to change. At the mid to late stage of development, when the anterior and posterior cortex was fully affected, the nucleus started to show changes anteriorly. Cortical fibres began to swell and become globular with subsequent breakdown. There was extensive vacuolation in the anterior epithelium, and migration of cells (similar to bladder

cells) posteriorly. The nuclear bow was disorganised. At its end stage, the lens was little more than a shrunken bag of proteinaceous debris. The epithelium was hyperplastic, with interspersed capsular material. Fibre structure was completely lost, except for small areas remaining at the equatorial cortex.

The end point for very mature cataracts can be either complete absorption of the lens (hypermature) or the escape of fluid which may cause blockage of the drainage angle resulting in glaucoma (Hogan and Zimmerman 1962). Reports of the spontaneous resorption of cataracts have been made in the ostrich (Ofri 1995), and a seal (Lilley 1997).

The aetiology of the bilateral cataracts in the foetal pygmy sperm whale is most likely to be either inheritance or maternal disease.

In the young minkes (25484-94 and 27926-97) with unilateral cataracts, these are unlikely to be congenital (related to maternal causes), inherited, or due to systemic disease since all of these causes tend to be bilateral. A local event, such as uveitis, is therefore the most likely cause. There was no histological evidence of this, but it may have occurred and been resolved.

The prevalence of cataracts in other animals including humans is variable, but one study found that at 43-54 years of age nuclear cataract occurred in 2.9% of the population, rising to 40% at 75 years or older. Corresponding values for cortical cataracts were 1.9% to 21.8%. Much lower values were found for posterior subcapsular cataracts (Klein *et al.* 1998). The fact that juvenile cataracts were seen at a much higher prevalence rate in young whales than in adults suggests that they are associated with early death. Their incidence at a higher rate than in humans raises the possibility that some of the known aetiologies may be of greater significance in whales than in humans.

Whales live to similar ages as humans so senile and environmental types of cataract may occur, but a larger population sample would be required to establish this. In older whales, environmental factors which could be implicated include ultraviolet light (UV) exposure, toxic effects of pollutants, or possibly exposure to an electric shock from rays and eels. Although most UV light is absorbed in the first few millimetres of surface water, traces can reach much greater depths than any other wavelength - enough to affect photographic film after prolonged (80 minute) exposure (Walls 1942). Light of 520 millimicrometre wavelength has been shown to penetrate best at depth (Beebe 1934). It is not known how prolonged exposure to UV light underwater may affect the lens. In species which migrate, long days of summer foraging at the poles and high UV exposure levels in winter due to low latitude may mean that the accumulated dose of UV is relatively high. In addition, ozone depletion and the tendency for the eye to remain open may further expose the lens and cornea to the effects of UV light.

Polychlorinated biphenyl (PCB) pollution of the sea is not believed to be a problem around New Zealand. However, since PCB's are similar to napthalene in

having an aromatic benzene ring, and napthalene is known to be cataractogenic, measurement of the PCB and other toxic agent levels in these animals should be undertaken.

Very little is known about systemic diseases such as diabetes, parathyroid deficiency or viral diseases (rubella, distemper) which may affect whales and produce secondary cataracts.

In order to thoroughly evaluate the incidence and causes of juvenile and adult cataracts in whales, it would be necessary to assess as many living and dead (stranded or bycatch) whales as possible using slit lamp illumination in live animals, and photographic and histopathogical techniques in dead animals. Full necropsy procedures for dead animals with cataracts should include examination for Chediak Higashi Syndrome, cardiomyopathy, other syndromes involving anatomical anomalies, hypoparathyroidism, viral disease (distemper, rubella), as well as analysis for PCB and other toxicological agent levels.

In the case of the bottlenose dolphin, it seems likely that a penetration wound (possibly as a result of a stingray attack) to the cornea had occurred in the past, which may have led to lens damage and uveitis, possibly of a phacolytic (leaking lens proteins) or phacoclastic (lens ruptured) type. This type of uveitis is described as intractable, even with therapy, in cats (Summers *et al.* 1995). Lens proteins are immunologically tissue and species specific, (Hogan and Zimmerman 1962) and elicit a particularly vigorous inflammatory response (Summers *et al.* 1995).

Cats, dogs and humans all have different responses to leaky or ruptured lenses, so it is no surprise that this whale displayed a unique response. The lesion was of very long standing but would have resulted in permanent blindness on the left side.

# SUMMARY DISCUSSION

One of the major tenets of this thesis is that if anatomical form can be observed but function is unknown, the function can often be hypothesised. In support of this hypothesis are the Darwinian theory of evolution and its subsequent discipline - comparative anatomy. In the former, the requirements of a particular habitat influence anatomical form. Typically, within an order the whole body evolves to meet the needs of the environment. In the order carnivora, for example, speed, agility, binocular vision and an appropriate dentition develop to assist predation and meat eating. In the cetacean order, all species have a similar shape apart from the head, which may have a melon or no melon, a beak or a snout, and teeth or baleen. Body size is very variable.

In this study, the eyes from 16 species were examined and a number of hypotheses developed. It is important to bear in mind that in most cases, only one or two individuals from each species were available for examination, and these may not have been representative of the entire species. General species differences are important to consider because the order cetacea inhabits a wide range of diverse habitats such as cold polar seas, muddy river estuaries and warm tropical oceans. This study has demonstrated a number of differences between the baleen and toothed whales, and the sperm whale was found to be exceptional among the toothed whales. Baleen whales were shown to have larger eyes with disproportionately thicker scleras and larger corneas than toothed whales. Their lens size increased in proportion to eye size. There were, however, no major differences in the uveal tract between baleen and toothed whales.
Sperm whales showed some interesting differences compared to other toothed whales. In sperm whales, the sclera that was disproportionately thick and lenses and corneas were disproportionately small. Histologically, the uveal tract was similar, but some ERs were atypical, occurring mainly as groups, and having an inner core which appeared to be devoid of any cellular structures apart from peripherally, where a number of nuclei were often loosely arranged in the form of a ring. It was the presence of these nuclei, and the diameter of the inner core, that precluded the classification of these structures as myelinated axons. Unfortunately, studies of sperm whale ERs with the electron microscope only captured the typical forms.

Optical findings in long-finned pilot whales were those of aerial and aquatic emmetropia by virtue of a cornea which is almost optically neutral in both water and air (slightly converging in air, slightly diverging in water), thus complementing similar findings for the cornea in a beluga and a narwhal by Pardue *et al.* (1993) and the finding of a mildly divergent cornea underwater in a

harbor porpoise by Kroger and Kirschfeld (1992). It would appear that previous work may be erroneous in describing the cornea as optically neutral due to the refractive index of the cornea being so similar to that of water (Walls 1942; Dawson 1972).

The possibility that accommodation is possible in the cetacean eye is supported by the discovery of an apparent bulge in the posterior lens surface, and variable capsular width around the lens. It may be necessary for rays to be restricted to the central area of the lens for emmetropic vision, which may relate to Dral's dorsotemporal 'emmetropic porthole' (1975), although he described this as an attribute of the cornea.

The present study confirms the presence of lens zones, as suggested in Rivamonte's hypothesis (1976) for emmetropia in both media, which proposes that in dimmer, underwater conditions the central lens of high refractive strength is responsible for image formation whereas in brighter aerial conditions the crescent-shaped pupil restricts light to the peripheral area of lower refractive strength. Both the area of increased central lens curvature and the lens RI gradient which were evident in the present study strongly support this theory. Rivamonte did not take into account the possibility that the cornea may be of considerably less significance for aerial vision than was originally thought, but his hypothesis would benefit in these circumstances.

The present study describes emmetropia in both media. This is in contrast to ophthalmoscopic and retinoscopic findings (Dawson *et al.*,1972; Dral 1975; Cronin *et al.*1998) which describe profound myopia in air, although an emmetropic porthole in air has also been described (Dral 1975; Pack and Herman 1995). In the cetacean lens, as with any lens, small changes in dioptric power would be required for near vision. This may be more appropriate for underwater vision since required viewing distance is likely to be shorter than in air, depending on water clarity. Any accommodative capability in whales is unlikely to be achieved by lens flexure from the release of zonule tension as a result of muscular action in the ciliary body since muscle was absent from all the animals studied. A mechanism for accommodation has been proposed which requires engorgement and enlargement of the vascular ciliary body, which would release zonule tension to allow 'rounding up' of the lens, and the subsequent rise in intraocular pressure would increase corneal curvature thereby increasing the dioptric power of the eye. This would probably occur in association with the dive reflex. The findings in dolphins of better near vision under water and better distant vision above water (Herman *et al.* 1975) supports the proposed hypothesis. The attributes necessary for the hypothesis to work are also present; a soft, flexible lens

(although *very fresh* specimens need to be examined to confirm this); a lens capsule with varying width; very large vascular capacity in the uveal tract; and a variable IOP from 'normal' mammalian values of 15-20mm Hg. (up to 33mm Hg has been demonstrated in experimental conditions by Dawson *et al.* 1992 a).

Future areas of research in similar areas to the present work might usefully address the following questions:

What is the significance of the thicker sclera in sperm and baleen whales as shown by biometrical analysis? If it is not related to the depth to which these animals dive (as already discussed in Chapter 4), what other function of their behaviour, their anatomy, or eye function, could this be related to? If the extra thickness is necessary for the maintenance of a particular shape, is this as a result of i] larger and more powerful extraocular muscles than other whales ii] compressive forces during dives due to the presence of air sacs in the region of the eyes in baleen and sperm whales or iii] higher intraocular pressures in these species than in terrestrial mammals?

With respect to the uveal tract, the proposed hypothesis of uveal tract engorgement leading to increased refractive power (i.e. increased intraocular pressure causing increased corneal curvature, and also possibly 'rounding up' of the lens), could be tested. This would require the observation of live animals in air and water to ascertain i] corneal curvature ii] intraocular pressure iii] the degree of engorgement of the uveal tract (although the difficulties involved in observing or imaging this would be enormous) and iv] further studies on refractive power in both media, since the literature to date is not definitive.

Further histological and ultrastructural studies of encapsulated receptors should reveal the prevalence of symmetrical, paciniform types compared to asymmetrical, Ruffini types. The atypical types observed in sperm whales, with their preponderance of myxomatous tissue, could be investigated through serial sections to their tips to confirm that they are ERs and not axons with unusual wrappings en route to innervate the limbus or iris. Large ERs with a preponderance of myxomatous tissue were found in some baleen, and in sperm whales. These whales were also unique in having thicker scleras than many other species. It is not known whether these two observations are linked.

The demonstration of near-emmetropia in air and water in NMR images of the eyes of two whales, due to the presence of a very weakly converging (in air) diverging (in water) cornea, and a powerful central lens area with increased curvature posteriorly, should be repeated in more, very fresh, or freshly fixed specimens to confirm that these observations are generally present.

The observation of a high prevalence of cataracts, particularly in young whales, should provide a rich departure point for a long term study of the incidence and aetiology of cataracts in whales. Live and dead animals should be examined

whenever possible. Affected animals at death should receive a full necropsy, with subsequent toxicological and microbiological investigations to establish whether there were any systemic causes. Evidence of local ocular pathology should be sought. Inheritance should be considered. Epidemiological factors such as habitat, latitude (related to UV exposure), and food sources should also be recorded, since aspects of these are increasingly being recognised as risk factors. Often abnormalities are not apparent because the original pathological condition has resolved, or the normal aging process of the lens has been accelerated.

The initial overall aims of this study were to estimate the importance of vision as a sense in whales and assess the implications of impaired vision with respect to the mass stranding phenomenon. In retrospect these aims were ambitious and could never be met absolutely without studying the retina, or by the use of visual assessment trials in live whales of different species. However, this study has demonstrated some optical attributes. In order to theorise about vision as a factor in strandings, it is necessary to combine the findings of this study with literature pertinent to retinal function.

The present study documents some indications of emmetropia in both air and water, and hypothesises a mechanism for accommodation which would enable a closer focus to be achieved underwater. The functional significance of these findings would be that good aerial vision would be achieved if the central lens area was utilised, so in the case of a constricted pupil, direction of view would be important. In reality, this does seem to be the case (Dawson 1972; Dral 1972; Herman and Pack 1995). With a larger pupil in an underwater situation, it may be more useful to have a closer focus because of variable water quality, and this could be achieved by the hypothetical accommodative mechanism described in Chapter 7.

The literature suggests that the retinal image in a cetacean eye would be small, bright and wide angled with only limited possibility for binocularity, particularly with independent eye movements and complete decussation of the optic tracts (Jacobs *et al.* 1975; Dawson *et al.* 1980).

