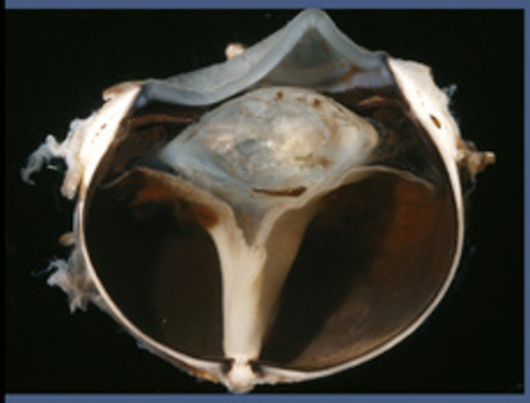
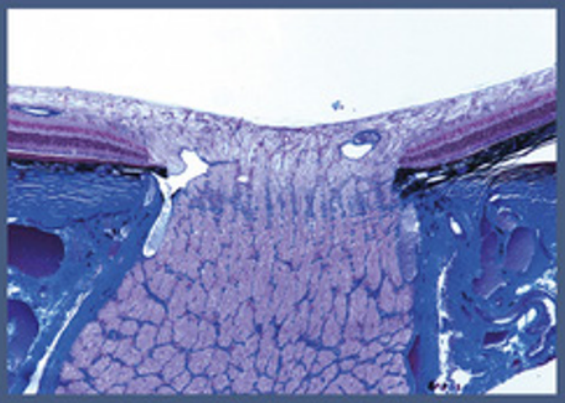
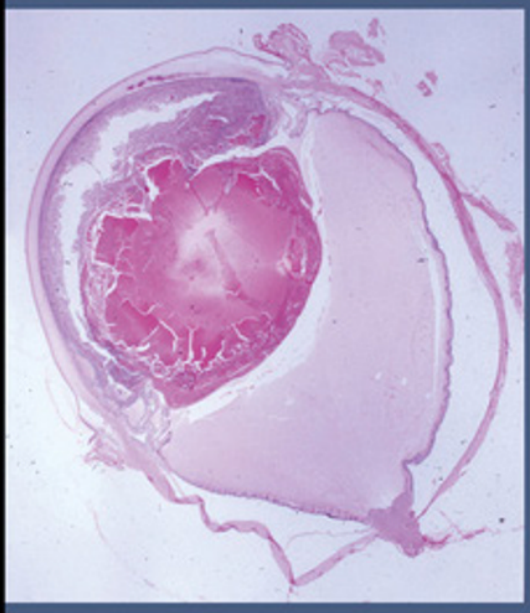
Histologic Basis of Ocular Disease in Animals



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WILEY Blackwell

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Foreword

The title of this valuable new book has been subtly well-chosen. The authors have avoided the word pathology with its implications of a speciality which always comes late in the management process and carries an overemphasis on tumor diagnosis. The book will indeed serve well as a surgical pathology bench book and it contains the best treatment I have seen on handling and processing of ophthalmic specimens. Its appeal and value, however, are far beyond that. As the title indicates what the authors are giving the reader is eye biology in disease states at the histological level and as such its appeal will be wide.

The work is comprehensive, thorough, and wide-ranging. As a result, it is substantial and longer than many clinical textbooks. The size of the work, however, is not at all intimidating and is no barrier to communication with the reader. Anyone familiar with previous published work by the authors will be aware of their communication skills and what to expect from their style. This book delivers as expected. The style is lucid, direct, even informal in places, and communicates specialist histological information in a way that does not feel specialist. The authors' backgrounds give them a unique perspective on the needs of all readers. Clinicians and others will readily relate to and learn from the contents. To give just two examples there are sections on trauma and on corneal wound healing and responses to insult, which are of daily importance to clinicians.

The authors and publisher have used the layout and illustrations well to support the text. The two-column format with the illustrations in line with the columns and closely tied to the text works well. The illustrations include clinical photographs, diagnostic images, sub-gross photographs, micrographs, and EMs and are well chosen. The judicious use of tables to convey some types of information is useful and well done.

This is a book that the whole veterinary ophthalmology community has been waiting for. Anyone who thinks of themselves as an eye biologist of any kind needs to understand how the cells are behaving. This book does that and will be a great service to all. This is a book to refer to, to learn from, and, if you are at all interested in eyes, quite simple to enjoy.

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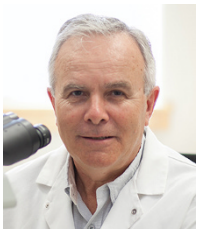
Acknowledgements



The seeds for this book were sown over three decades ago initially at the biannual Histologic Basis of Ocular Disease Course and later at the American College of Veterinary Ophthalmologists, William Magrane Basic Science Course. Several of the lecturers and many of the graduate students that participated in the Histologic Basis of Ocular Disease segment conversed of the need for a text that would couple the disciplines of clinical ophthalmology and anatomic pathology. Two decades later, Wiley and company expressed interest in publishing such a book and with the collaboration of my mentors, co-authors, and good friends Drs. Robert Peiffer and Brian Wilcock, the text evolved from the conceptual to the opus that follows.

There are many who deserve acknowledgement and recognition for their efforts during this project. My companion, soul mate, and wife for more than four decades, Mary Lou deserves first mention. She was there when the task was stressful and long, and at times seemed to have no end. Thanks for your support and continued love for which I am forever indebted. To my graduate students (Cheryl, Eric, Carey, Bianca, Marina, Stephanie, and Leila): your dedication to learning was inspirational, thanks for allowing me to be a part of your education. To my professor partners Lynne, and Bianca and Marina, for working with me and going the extra mile doing the many tasks that allowed me to complete this project. To my good friend Ian Shirley (technician extraordinaire) and LaRhonda Sobchishin for your brilliance and thoughtful insights, without which this project would not have been complete. To Julianna Duebner for your artistic skills, which are unequalled. To all the residents in Veterinary Ophthalmology and Pathology across the world who completed my graduate courses in an electronic classroom or lecture halls, your questions and attendance were never taken for granted. Special recognition is warranted to the department of Pathology at the Western College of Veterinary Medicine, who graciously accepted me into their fold and allowed me to interact and teach and research with them in the specialty Pathology. Finally, the University of Saskatchewan for promoting research and scholarly pursuits to all faculty, which has allowed me to complete this book.

Bruce Grahn

Here, then, is my travelers cloak, dusty and worn, for years it has known the road[1]… the journey immeasurably enriched by all who guided or accompanied. My parents, who instilled compassion for all things living and enabled a world-class education. My spouse Kathleen and our children, who shared me with other things with which I tried to maintain balance, not always successfully, and are still teaching me what love really is. Diverse educators, who fostered interest in literature and poetry and disciplines beyond the biologic as well as those who imparted a love for science and medicine. Charlotte Hill, who baptized me with the holy waters of ocular pathology in the bowels of the University of Minnesota Hospitals and Dave Eifrig, who courageously recruited a fledgling veterinary ophthalmologist to direct his new Department's ocular pathology and research laboratories at the UNC. The Georgiana Dvorak Theobald Society whose members warmly welcomed me into the fold. A litany of veterinary and medical students, residents, and colleagues, who fanned the embers of perpetual fascination and inquiry. My research collaborators whose depth of knowledge imparted great humility. Bill Carlton, who tutored my introduction to veterinary ocular pathology and MD pathologists Stan Lipper and Tom Bouldin at the UNC and Franz Fogt at Penn, we taught and learned together. My patients as well as my own Labradors, daily reminders of their sentience that elevates the meaning of it all.

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1 Grantland Rice's book, 1954. *The Tumult and the Shouting.* New York: A.S. Barnes & Co. p. 355.

Lastly my co-authors and our publisher without whom these words would not be penned. Both Brian and Bruce have been over decades wise and erudite mentors and counselors, but most importantly of all gentlemen and friends; I could not ask for better, and the patience and professionalism of the Wiley staff.

Equal appreciation of many omitted above by limitations of space. For all I hope that I have been found not to be lacking and that the destination has merited the journey.

Robert Peiffer

The trouble with having a long career is that the list of people to be acknowledged grows longer with each passing year. In many cases it is only later that you realize how many of your mentors and colleagues have had an influence on your life and career that might not have been obvious at the time. Any insights I have provided in this book reflect all of those influences.

The most immediate contributors to acknowledge are my co-authors. Bruce and Bob have been my friends and professional colleagues for many years, and I have no hesitation in acknowledging that their contribution to this book far exceeds mine.

Harvey Olander, the leading veterinary diagnostic pathologist of his era, was my friend and mentor during my training in pathology and my idol for many years thereafter.

Bob Peiffer introduced me as a young pathologist with an interest in ocular pathology to his wide circle of colleagues within the unfamiliar world of clinical veterinary ophthalmology. Sam Vainisi, Milt Wyman, Kerry Ketring, Cindy Cook, Cecil Moore, Charlie Martin, Dennis Brooks, and many others all extended the hand of friendship and each provided unique perspectives to enrich my career.

Leon Saunders was an intellectual giant with an absolute commitment to precision in the spoken and written word, and was a pioneer in veterinary ophthalmic pathology. He generously spent an hour of his time at the microscope with me as a young, newly-minted pathologist looking at some horse eyes. To this day I treasure it as one of the definitive moments of my career, and a reminder that a kind gesture is never wasted.

Dick Dubielzig is a treasured friend and colleague whose insight into ocular pathology has enriched all of us.

Finally, none of this would have been possible without Anne, my wife and life partner of 45 years. With characteristic patience and humor she has proofread manuscripts, managed correspondence, and attempted to explain that keyboards are not just musical instruments, mice do not require feeding, and the cloud is not really predictive of rain.

Brian Wilcock

1

Fixation and processing of ocular tissues

Fixed ocular tissues are submitted as (i) whole globes with attached orbital tissues from exenteration or transpalpebral enucleation (Figures 1.0 and 1.1); (ii) globes without orbital tissues (transconjunctival enucleation) (Figure 1.2); (iii) intraocular contents from evisceration specimens (Figures 1.3 and 1.4); (iv) prolapsed tissues following penetrating trauma; or (v) excisional or incisional biopsies of proliferative lesions (Figure 1.5). The clinical pathologist receives exfoliative (scrapings or impression smears) or aspirated specimens. Each of these samples has unique preparation requirements in order to optimize the pathologist's examination (Torcyznski, 1981).

Tissues other than globes are generally fixed as is routine for nonocular tissues with 10% neutral buffered formalin, while cytology specimens should be submitted as air-dried smears on glass slides. Globes require greater attention; it is important that they be fixed promptly to ensure rapid fixation of the internal structures and minimize autolytic changes. The ideal fixative would (i) penetrate cornea and sclera rapidly; (ii) preserve normal tissue relationships; (iii) allow for detailed gross examination; (iv) minimize embedding, trimming, and cutting artifacts; (v) enable any number of histochemical or immunohistochemical stains; and (vi) permit ultrastructural evaluation in those admittedly few cases in which it might be necessary. As no such perfect fixative exists, selection becomes a compromise influenced by availability, ease of use, and the quality of fixation required for the specific application (Yanoff et al., 1965; Yanoff and Fine, 1967; Yanoff, 1973; Menocal et al., 1980; Margo and Lee, 1995).

Fixatives

The most common method of fixation for light microscopic evaluation of globes is immersion in 10% neutral buffered formalin. This fixative has the advantages of being widely available even in private practice (supplied free by most diagnostic laboratories), and permits good macroscopic evaluation of submitted globes. It allows prolonged storage of globes without danger of excessive hardening, and the fixative is most often used with immunohistochemical protocols. Although it is claimed to penetrate tissue quite rapidly, in actuality fixation via cross-linking of proteins is quite slow (24–48 h) with resulting predisposition to retinal autolysis as well as other artifacts; although often referred to as "formalin artifacts," they are in fact a reflection of slow fixation and are not specifically related to formalin. These changes can be minimized by injecting a small volume of formalin into the vitreous prior to immersion, and they are not encountered in retinas from evisceration samples that are in immediate contact with the formalin.

Several other fixatives have specific advantages and disadvantages (Table 1.1). A mixture of 1% buffered formaldehyde/1.25% glutaraldehyde perhaps best approaches the requirements for an ideal fixative, but its limited stability, special storage requirements, and slow penetration of intact globes make it unsuitable as a fixative for use in private practice. Both Davidson's fixative and Bouin's fluid provide much more rapid penetration of the globe and thus much better retinal fixation than 10% formalin, and they harden the globe to greatly facilitate the trimming required for histologic processing. For eyes with boney ossicles, either of these acid fixatives provides the added convenience of simultaneous decalcification. Unfortunately, however, they both opacify the tissues and make macroscopic inspection and photography of the fixed globe less rewarding than with formalin (see Table 1.2). Because Bouin's fluid causes yellow discoloration of the tissue (and everything else it touches!), and because globes must be removed from the fixative and transferred to formalin within 24 h to prevent excessive hardening and poor subsequent histologic staining, we regard Davidson's fixative as the best ocular fixative for use in clinical ophthalmology practice and general diagnostic laboratories.

Histologic Basis of Ocular Disease in Animals, First Edition. Bruce Grahn, Robert Peiffer and Brian Wilcock.

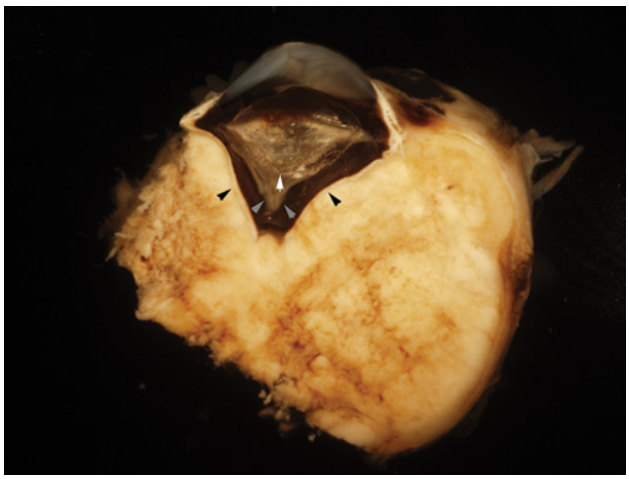


Figure 1.0 A bisected canine globe that was exenterated due to a large retrobulbar tumor that is indenting the sclera (black arrows), the retina is detached (gray arrows) and the vitreous is compressed and contains asteroid hyaloid bodies (white arrow) (formalin fixation).

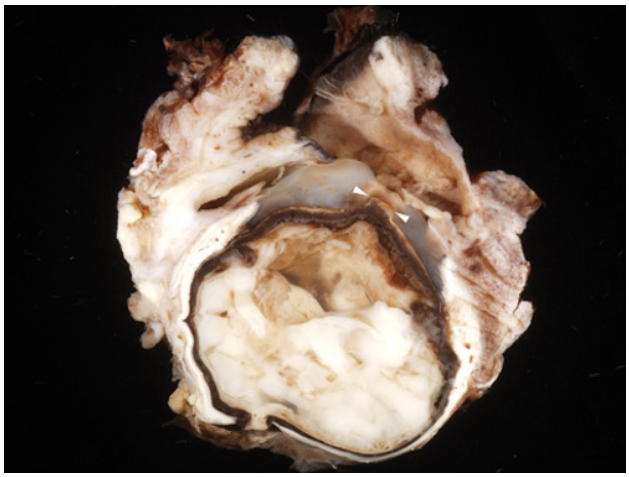


Figure 1.1 This bisected canine globe has endophthalmitis and was removed by transpalpebral enucleation. This globe contains white purulent exudate, and the iris is extending into a corneal defect (white arrows). Unless they are likely to contain lesions, the eyelids and other periocular soft tissues would ordinarily be removed during transconjunctival enucleations prior to fixation or if they are left on (transpalpebral enucleation and exenterations), they may be trimmed when the globe is sectioned (formalin fixation).

Recipe for davidson's fixative
4 parts 10% neutral buffered formalin. 3 parts 95% ethyl alcohol. 2 parts distilled water. 1 part glacial acetic acid.

A comparison of retinal histology resulting from the use of these various fixatives is presented in Figure 1.6a–f.

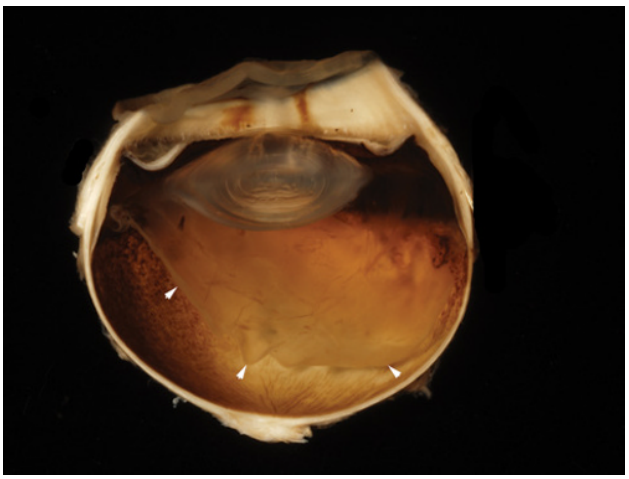


Figure 1.2 A bisected canine globe removed by transconjunctival enucleation, the technique used for the vast majority of globes submitted for histologic assessment. The retina is detached (white arrows) and noticeably thin (formalin fixation).

