Methods in Pharmacology and Toxicology (2014): 143–203
DOI 10.1007/7653_2013_7

Published online: 29 August 2013

The Ophthalmic Examination as It Pertains to General Ocular Toxicology: Basic and Advanced Techniques and Species-Associated Findings

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Abstract

Ocular toxicology pertains to toxicologic effects of drugs administered topically, intraocularly, or systemically. It should also include evaluation of adverse effects of ophthalmic devices such as contact lenses, intraocular lenses, and glaucoma implants. The ophthalmic examination is able to provide detailed in-life information and is used in combination with clinical observations, clinical pathology, and histopathology to assess potential toxicologic effects. The ophthalmologist must be familiar with the wide range of species used in the field of toxicology, be familiar with the anatomic variations associated with these species, be able to determine what is an inherited or a breed-related finding from a study-related effect, be competent with the required ophthalmic equipment, and be capable of examining this wide range of animals.

Key words Laboratory animal, Ophthalmology, Ocular toxicology

1 Introduction

The purpose of this chapter is to discuss laboratory animal ophthalmology as it pertains to industry, not to pocket pets. The industries of interest include contract toxicology laboratories and researchers in an academic environment that may require the expertise of a board-certified veterinary ophthalmologist.

Contract research organizations (CRO) evaluate products for pharmaceutical and agricultural use and are governed by Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) guidelines. In addition they may test cosmetics, contact lenses and associated materials, intraocular devices, and a host of other products that might have an ocular use, contact the eye or be applied topically, or be inhaled, ingested, or injected. The evaluation of potential drug effects and toxicity must integrate the disciplines of pharmacology, toxicology, pathology, and ophthalmology [1]. The CRO study is overseen by a study director and all study-associated personnel, including the consulting ophthalmologist, must be adequately trained and a quality assurance (QA) unit is responsible

for monitoring each study to ensure compliance. Toxicology studies conducted for regulatory purposes need to be conducted in compliance with good laboratory practice (GLP). All personnel, including the consulting ophthalmologist, involved in animal studies will be expected to be familiar with GLP and will usually be required to take annual GLP-refresher courses.

Systemic and ocular toxicity studies require evaluation of both systemic toxicity using clinical observations, body weight, and clinical and histologic pathology and ocular toxicity using detailed ophthalmic examinations [1]. The eye, as it pertains to toxicology, can be considered in one of the three ways. With respect to undesirable ophthalmic toxicologic effects the ophthalmologist is concerned with (1) undesirable ocular effects when the eye is the target organ of interest with the drug of interest applied to the eye, (2) undesirable systemic effects associated with an ocularly applied agent, and (3) undesirable ocular effects from an agent applied in a systemic manner (oral, dermal, injection, inhalation) with resulting ocular effects [2–5]. In addition to drug effects, animals may be used to evaluate the effects and side effects of a procedure or a device. With regard to the eye this may include evaluation of a new intraocular device such as an intraocular lens or viscoelastic agent or evaluation of a new surgical procedure.

These studies are designed to evaluate the potential for ocular toxicity or other adverse effects arising from the systemic, topical, or other administration of drugs or compounds, the application of medical devices, or certain surgical procedures. While in some cases the studies are designed to provide proof of concept as regards therapeutic efficacy, in the majority of cases the studies are being conducted specifically to enable an adequate assessment of safety of test materials and devices in consideration of meeting FDA and EPA (or other similar regulating agencies) approval for initiation of human clinical trials (supporting an investigational new drug (IND) and/or investigational device (IDE) application).

The eye, because of its large blood flow by organ weight, is a prime target for various systemic toxicities. In addition to the adnexal structures, vascularized intraocular structures include the retina and uveal tissues (iris, ciliary body, and choroid) [6]. The transparent nature of the eye and the ability to visualize arteries, veins, and neural tissue make the eye an organ where toxicities may be readily detectable. This makes the eye unique in that it is possible to conduct a detailed assessment during the in-life portion of a study [1].

As ophthalmologists, we must be familiar with the normal inter- and intraspecies variations that occur between the species involved in toxicologic studies. We must be able to examine all of these species given the limitations of size, temperament, and restraint. Finally we must be familiar with common naturally occurring abnormalities observed in each of these species and be able to

differentiate these from toxicologic effects. A discussion of the anatomy and physiology of the most common animals used in ocular research, including mice, rats, rabbits, guinea pigs, dogs, cats, pigs, and primates, is found in Chap. 2. This chapter emphasizes the routine ophthalmic examination of laboratory animals. It also provides information on more advanced ophthalmic diagnostic tools that are becoming more commonplace in the area of ocular toxicology.

2 Routine Examination

Prior to study initiation, the ophthalmologist should review all ophthalmic procedures, discuss with the study director and/or sponsor any issues or concerns with the study design or the examination procedures, and then follow standard operating procedures (SOPs) when conducting their examinations. It is the position of the American College of Veterinary Ophthalmologists (ACVO) that in order to ensure public safety the status of Diplomate of the ACVO is the minimum qualification for performing these ocular examinations and assessment of findings in a laboratory animal study that is intended to support applications to the FDA (or other similar regulating agencies) for entry into human clinical trials. Evaluation of toxicological effects of pharmaceutical agents involves assessment by a number of personnel, many of which are board-certified specialists, including pathologists, cardiologists, and others in addition to ophthalmologists. Sponsors engaging the services of a CRO must be advised of the participation of veterinary ophthalmologists and the potential limitations that may arise if such studies do not involve veterinary ophthalmologists.

A board-certified veterinary ophthalmologist is uniquely qualified to consult in the development of the experimental design (including the species selected, appropriate diagnostic tests, and frequency of exams) and the assessment of ocular effects of test materials being evaluated. Coordination between the testing agency and the veterinary ophthalmologist is essential throughout the process, to include protocol development, establishing SOPs, and the identification and assessment of ocular findings. If ocular abnormalities are identified, communication between the ophthalmologist and the pathologist will allow correlation of clinical and histopathologic findings.

The components of an ophthalmic examination may vary depending on the species involved and the specific objective of the study. However, if the purpose of such a study is to screen for adverse effects on any ocular tissue including, at a minimum, the adnexal structures (eyelids and conjunctiva), anterior segment (cornea, anterior chamber, iris, and lens), and posterior segment (vitreous and fundus), the following must be included:

1. Pharmacologic pupillary dilation
2. Darkened ambient light conditions
3. Indirect and/or direct ophthalmoscopy
4. Slit lamp biomicroscopy

Additional procedures may be included depending on the objective of the examination. These may include, but are not limited to, corneal staining, corneal esthesiometry, pachymetry, tonometry, fundus photography, fluorescein angiography, optical coherence tomography (OCT), and electrophysiological assessment of the visual system (e.g., electroretinography, multifocal electroretinography, visual evoked potentials (VEP)). Sedation or general anesthesia may or may not be required depending on the species, the procedure being performed, and individual animal.

The routine ophthalmic examination for all animals used in toxicologic studies should begin with the minimum database of both biomicroscopy and indirect ophthalmoscopy. Regardless of the species of interest, these two examination techniques are essential to ensure an accurate and complete examination of both the anterior and posterior segments of the eye. A comparison of techniques by Bellhorn found that of 100 rats with known lens abnormalities diagnosed by biomicroscopy only 65/100 of the lenticular lesions could be found using the direct ophthalmoscopy and only 35/100 were found using indirect ophthalmoscopy [7]. Together these two examinations must, at a minimum, include evaluation of the adnexal structures (eyelids and conjunctiva), anterior segment (cornea, anterior chamber, iris, and lens), and posterior segment (vitreous and fundus).

Ophthalmic examinations should be conducted on an eye that has been pharmacologically dilated and should be performed in a darkened examination room. While some have advocated the use of 10 % phenylephrine to aid in dilation of rodents [8, 9], this is generally not required. Pharmacologic dilation is most commonly performed using tropicamide at a concentration of 0.5 % for rodents and 1 % for larger mammals. The ophthalmologist should be familiar with the length of time required to achieve mydriasis and the duration of the mydriasis for the species being examined. In general, 10–15 min is the minimum time required to achieve acceptable mydriasis and this may be slightly longer in heavily pigmented eyes. The duration of mydriasis is directly related to the amount of intraocular melanin. In albinotic rodents, mydriasis will last no more than 1 h while in a pigmented eye of a dog or a primate the effect will persist for 3–5 h. This information is important so that the ophthalmologist knows when to begin dilation and how many animals should be dilated at one time. The later will depend on how many animals the ophthalmologist can examine in a given time period. For the basic examination, biomicroscopy, and indirect ophthalmoscopy, animals are either manually restrained

(rodent, dog, rabbit, pig, guinea pig, cat) or sedated or anesthetized (primates). Rats and mice may be held in a dose-hold and presented to the ophthalmologist with their heads restrained. Some ophthalmologists prefer the eyes to be slightly proptosed in rodents. Dogs and rabbits are most often examined on a table. Rabbits seem to do best if there is a towel on the table as this decreases their movements and may provide some comfort. For rabbits, the ophthalmologist should be seated slightly below the level of the restraint table to allow easy visualization of the rabbits' optic nerve and retinal vasculature which are located in the superior fundus. For dogs, the examiner may be seated or standing. Since primates will be anesthetized or heavily sedated, they may be manually restrained or placed on an examination table. If more extensive examinations such as electrodiagnostic testing or OCT are required sedation or anesthesia may be required regardless of the species.

Additional examination procedures such as direct ophthalmoscopy, corneal staining, tonometry, pachymetry, fluorescein angiography, photographic documentation (anterior or posterior segment), electrodiagnostic testing, ultrasonography, OCT, and other tests may be indicated depending on the study and toxicologic effects of interest. If any of these additional tests are required, the order in which tests are performed and when to perform pupil dilation must be considered. For example, determination of intraocular pressure (IOP) should be performed prior to pupil dilation. In addition, if repeated IOP measurements are required during a study they should be performed at the same time of day to avoid diurnal pressure fluctuation. When possible, examination of the cornea should be performed prior to procedures that may result in corneal changes as a result of corneal contact (pachymetry, tonometry) or the use of topical anesthesia. If sedation is required for the ophthalmic examination then consideration must be given to dosing and feeding schedules, clinical observations, and clinical pathology sampling. Finally, from an animal well-being standpoint, a balance must be struck between multiple procedures performed on the same day as compared with multiple repeated days requiring sedation [1].

Animals will be most commonly be identified by tattoo, ear tag, or microchip. If a tattoo or an ear tag is used, the animal handler will need to have enough light during the examination to read the identification to ensure accurate data collection. This may be provided by a separate light source elsewhere in the examination room or if possible by performing the ophthalmic examinations in a darkened anteroom allowing the handlers to keep the main animal room lights on. When identified by microchip, a computer scanner will allow animal identification to be linked to the computer program for data entry. To ensure accuracy, each animal must be identified to both the ophthalmologist and the data entry person at the time of the examination. The data entry person must verify

that the animal being examined and the animal for which the data is being entered correspond.

Depending on the compound being evaluated and the SOP, the ophthalmologist will be expected to wear shoe covers, a lab coat, or surgical scrubs and gloves at a minimum and may be required to wear a Tyvek® suit, surgical cap, mask, and occasionally a respirator. When working with nonhuman primates (NHPs), annual testing for tuberculosis (TB) using a TB intradermal PPD skin test or the new QuantiFERON®-TB blood test will generally be required of all personnel including the ophthalmologist.

The ophthalmologist must be familiar with what is normal for the species being examined and what are the common, spontaneous abnormalities for that species, age of animal, and breed/strain. Albino vs. pigmented eye may be a factor as is the type of retinal vasculature, ranging from anangiotic to merangiotic to holangiotic. The presence or the absence of a tapetum and whether the animal has a fovea should be considered. In addition, the examination techniques to be used, type of biomicroscope and indirect ophthalmoscope, size and diopter of the indirect lens, and number of animals that can be examined in an hour must be understood. The role of the veterinary ophthalmologist is to perform a pretest examination designed to eliminate those animals not suited to the study and to establish a baseline database to compare interim and end-of-study findings. Animals are then subsequently examined one or more times during the study, at the conclusion of the study, and possibly in a recovery phase depending on the study duration and design. Typically studies are divided into acute, subacute, subchronic, and chronic depending on the duration. The ophthalmologist must then interpret findings in light of the species examined, pretest data, compound evaluated, additional study procedures performed (anesthesia, orbital blood collection), and dose group outcome.

Since most laboratory studies involve a significant number of animals, organization and efficiency are essential. In general, most canine, primate, swine, feline, and rabbit studies involve 40–60 animals while rats and mice may involve 250–1,500 animals in a single study. A single ophthalmologist generally requires 2–3 animal handlers, a data entry individual, and in studies over 250 animals 1–2 individuals to go ahead of the animal handlers to dilate the pupils. For efficiency, an animal should be in front of the ophthalmologist at all times. The ophthalmologist's findings are reported verbally to the data entry individual who then either enters it into a computer program or records on a paper record for later entry into a computer database. This data is then verified at the end of the examination and both the ophthalmologist and data entry individual date and initial the accuracy of the report.

When an ophthalmic abnormality is observed, it must be characterized with respect to diagnosis, location, and severity. Depending on the laboratory, some will utilize a standardized scheme for recording of clinical observations such as Provantis® that has a set of

preloaded ophthalmic terms for organ location (cornea, lens, iris, etc.), clinical signs/diagnosis (opacity, coloboma, degeneration, hemorrhage, etc.), specific location (cortex, nucleus, tapetal, anterior, posterior, etc.), and severity (slight, moderate, severe). Other CROs may have their own in-house online or hand recording system. The ophthalmologist should be familiar with each laboratory's recording system and terminology to be consistent both within and between studies. When an abnormality is observed, correlation between dose groups is important when evaluating the incidence and severity of lesions so that any association with the test article can be assessed [1]. While it would be best if animals were examined out of dosing order so as to mask the ophthalmologist with respect to dose group being examined, this is often not possible given the way animals are housed and entered into the data collection system.

The ophthalmologist should also have a standardized scoring or grading scheme to assign a severity to any abnormalities seen. In general, a grading scheme of slight, moderate, and severe/marked is most common. When using this grading scheme for the transparent media (cornea, aqueous, lens, and vitreous) a grading of slight would imply a lesion that does not obstruct visualization of the deeper tissues past the lesion, a moderate grade implies a lesion that interferes with but does not fully obstruct the view of the tissues deep to the lesion, and a severe/marked lesion fully obstructs the view of structures deep to the lesion (Table 1). This is analogous to the terms incipient, immature, and mature when applied to a cataract. Lesions may also be characterized with respect to the

Table 1
Biomicroscopy grading criteria for cornea, aqueous, lens, and vitreous opacities

Grade	Definition
0	No observable lesion.
1+	Some loss of transparency. The underlying structures are clearly visible with diffuse illumination.
2+	Moderate loss of transparency. With diffuse illumination the underlying structures are barely visible, but can still be examined and graded.
3+	Severe loss of transparency. With diffuse illumination the underlying structures are not visible when viewed through the lesion and evaluation of them is impaired.

This grading system is based on the modified Hackett–McDonald scoring system
Grade 1—Slight or mild
Grade 2—Moderate
Grade 3—Marked, excessive, or severe

area of involvement with the terms most commonly used being focal, multifocal, and diffuse.

For studies involving topical ophthalmic application of a drug or an ocular/intraocular device a specific more detailed biomicroscopic examination protocol with standardized scoring or grading criteria is frequently used. This is most commonly the modified Hackett–McDonald scoring system (Tables 2 and 3) [10, 11]. Additionally, for studies involving intravitreal injection or intraocular procedures, the Standardization of Uveitis Nomenclature (SUN) grading system can be used or modified to evaluate aqueous flare and cells (Tables 4 and 5) [1, 12, 13]. When counting anterior chamber cells, the type of cell pigmented, white blood cell or red blood cell, should be noted [1]. For grading of intravitreal inflammation and cells, the National Eye Institute system can be used (Table 6) [14, 15].

2.1 Biomicroscopy

Biomicroscopy is used to examine the ocular anterior segment including the eyelids, conjunctiva, third eyelid, tear film, cornea, anterior chamber, iris, lens, and anterior vitreous. Biomicroscopy provides a magnified view of the living eye using a light that can be varied in intensity, width, height, and color. In general for laboratory animals, this is performed using a handheld slit lamp of the ophthalmologist's preference. The slit lamp used for routine examination should be portable, lightweight, and easy to use on a variety of species. The two most common portable slit lamps used for laboratory animals are the Zeiss HSO-10 (Fig. 1) and the Kowa SL-14/15 (Fig. 2). The Zeiss HSO-10 provides a 12× magnification with a 125 mm working distance and is both lightweight and easy to use on all species. The Kowa SL-14/15 has either a 10× or a 16× magnification with a 100 mm working distance. Unlike the Zeiss, the Kowa works from a battery pack and is re-charged in a stand. Both have fixed slit widths (0.15 and 0.75 mm Zeiss; 0.1, 0.2, and 0.8 mm Kowa) and both have a cobalt blue filter for fluorescein. If higher magnification or photographic documentation are required, a table-mounted slit lamp may be used (Fig. 3). Table slit lamps provide higher quality optics, increased magnification, and variable width and height of the slit beam and with additional attachments can allow for photographic documentation, gonioscopy, or specular microscopy. Table slit lamps are however significantly more expensive, less portable, and more difficult to use on a large number of animals or un-sedated animals.

