

Molecular and Integrative Toxicology

Andrea B. Weir
Margaret Collins *Editors*

Assessing Ocular Toxicology in Laboratory Animals

 Humana Press

Molecular and Integrative Toxicology

Series Editor

Rodney R. Dietert

For further volumes:

<http://www.springer.com/series/8792>

Andrea B. Weir • Margaret Collins
Editors

Assessing Ocular Toxicology in Laboratory Animals

 Humana Press

Editors

Andrea B. Weir
Charles River Laboratories
Preclinical Services
Reno, NV, USA

Margaret Collins
Charles River Laboratories
Preclinical Services
Reno, NV, USA

Series Editor:

Rodney R. Dietert
Department of Microbiology
and Immunology
College of Veterinary Medicine
Cornell University
Ithaca, New York, USA

ISBN 978-1-62703-163-9

ISBN 978-1-62703-164-6 (eBook)

DOI 10.1007/978-1-62703-164-6

Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2012951925

© Springer Science+Business Media, LLC 2013

This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed. Exempted from this legal reservation are brief excerpts in connection with reviews or scholarly analysis or material supplied specifically for the purpose of being entered and executed on a computer system, for exclusive use by the purchaser of the work. Duplication of this publication or parts thereof is permitted only under the provisions of the Copyright Law of the Publisher's location, in its current version, and permission for use must always be obtained from Springer. Permissions for use may be obtained through RightsLink at the Copyright Clearance Center. Violations are liable to prosecution under the respective Copyright Law.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

While the advice and information in this book are believed to be true and accurate at the date of publication, neither the authors nor the editors nor the publisher can accept any legal responsibility for any errors or omissions that may be made. The publisher makes no warranty, express or implied, with respect to the material contained herein.

Printed on acid-free paper

Humana Press is a brand of Springer
Springer is part of Springer Science+Business Media (www.springer.com)

*This book is dedicated to all toxicologists,
ophthalmologists and other scientists who
have furthered knowledge in the field of
ocular toxicology.*

Preface

The goal of this text is to provide a concise reference addressing ocular anatomy and physiology across species, approaches for assessing ocular toxicity and regulatory expectations regarding ocular toxicology. The text is intended for toxicologists and other scientists involved in conducting toxicology studies for regulatory purposes and/or reviewing data from such studies.

Ocular toxicity is known to occur following intended or unintended exposure of ocular tissues to xenobiotics. It can occur following local exposure of the eye to an agent or after exposure via oral or other routes of administration. In order to define the risks that pharmaceuticals, pesticides and other toxic substances pose to the eye, an assessment of ocular toxicity is routinely included in general toxicology studies conducted for regulatory purposes. Because anatomical and physiological differences between species can impact the nature of the ocular effects observed, understanding species differences is important. Although it is possible to detect some ocular effects, such as conjunctivitis, with the naked eye, more sensitive techniques are routinely used to assess ocular toxicity. Slit lamp biomicroscopy and indirect ophthalmoscopy are routinely utilized to more closely evaluate the anterior and posterior segments of the eye, respectively, during the course of toxicology studies. In some cases, more advanced diagnostic procedures that are not routinely performed in standard studies are needed. At the time of necropsy, ocular tissues are collected and processed for histopathological evaluation. More specialized endpoints, such as electroretinography, can be incorporated, as needed. The United States Food and Drug Administration (FDA) ensures the safety of medicinal products for human and animal use, food additives, cosmetics and other products. Similarly, the Environmental Protection Agency (EPA) ensures the safety of pesticides and other products. Toxicology studies are conducted to support the safety of FDA- and EPA-regulated products. The design of those studies includes an assessment of ocular toxicity, with the nature of the assessment dependent upon the regulatory authority, nature of the product and other factors.

We began this text with a discussion of ocular anatomy across various species of laboratory animals used in toxicology studies being conducted for regulatory purposes, which lays the groundwork for subsequent chapters. The next three chapters

address ocular diagnostic techniques, with Chap. 2 focusing on techniques that are routinely included in toxicology studies and Chaps. 3 and 4 on advanced diagnostics, including electrophysiology and imaging, which are used as scientifically warranted. Chapters 5 and 6 address ocular pathology and include a detailed description of appropriate techniques used to process ocular tissues as well as lesions that can be encountered in laboratory animals. Finally, Chaps. 7 and 8 focus on the regulatory expectations from FDA, EPA and other agencies for assessing ocular toxicity.

Acknowledgements

We would like to acknowledge the contributors for their efforts in creating this text. Each of the contributors has a full time career, and projects such as this one place even more demands on their limited time.

Contents

1	Comparative Ocular Anatomy in Commonly Used Laboratory Animals	1
	Mark Vézina	
2	Assessment of Ocular Toxicity Potential: Basic Theory and Techniques	23
	Robert J. Munger and Margaret Collins	
3	Emerging Imaging Technologies for Assessing Ocular Toxicity in Laboratory Animals.....	53
	T. Michael Nork, Carol A. Rasmussen, Brian J. Christian, Mary Ann Croft, and Christopher J. Murphy	
4	Emerging Electrophysiological Technologies for Assessing Ocular Toxicity in Laboratory Animals.....	123
	James N. Ver Hoeve, Robert J. Munger, Christopher J. Murphy, and T. Michael Nork	
5	Toxicologic Pathology of the Eye: Histologic Preparation and Alterations of the Anterior Segment.....	159
	Kenneth A. Schafer and James A. Render	
6	Toxicologic Pathology of the Eye: Alterations of the Lens and Posterior Segment.....	219
	Kenneth A. Schafer and James A. Render	
7	Nonclinical Regulatory Aspects for Ophthalmic Drugs	259
	Andrea B. Weir and Susan D. Wilson	