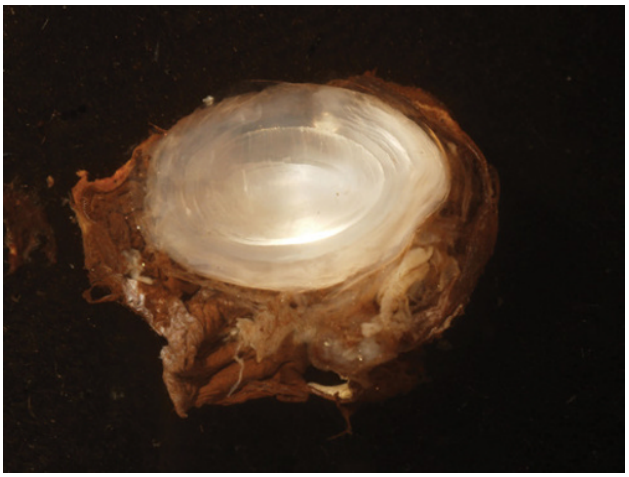


Figure 1.3 A canine evisceration sample bisected in preparation for histologic processing. Many are not as cohesive as this and arrive as multiple disconnected fragments. Evisceration samples like this should be sectioned in multiple planes to ensure that all intraocular tissues are present for histologic examination (retina, uvea, and lens) (formalin fixation).

Fixation and sectioning artifacts

The most common fixation artifacts are separation of the neurosensory retina from the retinal pigment epithelium, corneal swelling as the dehydrated cornea equilibrates with the isotonic fixative and therefore absorbs water, separation of the corneal endothelium from Descemet's membrane, and vacuolation of lens cortex. Contraction of the anterior chamber and vitreous cavities with resultant indentation of the cornea and sclera (Figures 1.7

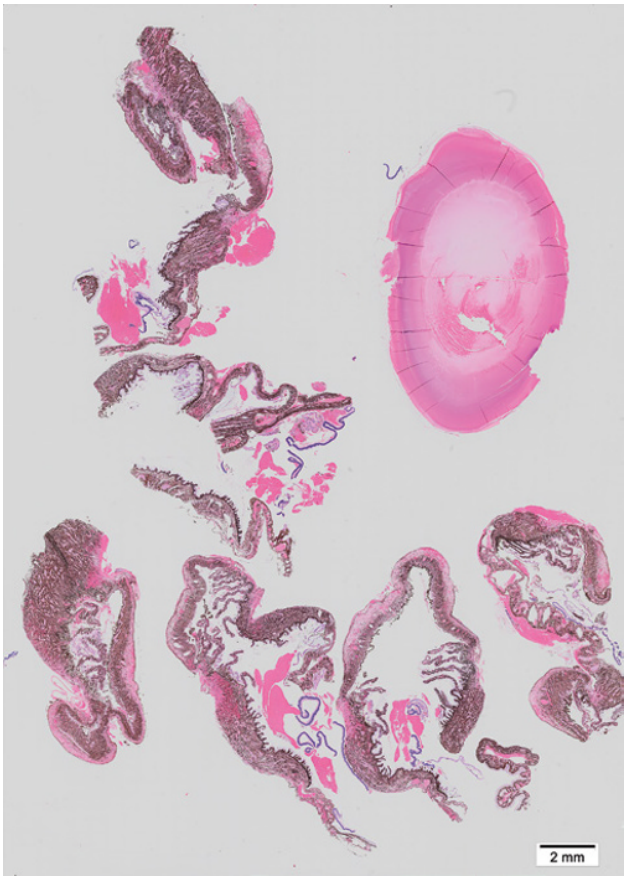


Figure 1.4 Low-magnification histologic image of a typical evisceration specimen. When contrasted to sections from enucleated globes, such samples provide limited (albeit sometimes still useful) diagnostic information. The predominance of pigmented uveal tissue of which the ciliary body is most abundant is routine (hematoxylin and eosin stain, formalin fixation, and subgross magnification).

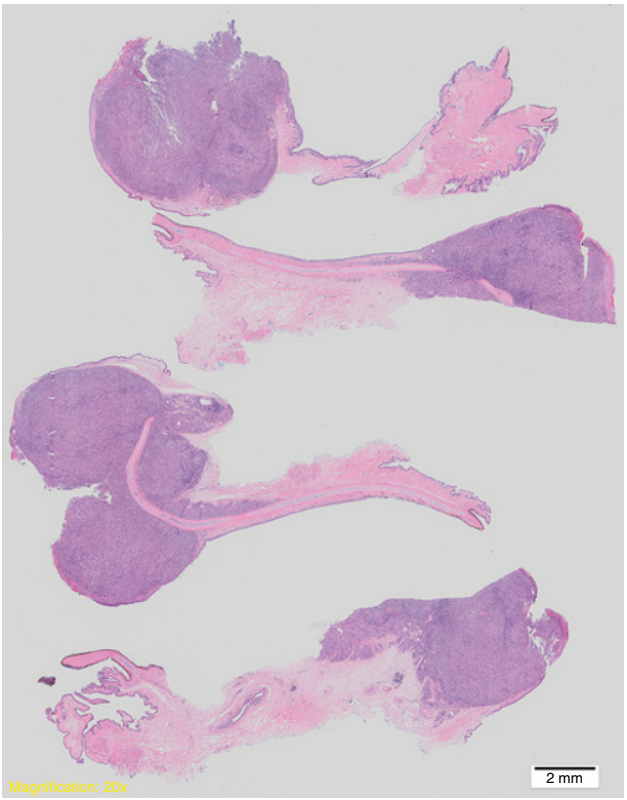


Figure 1.5 Low-magnification histologic section of a formalin-fixed third eyelid tumor in a dog, confirmed histologically as a minimally invasive adenocarcinoma arising from the gland of the third eyelid (hematoxylin and eosin stain, formalin fixation, subgross magnification).

and 1.8) are particularly common in canine and feline globes. The number and severity of artifacts will vary with the fixative being used and are less problematic with rapidly penetrating acid fixatives like Bouin's or Davidson's (Figures 1.9 and 1.10). Common sectioning artifacts (i.e. occurring during the cutting and slide preparation of the paraffin-embedded sections) include fragmentation and displacement of the lens, retinal elevation from retinal pigment epithelium, transverse wrinkling of the cornea, and separation of the corneal endothelium from Descemet's membrane (Figure 1.11a and b).

Fixation techniques

Unless their remaining in situ is critical to the diagnosis, all of the orbital and adnexal tissues adherent to the globe should be removed to optimize the speed of fixation. Ocular tissues are usually immersed at a ratio of 1 part tissue to 5–10 parts fixative. To enhance fixation of retina, globes that are to be immersed in buffered formalin should also receive an intravitreal formalin injection (0.5–1.0 ml depending on the size) via a 25 gauge needle inserted through the sclera. We prefer to insert the needle next to the optic nerve to ensure that the needle penetrates the vitreous and the formalin is dispersed evenly over the retina prior to immersing the globe in formalin. This allows for better fixation of the retina by compensating for the slow penetration by formalin through the sclera. Formalin-fixed globes benefit from subsequent immersion in graduated concentrations of 50–70% alcohol over the next 24–48 h. The alcohol hardens the globe and allows for better trimming in preparation for histologic processing.

For globes fixed with Bouin's fluid, sequential immersion in 2–3 rinses of 70% alcohol over a few hours will remove most of the picric acid and will improve histologic staining (especially when the sections are being made from wax blocks after prolonged storage). Globes fixed in Davidson's fixative require no additional special handling, but should not be stored in that fixative for longer than about 48 h.

Table 1.1 Ocular fixatives and their advantages and disadvantages.

	Formaldehyde	1% formaldehyde/ 1.25% glutaraldehyde	Bouin's fluid	Glutaraldehyde	Triple fixatives (paraformaldehyde, glutaraldehyde formaldehyde, and osmium)	Zenker's fluid	Davidson's fixative
Maintenance of tissue relationships (neurosensory retina–RPE)	Marginal	Good	Good	Poor by immersion of the globe as penetration is marginal, so globes must be sectioned and then small pieces immersed	Excellent; however, globes are typically opened	Good	Excellent
Preservation of membranes for ultrastructure	Poor	Very good	Poor	Excellent	Excellent	Good	Very good
Immuno compatibility	Good	Good	Good; however, different epitope retrieval is required	Good	Good; however, different epitope retrieval is required	Good; however, different epitope retrieval is required	Good
Advantages and disadvantages	Cost and, availability Poor penetration of the globe and marginal tissue fixation	Uncommonly used because of availability and stability	Better penetration of globe Obscuration of focal lesions at grossing Stains everything yellow	Excellent fixation of small tissue sections or small globes with thin sclera (mice, rats) destined for electron microscopy	Uncommonly used except in research; Osmium is toxic and expensive	Uncommonly Use limited by toxicity of mercury Obscuration of focal lesions at grossing	Very good immersion fixative

Table 1.2 Histochemical stains frequently used in ophthalmic pathology.

Target	Stain	Result
General purpose	Hematoxylin and eosin	Proteins – pink Nucleic acid – purplish blue Calcium – blue
Basement membranes (mucopolysaccharides and glycoproteins)	Periodic acid-Schiff (PAS)	Dark pink/purple
Collagen	Masson's trichrome	Blue
Acid mucopolysaccharides	Alcian blue	Blue
Iron	Perl's Prussian blue	Blue
Calcium	Von Kossa Alizarin red	Black Red
Myelin	Luxol fast blue	Aqua to blue
Axons	Bodian	Black
Lipid (fresh frozen tissue only)	Oil red-O Sudan black	Red Black
Amyloid	Congo red	Red (however, it imparts an "apple-green birefringence to amyloid fibrils when examined with polarized light")
Elastin	Verhoeff–Van Gieson	Black
Melanin	Fontana-Masson	Black
Fungi	PAS Gomori methenamine silver	Purple-pink Black
Acid-fast organisms	Ziehl–Neelsen	Red
	Fite-Faraco	Red
Chlamydia	Giemsa	Pink inclusions

Trimming the fixed globe

The vertical, horizontal, and anterior–posterior dimensions of the globe, vertical and horizontal dimensions of the cornea, and length of the optic nerve, as well as any notable external lesions are measured with calipers and the measurements recorded.

The globe may be trans-illuminated with a bright light to assist in identifying intraocular lesions or foreign bodies prior to orientation for sectioning. The globe for most species is orientated by identification of the shape of the cornea, the sites of insertion of the extraocular muscles, and the location of the vortex veins, ciliary arteries, and optic nerve (Figures 1.12–1.14). This allows the pathologist to transect the globe in a vertical plane from superior (12 o'clock) to inferior (6 o'clock). This orientation provides a section that will include the pupil, both tapetal and non-tapetal fundus, and optic nerve. Other planes of section may be required to enable inclusion of gross lesions or in globes from animals with a macula (primates and birds); these eyes should be sectioned horizontally (3 o'clock to 9 o'clock) to allow examination of the macula.

The globe is transected with a brain knife or microtome blade (Figures 1.15–1.18). Ordinary razor blades, in addition to being hard to find, are often too short to allow smooth cutting of anything but the smallest (i.e. rodent, small avian, and reptilian) globes. Scalpel blades do not offer the length or sharpness required for optimal sections.

The most common cause for persistently poor histologic sections of globes is the routine use of scalpel blades for trimming.

At this stage, there is a notable divergence between procedures used in research laboratories and those more likely to be used in high-volume general diagnostic laboratories. In a university or other research laboratory, the opened globe is likely to be examined with a dissecting microscope or magnifying head loupe, allowing gross findings to be recorded and photographed (Figure 1.19). In a general diagnostic laboratory, a globe is likely to be trimmed for histologic processing directly from the primary fixative (10% formalin or other) without any subsequent rinsing or hardening in alcohol. It is unlikely to be measured or photographed and the technician is likely to proceed immediately to the next step below.

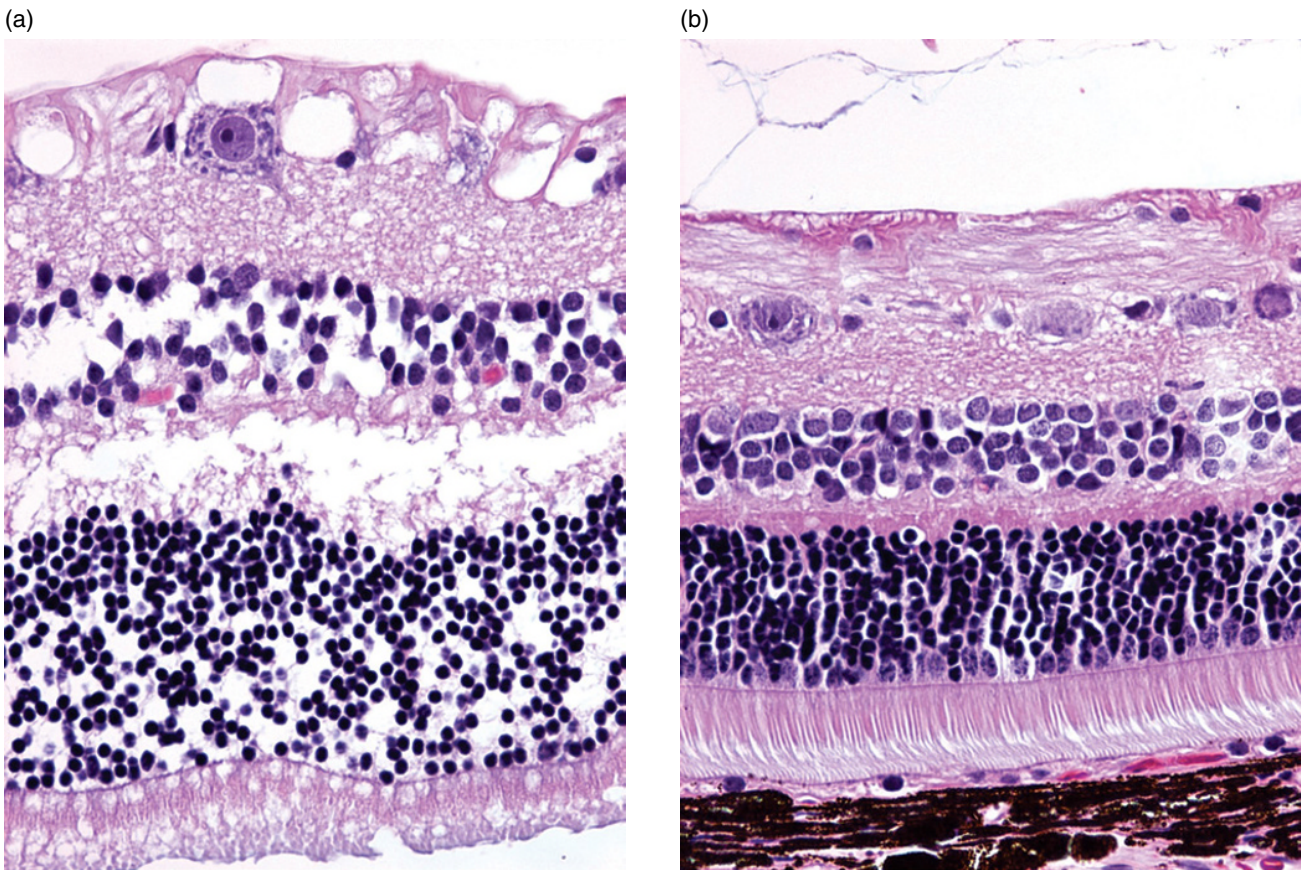


Figure 1.6 (a–f) Comparison of the effect of fixation on the normal canine retina. (a–d) are stained with hematoxylin and eosin, and have 40× magnification. (a) Fixed by immersion in 10% neutral buffered formalin. Note the marginal fixation of the photoreceptors and extensive pyknosis within the inner and outer nuclear layers. The watery clefting within the nuclear and plexiform layers is a common artifact. The retina has artifactual separation from the RPE, something that is almost impossible to avoid. (b) Fixed by injected with 0.5 ml of 10% formalin before being immersed in 10% neutral buffered formalin. This results in vastly improved retinal fixation when contrasted to immersion alone, although there is still separation and vacuolation within the inner and outer nuclear layers. (c) Fixed by immersion in Davidson's fixative. There is excellent preservation of photoreceptors and the various nuclear layers, with no artifactual clefting. This fixative also hardens the globe, making it much easier to trim. There is much less risk of artifactual retinal detachment when contrasted to formalin-fixed globes. (d) Fixed by immersion in Bouin's fluid. This results in excellent retinal fixation comparable to that achieved with Davidson's fixative. (e) A small piece of normal canine retina fixed in triple fixative (paraformaldehyde/glutaraldehyde/formaldehyde), post-fixed in osmium, embedded in epon, and stained with toluidine blue to achieve the best preservation for light microscopic examination. Note the exquisite cellular detail within the outer nuclear layer, photoreceptors, and RPE. This laborious procedure, suitable for only small pieces of tissue removed from the open globe, provides the superior preservation of the photoreceptor inner and outer segments as well as the RPE required for some research applications (Toluidine blue stain, 60× magnification). (f) A normal canine retina fixed by immersion in 10% neutral buffered formalin but processed as for (e) above. The preservation of the RPE, photoreceptors, and outer nuclear layer is poor (toluidine blue stain, 60× magnification).

The hemisphere containing the optic disc is then placed cut surface down and sectioned approximately parallel to the initial cut and at a distance sufficient to avoid a second contact with the lens (Figures 1.20–1.22). This thick central calotte is placed in an embedding chamber of appropriate size (Figure 1.23). The central calotte is embedded with the side closest to the optic disc down to minimize the need for rough-cutting of the wax block before obtaining the target section containing both pupil and optic nerve.

An alternative to the initial hemisection described above (which may dislocate the lens) is to slice away a medial and then a lateral cap to avoid any risk of dislocating the lens. This results in a very thick calotte in the wax block that will require additional rough trimming before reaching the optic disc. You will not be popular with the histotechnologists, but this does result in superior sections of lens and better maintains the anatomic relationships in the anterior half of the globe.