The slit lamp performs two major functions. First, it provides magnification for a more detailed examination of the eye. Secondly it makes use of the slit beam, decreasing the beam of light to a slit allowing an optical cross section of the eye to be obtained (Figs. 4 and 5). This allows precise localization of the depth of a lesion and allows visualization of subtle changes that cannot be seen with full illumination (Figs. 6, 7, 8, 9, and 10). The term for this type of illumination and examination is an optical section and it is the most

Table 2
Criteria used for a modified Hackett–McDonald scoring system [10]

Conjunctival congestion	
0=	Normal. May appear blanched to reddish pink without perilimbal injection (except at 12:00 and 6:00 o'clock positions) with vessels of the palpebral and bulbar conjunctiva easily observed.
1=	A flushed, reddish color predominantly confined to the palpebral conjunctiva with some perilimbal injection but primarily confined to the lower and upper parts of the eye from the 4:00, 7:00, 11:00, and 1:00 o'clock positions.
2=	Bright red color of the palpebral conjunctiva with accompanying perilimbal injection covering at least 75 % of the circumference of the perilimbal region.
3=	Dark, beefy red color with congestion of both the bulbar and the palpebral conjunctiva along with pronounced perilimbal injection and the presence of petechia on the conjunctiva. The petechia generally predominate along the nictitating membrane and the upper palpebral conjunctiva.
Conjunctival swelling (there are five divisions from 0 to 4)	
0=	Normal or no swelling of the conjunctival tissue.
1=	Swelling above normal without eversion of the lids (can be easily ascertained by noting that the upper and lower eyelids are positioned as in the normal eye); swelling generally starts in the lower cul-de-sac near the inner canthus, which needs slit lamp examination.
2=	Swelling with misalignment of the normal approximation of the lower and upper eyelids; primarily confined to the upper eyelid so that in the initial stages the misapproximation of the eyelids begins by partial eversion of the upper eyelid. In this stage, swelling is confined generally to the upper eyelid, although it exists in the lower cul-de-sac (observed best with the slit lamp).
3=	Swelling definite with partial eversion of the upper and lower eyelids essentially equivalent. This can be easily ascertained by looking at the animal head-on and noticing the positioning of the eyelids; if the eye margins do not meet, eversion has occurred.
4=	Eversion of the upper eyelid is pronounced with less pronounced eversion of the lower eyelid. It is difficult to retract the lids and observe the perilimbal region.
Conjunctival discharge—Discharge is defined as a whitish-gray precipitate, which should not be confused with the small amount of clear, inspissated, mucoid material that can be formed in the medial canthus of a substantial number of rabbit eyes. This material can be removed with a cotton swab before the animals are used.	
0=	Normal. No discharge.
1=	Discharge above normal and present on the inner portion of the eye but not on the lids or hairs of the eyelids. One can ignore the small amount that is in the inner and outer canthus if it has not been removed prior to starting the study.
2=	Discharge is abundant, easily observed, and has collected on the lids and around the hairs of the eyelids.
3=	Discharge has been flowing over the eyelids so as to wet the hairs substantially on the skin around the eye.
Aqueous flare—The intensity of the Tyndall phenomenon is scored by comparing the normal Tyndall effect observed when the slit lamp beam passes through the lens with that seen in the anterior chamber. The presence of aqueous flare is presumptive evidence of breakdown of the blood–aqueous barrier.	
0=	The absence of visible light beam in the anterior chamber (no Tyndall effect).

(continued)

Table 2
(continued)

1=	The Tyndall effect is barely discernible. The intensity of the light beam in the anterior chamber is less than the intensity of the slit beam as it passes through the lens.
2=	The Tyndall beam in the anterior chamber is easily discernible and is equal in intensity to the slit beam as it passes through the lens.
3=	The Tyndall beam in the anterior chamber is easily discernible; its intensity is greater than the intensity of the slit beam as it passes through the lens.
Pupillary light reflex—The pupillary diameter of the iris is controlled by the radial and sphincter muscles. Contraction of the radial muscle due to adrenergic stimulation results in mydriasis while contraction of the sphincter muscle due to cholinergic stimulation results in miosis. As an ophthalmic drug can exert potential effects on these neural pathways, it is important to assess the light reflex of an animal as part of the ophthalmic examination. Using full illumination with the slit lamp, the following scale is used.	
0=	Normal pupillary response.
1=	Sluggish pupillary response.
2=	Maximally impaired (i.e., fixed) pupillary response.
Iris involvement—In the following definitions, the primary, secondary, and tertiary vessels are utilized as an aid to determining a subjective ocular score for iris involvement. The assumption is made that the greater the hyperemia of the vessels and the more the secondary and tertiary vessels are involved, the greater the intensity of iris involvement. The scores range from 0 to 4.	
0=	Normal iris without any hyperemia of the iris vessels. Occasionally around the 12:00–1:00 o'clock position near the pupillary border and the 6:00 and 7:00 o'clock position near the pupillary border, there is a small area around 1–3 mm in diameter in which both the secondary and tertiary vessels are slightly hyperemic.
1=	Minimal injection of secondary vessels but not tertiary. Generally, it is uniform, but may be of greater intensity at the 1:00 or 6:00 o'clock position. If it is confined to the 1:00 or 6:00 o'clock position, the tertiary vessels must be substantially hyperemic.
2=	Minimal injection of tertiary vessels and minimal to moderate injection of the secondary vessels.
3=	Moderate injection of the secondary and tertiary vessels with slight swelling of the iris stroma (this gives the iris surface a slightly rugose appearance, which is usually most prominent near the 3:00 and 9:00 o'clock positions).
4=	Marked injection of the secondary and tertiary vessels with marked swelling of the iris stroma. The iris appears rugose; may be accompanied by hemorrhage (hyphema) in the anterior chamber.
Cornea cloudiness—The scoring scheme measures the severity of corneal cloudiness and the area of the cornea involved. Severity of corneal cloudiness is graded as follows:	
0=	Normal cornea. Appears with the slit lamp adjusting to a narrow slit image as having a bright gray line on the epithelial surface and a bright gray line on the endothelial surface with a marble-like gray appearance of the stroma.
1=	Some loss of transparency. Only the anterior half of the stroma is involved as observed with an optical section of the slit lamp. The underlying structures are clearly visible with diffuse illumination, although some cloudiness can be readily apparent with diffuse illumination.

(continued)

Table 2
(continued)

2=	Moderate loss of transparency. In addition to involving the anterior stroma, the cloudiness extends all the way to the endothelium. The stroma has lost its marble-like appearance and is homogeneously white. With diffuse illumination, underlying structures are clearly visible.
3=	Involvement of the entire thickness of the stroma. With the optical section, the endothelial surface is still visible. However, with diffuse illumination, the underlying structures are just barely visible (to the extent that the observer is still able to grade flare and iritis, observe for pupillary response, and note lenticular changes).
4=	Involvement of the entire thickness of the stroma. With the optical section, cannot clearly visualize the endothelium. With diffuse illumination, the underlying structures cannot be seen. Cloudiness removes the capability for judging and grading flare, iritis, lenticular changes, and pupillary response.
Corneal area—The surface area of the cornea relative to the area of cloudiness is divided into five grades from 0 to 4.	
0=	Normal cornea with no area of cloudiness.
1=	1–25 % area of stromal cloudiness.
2=	26–50 % area of stromal cloudiness.
3=	51–75 % area of stromal cloudiness.
4=	76–100 % area of stromal cloudiness.
Pannus—Pannus is vascularization or the penetration of new blood vessels into the corneal stroma. The vessels are derived from the limbal vascular loops. Pannus is divided into three grades.	
0=	No pannus.
1=	Vascularization is present but vessels have not invaded the entire corneal circumference. Where localized vessel invasion has occurred, they have not penetrated beyond 2 mm.
2=	Vessels have invaded 2 mm or more around the entire corneal circumference.
Fluorescein—For fluorescein staining, the area can be judged on a 0 to +4 scale using the same terminology as for corneal cloudiness. The intensity of fluorescein staining can be divided into 0–4 scale.	
0=	Absence of fluorescein staining.
1=	Slight fluorescein staining confined to a small focus. With diffuse illumination the underlying structures are easily visible. (The outline of the pupillary margin is as if there were no fluorescein staining.)
2=	Moderate fluorescein staining confined to a small focus. With diffuse illumination the underlying structures are clearly visible, although there is some loss of detail.
3=	Marked fluorescein staining. Staining may involve a larger portion of the cornea. With diffuse illumination the underlying structures are barely visible but are not completely obliterated.
4=	Extreme fluorescein staining. With diffuse illumination the underlying structures cannot be observed.

(continued)

Table 2
(continued)

Lens—The crystalline lens is readily observed with the aid of the slit lamp biomicroscope, and the location of lenticular opacity can readily be discerned by direct and retro illumination.
The lens is normal (N) or abnormal (A)
If abnormal, the location and severity of lenticular opacities are noted in the comments.

common method of biomicroscopic examination. Using a narrow slit beam, a highly magnified optical section of the eye is obtained. The direction of the slit beam may be varied so that the structures may be viewed using either direct illumination or retroillumination [1]. This will allow the examiner to detect and localize, with respect to depth, abnormalities in the anterior segment of the eye. For example, a corneal lesion can be localized to superficial, stromal, or endothelial; aqueous opacities such as cells (aqueous, vitreous), flare, or hemorrhage are detectable and quantifiable; and lesions of the lens may be localized to anterior, posterior, equatorial, and further to capsular, cortical, or nuclear. Interpretation of the findings on slit lamp biomicroscopy requires extensive knowledge of normal findings as well as background lesions that occur as incidental findings in the species and breed being examined [1].

2.2 Indirect/Direct Ophthalmoscopy

Indirect ophthalmoscopy is the preferred technique of choice for routine screening of the posterior segment in all laboratory animal species. Indirect ophthalmoscopy provides a binocular, inverted, and reversed aerial image with a wide field of view. It requires an indirect headset and a condensing lens. Once perfected, the technique of indirect examination also allows for a more rapid examination of the entire posterior segment as it provides a wider panoramic field of view, allowing the examiner to evaluate more animals, more accurately in a shorter period of time. The indirect headset of choice should be lightweight, comfortable, and easy to manipulate out of the way with one hand and have a small pupil setting. The ease of manipulation will allow the examiner to move efficiently between indirect and biomicroscopic examination techniques. While several excellent choices are available, the Keeler All Pupil® is best suited in the author's opinion (Fig. 11). Alternatively, the Heine indirect ophthalmoscope also offers excellent optics and can be fitted with a portable power supply, but is slightly heavier and more cumbersome for large number of animals (Fig. 12). The indirect condensing lens of choice varies by species examined and by the examiner's choice. In general, a 2.2 Pan Retinal or 30 diopter lens works well for routine screening examination of most

Table 3
Data recording sheet for a modified Hackett–McDonald scoring system

MICROSCOPIC GRADING DATA
(Hackett and McDonald)

Study Number: _____ Study Day: _____ Group: _____

	Animal No.	Conjunctival			Aqueous Flare	Iris		Corneal Cloudiness		Pannus	Time	Fluor. Exam	Time	Lens	Time
		Conges.	Swell	Dis.		Light Reflex	Involve-ment	Sev.	Area						
(Test) Left Eye															
(Control) Right Eye															

Comments: _____

_____

Tech Date/Initials: _____ Ophthalmologist Date/Initials: _____

Conges.=congestion Sev.=Severity
Swell-swelling Fluor, Exam=Fluorescein staining Dis.=Discharge

Table 4
Grading scheme for aqueous cells based on the SUN grading system using a biomicroscope and a 1 mm × 1 mm slit beam

Grade	# cells in the field
0	<1
0.5+	1–5
1+	6–15
2+	16–25
3+	26–50
4+	>50

Cells should be counted at the same location, usually the central anterior chamber [1, 12]

Table 5
Grading scheme for aqueous flare based on the SUN grading system using a biomicroscope [12]

Grade	Description
0	None
1+	Faint
2+	Moderate (iris and lens details clear)
3+	Marked (iris and lens details hazy)
4+	Intense (fibrin or plasmoid aqueous)

Table 6
Grading scheme for vitreous inflammation based on the National Eye Institutes grading system using an indirect ophthalmoscope [14]

Grade	Description
0	None
1+	Posterior pole clearly visible
2+	Posterior pole details slightly hazy
3+	Posterior pole details very hazy
4+	Posterior pole details barely visible
5+	Fundus details not visible

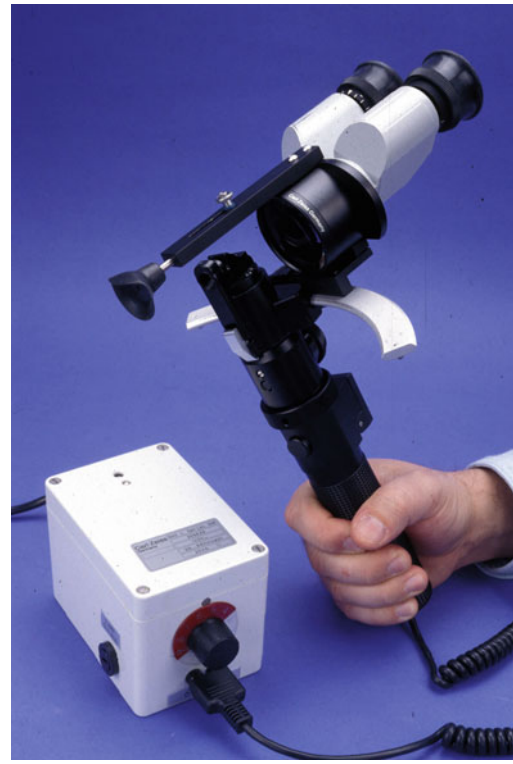

Fig. 1 A Zeiss HSO-10 slit lamp provides excellent optics and portability and is suited for use in all laboratory animal species

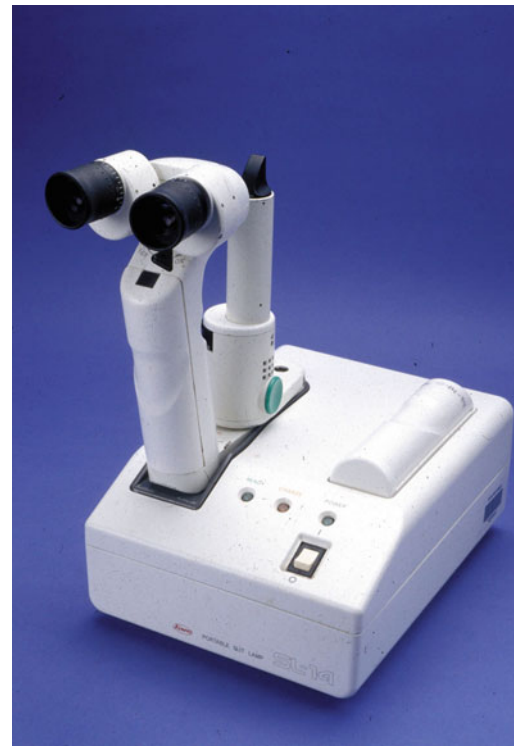

Fig. 2 A Kowa SL-14 or SL-15 handheld slit lamp are rechargeable and portable. As with the Zeiss HSO-10 they are suited for use in all laboratory animal species

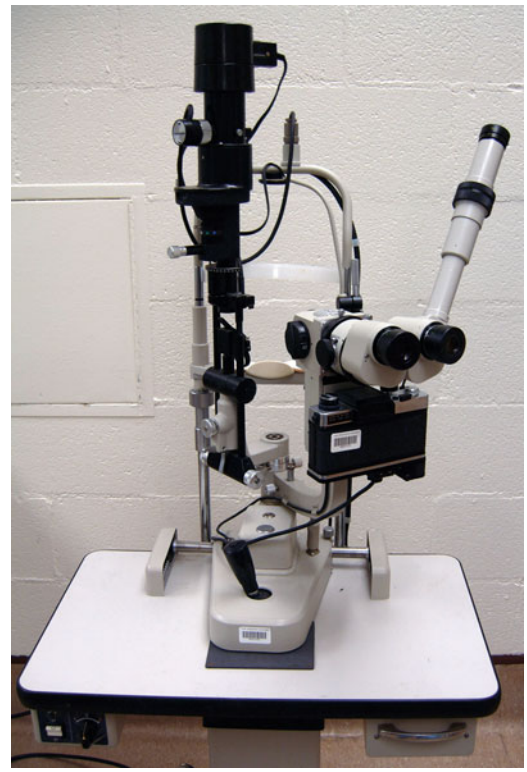

Fig. 3 A Topcon table-mounted slit lamp. This provides excellent optics, higher magnification, and variable slit beam width and height and additionally can be used for other procedures such as photographic documentation and specular microscopy

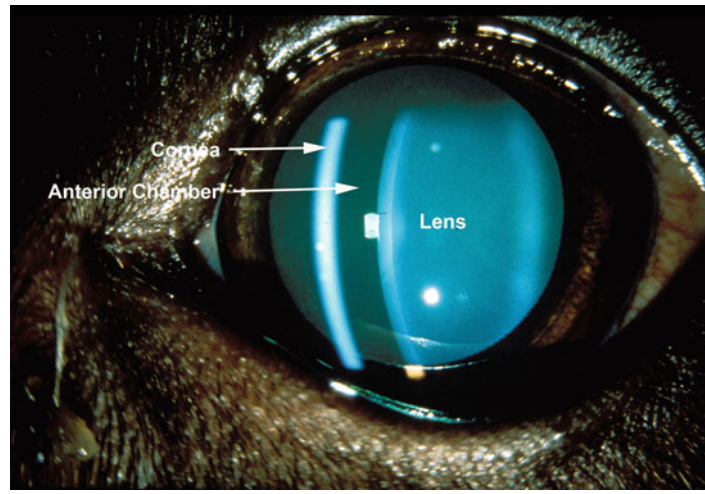

Fig. 4 A normal slit lamp examination using the technique of optical section to examine the anterior segment of a normal beagle

