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# Slit-Lamp Examination of Experimental Animal Eyes. I. Techniques of Illumination and the Normal Animal Eye

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**Synopsis**—The SLIT LAMP is an indispensable tool for the ophthalmologist and for the experimental researcher utilizing the eye. The purpose of this paper is to describe briefly the slit lamp and accessories, discuss uses and limitations of the type of slit-lamp ILLUMINATION for visualization of the eye, and describe the normal ANIMAL EYE. Since the use of the slit lamp can be easily mastered, its use in evaluating the eye in experimental studies should be implemented. Critical observation of the eye utilized in OCULAR IRRITATION studies for pharmaceuticals, cosmetics, and other formulations can be accomplished with the aid of the slit lamp.

## Introduction

The slit-lamp microscope is an instrument of this century (1, 2). It has evolved from an exclusive research device to an indispensable tool for the ophthalmic practitioner. Recently it has been adapted for use in the experimental ophthalmic area.

The slit lamp can become a useful tool for evaluation of formulations for ocular damage in animal test models. Since many cosmetic formulations may

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accidentally be introduced into the eye, the cosmetics should be and are routinely tested in animal eyes. The slit lamp becomes a tool which allows the investigator to make fine distinctions for ocular damage and between two or more comparative cosmetic materials.

This laboratory is part of an ophthalmic pharmaceutical manufacturing company; as such, the majority of the experimental and clinical research is in the ophthalmology area. Expertise has been developed in utilizing the slit lamp for the experimental ocular studies. Over the years, a grading system has been developed for toxicological and pharmacological evaluations of experimental studies in animals. This grading system depends upon the utilization of the slit-lamp biomicroscope as an indispensable experimental tool. Therefore, the general purpose of this and the next report is to present the uses and applications of the slit lamp in the experimental laboratory. The purpose of this endeavor is to describe the slit lamp and its use, and to illustrate the types of illumination employed.

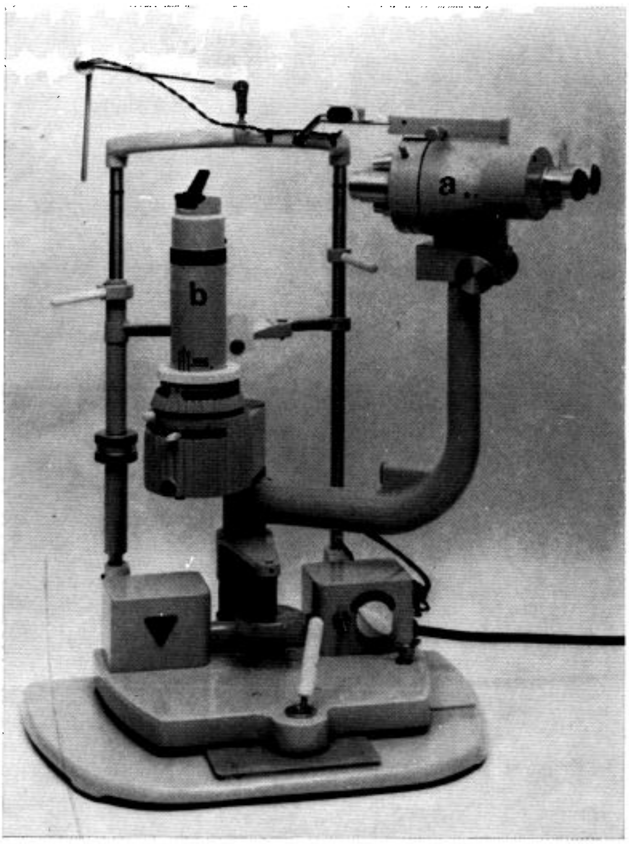


*Figure 1.* Slit lamp with corneal microscope (a) and slit illuminator (b). (Courtesy of Bausch and Lomb)

## Description of Slit Lamp

As shown in Fig. 1, the slit lamp is an upright instrument, which has a binocular eyepiece and a rather large ocular. The eyepiece and ocular are mounted on a movable arm so the slit lamp can be moved in a two-plane direction. There are two principal parts of a slit lamp. The optical part (a corneal microscope) includes the eyepiece and the different objectives for selecting the magnification and the slit illuminator. The slit illuminator allows the operator to adjust the width, the height, and for lateral displacement of the slit image for certain specific types of illumination. In addition, filters can be placed in the path of the light so that either cobalt blue light or a green beam can be used. The slit illuminator and corneal microscope are situated to allow free rotation of each part through a 180° arc.

A number of attachments are available for a slit lamp. The Hruby (3) lens (Fig. 2) is used to view the fundus. The Goldmann applanometer (4, 5) (Fig. 2) is used to measure intraocular pressure. However, the Goldmann applanometer is not as useful in experimental animals as it is in the clinic because the contour and wetting properties of the cornea of animals are apparently different from man. A pachometer (6) can be mounted onto the slit lamp (Fig. 3) and used for measuring the corneal thickness or anterior chamber depth. Cameras can be mounted in the path of the slit image and can be used for photographing the images seen by the observer or an observation tube

