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A comparative study of the corneal endothelium in vertebrates

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Introduction: In vertebrates, a corneal endothelium is essential for the maintenance of corneal transparency in a variety of environments, including aerial, terrestrial and aquatic. Knowledge of the surface structure of the corneal endothelium may assist our understanding of this unique tissue and its evolutionary development. Except for humans and some mammals, there have been few studies of other vertebrates, particularly the unique Australian species.
Methods: The field emission scanning electron microscope was used to study the corneal endothelium in representatives of four vertebrate classes: Teleostei (five species), Reptilia (two species), Aves (four species) and Mammalia (three species), including Marsupialia (two species). Endothelial cell densities were calculated from micrographs using computer-based image analysis.
Results: The cell densities varied considerably from 1,900 ± 197 cells per mm² for the bream to 11,734 ± 1,687 cells per mm² for the emu. Most of the corneal endothelia were similar to those reported for mammals. However, in some species such as the koala, the pattern was irregular. Some endothelial cells in birds possessed cilia.
Conclusions: The shape of the corneal endothelial cells of vertebrates is typically a mixture of hexagonal and pentagonal cells, in which the cell borders are irregular and interdigitating. An exception is the koala, in which the cells were markedly irregular. Many of the cells have surface microvilli but only in the birds are cilia found in the centre of many endothelial cells. In spite of the range of corneal environments, there are no systematic differences in the cell densities of the various classes and species.
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Key words: Australian vertebrates, cell densities, cilia, corneal endothelium, SEM

In mammals, the appropriate hydration levels of the cornea are maintained by the endothelium, which actively pumps bicarbonate ions and water out of the corneal stroma.[1] Hence, the role of the endothelium in maintaining corneal transparency is vital. In vertebrates, a transparent cornea is essential for clear vision which in turn is essential for feeding, mating and survival. However, vertebrates live in a great variety of environments including aerial, terrestrial and aquatic, which may have different demands on the cornea. The structure of the endothelium has been extensively reported for humans,[2] monkeys[3,4] and various experimental animals, including rabbit,[5] guinea pig[6,7] and rat.[8] However, there are few reports[8] on the endothelium of other vertebrates, particularly those unique to Australia. Similarly, except for humans, there are few reports of the corneal endothelial cell densities of vertebrates. A summary of these cell densities is shown in Table 1.

The aim of this field emission scanning electron microscopy study is to examine and compare the surface morphology and cell densities of the corneal endothelial cells of several aquatic and terrestrial vertebrates with particular emphasis on

Common name	Class	Cell density (cells/mm²)	Investigators
Dogfish	Elasmobranch	2,300	Yee and colleagues, 1987
Goldfish	Teleostei	431	Yee and colleagues, 1987
Trout	Teleostei	578	Yee and colleagues, 1987
Florida garfish	Teleostei	3,560	Collin and Collin, 1993
Bullfrog	Amphibia	550	Yee and colleagues, 1987
Gecko	Reptilia	481	Yee and colleagues, 1987
Goose	Aves	2,409	Yee and colleagues, 1987
Dog	Mammalia	2,545	Yee and colleagues, 1987
Rabbit	Mammalia	3,160	Yee and colleagues, 1987
Rat	Mammalia	2,211	Yee and colleagues, 1987
Guinea pig	Mammalia	3,425 ± 506	Collin, Grabsch and Johnston, 1982
Human (aged 3 years)	Mammalia (Primate)	4,450	Laule and colleagues, 1978
Human (aged < 10 years)	Mammalia (Primate)	3,476 ± 410	Bourne and O'Fallon, 1978
Human (aged 10-19 years)	Mammalia (Primate)	3,404 ± 40	Yee and colleagues, 1987
Human (aged < 15 years	Mammalia (Primate)	3,176	Binkhorst, Loones and Nygaard, 1977
Human (aged < 21 years)	Mammalia (Primate)	3,293 ± 375	Bourne and O'Fallon, 1978
Human (aged < 30 years)	Mammalia (Primate)	3,042 ± 313	Setälä, 1979
Human (aged > 30 years)	Mammalia (Primate)	2,716 ± 320	Setälä, 1979
Human (aged 66 years)	Mammalia (Primate)	2,387 ± 425	Hirst et al, 1977
Human (aged 80-89 years)	Mammalia (Primate)	2,316 ± 154	Yee and colleagues, 1987

Table 1. Published data on the corneal endothelial cell densities of various vertebrates

endemic Australian species. This information may demonstrate the presence of environmental adaptations and add to our knowledge of the evolutionary development of this unique tissue and of these species.