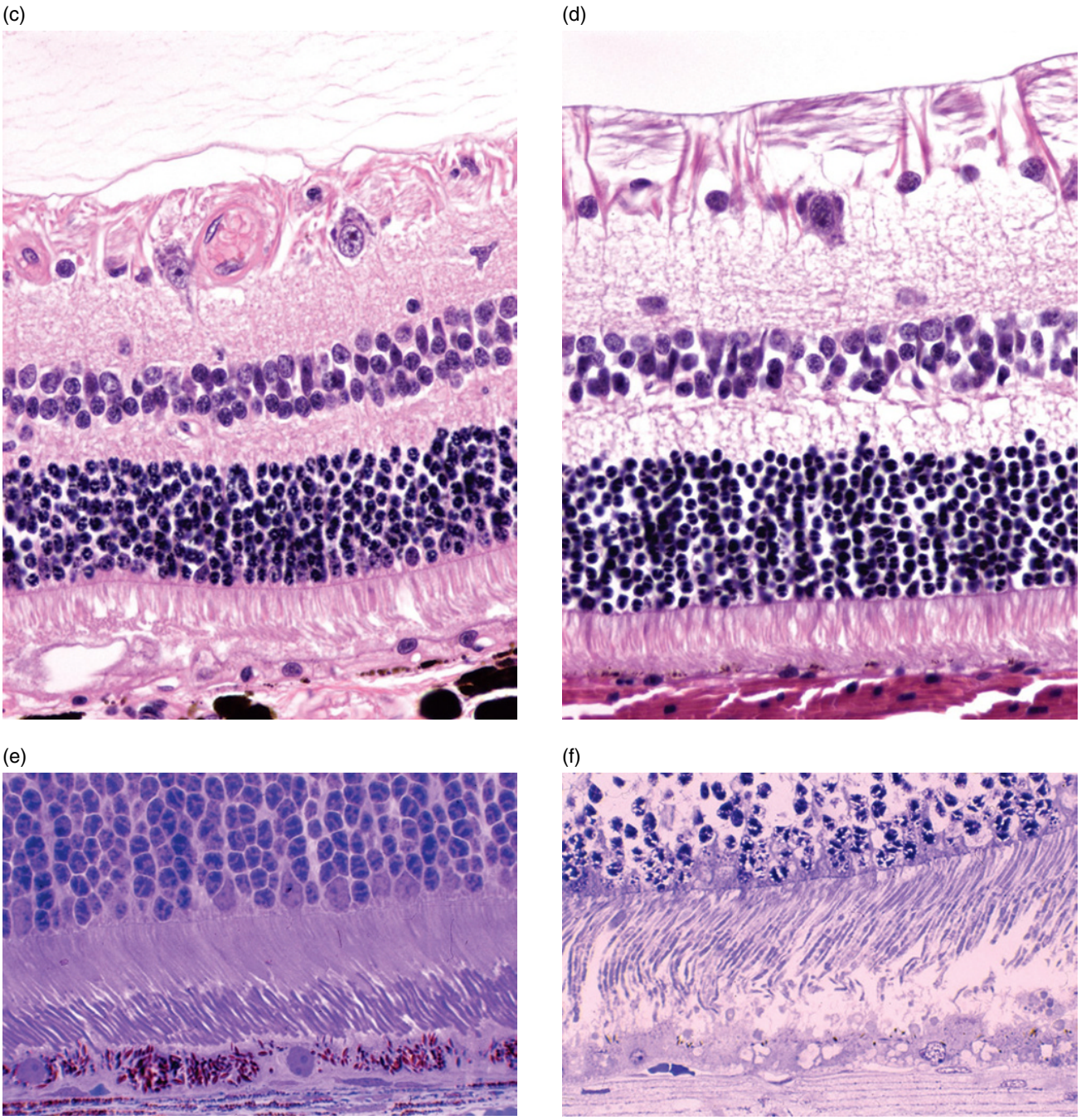


Figure 1.6 (*Continued*)

Not all commercial laboratories have the capacity to process the thick calottes described above. Although not ideal, under those circumstances gently remove the hemisected lens from the bisected globe before proceeding with your second cut, allowing you to produce a thinner calotte that will fit in an ordinary embedding cassette. The isolated lens can be sliced and replaced within the section of globe or processed separately. This will compromise the ability to evaluate lesions like posterior synechiae and various perilenticular membranes, but sometimes there will be no option.

Globes removed via **transpalpebral enucleation** (i.e. retaining conjunctiva and eyelids) are handled similarly to exenterated globes. The main indication for a transpalpebral enucleation is extensive neoplasia of the conjunctiva and eyelids, most commonly squamous cell carcinoma in the cats, cattle, and horses. Any excessive and diagnostically irrelevant tissues are removed (ideally

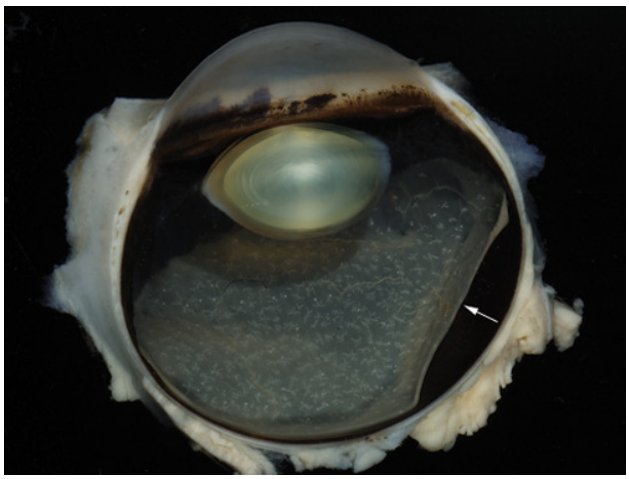


Figure 1.7 A bisected canine globe fixed by immersion in 10% neutral buffered formalin. Note the large artifactual retinal separation (white arrow). These artifacts are common and almost unavoidable with such fixation.

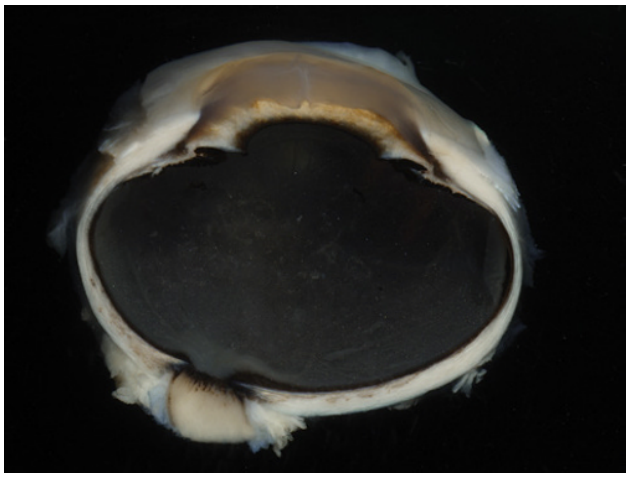


Figure 1.8 This is a section of the eye of a weanling female pig that was immersion fixed in 10% neutral buffered formaldehyde. Note the contracted anterior chamber. The lens was luxated during sectioning and it was removed and embedded in a separate block.

prior to fixation, and certainly after fixation if not already done so), and then the globe with the attached tissues of concern is processed as described above.

Globes removed via **transconjunctival enucleation** are the most common ocular specimens received by routine diagnostic laboratories. Such globes are those rendered blind and painful for any reason, which includes globes with intraocular neoplasia, glaucoma, refractory uveitis, or severe corneal ulceration. These globes have usually been fixed by immersion in 10% buffered formalin, Davidson's fixative, or Bouin's fluid. There are minimal attached orbital tissues which limit

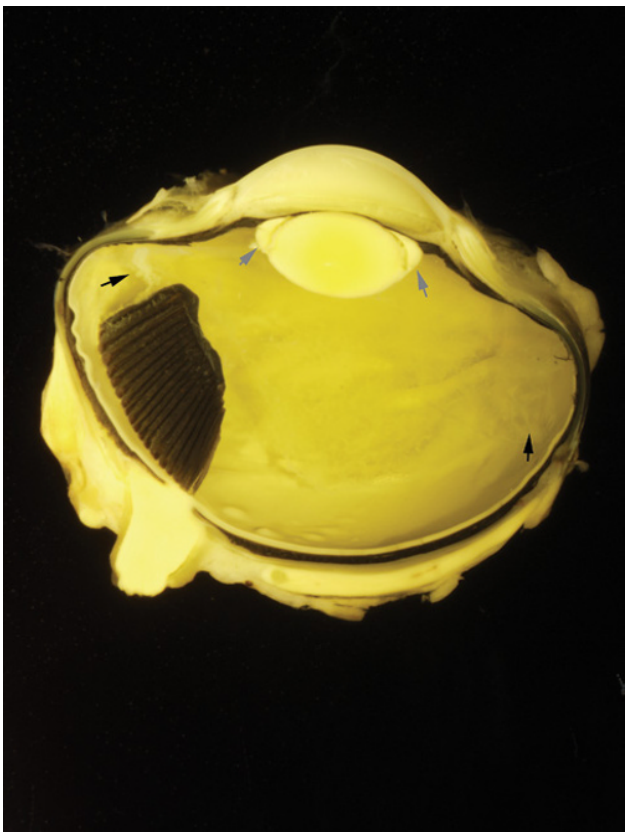


Figure 1.9 A Bouin's-fixed, bisected globe from a turkey. Acid fixatives coagulate protein and therefore accentuate inflammatory exudates, but they also cause opacity that interferes with lesion localization and photography. Note that the retina remains attached, and the mild vitreous degeneration (black arrows) and the mild separation of the lenticular occipital pads (gray arrows).

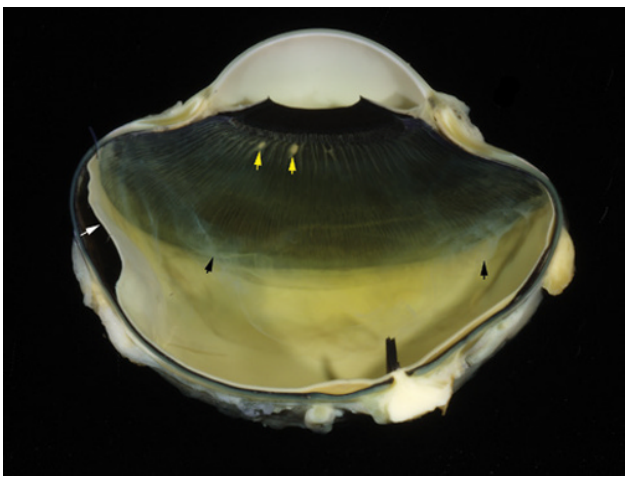
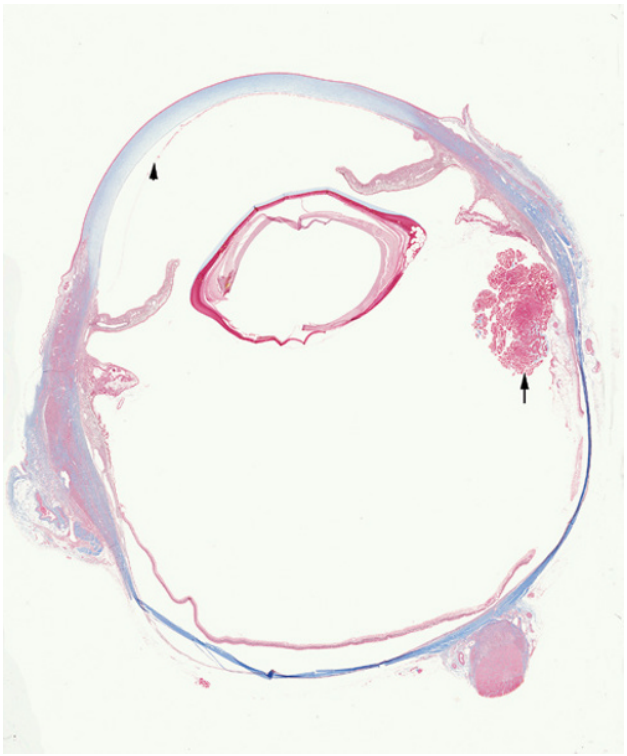


Figure 1.10 A formalin-fixed, bisected globe from a turkey. While this fixative allows better photography and the localization of lesions note the vitreous degeneration (black arrows) and the two focal yellow opacities on the ciliary processes (yellow arrows), however, there is an artifactual retinal separation that developed during sectioning (white arrow).

(a)



(b)

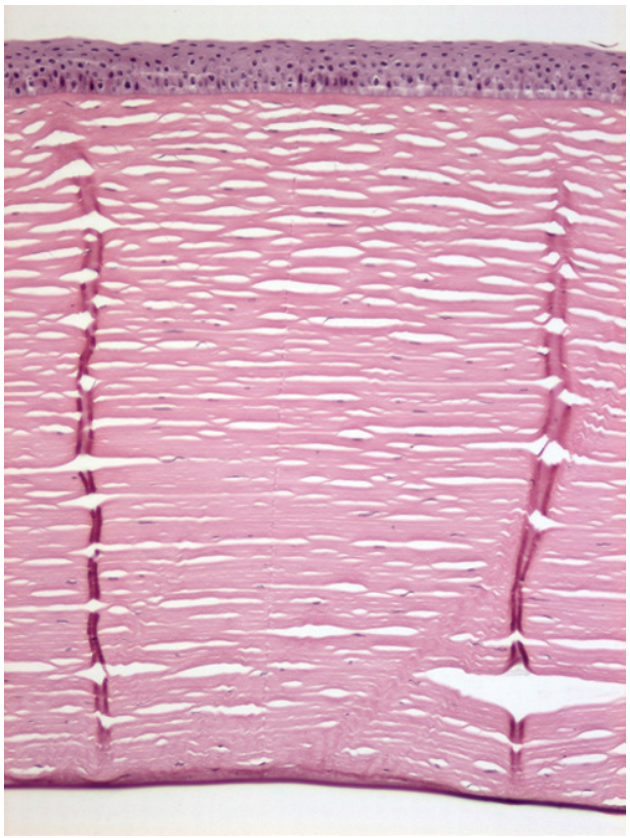


Figure 1.11 (a) A low-magnification photomicrograph of a canine globe. It is stained with Masson's trichrome stain and the collagen stains blue. Note some of the common artifacts like separation of the corneal endothelium (black arrow head), flat retinal separation, and fracturing of the lens. A fragment of tissue from another specimen (black arrow) accidentally attached itself to the specimen during histologic processing. Such "float-ons" occur in the water bath (Masson's trichrome stain, subgross magnification, and formalin fixation). (b) A histologic section of a normal feline cornea with horizontal clear clefts that are due to imbibition of water from the aqueous fixative and are unavoidable artifacts. The transverse wrinkling of the cornea is another common but avoidable artifact that occurs during slide preparation (formalin fixation, hematoxylin, and eosin stain, 20× magnification).

Figure 1.12 A canine globe removed via transpalpebral enucleation and subsequently fixed in formalin. Because of suspected lesions involving the anterior segment, the eyelids (red arrow) have been left in place after enucleation. Note the optic nerve (black arrow) and posterior ciliary artery (blue arrow) that will be used for orientation when bisecting this globe.

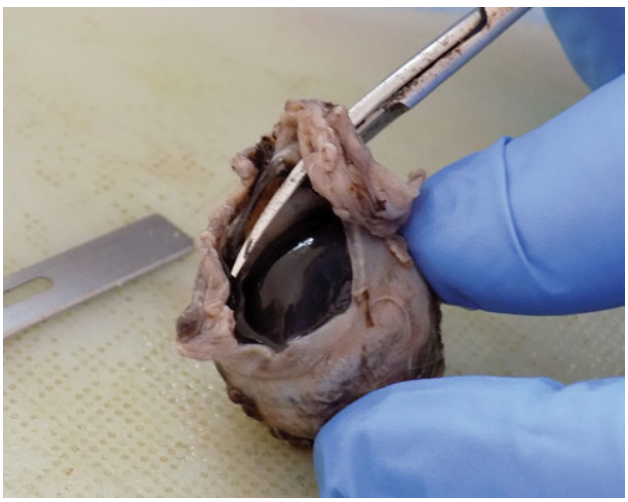


Figure 1.13 If not removed prior to fixation, the first step in trimming a globe is to remove the eyelids, conjunctiva, and extraocular muscles. At this time, the dimensions of the globe may then be measured with calipers and recorded.

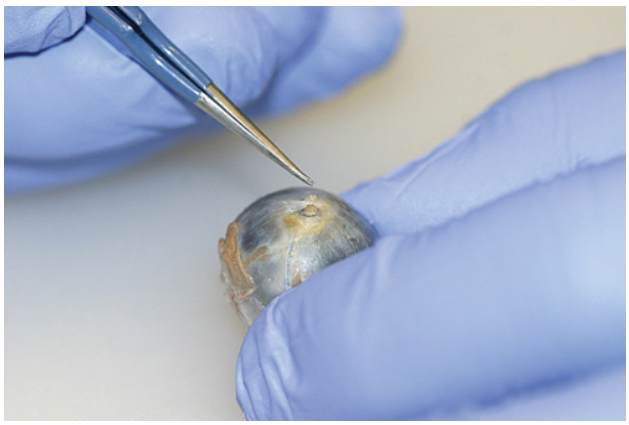


Figure 1.14 Next the globe is positioned with the cornea facing down on the cutting board. The optic nerve and posterior ciliary arteries are identified to guide the initial cut.

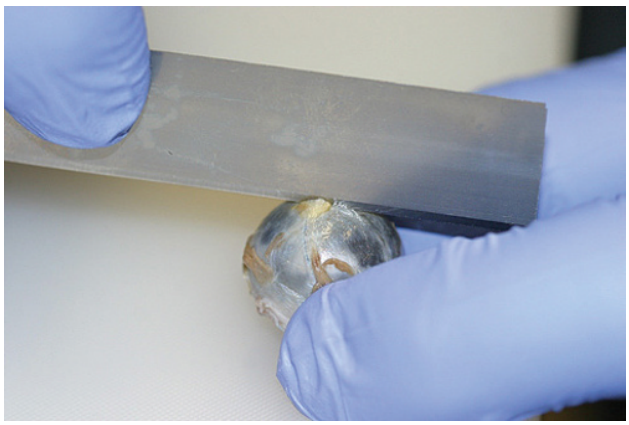


Figure 1.15 A new microtome blade or as depicted here, a brain blade is positioned perpendicular to the ciliary arteries just beside the optic nerve. The globe is stabilized between the index finger and thumb, and then is bisected using a smooth long slicing motion that avoids "sawing" as much as possible.