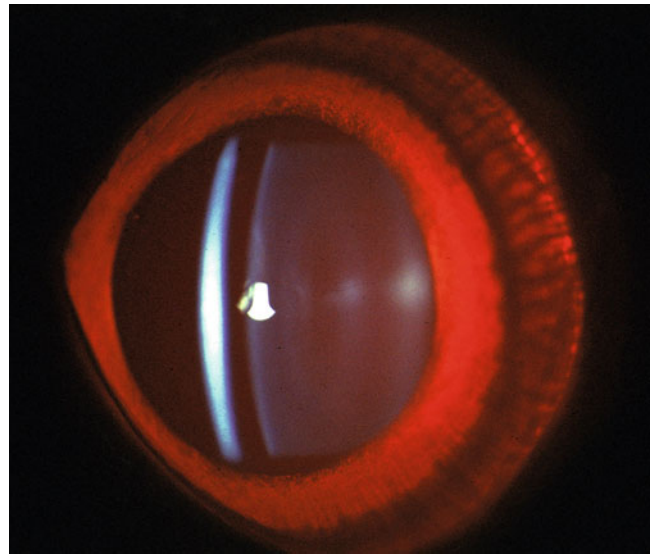

Fig. 5 A normal slit lamp examination using the technique of optical section to examine the anterior segment of a normal New Zealand white rabbit

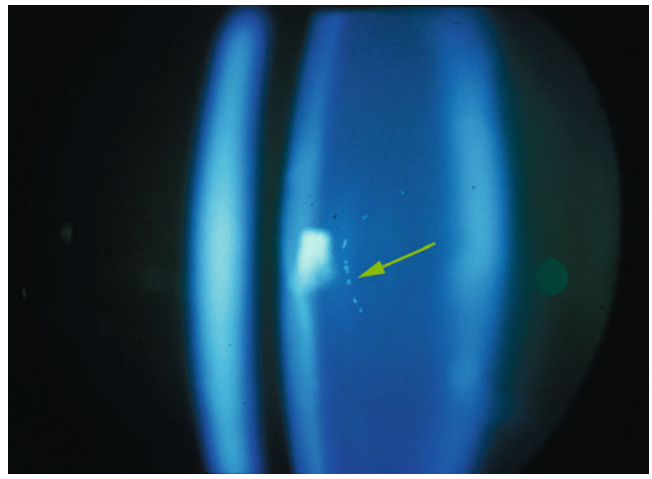

Fig. 6 Multiple anterior cortical suture opacities/cataract are noted (*arrow*) in a beagle in pretest examination

larger species and a 40 or a 60 diopter works best for rats and mice. The addition of a 20 diopter and/or a 15 diopter lens may be advised for higher magnification of the fundus in the canine and to examine the fovea and optic nerve in greater detail in NHPs. Alternately, a direct ophthalmoscope (Fig. 13) can be used to examine the optic nerve head and fovea in NHPs, but given its small field and monocular view, it is less than optimal in the author's opinion. In addition, when performing direct ophthalmoscopy any

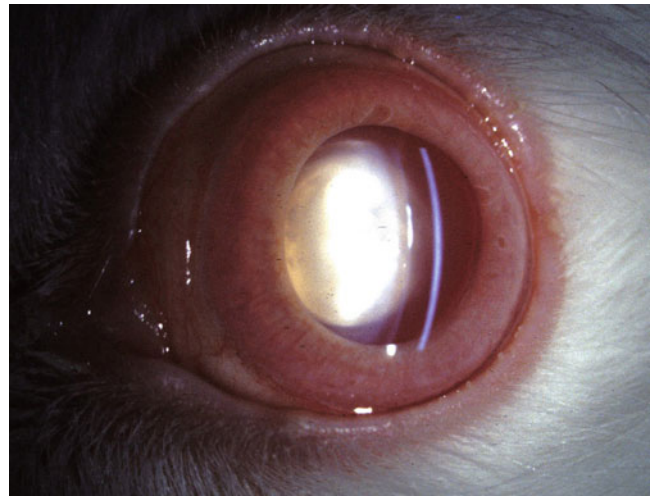

Fig. 7 An immature, nuclear cataract noted in a New Zealand white rabbit in pretest examination

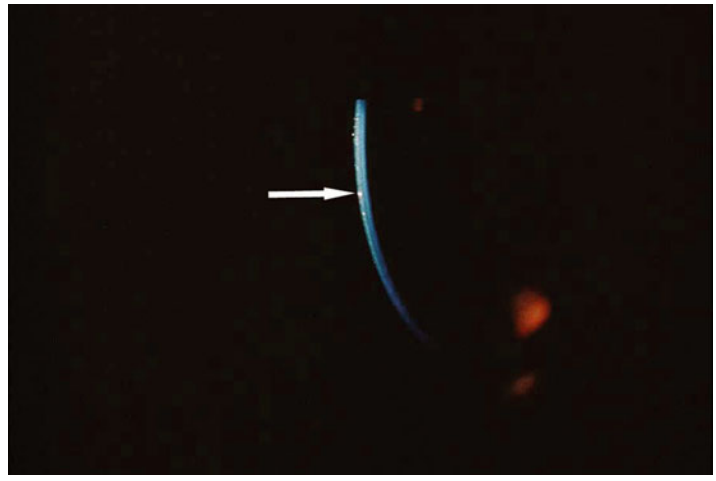

Fig. 8 Multifocal, corneal anterior stromal opacities (*arrow*) observed as a treatment-related effect in high-dose beagles during a chronic study

opacity of the cornea, aqueous, lens, or vitreous will severely impair or prevent visualization of the posterior segment.

Prior to indirect ophthalmoscopy, a short-acting mydriatic agent is required to dilate the pupil. Tropicamide 0.5–1.0 % is the mydriatic of choice. When examining using indirect ophthalmoscopy, the examiner remains at arm's length and places the condensing lens just anterior to the cornea. This technique is used to examine the posterior vitreous, optic nerve, retinal vasculature, retina, and choroid. In addition, opacities of the clear media

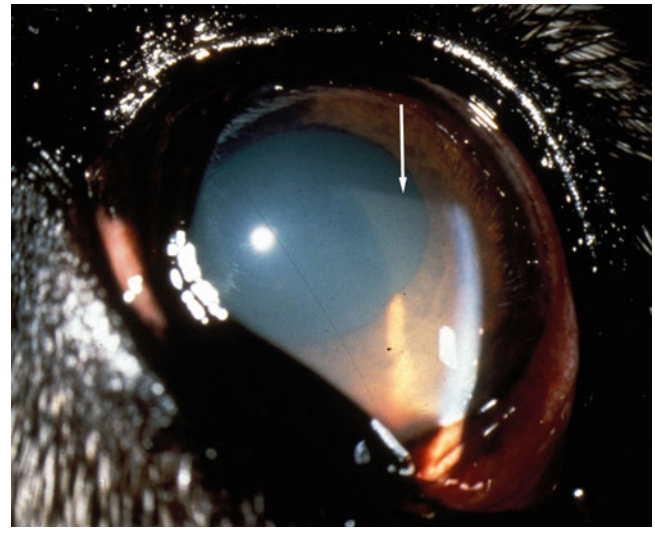

Fig. 9 Aqueous flare is noted in the anterior chamber as a cloudiness (*arrow*) in the normally transparent aqueous humor

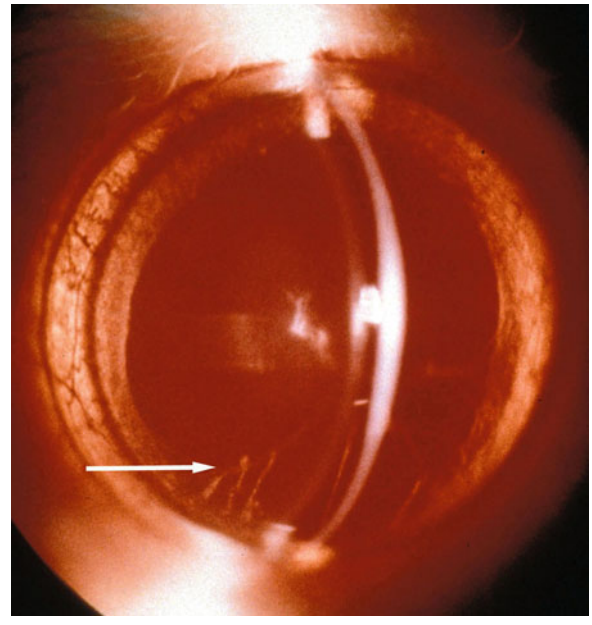

Fig. 10 A Sprague–Dawley rat with several persistent pupillary membranes (*arrow*) noted in pretest examination

(cornea, aqueous, lens, and vitreous) are readily detectable using retroillumination. This technique can be used to grade vitreous inflammation/opacification (Table 6). Given the wide variety of laboratory animals, the examiner must be familiar with the variation of normal anatomy and species variations. The retinal vasculature

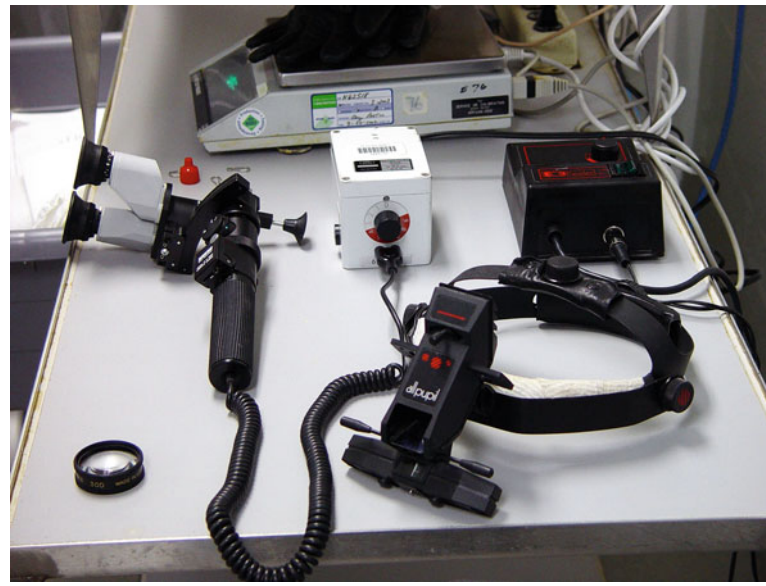

Fig. 11 A Keeler All-Pupil® indirect ophthalmoscope, 30D condensing lens, and a Zeiss HSO-10 slit lamp used for ophthalmic examination of laboratory animals

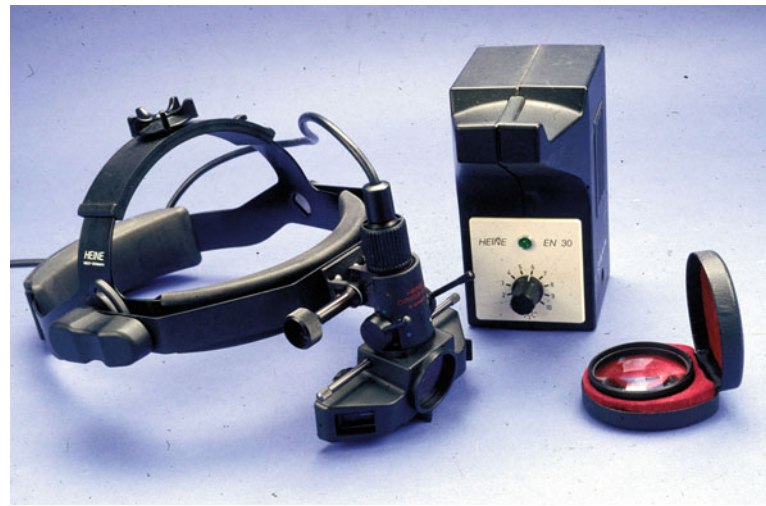

Fig. 12 A Heine® indirect ophthalmoscope with a power source and a 30 diopter indirect condensing lens

will vary with the species anangiotic (guinea pig), merangiotic (rabbit), and holangiotic (rodent, canine, swine, primate). The pigmentation of the retinal pigment epithelium (RPE) and the choroid vary between albinotic, sub-albinotic, and pigmented animals of the same species (mouse, rat, rabbit). Some species such as the canine will have a tapetum located in the superior choroid, but

Fig. 13 A Welch-Allyn direct ophthalmoscope

this can be lacking in color dilute or lemon beagles. Finally, NHPs are foveated and this region must be examined carefully for abnormalities.

Additional techniques to evaluate the retina may include fluorescein angiography, OCT, confocal scanning laser ophthalmoscopy, fundus photography, and electrodiagnostic testing. When these tests are used correctly and in combination they can provide additional en face, cross-sectional, and functional information of the retina that may be then correlated with histopathology.

2.3 Pretest Examination

Prior to study initiation, a pretest ophthalmic examination should be performed on all study animals. This is done for two reasons. The first is to eliminate from the study animals with current significant or potentially progressive ophthalmic abnormalities. The second is to establish a baseline of ocular findings to compare to as the study progresses and subsequent ophthalmic examinations are performed. Examples of pretest abnormalities that should automatically result in an animal's elimination from the study would include all ocular findings with a severity score of moderate or higher and all abnormalities that currently prevent or may prevent if progressive complete examination of intraocular structures. Examples of ocular findings that may be progressive during the course of the study and

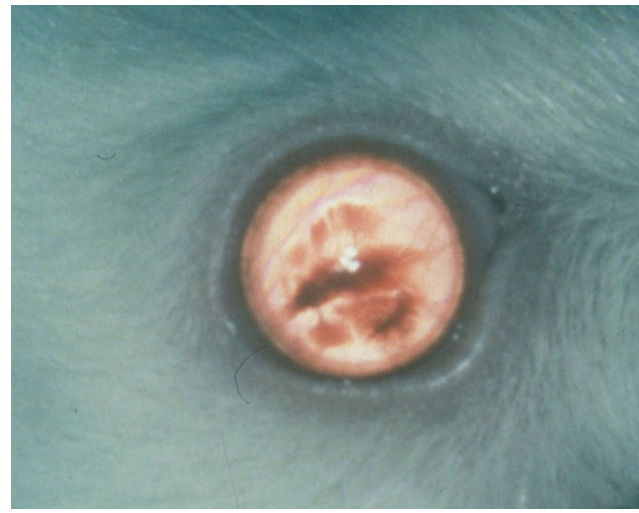

Fig. 14 A Sprague–Dawley rat with significant intravitreal extravasated blood associated with a persistent hyaloid. This was noted in pretest examination and the animal was eliminated from the study

would result in elimination would include cataract, intraocular hemorrhage, uveitis, and any other findings that may result in progressive opacification and interfere with a complete ophthalmic examination on subsequent examinations.

Common background abnormalities will vary by species, but may include ocular trauma associated with shipping, congenital embryonic remnants such as persistent pupillary membrane (PPM) and persistent hyaloid artery (PHA), extravasation of blood in association with a PHA, corneal opacity/dystrophy, coloboma (iris, lens, choroid), cataract, micropapilla, optic nerve hypoplasia, and retinal dysplasia [1, 7–9, 11, 16–28]. PPM and PHA are commonly observed in rodents (Figs. 10 and 14) and less frequently in the beagle (Figs. 15 and 16). In general, they are noted and graded. However, if they result in significant opacity of the cornea, lens, or vitreous at pretest examination elimination of the affected animal from study should be considered. If a persistent hyaloid has a significant component of extravasated blood, elimination from study is advised (Fig. 14).