PROCEDURES AND METHODS

The vertebrates examined in this study are listed in Table 2. Details of taxonomic classification, the source of each specimen and its habitat are also shown.

The five species of teleosts—the Western Australian seahorse, *Hippocampus angustus*; the yellow-tail trumpeter, *Amniataba caudavittatus*; the flying fish, *Exocoetus volitans*; the Perth herring, *Nematalosa vlaminghi*; and the black bream, *Acanthopagrus butcheri*—were anaesthetised and sacrificed using tricaine methane sulphonate (MS222) in accordance with the guidelines of the National Health and Medical Research Council of Australia.

After enucleation, the cornea was dissected free and fixed in Karnovsky's fixative (2.0 per cent paraformaldehyde, 2.5 per cent glutaraldehyde, 0.1 M sodium cacodylate buffer, 2.0 per cent sucrose and 0.1 per cent calcium chloride at pH 7.2) and rinsed in 0.1 M sodium cacodylate buffer.

All other specimens were obtained opportunistically. The range of fixatives is listed in Table 3. The specimens included corneas from the crocodile, *Crocodilus porosis*; the loggerhead turtle, *Caretta caretta*; the South African ostrich, *Struthio camelus*; the emu, *Dromaius novaehollandiae*; the barred owl, *Bubo strix*; the Australian galah, *Eolophus roseicapillus*; the sheep, *Ovis aries*; the Australian koala, *Phascoarctos cinereus;* and the fat-tailed dunnart, *Sminthopsis crassicaudata*. All corneas were rinsed in 0.9 per cent saline before being placed in two changes of 0.1 per cent sodium cacodylate buffer. In addition, there was a range of times between death and the fixation of corneal tissue for some specimens.

Post fixation of all specimens was in 0.1 per cent osmium tetroxide in 0.1 per cent cacodylate buffer followed by dehydration in a graded series of alcohols. Specimens were critical point dried in a Polaron critical point dryer and mounted on 10 mm aluminium stubs with double-sided tape. Each specimen was oriented and/or hemisected so that one half of the cornea was inverted and both sides were displayed. This enabled direct comparison and differentiation between epithelial and endothelial surfaces. The mounted specimens were coated with 12 to 15 nm of gold-palladium in a Polaron Sputter coater and placed in an oven at 40 degrees Centigrade overnight before being examined.

The corneal surfaces were examined using a Joel FSEM (field emission scanning electron microscope) with an accelerating voltage of 3 kv. Results were recorded both

Species	Common name	Family	Class	Size	Habitat
Hippocampus angustus	West Australian seahorse	Syngnathidae	Teleostei	1 cm (TL) (larval)	Marine. Moves slowly over eelgrass beds. Feeds on mobile phytoplankton.
Amniataba caudavittatus	Yellow-tail trumpeter	Teraponidae	Teleostei	25 cm (SL)	Estuarine benthic feeder over sand.
Exocoetus volitans	Flying fish	Exocoetidae	Teleostei	17.8 cm (SL)	Marine and aerial. Can glide over the water's surface for up to 100 m.
Nematalosa vlaminghi	Perth herring	Clupeidae	Teleostei	25 cm (SL)	Estuarine. Possesses adipose eyelids although central cornea is not covered.
Acanthopagrus butcheri	Black bream	Sparidae	Teleostei	15 cm (SL)	Estuarine bottom feeder of molluscs and crustaceans.
Crocodilus porosus	Crocodile	Crocodylidae	Reptilia	1.4 m in length	Estuarine bodies of water in Northern Australia.
Caretta caretta	Loggerhead turtle	Chelonidae	Reptilia	1 m in length (adult)	Predominantly marine. Breathes at surface and lays eggs on land.
Struthio camelus	South African ostrich (flightless)	Struthionidae	Aves	175 cm, 100 kg female	Lives in desert areas and has upper eyelids. Can run at up to 27 mph.
Dromaius novaehollandiae	Emu (flightless)	Dromaiidae	Aves	150 cm, 45 kg, female	Desert areas of Australia and has upper eyelids.
Bubo strix	Barred owl	Strigidae	Aves	25 cm, 420 g	Arborial, relying on camouflage to avoid predation. Captures prey at twilight.
Eolophus roseicapillus	Australian galah	Psittacidae	Aves	36 cm, 345 g male	Widespread habitat but prefers open scrubland.
Ovis aries	Sheep	Bovidae	Mammalia	50 kg, female	Herbivorous grazer
Phascolarctos cinereus	Australian koala	Phascolarctidae	Mammalia (Marsupialia)	9.2 kg	Arborial. Perches in eucalyptus trees grazing on Blue gum leaves.
Sminthopsis crassicaudata	Fat-tailed dunnart	Dasyuridae	Mammalia (Marsupialia)	15 g	Arid regions to low scrubland where it often lives among spinifex.