**8 Ocular Toxicity Regulatory Considerations for Nondrug
Food and Drug Administration (FDA) Products
and the Environmental Protection Agency (EPA) 295**
Christopher Bartlett

About the Editors 307

Index 309

Contributors

Christopher Bartlett, Ph.D. SciMetrika LLC, Durham, NC, USA

Brian J. Christian, Ph.D., DABT Covance Laboratories, Madison, WI, USA

Margaret Collins, M.S. Charles River Laboratories, Preclinical Services, Reno, NV, USA

Mary Ann Croft, M.S. Department of Ophthalmology and Visual Sciences, University of Wisconsin-Madison, Madison, WI, USA

Robert J. Munger, D.V.M., DACVO Animal Ophthalmology Clinic Inc., Dallas, TX, USA

Christopher J. Murphy, D.V.M., Ph.D, DACVO Ocular Services On Demand, LLC (OSOD) and Department of Ophthalmology & Vision Science, School of Medicine and Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California, Davis

T. Michael Nork, M.D., M.S., DABO, FARVO Ocular Services On Demand, LLC (OSOD) and Department of Ophthalmology and Visual Sciences, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA

Carol A. Rasmussen, M.S. Ocular Services On Demand, LLC (OSOD) and Department of Ophthalmology and Visual Sciences, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA

James A. Render, D.V.M., Ph.D., DACVP NAMSA, Northwood, OH, USA

Kenneth A. Schafer, D.V.M., DACVP, FIATP Vet Path Services Inc., Mason, OH, USA

James N. Ver Hoeve, M.S., Ph.D. Ocular Services On Demand, LLC (OSOD) and Department of Ophthalmology and Visual Sciences, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA

Mark Vézina, B.Sc Charles River Laboratories, Preclinical Services, Department of Ocular and Neuroscience, Senneville, QC, Canada

Andrea B. Weir, Ph.D., DABT Charles River Laboratories, Preclinical Services, Reno, NV, USA

Susan D. Wilson, D.V.M., Ph.D Aclairo Pharmaceutical Development Group, Inc., Vienna, VA, USA

Chapter 1

Comparative Ocular Anatomy in Commonly Used Laboratory Animals

Mark Vézina

Abstract Interspecies differences in ocular anatomy can alter the way a drug interacts locally within the eye, whether administered directly to the eye or systemically. It is therefore important to understand these differences and how they can influence the outcome and interpretation of safety or efficacy data for ocular therapeutics. The eye is a complex system of tissues integrated into a functional sense organ. Oriented toward the toxicologist or ocular researcher, this chapter will discuss the individual ocular tissues in commonly used laboratory animals in comparison with humans and will provide a basic understanding of ocular anatomy including quantitative comparisons when possible in these species. It will also act as a reference to the terminology that will be encountered in subsequent chapters.

1.1 Introduction

The eye is a complex organ system consisting of many specialized tissues that work in conjunction to make vision as we know it possible. Indeed, the malfunction of just one of these tissues can impair vision. In the clinical field, the eye's complexity has resulted in the development of specialists for individual tissues such as the cornea and retina. Since vision is arguably the most important of our senses, conducting ocular toxicology and tissue distribution studies in laboratory animals is essential to ensure the safety of therapeutics applied directly to or injected into the eye to treat ocular disease before they are administered to humans. Ocular toxicology is also assessed for drugs administered via non-ocular routes, such as oral and intravenous, to treat ocular

M. Vézina, B.Sc (✉)

Charles River Laboratories, Preclinical Services, Department of Ocular and Neuroscience,
Senneville, QC, Canada

e-mail: mark.vezina@crl.com

and other diseases. As well, an understanding of the pharmacodynamics or efficacy of an ocular therapeutic can be determined with the use of laboratory animals. Therefore, knowing the ocular anatomy of the species of laboratory animals commonly used for nonclinical studies (i.e., pharmacology, pharmacokinetics, and toxicology studies conducted in laboratory animals), such as those conducted to support drug development, is important because the anatomy can influence how the eye will react to a drug or foreign substance, whether administered systemically or directly onto or into the eye. Though many of the basic elements of the eye are conserved among species, significant anatomical diversity exists and has the potential to influence study results. However, there has been a lack of comprehensive information readily available to toxicologists or researchers to aid in the decision-making process when choosing a suitable species for ocular toxicology testing, or when evaluating the relevance of animal data to the human clinical situation. Therefore, this chapter is oriented toward the toxicologist, and the goal is to assemble the diverse anatomical characteristics of the eyes of mice, rats, rabbits, dogs, cats, minipigs, and nonhuman primates (NHP) in relation to humans to aid in study design and interpretation of results. In this chapter, references to mice, rats, cats, and rabbits pertain to normal, non-transgenic stock, dogs typically beagles or similar sized dogs, minipigs, Göttingen or Yucatan (occasionally a domestic strain or other minipig of similar size) and nonhuman primates (NHP), cynomolgus or rhesus. Anatomic terminology used in subsequent chapters of this book will also be covered. When relevant, quantitative anatomical comparisons have been included for certain tissues. However, when anatomical comparisons take on a quantitative nature, a range of measurements for the same tissue can often be found in the literature for the same species, primarily due to inter-laboratory differences in methodology. As technologies for making quantitative assessments advance, methods become more precise (although not always more accurate!) and these ranges may change, altering the “conventional wisdom.”

As an introduction, a gross view of the eye is presented in Fig. 1.1, illustrating the common structures possessed by all of the species that will be covered.