It has been demonstrated that some species such as orca, dolphins, minke (Spong *et al.* 1971; White *et al.* 1971; Murayama *et al.* 1995) have good visual acuity and anecdotal reports in humpbacks confirm this quite strongly (Madsen *et al.* 1980). The cetacean retina also has some unique and unusual features which enable it to be very sensitive, to both light and movement stimuli. The rod-rich retina is responsible for extreme sensitivity in dim light, and there is some suggestion that cone vision is also used in these conditions (Madsen 1976). Sensitivity to

movement is enhanced by a system of very large and 'giant' ganglion cells (cell bodies >60 microns) with very large axons (up to 8 microns) (Dawson and Perez 1973) and high conduction velocity, which appear to function in a similar way to the 'transient systems' described in terrestrial animals. Superior control over feedback loops in the plexiform layers is given by unusual amacrine cells, which span from the ganglion cells out to beyond the bipolar cells (Dawson and Perez 1973).

Studies to ascertain whether contrast, sensitivity or both of these are used for object detection underwater have been undertaken, with equivocal results (Watkins and Wartzok 1985). There is behavioural evidence to support the former (Lavigne and Ronald 1972; Wartzok 1979; Madsen and Herman 1980) but peak absorption from extracted pigments supports the latter (McFarland 1971; Lavigne and Ronald 1975).

In summary, cetacean vision in air and water is good (Spong *et al.* 1971; White *et al.* 1971; Dral 1975; Herman *et al.* 1975; Madsen *et al.* 1980; Murayama *et al.* 1995; Pack and Herman 1995; Cronin *et al.* 1998). The question of how it should be so has been a subject of some interest and speculation. The cetacean eye appears to be particularly useful for detecting bright, moving objects in dim light such as squid or small silvery fishes. It would also be capable of good vision in bright light by severely limiting the amount of light entering the eye. However, it is likely to be less well suited to conditions with poor contrast, particularly for stationary objects. Thus in turbid water (from turbulent tidal conditions causing excessive particulate matter dredged up from a gently shelving beach) or in stormy conditions, the topography of the sea floor would be difficult to assess, as would land and above water features, viewed either aerially, or from just below the surface. There does seem to be some epidemiological evidence to suggest that strandings occur more frequently in stormy conditions (Brabyn 1990). If vision is one of the variables in the stranding equation, then in such circumstances, stranding would be more likely.

# BIBLIOGRAPHY

Ascher K.W. 1942. Aqueous veins: A Preliminary Note. American Journal of Ophthalmology 25:31. Cited by Hogan M.J., J.A. Alvarado and J.E. Weddell. 1971. In Histology of the Human Eye. W. B. Saunders Co. Philadelphia, London, Toronto.

Ascher K.W. 1953. Aqueous veins: Their Status Eleven Years after their Detection. Archives in Ophthalmology 49:438. Cited by Hogan M.J., J.A. Alvarado and J.E. Weddell. 1971. In Histology of the Human Eye. W. B. Saunders Co. Philadelphia, London, Toronto.

Beebe W. 1934. Deep Sea Fishes of the Bermuda Oceanographic Expeditions, Family *Idiacanthidae*. Zoologica 166:149-241. Cited by Walls G.L. 1942. The Eye and its Adaptive Radiation. Hafner Publishing Co. New York.

Bettelheim F.A., and J.Y. Wang. 1974. Topographic Distribution of Refractive Indices in Bovine Lenses. Experimental Eye Research 18:351-356.

Bill A., and K. Hellsing. 1965. Production and Drainage of Aqueous Humour in the Cynomolgus Monkey (*Macaca irus*). Investigations in Ophthalmology 4:920. Cited by Hogan M.J., J.A. Alvarado and J.E. Weddell. 1971. In Histology of the Human Eye. W. B. Saunders Co. Philadelphia, London, Toronto.

Bill A. 1966. Formation and Drainage of Aqueous Humour in Cats. Experimental Eye Research 5:185. Cited by Hogan M.J., J.A. Alvarado and J.E. Weddell. 1971. In Histology of the Human Eye. W. B. Saunders Co. Philadelphia, London, Toronto.

Brabyn M.W. 1990. An Analysis of New Zealand Whale Strandings. MSc Thesis University of Canterbury. Pages 5, 15, 53.

Brooks H.V. 1981. Studies on an Inherited Cataract of Sheep. MVSc thesis Massey University. Pages 9, 27, 35, 43.

Bryden M.M., and G.S. Molyneux. 1986. Ultrastructure of Encapsulated Mechanoreceptor Organs in the Region of the Nares. Pages 99-107 *in* Bryden M.M., and R. Harrison, ed. Research on Dolphins. Clarendon Press, Oxford.

Burkitt G.H., B. Young, and J.W. Heath. 1993. Wheaters Functional Histology - A Text and Colour Atlas. Churchill Livingstone, Edinburgh. Pages 136-137.

Chan D.Y.C., J.P. Ennis, B.K. Pierscionek, and G. Smith. 1987. Determination and

Modelling of the 3-D Gradient of Refractive Index in Crystalline Lenses. Applied Optometry: 27:926-932. Cited by Pierscionek P.K., D.Y.C. Chan, J.P. Ennis, G. Smith, and R.C. Augusteyn. 1988. Nondestructive Method of Constructing Three Dimensional Gradient Index Models for Crystalline Lenses: 1. Theory and Experiment. American Journal of Optometry and Physiological Optics 65 (6): 481-491.

Chouchkov Ch. 1971. Ultrastructure of Pacinian Corpuscles in Man and Cats. Z. Mikrosk Anat. Forsch. 83:17-32. Cited by Chouchkov Ch. 1978. Cutaneous Receptors. Advances in Anatomy, Embryology and Cell Biology. Springer Verlag, Berlin.

Chouchkov Ch. 1978. Cutaneous Receptors. Advances in Anatomy, Embryology and Cell Biology. Springer Verlag, Berlin. Pages 9-17, 22-31.

Clark C.J., P.D. Hockings, D.C. Joyce, and R.A. Mazucco. Application of MRI to Post - harvest Studies of Fruits and Vegetables (in preparation).

Cronin T.W., J.I. Fasick, and H.C. Howland. 1998. Video Photoretinoscopy of the Eyes of Small Odontocetes *Tursiops truncatus, Phocoena phocoena and Kogia breviceps.* Marine Mammal Science 14(3):584-590.

Cruysberg J.R., R.C. Sengers, A. Pinckers, K. Kubat, and U.J. van Haelst. 1986. Features of a Syndrome with Congenital Cataract and Hypertrophic Cardiomyopathy. American Journal of Ophthalmology 102(6):740-999.

Culling C.F.A., R.T. Allison, and W.T. Barr. 1985. Cellular Pathology Technique, 4th Edition. Butterworths London, Boston, Durban, Singapore, Sydney, Toronto, Wellington. Page 387.

Dawson W.W., L. Bindorf, and J. Perez. 1972. Gross Anatomy and Optics of the Dolphin Eye. Cetology 10:1-12.

Dawson W.W., and J.M. Perez. 1973. Unusual Retinal Cells in the Dolphin Eye. Science 181:747.

Dawson W.W. 1980. The Cetacean Eye. Pages 53-100 in L.M. Herman, ed. Cetacean Behaviour, Mechanisms and Functions. Wiley Interscience, New York.

Dawson W.W., D. Carder, S. Ridgway, and E. Schmeisser. 1981. Synchrony of Dolphin Eye Movements and their Power Density Spectra. Comparative Biochemistry and Physiology 68(a):443-449. Pergamon Press Ltd. Great Britain.

Dawson W.W., J.P. Schroeder, J.C. Dawson , and P.E. Nachtigall. 1992 (a). Cyclic Ocular Hypertension in Cetaceans. Marine Mammal Science 8(2):135-142.

Dawson W.W., G.M. Hope, J.C. Dawson, P.E. Nachtigall, and J.P. Schroeder. 1992(b). Cetacean Lens Zones of Discontinuity - Indices of Health and Development. Marine Mammal Science 8(4):379-386.

Dawson W.W., J.P. Schroeder, and J.F. Dawson. 1987. The Ocular Fundus of Two Cetaceans. Marine Mammal Science 3(1):1-13.

Dawson W.W., J.P. Schroeder, and S.N. Sharp. 1987. Corneal Surface Properties of Two Marine Mammal Species. Marine Mammal Science 3(2):186-197.

Dawson W.W., C.K. Adams, M.C. Barris, and C.A. Litkow. 1979. Static and Kinetic Properties of the Dolphin Pupil. American Journal of Physiology 237(5):301-5.

Dawson S. 1985. The New Zealand Whale and Dolphin Digest. Brick Row Publishing Company Limited. Auckland. Page 1.

Donaldson B.J. 1977. The Tongue of the Bottlenose Dolphin (*Tursiops truncatus*). *In* R.J. Harrison, ed. Functional Anatomy of Marine Mammals, Vol. 3. Academic Press, San Francisco.

Douglas R., and M. Djamgoz. 1990. The Visual System of Fish. Chapman and Hall, London.

Dral A.D.G. 1975. Vision in Cetacea. Jounal of Zoo Animal Medicine 6:17-21.

Dral A.D.G. 1987. On the Optics of the Dolphin Eye. Aquatic Mammals 13(2): 61-64.

Dykes R.W. 1974. Factors Related to the Dive Reflex in Harbor Seals: Respiration, Immersion, Bradycardia, and Lability of the Heart Rate. Canadian Journal of Pharmacology 52:248-258. Cited by Kooyman G.L. 1989. Diverse Divers, Physiology and Behaviour. Springer Verlag, Berlin.

Dykes R.W. 1974. Factors Related to the Dive Reflex in Harbor Seals: Sensory Contributions from the Trigeminal Region. Canadian Journal of Pharmacology 52: 259-265. Cited by
Kooyman G.L. 1989. Diverse Divers, Physiology and Behaviour. Springer Verlag, Berlin.

Eccles C.D., C.J. Clark, S.L. Codd, and R. Dykstra. 1998. Construction of an MRI system for Horticultural and Materials Science Research, Proceedings of the Fifth New Zealand Electronics Conference, Dunedin.

Fernauld, R.D., and S.E. Wright. 1983. Maintenance of Optical Quality during Crytalline Lens Growth. Nature, London 301: 618-620.

Folgering H., P. Wijnheymer, and L. Geeraedts. 1983. Diving Bradycardia is not Related to the Oculocardiac Reflex. International Journal of Sports Medicine 4(3):166-99.

Ford T. J., and S.D. Kraus. 1992. A Rete in the Right Whale. Nature 359:680.

Glynn R.J., W.G. Christen, J.E. Manson, J. Bernheimer, and C.H. Hennekens. 1995. Body Mass Index - an Independent Predictor of Cataract. Archives of Ophthalmology 113(9):1131-1137.

Gooden B.A. 1994. Mechanisms of the Human Diving Response. Integrative Physiological and Behavioural Science 29(1):6-16.

Greenwood A. 1985. Prevalence of Anterior Segment Disease in Captive Pinnipeds. Aquatic Mammals 1:13-15

Greer H.D., and E.W. Massey. 1996. Neurological Injury From Undersea Diving. Pages 529-539 *in* R.W. Evans, ed. Neurology and Trauma. W.B. Saunders Company, Philadelphia.

Gripp K.W., L. Nicholson, and C.I. Scott Jr. 1996. Apparently New Syndrome of Congenital Cataracts, Down Syndrome-like Facial Appearance, Short Stature and Mental Retardation. American Journal of Medical Genetics. 61(4):382-386.

Guyton A.C. 1986. Textbook of Medical Physiology. W.B. Saunders Co. Philadelphia. Page 708.

Halata Z. 1977. The Ultrastructure of the Sensory Nerve Endings in the Articular Capsule of the Knee Joint of the Domestic Cat (Ruffini Corpuscles and Pacinian Corpuscles). Journal of Anatomy 124(3):717-729.

Halata Z., and B.L. Munger. 1980. The Ultrastructure of Ruffini and Herbst Corpuscles in the Articular Capsule of Domestic Pigeon. Anatomical Record 198(4):681-692.

Harding J.J., M. Egerton, R. van Heyningen, and R.S. Harding. 1993. Diabetes, Glaucoma, Sex and Cataract: Analysis of Combined Data from Two Case Control Studies. British Journal of Ophthalmology 77(1):2-6.

Harrison R.J., and G.L. Kooyman. 1971. *In* J.J. Head and O.E. Lowenstein, ed. Diving in Marine Mammals. Oxford Biology Readers.

Harrison R.J. 1977. Functional Anatomy of Marine Mammals. Academic Press, London.

Herman L.M., M.F. Peacock, M.P. Yunker, and C.J. Madsen. 1975. Bottle-nose Dolphin: Double Slit Pupil Yields Equivalent Aerial and Underwater Diurnal Acuity. Science, 189:650-652.