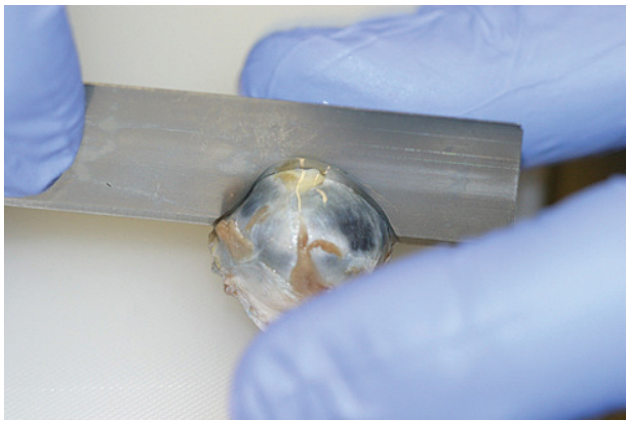


Figure 1.16 The moving blade penetrates the globe until it contacts the posterior surface of the lens. You will feel the increased resistance.

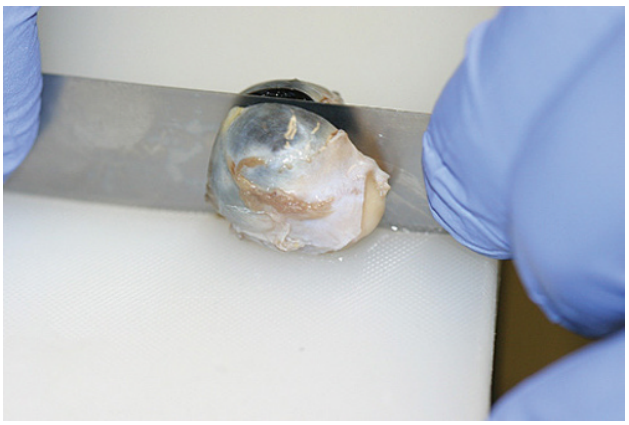


Figure 1.17 The blade is then grasped by both hands and the blade is pushed sharply ("guillotine like") through the lens and cornea to contact the cutting board.

Figure 1.18 Both halves of the bisected globe are examined for macroscopic lesions. Use of a dissecting microscope or a magnifying head loop increases the sensitivity of lesion detection. Any macroscopic lesions can now be photographed.

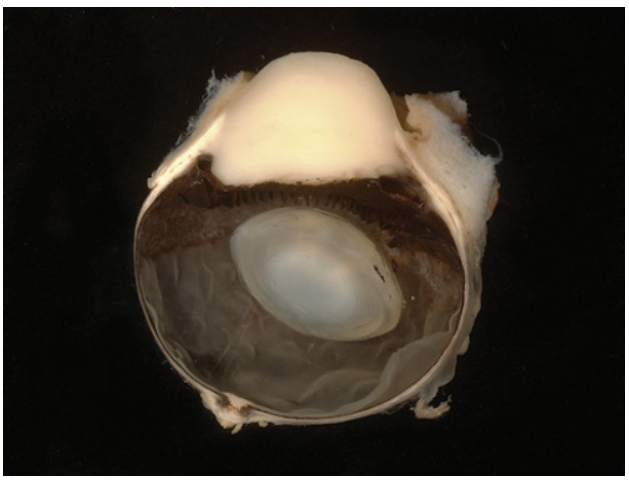


Figure 1.19 This bisected canine globe has a large corneal tumor, and it has been photographed under water after suspension on a black background. The retinal separations and the dislocation of the lens are processing artifacts. Note the difference in image quality from the section of the globe on the cutting block in Figure 1.18.

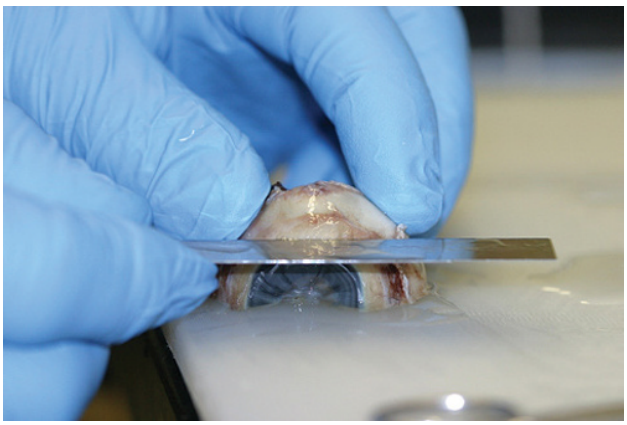


Figure 1.20 After inspection, the half of the globe with the lesion of interest is placed with the cut surface down and the second cut will be made from the cornea toward the posterior pole (to minimize any further artifactual retinal detachment).

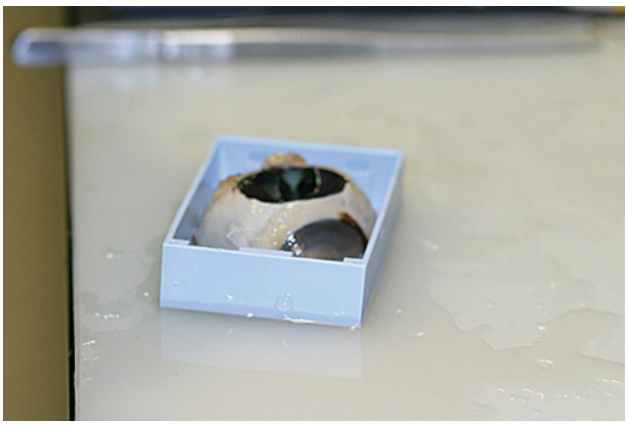


Figure 1.23 The central calotte, containing the bisected lens and the optic nerve, is placed in a plastic tissue cassette. The surface intended for cutting (ordinarily containing the pupil, lens, and optic disc) is placed face down in this cassette which is now ready for histologic processing.

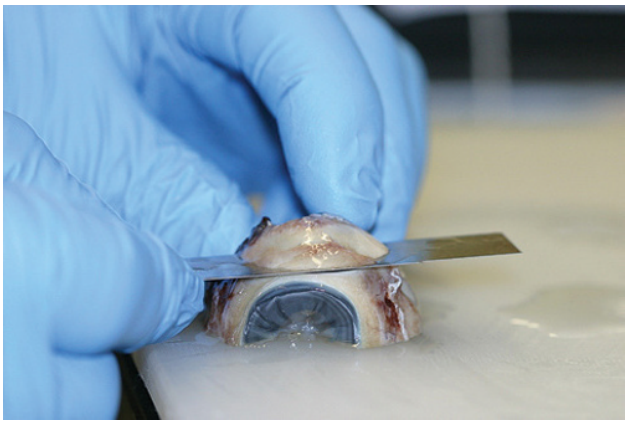


Figure 1.21 Once again the blade is drawn as smoothly as possible across the bisected globe parallel to the original cut, intended to create a thinner slice that avoids hitting the lens a second time (which will surely dislocate and further damage it). Take special care to avoid finger cuts during this stage of sectioning.

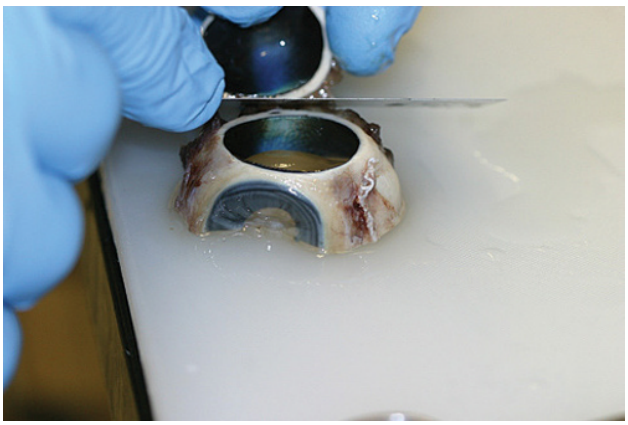


Figure 1.22 The second cut is now complete, removing a lateral or medial "cap" from the bisected globe to allow optimal penetration of histologic processing chemicals and paraffin.

the amount of trimming required by the pathologist. Examination, trimming, and processing are as described above.

Evisceration submissions are typically fixed in 10% formalin and sectioned at several levels to ensure a comprehensive examination.

Rodent and other small globes usually are fixed by immersion, oriented appropriately for embedding, and may be processed whole. Multiple anterior–posterior sections of these globes are taken until the appropriate area in the eye is located. Fetal rodents have very small globes that are difficult to remove intact due to their size and thin sclera. Fixation of the entire body in Bouin's fluid, and then serial sections of the skull horizontally or transversely to view both globes is the usual procedure. A similar approach can be used to examine very small globes in other species. Decalcification of the skull is usually a prerequisite.

Biopsies of masses involving eyelid, conjunctiva, and orbit are common submissions. They are usually submitted in 10% formalin and processed and stained routinely. It is important to identify surgical margins with India ink or sutures and labels to enable the pathologist to maintain proper orientation and to evaluate surgical margins.

Paraffin embedding and sectioning are performed using routine methodologies for all globes and biopsies (Wexler and Richardson, 1955; Ballou, 1966; Beemer, 1986). Some histotechnologists prefer to remove the lens prior to embedding the globe (Figure 1.8), embedding the lens in a separate block to improve the quality of the subsequent sections. In our view, the risk of destroying significant perilenticular lesions outweighs any technical advantages and we discourage this practice. Embedding tissues in plastic allows for crisp thin sections for research application (Anderson and Shearer, 1986).

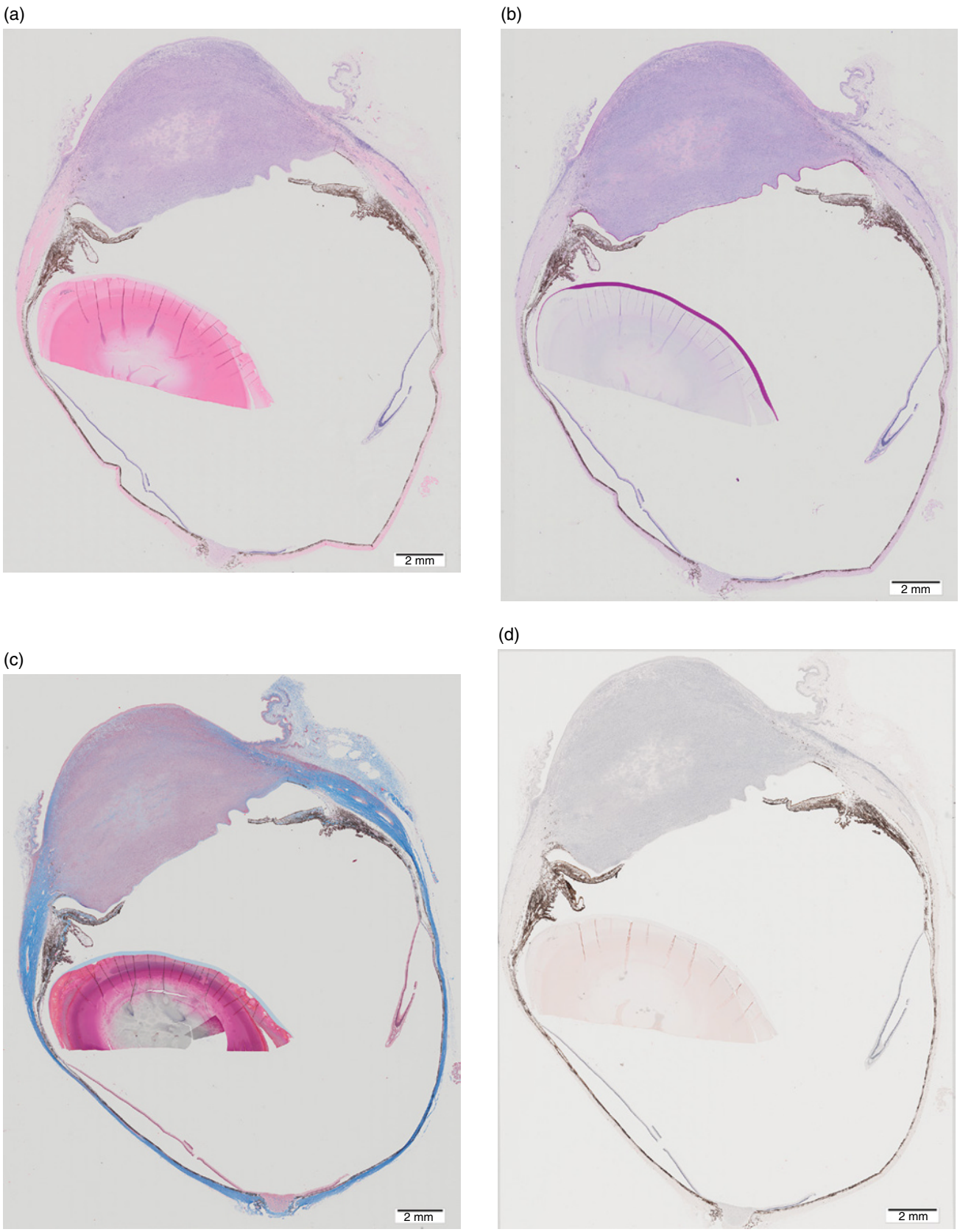


Figure 1.24 (a–d) These are a routine histologic sections of the same formalin-fixed globe as depicted in Figure 1.19. They have been stained routinely with (a) hematoxylin and eosin; (b) periodic acid-Schiff reagent to accentuate Descemet's membrane, lens capsule, and other basement membranes; and (c) Masson's trichrome stain to stain collagen (blue). (d) Congo red stain that identifies amyloid fibrils. This is one of several other histochemical stains that are occasionally used to identify specific exudates and cellular materials within ocular tissues.

Fine needle aspirates or scrapings are usually submitted as smears on glass slides and are usually stained with automated systems that use routine hematology stains. Aspirates from aqueous, vitreous, or sub-retinal fluid are usually only sparsely cellular and best placed in EDTA (purple topped) tube and submitted for cytospin concentration prior to evaluation.

The routine stain for light microscopic exam of ocular tissues is hematoxylin and eosin. Additional stains that are particularly useful are periodic acid-Schiff reagent (PAS) and Masson's trichrome stain (Figure 1.24 a–c). The PAS accentuates basement membranes like Descemet's membrane and lens capsule, and also greatly increases the sensitivity of detecting intraocular fungi and protozoa. The trichrome stain allows better characterization of scarring and fibroblastic metaplasia, and is a particularly photogenic stain. Occasionally, additional special stains with specific but less frequent applications are required including Congo red stain (for amyloid) (Figure 1.24 d) and Luxol fast blue (for myelin) (Table 1.2).

Immunohistochemistry is commonly used as an adjunct to routine histopathology in identifying primitive neoplasms, enhancing detection of some infectious agents, and in characterizing the lineage of various proliferative lesions including retrocorneal and subretinal membranes. Only a few of these are suitable for use with fixed tissues, and none of these has absolute specificity and sensitivity. A myriad of markers is available (the list grows almost daily), and the proper selection and interpretation of results may be complex. Commonly used immunohistochemical stains are summarized in Table 1.3, the majority of which are used when trying to identify anaplastic malignancies for which precise identification has important prognostic or therapeutic implications.

Electron microscopy

Transmission and scanning electron microscopy are rarely used in a diagnostic setting. Nonetheless, they are still useful and sometimes vital in some research applications. They can identify morphological change at the cellular and subcellular levels and are particularly useful in identifying subtle degenerative changes that may not be evident by light microscopy. Specimens from the eye and adnexa destined for electron microscopy have specific fixation, embedding, sectioning, and staining requirements. Small biopsies of eyelid skin, conjunctiva, sclera, or cornea may be adequately fixed by immersion in glutaraldehyde. Although samples of the intraocular tissues from globes that were fixed by routine immersion in 10% formalin can be processed for electron microscopy, membranes are not preserved and the quality of the results is usually marginal. In a research setting, fixation via perfusion with glutaraldehyde or similar fixative provides excellent fixation. Enucleated globes may be immediately immersed in chilled fixative for several minutes; the globe is then opened with a circumferential pars plana incision to allow direct exposure of the intraocular tissues to glutaraldehyde-based fixatives and buffers. Subsequent fixation with osmium is a frequent component of protocols to study photoreceptor outer segments and retinal pigment epithelium (Figure 1.6e and f).

Table 1.3 Some of the more common immunohistochemical labels that may be used to aid the pathologist in diagnosis of specific ophthalmic neoplasms.

Application	Antibody
Suspected amelanotic melanoma	PNL2/melan A, TRP-1, TRP-2 combinations
Suspected astrocytoma or other glial tumor	Glial fibrillary acid protein (GFAP)
Round cell tumor suspicious for lymphoma	CD3 (T cells), CD 20, or CD79a (B-cells)
Suspected metastatic carcinoma	Pancytokeratin
Suspected histiocytic tumor	CD 18, MHC II
Suspected hemangiosarcoma	CD 31, factor VIII related antigen
Suspected primary neuroectodermal neoplasia retinoblastoma (medulloepithelioma will be negative for these)	Opsin, S-antigen, and interphotoreceptor binding protein
Suspected iridociliary epithelial carcinoma (versus metastatic carcinoma)	Vimentin, neurospecific enolase
Anterior uveal schwannoma (of blue-eyed dogs)	GFAP, desmin, and vimentin
fibrous metaplasia or neoplasia of lens epithelium (in phacoclastic uveitis or primary ocular sarcoma, ciliary adenocarcinomas)	Pancytokeratin (lens epithelium), neurospecific enolase (ciliary adenocarcinomas) leucocyte markers (rule out lymphosarcoma and histiocytic neoplasms)

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2

General pathology of the eye

General pathology is the study of how living tissue responds to injury. It is built on a foundation of cell biology, physiology, and anatomy that defines normal structure and function. General pathology studies the causes and consequences of tissue injury that alter normal homeostasis, and the mechanisms by which that homeostasis is restored.