1.2 Eyelids

The eyelids are often ignored in ocular studies being considered “in the way” of the true region of interest, the eye itself. However, the eyelids do play a key role in ocular maintenance in the form of the blink. The true mechanism of blink control is not fully understood. It is likely a combination of a central nervous system mediated “blink center” that receives sensory input from the ocular surface as well as reflex from visual and mental sources. In humans, blinking can occur automatically (but not at truly fixed intervals), as a reflex to a visual or sound stimulus and at will. Most mammals can do the same. Blinking cleans the surface of the eye, results in the application of the lubricating tear film to the ocular surface, and prevents complete photobleaching of the photoreceptors by providing brief instances of darkness. In humans, the duration

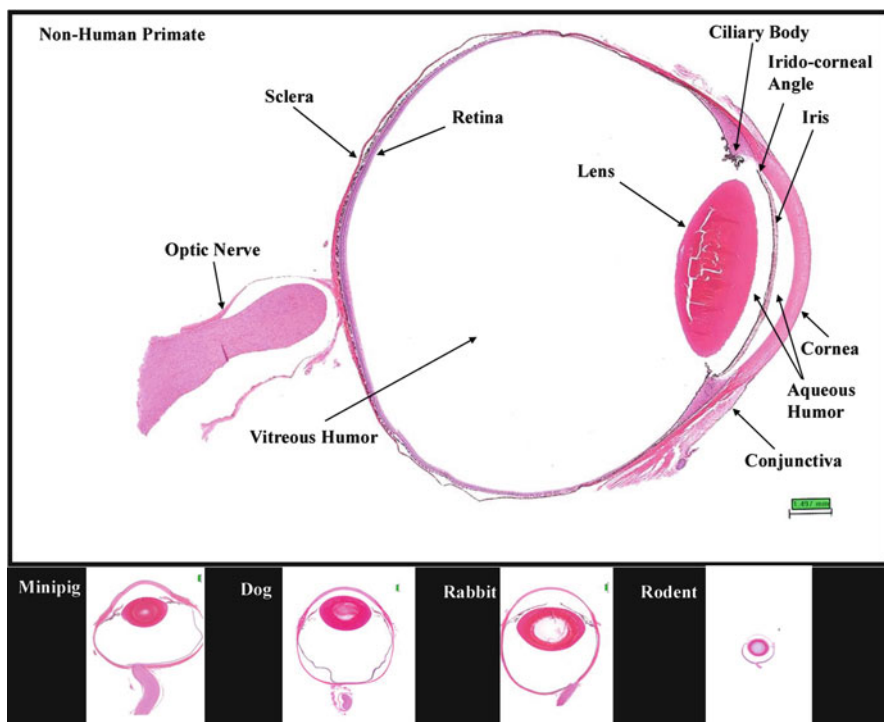


Fig. 1.1 Gross ocular anatomy. Human and nonhuman primate eyes are similar, with the NHP eye at approximately half the scale of the human eye. Other species presented for comparison demonstrate the more obvious differences such as lens size, vitreous and aqueous chamber size, and corneal thickness

of a blink is approximately 250–400 ms [43], which translates into an additional 5–7 min of “darkness” each day during normal waking hours for humans. Although blinking speed has not been well studied in laboratory animals, it has been observed in dogs and nonhuman primates to be in the range of approximately 100–300 ms [11], which is not dissimilar to humans. In addition to applying the tear film, the eyelids are also involved in draining excess tear film, or topical drops for that matter, from the ocular surface. All mammals have two functional eyelids; however, they are not all created equally. Of interest to the toxicologist is the drainage system since it will impact on the duration of the presence of a liquid eye drop on the surface of the eye and by inference the amount of systemic exposure that might occur. Normal tear film is drained from the eye by small openings in the eyelids called puncta. The puncta are connected to nasolacrimal ducts which drain into the sinus onto the nasal mucosa. Their location on the eyelid can vary somewhat, but in general they are located in the medial canthal area, near the edge of the eyelid where the conjunctiva and skin meet. Most laboratory species as well as humans have one punctum on

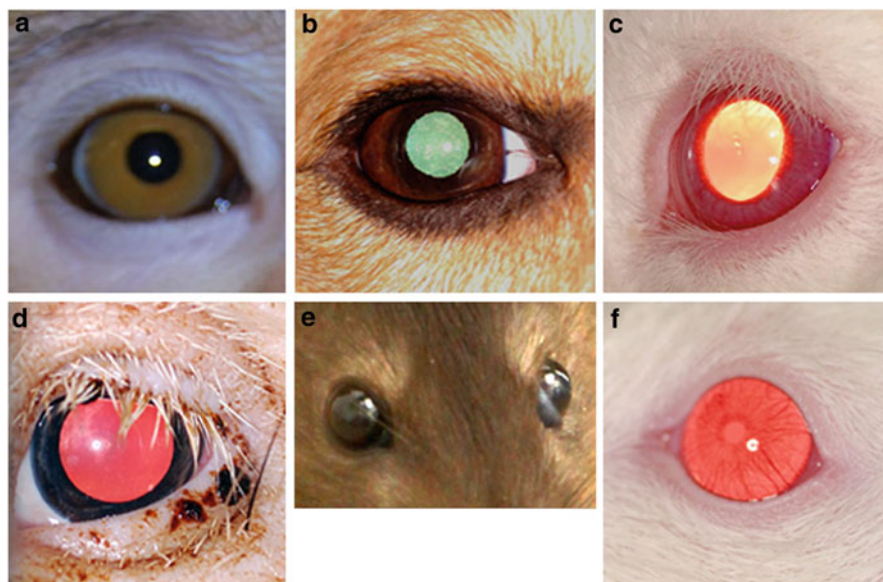


Fig. 1.2 Relative eyelid configuration and eyeball exposure. (a) Cynomolgus monkey, (b) dog, (c) New Zealand White rabbit, (d) Göttingen minipig, (e) Brown Norway rat, (f) albino mouse

