J. Soc. Cosmet. Chem., 24, 181–195 (March 2, 1973)

Slit-Lamp Examination of Experimental Animal Eyes. II. Grading Scales and Photographic Evaluation of Induced Pathological Conditions

H. A. BALDWIN, B.S.,* T. O. McDONALD, M.S.,* and C. H. BEASLEY, M.D.†

Presented May 25, 1972, Seminar, Los Angeles, Calif.

Synopsis—The SLIT LAMP has been shown to be a valuable tool for the examination of experimental animal EYES. With use of various techniques previously described, subtle as well as gross PATHOLOGICAL CHANGES in experimental eyes may be easily identified. These ocular pathological changes can be quantitated with numerical GRADING systems as outlined in this manuscript. These subjective grades center around the cornea, anterior chamber, iris, and lens. Ocular pathological changes can be documented by PHOTOGRAPHING with the aid of a photoslit lamp. The use of stereophotography can be most useful because it gives the illusion of depth as one normally sees with the stereoptic vision of the slit lamp.

Introduction

Throughout the years various chemical entities have been studied in animals prior to becoming either a useful therapeutic medicament or one of many thousands of compounds found to be too toxic or ineffective for use in humans. Animal models have also been devised in order to study similarities and differences between the natural or induced disease state and similar dis-

* Science and Technology Division, Alcon Laboratories, Inc., P.O. Box 1959, Fort Worth, Tex. 76101.
† 1212 West Presidio, Fort Worth, Tex.

eases in man. Through very carefully controlled experiments in animals, investigators have gained a tremendous amount of knowledge which has been ultimately useful in the care and treatment of diseases in man.

In the area of ophthalmology, animal test models are also an integral part of the study of disease processes of the eye. In this laboratory, in addition to the study of mechanisms of conditions similar to clinical eye diseases, each new therapeutic drug entity or cosmetic proposed for human use is carefully evaluated in animals as an extra measure of consumer protection. And, as the scope of regulatory requirements broadens, other agents not specifically designed for direct ocular application but for periocular use must now be evaluated for effects on this sensitive organ. Ophthalmic, dermatologic, and cosmetic formulations are routinely evaluated in our laboratories for their potential to induce ocular damage in animal species prior to use in man. The use of the slit lamp as an aid in examination allows the investigator to make fine distinctions of ocular pathology. Thus, the investigator is able to make better judgments for selection of a formulation which possesses a low potential for ocular irritation.

Of primary concern, regardless of the categories of interest, is that the investigator is ultimately faced with documentation and quantitation of the experimental results. Of course, the animal investigator usually has an option not available to the clinician of terminal histopathologic ocular examination. Indeed, this is an extremely valuable tool but is not always optimal for following the course of a disease process. Therefore, appropriate methods for following the disease and recording changes must be developed to evaluate *in situ* pathology.

Ocular Irritation Grading Scales

In 1944, Draize and others (1) devised a system for evaluating ocular lesions induced by topical applications of test chemicals (Table I). The Draize scale has thus become the mainstay for the evaluation of ocular lesions in experimental animals and has been widely utilized as the basis for devising other grading systems. Basically, the Draize scale is a subjective macroscopic evaluation of the conjunctiva, cornea, and iris. Although originally intended for use in albino rabbits, modifications will allow use in other animal species and in specialized situations. As an outgrowth of the original Draize scoring procedure, our group as well as others have adopted the use of the slit lamp to the routine procedures of examination of experimental animal eyes. The slit lamp, developed by Gullstrand in 1911, was originally an exclusive research device which has now evolved to an indispensable tool for the ophthalmic practitioner. We have found that this instrument is also an invaluable aid for the examination of experimental animal eyes in that gross as well as minute pathological changes may be identified and subjectively quantitated in an *in situ* situation. With the development of the photographic capabilities

Table I
Draize Scale[a] for Scoring Ocular Lesions

Cornea	
A. Opacity—degree of density	Grades 0 — 4
B. Area of cornea involved	Grades 1 — 4
A × B × 5	Maximum score = 80
Iris	
A. Values	Grades 0 — 2
A × 5	Maximum score = 10
Conjunctiva	
A. Redness	Grades 0 — 3
B. Chemosis	Grades 0 — 4
C. Discharge	Grades 0 — 3
(A + B + C) × 2	Maximum score = 20
The total score for the eye = sum of all scores	

[a]The Draize scale has been abbreviated in this table. Consult Draize *et al.* (1) for complete table.

now possible with the slit lamp, these induced pathological changes in the experimental animal eye may now be recorded in stereopsis by the investigator.