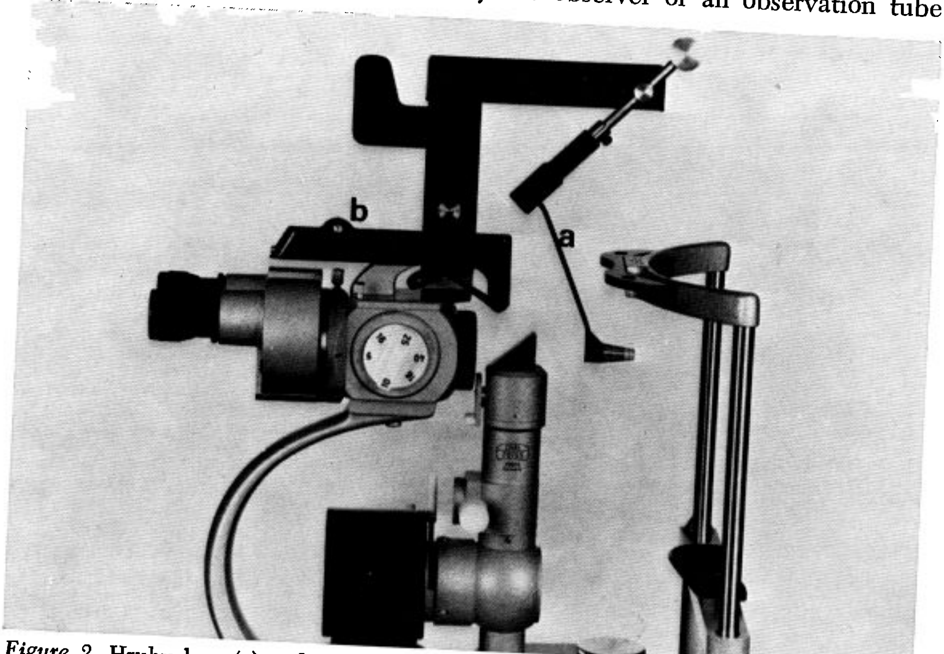


*Figure 2.* Hruby lens (a) and applanometer (b) attached to slit lamp. (Courtesy of Carl Zeiss, Inc.)

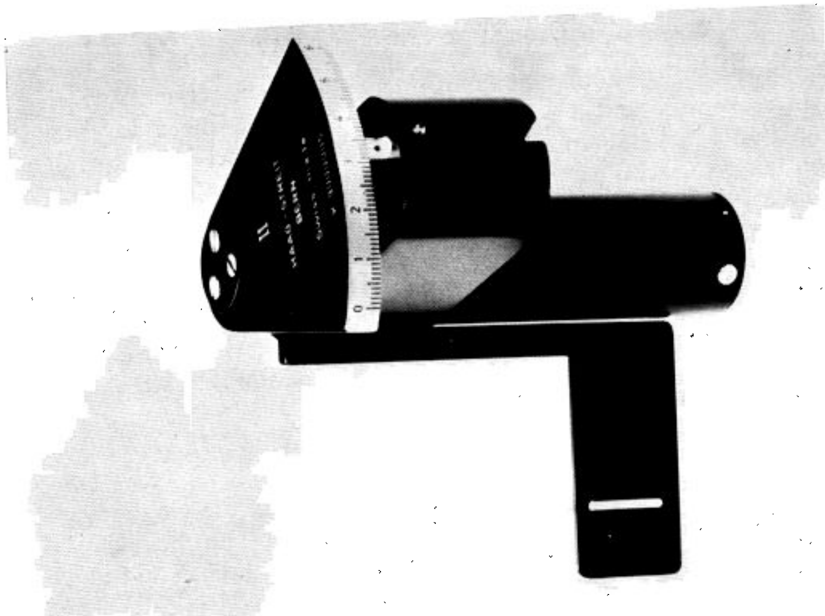

*Figure* 3. Pachometer. (Courtesy of Haig-Streit, Inc.)

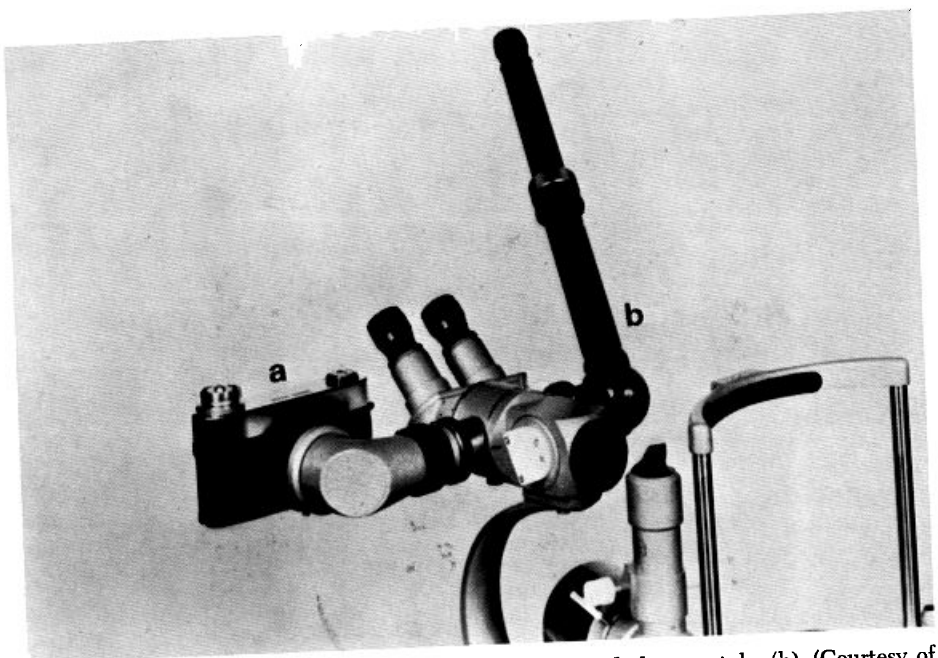


*Figure 4*. Camera (a) for anterior segment photography and observer tube (b). (Courtesy of Carl Zeiss, Inc.)

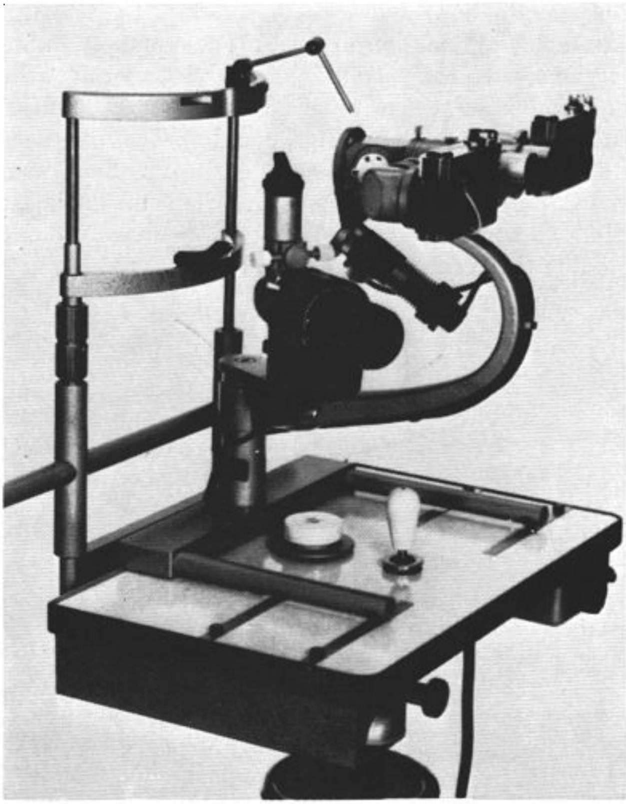

*Figure* 5. Camera set-up for slit-image stereophotography. (Courtesy of Carl Zeiss, Inc.)