It is not our intention here to present the huge amount of information about the fundamental causes and manifestations of disease contained in innumerable textbooks devoted to general pathology. Any attempt for us to do so in a textbook intended to explain the histologic basis for clinical ocular disease would be unnecessary and woefully inadequate for any pathologist reading this chapter. For clinicians, such detailed review would be seen as irrelevant and an excuse to quickly move on. We have, therefore, tried to walk the fine line between being excessively simplistic and being simply excessive. We have tried to explain the most important general pathology phenomena using examples relevant today in clinical ophthalmology or ophthalmic diagnostic pathology. These phenomena range from abnormalities in growth to mechanisms of tissue injury and the ways in which ocular tissues attempt to reestablish normal homeostasis following such injury.

The general pathology of the globe includes all of the same phenomena as seen in other tissues, but there are a few aspects of ocular anatomy and physiology that have a powerful effect on how these phenomena are manifested within the globe. These "peculiarities" are fundamental to the understanding of ocular pathology.

1) The globe relies on an extremely precise set of anatomic interrelationships to maintain its function. Even minor changes in anatomic relationships caused by edema, leukocytic infiltration, or fibrosis that might have negligible effects on most other body systems can have a powerful effect on ocular function.
2) The globe is a closed sphere in which events in one part of the globe are inevitably shared with all parts of the globe via diffusion of mediators or shared vascular or neural networks. Although the fact that the globe is a closed and isolated sphere which often protects it from injury from circulating infectious agents or toxins, it also means that exudates, inflammatory mediators, and hemorrhage are not easily removed from the globe, thereby prolonging and therefore may intensify their damaging effects.
3) Although the globe is well protected by eyelids and a bony orbit, it seems ill-equipped to deal with injury that manages to evade these structural protections. It is relatively slow to mount a protective cellular inflammatory reaction because there are virtually no resident leukocytes, and its ability to regenerate following injury is very limited when contrasted to most other tissues.
4) The eye has almost no redundancy and the concept of "insignificant" tissue injury so familiar in lungs, liver, kidneys, or intestine essentially does not apply to the globe. It requires a substantial readjustment by pathologists accustomed to essentially ignoring minor degrees of fibrosis or inflammation in other tissues because the same phenomena are almost always functionally significant within the globe.

We have organized this chapter by looking first at more or less successful adaptation to sublethal injury (often manifested as alterations in tissue volume like hypoplasia, atrophy, and hyperplasia), unsuccessful responses manifested as degeneration and necrosis, and finally at the phenomena of inflammation and repair that represent attempts to reestablish homeostasis. There are admittedly some phenomena that do not easily fit into just one of these categories (aplasia or neoplasia, for example), so we have discussed them where they seem to make the most sense.

Adaptations characterized by changes in cell size, number, or appearance

Aplasia and hypoplasia are not really adaptive responses, but are included here for completeness. Aplasia is an extremely rare phenomenon defined as a

Histologic Basis of Ocular Disease in Animals, First Edition. Bruce Grahn, Robert Peiffer and Brian Wilcock.

complete lack of development of a tissue. Almost all examples of so-called aplasia are more correctly classified as profound hypoplasia in which the tissue is so lacking that it has eluded gross and sometimes even casual microscopic detection. Most examples of complete ocular aplasia diagnosed clinically are eventually identified as profound hypoplasia after careful microscopic evaluation of orbital content. True aplasia within the globe is usually focal or segmental, and the only common example in the context of ocular disease is coloboma (Figure 2.0a–d). The eyelids, iris, choroid, and peripapillary optic nerve are common ocular structures affected by colobomas in which there is a focal defect in tissue continuity. They differ in pathogenesis and not all examples represent true aplasias. Choroidal and scleral colobomas, for example, usually represent incomplete closure of the edges of the optic fissure rather than actual failure of tissue development. Lenticular colobomas are actually a secondary change related to segmental ciliary body and zonular aplasia. Most ocular colobomas do not significantly affect ocular function and are incidental findings in globes submitted for other disorders.

Hypoplasia is defined as congenital underdevelopment of an organ or tissue due to inadequate production of stimulatory cytokines, lack of specific cellular receptors for those cytokines, or damage to progenitor cells. A common example in the eye occurs in so-called "subalbinotic" animals in which the uvea (including tapetum) is hypoplastic because of deficient-inductive signaling by pigmented uveal melanocytes of neural crest origin (Figure 2.1a–e). The exact nature of the interdependence of uveal pigmentation with normal uveal development remains unknown. Other examples of hypoplasia within the globe include profound iris hypoplasia usually seen as part of anterior segment dysgenesis, and premature cessation of the remodeling of the filtration angle with a predisposition to glaucoma (Figure 2.1f).

Dysplasia is disorganized tissue growth in response to previous injury, especially if the regeneration process is occurring in the presence of ongoing injury or in an abnormal environment. The term is used in three different contexts. In the context of embryogenesis, it describes a developmental anomaly characterized by tissue disorganization and presumably reflects a response to some kind of in-utero or neonatal injury that did not result in destruction of all of the progenitor cells (Figure 2.2a–e). There are many examples of developmental dysplasia in veterinary ophthalmology; most of them are inherited genetic defects in dogs. Examples include photoreceptor dysplasia, retinal pigment epithelial dysplasia, and a few examples of idiopathic retinal dysplasia (Figure 2.2a–d). However, retinal dysplasia also occurs as a manifestation of defective wound healing following injury to the developing retina by a virus, toxin, or nutritional deficiency. Finally, dysplasia is also used as a purely descriptive word when describing disorganized wound healing with cellular jumbling, and more specifically in the context of preneoplastic change where repeated tissue injury and imperfect wound healing lead to dysplasia as a prelude to malignant transformation (Figure 2.2e). In the context of ocular pathology, this last phenomena is most commonly encountered in the stepwise transition from corneal or conjunctival epithelial hyperplasia to dysplasia and eventual squamous cell carcinoma in response to chronic solar injury.

Dystrophy is a somewhat ambiguous term usually used to indicate an inherited metabolic abnormality of dysfunctional mature cells that manifests later in life. The most common ocular dystrophies include mutations that result in corneal stromal (Figure 2.3), and endothelial disease, the former characterized by the deposition of lipid and the latter by an inability to maintain corneal dehydration.

Atrophy is a reduction of tissue mass after the cells are mature, and is reflected histologically by a decrease in cell size, a decrease in cell number, or a combination of the two. This response is common in ocular tissues and reflects diverse pathogeneses including ischemia, denervation, nutritional deficiencies, advanced age, and prolonged elevations of intraocular pressure. These same pathogeneses, if applied with greater severity or duration, may induce degeneration, necrosis, or apoptosis rather than just atrophy. The term is often used with considerable imprecision, especially in the context of macroscopic changes. The optic nerve "atrophy" of glaucoma, for example, is actually the result of axonal necrosis. The same is true for various retinal "atrophies" that usually involve progressive degeneration of photoreceptors and eventual necrosis of the associated neurons within the outer nuclear layer.

Common examples of atrophy include senile iris atrophy (Figure 2.4a and b), photoreceptor atrophy subsequent to neurosensory retinal detachment, and at least some of the manifestations of uveal, retinal, and optic nerve atrophy associated with glaucoma (Figure 2.4c–f). Histologic evidence may be subtle because it is difficult to accurately detect subtle decreases in cell size associated with malnutrition or marginal ischemia.

Hypertrophy is an increase in cell size and, therefore, overall tissue mass because of an increase in intracellular organelles in response to increased workload or some other stimulus that does not trigger cellular replication. Hypertrophy is most commonly seen in tissues with limited or no mitotic capability (so that hyperplasia is not an option). Within the globe, it is a relatively uncommon reaction, exemplified by hypertrophy of the retinal pigment epithelium (Figure 2.5a and b), which occurs within hours following retinal detachment.

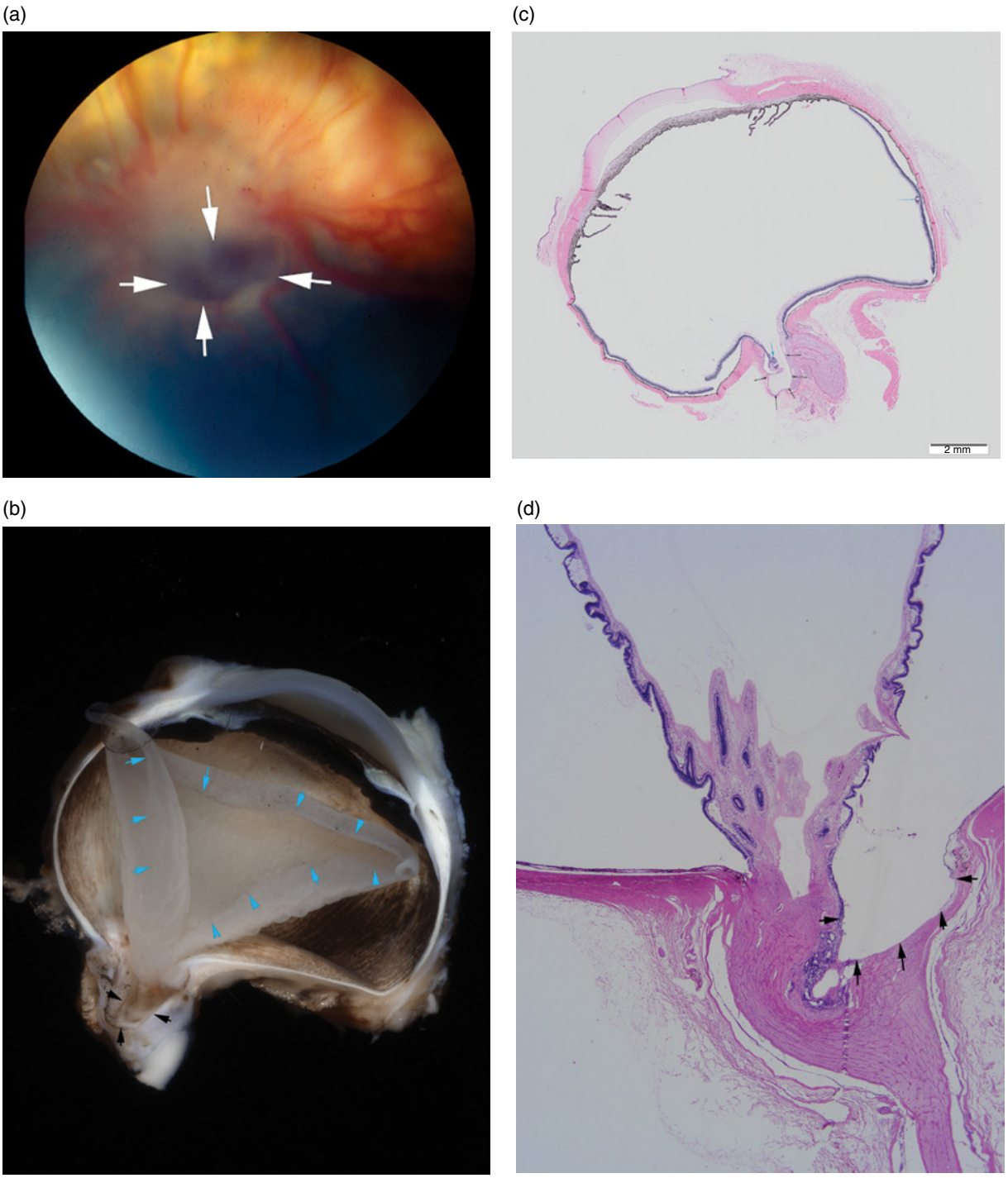


Figure 2.0 (a) A fundic photograph of a collie dog with collie eye anomaly with focal temporal choroidal hypoplasia and a typical peripapillary coloboma (white arrows). (b) This is a gross section of an Australian shepherd dog with optic nerve coloboma (black arrows), retinal nonattachment with folded edges (blue arrows), and generalized uveal hypoplasia. (c) Histologic section of the globe of an Australian shepherd dog with a peripapillary optic nerve coloboma (black arrows). This is an off-center section to explain the lack of lens and pupil. Two focal retinal rosettes are present (blue arrows), barely visible with this low magnification (subgross magnification, hematoxylin and eosin stain). (d) A higher power histologic section of an optic nerve coloboma and staphyloma of the globe from a collie dog (black arrows) (hematoxylin and eosin stain, 4× magnification).

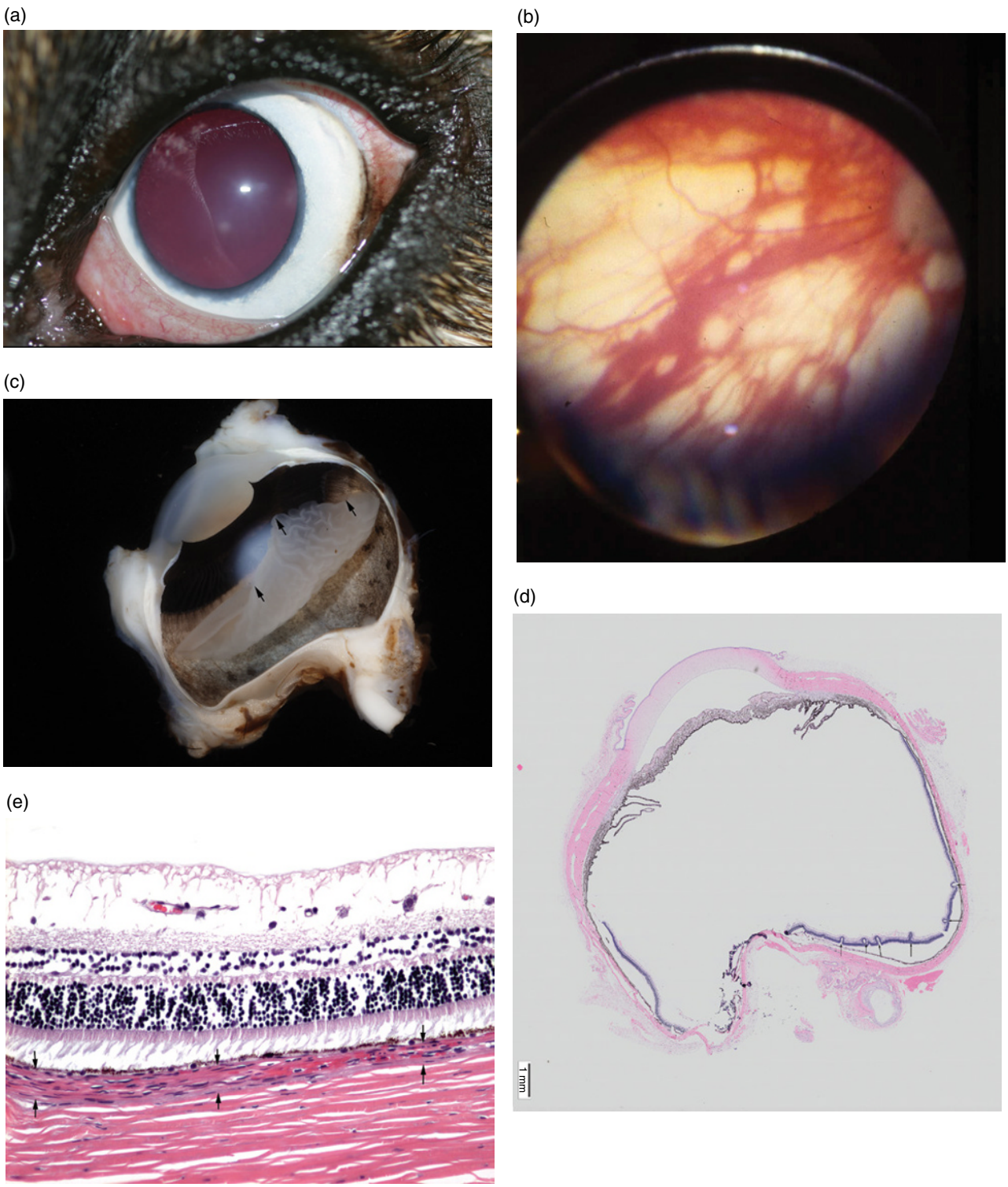


Figure 2.1 (a) The light uveal pigmentation noted here in the blue iris of this Siberian husky dog is predictive of histologic hypoplasia of choroid and tapetum ("subalbinism"). (b) This is a fundic photograph of the husky in (a). Note the lack of tapetal development and minimal choroidal pigment, which allows the examiner to visualize all the choroidal blood vessels and the underlying sclera. Note also the patches of more extensive hypoplasia where minimal choroidal blood vessels are present. (c) A bisected eye of an Australian shepherd dog with uveal hypoplasia and a nonattached retina (black arrows) with multiple retinal folds. Note the lack of choroidal pigment and the paucity of choroidal blood vessels. (d) Histologic examination of the globe in (c) reveals retinal separation, retinal folds (black arrows), and generalized uveal hypoplasia (hematoxylin and eosin stain, subgross magnification). (e) Choroidal hypoplasia is present in this histologic section. The choroid is very poorly pigmented and very thin (black arrows) (hematoxylin and eosin stain, 10× magnification). (f) Trabecular hypoplasia in a 10-month-old dachshund. What should by now be a fenestrated trabecular meshwork has remained as primitive iris stroma (red arrows), which insert near the end of Descemet's membrane (black arrow), reflecting an arrest in late neonatal or early postnatal remodeling (hematoxylin and eosin stain, 10× magnification).