each upper and lower eyelid. Minipigs (pigs in general) only have puncta on the upper eyelid and rabbits only on the lower eyelid. In humans, approximately 75–80% of tear volume is drained with each blink. It has been estimated that up to 80% of a topical drop can be absorbed systemically [32, 51], with some absorption through the conjunctival vasculature and most of the fluid arriving directly on the highly absorbent nasal mucosa via the nasolacrimal duct system. Mechanically, the drainage occurs by the suction force created when the upper and lower eyelids part, when fluid is drawn into the nasolacrimal drainage system. The size and shape of the eyelids also plays a role in systemic absorption. A species with looser eyelids and larger conjunctival sac such as a rabbit may have more “drop” available for absorption on a subsequent blink than a nonhuman primate which has eyelids more tightly pressed to the eye, where runoff onto the surrounding skin and/or fur from the initial blink is likely to be higher (refer to Fig. 1.2). Similar systemic absorption after topical ocular instillation has been observed in dogs and rabbits [11]. The concern with the systemic absorption is the potential for unwanted systemic side effects. In adult humans, the unintended systemic dosage is usually relatively low on a body weight basis, and consequently, the risk of side effects in the general population is also low, with exceptions for certain susceptible populations. However, when the dose/body weight ratio becomes higher such as in children, the side effects can be more serious [23]. Scale that effect to a 2-kg rabbit or nonhuman primate and the potential for systemic toxicity increases further.

Because blinking can influence the residence time of a topically applied test substance in the eye as well as the tear film, the blink rate may provide some insight

Table 1.1 Average blink rates by species

Human	Every 5 s (task dependent) [60]
Nonhuman primate	Every 6 s [31]
Pig	Every 20–30 s [11]
Dog	Partial blink: Every 4 s [10] Complete blink: Every 10–20 s [10]
Cat	Every 18 s [9]
Rabbit	Every 6 min [59]
Rat	Every 5 min [58]
Mouse	Similar to rats

Table 1.2 Species with nictitating membranes

Human	Nonhuman primate	Minipig	Dog	Cat	Rabbit	Rat	Mouse
No	No	Yes	Yes	Yes	Yes	Yes ^a	Yes ^a

^aThe nictitating membrane in rodents is effectively nonfunctional

into interspecies local or systemic reaction to treatment. A faster blink rate can result in decreased residence time on the eye as well as alter the systemic absorption characteristics of a topically applied product compared to a slower blink rate. Blink rates for the various species are presented in Table 1.1.

The nictitating membrane, or 3rd eyelid, is a translucent to opaque structure that supplies additional lubrication and cleaning of the corneal surface. It is not easily visible in most laboratory species, and though it has some sympathetic innervation with minor musculature in some species (like cats), its motion is mostly passive occurring with slight retraction of the eyeball and/or the action of blinking. It is also thought to be a protective structure in the animals' natural environment. The presence of this structure in laboratory species needs to be considered when conducting certain ocular evaluations such as scoring of local irritation or when comparing tear film or corneal changes between species. For example, a protruding or inflamed nictitating membrane may be confused for severe conjunctival hyperemia or may physically mask other ocular changes, and the absence or presence of the third eyelid could be the difference in why one species exhibits symptoms of ocular dryness or erosion and another does not after receiving the same topical drug (Table 1.2).

1.3 Conjunctiva

The conjunctivae are the transparent membranes that line the underside of the eyelids and cover the sclera. There are three primary classifications: (1) the *palpebral*, covering the underside of the eyelids, is thick and can be reddish in appearance; (2) the *bulbar*, covering the sclera, is thinner, vascularized, and transparent but may contain

some pigment in more heavily pigmented animals, such as nonhuman primates; and (3) the *fornix*, forming the junction where the palpebral turns to meet the bulbar. The conjunctival sac or cul-de-sac is the space formed by this arrangement.

The conjunctivae serve several purposes. They provide lubrication, help to hold the eye in place, and allow it to move smoothly within the eye socket.

The amount of conjunctiva visible when observing an eye varies by species (as seen in Fig. 1.2), with a larger amount visible in rabbits compared to nonhuman primates. The differences in the amount of visible conjunctiva can make it more or less difficult to evaluate surface irritation (conjunctivitis) after administration of a test substance.

1.4 Pre-corneal Tear Film and Ocular Glands

The pre-corneal tear film is composed of aqueous and lipid layers and is secreted by several glands mostly located around the eye. The tear film is spread over the cornea during blinking, and its composition is related to the blink rate of the various species. A more aqueous tear film is subject to more evaporation and requires more frequent reapplication than a more lipid-based tear film. The glands involved in the secretion of the tear film include the lacrimal gland (located in the orbit), the Harderian gland (located on or near the nictitating membrane and therefore not found in the primates), accessory lacrimal glands, Meibomian glands (located on the eyelid margin), and goblet cells (located in the conjunctiva (palpebral and fornix). In general, the tear film has three layers. A mucin layer secreted by the goblet cells is the innermost layer. The middle layer is more aqueous and is secreted by the lacrimal glands with contributions from the Harderian gland in some species such as dogs and cats (where the Harderian gland is more similar to a lacrimal gland). The outermost layer is a lipid layer secreted by Meibomian glands and Harderian glands. In rodents, the Harderian glands also secrete porphyrins, which when over-secreted can cause a reddish deposit around the eye [16]. The reason for the presence of porphyrins in the Harderian gland of rodents is unknown, but it suggests a sensitivity to light and a possible relationship to the pineal gland [8].