may be employed for a second observer (Fig. 4). Camera attachments are available for both anterior segment photography and for slit image photography (7). Stereophotography (8) can be accomplished with the aid of two cameras (Fig. 5).

## Type of Slit-Lamp Illumination

There are two basic types of slit images used in biomicroscopy: a parallelepiped and optical section. When employing the parallelepiped, a rectangular beam (1–2 mm wide and 5–10 mm high) is projected through the cornea with the slit illuminator at a 45° angle. The shape of the illuminated area is similar to a parallelepiped prism whose outer and inner surfaces are curved due to the curvature of the cornea. When using the optical section slit image, the width of the beam is narrowed to almost its minimum and is projected from an angle of about 45° through the cornea. The result is a sagittal view or optical section similar to a thin histological section. The width of the anterior and posterior faces of the optical section should be about 20 $\mu$.

The types of illumination used with the described slit images are: direct illumination, diffuse illumination, direct retro-illumination, indirect retro-illumination, sclerotic scatter, indirect illumination, and specular reflection (9).

With direct illumination, the beam and the microscope are sharply focused at the same area in the same plane (Figs. 6 and 7). The microscope is directly in front of the eye and the angle between the slit illuminator and the corneal microscope is 45°. A rectangular beam, with no greater thickness than 2 mm, is projected from an angle of 45° to the optical medium. When the parallelepiped is formed on the cornea, three general areas may be observed: the epi-

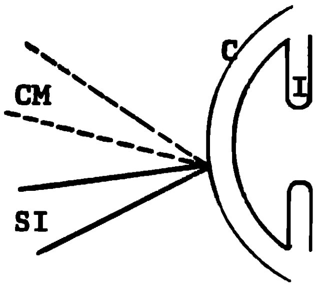


*Figure 6.* Direct illumination. Slit illuminator beam and corneal microscope focused on same area and plane. CM = corneal microscope; SI = slit illuminator; C = cornea; I = iris

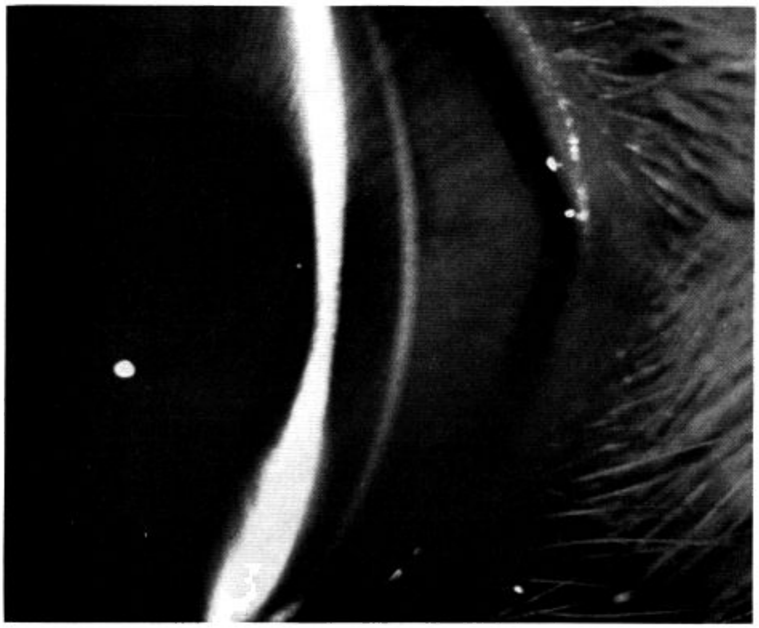

*Figure 7.* Direct illumination of normal rabbit cornea

thelium (which appears as an anterior bright line), the stroma (a marblelike area), and endothelium (a posterior thin white line). When an optical section is formed on the cornea, it has four general layers which may be observed. From anterior to posterior, they are: (a) a thin, bright layer representing the precorneal film; (b) a thin, dark layer representing the epithelium; (c) a thin, granular area representing the stroma; and (d) a thin, bright layer representing the endothelium. Direct illumination is used to study any tissue of the anterior segment. A conical beam is used to detect aqueous flare, which appears as a Tyndall effect and results from light being reflected from cellular or proteinaceous debris in the anterior chamber.

In diffuse illumination, the beam is not sharply focused in the plane of the area being studied but is either converging or diverging. The wide aperture illuminates the interorbital area when the microscope is focused in the plane of the area being studied (Figs. 8 and 9). The microscope is directly in front of the eye being examined and the angle between the microscope and the slit illuminator is about 45°. Any size beam may be used; however, it is usually optimal to have a wide aperture so that a large area is illuminated. The diaphragm can be adjusted to produce a wide rectangular or circular beam. Lack of focus in the plane of observation and the width of aperture thus produces a large illuminated area. This type of illumination is useful for viewing a large area with a relatively intense uniform illumination and under conditions of stereoscopic magnification. A general view of the cornea, lids, sclera, conjunctiva, and lacrimal drainage system is seen by diffuse illumination.