Table 2. The species, family, class and common names of vertebrates examined in this study. The size and habitat of the specimens are also shown. SL: standard length, TL: total length.

on 35 mm film and digitally. The areas of individual endothelial cells were obtained by digital analysis of the computer images using Image Slave software. Using this procedure, the mean endothelial cell density and standard deviation were calculated, providing the extent of variation in cell area and a measure of polymegathism.

Measurements of cilia and microvilli were made on the photographic prints using a magnifier and graticule. There were insufficient cilia observed to calculate standard deviations of these measurements.

RESULTS

The integrity of the surface of the corneal endothelium as viewed by field emission scanning electron microscopy is dependent on the effectiveness of the tissue preservation which in turn depends on the nature of the fixative and the time between death of the animal and the fixation of the tissue. Those specimens which were prepared specifically for this investigation and those obtained immediately after death led to useful results. In some specimens obtained opportunistically, the preservation of the tissue is less than ideal. However, all specimens reported here were of sufficient quality to permit measurement of cell area and the calculation

Common name	Source	Fixative	Time from death to fixation
West Australian seahorse	Donated by Mr Glenn Moore, Dept of Zoology, University of Western Australia	Karnovsky EM fixative Stored in fixative for 2 years	2 minutes
Yellow-tail trumpeter	Collected by seine net in the Swan River, Western Australia	Karnovsky EM fixative	2 minutes
Flying fish	Collected on RRS Discovery Cruise 204 to the Eastern Atlantic Ocean between 15°–25°N latitude and 20°–30°W longitude.	Karnovsky EM fixative Stored in fixative for 2 years	1 hour
Perth herring	Collected by seine net in the Swan River, Western Australia	Karnovsky EM fixative	5 minutes
Black bream	Fishing and Aquaculture Centre of TAFE, Fremantle, Western Australia	Karnovsky EM fixative	2 minutes
Crocodile	Fremantle Crocodile Farm, Western Australia	Karnovsky EM fixative	10 minutes
Loggerhead turtle	Stranded off Northwest Shelf, Western Australia	10% formalin	1 hour (approx)
South African ostrich (flightless)	Donated by San Diego Zoo, California, USA	4% paraformaldehyde in phosphate buffer. Stored in fixative for years	15 to 30 minutes
Emu (flightless)	Collected from the Dromaius Farm, Western Australia	Karnovsky EM fixative Stored in fixative for years	2 minutes
Barred owl	Donated by Prof John D Pettigrew, Vision, Touch and Hearing Research Centre, University of Queensland	Karnovsky EM fixative Stored in fixative for years	10 minutes
Australian galah	Donated by Dr Ken Richardson, Murdoch University, Western Australia	EM fixative: 5% glutaraldehyde in Sorenson's phosphate buffer Stored in buffer for 2 years	2 minutes
Sheep	Collected at Allandale Farm, University of Western Australia	Karnovsky EM fixative Stored in fixative for 1 week	15 minutes
Australian koala	Donated by the Dept of Anatomy, University of Adelaide, Australia	EM fixative: 3% glutaraldehyde, 3% paraformaldehyde in phosphate buffer. Stored in fixative for 2 months	10 minutes
Fat-tailed dunnart	Colony based in the Dept of Zoology, University of Western Australia	4% paraformaldehyde in 0.1M phosphate buffer. Stored in fixative for 1 week	10 minutes

Table 3. The tissue source and fixative for the specimens examined in this study. As can be noted, some specimens were obtained opportunistically.

of endothelial cell densities from the electron micrographs.

As all specimens described in this investigation were fixed, processed and freeze-dried prior to examination in the electron microscope, some shrinkage of the corneal tissue would have occurred. Hence, the cell densities provided in this study would be considerably greater than those obtained using specular microscopy.

