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OCULAR ANTERIOR SEGMENT DISEASE IN CAPTIVE PINNIPEDS [1)]

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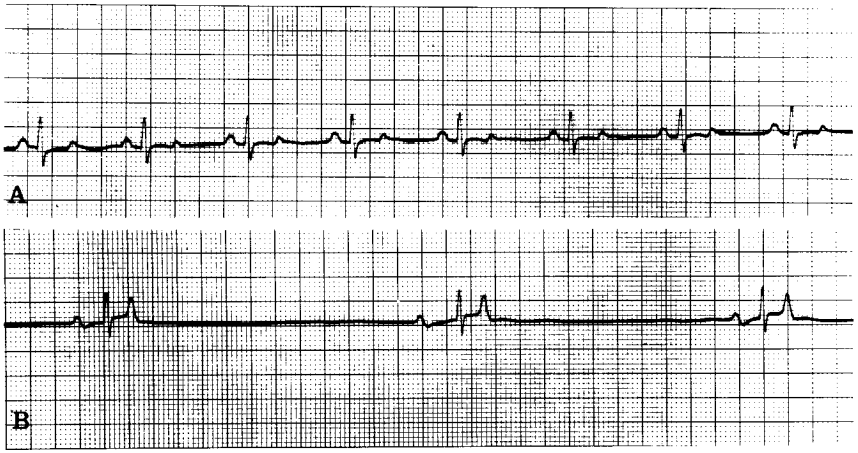
Introduction

Corneal opacities, corneal ulcers and cataracts are frequently observed in captive pinnipeds held in a variety of environmental conditions, as well as in free living wild animals (DRAL et al, 1980; NEEDHAM, 1978; SWEENEY, 1973; SWEENEY, 1974). Despite the ubiquitous nature of these eye problems, the etiology, diagnosis and treatment of anterior segment disease in the pinniped remains poorly understood. Major problems encountered in performing a complete eye examination and the inability to closely follow the progression of lesions, contribute to this situation. The potential for multiple etiologies for a single type of lesion complicates the picture even further (BELLHORN, 1977; NEEDHAM, 1978; RIDGEWAY et al, 1975).
Development of clinical and pathological techniques to improve the veterinarian's ability to observe the pathogenesis of pinniped ocular diseases and to differentiate the effects of contributing etiologies, is the first step towards prevention. This paper presents a protocol for complete ocular examinations of pinnipeds, emphasizing the anterior segment of the eye, and utilizing current ophthalmological technology. The ability of this protocol to differentiate several proposed etiologies of corneal and lenticular disease in clinical investigations is discussed emphasizing clinical findings in Harbour Seals *(Phoca vitulina)* and California Sea Lions *(Zalophus californianus).*