In retro-illumination, the beam is reflected from a structure (iris or lens) posterior to the plane on which the microscope is focused. The structures in the anterior plane are studied in the reflected light. This type of illumination is most commonly used when the beam reflects from the iris and the microscope is focused on the cornea. Unless some structure in the media obstructs or scatters this reflected light, no special details are observed. Scars, pigment, and vessels containing blood are opaque to light and appear dark on the

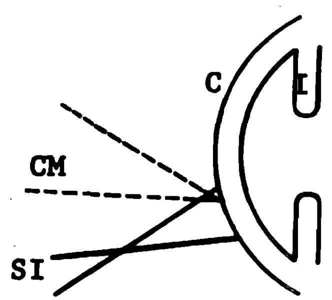


*Figure 8.* Diffuse illumination. Wide slit illuminator beam not focused in area and plane corneal microscope is

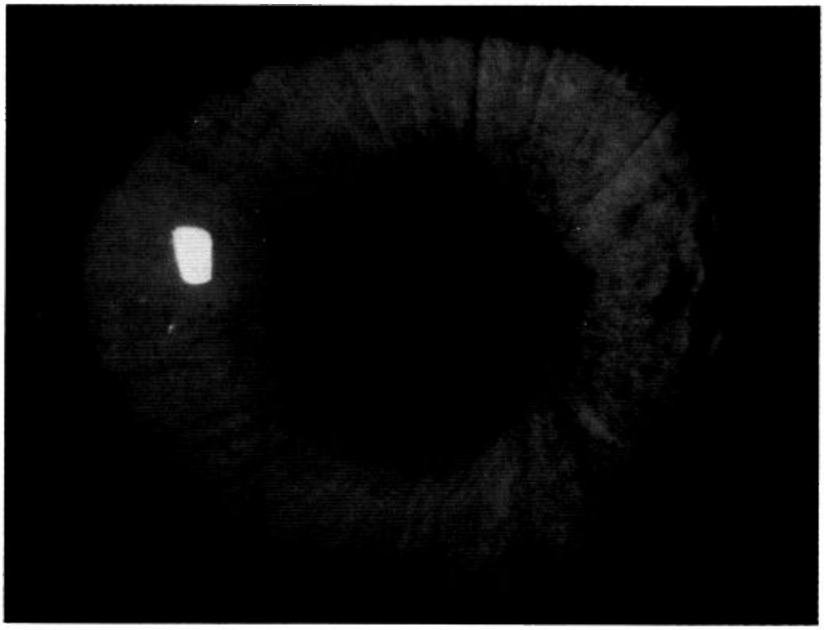

*Figure 9.* Diffuse illumination of normal rabbit corneal epithelium

brighter background when viewed with retro-illumination. Edema and corneal precipitates scatter light and appear bright on a darker background when viewed with retro-illumination.

There are two types of retro-illumination: indirect and direct. In indirect retro-illumination, the structure, microscope, and reflected light do not lie in the same visual line. The structure is at the side of the path of the reflected light and the microscope is moved away from the path so that the structure is observed against a dark background such as the pupil or the iris. In direct retro-illumination, the structure, microscope, and reflected light all lie in the same visual line. Scars, pigment, and the vessels with blood are best seen with direct retro-illumination. Edema and precipitates are best seen with indirect retro-illumination. A slit 1–2 mm wide may be used to form the parallelepiped. For indirect retro-illumination, the microscope is directly in front of the eye being examined and the angle between the illumination and the observer is set at 45° (Figs. 10 and 11). In direct retro-illumination, both the microscope and the illumination system are set at about 45° to the eye being examined. The angle between the microscope and the illuminator is, therefore, 90° (Figs. 12 and 13). Retro-illumination is useful in studying most types of epithelial edema, vacuoles, scars, posterior precipitates, and channels from the blood vessels that infringe upon the cornea.

In sclerotic scatter, the beam is focused on the corneal limbus. The scattering or dispersion of the light from the perilimbal sclera produces a halo

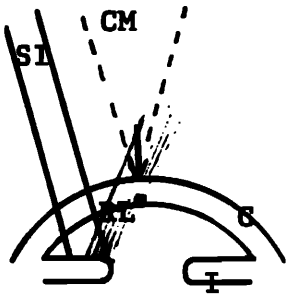


*Figure 10.* Indirect retro-illumination. Slit illuminator beam set at 45° angle with corneal microscope tangent to and focused on corneal surface. RL = reflected light

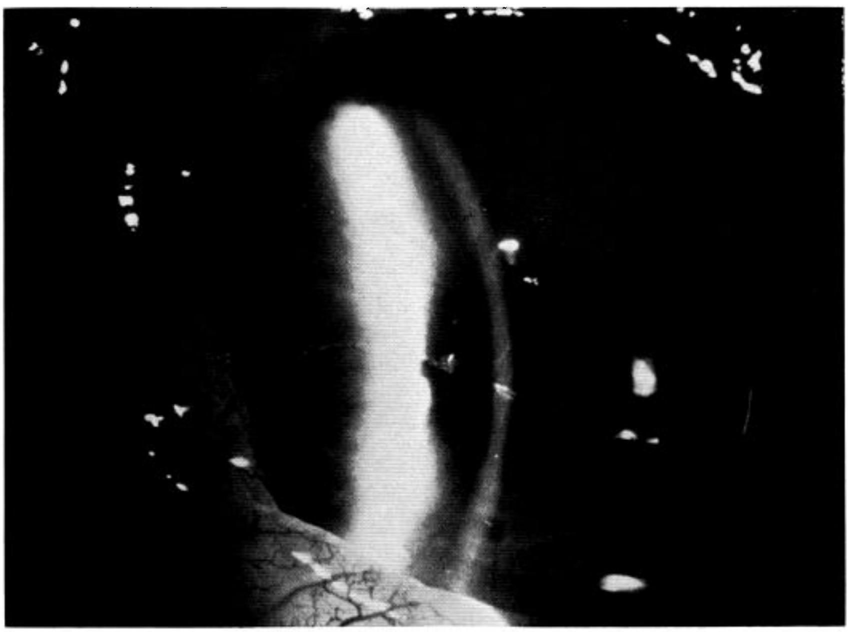

*Figure 11.* Indirect retro-illumination of normal rabbit eye

around the cornea. The halo is the brightest on the side of the cornea opposite from the focused beam. Internal reflection of the light transverses the cornea and illuminates it. Unless there are abnormalities of the cornea which impede or scatter this light, nothing is seen. The microscope is focused at the plane of the cornea in the area to be examined and is directly in front of the eye being examined. The angle between the beam of light and the microscope should be at least 45° (Figs. 14 and 15). The diaphragm is adjusted to produce the desired slit width (1–2 mm) and the beam is focused at the temporal limbus.