All endothelia were composed of mainly hexagonal with some pentagonal cells. Only the endothelium of the koala had markedly irregular cells. In some specimens, in which the fixation was not optimal, the shape of the individual cells was less defined. However, the assessments of cell density were considered to be only minimally affected.

The endothelial cell densities found in this study are summarised in Table 4. There were marked variations in cell densities even within each vertebrate class. However, there was no evidence of systematic differences either between or within classes.

Teleostei

The corneal endothelial cells of the various teleosts were similar in appearance, although they showed marked variations in cell densities, ranging from 1,900 ± 197 cells per mm^2 in the bream, *Acanthopagrus butcheri*, to 11,133 ± 523 cells per mm^2 in

Common Name	Class	Cell density (cells/mm² ± SD)
West Aust seahorse	Teleostei	11,133 ± 523
Yellow tailed trumpeter	Teleostei	3,540 ± 1,147
Flying fish	Teleostei	2,246 ± 522
Perth herring	Teleostei	4,464 ± 4,310
Bream	Teleostei	1,900 ± 197
Crocodile	Reptilia	3,687 ± 574
Loggerhead turtle	Reptilia	3,179 ± 1,002
South African ostrich	Aves	9,250 ± 1,080
Emu	Aves	11,734 ± 1,687
Barred owl	Aves	4,713 ± 766
Australian galah	Aves	9,905 ± 873
Sheep	Mammalia	11,319 ± 2,081
Koala	Mammalia (Marsupialia)	5,754 ± 1,094
Dunnart	Mammalia (Marsupialia)	3,476 ± 709

Table 4. The corneal endothelial cell densities found in this study. As the areas of individual endothelial cells were obtained by digital analysis of the computer images using Image Slave software, the standard deviation may represent a measure of polymegathism.

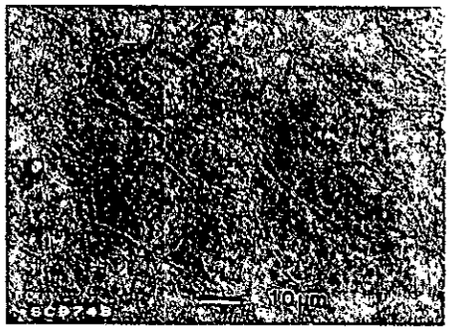

Figure 1. The corneal endothelium of the bream, *Acanthopagrus butcheri*, showing the mixture of hexagonal and pentagonal cells

Figure 2a. Micrograph of the corneal endothelium of the crocodile, *Crocodilus porosis*, showing somewhat irregular polygonal cells, many of which are quadrilateral or pentagonal in shape

Figure 2b. High power micrograph of the endothelial cells in the crocodile. The cell margins show few interdigitations and few microvilli.

the Western Australian seahorse, *Hippocampus angustus*. The endothelial cell surface of the bream is shown in Figure 1. Cilia were not found in the teleost endothelial cells.

Reptilia

The endothelial cells of the crocodile, *Crocodilus porosis*, (Figure 2) and loggerhead turtle, *Caretta caretta*, were mainly hexagonal and pentagonal in shape although they were less regular than in some other species. The cell densities for the crocodile (3,687 ± 574) and turtle (3,179 ± 1,002) were similar.

Aves

The endothelial cells of the representative species of birds were mostly hexagonal, while some were pentagonal. The cell densities ranged from 4,713 ± 766 cells per mm² to 11,734 ± 1,687 cells per mm².

In some areas of the endothelium, particularly in the emu, *Dromaius novaehollandiae*, (Figure 3) and the galah, *Eolophus roseicapillus* (Figure 4), there was a slight separation of the cells and an apparent reduction of interdigitating cell processes, possibly the result of poor fixation.

Each of the cells of the endothelial surface of the emu, a large flightless bird, had up to 30 microvilli which were mostly confined to the central region of the cell surface (Figure 3a). In the centre of approximately one third of these cells, there was also a single cilium, measuring up to 0.2 μm in width and 1.2 μm in length (Figures 3b and 3c).

Very few surface microvilli were visible on the surface of the corneal endothelial cells of the galah. Cilia were observed in approximately one third of the cells and these measured about 0.42 μm in width and 1.3 μm in length (Figure 4c).