Clinical examination

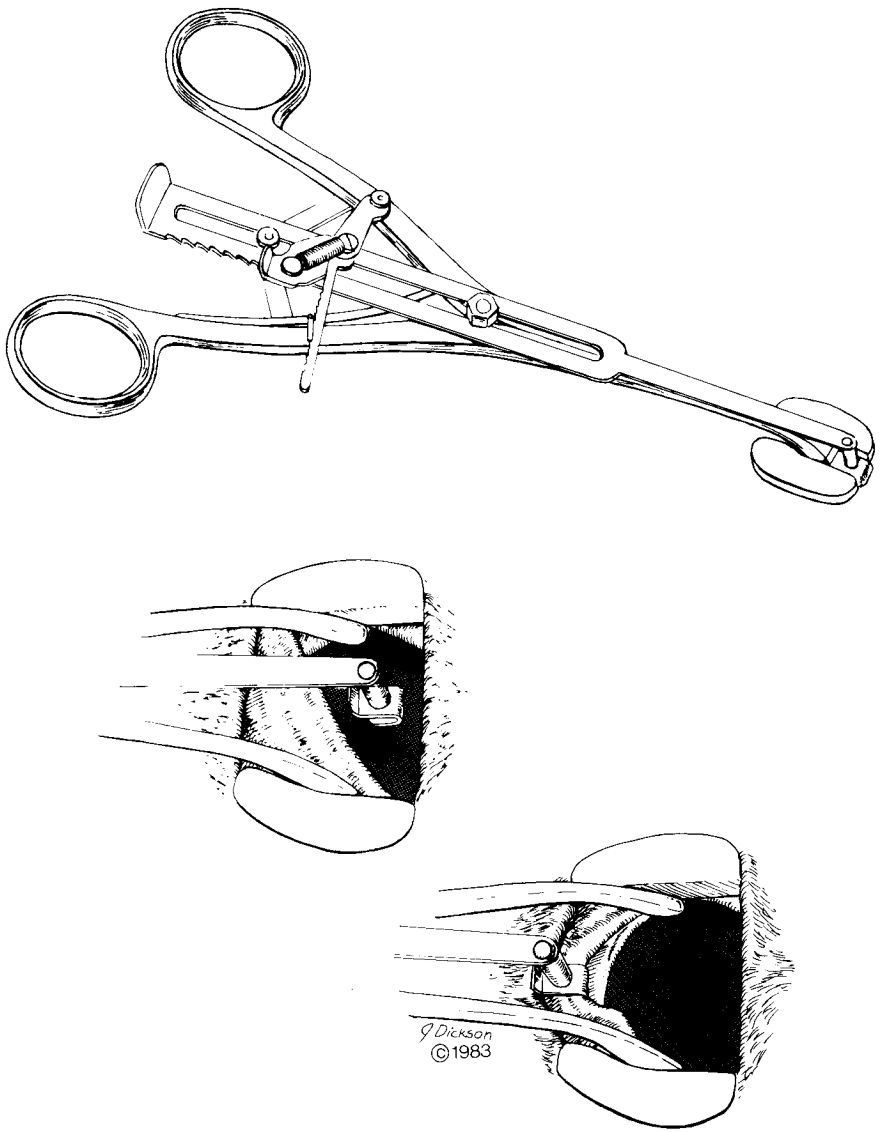
A specific order of examination provides the basis for logical, accurate diagnoses. Captive pinnipeds present a formidable obstacle to clinicians interested in performing manipulative diagnostic examinations. Training animals to target permits the preliminary examination of performing animals but general anaesthesia is required for a complete examination. The examination should begin prior to immobilization by checking the blink reflexes and the presence of adequate tear film. This should be followed by a careful evaluation of the lids for proper closure and good apposition to the globe. The nictitans should also have good movement and apposition which can occasionally be demonstrated without immobilizing the animal.
There is an inherent risk of anaesthetizing pinnipeds (SINNETT et al, 1981; TRILLMICH & WIESNER, 1979). They do not tolerate many anaesthetic agents, and complex physiological responses based upon the normal dive reflexes, make general anaesthesia dangerous. Proper



1. Electrocardiographic tracing of a Harbor Seal (*Phoca vitulina).* A. Normal tracing under Halothane anaesthesia. B. High amplitude T waves indicative of hypoxia.

instrumentation minimizes the hazard. Cardiac electrical activity must be monitored continuously, particularly during induction and recovery. Induction with gaseous anaesthetics such as halothane should be as rapid as possible to avoid hypoxic situations created by repeated dive reflexes. This requires special vaporizers capable of providing concentrations of halothane exceeding 5%. Once anaesthetized, pinnipeds must be properly ventilated via an endotracheal tube, using an apneustic rhythm, to avoid severe post-anaesthetic complications. Lung congestion due to improper ventilation mimics pulmonary hypertension electrocardiographically. Large amplitude T waves or Ta waves are indicative of hypoxia (figure 1). Failure to improve ventilation within minutes can result in severe acidosis and failure to recover at the termination of the procedure. If elevated T waves persist, the procedure should be aborted and the patient oxygenated for at least twenty minutes before extubation.