(f)

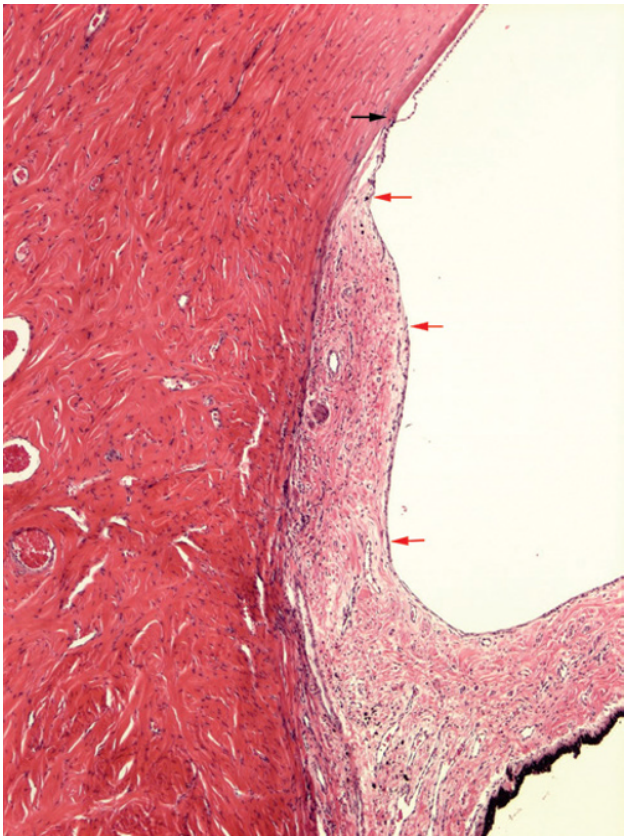


Figure 2.1 (*Continued*)

Hyperplasia is an increase in tissue mass resulting from an increase in cell number. There may be concurrent hypertrophy. Hyperplasia is traditionally divided into physiologic and pathologic. Physiologic hyperplasia is most obvious in tissues like uterus and mammary gland. No obvious examples occur within the globe, although one could argue that conjunctival lymphofollicular hyperplasia is a physiologic adaptation to minor alterations in conjunctival microbial flora. Pathologic hyperplasia is a manifestation of sublethal injury triggering an overzealous regenerative response that is usually transient and fully reversible once the stimulus is removed. Common examples within the globe include hyperplasia of lens epithelium in cataractogenesis, hyperplasia of retinal pigment epithelium (RPE) in the face of chronic retinal detachment (occurring later than hypertrophy) (Figure 2.5c), and corneal epithelial hyperplasia occurring in response to mild superficial irritation as in corneal desiccation or entropion (Figure 2.5d).

Metaplasia is an adaptation response to stress in which a mature differentiated tissue transforms into another mature cell type, ordinarily of the same germ line (i.e. epithelial to epithelial, mesenchymal to mesenchymal) (Figure 2.6a and b). Metaplasia is relatively more common in the globe than in any other tissue. The most common and important examples are fibrous metaplasia of the cornea, lens, and RPE that seems to occur in response to chronic low-grade injury.

Neoplasia

Neoplasia is the most dramatic of the growth disorders. It can be viewed as an extreme version of adaptation to chronic insult, representing an extreme progression of hyperplasia, dysplasia, or metaplasia in response to chronic tissue injury. Indeed, many neoplasms evolve in very deliberate stepwise fashion from these preneoplastic proliferations. Any attempt at a thorough discussion of neoplasia here is doomed to failure because the topic is so complex. Nonetheless, neoplasia is important to veterinary ophthalmologists and it is especially important to veterinary pathologists because it represents such a large portion of their ocular caseload. In keeping with the title of this book, here we will present only those fundamental concepts of the evolution, nomenclature, and prognostication of neoplasia that will enable clinicians to make correct clinical judgments about ocular neoplasia. That includes an understanding of the fallible criteria used by pathologists to classify and prognosticate those neoplasms.

At its most fundamental basis, neoplasia is dysregulated repair following repeated injury. The accumulation of those injuries results in heritable mutations in cellular DNA resulting in abnormalities of cellular form and cellular function that give those transformed cells a selective growth and survival advantage over their normal predecessors. When the neoplastic transformation gives rise to cells that are similar to both appearance and behavior from the parent cells, we refer to the neoplasm as benign; these tumors resemble the tissue of origin and typically grow in a slow, expansile fashion, and are not capable of local invasion or of survival in distant locations (i.e. metastasis) (Figure 2.7a–c).

As neoplasms accumulate more and more mutations, some of those mutations impart a selective survival advantage and so those clones proliferate more successfully and therefore become increasingly predominate within the tumor population. This notion of genetic pleomorphism, manifested as increasing clonal diversification within a tumor population, is central to the concept of malignancy. There is no sharp line at which a benign tumor suddenly becomes malignant (malignant means "life-threatening"). Malignant progression takes months or years and is characterized by decreasing responsiveness to the normal cytokine stop signals that control the growth of normal cells. It is also characterized by loss of cellular cohesion, increased expression of proteolytic enzymes to destroy tissue barriers and therefore allow local invasion, and by an increased ability by these

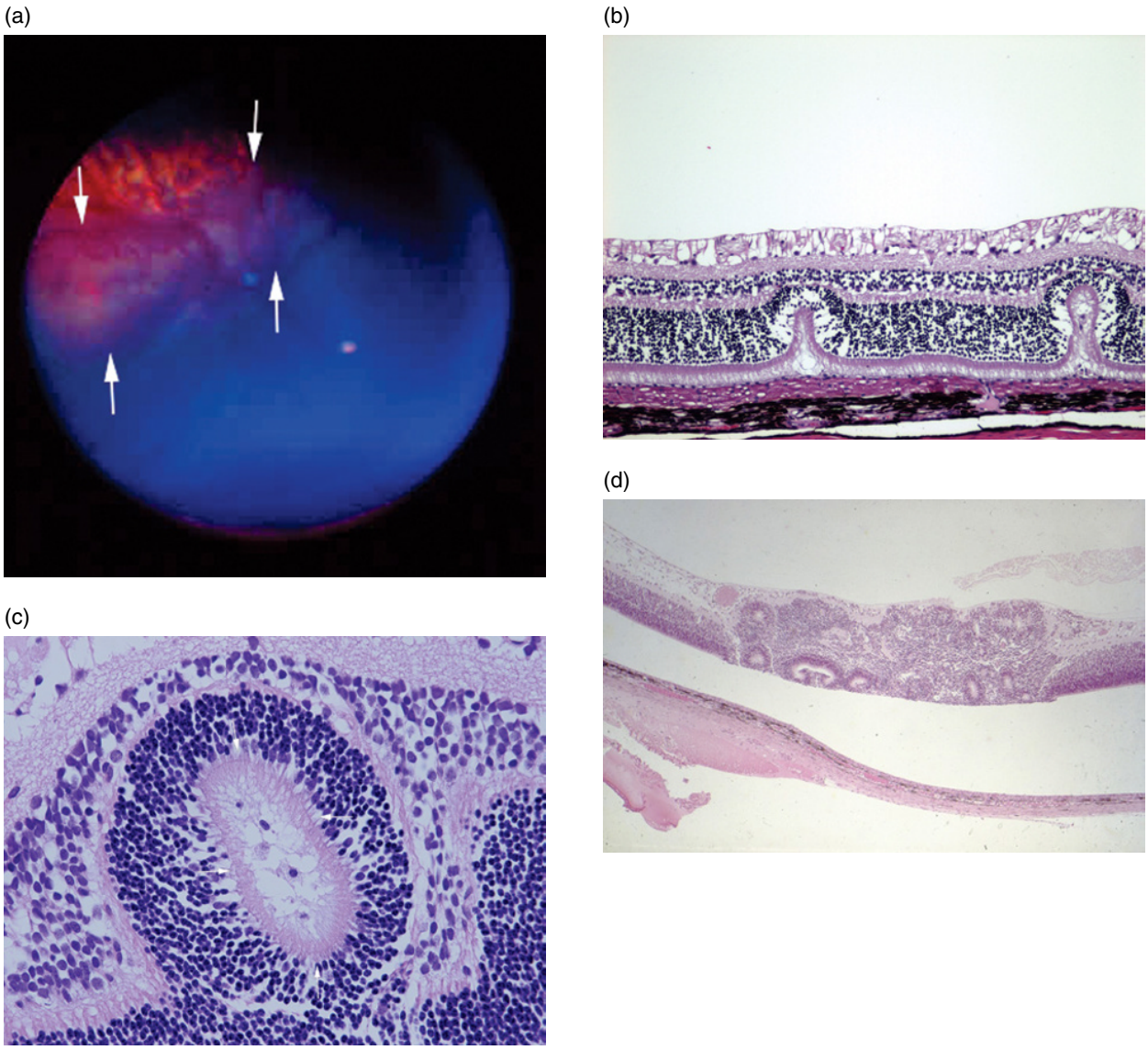


Figure 2.2 (a) Geographic retinal dysplasia usually can be detected during clinical examination. The retina often seems thicker than normal and has gray to green serpentine tracts or rolls within the retina as outlined by the white arrows. (b) Some focal retinal dysplasia diagnosed clinically are, in fact, just retinal folds as a reflection of the transient or permanent imbalance between the amount of retina and the size of the scleral shell to accommodate that retina in young animals as noted in this histologic section. A diagnosis of true retinal dysplasia requires the observation of jumbling of the retinal layers with rosette formation (hematoxylin and eosin stain, 10× magnification). (c) A higher magnification of a typical retinal rosette. Note the ring of outer nuclear cells surrounding an intact external limiting membrane (white arrows) encasing a lumen containing slightly degenerate photoreceptors (hematoxylin and eosin stain, 60× magnification). (d) Geographic retinal dysplasia is a relatively common ophthalmologic diagnosis and this lower power histologic section reveals such at the edge of the optic disc. In contrast to more common single retinal rosettes, here there is true jumbling of the retinal layers and multiple rosettes (hematoxylin and eosin stain, 4× magnification). (e) Corneal epithelial dysplasia as a prelude to squamous cell carcinoma in a pug with underlying chronic corneal desiccation and corneal cutaneous metaplasia. There is hyperchromasia, jumbling, and defective maturation, but as yet no invasion across the basement membrane to signal transition to genuine squamous cell carcinoma (hematoxylin and eosin stain, 40× magnification).

(e)

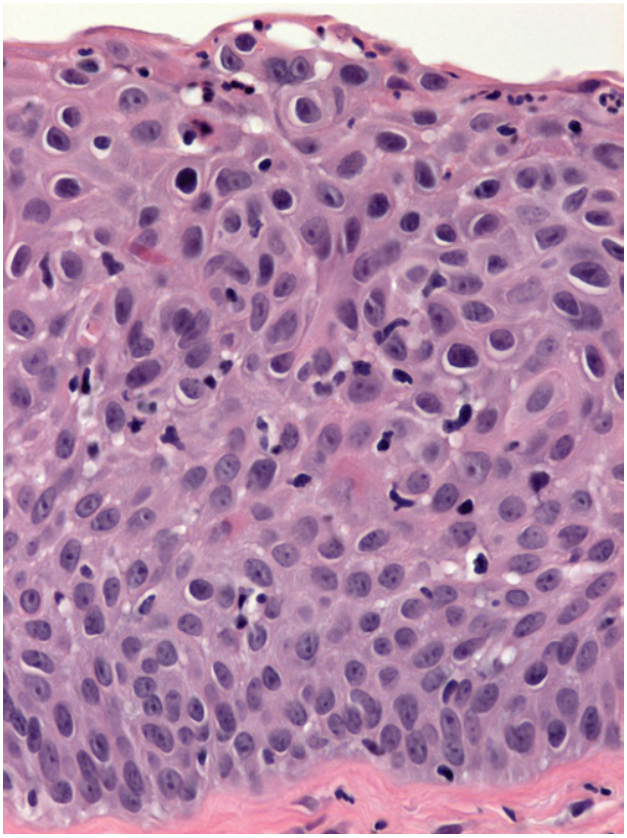


Figure 2.2 (*Continued*)

malignant cells to survive and prosper in distant organs. It is worth mentioning here that there is a difference between tumor embolism and true metastasis. In the globe, tumor cells will easily exfoliate into the aqueous tumor and leave the globe via the normal aqueous outflow pathways. That by no means guarantees, however, that those cells will survive in distant locations. It is only when those cells have become truly primitive ("anaplastic") that they will successfully colonize distant locations and create clinically-significant metastatic foci. It is important to understand this when trying to predict the significance, for example, of tumor cells seen within the trabecular meshwork which does not necessarily foretell the development of clinically detectable metastatic disease.

Malignant cells continue to mutate and create ever-increasing atypia in both cellular appearance and function (Figure 2.7d). This atypia is manifested by increasingly obvious variation in cellular and nuclear characteristics (excessive variation in cellular and nuclear size, hyperchromasia, and irregular nuclear shape), by the production of unexpected secretory products as random portions of the genome are abnormally expressed

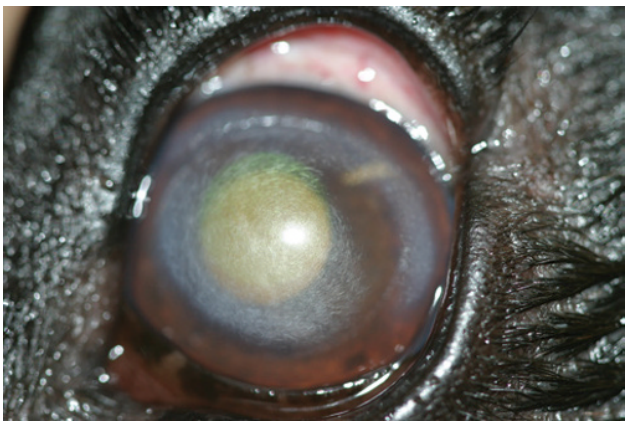


Figure 2.3 Corneal lipid dystrophy manifests with white to gray spicules that are arranged in a ring in the superficial corneal stroma. Corneal dystrophy is a bilateral symmetrical inherited corneal disorder that is most common in dogs. It is seldom available for histologic examination.

The stepwise progression from hyperplasia to dysplasia and eventual neoplasia is illustrated in two of the most common ocular tumors: sunlight-induced conjunctival squamous cell carcinoma in various species and diffuse iris melanoma of cats.

With squamous cell carcinoma, the epithelium responds to persistent actinic injury by ever-increasing hyperplasia and cytologic atypia ("dysplasia"). This atypia progresses through several well-recognized stages of squamous plaque, squamous papilloma, carcinoma in situ, and finally to truly invasive squamous cell carcinoma. This is a continuum and these divisions are entirely arbitrary. The only absolutely convincing indicator of malignant transition is invasion by atypical squamous cells across the basement membrane and into the underlying dermis or lamina propria.

With diffuse iris melanoma, the tumor begins as hyperplasia and hypertrophy of melanocytes along the anterior border layer of the iris. Over time (often many years), the cells increase in number and in cytologic atypia. The cells eventually replace the iris stroma with a population of cells so different from normal melanocytes that they might not even be recognized as melanocytes by those not familiar with this uniquely feline tumor.

or suppressed, and by cellular immortality because the cells no longer respond to the normal signals that trigger apoptosis.