Whenever possible, animals with ocular abnormalities are eliminated from inclusion in the study. This is especially true if the lesion may be progressive during the study. However, some abnormalities are so common as to preclude elimination. The most common example of this would be corneal dystrophy in the rat and mouse [23, 29–38]. The prevalence of corneal dystrophy varies by stain, age, and sex of the rat. In the Sprague–Dawley, males are more commonly affected than females and prevalence will increase with age and may vary from 20 to 50 % (Fig. 17). In the Wistar, corneal

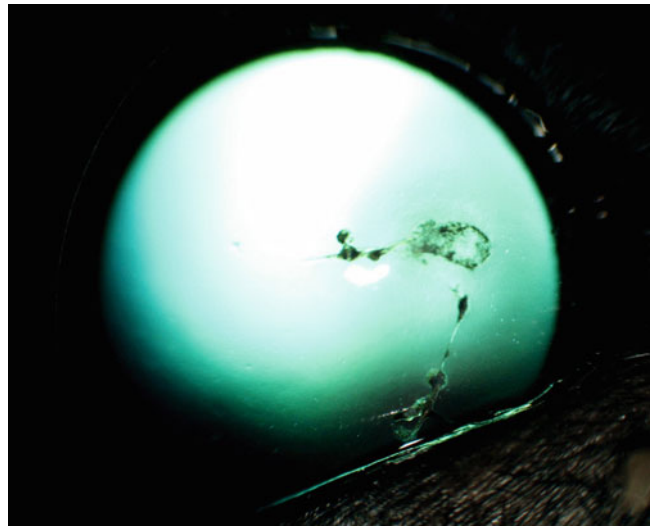

Fig. 15 A persistent pupillary membrane with attachment to the corneal endothelium is observed using retroillumination in a beagle

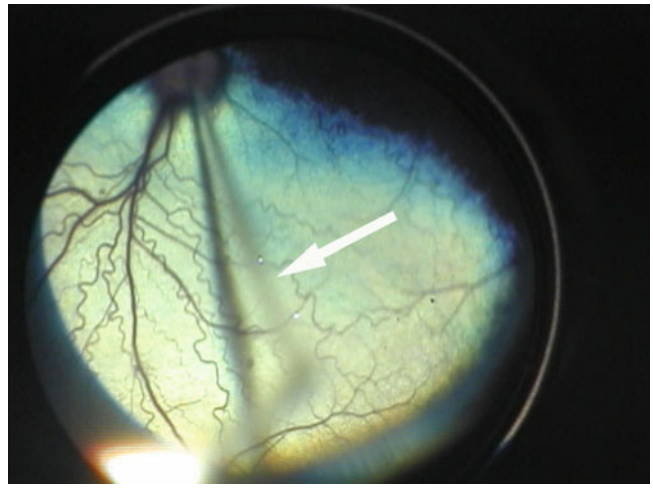

Fig. 16 A persistent hyaloid remnant (*arrow*) extends from the optic nerve to the posterior lens in a beagle

dystrophy has been reported to affect >65 % (Fig. 18) and in the Fisher 344 (Fig. 19), the prevalence is generally 100 %. As a result of such a high prevalence, affected animals cannot be eliminated from inclusion in study. However, animals affected with corneal dystrophy with a severity grade of greater than slight or with secondary keratitis should be eliminated at pretest. Corneal dystrophy lesions are also observed in other species including the beagle (Fig. 20) and Dutch Belted rabbit (Fig. 21). It should also be noted that lesions such as corneal dystrophy can be exacerbated in both

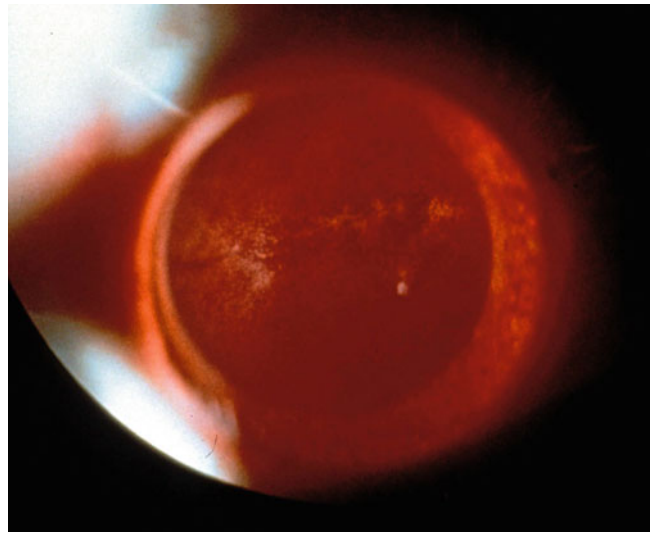

Fig. 17 Corneal dystrophy in a Sprague–Dawley rat

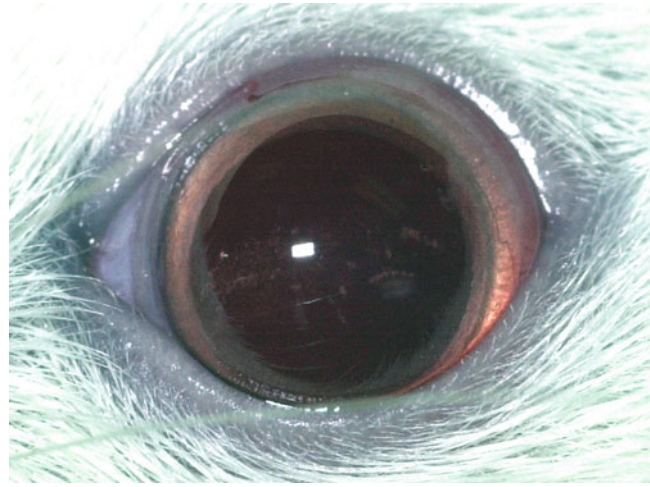

Fig. 18 Corneal dystrophy in a Wistar rat

prevalence and severity by corneal exposure caused by desiccation, sedation/anesthesia, orbital bleeding, environmental irritants, and the test article itself.

2.4 Species-Specific Ophthalmic Findings

The ophthalmologist must be familiar with what is normal for the species in question and what are the common spontaneous abnormalities for that species, age of animal, and breed/strain [39]. Common laboratory species of interest may include mice, rats, guinea pigs, cats, rabbits, dogs, Gottingen mini-pigs, and NHPs (cynomolgus, rhesus). For the common laboratory species

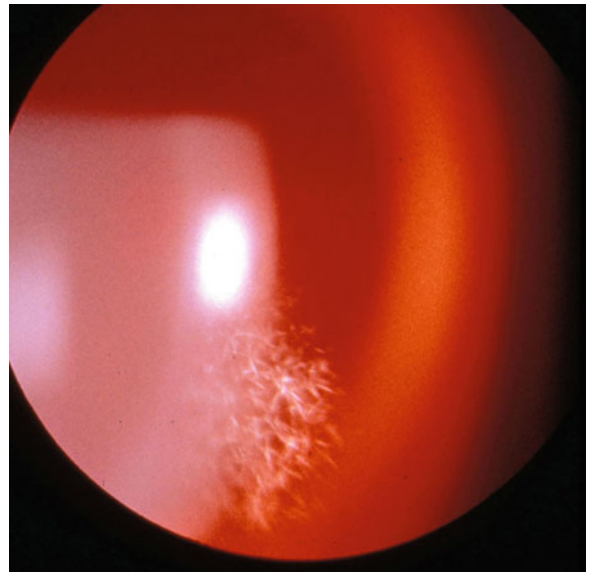

Fig. 19 Corneal dystrophy in a Fisher 344 rat

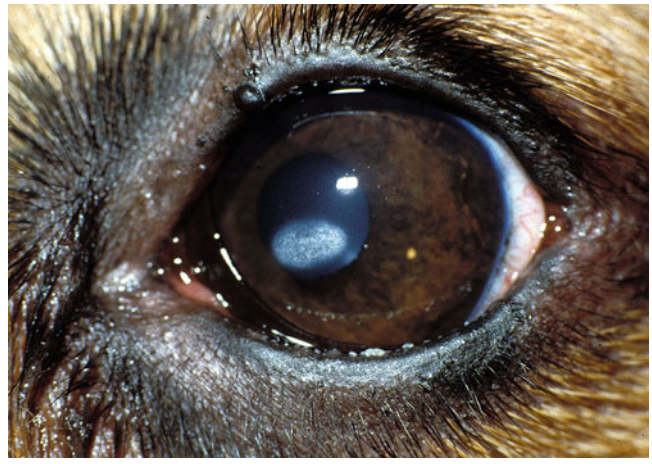

Fig. 20 Corneal dystrophy in a Marshall Farms beagle

examined, the type of retinal vasculature includes anangiotic (guinea pig), merangiotic (rabbit), and holangiotic (rodent, feline, canine, swine, primate) (Figs. 22, 23, 24, 25, 26, 27, 28, and 29). An albinotic vs. pigmented eye may be a factor in clinical findings as well as response to mydriasis or test article effect. The presence of a nictitating membrane (third eyelid), number, location, and type of lacrimal/orbital glands, location and number or lacrimal puncta, presence or absence of a tapetum, myelination of the optic nerve, anatomy and physiology of aqueous outflow, and whether the animal has a fovea should all be understood and considered [39].

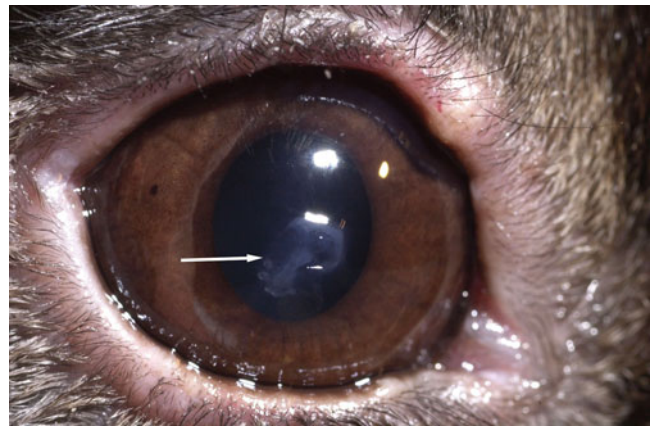

Fig. 21 Corneal dystrophy in a Dutch Belted rabbit (*arrow*). This was not present in pretest examination, but appeared during the study

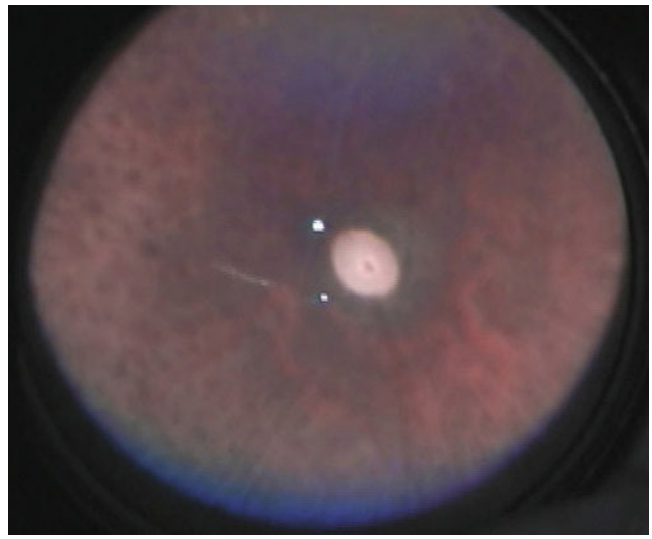

Fig. 22 Normal anangiotic fundus of the guinea pig

For intraocular procedures or intraocular dosing the specific size of the globe and the size and volume of all intraocular structures and the aqueous and vitreous must be understood in order to avoid inadvertent trauma during the procedure [39]. For topical ophthalmic dosing, the volume capacity of the conjunctival cul-de-sac and volume of the pre-corneal tear film must be known.

In addition, the examination and restraint techniques, need for sedation, type of biomicroscope and indirect ophthalmoscope, diopter power of the indirect lens, and number of animals that can be examined in the time period that the mydriatic effect will persist must be understood. The role of the veterinary

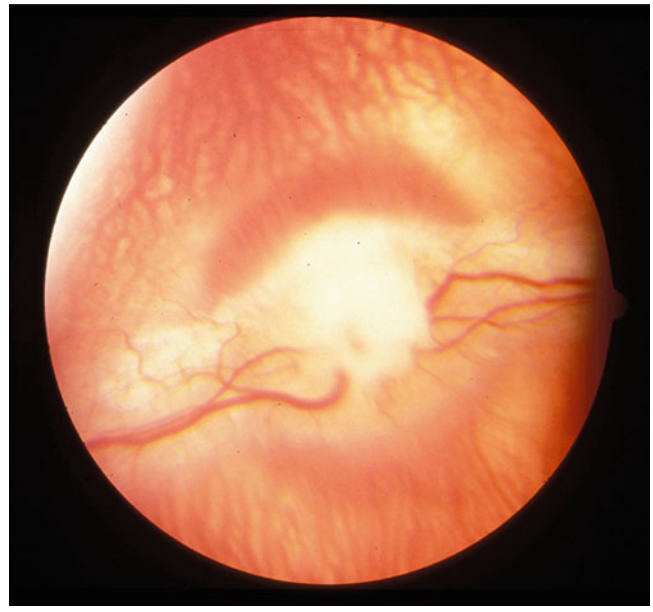

Fig. 23 Normal merangiotic fundus of an albinotic New Zealand white rabbit

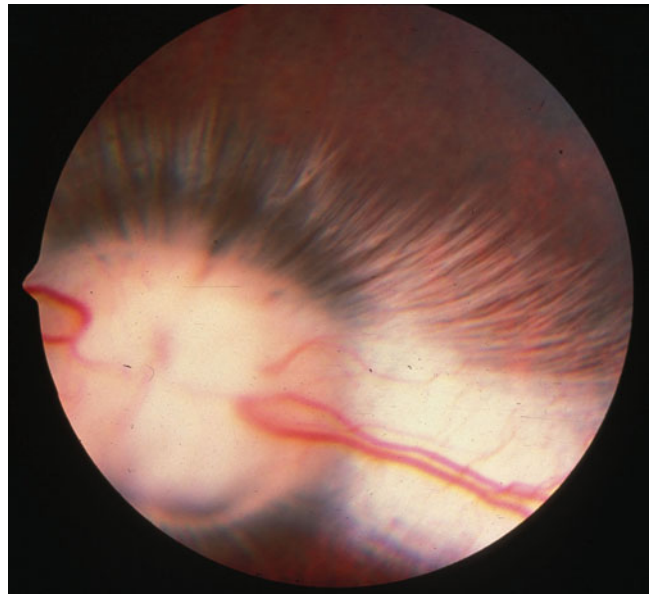

Fig. 24 Normal merangiotic fundus of a pigmented Dutch Belted rabbit. Note the prominent myelinated medullary rays and optic cup

ophthalmologist is to perform a pretest examination designed to eliminate those animals not suited to the study and to establish a baseline database to compare to. Animals are then subsequently examined one or more times during and at the conclusion of the

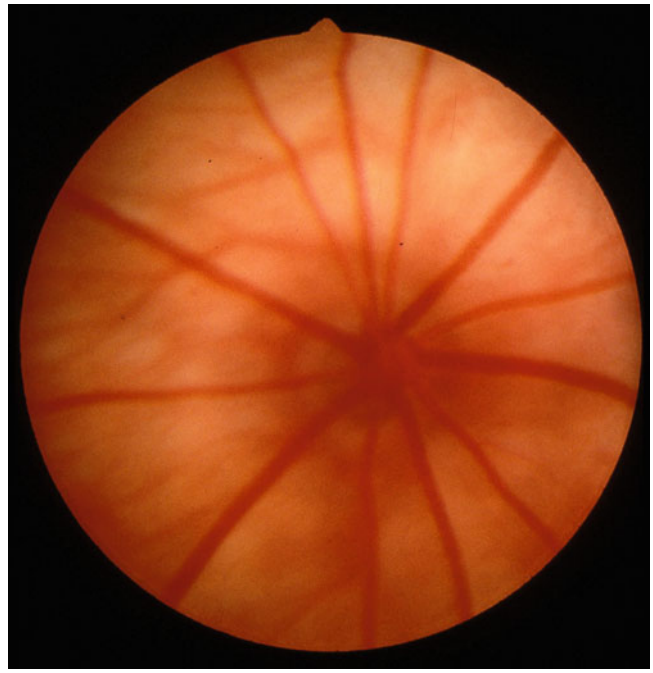

Fig. 25 Normal holangiotic fundus of an albinotic Sprague–Dawley rat

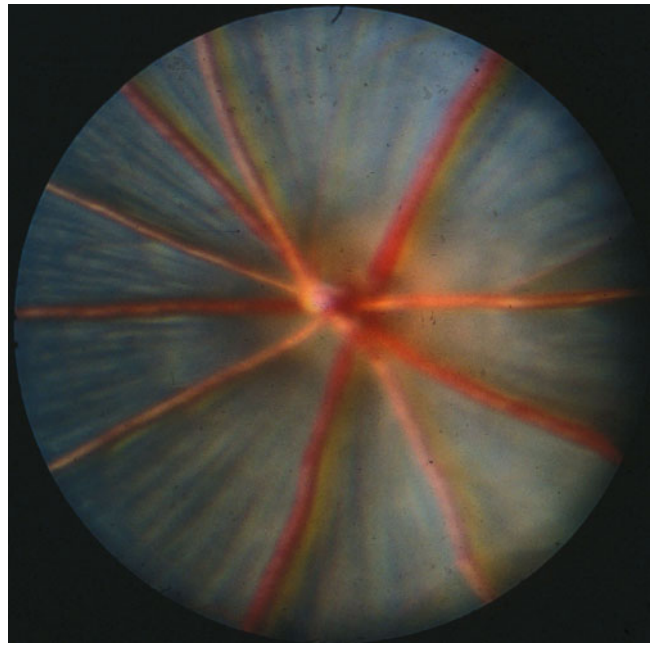

Fig. 26 Normal holangiotic fundus of a pigmented Long–Evans rat

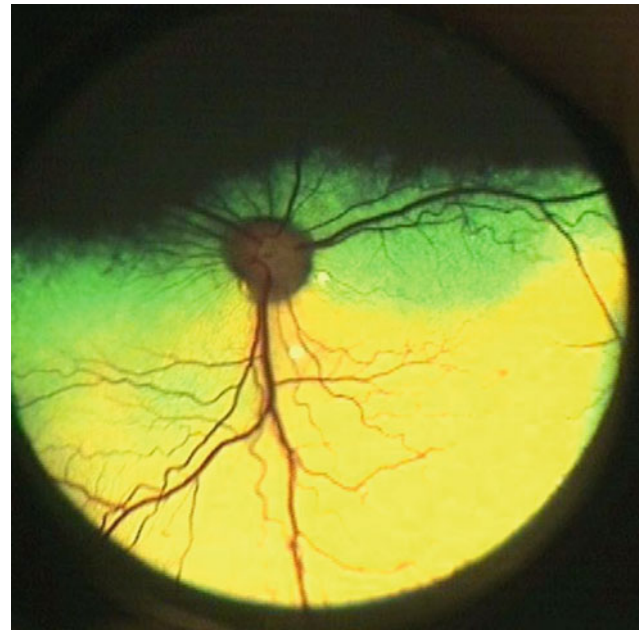

Fig. 27 Normal holangiotic fundus of a beagle. Note the yellow-green tapetum which appears ventral as the indirect image is inverted and reversed