Hodge W.G., J.P. Witcher, and W. Satariano. 1995. Risk Factors for Age Related Cataracts. Epidemiological Reviews 17(2):336-46

Hogan M.J., and L.E. Zimmerman. 1962. Ophthalmic Pathology: An Atlas and Textbook. W.B. Saunders Company, Philadelphia. Pages 661-685.

Hogan M.J., J.A. Alvarado, and J.E. Weddell. 1971. Histology of the Human Eye W. B. Saunders Co. Philadelphia, London, Toronto. Pages 60-62, 126-178, 210, 260-274, 321-336, 611, 645-653.

Holmes, W. 1947. Recent Advances in Clinical Pathology Ed. Dyke. SC. Churchill, London. Cited by Culling C.F.A., R.T. Allison, and W.T. Barr. 1985. Cellular Pathology Technique 4th Edition. Butterworths London, Boston, Durban, Singapore, Sydney, Toronto, Wellington. Page 387.

Ide C., and S. Hayashi. 1988. Specialisations of Plasma Membranes in Pacinian Corpuscles: Implications of Mechano-electric Transduction. Journal Neurocytology. Cited by Munger B.L., and C. Ide . 1988. The Structure and Function of Cutaneous Sensory Receptors. Archives of Histology and Cytology 51(1):1-34.

Iggo, A. 1976. Is the Physiology of Cutaneous Receptors determined by Morphology? Progress in Brain Research 43:15-31. Cited by Bryden M.M., and G.S. Molyneux. 1986. Ultrastructure of Encapsulated Mechanoreceptor Organs in the Region of the Nares. In Bryden M.M., and R. Harrison, ed. Research on Dolphins. Clarendon Press, Oxford.

Jacobs M., P. Morgane, and W. McFarland. 1975. Degeneration of Visual Pathways in the Bottlenose Dolphin Brain. Brain Research 88:346-352. Cited by Dawson W.W. 1980. Pages 53-100 in L.M. Herman ed. Cetacean Behaviour; Mechanisms and Functions. Wiley Interscience, New York.

Jain K.K. 1994. High Pressure Neurological Syndrome (HPNS). Acta Neurologica Scandinavica 90(1):45-50.

Jansen J., and J.K.S. Jansen. 1969. The Nervous System of Cetacea. Pages 187-201 in H.T. Anderson, ed. The Biology of Marine Mammals. Academic Press, New York.

Javitt J.C., and H.R. Taylor. 1994. Cataract and Latitude. Documenta Ophthalmologica 88(3-4):307-25.

Jenkins F.A., and H.E. White. 1957. In Fundamentals of Optics, Mc Graw Hill Book Company Inc. New York. Page 28

Jocson V. L., and W.M. Grant. 1965. Interconnections of Blood Vessels and Aqueous Vessels in Human Eyes. Archives Ophthalmology 73:707. Cited by Hogan M.J., J.A. Alvarado, and J.E. Weddell. 1971. Histology of the Human Eye. W. B. Saunders Co. Philadelphia, London, Toronto.

Johnson G.L. 1901. Contributions to the Comparative Anatomy of the Mammalian Eye, based chiefly on Ophthalmoscopic Examination. Philosophical Transactions - Royal Society of London 194:1-82.

Jonah. The Old Testament. The Holy Bible. Eyre and Spottiswoode, London 1897 (antique edition). Page 764.

Jubb K.V.F., P.C. Kennedy, and N. Palmer. 1993. Pathology of Domestic Animals. Vol. 1, 4th Edition. Academic Press Inc. Harcourt Brace Janovich, San Diego.

Kannan N., S. Tanabe, M. Ono, and R. Tatsukawa. 1989. Critical Evaluation of Polychlorinated Biphenyl Toxicity in Terrestrial and Marine Mammals: Increasing Impact of Non- ortho and Mono- ortho Coplanar Polychlorinated Biphenyls from Land to Ocean. Archives of Environmental Contamination and Toxicology 18(6):850-857.

Kastelain R.A., Ruud C.J.V. Zweypfennig, and H. Spekreijse. 1990. Anatomical and Histological Characteristics of the Eyes of a Month Old and an Adult Harbour Porpoise (*Phocoena phocoena*). Pages 463-480 *in* J. Thomas and P. Kastelein, ed. Sensory Abilities of Cetaceans. Plenum Press, New York.

Khomenko B.H. 1970. [The Histostructure and Innervation of the Sound Apparatus - nasal sacs in Dolphins]. Dopovidi Akad. Nauk. Ukrayinskoy, R.S.R. 32, 83-7. In Russian, English abstract seen and cited by Bryden M.M., and G.S. Molyneux. 1986. Ultrastructure of Encapsulated Mechanoreceptor Organs in the Region of the Nares. Pages 99-107 in M.M. Bryden and R. Harrison, eds. Research on Dolphins. Clarendon Press, Oxford.

Kirschvink J.L., A.E. Dizon, and J.A. Westphal. 1986. Evidence from Stranding for Geomagnetic Sensitivity in Cetaceans, Journal of Experimental Biology 120:1-24.

Klein B.E., R. Klein, and K.E. Lee. 1998. Incidence of Age Related Cataract: the Beaver Dam Eye Study. Archives of Ophthalmology 116(2):219-25.

Klein B.E., R. Klein, and L.L. Ritter. 1994. Is there Evidence of an Oestrogen Effect on Age Related Lens Opacities? The Beaver Dam Eye Study. Archives of Ophthalmology 112(1):85-91.

Klinowska M. 1985. Cetacean Live Stranding Sites Relate to Geomagnetic Topography. Aquatic Mammals 11:27-32. Cited by Wiltschko R., and W. Wiltschko. 1995. Magnetic Orientation in Animals. Springer Verlag.

Klinowska, M. 1985. Cetacean Live Stranding Dates Relate to Geomagnetic Disturbances. Aquatic Mammals 11:109-119.

Kooyman G.L., and H.T. Anderson. 1969. Pages 77-80 in H.T. Anderson ed. The Biology of Marine Mammals. Academic Press, New York.

Kooyman G.L. 1989. Diverse Divers, Physiology and Behaviour. Springer Verlag, Berlin. Pages 18, 30.

Kooyman G.L., and P.J. Ponganis. 1998. The Physiological Basis of Diving to Depth: Birds and Mammals. Annual Review of Physiology 60:19-32.

Kroger R.H.H., and K. Kirschfeld. 1992. The Cornea as an Optical Element in the Cetacean Eye. Pages 97-106 *in* J. Thomas, P. Kastelein, and Supin, eds. Marine Mammal Sensory Systems. Plenum Press, New York.

Lavigne D.M., and K. Ronald. 1972. The Harp Seal; *Pagophilus groenlandicus* (Erxleben 1977) XXIII Spectral Sensitivity. Canadian Journal of Zoology 500:1197-1206. Cited by Watkins W.A., and D. Wartzok. 1985. Sensory Biophysics of Marine Mammals. Marine Mammal Science 1(3);219-260.

Lilley, J. 1997. The Misadventures of Dumbo - a Vagrant Southern Elephant Seal. Marine Watch New Zealand (personal communication).

Lowenstein W.R., Y. Kanno, and S. Socolar. 1978. The Cell-to-Cell Channel. Federation Proceedings 37. Munger B.L., and C. Ide. 1988. The Structure and Function of Cutaneous Sensory Receptors. Archives of Histology and Cytology 51(1):1-34.

Lythgoe J.N. 1979. The Ecology of Vision. Clarendon Press, Oxford. Cited by Douglas R.,and M. Djamgoz. 1990. The Visual System of Fish. Chapman and Hall, London.

Lythgoe J.N. 1968. Visual Pigments and Visual Range Underwater. Vision Research

88:997-1011. Cited by Watkins W.A., and D.Wartzok. 1985. Sensory Biophysics of Marine Mammals. Marine Mammal Science, 1(3):219-260.

Madsen C. 1976. Tests for Colour Discrimination and Spectral Sensitivity in the Bottlenose Dolphin *Tursiops truncatus*. PhD thesis, University of Hawaii. Cited by Dawson W.W. 1980. The Cetacean Eye. Pages 53-100 in L.M. Herman, ed. Cetacean Behaviour, Mechanisms and Functions. Wiley Interscience, New York.

Madsen C.J., and L.M. Herman. 1980. Social and Ecological Correlates of Cetacean Vision and Visual Appearance. Pages 101-149 in L.M. Herman, ed. Cetacean Behaviour, Mechanisms and Functions. Wiley Interscience, New York.

Matthiessen L. 1886. Uber den physikalisch- optischen Bau des Auges der cetacean under Fische Pflugers Archiv.38:521 1886. Cited by Kroger R.H.H., and K. Kirschfeld. 1992. The Cornea as an Optical Element in the Cetacean Eye. Pages 97-106 in J. Thomas, P. Kastelein, and Supin, eds. Marine Mammal Sensory Systems. Plenum Press, New York.

Matthiessen L. 1893. Uber den physikaish-optischen bau der angenoom knowal und finwal. Z. Verl Augenheilk,7, 94. 1893. Cited by Dawson W.W. 1980. The Cetacean Eye. Pages 53-100 *in* L.M. Herman, ed. Cetacean Behaviour, Mechanisms and Functions. Wiley Interscience, New York.

Maurice D.M. 1957. The Structure and Transparency of the Cornea. Journal of Physiology, London 136:263-286.

McFarland W.N. 1971. Cetacean Visual Pigments. Vision Research 11:1065-1076. Cited by Watkins W.A. and D. Wartzok. 1985. Sensory Biophysics of Marine Mammals. Marine Mammal Science 1(3):219-260.

Melville, H. 1942. Moby Dick. Dodd, Mead and Co., New York.

Merriam J.C. 1996. The Concentration of Light in the Human Lens. Transactions of the American Ophthalmological Society 94:803-918.

Meyer W., and K. Neurand. 1982. The Demonstration of Krause End Bulbs (Paciniform Corpuscles) in the Hairy Skin of the Pig. Anatomica, Histologica, Embryologia 11(3):283-8.

Miller M.E. 1964. The Anatomy of the Dog. W. B. Saunders Co., Philadelphia, London.

Munger B.L., and C. Ide. 1987. The Enigma of Sensitivity in Pacinian Corpuscles - A Critical Review and Hypothesis of Mechanoelectric Transduction. Neuroscience

Research 5:1-15. Cited by Munger B.L., and C. Ide. 1988. The Structure and Function of Cutaneous Sensory Receptors. Archives of Histology and Cytology 51(1):1-34.

Munger B.L., and C. Ide. 1988. The Structure and Function of Cutaneous Sensory Receptors. Archives of Histology and Cytology 51(1):1-34.

Munz F.W. 1965. Adaptation of Visual Pigments to the Photic Environment. Pages 27-51 *in* A.V.S. de Reuck and J. Knight, eds. Colour Vision: Physiology and Experimental Psychology. CIBA Foundation Symposium. J.A. Churchill, London. Cited by Watkins W.A., and D. Wartzok. 1985. Sensory Biophysics of Marine Mammals. Marine Mammal Science, 1(3):219-260.

Murayama T.M., H. Somiya, I. Aokii, and T. Ishii. 1995. Retinal Ganglion Cell Size and Distribution Predict Visual Capabilities of Dall's Porpoise. Marine Mammal Science 11(2):136-149.

Murphy C. J., and H.C. Howland. 1983. Owl Eyes: Accommodation, Corneal Curvature and Refractive State. Journal of Comparative Physiology 151:277-284.

Murphy C.J., R.W. Bellhorn, T. Williams, M.S. Burns, F. Schaeffel, and H. Howland. 1990. Refractive State, Ocular Anatomy and Accommodative Range of the Sea Otter. Vision Research 1:23-32.

Murphy C.J., T.J. Kern, and H. Howland. 1992. Refractive State, Accommodative Range and Ocular Anatomy of the Asian elephant (*Elephas maximus*). Vision Research 32:2013-2021.

Necker R. 1974. Temperature Sensitivity of Slowly Adapting Mechanoreceptors on the Beaks of Pigeons. Ed. Scwartzkopff J. Symposium: Mechanoreception. Abhandlungen der Rheinisch-Westfalischennn Akad Wissennnschaftenn. Westdeutscher Verlag Opladen Bd 53. Cited by Wickham M.G. 1980. Irido-corneal Angle of Mammalian Eyes: Comparative Morphology of Encapsulated Corpuscles in Odontocete Cetaceans. Cell Tissue Research 210:501-515.

Newell F.W., and J.T. Ernest. 1974. The Lens. Pages 313-327. In Ophthalmology, Principles and Concepts, 3rd. ed. C.V. Mosby Co., St Louis. Cited by Brooks H.V. 1981. Studies on an Inherited Cataract of Sheep. MVSc thesis Massey University.