As a basis for establishing grading scales, the investigator must become completely familiar with the normal animal eye as viewed with the slit lamp. Then appropriate numerical scores may be arbitrarily assigned between the normal eye and that which the investigator considers maximal pathologic change for any given test situation. Although similar to the original Draize method of subjective scoring, as previously stated, minute changes may be identified and quantitated which could not be detected by macroscopic examination. We have found this method extremely valuable with regard to examination of the cornea, anterior chamber, iris, and lens. Due to anatomical features in many animal species, the Draize method of scoring conjunctival pathology has proved satisfactory for quantitating reaction in this ocular tissue.

Although slit-lamp examination is readily adaptable to various animal species, for the purposes intended in this communication, comments are confined to the albino rabbit eye to enable comparison to the original Draize method.

Study of Albino Rabbit Eye

Cornea

The rabbit cornea is examined first (Table II). With the aid of the slit lamp one may easily visualize three distinct layers: epithelium, stroma, and endothelium. Contrary to the Draize method, one is capable of separately examining each layer in most instances. With the aid of fluorescein staining, epithelial defects may be quantitated with regard to both intensity and area

Table II
Slit-Lamp Examinations of the Cornea

Epithelial staining (fluorescein)	
Intensity of staining	
Grade 1	Slight fluorescein staining in a given area
Grade 2	Moderate fluorescein staining in a given area
Grade 3	Marked fluorescein staining in a given area, underlying structures still visible
Grade 4	Extreme fluorescein staining, masking underlying structures
Area of staining	
Grade 1	25% or less of cornea stained
Grade 2	50% of cornea stained
Grade 3	75% of cornea stained
Grade 4	All of cornea stained
Opacities	
Grade 1	Slight clouding
Grade 2	Moderate clouding
Grade 3	Marked clouding of the cornea with underlying structures still visible
Grade 4	Complete opacity, obscuring underlying structures
Panus	
Grade 1	Vascularization is present but is not invading from around the entire circumference of the cornea
Grade 2	Panus has invaded 2 or more mm from the entire circumference of the cornea

of staining (Figs. 1 and 2).* Opacities of the stromal layer are commonly observed in rabbits and generally these may be graded by the degree of stromal clouding and/or corneal thickness (Figs. 3–7). Occasionally observed in rabbits are small areas of opacity in the corneal endothelium. It has been found that these opacities are usually masked by other corneal pathologies but should be noted when observed. If a severe corneal inflammatory process is allowed to progress, neovascularization often occurs, thus necessitating the grading of panus (Fig. 8).

Anterior Chamber

The anterior chamber is examined next (Table III). This normally optically transparent chamber was not included in the original Draize scale for obvious reasons. However, aqueous flare and cells may be easily identified with the aid of the slit lamp and may be quantitated either on the basis of cell count or optical density judgments (Figs. 9–12).

* Ocular pathologic changes were induced by various means. These include immunologic reactions (uveitis and corneal transplantation), formalin, detergents, shampoos, and various preservatives at concentrations to cause toxicity.

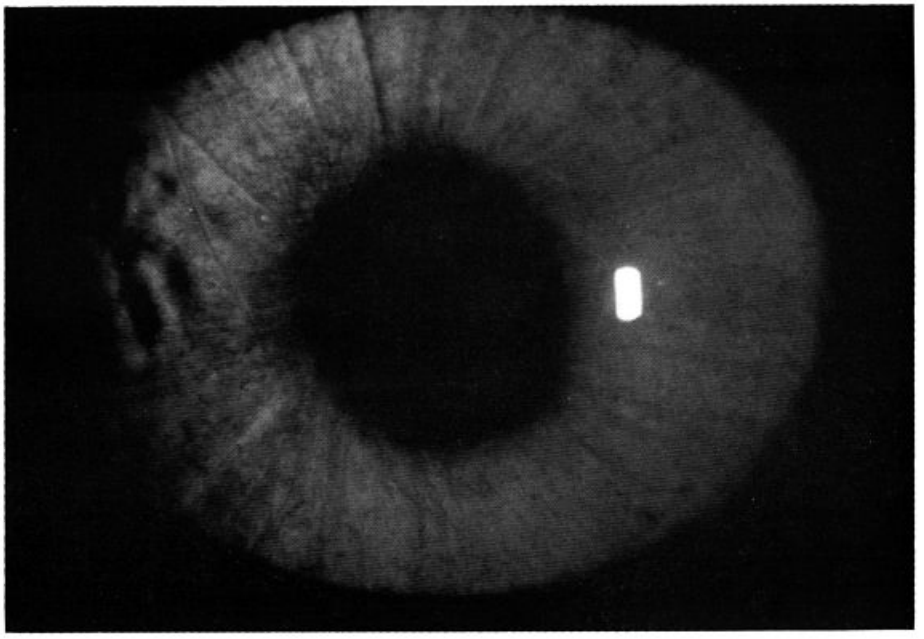

Figure 1. Normal rabbit corneal epithelial fluorescein staining (Grade = 0; area = 0)