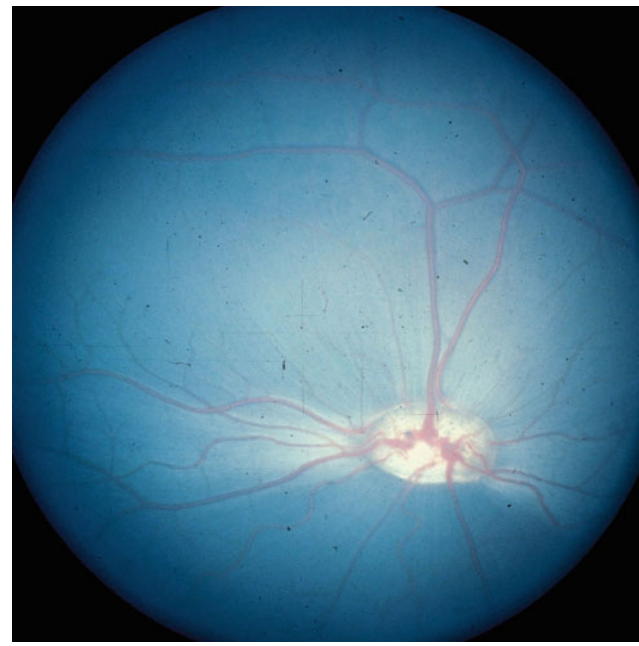

Fig. 28 Normal holangiotic fundus of a Gottingen pig. Observe the lack of a tapetum

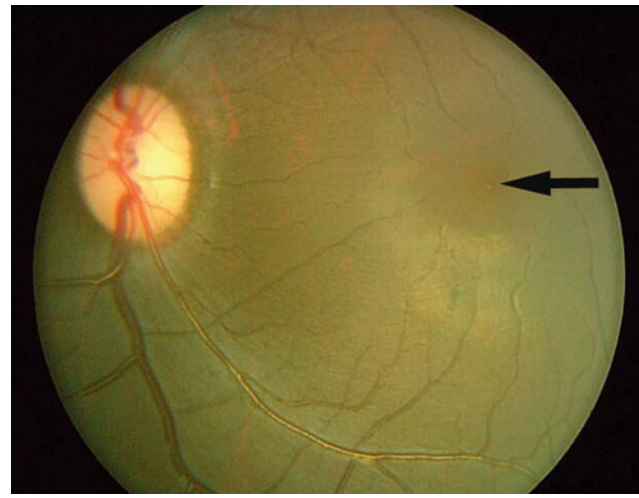

Fig. 29 Normal holangiotic fundus of a cynomolgus monkey. The fovea is denoted by the *arrow*

study depending on the study duration. Typically studies are divided into acute, subacute, subchronic, and chronic depending on the duration. The ophthalmologist must then interpret findings in light of the species examined, pretest data, compound evaluated, and dose group outcome.

2.4.1 *Beagle*

The beagle is a canine like any other and most veterinary ophthalmologists are both familiar and comfortable with the examination and abnormalities of this breed [16–18, 23]. The breed-related abnormalities will vary according to the supplier and are often seen with a prevalence that waxes and wanes according to the current sires and dams. For example in the author's experience, micropapilla is more common in a specific supplier's beagles, but rare in beagles from other suppliers. Keep in mind that these are tightly controlled breeding programs to yield a beagle with specific characteristics and their ocular lesions while similar in kind may differ in prevalence from the pet beagles seen in a clinical setting. In the author's experience, prolapse of the gland of the nictitans (Fig. 30), retinal dysplasia (folds) (Figs. 31 and 32), optic nerve micropapilla/hypoplasia (Fig. 33), and incipient posterior cortical or anterior suture cataract (Figs. 6, 34, and 35) are the most common breed-related findings in the laboratory beagle [17, 18, 21, 40]. These are similar to the previously reported prevalence of ophthalmic findings in 8–10-month-old beagles (Table 7) [17]. Corneal dystrophy is also observed in the laboratory beagle [19, 20]. It should be noted that corneal dystrophy, cataracts, and retinal degeneration may also be noted as a treatment-related finding (Figs. 36, 37, and 38).

The beagle has a holangiotic retina and, unlike most laboratory animal species, has a tapetum located in the superior choroid

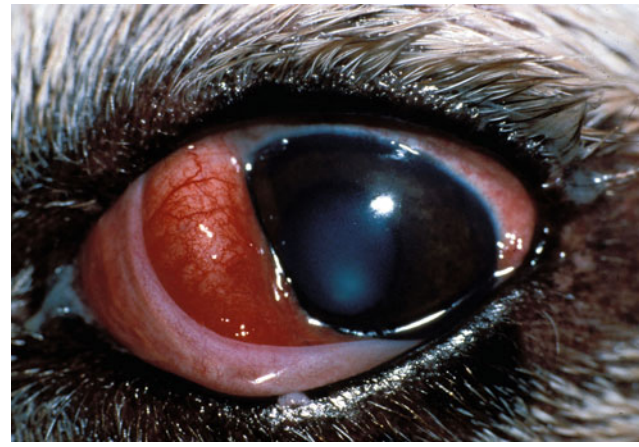

Fig. 30 Prolapse of the gland of the nictitans with associated mucoid epiphora, conjunctival hyperemia, and a focal corneal opacity

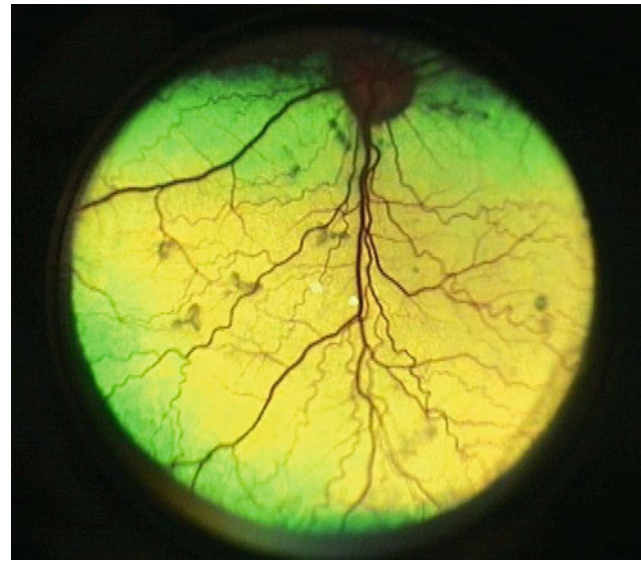

Fig. 31 Multiple, focal retinal folds are observed in the tapetal fundus. They appear as linear hyporeflective areas obscuring the underlying tapetum

(Fig. 27). The tapetum is cellular in nature and is located between the large choroidal vessels and the choriocapillaris in the dorsal aspect of the fundus. The tapetal color is variable with blue, green, orange, and yellow the most common variations. There are also atapetal beagles, usually color dilute, which are reported to have tapetal cells that are deficient in tapetal rodlets [41]. The tapetum can have a role in ocular toxicity [42] and in such instances atapetal beagles can be used. The tapetum of the dog contains a high concentration of zinc and as such zinc chelators may result in

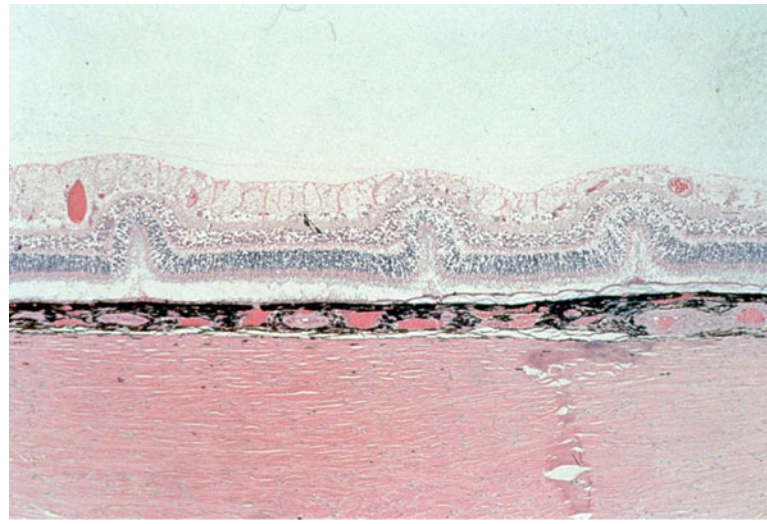

Fig. 32 Histopathology of retinal folds. H&E 100×

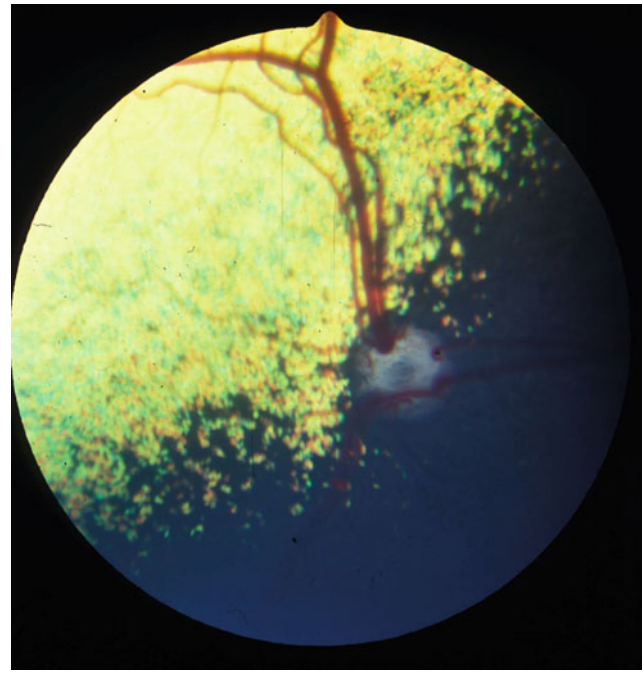

Fig. 33 Optic nerve micropapilla is present as a congenital lesion in a beagle

tapetal necrosis and secondary retinopathy [43]. Additional drug-associated lens and retinal changes have also been described in the beagle (Figs. 37 and 38) [44, 45].

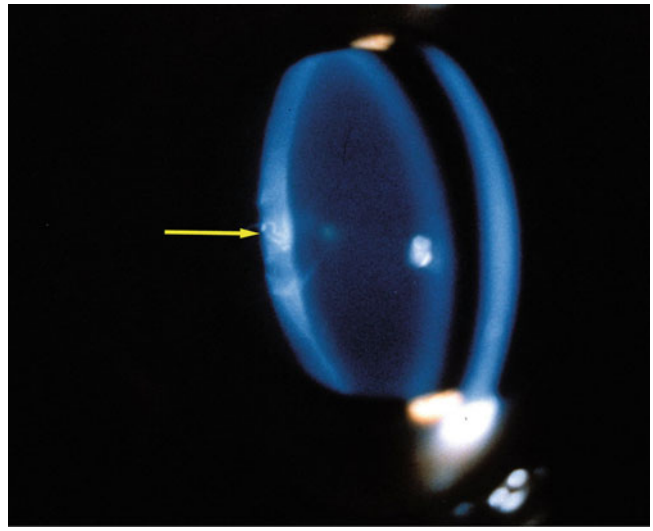

Fig. 34 An axial incipient, posterior cortical cataract is noted in pretest examination

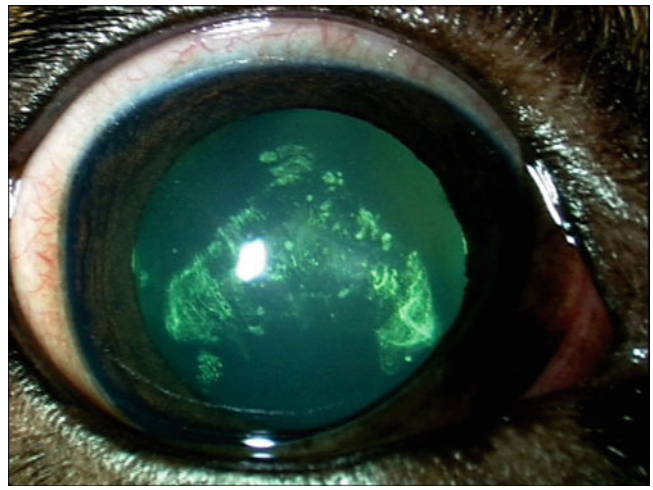

Fig. 35 An immature posterior cataract is noted in pretest examination

2.4.2 Rat/Mouse

In general, spontaneous ocular lesions in rodents may include microophthalmos, buphthalmos, corneal dystrophy, keratitis, persistent hyaloid (PH) (with or without hemorrhage), PPM, peripheral anterior synechiae, cataract (often nuclear), retinal hemorrhage, coloboma, saccular aneurysm of retinal vessels, and choroidal and optic nerve coloboma as the most common species-associated findings in laboratory rats and mice (Figs. 39 and 40) [9, 23, 27, 29, 35, 46–51].

Table 7
Summary of findings in 8–10-month-old laboratory beagles [17]

Finding	# dogs (479 examined)	# dogs (479 examined)
Prolapsed gland	4	0.83
Corneal opacity	7	1.46
Persistent pupillary membrane	1	0.21
Lens cortical vacuoles/opacity	23	4.8
Fetal nucleus opacity	7	1.46
Prominent nucleus	2	0.42
Peripheral capsular opacity	1	0.21
Prominent posterior lens sutures	25	5.23
Posterior capsular opacity	27	5.64
Persistent hyaloid remnant	15	3.13
Tapetal scar/pigment	13	2.71
Micropapilla	22	4.59

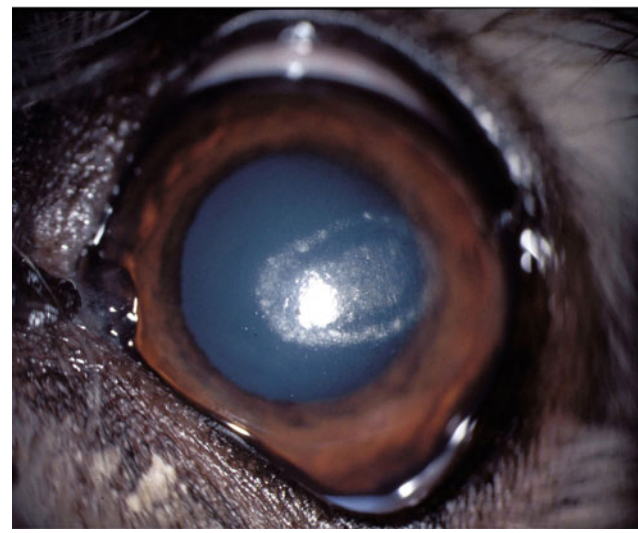

Fig. 36 Corneal opacity with a dystrophy-like appearance secondary to a treatment-related effect

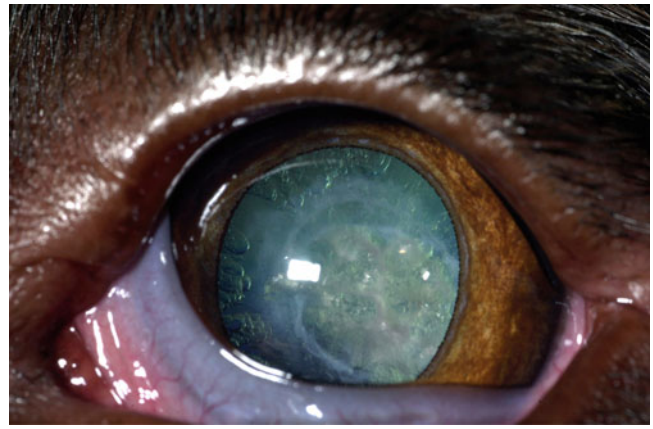

Fig. 37 Acute-onset, immature anterior and posterior cortical cataract with vacuolization secondary to a treatment-related effect

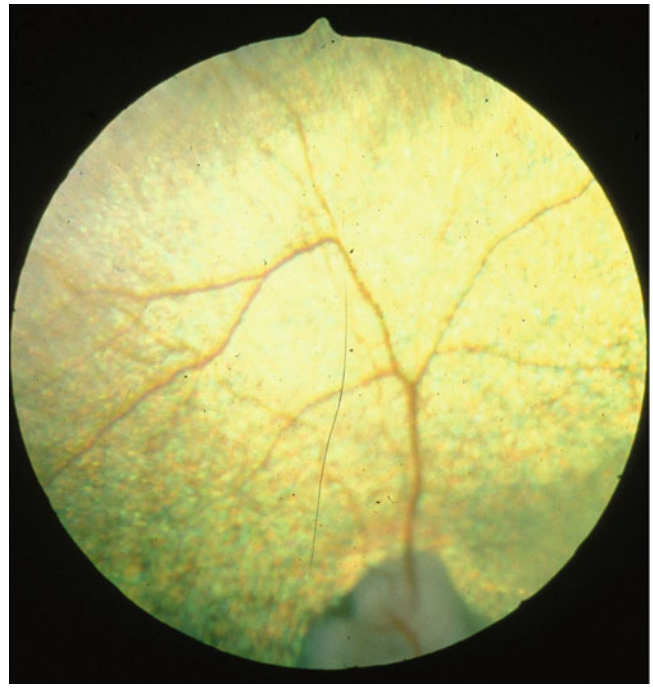

Fig. 38 Retinal degeneration characterized by vascular attenuation, tapetal hyper-reflectivity, and optic nerve pallor secondary to a treatment-related effect