Ofri R., and I. Horowitz. 1995. Spontaneous Cataract Resorption in an Ostrich. Veterinary Record. 136(11):276.

Pack A.A., and L.M. Herman. 1998. Sensory Integration in the Bottlenose Dolphin: Immediate Recognition of Complex Shapes Across the Senses of Echolocation and

Vision. Journal of the Acoustical Society of America 98:722-733.

Palmer A.C., I.M. Calder, and J.T. Hughes. 1987. Spinal Cord Degeneration in Divers. Lancet, 2(8572):1365-1366.

Palmer E., and G. Weddell. 1964. The Relationship between Structure, Innervation and Function of the Skin of the Bottlenose Dolphin (*Tursiops truncatus*). Proceedings of the Zoological Society of London 143:553-67.

Panilov B.G. 1975. Fin Whale (*Balaenoptera physalus*) with Complete Cataract of Both Eyes. Zoologicheski Zhurnal 54.11:1739-1740. (Russian).

Pardue M.T., J.G. Sivak, and K.M. Kovacs. 1993. Corneal Anatomy of Marine Mammals Canadian Journal of Zoology 71:2282-2290.

Paterson C. A. 1979. Crystalline Lens. Pages 232-260 in R.E. Records, ed. Physiology of the Human Eye and Visual System. Harper and Row, Hagerstown, Maryland. Cited by Brooks H.V. 1981. Studies on an Inherited Cataract of Sheep. MVSc thesis Massey University.

Peterson Jones, SM. 1989. Pages 13-26 in S. Wheeler, ed. Manual of Small Animal Neurology. BSAVA Cheltenham.

Pierscionek P.K., D.Y.C. Chan, J.P. Ennis, G. Smith, and R.C. Augusteyn. 1988. Nondestructive Method of Constructing Three Dimensional Gradient Index Models for Crystalline Lenses: 1. Theory and Experiment. American Journal of Optometry and Physiological Optics 65(6):481-491.

Pilleri G., and A. Wandeler. 1964. Developmental and Functional Anatomy of the Eyes of the Fin Whale *Balaenoptera physlus*. Acta anatomica 57(Suppl. 57):1-74.

Polacek P, and K. Mazanek. 1966. Ultrastructure of Mature Pacinian Corpuscle from Mesentry of Adult Cat. Z. Miikr. Anat. Forsch. 74:343-354. Cited by Chouchkov Ch. 1978. Receptors. Advances in Anatomy, Embryology and Cell Biology. Springer Verlag, Berlin.

Prince J.H. 1956. Comparative Anatomy of the Eye. C.C. Thomas, Springfield, USA.

Prince J.H., C.D. Diesem, I. Eglitis, and G.L. Ruskell. 1960. Anatomy and Histology of the Eye and Orbit in Domestic Animals. C.C. Thomas, Springfield, USA.

Putter, A. 1903. Die Augen der Wassersaugetiere. Zoologische Jahrbucher (Anat.), 17:232-247. Cited by Waller G.N.H. 1992. Ciliary Muscles in the Eye of the Long

Finned Pilot Whale *Globiocephala melaena* (Traill, 1809). Aquatic Mammals 18(2):36-39.

Ridgway S.H. 1988. The Cetacean Nervous System. Pages 20-24 in L.N. Irwin ed. Comparative Neuroscience and Neurobiology. Birkhauser, Boston Inc. Boston.

Risavi A., A. Muniti, and S. Pitpovic. 1983. Lesions in the Inner Ear of a Diver. Vojnoanitetski Pregled 40(5):371-374.

Rivamonte L. 1976. Eye Model to Account for Comparable Aerial and Underwater Acuities of the Bottlenose Dolphin. Netherlands Journal Sea Research 10:491-498.

Rochon-Duvigneaud A. 1940. L'oeil des cetaces. Arch. Mus. Nat. Hist. Paris 16:57-90. Cited by Dawson W.W. 1980. The Cetacean Eye. Pages 53-100 in L.M. Herman, ed. Cetacean Behaviour, Mechanisms and Functions. Wiley Interscience, New York.

Ruttum M.S., D.B. Nelson, M.J. Wamser, and M. Balliff. 1987. Detection of Congenital Cataracts and Other Media Ocular Opacities. Pediatrics 79(5):814-877.

Sassani J.W., and M.D. Osbakken. 1984. Anatomic features of the Eye disclosed with NMR Imaging. Archives of Ophthalmology 102(4):541-546.

Schmidt-Nielson, K. 1990. Animal Physiology: Adaptation and Environment. Cambridge University Press, USA. Page 192.

Schoon H. A., and D. Schoon. 1992. Lenticular Lesions in Harbour seals. Journal of Comparative Pathology 107:379-388.

Shanthaveerappa T.R,, and G.H. Bourne. 1963. New Observations on the Structure in Pacinian Corpuscles and its Relationship to the Epithelium of Peripheral Nerves. American Journal of Anatomy 112:97-109. Cited by Munger B.L. and C. Ide. 1988. The Structure and Function of Cutaneous Sensory Receptors. Archives of Histology and Cytology 51(1):1-34.

Sivak J.G. 1980. Accommodation in Vertebrates - a Contemporary Survey. Pages 281-327 *in* J.A. Zadunaisky and H. Davson, eds. Current Topics on Eye Research Vol. 3. Academic Press, New York.

Sivak J.G. 1991. Shape and Focal Properties of the Cephalopod Ocular Lens. Canadian Journal of Zoology 69:2501-2506.

Slijper E.J. 1962. Whales, Hutchinson, London. Cited by Waller G.N.H. 1992. Ciliary Muscles in the Eye of the Long Finned Pilot Whale *Globiocephala melaena* (Traill,

1809). Aquatic Mammals 18(2):36-39.

Smythe R.H. 1958. Veterinary Ophthalmology. Balliere, Tindall and Cox, London. Page 47.

Spooner J.D. 1957. Ocular Anatomy. Butterworths, London, Boston. Pages 11, 35, 177.

Spong P., and W. White. 1971. Visual Acuity and Discrimination Learning in the Dolphin *(Lagenorhynchus obliquidens)*. Experimental Neurology 31:431-436.

Summers P.A., J.F. Cummings, and A. de Lahunta. 1995. Veterinary Neuropathology. Mosby Year Book Inc., St Louis. Pages 408-410.

Stoskopf M.K., S. Zimmerman, L.W. Hirst, and R. Green. 1985. Ocular Anterior Segment Disease in Northern Fur Seals. Journal of the American Veterinary Association 187(11):1141-1144.

Sweeney J.C., and S.H. Ridgway. 1975. Common Diseases of Small Cetaceans. Journal of the American Veterinary Medical Association 167(7):533-540.

Tachibana T., N. Fujwara, and T. Nawa. 1989. Intraperineural Localisation of Lamellated Sensory Corpuscles in the Skin and Oral Mucosae of the Adult Cat and Miniature Pig. Journal of Neurocytology 18(1):61-69.

Tamm E.R., and E. Lutjen-Drecoll. 1996. Ciliary body Microscopy Research and Technique 33(5):390-439.

Tansley K. 1965. Vision in Vertebrates. Chapman Hall, London. Cited by Douglas R., and M. Djamgoz. 1990. The Visual System of Fish. Chapman and Hall, London.

Tavani A., E. Negri, and C. La Vecchia. 1996. Food and Nutrient Intake and Risk of Cataract. Annals of Epidemiology 6(1):41-6.

Tinker S.W. 1988. Pages 79-80 *in* N.Y. Leiden and E.J. Brill, eds. Whales of the World. Bess Press, Honolulu.

Vogl A.W., and H.D. Fisher. 1982. Arterial retia to Supply the Central Nervous System in Two Small Toothed Whales - Narwhal (*Monodon monoceros*) and Beluga (*Delphinapterus leucas*). Journal of Morphology 174:41-56.

Vrabec F. 1961. The Topography of Encapsulated Terminal Sensory Corpuscles of the Angle of the Goose Eye. Pages 325-333 *in* G. Smelser, ed. The Structure of the Eye. Academic Press, New York.

Vrabec F. 1972. Encapsulated Sensory Corpuscles in the Sclerocorneal Boundary Tissues of the Killer Whale *Orcinus orca.* Acta Anatomica 81:23-29.

Waller G.N.H. 1980. The Visual System of Toothed Whales (Mammalia, cetacea, odontoceti). PhD thesis, Cambridge University. Cited by Waller G.N.H. 1984. The Ocular Anatomy of Cetacea - An Historical Perspective. Pages 137-148 in G. Pilleri, ed. Investigations on Cetacea Vol. XVI. University of Berne Press, Berne.

Waller G.N.H. 1983. Is the Blind River Dolphin Sightless? Aquatic Mammals 10(3):106-108.

Waller G.N.H. 1984. The Ocular Anatomy of Cetacea - An Historical Perspective. Pages 136-146 *in* G Pilleri ed. Investigations on Cetacea Vol. XVI. University of Berne Press.

Waller G.N.H. 1992. Ciliary Muscles in the Eye of the Long Finned Pilot Whale *Globiocephala melaena* (Traill, 1809). Aquatic Mammals 18(2):36-39.

Walls G.L. 1942. The Eye and its Adaptive Radiation. Hafner Publishing Co. New York. Pages 210-212, 373, 410-412, 414-416, 426, 445-446.

Watkins W.A., and D. Wartzok. 1985. Sensory Biophysics of Marine Mammals. Marine Mammal Science 1(3):219-260.

Wartzok D. 1979. Phocid Spectral Sensitivity Curves. Abstract. Third Biennial Conference on the Biology of Marine Mammals, Seattle. Cited by Watkins W.A. and D. Wartzok. 1985. Sensory Biophysics of Marine Mammals. Marine Mammal Science, 1(3):219-260.

White D., N. Cameron, P. Spong, and J. Bradford. 1971. Visual Acuity of the Killer Whale (*orcinus orca*). Experimental Neurology 32:230-236.

West J.A., J.G. Sivak, C.J. Murphy, and K.M. Kovacs. 1991. A Comparative Study of the Iris and Ciliary Body in Aquatic Mammals. Canadian Journal of Zoology 69:2594-2607.

West S.K., and C.T. Valmadrid. 1995. Epidemiology of Risk Factors for Age Related Cataract. Survey of Ophthalmology 39(4):323-34.

Worthington C.R. 1984. The Structure of Cornea. Quarterly Review of Biophysics 17(4):423-451.

Wickham M.G. 1980. Irido-corneal Angle of Mammalian Eyes: Comparative Morphology of Encapsulated Corpuscles in Odontocete Cetaceans. Cell Tissue Research 210:501-515.

Wiltschko R., and W. Wiltschko. 1995. Magnetic Orientation in Animals. Springer Verlag, Berlin.

Young N., G.M. Hope, W.W. Dawson, and R.L. Jenkins. 1988. The Tapetum Fibrosum in the Eyes of Two Small Whales. Marine Mammal Science 4(4):281-290.

Zimmer E.Z., M. Bronshtein, E. Ophir, I. Meizner, R. Auslender, G. Groisman, and H. Meyer. 1993. Sonographic Diagnosis of Foetal Congenital Cataracts. Prenatal Diagnosis 13(6):503-511.

Zoeger J., J.R. Dunn, and M. Fuller. 1981. Magnetic Material in the Head of the Common Pacific Dolphin. Science 213:892-894.

## APPENDIX 4-1.

TABLE A4-1.