On the endothelial surface of the cornea of the barred owl, *Bubo strix*, there were a few microvilli (up to approximately 20 per cell) and these were

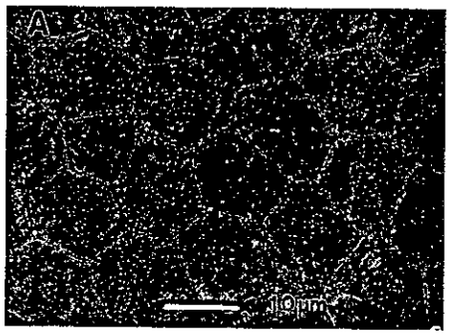

Figure 3a. The regular array of hexagonal and pentagonal cells in the corneal endothelium of the flightless emu, *Dromaius novaehollandiae*. There are microvilli and a central cilium in most cells.

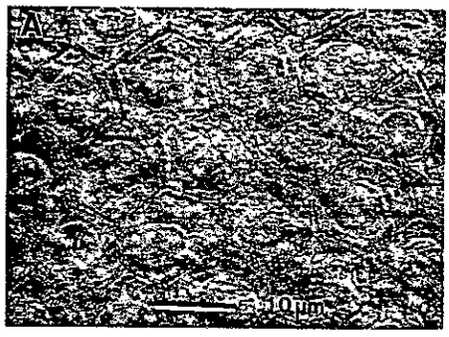

Figure 4a. The corneal endothelium of the Australian galah, *Eolophus roseicapillus*. The endothelial cells form a regular pattern of mostly hexagonal cells. The central nuclei are slightly raised and a few microvilli and cilia are present.

Figure 5a. The corneal endothelium of the barred owl, *Bubo strix*, showing the regular array of hexagonal and pentagonal cells. Microvilli are present in the central and peripheral regions of most cells.

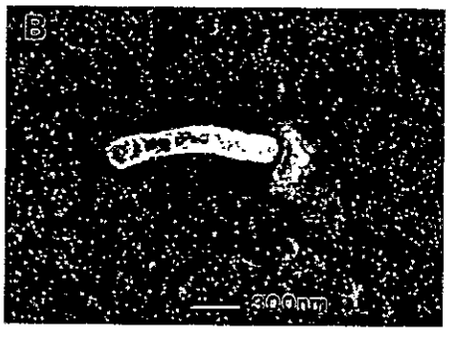

Figure 3b. High power micrographs of the central cilium from a corneal endothelial cell of the emu

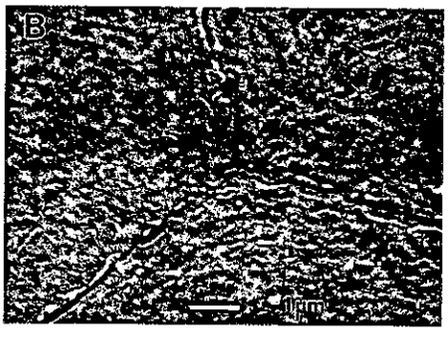

Figure 4b. The endothelial cell borders of the galah showing few interdigitations or microvilli

Figure 5b. The junction of three cells in the corneal endothelium of the barred owl. There are numerous peripheral microvilli and many interdigitations.

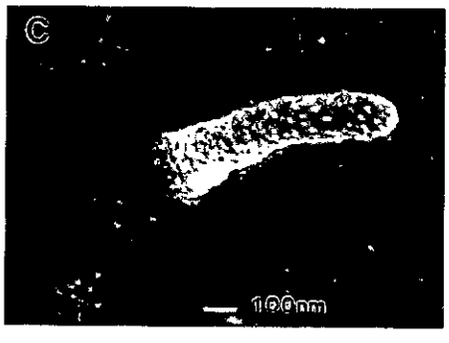

Figure 3c. High power micrographs of the central cilium from a corneal endothelial cell of the emu

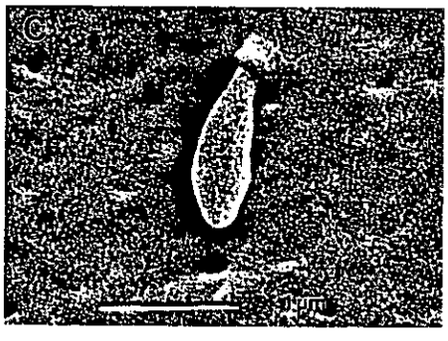

Figure 4c. A cilium from the centre of one of the endothelial cells shown in Figure 4a. The cilium and the cell surface are covered with a fine granular material, which may be the result of the preparation procedure.