The strong eyelids of marine mammals are difficult to open without excessive force which can further damage an already diseased globe. Abundant chemotic conjunctiva and a prominent nictitating membrane also obscure adequate visualization of the eye. A special retractor designed specifically for opening and retaining the three lids is a great advantage in overcoming this problem (Fig. 2). With the speculum in place, harderian gland inflammation or proliferation should be ruled out. The conjunctiva should be examined for signs of redness or inflammation, and the culdesacs of the eye checked for parasites or foreign bodies.
The cornea and anterior chamber are best examined with a portable slit lamp to determine the clarity of the aqueous humor and estimate the depth of the chamber. A shallow anterior chamber may indicate the presence of a soft eye which can be confirmed by tonometry. The slit lamp also allows examination of the iris and the small portion of the lens visible through the constricted pupil. The iris should be examined for normal pigmentation and vasculature, and the pupil for regularity and normal configuration. A focal light source together with a magnifying loupe or a direct ophtalmoscope may also be used to examine the lens and iris. Direct examination of the lens or the posterior segment of the eye requires dilatation of the slit-like pupil which closes almost completely when subjected to diagnostic lights. Attempts to dilate the pupil within the time restraints of safe anaesthesia with commonly used mydriatics



2. Specially constructed eyelid retractor for use in pinniped eyes. (Illustration by J. Dickson)

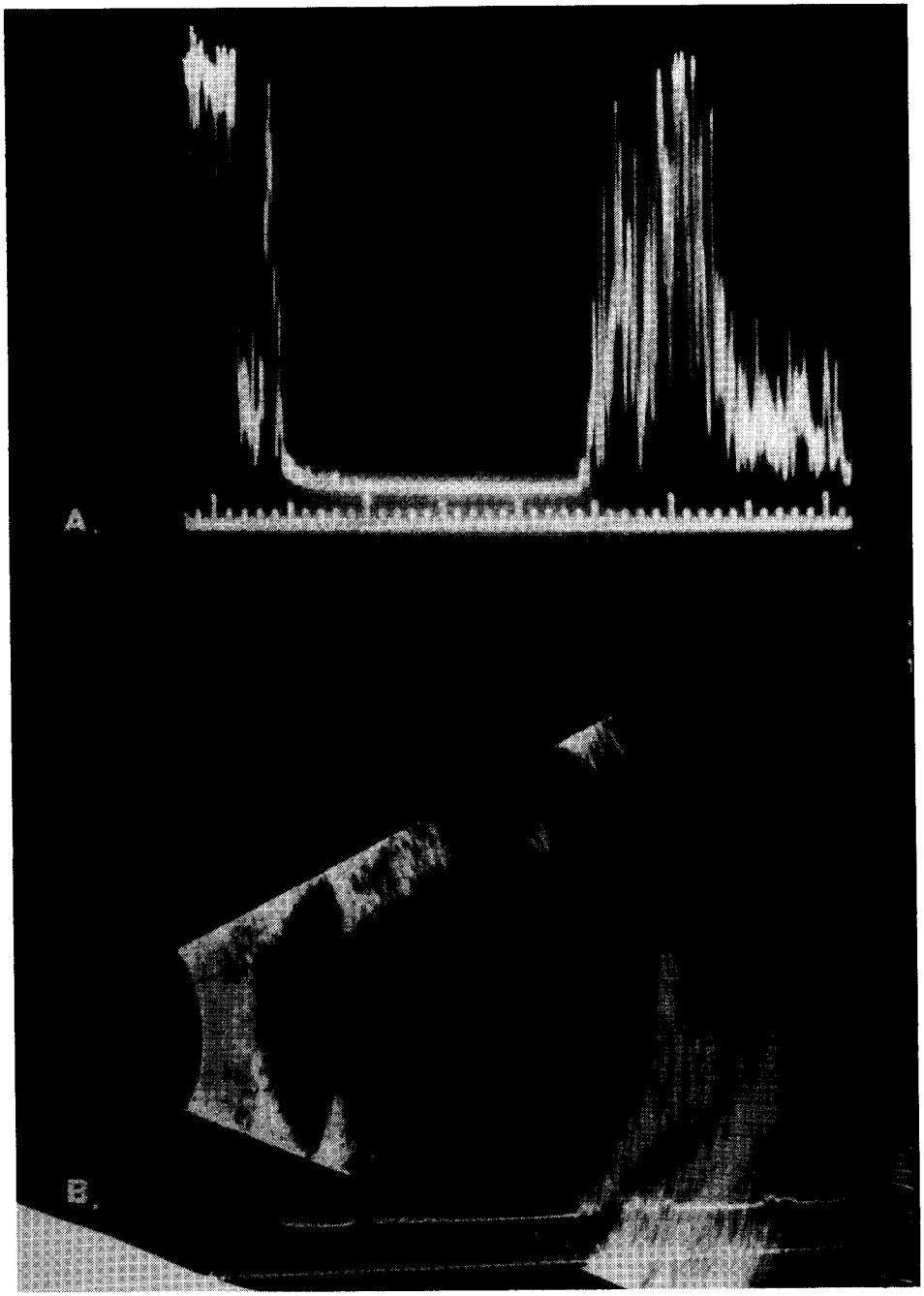
including atropine sulfate, tropicamide, neosynepherine, or cyclopentolate, are rarely successful. Better results are obtained if mydriatic drops can be applied repeatedly at intervals over a six to eight hour period prior to anaesthesia. This is possible if the patient has been trained to accept such ministrations calmly. Otherwise, examination of the posterior segment is best achieved with specialized ocular ultrasound equipment used in humans with opaque media. This equipment allows identification of retinal detachments, lens displacements and deformations of the posterior globe (Fig. 3).