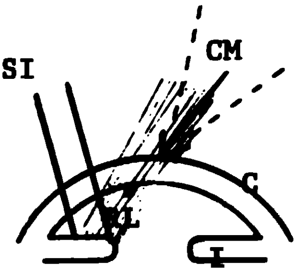


*Figure 12.* Direct retro-illumination. Slit illuminator beam set at 45° with a 90° angle between corneal microscope, focused on cornea, and slit illuminator. Observer looks parallel to reflected light rays. RL = reflected light

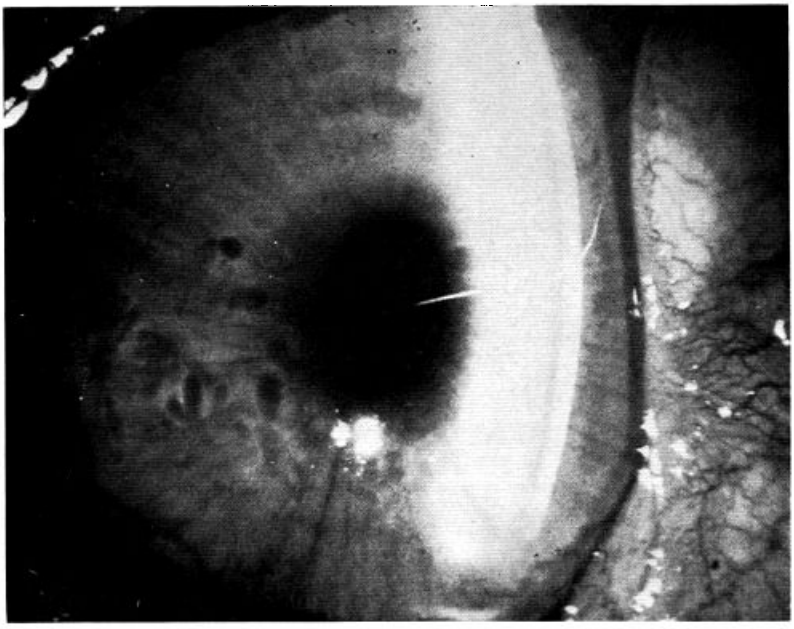

*Figure 13.* Direct retro-illumination of normal rabbit eye

Sclerotic scatter illumination aids in detecting very slight and early changes in the corneal tissue which only mildly obstruct, but significantly scatter, light. Central circular clouding of the cornea is probably best determined by this method.

In indirect illumination, a narrow beam is focused on nontransparent, translucent tissue such as sclera, iris, or leukoma of the cornea. The microscope is focused at the same plane, slightly to the side of the beam. The angle between the corneal microscope and the slit illuminator is 60° or more, thus increasing the amount of internal reflection and the amount of light scattered (Figs. 16

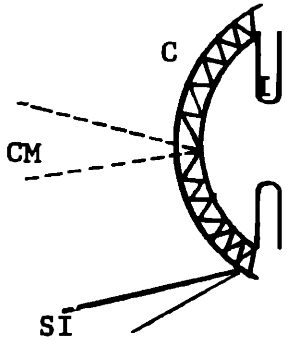


*Figure 14.* Sclerotic scatter. Slit illuminator beam is focused at limbus with corneal microscope focused on corneal plane

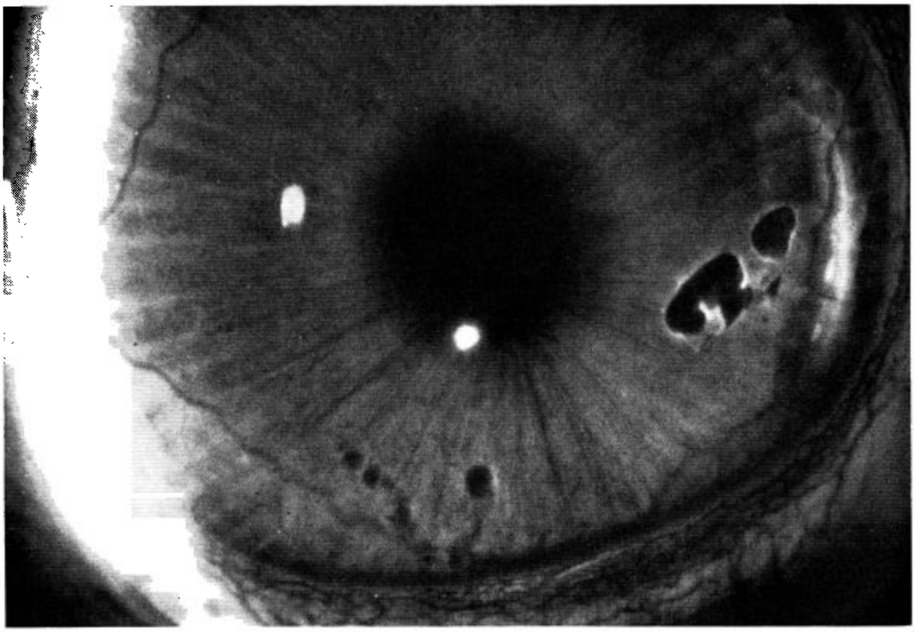

*Figure 15.* Sclerotic scatter of normal rabbit eye

and 17). A beam of about 1 mm wide is used. A narrow slit is focused on one of the tissues mentioned. The microscope is focused adjacent to the illuminated area. This type of illumination is most useful in studying the iris.