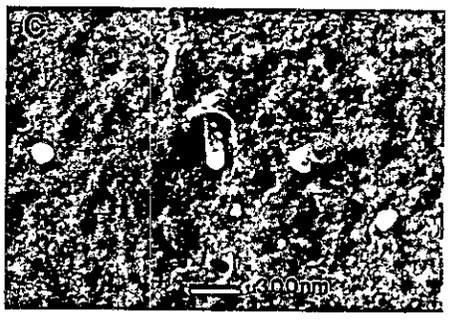

Figure 5c. A cilium in the centre of a corneal endothelial cell of the barred owl. Most of the cilium appears to be beneath the cell membrane.

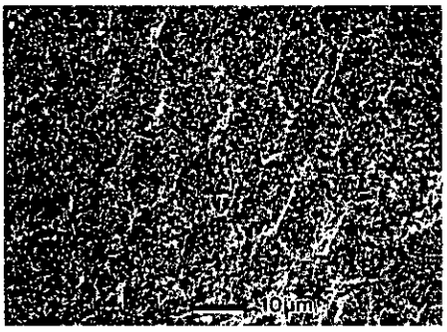

Figure 6. The corneal endothelium of the South African ostrich, *Struthio camelus*. There is a regular array of mostly hexagonal cells. The whole of the endothelial surface is covered with particulate material. This resembles a precipitate and may be the result of the paraformaldehyde fixative and subsequent changes of buffer solution.

Figure 7. The corneal endothelium of the sheep, *Ovis aries*. The polygonal cells are somewhat irregular in shape and the cell borders appear raised.

mostly located in the central region of the cell (Figure 5a). At the cell borders there were numerous microvilli and/or interdigitating cell processes (Figure 5b). Cilia were found in the centre of some of the cells and measured approximately 0.14 μm in width and 0.5 μm in length (Figure 5c).

The corneal endothelium of the South African ostrich, *Struthio camelus*, showed very regular mostly hexagonal cells (Figure 6). However, the presence of a deposit or precipitate on the surface made further details difficult to discern. The deposit may be due to the paraformalaldehyde fixation and the long period of storage.

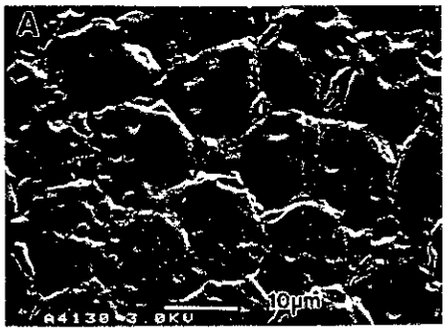

Figure 8a. The corneal endothelium of the Australian koala, *Phascoarctos cinereus*. The cells are irregular rather than polygonal in shape.

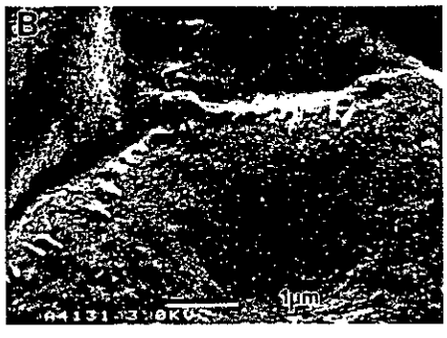

Figure 8b. The junctional region of three endothelial cells of the koala showing the interdigitations. Microvilli are rare.

Mammalia

The endothelium of the sheep, *Ovis aries*, has very small cells (11,319 ± 2,081 cells per mm^2). The cell surface was rough and was covered with microvilli and what appeared to be a deposit. Cilia were not apparent (Figure 7).

Marsupialia

The endothelial cells of the dunnart (3,476 ± 709 cells per mm^2) are mainly hexagonal and pentagonal, while those of the koala (5,754 ± 1,094 cells per mm^2) are more irregular with rounded cell borders (Figures 8a and 8b).

DISCUSSION

A corneal endothelium has previously been described in some species of teleosts,[8-12] elasmobranchs,[8] amphibians,[8] reptiles,[8] birds,[8] and mammals,[6-8] including primates[11-18] (Table 1). This study confirms the ubiquitous presence of a complete endothelial cell layer in at least the four representative classes examined.