Atypia in cytologic character and/or in tissue architecture form the basis for the assumption that a population of cells is neoplastic. The assumption is also

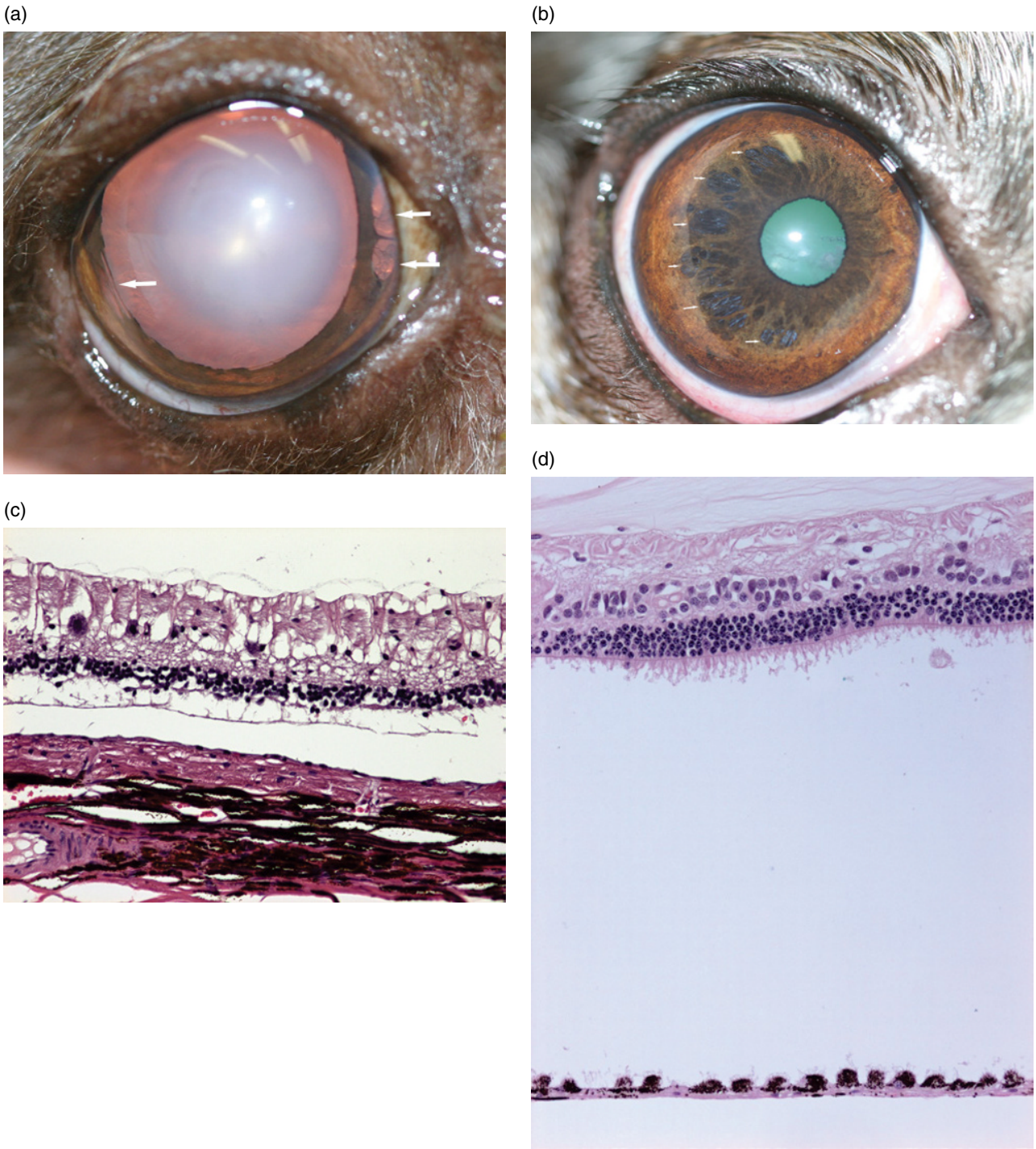
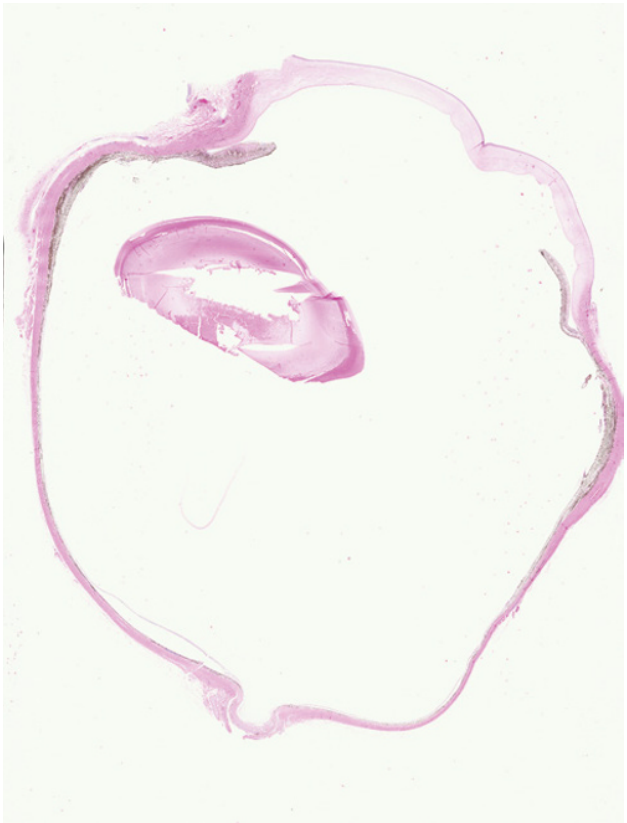


Figure 2.4 (a) The clinical manifestations of iridal atrophy can be extensive and easy to recognize or more subtle such as noted here. Note the cataractous lens is visible through a full thickness peripheral nasal atrophic iridal hole (white arrows). Iridal colobomas is an important differential diagnoses for these focal areas of atrophy; the former are congenital and static and present since birth, while iridal atrophy in the pupillary constrictor region is acquired and progressive and eventually induces pupillary dilatation such as noted in this globe (white arrow). (b) Iris atrophy usually affects the iridal surface initially as noted in this dog where the iris stroma is beginning to thin and cavitates (white arrows). (c) Advanced atrophy of the photoreceptors and outer nuclear layer in a cat with presumed nutritional retinal atrophy (hematoxylin and eosin stain, 20× magnification). (d) Photoreceptor atrophy has developed in this instance as a result of ischemic injury secondary to retinal detachment. In this instance, mostly just the outer segments of the photoreceptors are affected, with most of the inner segments and their nuclei (i.e. the outer nuclear layer) remaining histologically normal for the moment. Note the hypertrophy of the RPE, which is a rapid response to retinal detachment of any pathogenesis (hematoxylin and eosin stain, 20× magnification). (e) Chronic canine glaucoma has caused extensive retinal and optic nerve atrophy as well as atrophy of the ciliary processes and marked scleral thinning (hematoxylin and eosin stain, subgross magnification). (f) Higher magnification of classical glaucomatous retinal atrophy which typically results in disappearance of the nerve fiber layer, ganglion cells, and most of the inner nuclear layer, while leaving the most of the outer nuclear layer and photoreceptors unaffected. In this particular example there is substantial gliosis within the inner half of this retina (hematoxylin and eosin stain, 20× magnification).

(e)



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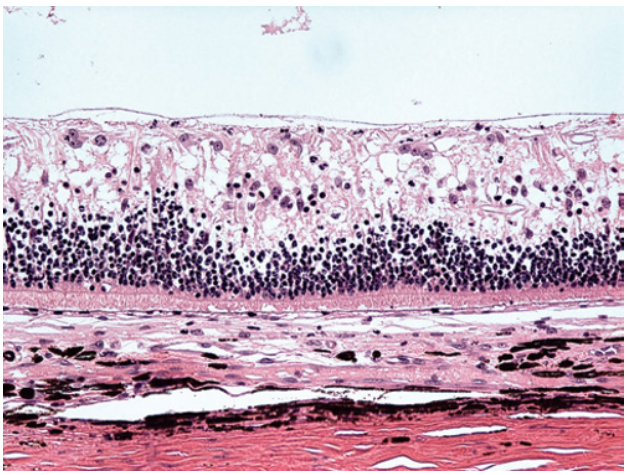


Figure 2.4 (*Continued*)

made that increasing degrees of structural atypia correlates with an increasing risk of behavioral atypia like invasion and metastasis. Some mutations, however, result only in functional atypia, and those mutations are the basis for excessive secretion of normal cell products including hormones, cytokines, or matrix material like osteoid. Sometimes, however, the mutations are so profound that the result is the unexpected production of products not normally associated with that cell type (resulting in "paraneoplastic syndromes" like production of parathyroid hormone by adenocarcinomas arising from the anal sac). We are not aware of any paraneoplastic syndromes reported for any of the primary ocular tumors.

These fundamental concepts of malignant transformation form the basis for the histologic classification and prognostication of neoplasms as outlined below. They also explain frequent failures by malignant neoplasms to adhere to the rules of behavior that we foolishly attempt to impose upon them. Malignant neoplasms, at their very core, are behavioral "wild cards" that have no obligation to behave in a predictable fashion. The more primitive ("anaplastic") the tumor, the less likely it is to follow any of the rules of behavior that we attempt to impose in a desperate effort to create order from chaos. It also means that anaplastic tumors have no obligation to follow the "usual" behavior for tumors of similar origin that are less anaplastic and therefore are more predictable.

These very basic concepts of neoplasia have very pragmatic clinical correlations affecting everything from the nomenclature of neoplasms to their clinical manifestations and response to therapy.

Nomenclature

It is widely assumed that neoplasms are named based on the tissue of origin, but, in truth, they are named based upon the tissue that they most closely resemble. With very malignant neoplasms, that resemblance may not indicate the tissue of origin but simply the histologic outcome of the many genetic mutations that influence the current appearance of the cells. Epithelial tumors, for example, frequently undergo squamous metaplasia, however, that does not mean they originated from squamous epithelium. Optic nerve meningiomas often make bone or cartilage, yet have an uncanny resemblance to squamous epithelial cells in other areas of the same tumor. Posttraumatic sarcomas in cats, thought to originate from lens epithelium, have a histologic appearance identical to a high-grade fibrosarcoma.

Benign epithelial tumors are given the suffix "adenoma." Those judged to have a substantial risk of malignant behavior are given the suffix "carcinoma." If they are thought to originate from glands, the suffix is amended to "adenocarcinoma." In the context of ocular disease, we

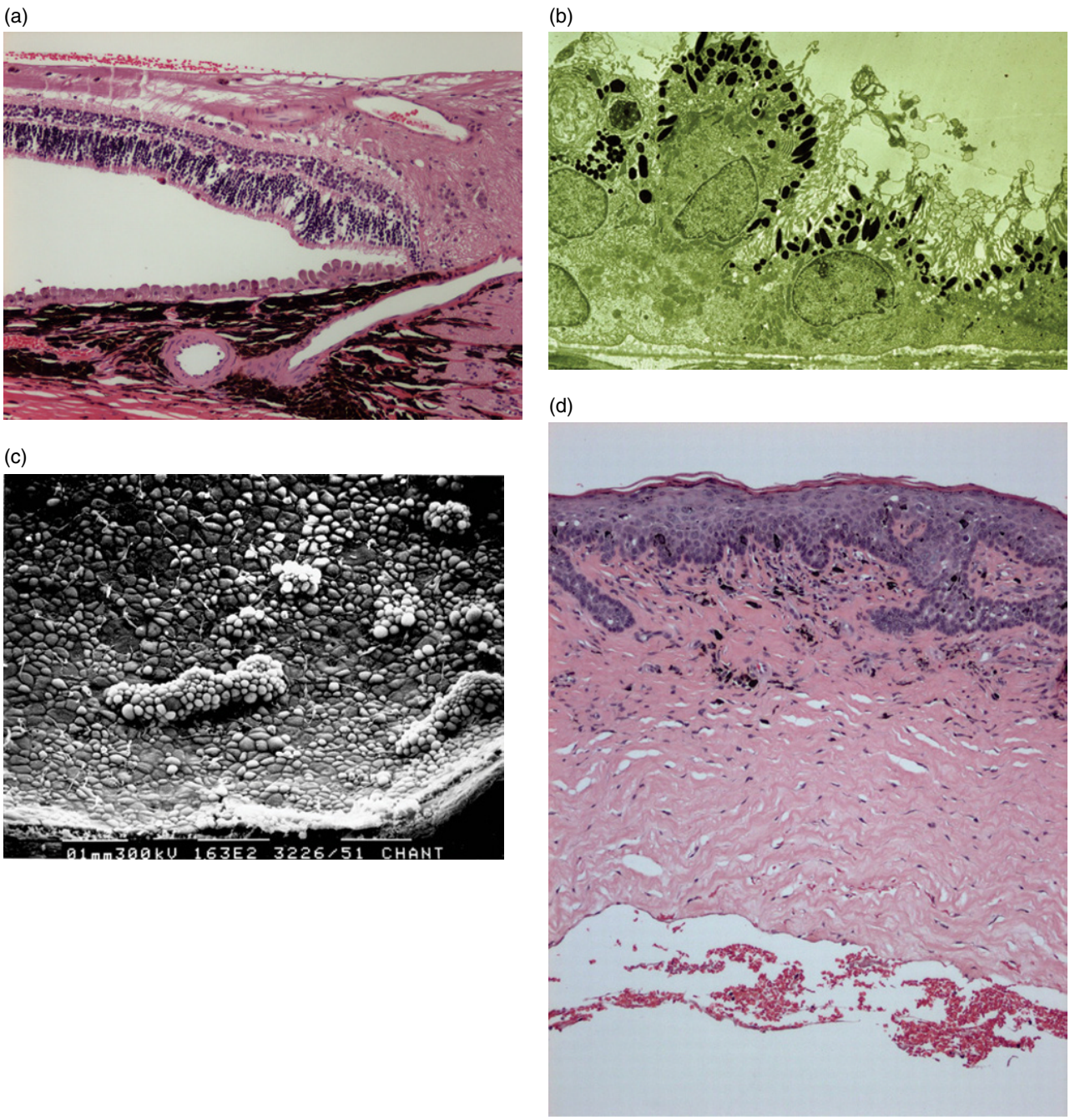
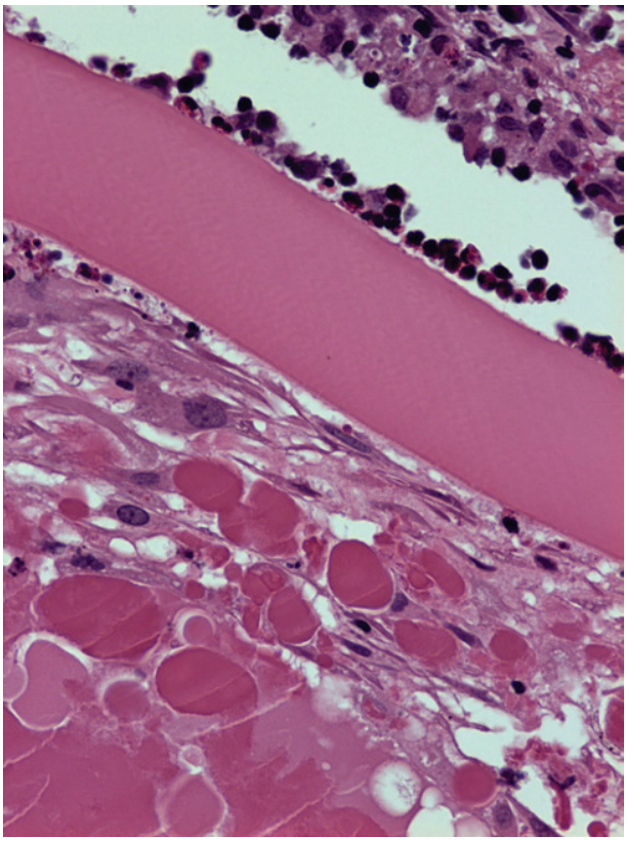


Figure 2.5 (a) Profound hypertrophy of the RPE following retinal detachment. In this instance there is subtle degeneration of the photoreceptors, but that occurs more slowly than the RPE hypertrophy and, therefore, may not be seen in all cases (hematoxylin and eosin stain, 40× magnification). (b) Retinal pigment epithelium hypertrophy visualized in a thin epon-embedded section (60× magnification). (c) Retinal pigment epithelial hypertrophy is often accompanied by hyperplasia as readily seen in this scanning electron microscopy image. (d) Corneal epithelial hyperplasia is a routine response to chronic low-grade irritation, in this case chronic desiccation. Depending on the cause and duration, there may be concurrent metaplastic changes including keratinization and pigmentation as seen here (hematoxylin and eosin stain, 20× magnification).

(a)



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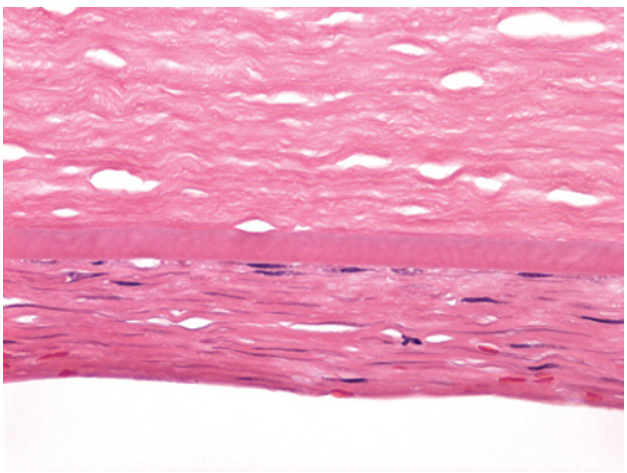

Figure 2.6 (a) Fibroblast-like metaplasia of lens epithelium as part of cortical cataract in a dog (hematoxylin and eosin stain, 40× magnification). (b) Fibroblast-like metaplasia of corneal endothelium secondary to injury from anterior lens luxation in a 10-year-old Pomeranian dog. The layers of collagen separating the slender fibroblast-like corneal endothelial cells are basement membrane material similar to Descemet's membrane (hematoxylin and eosin stain, 40× magnification).

thus have ciliary body adenomas and also the less common ciliary body adenocarcinomas. In eyelid, we have benign and purely expansile Meibomian adenomas, and very rarely an invasive Meibomian adenocarcinoma.

Benign mesenchymal tumors are given the suffix "oma"; those judged to be malignant are termed "sarcomas." There are numerous inconsistencies and exceptions to this general scheme, and purely by chance many of these exceptions are relevant to the globe. The logic behind many of these exceptions is elusive. A benign melanocytic tumor is inexplicably known as melanocytoma, perhaps because its malignant counterpart had already usurped the term "melanoma," even though that name would ordinarily implied benign disease. Similarly, a malignant tumor of lymphocytes is known as malignant lymphoma. A malignant tumor of squamous epithelium is squamous cell carcinoma, but its benign counterpart is usually termed squamous papilloma in deference to its papillary, exophytic growth habit.

Although most tumors are identified based on histologic resemblance to some normal tissue, malignancies that are increasingly atypical may require other diagnostic strategies. Chief among these is immunohistochemistry, in which highly conserved cellular antigens are identified by the application of colored antibody markers that attach themselves to those antigens to make them histologically visible. The perfect antigen for such targeting is one that is specific for a single cell type, is highly conserved even when the cell is very primitive, and is unlikely to be expressed as a manifestation of mutation in any other cell type. That "perfect" antigen does not exist, and there is no immunohistochemical marker that has perfect sensitivity and specificity. Like any other test, immunohistochemistry has to be interpreted in the context of all the other test results including histopathology.