In specular reflection, a narrow slit beam is focused at the corneal surface and diffuse reflection produces a corneal parallelepiped. There is a significant difference between the refractive indices of the air in the precorneal film and to a lesser degree between the indices of the aqueous and the endothelium. These differences in refractive indices produce so-called "zones of discontinuity." Regular and irregular reflections of the light occur at each zone of dis-

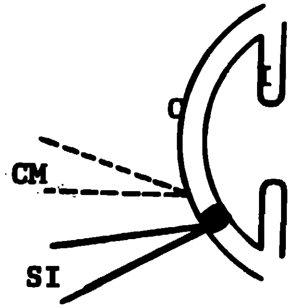


*Figure 16.* Indirect illumination. Corneal microscope is focused in same plane as slit illuminator beam but slightly to side

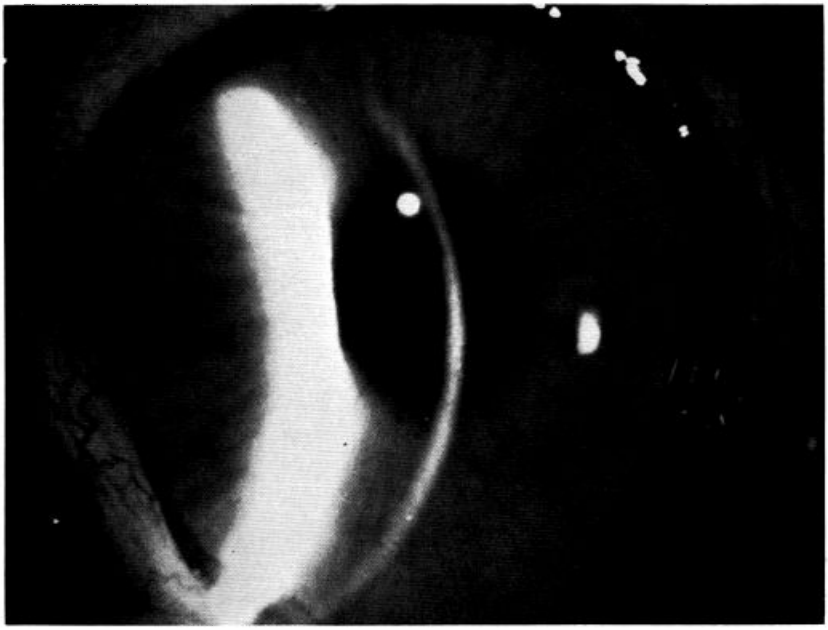

*Figure 17.* Indirect illumination of normal rabbit iris

continuity. As a result of regular (specular) reflections, a reflecting light image of the illuminating lens of the slit lamp is formed by the convex, mirror-like anterior corneal surface. Because of the irregular reflection, details of the reflecting surface itself can be seen, but only when the eye of the observer is in the direct path of the reflecting beam. Irregularities of the surface absorb much light, and therefore, appear as dark spots on the background of the dazzling reflex produced by the regularly reflected light. The microscope is focused on the image produced by a regular reflection. The angle between the corneal microscope and the slit illuminator is 60° with a slit beam of 1–2 mm

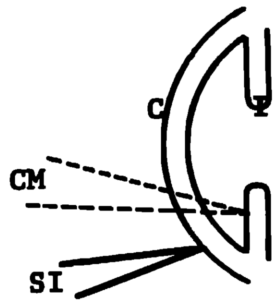


*Figure 18.* Specular reflection. Slit illuminator beam focused on corneal surface with corneal microscope focused on reflected light image of iris

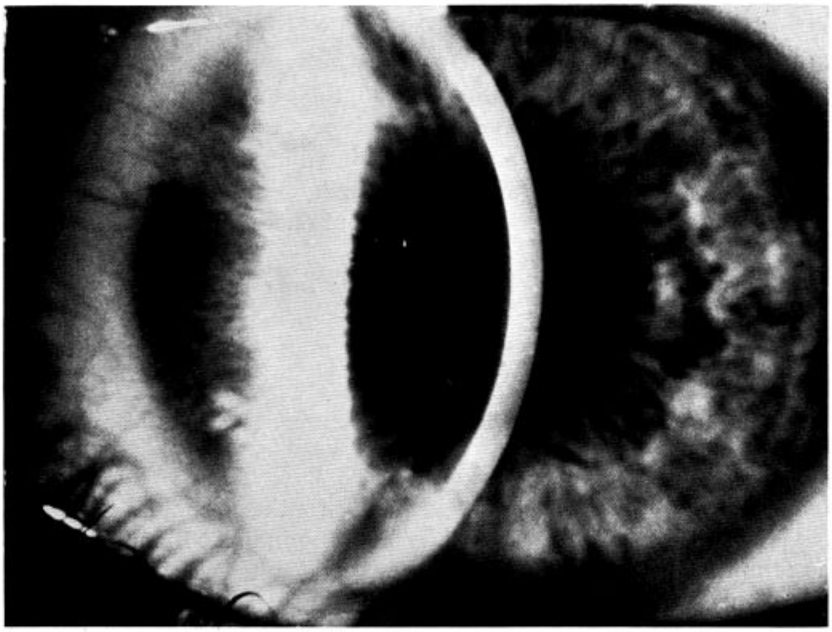

*Figure 19.* Specular reflection of normal human eye

wide being used (Figs. 18 and 19). The vertical height of the slit is reduced to about 4–5 mm. When the slit beam travels through the cornea, it forms an illuminated corneal area, an illuminated iris area, and a reflected light image of the illuminated lens in the aqueous. If the microscope is on its common axis, its own specular reflection comes into view. Specular reflection is most useful for viewing surface irregularities of the precorneal film, corneal epithelium, and corneal endothelium. Blinking helps to differentiate between fixed and movable particles. The endothelium mosaic appears as a dark depressed spot and endothelium deposits can be seen. Folds or tears of Descement's membrane can be seen because they disturb the endothelial contour.

## Normal Rabbit Eye

The rabbit has been the animal of choice for the pharmacological and toxicological evaluations carried out in our laboratories. However, other species, such as the monkey, dog, rat, and mouse may be used for experimental studies. For the purpose of this presentation and because a grading scale was designed for the rabbit, it will be utilized for discussion.