1.5 Cornea

The cornea is a transparent multilayered structure at the front of the eye that is responsible for allowing light to enter the eye as well as for approximately 2/3 of light refraction (focusing). It joins the sclera in a zone called the limbus. The cornea is avascular, but it has the highest concentration of nerve endings in the body.

The layers of the cornea from outer to inner generally consist of:

Epithelium: The corneal epithelium is several cell layers thick and is continuous with the bulbar conjunctiva. It desquamates at the surface and rapidly regenerates. The epithelium is the primary barrier within the cornea to drugs and bacteria. It is

Table 1.3 Average central corneal thickness (mm)

Mouse	Rat	Rabbit	Dog	Cat	Pig	NHP	Human
0.089–0.123 [36, 52]	0.16–2 [11, 52]	0.36 [52]	0.5–0.66 [24, 37]	0.57 [55]	0.8 [54]	0.42 [39]	0.54 [17]

damaged easily and, therefore, alterations to the corneal epithelium may alter drug penetration into the anterior section of the eye. It does not present a uniform surface, and the mucin layer of the pre-corneal tear film fills in the gaps to provide the required optical quality.

Bowman’s membrane: This layer underlies the epithelial layer and acts as a barrier protecting the stroma. Not all species have this structure, including rabbits, dogs, cats, and rodents [30, 56].

Stroma: This is the thickest layer. It is composed of parallel collagen fibrils and is responsible for the refractive power of the cornea.

Descemet’s membrane: This layer underlies and supports the stroma. It is collagenous and elastic and acts as the basement membrane of the corneal endothelium.

Endothelium: The corneal endothelium is a single layer of cells that maintains the proper relative water content of the stroma. Additionally, it transports nutrients to the stromal cells from the aqueous humor and removes waste. It is effectively non-regenerative. In the event of damage to this layer, some cells will enlarge to fill in gaps left by dead cells. A healthy endothelial layer is critical for corneal function. An unhealthy endothelial layer will eventually result in corneal edema (thickening due to increased water content) which will interfere with vision.

The cornea, being avascular, obtains its nourishment from sources such as the tear film, aqueous humor, and a ring of perilimbal vessels located approximately 1–3 mm from the edge of the corneoscleral junction (limbus).

Corneal thickness varies by regions within the cornea, with the time of day, with age, with external influences (e.g., contact lenses), damage, disease, and species. Because of this variability, average central corneal thickness is usually the parameter that is measured and quoted for comparison purposes. Some measurements for laboratory species are presented in Table 1.3.

1.6 Sclera

The sclera is the protective white fibrous sheath around the eye. It is continuous with the cornea and is composed of the same type of collagen fibrils as the stroma. However, as opposed to being aligned in a parallel fashion, the fibrils are in a cross-matrix pattern, which results in the white reflective appearance. Other scleral components include proteoglycans and mucopolysaccharides.

Scleral thickness varies over the ocular surface as well as among species. However, direct comparison of quantitative data is difficult due to the inconsistent

methods used to determine thickness. Methods used include measuring thickness on excised fresh tissue with calipers, measuring fixed tissue with calipers or microscopically, as well as *in vivo* with various imaging techniques. In many species, including NHPs, dogs, and cats, the sclera is thickest at the limbus and thinnest at the equator and somewhere in between near the optic nerve. In humans, it is thickest near the optic nerve, thinnest at the equator, and thicker again near the limbus. In pigs, however, the thickest region is approximately 5–6 mm from the limbus [45], being otherwise comparable to human. In rabbits, measurements are about half as thick as human over most of the sclera surface, thickening only at the limbus [44, 48]. Rodents tend to have thinner scleras than the larger-eyed species.

Much has been said about the relative sclera thickness of various species compared to human and its relationship to penetration of externally applied drugs into the eye. However, though the sclera does play a role in this respect, hydrophilic molecules pass through the sclera fairly easily. An additional significant barrier to ocular penetration appears to be Bruch's membrane and the vascular choroidal layer which can easily carry away a drug in the circulation.

1.7 Aqueous Humor (Part I)

The aqueous humor is a clear, watery fluid that contains ions, proteins, and other nutrients. It provides nutrients to avascular structures such as the cornea, lens, and trabecular meshwork and removes waste products. It plays a significant role in ocular pressure and maintaining the shape of the globe and therefore the optical quality of the eye.

Aqueous humor is in a constant state of relatively rapid flow and is subject to diurnal fluctuations. The flow rate is an important consideration when evaluating the relative kinetics of a drug in the anterior portion of the eye. A higher flow rate may contribute to increased clearance. Complete turnover can take as little as an hour. Average aqueous humor flow rates are presented in Table 1.4 and estimated aqueous volumes are presented in Table 1.5.

1.8 Iris, Ciliary Body, Trabecular Meshwork, and Aqueous Humor (Part II)

The iris is the muscular diaphragm that controls the amount of light entering the eye by enlarging or narrowing the pupil. It is circular in shape except for cats where it is in the form of a vertical slit-shaped oval. The iris separates two chambers in the anterior segment of the eye. The *anterior chamber* represents the space between the cornea and the iris and the *posterior chamber* the space between the lens and the iris. The iris joins the cornea at the irido-corneal angle.