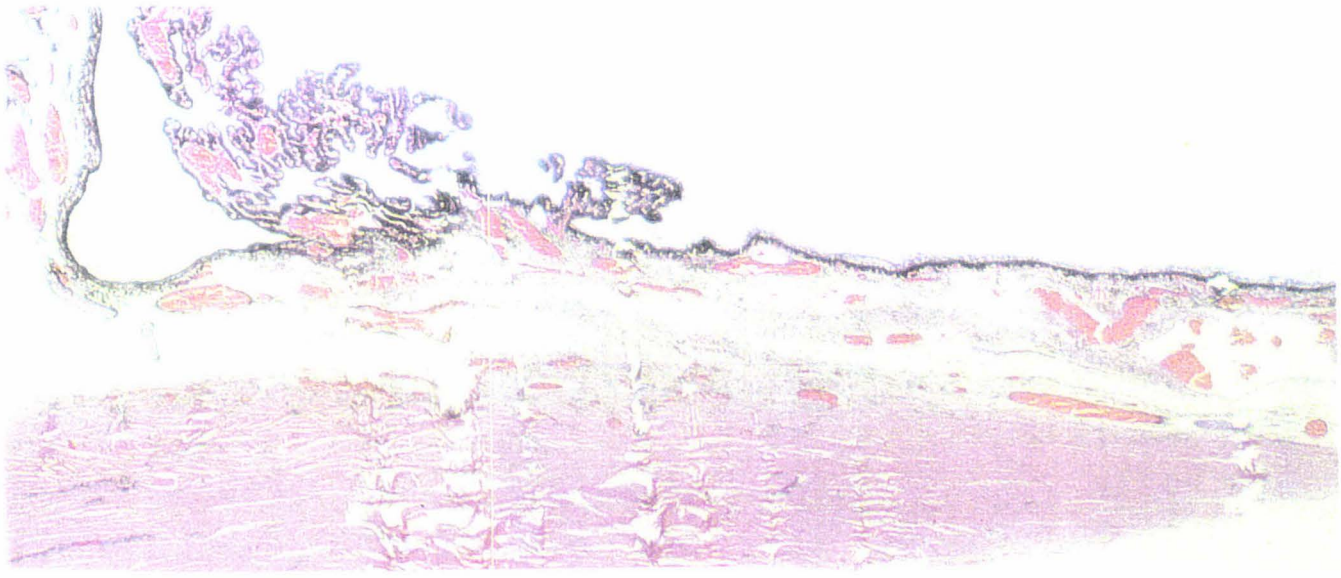
FORTY FIVE WHALES EXAMINED IN STUDIES

\# Chapter 8, Pathology
* Chapters 5 and 6, Histology of Uveal Tract
" " Chapter 7, Optics

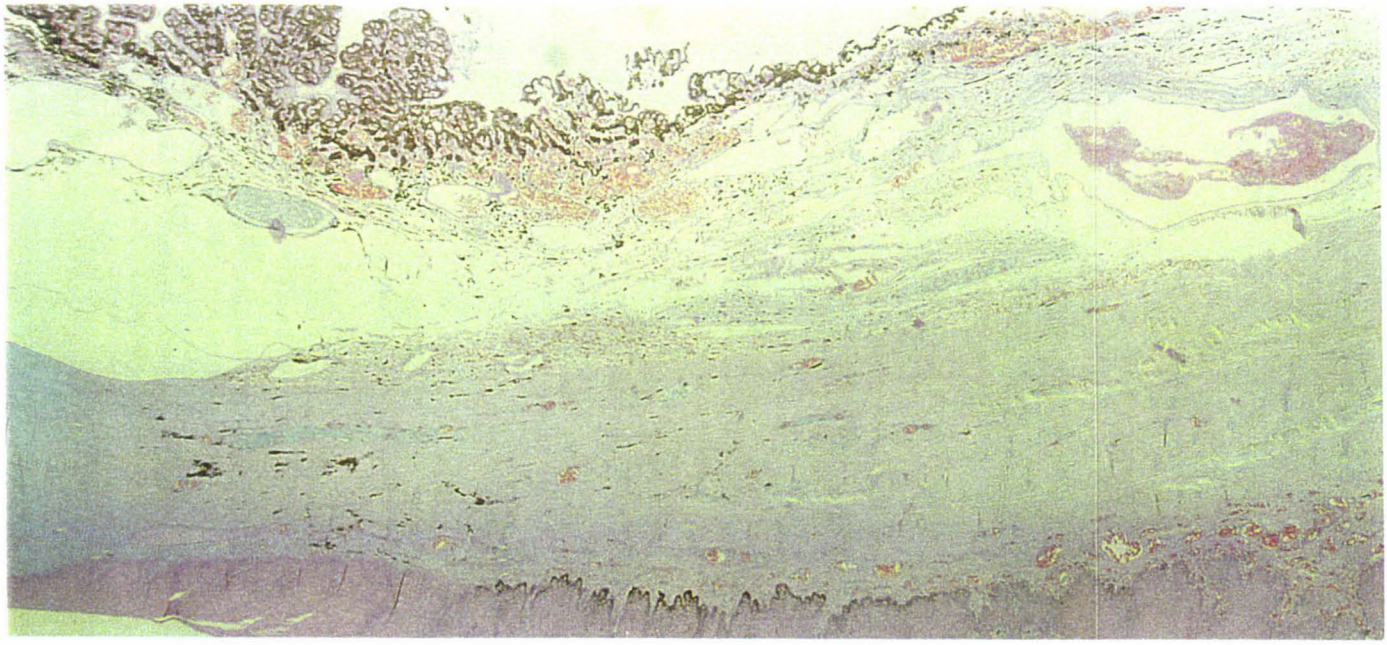
| BIOMETRICS REFERENCE | WHALE | PATHOLOGY/HISTOLOGY NO. | cornea ml | globe ml | cornea dv | globe dv | globe int dv | globe axial | cornea thickness perioheral | cornea thickness central | scleral thickness | nerve diameter | lens diameter horizontal | lens diameter vertical |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Pygmy right (adult) | | 30 | 80 | 20 | 70 | | 30 | 1 | 0.5 | 19 | 8 | - | - |
| 2 | Brydes | 25486-95 E16-97 | 40 | 90 | 30 | 75 | | 40 | - | - | 25 | 8 | - | - |
| 3 | Minke# | 25485-95 | 40 | 74 | 30 | 65 | | 35 | 2 | 1 | 11 | 4 | - | - |
| 4 | Minke * | 28609-97 | 42 | 85 | 30 | 75 | 58 | 38 | | | 17 | | 12 | 15 |
| 5 | Minke * | 28831-98 | 45 | 95 | 30 | 85 | 60 | 40 | | | 20 | | 15 | 20 |
| 6 | Dwarf minke# | 27926-97 | 32 | 60 | 22 | 48 | 38 | 26 | | | 10 | | | |
| 7 | Sperm | ------ | 27 | 70 | 19 | 60 | | 25 | - | - | - | 6 | - | - |
| 8 | Sperm * | E430-95 | 24 | 65 | 18 | 54 | | 35 | 1 | 0.5 | 19 | 6 | 10 | 12 |
| 9 | Sperm | E423-95 | 25 | 66 | 19 | 59 | | 20 | 2 | 1 | 19 | 3.5 | 9 | 11 |
| 10 | Sperm * | E90-97 | 30 | 68 | 23 | 58 | 35 | 27 | | | 20 | | 10 | 12 |
| 11 | Pygmy Sperm * | 25484-95 E413-95 | 25 | 50 | 17 | 43 | | 17 | 2.5 | - | 6 | 5 | - | - |
| 12 | Pygmy Sperm | E414-95 | 30 | 50 | 20 | 45 | | 17 | 1 | - | 5 | 5 | - | - |
| 13 | Pygmy Sperm | 27821-97 | 34 | 47 | 29 | 45 | | 29 | 2 | 1 | 8 | 5 | 13 | 13 |
| 14 | Pygmy sperm * | 27961-97 | 25 | 40 | 18 | 36 | 32 | 24 | | | 3 | | 8 | 10 |
| 15 | Pygmy sperm | 29069-98 | 24 | 45 | 28 | 50 | | | | | | | | |
| 16 | Shepherds | -------- | 35 | 65 | 25 | 52 | | 25 | 1 | - | 9 | 3 | 4 | 7 |
| 17 | Straptoothed | E429/96 | 38 | 60 | 30 | 55 | | - | 3 | 1 | 7 | 4 | - | - |
| 18 | Grays | E411-95 | 37 | 60 | 30 | 55 | | 26 | 4 | - | 7 | 5 | - | - |
| 19 | Grays * | E433-95 | 37 | 60 | 30 | 50 | | - | 2 | 1 | 7 | 4 | 7 | 10 |
| 20 | Grays | E431-95 | 28 | 55 | 24 | 47 | | 28 | 2 | 1 | 7 | 6 | 12 | 15 |
| 21 | Southern right bottlenosed | E412-95 | 40 | 60 | 30 | 55 | | 24 | 2 | 1 | 10 | 4 | - | - |
| 22 | Cuviers * | E15/97 | 31 | 70 | 26 | 60 | | 32 | 3 | - | 11 | 6 | - | - |
| 23 | Cuviers * | 28543-97 | 31 | 75 | 25 | 65 | 47 | 34 | | | 11 | | 13 | 15 |
| 24 | Splaytoothed | 29355-98 | 32 | 62 | 26 | 55 | 44 | 30 | | | | 4 | | |
| 25 | Long finned pilot * | E415/E425 | 30 | 40 | 22 | 35 | | 16 | 2 | 0.5 | 6 | 4 | - | - |
| 26 | Long finned pilot * | E432-95 | 27 | 44 | 23 | 40 | | - | - | - | - | - | - | - |
| 27 | Long finned pilot"" | 30-98 | 25 | 42 | 23 | 38 | | | | | | | | |
| 28 | Long finned pilot"" | 38-98 | 29 | 46 | 25 | 42 | 36 | 24 | | | 6 | | 10 | 12 |
| 29 | Long finned pilot"" | 44-98 | 27 | 40 | 20 | 36 | 32 | 21 | | | 6 | | 8 | 10 |
| | Long finned pilot * | 189-98 | | | | | | | | | | | | |
| | Long finned pilot * | 195-98 | | | | | | | | | | | | |
| | Long finned pilot * | 198-98 | | | | | | | | | | | | |
| | Long finned pilot * | 199-98 | | | | | | | | | | | | |
| | Long finned pilot * | 200-98 | | | | | | | | | | | | |
| 30 | orca | 29805/98 | 25 | 40 | 20 | 35 | 25 | 17.5 | | | 10 | | 10 | 10 |
| | Spectacled porpoise | | | | | | | | | | | | | |
| 31 | Bottlenosed dolphin# | 27201/97 | 25 | 38 | 22 | 25 | | 31 | 1.5 | 0.5 | 5 | - | - | - |
| 32 | Dolphin * | 27865/97 | 18 | 28 | 15 | 25 | | 14 | 2 | 1 | 2.5 | 3 | 6 | 7 |
| 33 | Dolphin | 27866/97 | 28 | 30 | 17 | 27 | | 17 | 2 | 1 | 3 | 2.5 | 7 | 9 |
| 34 | Dolphin * | 27796/97 | 19 | 28 | 16 | 25 | | 16 | | 0.5 | 2 | 2.5 | - | - |
| | Dolphin * | 28112/97 | | | | | | | | | | | | |
| 35 | Dolphin | 28782/98 | 20 | 34 | 15 | 30 | 26 | 17 | | | 4 | | 9 | 12 |
| 36 | Juvenile pygmy right | E428-95 | 32 | 50 | 21 | 43 | | 22 | 2 | 1 | 7 | 4 | - | - |
| 37 | Juvenile long finned | 27471Raumati | 22 | 27 | 22 | 31 | | 18 | 2 | 1 | 4 | 4.5 | - | - |
| 38 | Foetal pyg. sperm # | 27685/97 | 25 | 46 | 18 | 34 | | 28 | 2.5 | 1 | 3 | 3 | - | - |