In general, young animals are more likely to have PH or PPM (Figs. 10, 14, and 41). In one report the prevalence of hyaloid remnants may be as high as 60 % at 6 weeks of age, but decrease to less than 17 % by 1 year of age [26, 52, 53]. In mice, the prevalence of a PH has been reported to range between 28 and 32 % with a

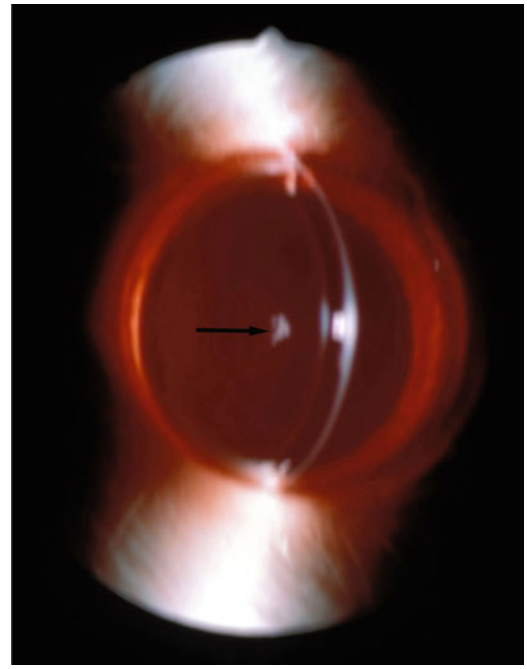

Fig. 39 A focal, incipient cataract (*arrow*) is noted in pretest examination

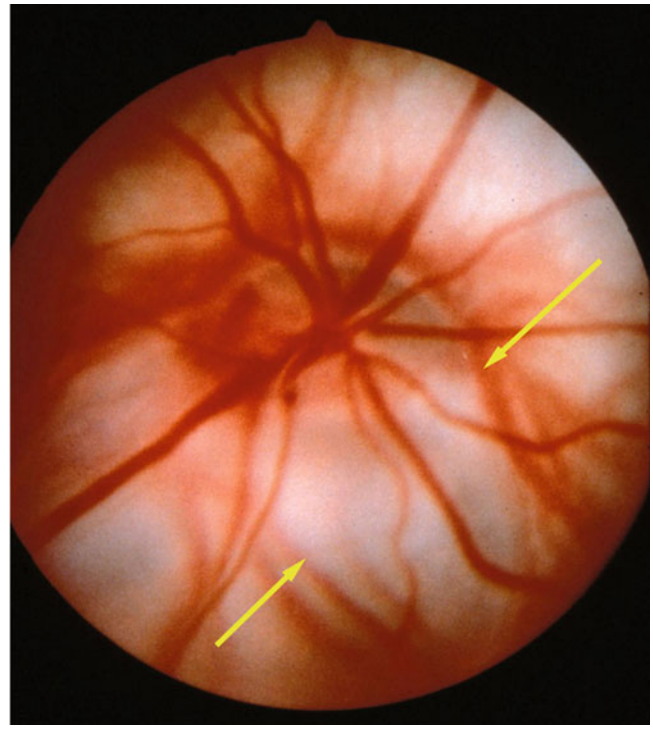

Fig. 40 A coloboma involving the optic nerve and adjacent structures is noted in pretest examination (*arrows*)

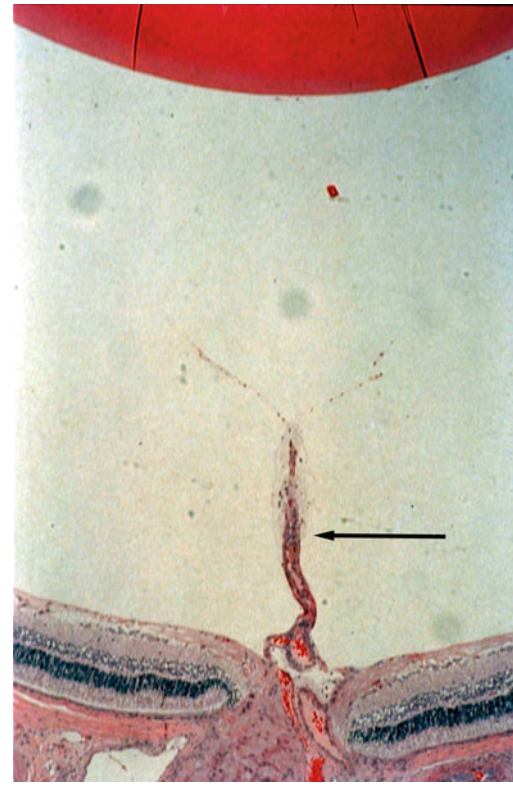

Fig. 41 Histologic section of the posterior globe in a rat demonstrating a persistent hyaloid remnant (*arrow*)

higher prevalence in females [48]. On a pretest examination, animals with PPM/PH and associated hemorrhage (vitreous, aqueous) should typically be eliminated from the study if possible (Fig. 14). Anterior synechia, microophthalmos, and anterior cleavage anomalies are also occasionally seen as congenital ocular anomalies in rats and mice.

The term corneal dystrophy implies a bilateral, noninflammatory inherited, degenerative disorder and is a specific term. Unfortunately, the terms corneal opacity or corneal crystals are also used and are less specific and more general terms. The term calcific band keratopathy has also been applied to the lesions observed in rodents [43]. Clinically, the lesions of corneal dystrophy most commonly appear as fine granular or linear opacities in the nasal and axial palpebral fissure (Figs. 17–19). They are most frequently bilateral. Histologically, corneal dystrophy in rodents is typically a basement membrane, anterior stromal corneal defect resulting in deposits of mineral and phospholipid in and adjacent to the epithelial basement membrane (Fig. 42) [34, 43]. Corneal dystrophy, while common in a pretest examination, will be observed to increase in prevalence and severity with age. Corneal dystrophy is common in rats and less

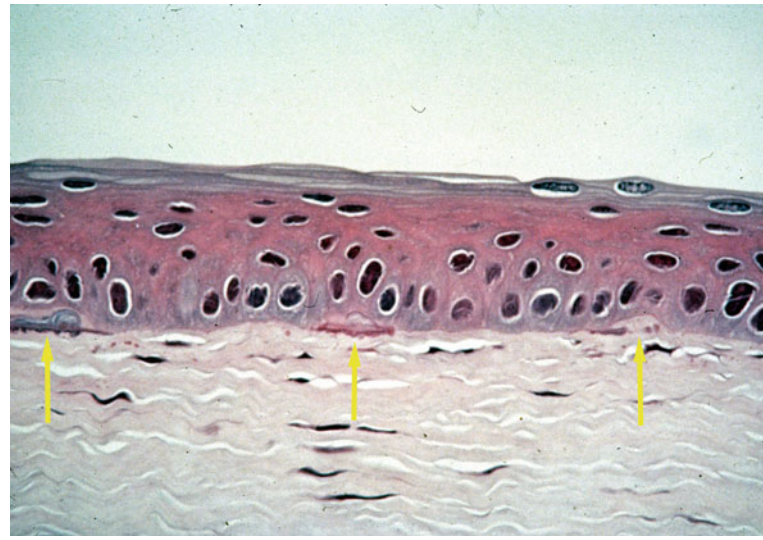

Fig. 42 Histologic section of the anterior cornea of a Sprague–Dawley rat with corneal dystrophy. The areas of basement membrane thickening, disorganization, and mineralization are indicated by the *arrows*. H&E 100×

common in mice, and varies in prevalence, appearance, and severity by strain of rat/mouse, sex, age, and shipment and supplier. It is more prevalent in males and increases in prevalence and in some species severity with age [23]. The prevalence will vary from 5 to 100 % [30–36]. It has been variously termed corneal dystrophy, calcific keratopathy, corneal opacity, corneal calcification, and spontaneous corneal degeneration. It has also been reproduced experimentally in rats by corneal desiccation which can occur secondary to dehydration, a decrease in blink rate, sedation, or anesthesia. As it increases in severity with age it can be associated with secondary keratitis (typically nasal), especially in the Fischer 344 rat (Fig. 43). In addition, keratitis can occur secondary to anesthesia, corneal exposure, keratoconjunctivitis sicca, sialodacryoadenitis (SDA), environment (dust, irritants), and conjunctivitis.

Cataracts may be seen during pretest screening and are most often nuclear (Fig. 39). They can be unilateral or bilateral and if possible, these animals are eliminated from the study. Posterior cortical cataracts are also seen, often associated with a PH with or without hemorrhage. Cataracts may also be spontaneous, associated with age, secondary to retinal degeneration, or associated with trauma, anesthesia, or other external factors (Fig. 44) [29, 54–56]. Acquired cataracts may also be treatment related and the result of a toxicologic effect. The examiner should always ascertain if orbital bleeding was performed during the study in question, especially if the cataracts are unilateral and typically in the same eye within the study animals. Reversible lens opacities may be

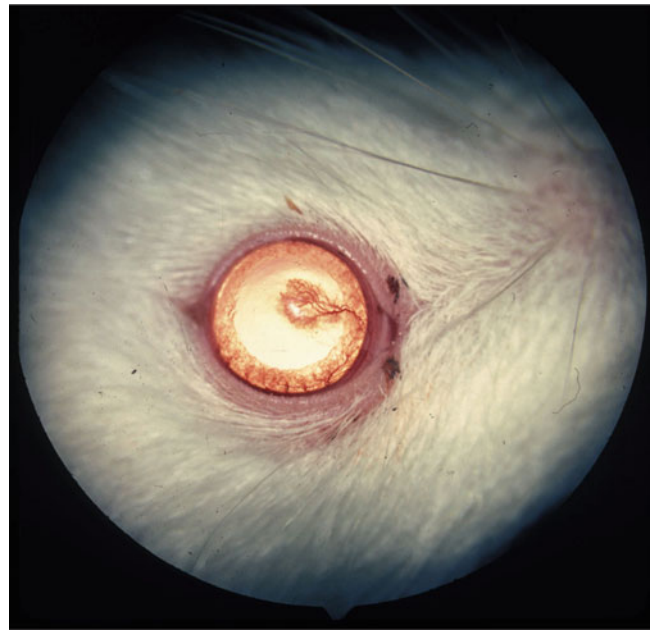

Fig. 43 Nasal corneal vascularization and keratitis in a Fisher 344 rat secondary to corneal dystrophy

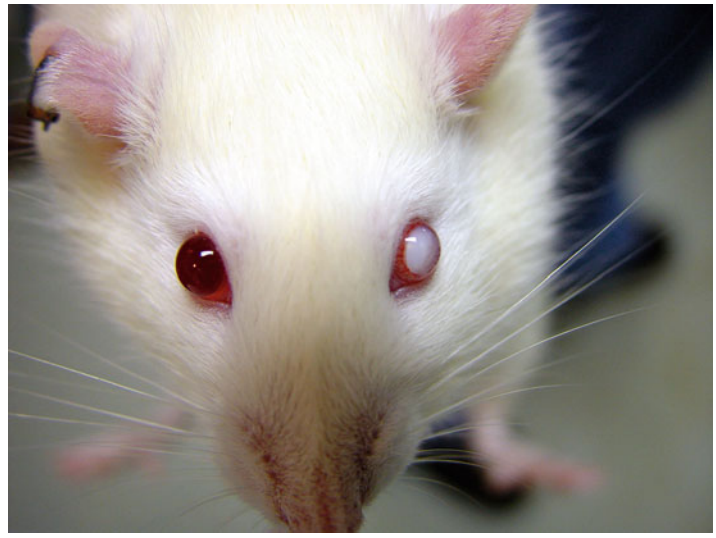

Fig. 44 A unilateral, mature cataract is seen secondary to the trauma of orbital bleeding

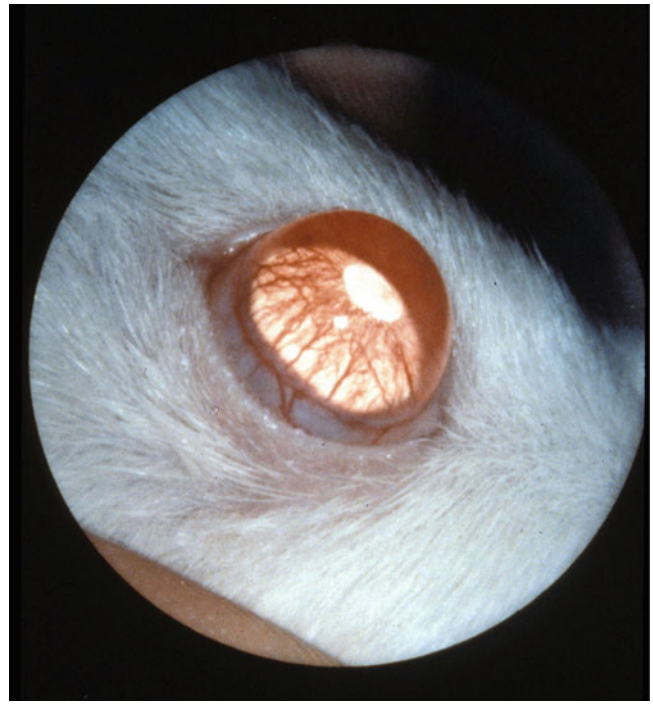

Fig. 45 Slit lamp examination of a rat with buphthalmos noted in pretest examination

induced in the rat by temperature (cold), dehydration, anoxia, and specific drugs (opiates, opioids, phenothiazine) [53, 57, 58].

Buphthalmos and glaucoma have been observed and are typically congenital (Fig. 45) [49]. Unfortunately, the intraocular pressure is generally not determined in affected animals as this is most often noted in young rats in a pretest examination and they are usually eliminated from study without further diagnostics or follow-up.

Retinal dysplasia may be noted in rats and can be unilateral or bilateral [27]. Retinal degeneration may occur spontaneously, be associated with aging, occur as a result of orbital bleeding techniques, occur secondary to phototoxicity, or be inherited [27, 47, 59–66]. It is reported that the prevalence of senile retinal degeneration in the 2-year-old Wistar rat may be as high as 10 % [60]. In addition, retinal degeneration can be a toxicologic effect [53]. Care should be taken to evaluate retinal "blanching" in combination with the temperament and restraint required to examine a particular animal. Excessive restraint will result in apparent retinal degeneration.

As rats age, the prevalence of corneal, lenticular, and retinal abnormalities will increase and in a 2-year chronic study abnormalities may be found in >50 % of the animals examined [29, 38].

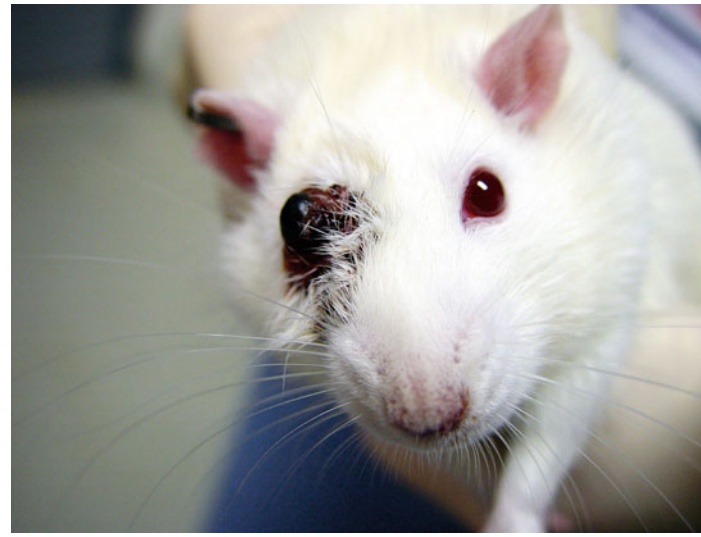

Fig. 46 Marked exophthalmos and exposure keratitis are observed secondary to the trauma of orbital bleeding

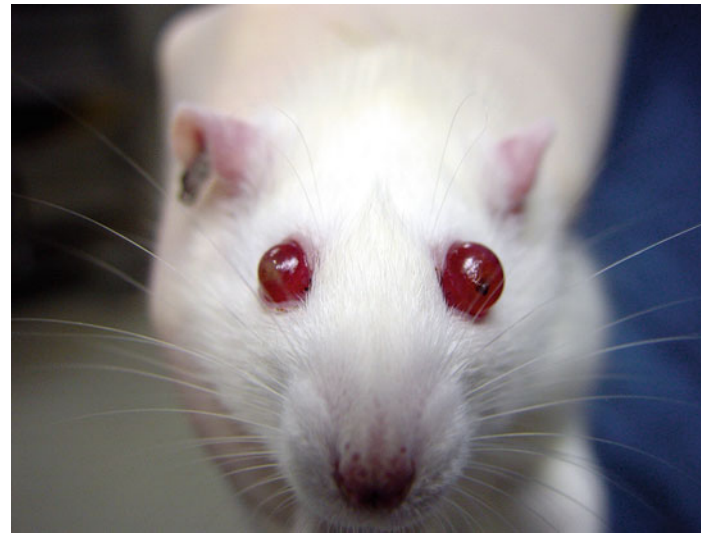

Fig. 47 Bilateral exophthalmos secondary to orbital bleeding

There is also often a sex predilection with corneal and possibly lens abnormalities seen more often in males than females.