Although no data are presented for the Class Elasmobranchii, Yee and his colleagues[8] also described an endothelium in the dogfish, *Squalis acanthias*, and claimed a cell density of 2,300 cells per mm^2. However, Keller and Pouliquen[20,21] found no endothelial cells in 11 species of cartilaginous fishes, which included representatives of the Holocephali. This was in agreement with the work of Faure,[22] who found no endothelial cells in the cornea of a galeomorph shark, *Scyliorhinus canicula*. Keller and Pouliquen[20,21] claimed that an endothelium appears unnecessary as these corneas swell very little and retain their transparency when immersed in distilled water. This could explain the absence of any regular array of endothelial cells in the corneas of the smooth dogfish, *Mustelus canis*, the tiger shark, *Galeocerdo cuvier*, the deep-sea black shark, *Dalatias licha* and the holocephalomorph, *Hydrolagus colliei* examined by the same technique, although the results are not presented here (SP Collin and HB Collin, unpublished data). In contrast, in the ray, *Torpedo ocellata*, endothelial cells were found.[21]

Yee and his colleagues[8] also claimed that teleost corneas lack Desçemet's membrane and a well-developed corneal endothelium, in that they lack well-developed endoplasmic reticulum and have few mitochondria. However, although Desçemet's membrane is irregular and sometimes incomplete in the Florida garfish, *Lepisosteus platyrhincus*,[9] it is thin and irregular in the salamanderfish, *Lepidogalaxias salamandroides*, (75 to 100 nm centrally and 200 nm peripherally),[11] and complete and well-developed in the sandlance, *Limnichthyes fasciatus*, (270 nm),[12] the Pacific tomcod, *Microgadus proximus* (400 nm)[19] and the pipefish,

Corythoichthyes paxtoni, (2 μm).[10] In addition, the endothelium was complete in all of the teleost species examined in this study and at least in the Pacific tomcod, it has been shown to be rich in endoplasmic reticulum.[19]

The shape of the corneal endothelial cells of vertebrates is typically a mixture of hexagonal and pentagonal cells, in which the cell borders are irregular and interdigitating.[23] This is confirmed in this study where, in all of the vertebrates examined, except the koala, the cells were regular and the cell borders were markedly irregular.

The endothelial cells of the teleosts described here are mostly hexagonal or pentagonal. This is similar to findings of a regular array of either hexagonal or pentagonal cells bordered by a ruffle of interdigitations for the Florida garfish, *Lepisosteus platyrhincus,*[9] or an irregular array in the trout, *Salmo gairdneri.*[8] However, in the goldfish, *Carassius auratus,* endothelial cells have been described as non-polygonal with rounded borders like those of a jigsaw pattern.[8]

The cell pattern is irregular in the bullfrog, *Rana catesbeiana,* and the gecko, *Gekko gekko*[8] but regular in the goose, *Anser domestica* and many of the mammals examined.[8] However, the shape of these cells may be altered either *in vivo*[24-27] or *in vitro*[28,29] or even disrupted[30] as a result of contact with toxic substances *in vivo* or during tissue preparation. Similarly, superficial pores located at the junctions, where three apposing endothelial cells meet, may become dramatically extended after exposure to hypertonic solutions, such as 1580 mOsmol sucrose.[31]

The size and regularity of the shape of endothelial cells are influenced by their ability to regenerate. In many vertebrates, the corneal endothelial cells mitose and regenerate. In some mammals, there is a mitotic turnover of endothelial cells and a mitotic response to endothelial injury.[2] However, in both human and non-human primates, although mitosis occurs in the young,[32] the endothelial cell population does not regenerate in any significant fashion in the adult.[2,32] This gives rise to a decreasing cell density with age.[15,17,18] It is unknown if this is true for the majority of the species reported here.

The cell densities for teleost corneal endothelium reported by Yee and colleagues[8] are markedly different from the findings of this study. The sources of this discrepancy are unknown. However, different techniques were employed in the two studies. Yee and his colleagues[8] used specular microscopy on the anaesthetised fish, while we used field emission scanning electron microscopy following fixation, alcohol dehydration and critical point drying, which would result in some tissue shrinkage. Binder and his coworkers[33] found an average shrinkage of 31 per cent when they measured the same corneal endothelial cells before fixation, during fixation with glutaraldehyde and after critical point processing for scanning electron microscopy. Hence, the true cell densities will be considerably less, perhaps about 30 per cent, than those reported here.