## Appendix 5-1

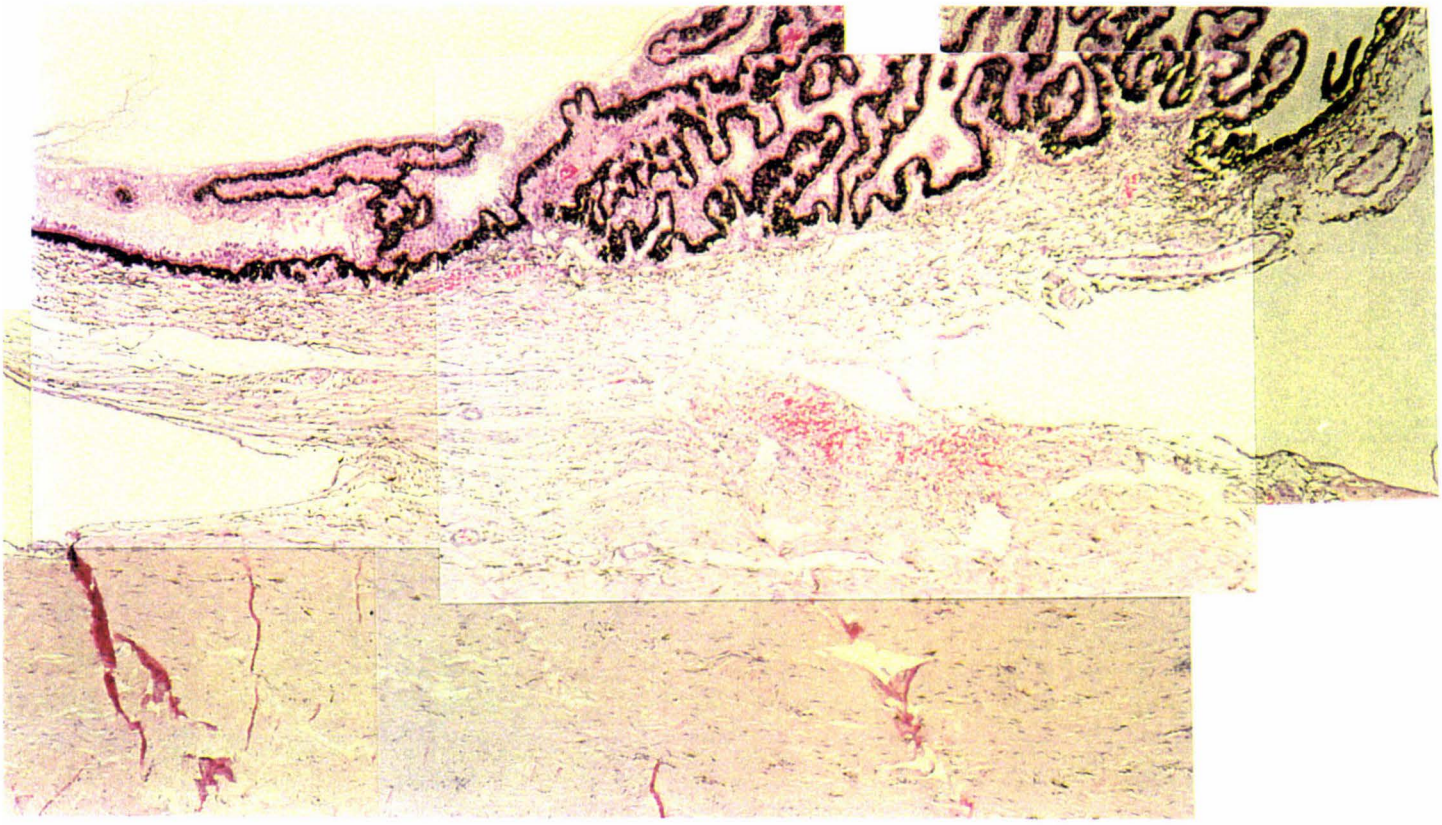
Photomontages to illustrate comparative microanatomy of whales and herbivores as referred to in text Chapter 5 page 50.



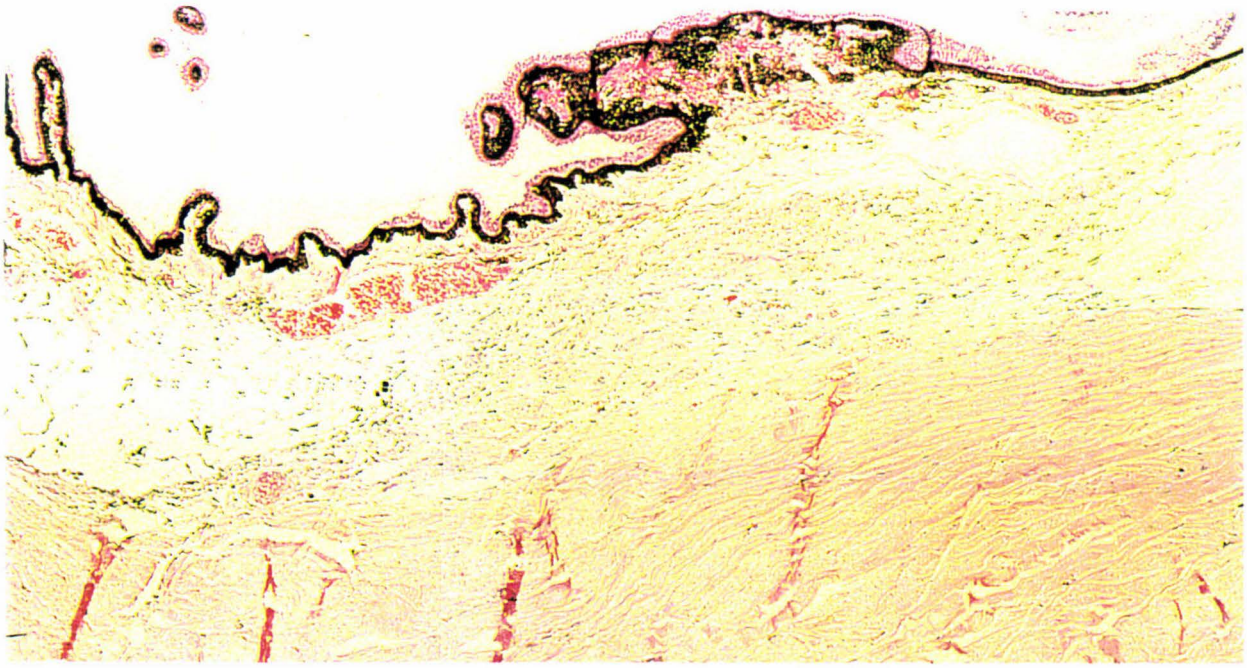
A5-1 Ciliary body of minke 28609-97 H&E x 20



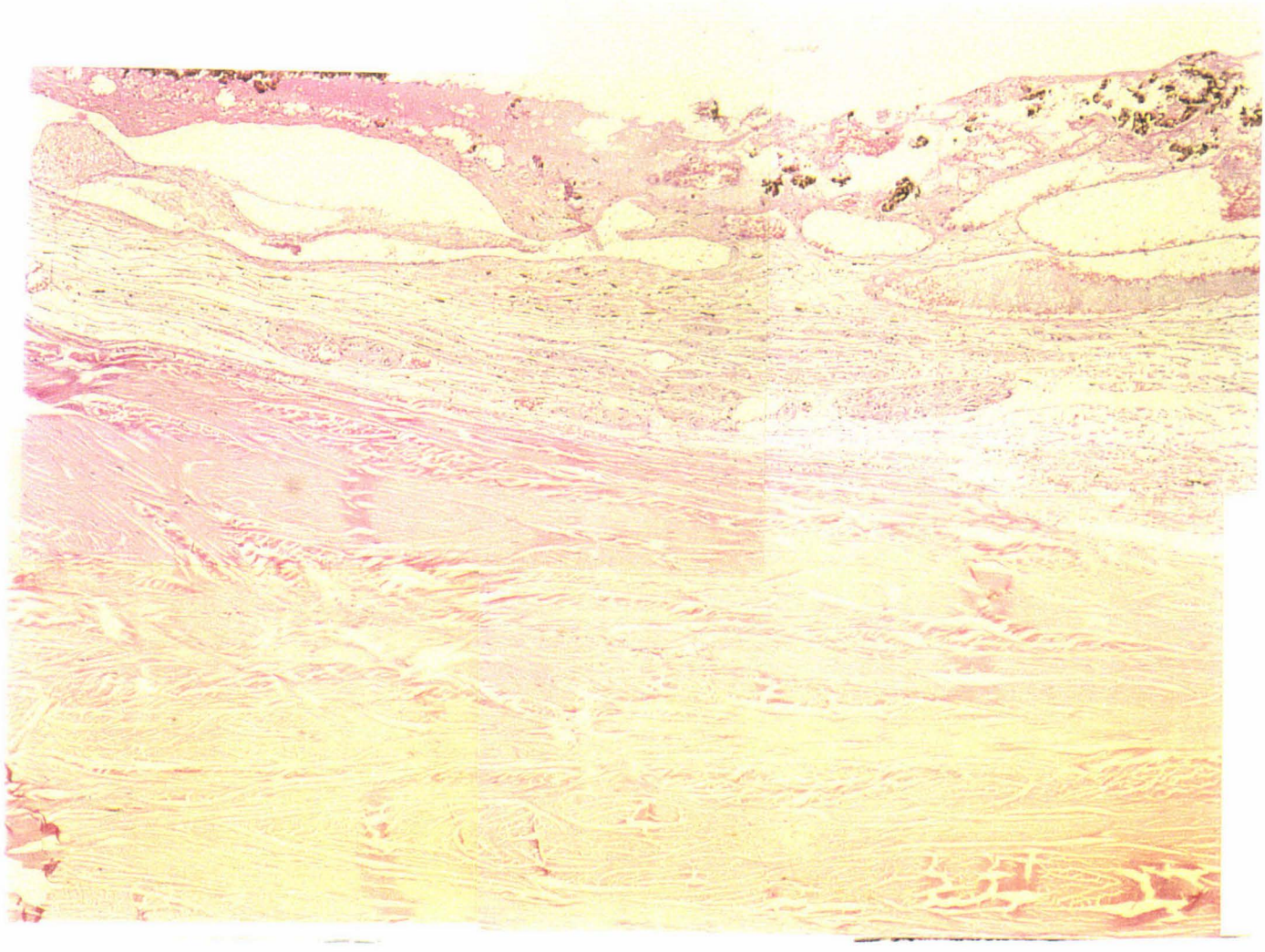
A5-2 Ciliary body of sperm whale E90-97 H&E&alcian blue stain x20



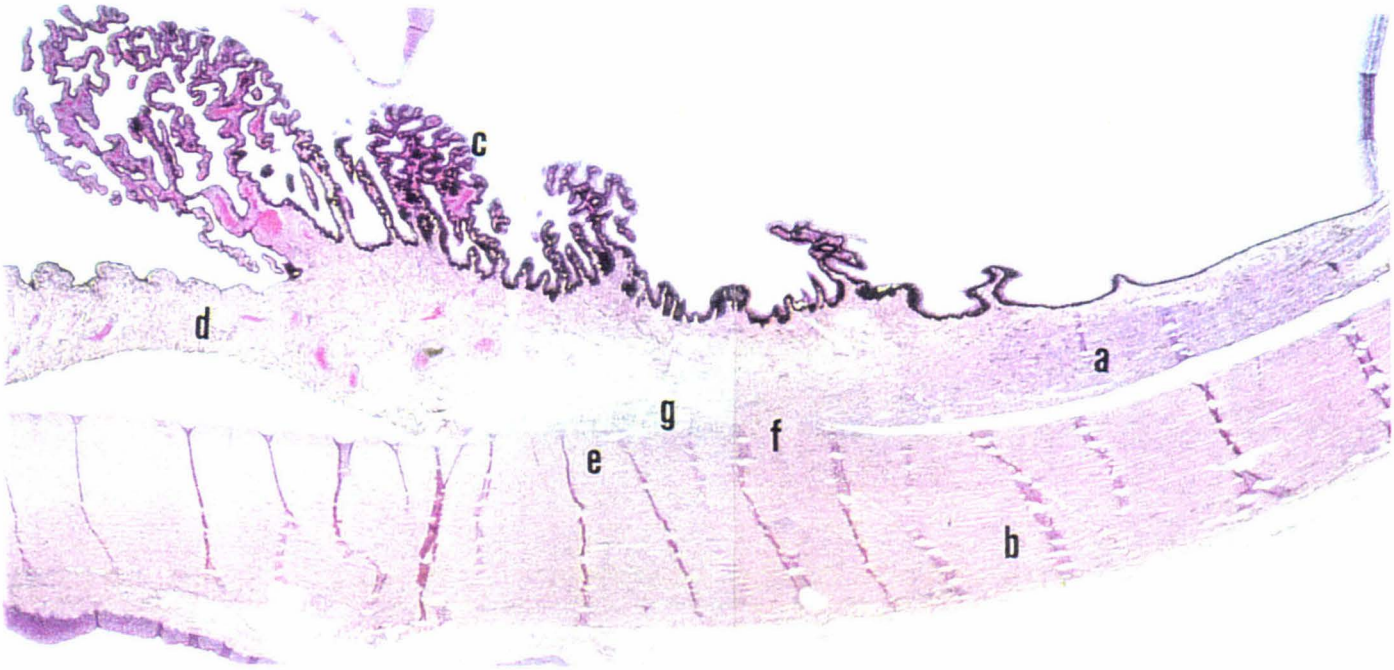
A5-3 Ciliary body of pygmy sperm whale 27961-97 H&E x52



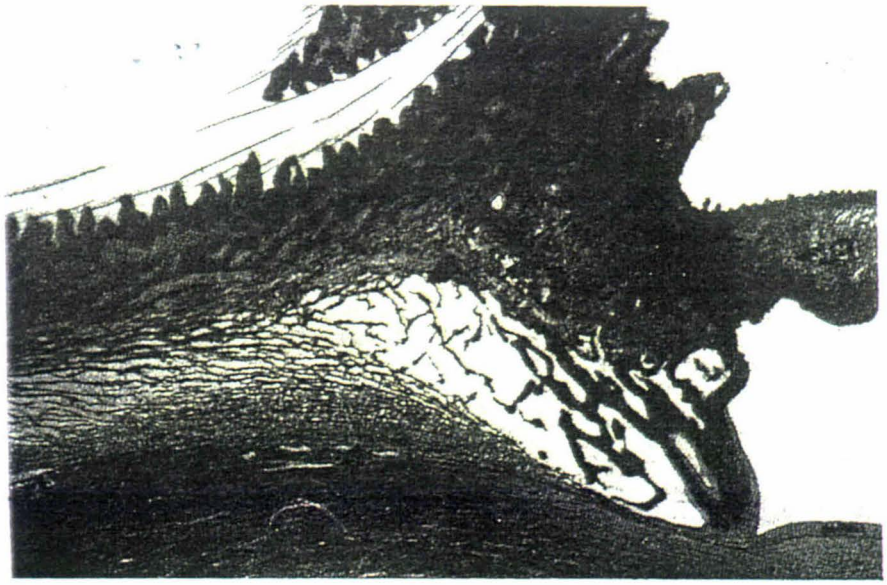
A5-4 Ciliary body of long-finned pilot whale E432-95 H&E x52