The ciliary body lies in the posterior chamber and is responsible for production of aqueous humor, lens accommodation, and uveoscleral outflow. It consists primarily of ciliary muscle but has extended villus-like components called ciliary processes that are responsible for the production of the aqueous humor. The ciliary processes

Table 1.4 Average aqueous humor flow ($\mu\text{L}/\text{min}$)

Mouse	Rat	Rabbit	Dog	Cat	Pig	NHP	Human
0.18 [1]	0.35 [41]	2.7 [19]	4.5 [62]	5.5–8.5 [13, 33]	^a	1.95 [47]	2.8 [46]

^aUndetermined *in vivo***Table 1.5** Average aqueous humor volume (μL)^b

Mouse	Rat	Rabbit	Dog	Cat	Pig	NHP	Human
5.9 [1]	13.6 [27]	287 [12]	770 [22]	853 [38]	^a	123 [7]	310 [57]

^aUndetermined *in vivo*^bVolumes derived from direct aspiration or anterior/posterior chamber measurements

are also connected to the lens via proteinaceous filaments called zonules. The zonules hold the lens in place and allow the ciliary muscle to exert force on the lens for accommodation.

The plasma-derived aqueous humor is secreted from the epithelial cells of the ciliary processes into the posterior chamber. It flows through the pupil into the anterior chamber where most of it flows out of the eye at the irido-corneal angle via the trabecular meshwork.

The trabecular meshwork, located in the irido-corneal angle in the anterior chamber, consists of a net of cross-linked collagen fibers with some endothelial-like cells. It filters the aqueous humor into Schlemm's canal (not specifically present in all species), scleral collector channels, the episcleral veins, and finally into the general venous circulation. This constitutes the conventional outflow pathway and accounts for most of the aqueous humor drainage. Damage to the trabecular meshwork or narrowing of the irido-corneal angle can result in reduced outflow and subsequent increased intraocular pressure.

The unconventional pathway, also known as uveoscleral drainage, consists of drainage of aqueous humor through the supraciliary spaces in the ciliary body through to the sclera and choroid. It is difficult to measure, and therefore, there are a wide range of values associated with the amount of aqueous it actually drains. Some estimates place it as a major contributor. For example, in humans, estimated uveoscleral drainage can account for as little as 4% and as much as 60% of total outflow [64]. In rabbits, 3–8% has been reported [6] and in nonhuman primates, up to 60% has been reported [5]. Currently, it is considered to be a secondary outflow pathway in laboratory species.

1.9 Lens

The lens provides the final fine tuning for focus of incoming light and is comprised of three major components:

The capsule: The lens capsule is a collagenous membrane that surrounds the lens and provides support by elastic tension.

The lens epithelium: This structure is located in a layer beneath the anterior capsule. The cells of this structure provide homeostatic support and are regenerative.

As they age, they migrate to the lens equator, compress into an elongated form, lose their nucleus, and become new lens fibers.

The lens fibers: The lens fibers are elongated transparent cells that contain the crystallins, which are essential for the refractive properties of the lens. The lens fibers have no light-scattering internal organelles such as a nucleus, endoplasmic reticulum, and mitochondria. They rely on the aqueous humor for nutrients and waste removal. The lens fibers are divided into cortex and nucleus. Crystallins are a complex group of structural water-soluble proteins that are organized within the lens fibers in such a way as to increase the refractive index of the lens while maintaining transparency. The lens fibers are classified as cortical and nuclear based on their age and location. The cortical fibers are the newer, softer lens fibers in the outer regions of the lens. As they age and become more compressed by the development of new cortical fibers, they locate more centrally and become part of the lens nucleus which itself becomes larger and harder with age as more cells are incorporated in this region.

The size and shape of the lens varies with species, as demonstrated in Table 1.6 (to scale). In rodents, the lens occupies approximately 70% of the entire volume of the eye.









When discussing general regions of the lens, terminology is similar to that used to describe the Earth. The *anterior pole* refers to the center of the anterior surface and the *posterior pole* the center of the posterior surface. The lens is divided into two hemispheres at the *equator* which is the circumference between the two poles.

1.10 Vitreous Humor

With the exception of mice and rats, the vitreous humor occupies the majority of the volume of the eye. Its clear, gel-like consistency is composed primarily of water with some hyaluronic acid, a small amount of salts, and a few cells. It also has an ultrastructure composed of collagen and some proteins that give it the gel-like consistency. As the vitreous ages, it becomes less gel-like and more aqueous. It is generally non-regenerative. Vitreous that has been removed will be replaced eventually with aqueous humor. Vitreous flow has been reported, but there is controversy over whether a front-to-back convection-related flow exists or whether the flow is the result of the shear-related forces associated with saccadic eye movements [15, 20]. In any case, the vitreous humor is not completely stagnant.

Average vitreous volumes are presented in Table 1.7. For humans, the reported range is approximately 3.5–5.4 mL [3]; however, the currently accepted average is 4 mL as presented in the table. Knowledge of the vitreous volume (and hence general shape of the vitreous chamber) is important for determining scaling comparisons of animals to humans, as well as in the consideration of the diffusion of a material within the vitreous and its inevitable contact with the sensory retina in pharmacology and toxicology studies using intravitreal injection.

Table 1.6 Comparative lens shape and dimensions

Species	Mouse	Rat	Rabbit	Dog	Cat ^b	Minipig	NHP	Human
Shape								
Axial length (mm) ^a	2.15 [49]	3.87 [40]	7.9 [26]	6.7 [65]	8.5 [61]	9 [11]	2.98 [34]	4 [25]

^a Axial length measured anterior to posterior. The anterior face of the lens is the upper surface in this diagram

^b Cat lens image courtesy of Julia Baker

Table 1.7 Average vitreous volumes by species (mL)

Mouse	Rat	Rabbit	Dog	Cat	Minipig	NHP	Human
0.0053 [50]	0.013–0.054 [18, 27]	1.5–1.8 [2, 11]	3 [2, 11]	2.4–2.7 [11, 63]	3–3.2 [2, 11] 2–2.7 [53]	1.8–2.0 [29]	4

1.11 Fundus, Retina, and Choroid

The term “fundus” represents all of the structures that can be seen at the back of the eye when looking directly through the pupil with an adequate light source. This includes the optic nerve head (ONH), retinal vessels, sensory retina, underlying pigmented layer, reflective tapetal layer (for those species that have one), and choroidal vessels (when not obscured by overlying pigment). Examples of the diversity of the view of the fundus are demonstrated in Fig. 1.3.