Observation of the corneal endothelium through the anterior corneal tissue could modulate the size of the image in comparison to direct observation of the endothelial surface as in our study. In fact, Sperling[34] found that estimates of endothelial cell densities in intact eyes were 9.7 per cent less than the estimates in excised eyes. Although it appears not to have been studied in most teleosts, the effect of the corneal optics on the image of the endothelium obtained in specular microscopy should be minimal. However, an exception to this may be the cornea of the sandlance, *Limnichthyes fasciatus,*[12,35] in which the cornea contributes approximately 200 dioptres to the refractive power of the eye and hence, would have a magnifying effect on the appearance of the corneal endothelium as viewed from the anterior surface.

In mammals, the function of the corneal endothelium is to pump bicarbonate and water out of the cornea to maintain corneal transparency.[1] However, there is no evidence that the efficiency of this pump is proportional to the endothelial cell density. Mishima[36] reported that in humans there is a critical cell density, which is likely to be several hundred cells per mm^2, below which the endothelium decompensates and cannot maintain corneal transparency. In this study, in which the lowest density is 1,900 cells per mm^2, there are no systematic differences among any of the cell densities of the various classes and species. This is in spite of the range of corneal environments represented, namely, aerial, terrestrial and aquatic.

Consistent with the findings in previous studies, cilia do not appear to have been observed in the teleost corneal endothelium and this appears to be the first report of cilia in the corneal endothelia of birds.

The presence of cilia has been reported in various vertebrates. A single primary cilium, which protrudes into the anterior chamber, is occasionally found in the endothelial cells of humans.[2,3,31] The cilia may be sparse in the central region but are present in approximately 50 per cent of peripheral endothelial cells.[5] There are a few single cilia in the centrally-located endothelial cells of the Japanese,[4] cynomolgus *(Macaca irus)*[5] and stumptail (*Macaca speciosa*) monkeys.[5] Svedbergh and Bill[5] also describe one human endothelial cell with three cilia.

In the rabbit, Gallagher[5] found that a primary cilium is a normal component of every rabbit corneal endothelial cell rather than a rare or sporadic organelle as some previously believed. Her investigation suggested that the cilia are easily broken off during the mounting procedure and that care must be taken in flushing the tissue with fluids. The cilia seem to be capable of immediate regeneration after being broken off.[5] The fact that cilia were observed in only a few of the endothelial cells of birds, and were not found in the cells of the other vertebrates, may be due to the tissue preparation procedures involved.

The average length of the cilia in the rabbit corneal endothelium is 3.9 ± 0.5 μm.[5] The cilia appear to project at a preferred angle, which may be dependent on the physiological activity of the endothelial layer.[5] Under physiological stress, the cilia appear to retract within the cell and may not be visible at the cell surface.[5]

Thus, the smaller length of the cilia described here (up to approximately 1.3 μm) may be due to species-specific differences, tissue shrinkage and/or the result of physiological stress associated with tissue preparation resulting in the incomplete protrusion of the cilia through the cell membrane.

The endothelial cilia have a structure similar to other primary cilia.[5,37] In rabbits, the ciliary shaft is composed of nine peripheral doublets without central fibrils (9+0 axoneme), which converts to an 8+1 when a doublet, which was located peripherally near the base, shifts towards the centre closer to the tip.[5] At the base of the cilium is a centriole pair.[5]

The function of the cilium is unclear.[3,5] It does not appear to be motile and is structurally associated with the cell centriole pair.[37] Ciliated centrioles are present in quiescent cells stopped in the G1 or presynthetic phase of the mitotic or cell cycle. However, when the cells are stimulated to enter DNA synthesis, the centrioles lose their cilia, which regrow in six to eight hours.[38]

In the corneal endothelium of rabbits, the centriole may form cilia during G1 and perhaps utilises the microtubules for construction of the spindle apparatus prior to mitosis.[5] Unlike that of most mammals, the corneal endothelium of humans is not a self-renewing cell layer.[39] Klyce and his colleagues[37] suggested that in the human, the cilium may be related to the inability of the cells to undergo mitosis in the adult. Other postulated functions for the cilia include chemoreception, osmoregulation and/or pressure detection.[5]

Microvilli were observed on many of the endothelial cells reported here. They have been described in many species. In both the rabbit[23] and human,[37] there may be 20 to 30 per cell. In the rabbit, they are typically 0.2 to 0.4 μm long and 0.1 to 0.2 μm thick[23] and in the human 0.5 to 0.6 μm high and 0.1 to 0.2 μm wide.[37] In the garfish, there are numerous microvilli about 0.2 μm in diameter.[9] The number of microvilli may be markedly changed, usually by an increase in the number and length of the microvilli, following application of toxic substances.[24-26]

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