It is very important that the examination of the cornea proceed rapidly to avoid drying which can cause artifacts which are easily misinterpreted. Staining the cornea with fluorescein dye is a common adjunct to examination of the corneal epithelium. Sterile strip dye applicators are preferable to concentrated drops since they decrease the risks of bacterial contemination, and overapplication of dye. Overapplication is a common error which results in an excessive concentration dye in the tear film. This is often misinterpreted as extensive disruption of the entire corneal epithelium. Only one drop of sterile saline should be applied to the sterile fluorescein strip, and the strip applied to the conjunctiva, not the cornea. Moving the eyelids will disperse the dye. The eye can then be examined with a cobalt blue light.
Incorrect interpretations usually involve over diagnosis. Mottled staining from corneal drying can be mistaken for multifocal diffuse epithelial defects. Dye pooling in depressions caused by old healed defects imitate the appearance of new epithelial lesions. Failure to examine the stained eye immediately allows fluorescein to diffuse rapidly (5-10 minutes) through a small epithelial defect, staining the entire corneal stroma.
Examination of the corneal endothelium requires specialized specular microscopes (BOURNE & KAUFMAN, 1976). Damage to this layer has been implicated as an etiology of corneal opacity in captive pinnipeds (BELLHORN, 1977). When viewed through the specular microscope, the endothelial layer of the normal cornea should have a regular polygonal pattern, free of disruption, and a cellular density of between 1500 and 3000 cells per square millimeter (Fig. 4), Endothelial wrinkling is reported not to occur in pinnipeds (SHERRARD & BUCKLEY, 1981).

Tonometry is usually the final procedure of the examination. The application of the tonometer to the cornea requires the administration of a topical ophthalmic anaesthetic such as proparacaine HCl even with the animal under general anaesthesia. Pressure readings in harbor seals and california sea lions are very similar to normal human values, and interpretation of abnormal values should follow the algorithms of human diagnosis.

Follow up and pathological examinations

A single examination provides relatively limited information concerning the pathogenesis of lesions or the progression of healing. The logistics and risk of anaesthesia limit the frequency of follow up examinations in pinnipeds, but every attempt should be made to periodically examine animals which have been affected by eye disease. This can be accomplished during routine complete semiannual or annual physical examinations. Multiple examinations throughout the course of active disease are justified by the need to evaluate the effectiveness of therapeutic measures, particularly in animals threatened with the loss of a functional eye.
Careful postmortem examination of all pinniped eyes is also important to the understanding of ophthalmic disease in pinnipeds. Both eyes should be taken for dissection and identified as left and right for correlation with clinical notes on the eyes. If facilities for biochemical



3. Ultrasonograms of the eye of normal Harbor Seal *(Phoca vitulina),* demonstrating an anterior chamber depth of 3mm. A. A-mode scan, corneal surface to left. B. B-mode scan in same orientation.

examination of the cornea and lens are available, careful fresh dissection of eyes obtained shortly after death can provide insight into the validity of proposed biochemical and metabolic mechanisms. Corneas and lenses placed in ice cold 0.25 Molar sucrose are suitable for several hours for examination of enzyme activities and metabolite content by a biochemist. Otherwise, enucleated eyes should be carefully fixed in separate containers of gluteraldehyde-formalin, and supported by gauze packing to avoid deformation. Multiple incisions through tenon's capsule of the conjunctiva, perpendicular to and near the limbal junction, allow better fixation of internal structures.