Clearly, there are some major differences between species that could be important for both species selection and interpretation of drug-related ocular changes. The human fundus most closely resembles that of the nonhuman primate species commonly used in the laboratory, including specialized structures not present in the other laboratory species such as the macula and fovea.

The optic nerve head, also known as optic disc or the blind spot (as there is no sensory retina in the region), is the point of entry into the eye for the optic nerve as well as the major retinal veins and arteries. Its myelination (in most cases) gives it a white appearance, and its location in the eye can vary somewhat, usually out of the way of central vision (see Fig. 1.3). In rabbits, the myelination extends into the nerve fibers in a horizontal plane on either side of the optic nerve head. The optic nerve head is also cup shaped, with rabbits having one of the deeper cups of all the species used in the laboratory. Alterations in the shape and orientation of the ONH can be affected by chronic elevations in intraocular pressure, a hallmark of glaucoma.

The retinal vessels provide nourishment to the inner retinal components such as the interstitial and ganglion cells. As well, the retinal vascular system provides structural support to the retina and helps dissipate the heat associated with the light energy entering the eye. Most of the laboratory species as well as humans have a well-developed retinal vasculature throughout the inner retina termed holangiotic. Rabbits are the exception with a limited retinal vasculature known as merangiotic, where the retinal vasculature is limited to axial vessels in a horizontal plane radiating from the optic nerve over top of the myelinated nerve fibers (Fig. 1.4).

The sensory retina itself consists of several layers (Fig. 1.5). In the outermost layer are the photoreceptors, the rods and cones that contain the pigment rhodopsin, which reacts with light and makes vision possible. In very general terms, cones are responsible for daytime color vision (the wavelengths determined by the subtype of rhodopsin within the cone outer segment) and higher visual acuity. They require substantial light to function. The rods are designed for low-light function, have less visual acuity, and are not sensitive to color. Rods outnumber cones in most vertebrate species. Subsequent layers include the outer nuclear layer, comprised of the nuclei

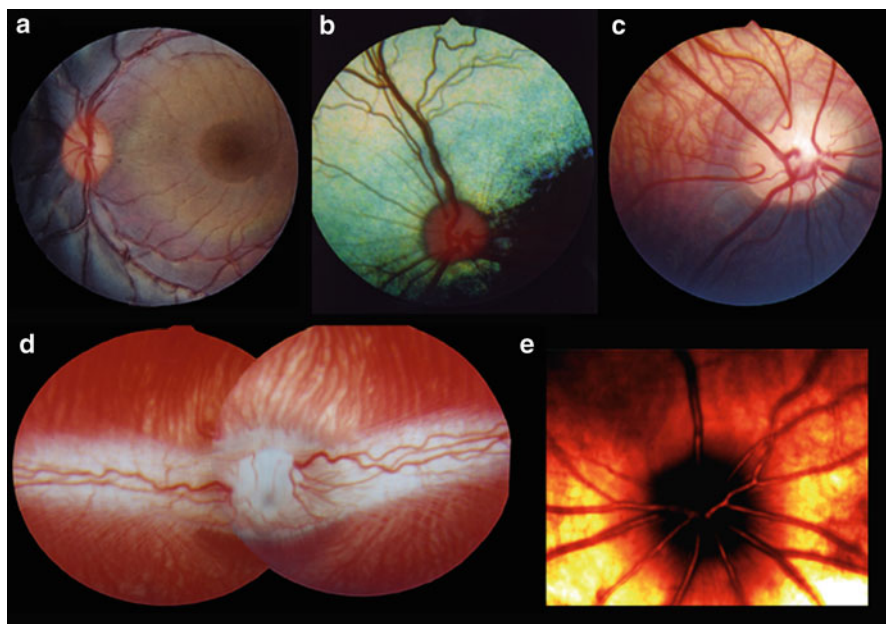


Fig. 1.3 (a) Nonhuman primate showing macula and fovea. The vascular pattern cradles this region and is similar to human fundus. (b) Dog fundus showing reflective tapetal layer. Cat is similar. (c) Minipig fundus with pigment variation and choroidal vessels visible in a nonpigmented region superior to the optic nerve head (ONH). (d) Albino rabbit with myelinated nerve fibers and merangiotic vasculature with clearly visible choroidal vessels inferior and superior to the ONH. (e) Albino rat with radial vasculature extending from optic nerve head. Mouse is similar

of the photoreceptors, followed by the outer plexiform layer, the synapses between the photoreceptors and the bipolar cells. The bipolar, amacrine, horizontal, and Müller cells are located in the inner nuclear layer. These cells are involved in chaperoning and secondary signal processing. The synapses between these cells and the ganglion cell layer are the inner plexiform layer. The ganglion cell layer contains the bodies of the ganglion cells that further process the signal before it is transmitted to the visual cortex in the brain. The innermost layer, the nerve fiber layer, is comprised of the axons of the ganglion cells which run toward and become the optic nerve. Covering the nerve fiber layer is a protective membrane called the inner limiting membrane. This membrane can be a barrier to intravitreally injected therapeutics, such as certain adeno-associated viral (AAV) vectors [14], preventing exposure to the underlying cellular structures. The thickness or number of cells within these retinal layers varies by location, from central (generally a higher concentration of cells for more acute vision) to peripheral (generally fewer signal processing cells/photoreceptor). The constituency of these layers can also vary significantly by species. Figure 1.5 compares a microscopic view of a cynomolgus monkey retina and a Sprague Dawley rat retina. The monkey is primarily a visually oriented animal and