Distinguishing benign from malignant

A neoplasm is likely to be classified as benign if it is populated by cells that closely resemble the parent tissue, by cells that lack cytologic criteria of malignancy (see below), and by cells that create a discrete mass with a purely expansile growth habit. In contrast, a tumor is likely to be classified as malignant if the cells no longer resemble the parent tissue, if they have cytologic criteria of malignancy, and especially if they exhibit invasive behavior. In truth, however, the only absolutely reliable criterion that identifies a malignant neoplasm is metastasis. Everything else is just a statistical predictor based

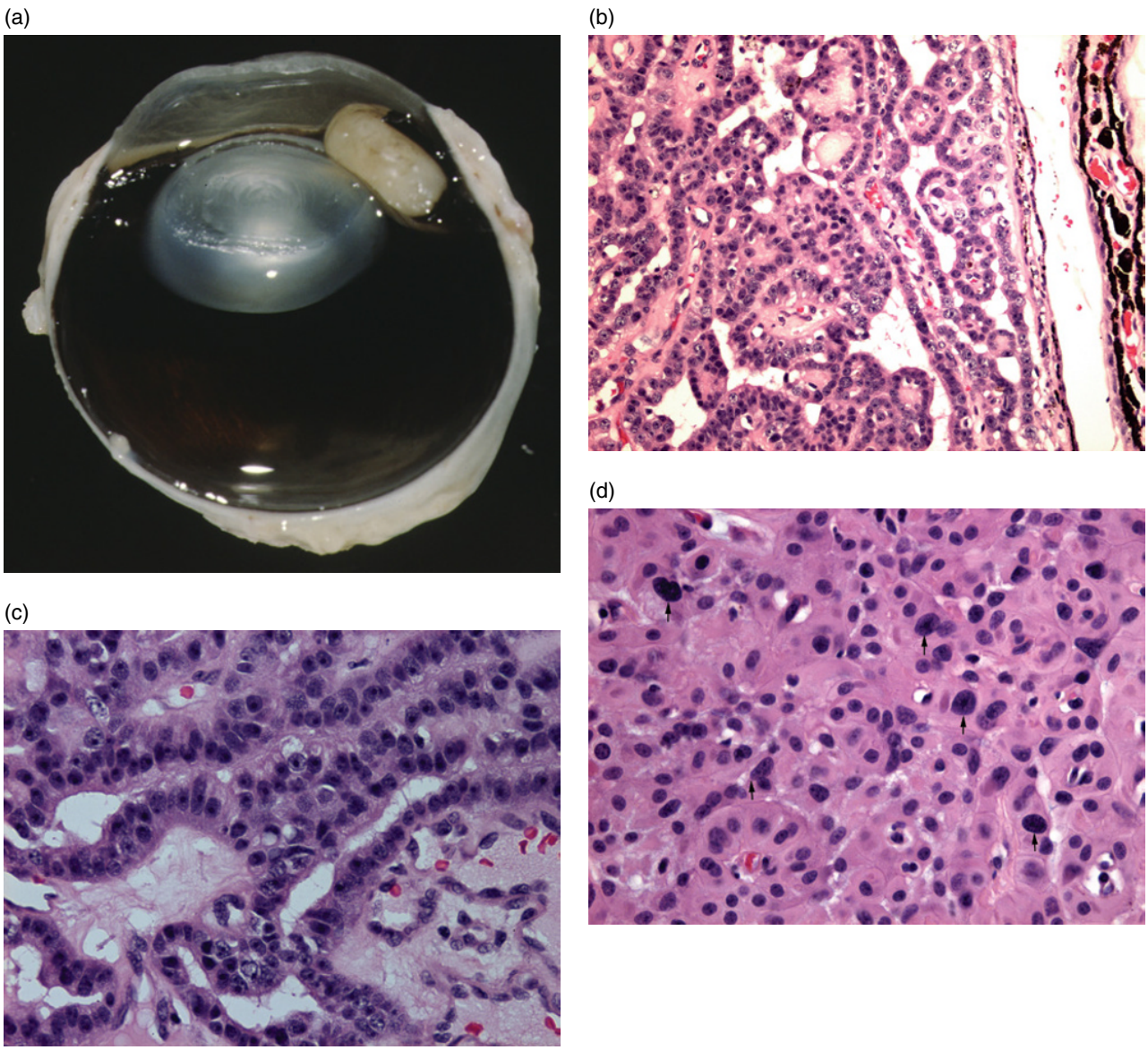

Figure 2.7 (a) A bisected canine globe with a discrete and purely expansile white tumor within the posterior chamber arising from the pars plicata of the ciliary body, histologically confirmed as ciliary adenoma. (b) Canine ciliary adenoma, note the tumor cells retain a very high degree of differentiation and closely resemble the non-pigmented ciliary epithelium covering an adjacent normal ciliary process (hematoxylin and eosin stain, 10× magnification). (c) Higher magnification of classical iridociliary adenoma in which the cells retain a high degree of differentiation resembling normal ciliary epithelium with a hint of differentiation into ciliary processes, and have no cytologic criteria of malignancy (hematoxylin and eosin stain, 40× magnification). (d) This more primitive ciliary tumor has poor architectural maturation with only a hint of maturation into ciliary processes, and primitive cytologic characteristics including hyperchromasia and threefold variation in nuclear size, note the large nuclei identified with black arrows, compared to the remainder of the cells. These cytologic features and local invasion justify a diagnosis of ciliary carcinoma. The metastatic risk remains exceedingly low (hematoxylin and eosin stain, 40× magnification).

on correlation between the histologic/cytologic appearance of tumor cells and the eventual behavior of the tumor documented in retrospective studies.

The traditional cytologic criteria of malignancy, observed both in cytologic preparations or in histologic sections, include nuclear enlargement, an increase in the nuclear: cytoplasmic ratio, hyperchromasia, excessive variation in nuclear and cellular size, and an increase in the number of mitotic figures when contrasted to what one would expect in the parent tissue. None of these is absolutely reliable. There are many examples of neoplasms that consistently behave very badly even when the tumor looks "histologically benign," or tumors that look histologically very malignant and yet behave in a harmless fashion. There is simply no substitute for experience. In the context of ocular neoplasms, virtually all ciliary epithelial tumors are behaviorally benign regardless of the histologic appearance. Diffuse iris melanomas in cats often have extreme cytologic atypia that ordinarily would predict a rapid onset of metastatic disease, but that is just not true. At the opposite end of the spectrum, many malignant lymphomas are populated by cells that have essentially none of the traditional cytologic or histologic criteria of malignancy, and yet, of course, all of these behave as aggressive metastatic malignancies.

Prognostication

Ultimately, every clinician is much more interested in the expected behavior of a neoplasm than in anything to do with its eloquent histologic description or its multisyllabic name. Ultimately, prognostication is based upon statistics derived from retrospective studies of tumors of the same type. In some instances, naming the tumor based on histologic characteristics is all that we need because all tumors with that name have the same behavior. In other instances, however, the histologic name for the tumor imparts only the range of behavior (and sometimes ranging from very benign to extremely malignant). To predict the behavior of an individual tumor, we then look for additional histologic or cytologic features that correlate with specific behaviors like metastatic risk, survival time, or response to therapy. This subclassification designed primarily to predict behavior is known as **tumor grading**. Based primarily on retrospective studies, we try to correlate specific histologic or cytologic features (most commonly, mitotic index and the degree of nuclear variability) with eventual biological behavior. Unfortunately, there are no "universal" predictors and so the relevant grading characteristics must be determined separately for each and every tumor type. It is a very slow process. Because most malignant tumors have lots of histologic and cytologic variations within tumors of the same name, there is the assumption that at least some of those variations will be prognostically relevant. For most tumors, translating those variations into a workable grading scheme remains a distant goal!

Useful grading schemes do not exist for most of the important intraocular tumors. In dogs, the most common tumors like anterior uveal melanocytomas and ciliary body epithelial tumors are almost all benign and so the need for grading is minimal.

Primary conjunctival melanocytic tumors are excellent candidates for grading, using reliable criteria adapted from oral melanomas. The problem to date is a paucity of cases with adequate follow-up information to know whether those grading criteria accurately predict behavior.

In cats, diffuse iris melanomas have proven to be frustrating and their behavior has not yet been linked to any identifiable histologic or cytologic variables like mitotic index or the degree of nuclear gigantism. The same is true for posttraumatic sarcomas, which certainly exhibit a wide range in histologic character and are assumed to metastasize although a definitive study documenting the risk of metastasis or invasion of brain and other organs is lacking.

Unsuccessful adaptation: cellular degeneration, necrosis, and apoptosis

When tissues encounter a "stress" that exceeds the threshold for cellular adaptation, the result is deterioration in cellular structure and function usually culminating in cell death. While degeneration is strictly defined as reversible cell injury, in practical terms, we use it to indicate degenerative cellular changes that are not yet recognizable as having the hallmarks of necrosis or apoptosis. Some of these changes may be reversible but we can rarely make that judgment based on histologic characterization of the lesion in routine sections. There is nothing unique about the causes, histologic manifestations, or sequels to degeneration of ocular or periocular tissues as contrasted to any other tissues. The changes of hydropic cell swelling, injury to cell membranes, and the biochemical events of impending metabolic failure are exactly the same as in other tissues and will not be reviewed here.

The most obvious histologic manifestations of **sublethal cell injury** resulting in cell swelling within the globe occur within corneal epithelium in the early stages of bullous keratopathy, and in lenticular bladder cells which become massively distended with fluid. As stated above, many examples of "degeneration" are really just preludes to necrosis.

Cell death comes in two forms: necrosis and apoptosis. Necrosis is most commonly a manifestation of irreversible cell injury triggered by hypoxia or direct cell membrane injury from toxic chemicals, including those of microbial origin. The essential steps in cell necrosis that determine the "irreversibility" of the injury involve increased membrane permeability that allows an influx of extracellular calcium into the intracellular environment where it (among many other things) triggers activation of various intracellular enzymes resulting in destruction of mitochondrial and other intracellular membranes. Destruction of the mitochondria is the kiss of death. Also important in the irreversibility of cellular injury is the generation of free radicals within the intracellular environment, which further damages cell membranes resulting in a profound drop in intracellular oxidative metabolism and release of destructive lysosomal enzymes.

Cells undergoing necrosis have characteristic nuclear and cytoplasmic changes. The nuclear changes are pyknosis (shrunken, darkly stained, and homogenous), karyorrhexis (nuclear fragmentation), and karyolysis (swelling and pallor caused by dissolution of chromatin). Simultaneously, the cytoplasm becomes excessively homogenous and eosinophilic, followed by rupture of the cellular membrane. Necrotic cells often become detached from neighboring cells because of the dissolution the junctional complexes as a prelude to complete dissolution of the cell membrane.

Descriptively, necrosis has historically been classified into three major types: coagulation necrosis, caseous necrosis, and liquefactive necrosis. The distinction is of limited utility and it is not strongly linked to differences in pathogenesis or significance. In the context of ocular pathology, these traditional descriptive terms have relatively little predictive value in terms of determining what has caused the necrosis. They are included here mostly just so that the terms, which often appear in histopathology reports, will not be mysterious.

Coagulation (coagulative) necrosis is characterized by preservation of cellular and tissue outlines so that everything looks like a dead "ghost" with preservation of cellular and tissue architecture. The dead cells are homogenous and eosinophilic, resistant to immediate liquefaction because whatever caused the necrosis also denatured the intracellular enzymes responsible for postmortem cellular liquefaction (Figure 2.8a and b). This coagulated, hypereosinophilic, ghost-like appearance does not persist indefinitely, and after a few days the tissue will begin to liquefy. This will be accelerated if the necrosis has attracted leukocytes to contribute their proteolytic enzymes. The most common pathogenesis for coagulation necrosis is hypoxic/ischemic injury, but it is by no means the only one.

In contrast, in **caseous necrosis** (as the name implies) the dead tissue becomes granular and friable to resemble cottage cheese. Its histologic counterpart is disruption of cells resulting in the accumulation of granular debris. In most cases, the cellular debris is mixed with live or dead leukocytes. Caseous necrosis is the hallmark of necrosis associated with bacterial infection.

Liquefactive necrosis is exactly as the name would imply: rapid liquefaction of dead tissue (Figure 2.8c and d). In some tissues, particularly in brain, this is the traditional pattern of necrosis regardless of the cause. It may be the initial pattern of necrosis in ocular tissues, but most often is a sequel to coagulation or caseous necrosis as the necrotic foci age and the cells disintegrate.

All of the above traditional patterns of necrosis are encountered within ocular disease. A superficial corneal sequestrum is coagulation necrosis of corneal stroma so that the dead stroma is hypereosinophilic, hyalinized, and acellular. Because leukocytes typically do not infiltrate such lesions, the coagulation may persist for a long time rather than undergoing liquefaction. In contrast, most examples of stromal necrosis rapidly undergo liquefaction to create what is known as keratomalacia. Sometimes this can be blamed on the influx of neutrophils to contribute their degradative proteolytic enzymes, but sometimes keratomalacia happens with no apparent leukocytic infiltration and remains a clinical mystery. Retinal infarction secondary to thromboembolism or obstructive tumor emboli results in transient coagulation necrosis that rapidly gives way to liquefaction and dissolution. Neuronal necrosis in glaucoma or optic nerve trauma also results in transient coagulation necrosis of neurons which become swollen and hypereosinophilic, but again this is followed quite quickly by liquefaction and disappearance.

Virtually all examples of necrosis trigger localized inflammation in response to the release of inflammatory mediators from injured cells. In most cases, the inflammation is proportionate to the amount of tissue destruction and is designed to ingest and remove the dead tissue in preparation for tissue regeneration. When the inflammation seems excessive for the amount of necrosis, one should suspect that the necrosis is caused by an infectious agent.

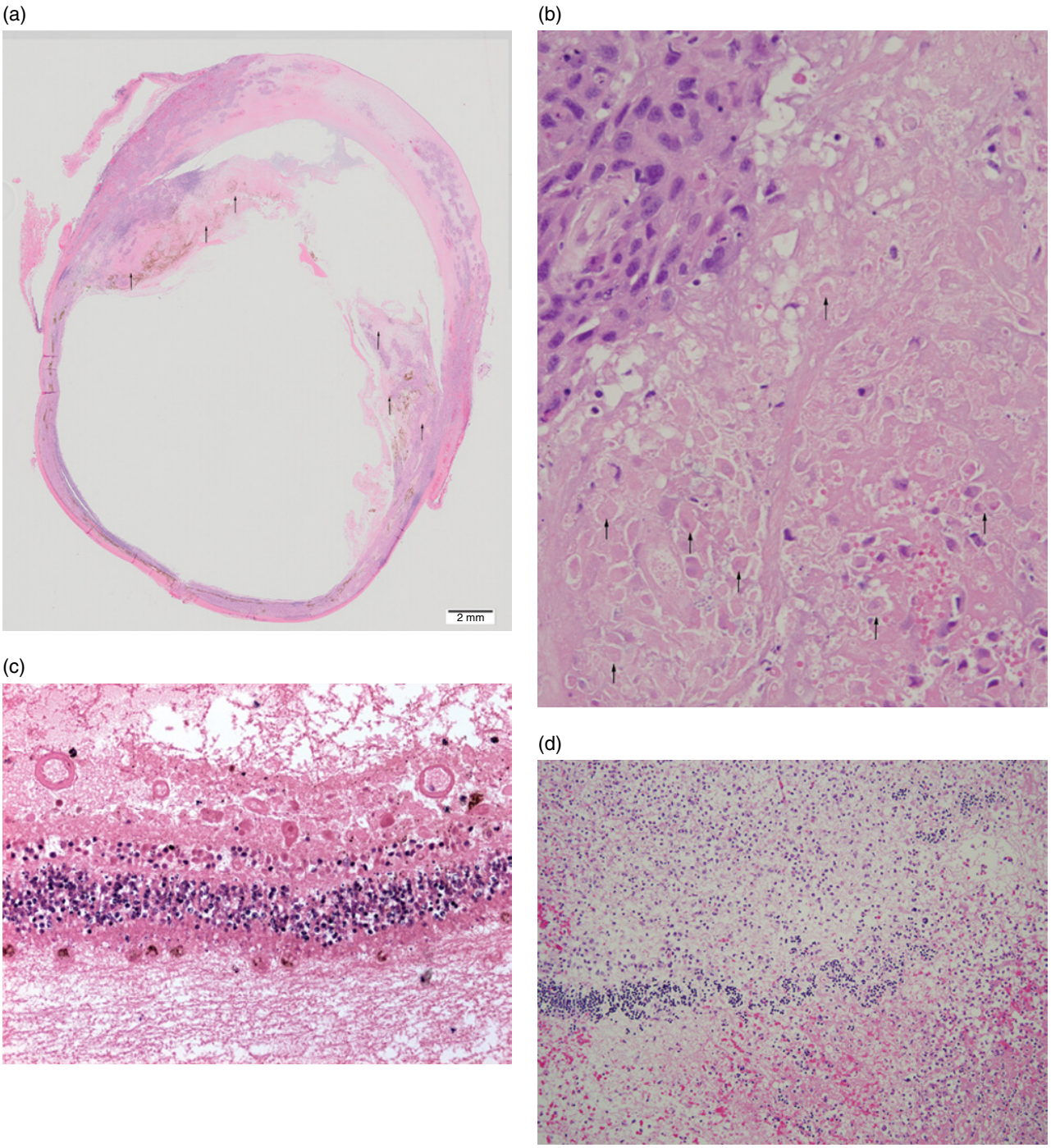

Figure 2.8 (a) Coagulation necrosis is noticeable on these subgross as pale pink staining areas (black arrows) within this globe with a malignant neoplasm (hematoxylin and eosin stain, subgross magnification). (b) This intraocular tumor has undergone regional coagulation necrosis, resulting in ghost-like outlines of the previous tumor cells with pyknotic nuclei and swollen, hyper-eosinophilic cytoplasm (black arrows) (hematoxylin and eosin stain, 40× magnification). (c) Acute retinal infarct with transient coagulation necrosis and karyolysis of ganglion cells. However, this lesion will rapidly progress to liquefaction (hematoxylin and eosin stain, 40× magnification). (d) Caseous necrosis progressing to liquefaction of the retina and the accumulating neutrophils in an example of embolic bacterial retinitis (hematoxylin and eosin stain, 40× magnification). (e) Equine globe with liquefactive collagenolysis of the cornea (suppurative keratomalacia) as a result of opportunistic corneal aspergillosis. (f) Corneal ulcer now complicated by an influx of neutrophils triggering stromal liquefactive collagenolysis (hematoxylin and eosin stain, 10× magnification).