Corneal disease

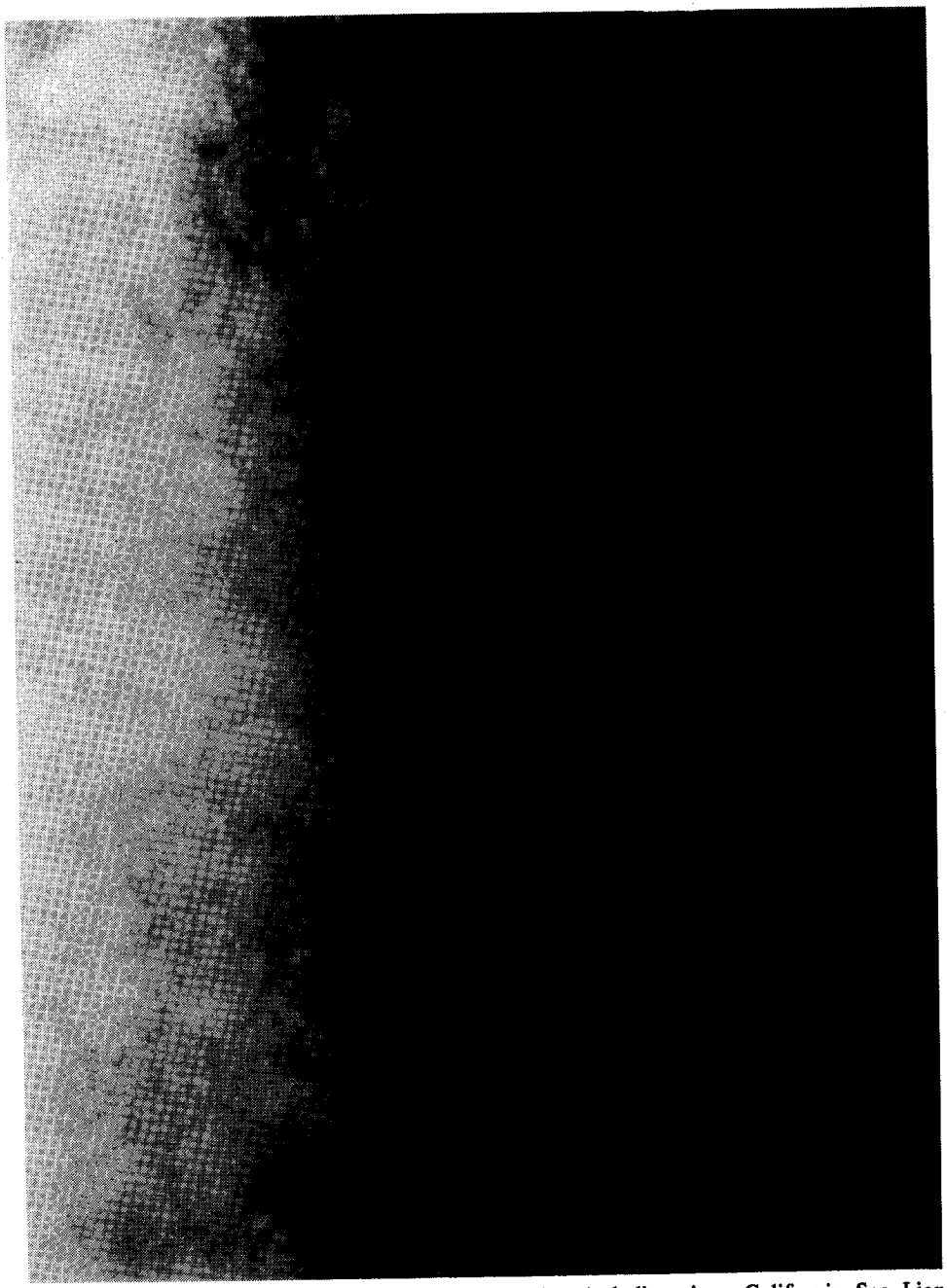
Cloudy and opaque corneas can and do result from a number of completely different etiologies, a fact which can have profound impact on therapeutics (BELLHORN, 1977; RIDGEWAY et al, 1975). A cornea completely denuded of epithelium can reepithelialize in just 24 to 48 hours upon removal of the specific insult initiating the damage (LERMAN, 1980). Identification of the offending agent and protecting the eye from further exposure is paramount to this recovery.