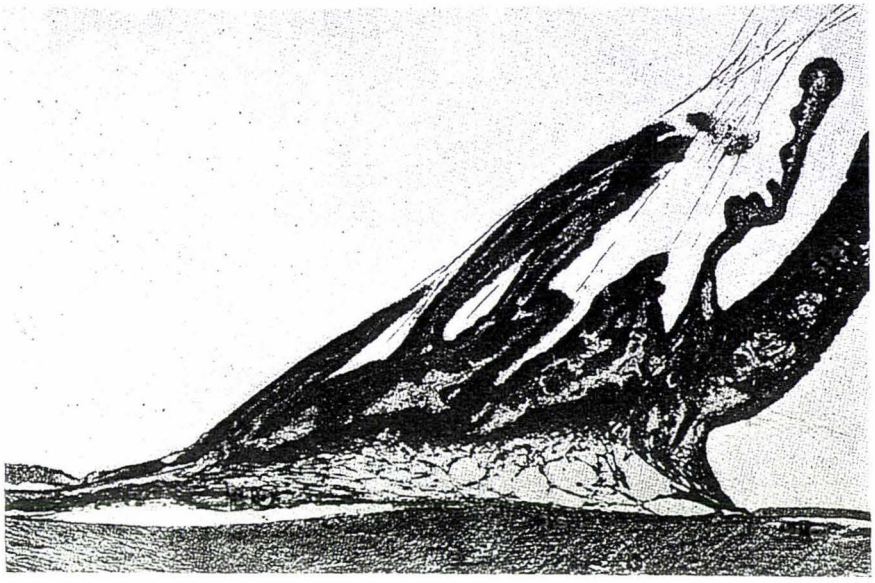
A5-5. Ciliary body of Cuvier's beaked whale E15-97. H&E x52



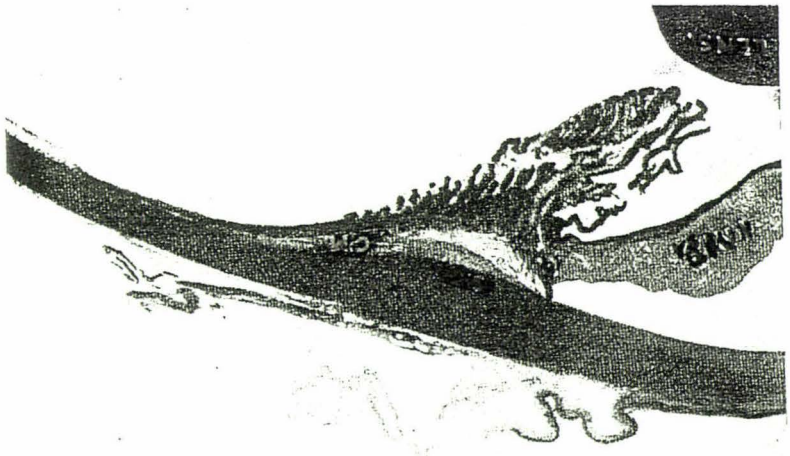

A5-6 Ciliary body of a steer. a, muscle; b, sclera; c, ciliary processes; d, iris; e, canal of Schlemm; f, scleral spur; g, trabecular meshwork. H&E x20



A5-7 Ciliary body of a zebra x24 . Taken from Smythe 1958.



A5-8 Ciliary body of a kangaroo. Taken from Smythe 1958.



A5-9 Ciliary body of a sheep. Taken from Smythe 1958.

## APPENDIX 6.1

**Method For Holmes'Silver Stain** (From Culling 1985)

**Reagents**

Impregnating solution

Dilute 100ml of Holmes' boric acid-borax buffer of pH 8.4 to 494ml with distilled water. Add 1ml of 1% aqueous silver nitrate, then 5ml of 1% aqueous solution of pure pyridine. Mix well. This solution should be freshly prepared.

Reducing solution

| | |
|---|---|
| Hydroquinone | 1 g |
| Sodium sulphite(cryst.) | 10g |
| Distilled water | to 100ml |

**Method**

1] Bring formalin fixed paraffin sections to water.

2] Place in 20% aqueous silver nitrate in the dark at room temperature for 1 hour.

3] Wash in distilled water (three changes) for ten minutes.

4] Place in impregnating solution, cover container and leave overnight at 37 degrees centigrade. There should be not less than 20ml of solution for each slide.

5] Remove slides from impregnating solution, shake off excess fluids and place in reducing solution for 2-3 minutes.

6] Wash in running water for 3 minutes.

7] Rinse in distilled water.

8] Tone in 0.2% gold chloride for 3 minutes.

9] Rinse in distilled water.

10] Place in 2% oxalic acid for 3-10 minutes. The impregnation of the neurons is controlled at this stage, they become progressively pale red, deep red, then black. If Luxol fast blue is being used to countestain the myelin the impregnation should be stopped while axons are reddish black.

11] Rinse in distilled water.

12] Place in 5% sodium thiosulphate for 5 minutes.

13] Wash in tap water.

14] Dehydrate, clear and mount in synthetic resin.

Or:-

14] Rinse in 95% alcohol.

15] For myelin stain, use Luxol fast blue at this stage.

16] Dehydrate, clear and mount in synthetic resin..

**Results**

| | |
|---|---|
| Neurons | Red-black |
| Myelin (if stained) | Blue |

## ENCAPSULATED RECEPTORS; ADDITIONAL NOTES ON MORPHOLOGY

Mechanoreceptors have received much attention in the last forty years in the form of EM studies. Prior to this, in the 1800's Pacini, Herbst, Meissner Krause, Merkel Grandry and Ruffini all lent their names to encapsulated nerve endings of various shapes and sizes ( Chouchkov 1978 ).
In particular, Halata (1978, 1980 ) described the ultrastructure and topography of lamellated corpuscles in the articular capsule of cats and pigeons.
In the cat, Ruffini corpuscles were sited in the dense stratum fibrosum layer of the articular capsule and Pacinian corpuscles in the stratum synovale. The larger Ruffini corpuscles were 300-800u long and 300u wide, consisting of 2-6 cylinders, each with a structure similar to that illustrated in Figure A6-1 with non lamellar Schwann cell wrappings , a small subcapsular space and a perineurial capsule 2-8 layers wide. A wide (13-17u ) myelinated axon supplied the corpuscle and bifurcated repeatedly to smaller (4-5u) branches which entered cylinders. Further ramification occurred within the cylinder and cylinders frequently perforated the Ruffini capsule to allow naked axonal processes to extend into the collagen bundles of the stratum fibrosum. Similarly, fascicles of collagen fibres entered the cylinder to mingle with the collagen of the cylinder, and cylinders tended to lie parallel to the direction of collagen in the stratum fibrosum.
The smaller Pacinian corpuscles ( 150-250u x 20-40u ) lie at the stratum fibrosum-synoviale junction, either singly or in groups, ocassionally inside a nerve. The inner Schwann cell core was lamellar as was the perineural capsule. The number of layers dertermined the size of the corpuscle. The single, myelinated axon (8-12u ) branched to supply several corpuscles but each corpuscle had just one central unmyelinated axon. However, this did extend axonal processes to touch collagen fibres between the lamellae of the inner core.

In the pigeon, Ruffini corpuscles occurred in a similar site to that of the cat (ie. in articular capsule, stratum fibrosum layer), with Herbst corpuscles in the stratum synovale. Ruffinis consisted of 2-4 cylinders, 80u long and 30u wide, entwined together and supplied by a single nerve which branched repeatedly so that nerve terminals and their associated Schwann cells encircled bundles of collagen fibres. Herbst corpuscles have only been described in birds. Their architecture is quite specific.

Tachibana *et al.* (1988) described two types of lamellated corpuscle in pig and cat skin of Paciniform and Meissner type, some within nerve bundles, some occurring singly and others together in groups.
Meyer and Neurand (1982), using histochemical (cholinesterase ) staining and light microscopy, described paciniform corpuscles in the hairy skin of the pig though these had already been demonstrated in the skin of other mammals and in

the nasal skin of the pig. Their small size (90 x 30 microns) and association with blood vessels is noteworthy. It is suggested that, since a similar relationships between paciniform and Pacinian corpuscles with blood vessels are recorded in other mammals, that their function may be to sense local changes in blood flow.

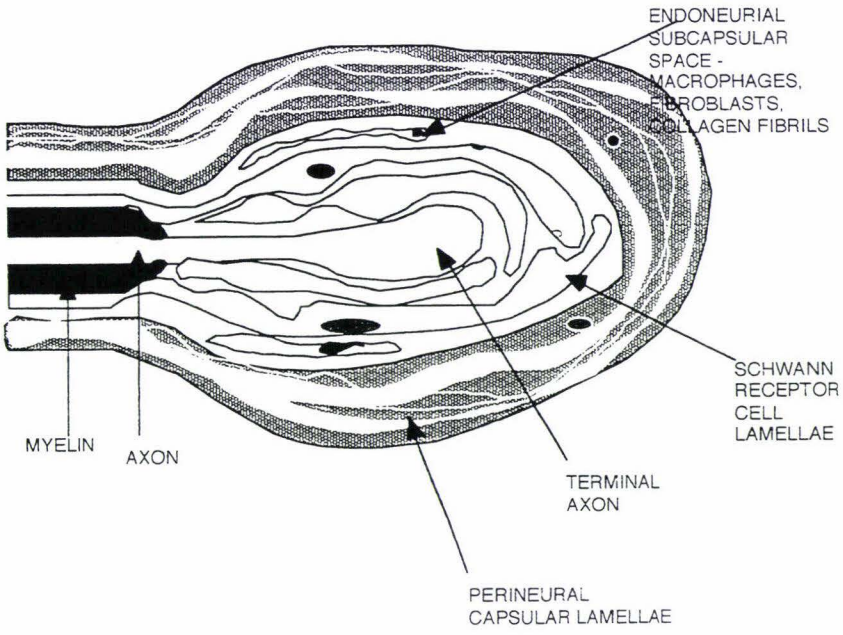



**Figure A6-1 Longitudinal cross section of a simple symmetrically lamellated corpuscle. From Chouchkov 1978.**

Reviews of the vast body of work in this area have been carried out by Chouchkov (1978) and Munger and Ide (1988). Chouchkov described the ultrastructure of the component cells of an encapsulated nerve ending, and a morphological classification of the many types. The five basic components are:-

1) Nerve fibre and ending

Myelinated axon, becomes unmyelinated on entering capsule. Axon of variable width, contains microtubules (transport channels) neurofilaments (for structure) smooth ER and occasionally lysosomes. The preterminal is completely surrounded by Schwann cell cytoplasmic lamellae, as described in unencapsulated endings. Desmosome like junctions fix the Schwann plasmalemma to the axolemma.

The terminal or nerve endings are large and bulbous, often flattened. Whereas in the preterminal the organelles pallisaded around the edge, in the terminal the mitochondria are prolific and lie centrally. There is also a large number of lysosomes, vesicles, and glycogen granules.

2) Schwann receptor cells

Ubiquitous for all sensory neurones except intrepidermal. These form the basis of the inner core of encapsulted receptors. Many researchers have disputed their Schwann cell origin , but Chouchkov considers the body of evidence to favour this origin.

3) Endoneural receptor cells; subcapsular cells

Endoneurium is equivalent to the subcapsular space sandwiched between two basal laminae of inner core and outer capsule, consisting of fibroblasts, macrophages, connective tissue ground substance and collagen fibrils.