In order to become proficient in the use of the slit lamp for animal experimentation, one must become familiar with the normal anatomy of the animal eye as it appears with the aid of the slit lamp. The techniques which are discussed for the rabbit are applicable to other species. In test conditions, ocular changes from normal to most severe may be visualized.

The first structure to be observed with the aid of the slit lamp is the cornea (Fig. 7). When utilizing direct illumination, the microscope is focused on the surface of the cornea. Epithelial defects are easily observed with this type of illumination. Switching to retro-illumination, the observer quickly looks at the layers of the cornea for any changes with respect to transparency, hydration, or epithelial defects. In switching to specular illumination, the endothelium pattern of the cornea is observed. At first, the mosaic pattern of the endothelium may not be recognized by the observer but once observed it is recognized easily.

The anterior chamber, which is posterior to the cornea, is the next area to be observed. The normal anterior chamber is invisible to light of the slit lamp because there are no discrete cellular units or reflective characteristics therein (Fig. 20). Merely by localizing the beam, i.e., restricting the beam to a small, conical unit, the integrity of the anterior chamber can be observed. In the abnormal anterior chamber, the presence of light which reflects (Tyndall effect) is indicative of a pathological change.

The next structure to be visualized in the routine examination is the iris (Fig. 17). This can be accomplished by utilizing indirect illumination. In the albino rabbit eye, blood vessels may be observed quite easily with all magnifications of the slit lamp due to the lack of iridal pigmentation. However, if the observer looks at another species, such as the dog or cat, pigmentation of the iris usually obscures the presence of small vessels of the iris. It is fortunate that the albino rabbit has easily seen vessels, since congestion of the iris blood vessels is indicative of a pathological change. In very young rabbits, the vestigial ends of blood vessels may be seen protruding into the pupillary area. Normally, although the iris of the rabbit will appear pink, these blood vessels will not be congested. In very young rabbits, slight amount of congestion of iridal vessel is normal. Pupillary response to light may be observed by noting changes in pupillary size with the slit image on the pupil.

Within the pupillary perimeter, the anterior capsule of the lens is the next structure to be examined using direct and indirect illumination. The anterior capsule will appear as a slightly opaque, rough-textured surface. The observer

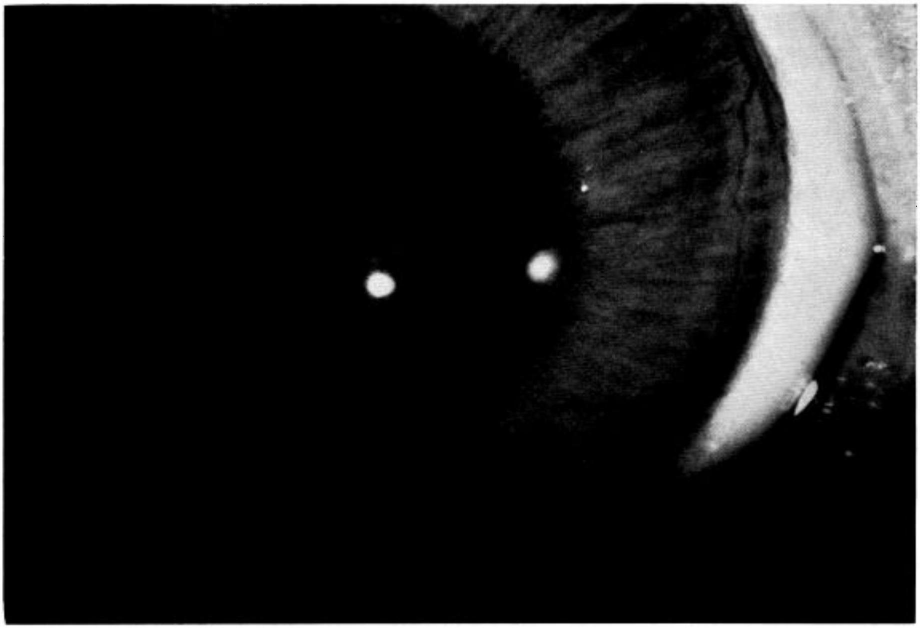

*Figure 20.* Normal rabbit anterior chamber as detected by small conical beam

will see a Tyndall effect within the lens and by close observation the capsule of the anterior and posterior surfaces (Figs. 21 and 22) can be easily distinguished from the cortex by noting when the Tyndall effect starts (anterior capsule) and when the Tyndall effect ends (posterior capsule). By noticing the changes of opaqueness within the lens as the light image passes through the lens, pathological changes can be detected. In order to examine thoroughly the lens of normal and pathological eyes, the pupil should be dilated. This is accomplished by pretreating the animal approximately 15–30 min prior to examination with a mydriatic, such as phenylephrine, tropicamide, cyclopentolate, or atropine.

Following examination of the lens, the vitreous is observed. This is somewhat difficult, since only the first one-third of the vitreous can be seen by direct illumination. The remaining vitreous body must be observed with the aid of the ophthalmoscope or with the Hruby (3) lens attached to the slit lamp. Other attachments, such as the Braley-Allen Fundus (8) lens are most helpful in observing the vitreous and the fundus. The fundus is the last intraocular structure examined (Fig. 23). A mydriatic eye greatly facilitates the observations of the vitreous and fundus. Using direct or diffuse illumination, the vitreous and fundus can be easily observed for changes from a normal condition.