The optical properties of the cornea are determined by several factors. Growth and development of the eye determine the radius and curvature of the cornea, while chemical composition and avascularity determine refractive index and transparency. The cornea is about 75% water, a composition closely regulated by the corneal epithelium and endothelium. Even small changes in water content cause loss of transparency, so any factors which impair the ability of the epithelium or endothelium to regulate corneal water content are potential etiologies of corneal clouding or edema (LERMAN, 1980).

Traumatic lesions resulting in damaged epithelium and ulcers are an excellent example. Inflammatory response to the injury further complicates the picture. Corneal opacity due to trauma is often associated with distinct circumscribed abrasions or ulcers. These are frequently centrally located, and are the result of accidental collisions with objects in the enclosure. Careful examination of the rest of the eye in these cases often reveals other problems which impair ocular acuity and predispose the patient to these accidents. If this is not the case, close examination of behavioral interactions between members of the group and the patient will often show aggression or sudden flight responses which result in reckless swimming. Other traumatic injuries, such as piercing wounds, need not be central, but are usually well defined and circumscribed. The healing of traumatic lesions or ulcers of various etiology can result in permanent defects in the transparency of the cornea through the deposition of scar tissue.

Little, if any, work has been done concerning nutritional and metabolic causes of corneal opacity in pinnipeds although they may well contribute to the pathogenesis. We have not definitely identified either factor to be a cause of corneal opacity in the cases we have examined. The observation of corneal clouding with no visible defect in either epithelial or endothelial layers is an indication for a complete physical workup and analysis of feed composition and handling.

Infectious diseases including viral, bacterial and protozoal infections must be considered in the differential diagnosis of corneal opacity. The significance of these problems in the pinniped eye has not been well studied. The constant exposure of the eyes to possibly contaminated water greatly complicates the interpretation of simple culture results. Examination of the eyes should be accompanied by carefully collected water cultures and conjunctival or corneal cultures. No corneal disease of infectious etiology has been identified in the captive seals we have examined.



4. Contact specular micrograph of normal corneal endothelium in a California Sea Lion *(Zalophus californianus)*, showing a regular hexagonal pattern and a cellular density of between 1500 and 3000 cells per square millimeter.