4) Perineural cells

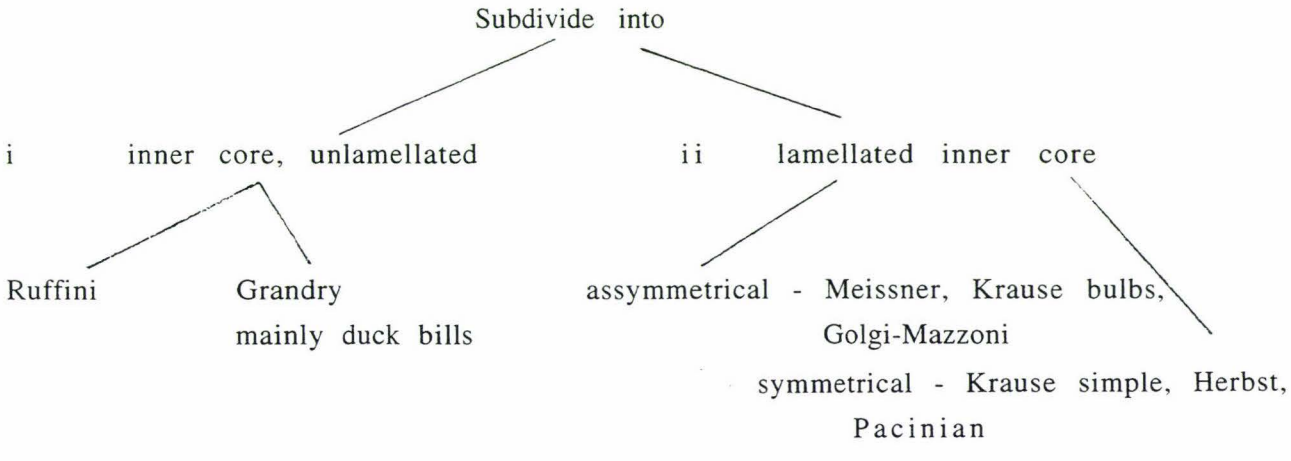
Recognisable by their elliptical nucleus and elongated cytoplasm. These form lamellae linked by desmosomes. Space between layers contains collagen fibrils. The thickness of the capsule depends on size of corpuscle eg. a large Pacinian has 70 layers (Chouchkov 1971) . Also the deeper it lies the more layers in the capsule (Polacek and Mazanek 1966). In Meissner and Grandry copuscles endo- and perineurium are mixed .

Receptor nerve endings can be classified into unencapsulated and encapsulated types:

## 1 UNENCAPSULATED

Free unmyelinated nerve endings in epidermis or subepidermis, often invested in schwann cells, often ramify repeatedly, in certain situations associated with a specialised cell - Merkel cell- in hair follicles unmyelinated ends invested in schwann cells pallisade along the follicle.

## 2 ENCAPSULATED



These types are described in more detail in Table A6-1.

**TABLE A6-1. CLASSIFICATION OF SYMMETRICAL AND SYMMETRICAL RECEPTORS (FROM CHOUCHKOV, 1978).**

| INNER CORE "UNLAMELLATED" | INNER CORE LAMELLATED - ASYMMETRICAL | INNER CORE LAMELLATED - SYMMETRICAL |
|---|---|---|
| RUFFINI CORPUSCLES - 150UX500-2000U -- SPINDLE SHAPED - THICK CAPSULE - LARGE SUBCAPSULAR SPACE - PARALLEL TO EPIDERMIS - MANY AXONAL PROFILES - 1 OR 2 SCHWANN RECEPTOR LAMELLAE | MEISSNER CORPUSCLES -40-70X100U -SPINDLE SHAPED - THICK CAPSULE, UNLAYERED - NO SUBCAPSULAR SPACE - PERP TO EPIDERMIS - AXONS RAMIFY - STACKS OF SRCELLS WITH NUCLEI TO ONE SIDE | KRAUSE SMALL PACINIFORM CORPUSCLES ELLIPSOIDAL OR SPHERICAL SHALLOW30X100DEEP50X200 NUMBER OF INNER CORE AND CAPSULAR LAMELLAE INCREASES WITH SIZE AND DEPTH AXON USUALLY SINGLE |
| GRANDRY | KRAUSE BULBS OVOID MUCOSA OF PHARYNX, CONJUNTIVA AND GENITALS OF MAN AND PRIMATES CAPSULE THIN AXON COILED OR UNCOILED | HERBST - BIRDS 50-100X300U OVOID THICK CAPSULE VERY LARGE SUBCAPSULAR SPACE INNER CORE IN TWIN ROWS OF SR CELLS AXON SINGLE |
| | GOLGI MAZZONI CORPUSCLES | PACINIAN CORPUSCLE 0.7X1MM OVOID THICK CAPSULE(20-70 LAYERS) SMALL SPACE 30-60 SR CELL LAMELLAE SINGLE AXON |

## APPENDIX 7

### TABLE 7-1A. THICKNESS OF CORNEAL LAYERS AT INTERVALS THROUGHOUT AVAILABLE LENGTH OF CORNEA.

| EFERENCE | TOTAL | AVERAGE TOTAL | EPITHELIUM | AVERAGE EPITHELIUM | BOWMANS MEMBRANE | AVERAGE BOWMANS | STROMA | DESCEMETS MEMBRANE | AVERAGE DESCEMETS | ENDOTHELIUM | AVERAGE ENDOTHELIUM |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | E432 LONG FINNED PILOT E432-95 | | | | | | | | |
| FP432 | 1950 | | 150 | | 30 | | | 1 | | 2 | |
| ERIPHERAL | 2000 | | 122 | | 30 | | | 1 | | 2 | |
| | 1900 | 1875 | 155 | 143 | 27 | 28.6 | 1700 | 1 | 1 | 1.5 | 2 |
| | 1850 | | 149 | | 28 | | | 1 | | 2 | |
| | 1675 | | 139 | | 28 | | | 1 | | 2 | |
| ENTRAL | 1125 | | NO EPI | | 15 | | | 2 | | 2 | |
| | 1125 | 1000 | " | | 18 | | | 1 | | 2 | |
| | 1000 | | " | | 17 | 15.2 | | 1 | 1 | 2 | 2 |
| | 750 | | " | | 14 | | | 1 | | 2 | |
| | | | " | | 12 | | | 1 | | 2 | |
| | | | JUVENILE LONG FINNED PILOT | | | | | | | | |
| ERIPHERAL | 1900 | | 130 | | 25 | | | NOT VIS | | 7 | |
| | 1900 | | 125 | | 34 | | | " | | 6 | |
| | 1900 | 1805 | 115 | 117 | 34 | 31 | 1696 | " | | 6 | 6 |
| | 1700 | | 110 | | 32 | | | " | | 6 | |
| | 1625 | | 105 | | 30 | | | " | | 6 | |
| ENTRAL | 925 | | 278 | | 22 | | | " | | 4 | |
| | 1200 | | 228 | | 23 | | | " | | 4 | |
| | 1075 | 1030 | 190 | 205.2 | 21 | 22.2 | 798.6 | " | | 4 | 4 |
| | 950 | | 165 | | 23 | | | " | | 4 | |
| | 1000 | | 165 | | 22 | | | 2 | | 4 | |
| | | | STRAPTOOTHED E429-95 | | | | | | | | |
| ERIPHERAL | 2250 | | 70 | | 20 | | | 3 | | 3 | |
| | 2437 | 2320.666 | 75 | | 17 | | | 2.5 | | 2.5 | |
| | 2275 | | 70 | 76.2 | 16 | 17.5 | 2219.7 | 4 | 3.5 | 5 | 3.7 |
| | | | 86 | | 17 | | | 4 | | 3 | |
| | | | 80 | | | | | 4 | | 5 | |
| | 1950 | | 72 | | 18 | | | 4 | | 4 | |
| | 2200 | 2083.3 | 70 | | 18 | | | 4 | | 3 | |
| | 2100 | | 75 | 73 | 19 | 18.4 | 1983.4 | 5 | 4.4 | 4 | 3.8 |
| | | | 74 | | 19 | | | 5 | | 4 | |
| | | | 74 | | 18 | | | 4 | | 4 | |
| | | | PYGMY SPERM E413-95 | | | | | | | | |
| PERIPH | 1625 | | 280 | | 7 | | | 1 | | 4 | |
| | 1750 | | 285 | | 5 | | | 1 | | 4 | |
| | 1750 | 1718 | 250 | 271 | 5 | 5 | 1437 | 1 | 1 | 4 | 4 |
| | 1550 | | 240 | | 4 | | | 1 | | 3 | |
| | | | 300 | | 4 | | | 1 | | 4 | |
| CENTRAL | 875 | | 300? | | 5 | | | 1 | | 4 | |
| | 850 | | 3250? | | 5 | | | 1 | | | 4 |
| | 825 | 856 | 3500? | 345 | 5 | 5 | 501 | 1 | 1 | 4 | |
| | 875 | | 3750? | | 5 | | | 1 | | | |
| | | | 3750 | | 5 | | | 1 | | | |

**TABLE 7-1B. TOTAL CORNEAL THICKNESS AT INTERVALS THROUGHOUT AVAILABLE LENGTH OF CORNEA.**

| | | | | |
|---|---|---|---|---|
| CUVIERS BEAKED E15-97 | | | | |
| | 2175 | | | |
| | 2350 | | | |
| | 1500 | | | |
| | 950 | | | |
| | 625 | | | |
| | 700 | | | |
| length 26mm | | | | |
| MINKE E18-97 | | | | |
| | 2500 | | | |
| | 1750 | | | |
| | 1500 | | | |
| | 1625 | | | |
| | 1300 | | | |
| | 1200 | | | |
| | 1200 | | | |
| length 30mm | | | | |
| | | | | |
| GRAYS BEAKED E433-95 | | | | |
| | 3000 | | | |
| | 3000 | | | |
| | 2750 | | | |
| | 1700 | | | |
| | 1375 | | | |
| | 1500 | | | |
| | 1300 | | | |
| | 1300 | | | |
| | 1500 | | | |
| | 1500 | | | |
| | 3000 | | | |
| | 3000 | | | |
| | 3000 | | | |
| | 3000 | | | |
| length 30mm | | | | |

## APPENDIX 7-2. THE GENERALISED LENSMAKERS FORMULA

The lens maker's formula enables the prediction of image positions when the object position, radius of curvature of the refracting surfaces, and refractive indices of all media are known. The following introduces this formula.

Note that this analysis only applies to thin lenses and small angles of incidence.

Assume light leaves an object at position $s_O$ to the left of the lens. An image will be formed at the position $s_I$ to the right of the lens according to the formula:

$$\frac{n_1}{s_O} + \frac{n_3}{s_I} = \frac{n_2 - n_1}{r_1} + \frac{n_3 - n_2}{r_2}$$

(for a detailed derivation see Jenkins and White, p57, 1957).

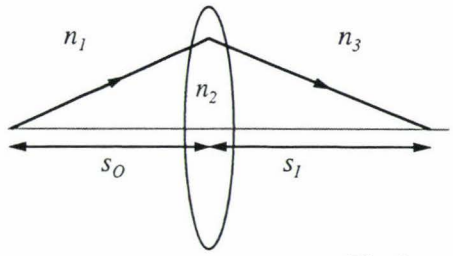



**Fig. 1**

In this formula $n_1$, $n_2$ and $n_3$ refer to the refractive indicies of the 3 media as indicated in figure 1. $r_1$ and $r_2$ are the radii of curvature for the two lens surfaces. $r$ is positive if the lens centre of curvature is to the right of the lens vertex (see figures 2 for examples).

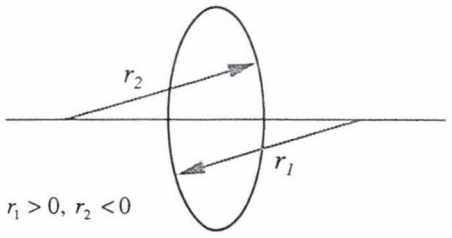
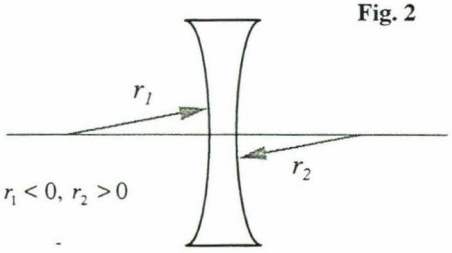



**Fig. 2**




For the special case of an object at infinity the above equation allows the dioptric power $1/f$ to be determined:

$$\frac{1}{f} = \frac{1}{s_I} = \frac{n_2 - n_1}{n_3}\frac{1}{r_1} + \frac{n_3 - n_2}{n_3}\frac{1}{r_2}$$

where $f$ is the focal length as indicated in figure 3. Note that if all distances are expressed in metres then $1/f$ will be in dioptres.

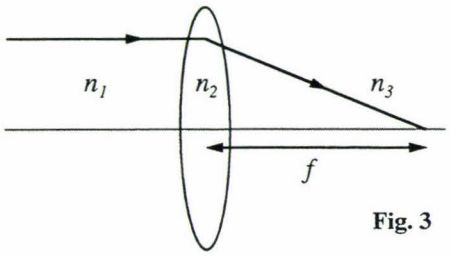



**Fig. 3**