The technique of orbital bleeding may be used to collect venous blood from the retrobulbar sinus of rodents during a study [8, 9]. Most often one side is preferred, usually the right. The side effects of orbital bleeding can be severe and include exophthalmos, corneal rupture, exposure keratitis, retinal degeneration, hyphema, cataract, and phthisis bulbi (Figs. 44, 46, 47, and 48). The frequency of these

Fig. 48 Unilateral phthisis bulbi secondary to orbital bleeding

complications likely relates directly to the skill of the individual performing the procedure. However, complications are frequent enough that the technique should be discouraged, especially in studies that require ophthalmic examination.

If a rodent study requires general anesthesia the ophthalmologist can expect exacerbation of corneal dystrophy and in some instances cataract formation. Xylazine has been incriminated as being cataractogenic in one study [58].

Chromodacryorrhea appears as a red-brown periorbital discharge (Fig. 49) [8, 9, 47]. Ocular irritation, respiratory infection, stress, and SDA (coronavirus) can all result in chromodacryorrhea [67]. Irritation can occur from something as simple as a diet formulation change in an effort to get a test article into the feed.

Sprague–Dawley

Corneal dystrophy is common affecting 20–50 % of animals in the author's clinical experience. This is higher than what is reported in the literature most likely due to the failure to use biomicroscopy in several published reports. Corneal dystrophy appears more prevalent in males than females and increases in prevalence with age [23, 35, 36, 46]. In the Sprague–Dawley, the lesions of corneal dystrophy involve the axial cornea and appear as fine granules or crystals in the subepithelial stroma (Fig. 17). A basement membrane dystrophy and calcium deposits are noted histologically and ultrastructurally (Fig. 42). The severity is generally mild, keratitis is uncommon, and prevalence increases with age. Rats as young as a few weeks of age can be affected.

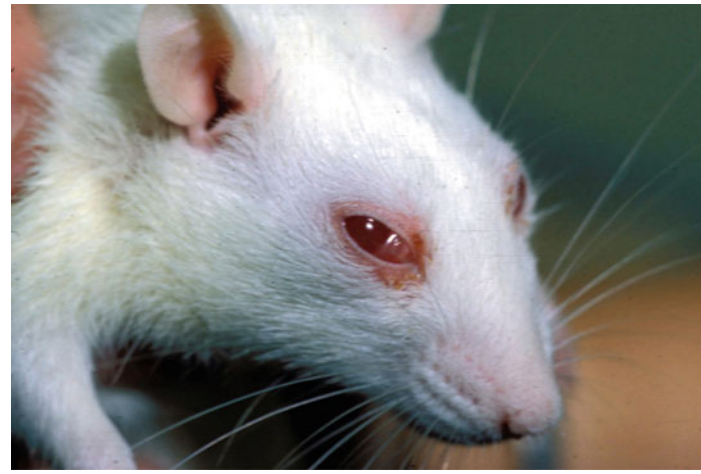

Fig. 49 Conjunctivitis and chromodacryorhea in a Sprague–Dawley rat

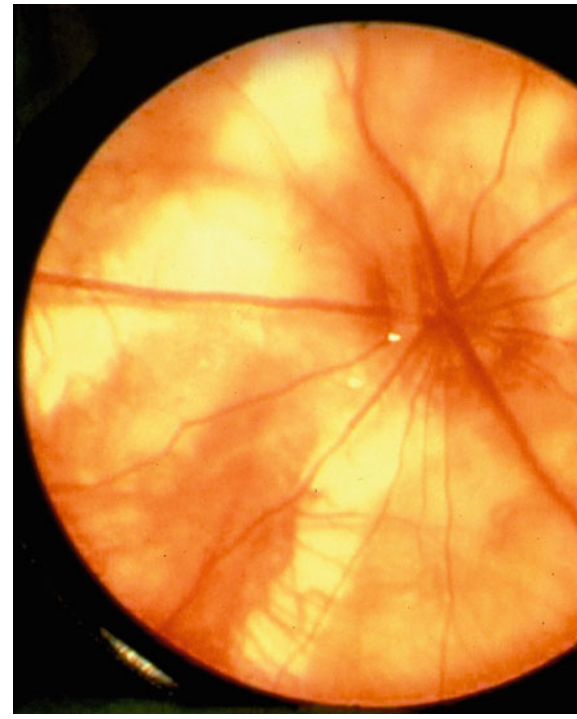

Fig. 50 Linear retinopathy in a Sprague–Dawley rat

Lens opacities in the Sprague–Dawley aged 101–134 weeks have been reported to be 18–21 % [54, 55]. A chorioretinal abnormality, termed linear retinopathy, retinochoroidal degeneration/atrophy, retinal dysplasia, or choroidal coloboma, is described in 7–10-week-old Sprague–Dawley rats (Fig. 50) [23, 27, 29, 35, 68]. Histologically the lesion is characterized by thinning of the outer

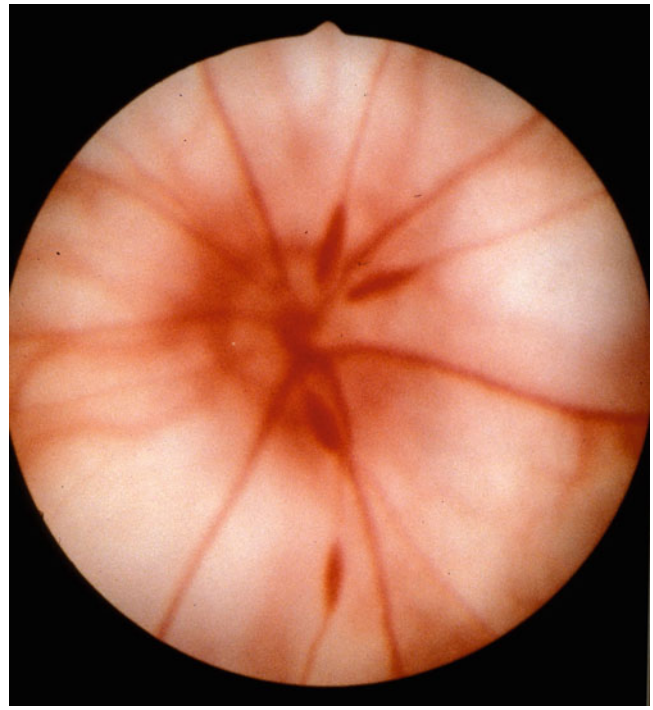

Fig. 51 Saccular aneurysms in a Sprague–Dawley rat

retina and choroid [53]. Posterior segment vascular anomalies have been described in the Sprague–Dawley and may include a preretinal vascular loop and saccular aneurysms (Fig. 51) [51]. In one study this was observed in 12 % of rats examined [27].

Fisher 344

I have never examined a Fisher 344 that did not have corneal dystrophy (Fig. 19); in my clinical experience the prevalence is 100 %. It should be noted that papers citing prevalence below this often fail to examine using biomicroscopy [33, 34]. When compared to the Sprague–Dawley the lesions in the Fischer 344 are more numerous and larger and may have a linear pattern in the axial cornea. As the Fischer 344 rat ages, the corneal dystrophy severity score is expected to increase and keratitis, corneal vascularization, and corneal ulceration may occur in association with corneal dystrophy. In addition to corneal dystrophy, in one report affected rats were also found to have basement membrane changes in other organs [34]. Corneal changes are characterized histologically by thickened, fragmented, and mineralized corneal basement membranes. These lesions have also been described to be associated with limbal inflammatory reaction which may be the precursor to the keratitis and vascularization. It should be noted that as corneal dystrophy in the Fisher 344 worsens clinically the opacities increase in size and density. The overlying epithelium will become elevated,

as the dystrophy becomes a space-occupying lesion. Histologically, the epithelium overlying a severe area of dystrophy will be thin and possibly keratinizing indicating irritation. It is this space-occupying, foreign-body effect combined with the disruption of the corneal epithelial basement membrane that results in corneal vascularization and keratitis. It has been the author's experience that in some instances dystrophy will even result in the loss of the overlying corneal epithelium exposing the underlying basement membrane and possibly the corneal stroma. Severity may be more severe in males.

Wistar

Corneal dystrophy has been reported to affect >65 % of Wistar rats and to be composed of extracellular calcium and phosphorous at the level of the corneal epithelial basement membrane (Fig. 18). In one clinical study, 40/43 males and 26/29 females were affected with an interpalpebral corneal opacity [32, 36, 37]. Histologically, the lesions are located at the level of the basement membrane and consist of calcium and phosphorus [32]. Clinically, when compared to the Sprague–Dawley rat the axial corneal lesions in the Wistar are larger, linear, and more opaque.

Another biomicroscopy study found lenticular water clefts in 5 and 13 % of 1-year-old female and male Wistar rats and in 30 and 48 % of 2-year-old female and male Wistar rats. The same study found cortical/nuclear lens opacities in approximately 20 % of 2-year-old male/female Wistars and posterior capsular opacities in up to 37 and 67 % of 2-year-old female and male Wistars [38].

A retinal dystrophy/degeneration has been reported in the Wistar rat [69]. It is reported that the prevalence of senile retinal degeneration in the 2-year-old Wistar rat may be as high as 10 % [60].

2.4.3 Rabbit

Rabbits have long been used in ophthalmic and toxicologic research for topical irritancy testing (Draize, modified Hackett–McDonald) [10, 70], contact lens evaluation, ocular pharmacology, intraocular device biocompatibility, and intravitreal injection protocols as well as in systemic toxicity studies. The most common rabbits examined in toxicologic studies are New Zealand white (NZW) and American Dutch Belted. Most rabbits are found to be normal on routine ophthalmic examination. The eye of the rabbit may be albinotic or pigmented and has a single nasolacrimal punctum, a merangiotic fundus, and deep physiologic optic disc cup with a heavily myelinated optic nerve termed a medullary ray [71].

Congenital and spontaneous sporadic findings include optic nerve coloboma, corneal dystrophy, cataract, glaucoma, epiphora, pseudopterygium, and dacryocytitis (Figs. 7, 52, 53, and 54). Corneal dystrophy has been reported in American Dutch Belted and NZW rabbit (Fig. 21) [25, 72] and can also occur as a result of diet.

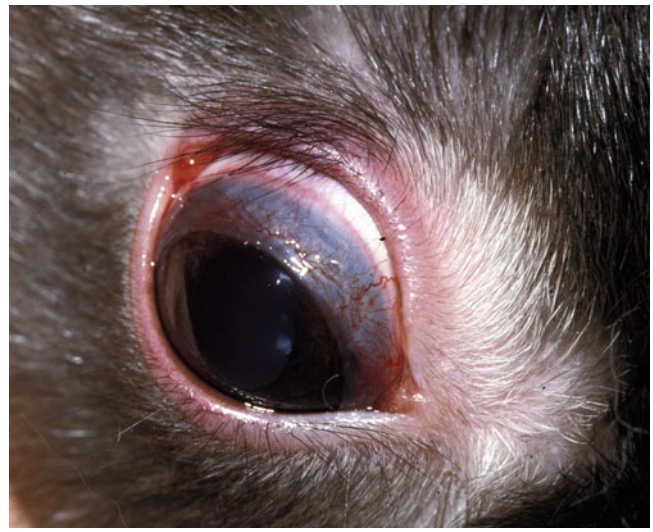

Fig. 52 Conjunctival overgrowth in a Dutch Belted rabbit

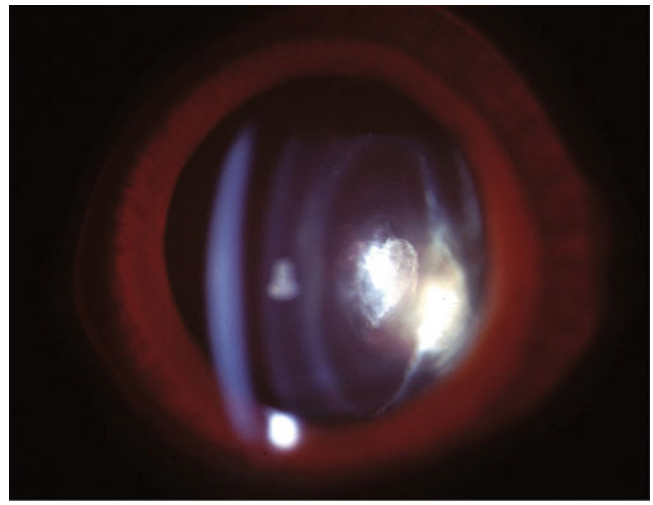

Fig. 53 Slit lamp of a posterior cortical immature cataract in a New Zealand white rabbit

In corneal dystrophy of the Dutch Belted rabbit there is a thickening of the corneal basement membrane and a thinning and disorganization of the overlying corneal epithelium [25]. In the Watanabe rabbit, altered lipid metabolism may be associated with corneal lipidosis especially when fed a high-fat diet (Fig. 55) [43, 73]. Inherited glaucoma and associated buphthalmia are seen in the NZW rabbit and occur as the result of goniodysgenesis with the mode of inheritance being autosomal recessive (bu/bu gene) [74–76]. IOP increases beginning at 1–3 months of age with resulting buphthalmia [77].

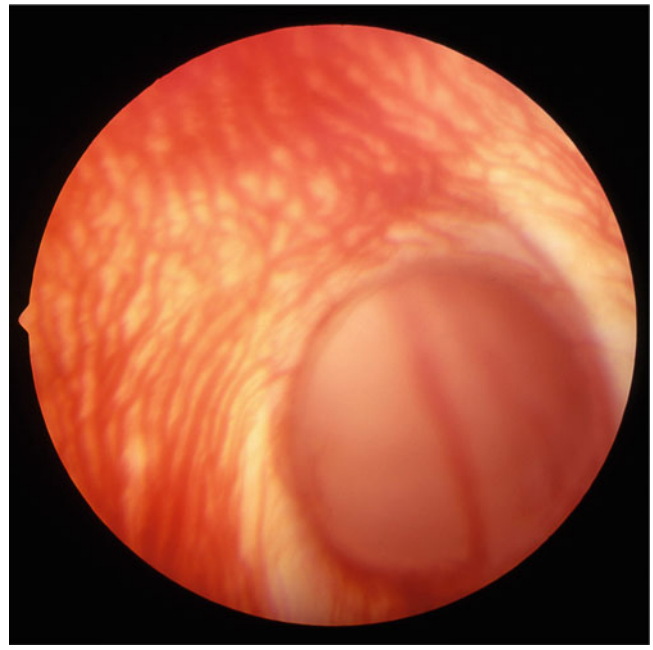

Fig. 54 A coloboma involving the optic nerve and adjacent structures is noted in pretest examination

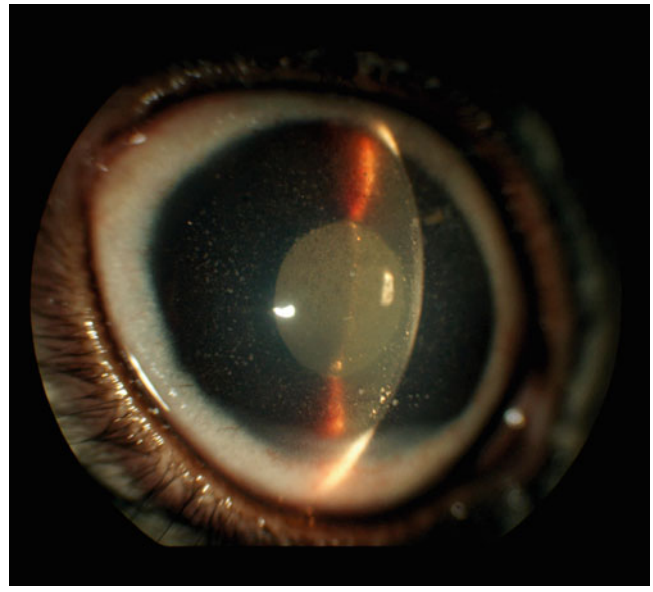

Fig. 55 Slit lamp examination of a Watanabe rabbit with lipid aqueous flare and corneal lipidosis

2.4.4 Nonhuman Primate

The use of NHPs in toxicologic research seems to have become more common over the past 10 years with cynomolgus and rhesus monkeys the most commonly examined. The ocular anatomy of the NHP is similar to that of man with a canal of Schlemm for aqueous outflow, well-developed accommodative abilities, a central retinal artery, and a fovea resulting in a visual acuity and color vision similar to humans (Fig. 29) [9, 78, 79]. As with other species, there can be variations in pigment distribution and amount in the fundus and the examiner needs to be familiar with normal variations [80].

For safety purposes, NHPs are generally examined under a short-acting general anesthetic such as ketamine with the mydriatic administered following sedation. The examiner will generally be required to wear gloves (usually double glove), a Tyvec® jump suit, surgical bouffant, and a mask. Safety goggles are advised until the animals are anesthetized and you are examining the eyes.

Traumatic lesions are most common and may include eyelid lacerations and corneal scars. In addition, traumatic cataract and retinal scars have been noted. Focal iris nevi are not considered abnormal and are observed not infrequently. In one study of 2,100 wild-caught cynomolgus monkeys 167 animals (7.95 %) had 185 findings, the majority of which involved the posterior segment [24]. The lesions of the posterior segment are predominately chorioretinal scars [28]. Glaucoma has been described in several species of NHPs [81] as has macular degeneration [82, 83] and cataract [84, 85].