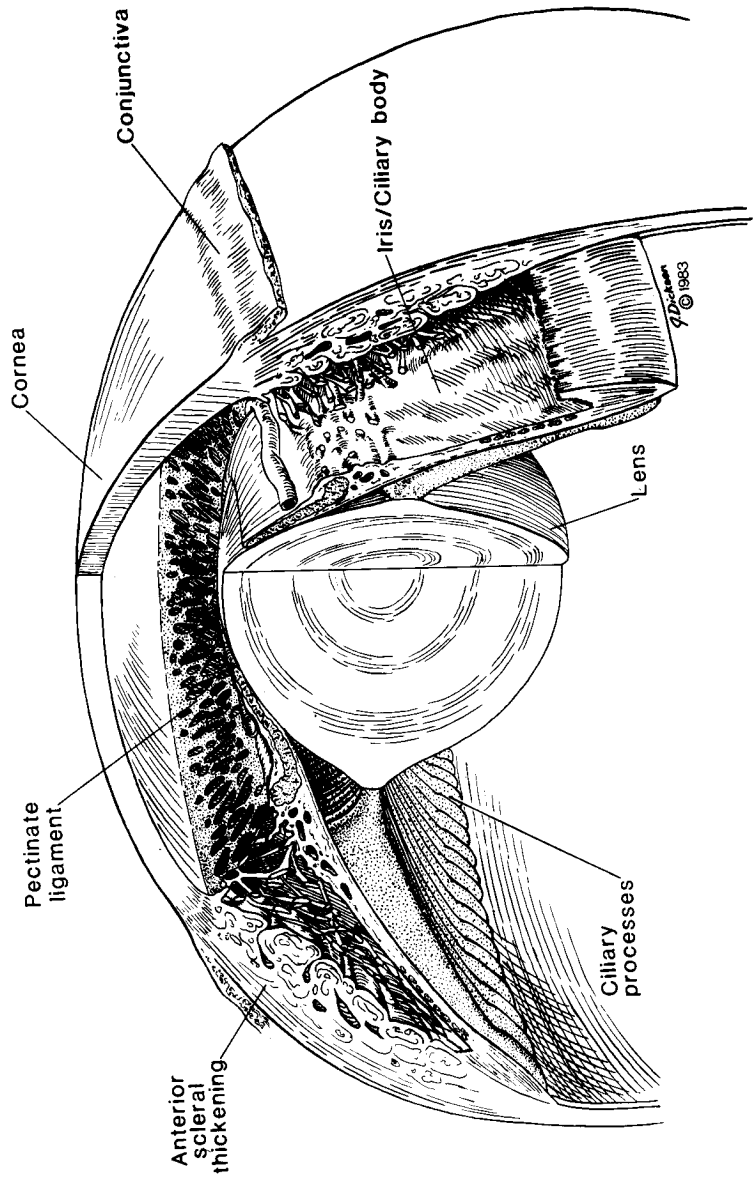
Several authors have incriminated chemical contaminations or imbalances in water quality as a cause of corneal opacity (BELLHORN, 1977; NEEDHAM, 1978; RIDGEWAY et al, 1975). One major aspect of chemical water quality is osmolarity. A credenda in the management of captive pinnipeds is that animals kept continuously in fresh water will develop corneal opacities if not provided with salt water head dips or surplus dietary salt. This factor may indeed play a role in the pathogenesis of corneal disease, but the evidence for it being a primary mechanism is not overwhelming. Many animals have been kept in fresh water without supplementary salt without developing corneal edema (RIDGEWAY et al, 1975; WARTZOG, 1982). The highly developed superficial layer of the corneal epithelium in pinniped corneas, thought to protect the cornea from the excessive osmotic pressures of sea water (EHLERS, 1970), should be adequate for protection against low osmotic pressure, through metabolic adaptation. This hypothesis is supported by a case we examined in which an animal acclimated to freshwater for years and suddenly introduced to salt water developed acute osmotic corneal edema. The role of osmolarity in the pathogenesis of corneal disease merits continued investigation.

Another aspect of water quality which has proven to have a major impact upon the transparency of the cornea is the presence of oxidative compounds provided for the purpose of disinfection and sanitation. The eye is normally subjected to extensive oxidative pressure, and the corneal epithelium produces massive quantities of free radical scavengers in the form of glutathione and ascorbic acid to minimize these effects (LERMAN, 1980). The addition of excessive amount of oxidative disinfectants such as chlorines, iodines, and ozone can overload the protective and adaptive mechanisms of the cornea and become more dangerous than elevated ammonia levels. Corneal lesions from this type of insult tend to be generalized over the entire cornea and demonstrate a roughened but intact epithelium. The lesions need not be bilateral, an enigma which may relate to animals using a dominate eye for vision while protecting the other by closing the lids, or to metabolic differences between eyes.

Careful observers have noticed that the incidence of corneal edema in some animals is well correlated with exposure to bright light (BELLHORN, 1977; NEEDHAM, 1978; RIDGEWAY et al, 1975). In these animals the corneal opacity can be reversed by merely transferring the animal to a darkened room. We have seen this phenomenon when called to examine animals with severe opacification of the cornea held overnight in facilities utilizing the same water system. On close examination of these animals small focal regions of opacification remained under a completely intact epithelium.