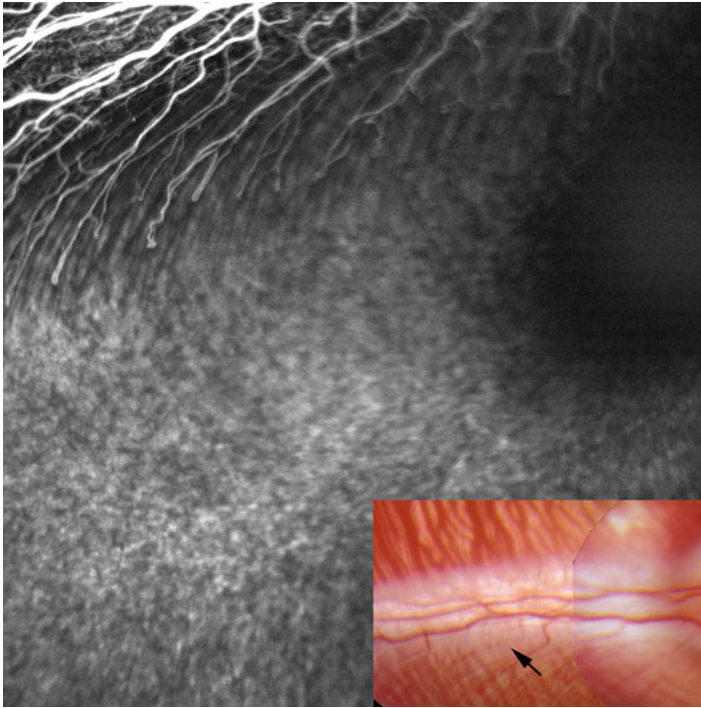


Fig. 1.4 Fluorescein angiogram of rabbit fundus demonstrating the merangiotic vascular arrangement. Retinal vessels are limited to a small region of the sensory retina (*upper left corner*). *Color inset and arrow* indicate general region of vessels in the angiogram

the nocturnal rat has rather poor vision (it does not need good vision in a dark environment), which is evident in the relative difference in complexity of the two eyes.

Most animals have a region in the retina with a higher concentration of cones for increased visual acuity. It is most often, but not always, in the central region of the retina, central vision usually being the most important for survival (rabbits being an exception). In humans and nonhuman primates, this region is called the macula and within the macula is a smaller (1–2 mm) region called the fovea, exclusively containing cones, that is responsible for the majority of our high-resolution daytime vision. The macula is easy to detect due to its yellowish pigmentation. The pigment is protective, acting as a UV filter. The area overlying the fovea is devoid of the usual retinal cells and retinal vessels in order to allow light to reach the photoreceptors with as little interruption as possible (Fig. 1.6). This results in a depression in the retina known as the foveal pit (Fig. 1.7).

In the other species, the region of concentrated cones is most often referred to as the area centralis or visual streak depending on its shape. Figure 1.8 shows some examples of its shape and general location. It is difficult to detect visually because it lacks the yellow pigment that is present in the macula.

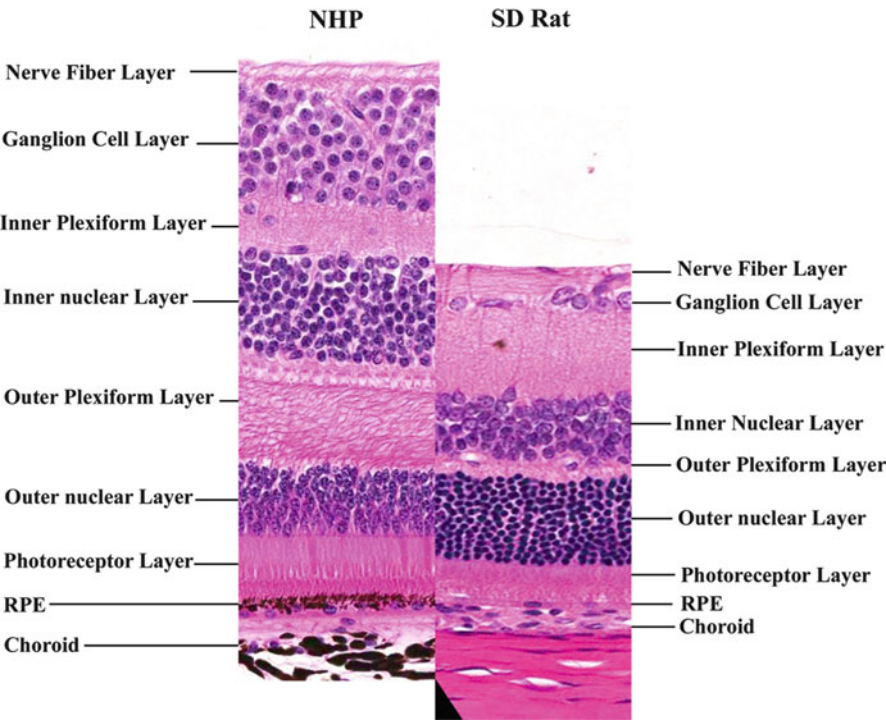


Fig. 1.5 Comparison of the cellular anatomy of the retina of a nonhuman primate (*NHP*) and Sprague Dawley albino rat, aligned at the retinal pigment epithelium (*RPE*). Note the significantly higher number of signal processing cells in the inner nuclear and ganglion cell layers of the NHP



Fig. 1.6 Fluorescein angiogram of the macular region of a cynomolgus monkey showing the avascular foveal region in the center (approximately 1-mm diameter)