The last feature of the eye to be observed by the slit-lamp operator is the integrity of the epithelium of the cornea. This is accomplished by placing a limited amount of fluorescein into the cul-de-sac, allowing the fluorescein to

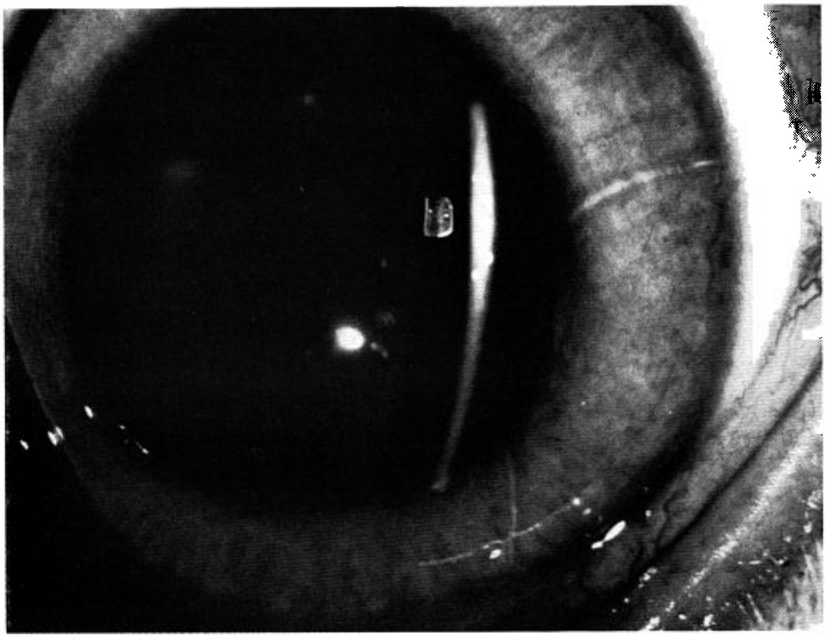

*Figure 21.* Anterior capsule of normal rabbit lens

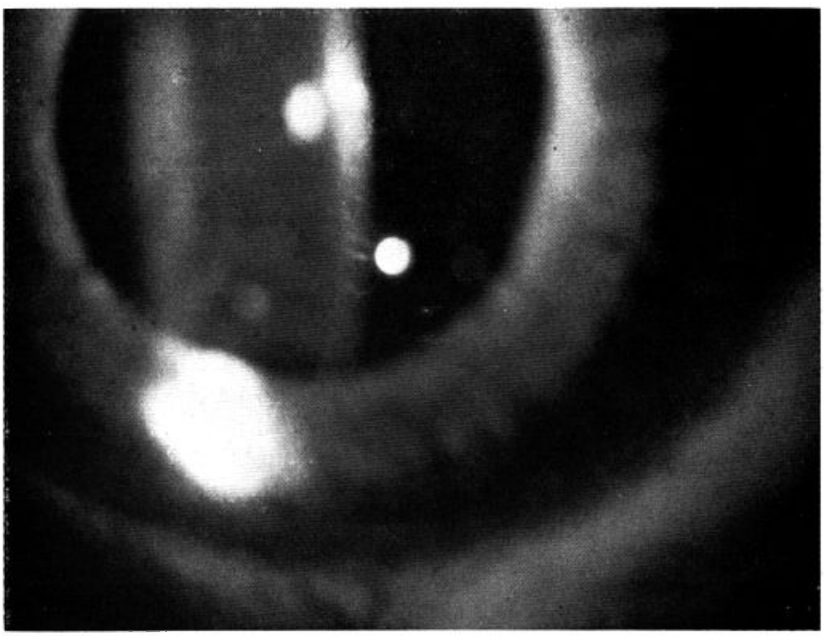

*Figure 22.* Posterior surface of capsule of normal rabbit lens

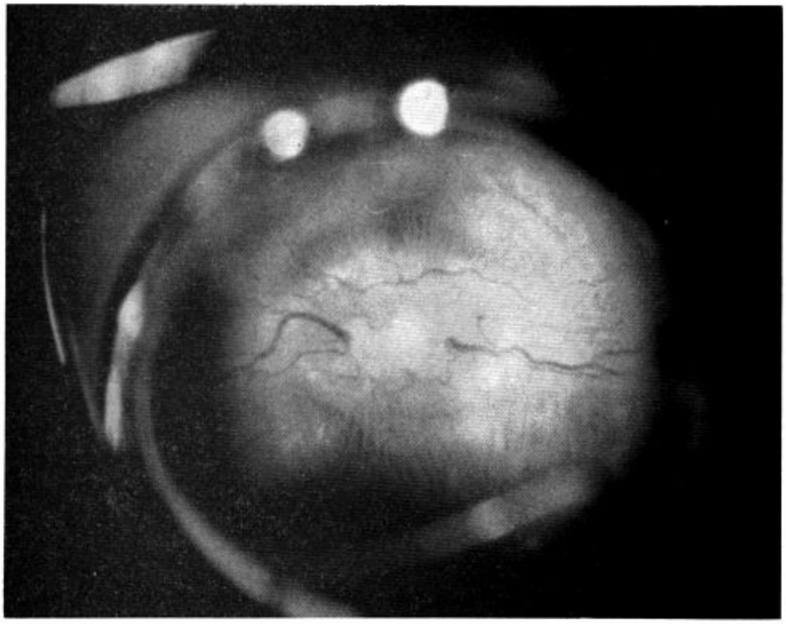

*Figure 23.* Fundus of normal rabbit eye

spread evenly in the precorneal film and cover the entire surface of the cornea. A cotton-tipped swab dipped in 2% fluorescein and lightly applied to the upper conjunctiva, followed by eye-manipulated blinking, is required. Using diffuse illumination with a blue cobalt filter, the observer checks for epithelial defects (Fig. 9). An epithelial defect is present when fluorescein penetrates an area in which the squamous epithelial cells have been removed and stain the living cells of the stroma. It is not uncommon for an infrequent slight corneal staining to be observed in a rabbit population.

## Summary

The preceding discussion has centered around the utilization of the slit lamp in experimental situations. Ocular pharmacological and toxicological evaluations of pharmaceutical, cosmetic, and other formulations under investigation in the experimental animals are easily and routinely made with the slit lamp. Experience in the past has demonstrated that the slit-lamp biomicroscope has become an indispensable tool in aiding in investigations. The different types of illumination used in the clinical situation can be easily adapted to the experimental situation, and as one becomes familiar with the types of illumination, they can be utilized in routine examinations of the experimental animal eye.

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