BELLHORN (1977) proposed that since the epithelium was intact, endothelial damage was the cause of osmoregulatory difficulties in these cases. The source of this damage was hypothesized to be the result of pressure damage inflicted by iridial contact with the endothelial surface of the cornea. A shallow anterior chamber was postulated, allowing the thick muscular iris to span the chamber and contact the cornea (BELLHORN, 1977). In testing this theory, our examinations and those of others (DRAL et al, 1980; JAMIESON & FISHER, 1972; PÜTTER, 1903), reveal a relatively deep anterior chamber (approx. 3mm) in the harbor seal and california sea lion (fig. 5). Furthermore examination of the endothelium in cases with histories compatible with light-induced damage did not reveal any evidence of endothelial disruption by specular microscopy. Regular polygonal patterns with "normal" cell densities were observed. Direct examination of anaesthetized seals showing maximum constriction of the pupil in response to strong light sources did not show any evidence of corneal contact by the iris.

An alternative hypothesis to explain the strong correlation between light and corneal edema is needed. Ultraviolet light affects cellular permeability through the generation of pyrimidine dimers, the crosslinking of macromolecules, the photoinactivation of important enzymes and the generation of free radicals through the formation of photosensitization products of



5. Diagram of the spatial relationships of structures of the anterior segment of the pinniped eye. (Illustration by J. Dickson)

aromatic amino acids (LERMAN, 1980). Ultraviolet light in the range of 200 to 295 nanometers is selectively absorbed by corneal tissues which have massive concentrations of radical scavenging compounds generated by the epithelial cytosolic enzymes. This theory explains the frequently observed relationship between exposure to strong light and the appearance of corneal opacities in pinnipeds. It also offers an explanation for the failure of strong light exposure to induce lesions in all exposed animals since it postulates damage which would be cumulative as well as additive to damage caused by other agents which deplete the pool of free radical scavengers and protective proteins produced by the cornea. The dynamics of the maintenance of this pool should provide insight into the recovery of animals from oxidative insults. This would include the integration of dietary factors including the availability of ascorbic acid precursors and sulfur containing amino acids into the overall mechanism.

Lenticular disease

During routine examinations of otherwise healthy animals, we encountered a disproportionate number of cataractous lenses in harbor seals. The majority were quite mature and usually bilateral in animals with two functional eyes. Remarkably the seals examined had no history of visual deficits and the discovery of cataracts came as a surprise to their trainers. Nutritional cataracts have been reported in infant stellar sea lions as a result of lactose intolerance, but the metabolism of harbor seals apparently allows them to utilize lactose, (normally a constituent of their milk), without untoward effects (HUBBARD & POULTER, 1968). The wide genetic base of the stock examined decreases the probability that the cataracts being observed were heritable, and points to other causes.

The lens is in a more protected position than the cornea, but many of the same factors which affect the corneal transparency are known to affect the lens, including trauma, metabolic disease and nutritional imbalance. Direct effects of water quality seem less likely. The cornea effectively filters all ultraviolet radiation in the 200 to 295 nanometer range, but considerable radiation in the 295 to 400 nanometer range reaches the lens with its large amounts of tryptophan held in nonpolar environments (LERMAN, 1980). This may allow the production of fluorescent emission products capable of initiating free radical formation and affecting the permeability and transparency of the lens. Biochemical studies of pinniped lenses are necessary to examine this theory.

Conclusions

The incidence of eye disease in captive pinnipeds demands that detailed investigations be made into the etiology and therapy of the important conditions. Towards this end it is important that opportunities to study the eyes of these animals be utilized fully by being prepared to perform a thorough examination of the eye whenever an animal is being anaesthetized. Proper preparation and planning of this examination can make it more productive. Once lesions are identified, good temporal follow up is required to illuminate the success of any therapy or to document the progression in untreated cases.

Similarly, pathological examination of the eyes of dead animals should become routine. Much of the confusion about the pinniped eye can be answered by detailed anatomical, histological and biochemical evaluations of the various components of the eye. Proper attention to fixation and preparation can greatly improve the amount of information obtained from an individual specimen.

Acknowledgements

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