3 Additional Ophthalmic Diagnostic Procedures

There are numerous noninvasive ophthalmic diagnostic techniques that, depending on the tissue of interest, can provide both structural and functional information of both the anterior and posterior segments of the eye. Some of the more common techniques are discussed below and a more detailed discussion of these and additional techniques as they apply to toxicologic, ophthalmic research and clinical application has recently been published [86, 87].

3.1 Tonometry

Tonometry is used to measure and obtain an indirect measurement of the IOP. There are several methods that are considered portable and they include indentation, applanation, and rebound tonometry. Of these, indentation tonometry, utilizing the Schiotz tonometer, would be considered inaccurate and unreliable and so it should not be used for laboratory studies. As all tonometers are originally designed for the human cornea, readings in animals may be slightly inaccurate, but provided the same tonometer is used throughout a study, the changes in IOP will still remain valid [88–97].

The IOP in most laboratory animals will range between 12 and 25 mmHg and there should be $\leq$5 mmHg difference between the two eyes. The IOP can be affected by restraint techniques, animal

Fig. 56 A Tonopen Vet® being calibrated prior to use

stress, diurnal or circadian rhythm [92, 98], eye position, sedation or anesthesia, corneal thickness, and several other variables. If possible, the IOP should be obtained for all animals at the same general time of day throughout the study and by the same examiner, using the same tonometer, same handling personnel, and same technique each time. Determination of IOP should be performed prior to pharmacologic dilation. When IOP is a critical aspect of a study, it is also advisable for the animals to be acclimatized to both the procedure and restraint techniques prior to study initiation.

Applanation tonometry is most commonly performed using the Tonpen XL®, Tonopen Vet®, Tonopen Avia®, or pneumotonograph. It requires topical anesthesia of which 0.5 % proparacaine is the most common topical ophthalmic anesthetic of choice. This technique measures the force required to applanate or flatten a given area of cornea and then converts this into an IOP value in mmHg. These tonometers have the advantage of being able to be self-calibrated for a GLP study (Fig. 56) and the pneumotonograph can also provide a hard paper copy for record keeping. The Tonpen XL and Tonopen Vet® obtain four independent readings, average them, and indicate both the IOP and the % error indicating the variability between the four readings obtained. The Tonopen Avia® obtains ten independent readings, averages them, and reports the IOP and reports the variability in readings as a % confidence. For the Tonopen XL® the % error should be <5 % and for the Tonopen Avia® the % confidence should be >95 %.

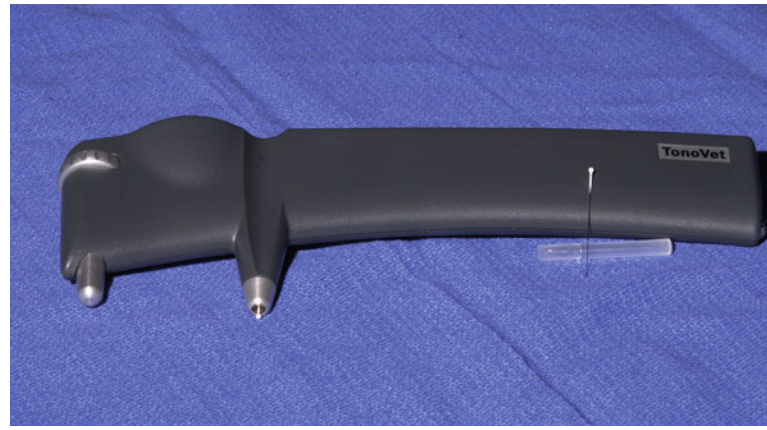

Fig. 57 A Tonovet® rebound tonometer

Rebound tonometry determines IOP by firing a small plastic tip against the cornea. The tip then rebounds back into the device creating an induction current from which the IOP is calculated. The probe must be fired at the cornea in the horizontal position, parallel to the floor to be accurate. The most common rebound tonometer for laboratory animals is the Tonovet® (Fig. 57). Unfortunately the Tonovet® is most specifically calibrated for the dog and horse, but it has been used reliably in other species [95–97, 99–103]. It has the advantage of not requiring topical anesthesia and seems to obtain the IOP more easily than the Tonpen® in many laboratory animals including the dog, rabbit, and rat. Its disadvantage is that it cannot be self-calibrated prior to use. Like the Tonopen® the Tonovet® also averages six readings and gives an indication of % error using a bar at the left of the IOP value. There should be no error bar or the bar should be at the left ventral aspect of the screen for a reading to be acceptable.

Regardless of the tonometer used, typically a minimum of 2–3 final averaged readings per eye should be obtained and recorded. As both the Tonpen® and Tonovet® give only digital readouts, the IOP must be either hand recorded or entered into a computer database as no permanent record is created by the device.

3.2 Pachymetry

Pachymetry is the evaluation of corneal thickness. It is most commonly performed by use of a contact ultrasound specifically designed for this purpose, but the corneal thickness measurement can also be obtained by high-resolution ultrasound or OCT. Pachymetry allows evaluation of subtle changes in corneal thickness prior to the appearance of clinically detectable corneal edema on biomicroscopy. The corneal thickness varies between species, but also varies by region of the cornea (axial vs. peripheral). As a result of the regional variation readings must be obtained from the same region of the cornea, usually axially, at each time point.

3.3 Fluorescein Staining

Sodium fluorescein to evaluate the cornea is routinely used in studies involving topical ophthalmic drug administration and contact lens evaluation and other studies that use the modified Hackett–McDonald scoring system [10]. Fluorescein is a water-soluble dye that is retained by the hydrophilic corneal stroma, but not by the corneal epithelium. It is used to evaluate for corneal epithelial defects and can also be used in evaluation of the pre-corneal tear film. Fluorescein is available in individual impregnated strips that are moistened at the time of use using sterile saline. The moistened strip is gently applied to the dorsal sclera taking care not to contact the cornea. The excess fluorescein is then gently irrigated from the eye using a gravity-fed stream of saline rather than a forced high-velocity stream. The eye is then examined by the ophthalmologist using a biomicroscope and the cobalt blue filter to excite the fluorescein should any remain following irrigation.

3.4 Photographic Documentation

Ophthalmic photography may be used to demonstrate a lack of change in an area like the fundus, to document abnormalities, or to monitor progression of a lesion. Serial photographs taken at various time points during a study will allow comparison to accurately establish whether an abnormality is static or progressive. As photography adds additional time, cost, and animal stress, it is not routinely performed in all studies. Rather it is in a study protocol as an option to be used to document a lesion when observed or for studies where abnormalities are more likely to occur, such as with an intraocular implant, or for intravitreal injection studies.

Photography can be divided into external and intraocular. External photography can be performed using a standard SLR digital camera with a macro lens or with a digital Kowa Genesis-D fundus camera with the diopter settings adjusted to allow external and anterior segment imaging (Fig. 58). Photography of the posterior segment requires some type of fundus camera and the digital Kowa Genesis-D camera is suitable for most routine laboratory animal photography. The Kowa Genesis-D can also be adapted for indirect ophthalmic photography, rodent fundus photography, and fluorescein angiography [61]. In addition, alternate methods for fundus photography in small rodents have been described [104–108]. The advantages of fundus photography are the ability to have a permanent stored record to compare potential study-related findings and if indicated to obtain an independent review by another ophthalmologist [86].

When obtaining photographs, the magnification and illumination settings should be standardized for all images. In addition, the eyes (left vs. right) should be photographed in the same order and all photographs should be accompanied by an animal identification photograph and a photographic log should be maintained as part of SOP.

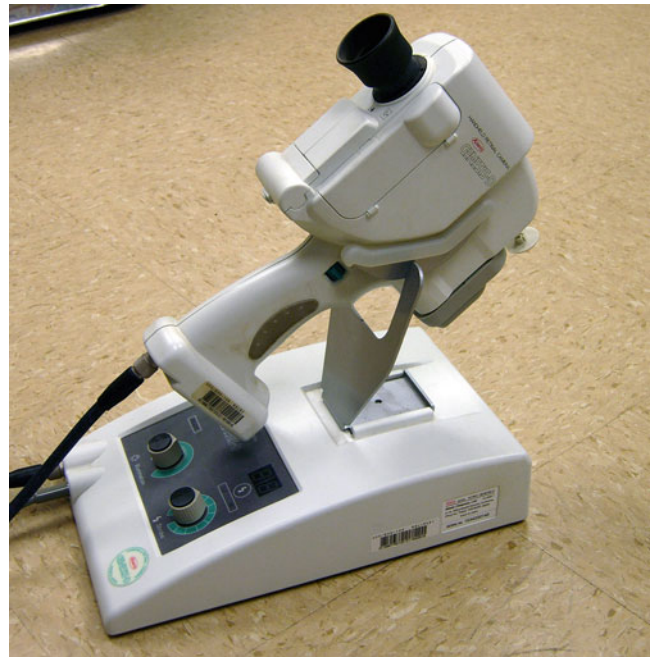

Fig. 58 A Kowa Genesis-D® fundus camera

3.5 Fluorescein Angiography

Fluorescein angiography is used to evaluate the vascular integrity of the intraocular arteriolar and venous vasculature. While it is most commonly used for examination of the retinal and choroidal vessels, it can be applied to iris vasculature as well. It is most frequently used in toxicology studies to evaluate a compounds effect on neovascularization. It has been applied to various laboratory animals with 10 % fluorescein most commonly used, but with the use of indocyanine green also described [61, 82, 108–117].

The technique of fluorescein angiography requires sedation or anesthesia and pupil dilation [115]. The excitable compound, fluorescein, is injected intravenously and a series of timed images of the tissue of interest (chorioretinal, iris) are obtained. Complications associated with fluorescein injection may include extravasation and tissue irritation, vomiting, and anyphylaxis. An excitation filter (490 nm) and a barrier filter (520–520 nm) must be used on a fundus camera that is capable of taking multiple, rapid sequenced images. The Kowa Genesis-Df is designed for fluorescein angiography and is portable. Prior to injection a baseline color image is obtained and then sequential black and white images are taken every 20 s. As the fluorescein fills the chorioretinal vasculature various phases of vascular filling are described. They include the prearteriolar, retinal arteriolar, capillary, early venous, late venous, and recirculation. Abnormalities noted on fluorescein angiography may include vascular anomalies (aneurysms, neovascularization), blocked fluorescence, leakage of fluorescein, hypofluorescence, and hyperfluorescence.

3.6 Electroretinography/Visual Evoked Potential

Depending on the toxicologic study and the specific aspect of the visual system that may be affected, there are various electrophysiologic tests that are available to evaluate the retina and visual pathways. Electroretinography (ERG) is the measurement of the electrical potential generated by the retina when stimulated by light. The standard ERG is a full-field stimulation that provides information about the retina as a whole and is a mass response of the retinal pigment epithelium, photoreceptors, and inner retinal layer [87]. For localized retinal evaluation the multifocal electroretinogram (mfERG) and for evaluation of macular ganglion cells the pattern reversal electroretinogram (PERG) are indicated [87]. To evaluate the entire visual pathway from the retina to the visual cortex, a VEP is the technique of choice [87]. Of these tests, the full-field ERG is most common for preclinical toxicologic testing. The ERG provides a noninvasive means of repeatedly assessing retinal function that in combination with indirect ophthalmoscopy and histology provides integrated assessment of retinal anatomy and function.

The ERG should be conducted in a standardized manner following pupil dilation and there are standardized protocols developed for human and canine ERGs that can serve as a study design guide [118, 119]. The conditions for obtaining a full-field ERG must be consistent with respect to room illumination, dark adaptation, flash intensity and frequency, and sedation or anesthetic used and their dosage. Discussion of these details and protocols is provided elsewhere and is beyond the scope of this chapter [87, 118, 119].

3.7 Optical Coherence Tomography

OCT is a high-resolution, noninvasive imaging technique that can provide a real-time cross-sectional imaging of ocular structures, most commonly retina and optic nerve, at an axial resolution of 2–10 μm [1, 120–122]. It can also be used to image the anterior segment of the eye. Like many advanced imaging techniques it requires sedation or anesthesia, pupil dilation, and specialized equipment. When imaged by OCT, all individual retinal layers can be seen and their thickness measured to allow a quantitative and repeated evaluation of all retinal layers over time. The optic disc can be measured with respect to the cup area, disc area, cup diameter, disc diameter, and rim area [121]. Evaluation of the anterior segment by OCT provides structural information of the cornea, anterior chamber, iris, and iridocorneal angle without the need for corneal contact as is required for ultrasound biomicroscopy (UBM) [121]. It also provides greater axial resolution than that provided by UBM [121]. The use of OCT in laboratory animals has been well described in a variety of species and its use is increasing in animal models of human disease and preclinical trials [122–136].

A recent advancement, spectral-domain OCT (SD-OCT), uses a significantly faster, nonmechanical technology than traditional OCT or time-domain OCT (TD-OCT) [122, 124, 137]. SD-OCT

simultaneously measures multiple wavelengths of reflected light across a spectrum, hence the name spectral-domain. SD-OCT is 100 times faster than TD-OCT and acquires 40,000 A-scans per second. The increased speed and number of scans translate into higher resolution.

3.8 Specular Microscopy

Specular microscopy provides in vivo, noninvasive imaging of the corneal endothelial cells [86, 138]. It can be performed using a contact or non-contact method. Once visualized, the corneal endothelial cells can be evaluated with regard to cell morphology and can be quantified as to the number of cells per mm [2]. Normal corneal endothelial cells are regular in arrangement and hexagonal in shape. Cells are evaluated for cell density, pleomorphism, and polymegethism. As cell counts vary by age of the animal and region of the cornea these variables must be standardized using animals of the same age and examining the axial cornea. Animals must be sedated or anesthetized to obtain an accurate image and automated systems are available that simplify the technique. Guidelines for specular microscopy in human FDA clinical trials have been established and these can be used as a guideline for preclinical study design [138].

3.9 Confocal Microscopy

In vivo confocal microscopy is a noninvasive method for the microscopic imaging of the living tissues that allows optical sectioning of almost any material with increased axial and lateral spatial resolution and better image contrast [86, 139, 140]. It allows in vivo, noninvasive, real-time images of the eye at magnifications (630×) which allow resolution of anatomical detail at the cellular level [139]. Three-dimensional confocal microscopy of the eye has also been described [141, 142]. Confocal microscopy has been used to image the cornea of various laboratory species including rabbits, rats, and mice [143]. Its use has also been described in dogs, cat, birds, guinea pigs, and horses [144–148]. Confocal microscopy can provide detailed imaging of the corneal architecture at the cellular level of each corneal epithelial cell layer, the epithelial basement membrane, corneal stroma including nerve fibers and keratocytes, Descemet's membrane, and endothelium [86]. While confocal microscopy is most commonly used in the clinical arena [149–153], its use may be indicated to evaluate the cornea and corneal thickness in contact lens studies, to evaluate the stromal keratocytes or corneal endothelium for toxicity, or to monitor wound healing [86, 144].

3.10 Confocal Scanning Laser Ophthalmoscopy

Confocal scanning laser ophthalmoscopy (cSLO) is an ophthalmic imaging technology that uses laser light instead of a bright flash of white light to illuminate the retina en face [154]. The advantages of using cSLO over traditional fundus photography include improved image quality, small depth of focus, suppression of scattered light, patient comfort through less bright light, 3D imaging capability,

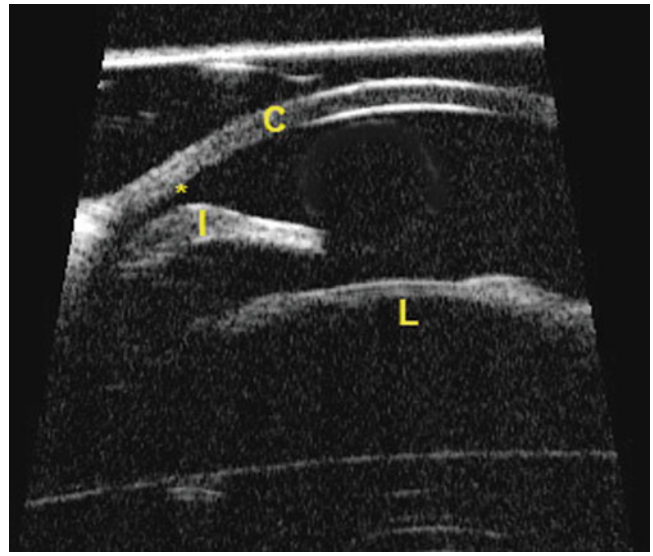

Fig. 59 Ultrasound biomicroscopy of the anterior segment of a canine eye using a 35 mHz transducer. C = cornea, I = iris, L = lens, * = iridocorneal angle

video capability, and effective imaging of patients who do not dilate well. cSLO has been used in several laboratory animal models [124]. When cSLO is combined with SD-OCT the combination provides both en face and cross-sectional imaging of the retina [124].

3.11 Ultrasound and Ultrasound Biomicroscopy

Traditional ocular ultrasound uses frequencies ranging from 7.5 to 20 mHz and is used to image the entire globe and orbit. UBM utilizes higher frequencies (35–50 mHz) to image the anterior segment of the eye, specifically the cornea, iridocorneal angle, iris, ciliary body, and lens (Fig. 59) [86, 155–157]. It can be used to determine corneal thickness, document, and monitor changes in the iridocorneal angle, ciliary cleft, angle opening distance, and anterior chamber depth in response to various pharmacologic agents and in studies of accommodation [86, 158].

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