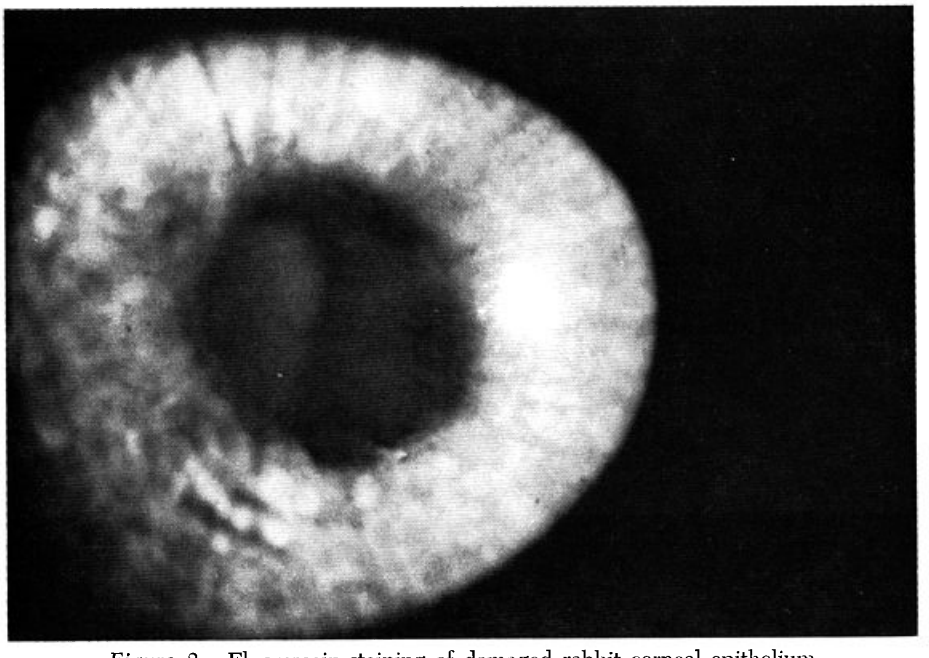

Figure 2. Fluorescein staining of damaged rabbit corneal epithelium (Grade = 2; area = 1)

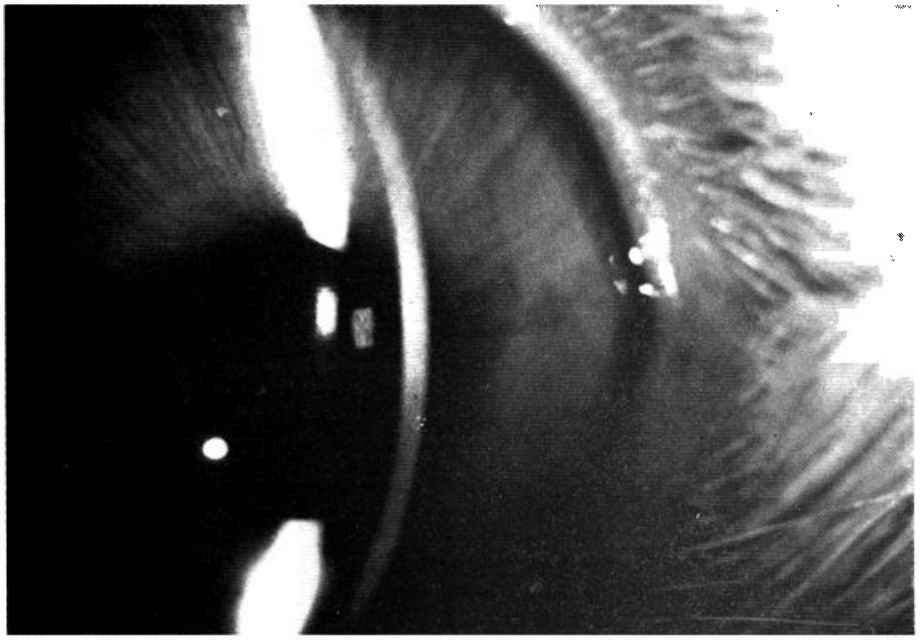

Figure 3. Normal rabbit cornea stroma (Grade = 0). Epithelium is at right of slit image; stroma is central position of slit-image; endothelium at left of slit-image

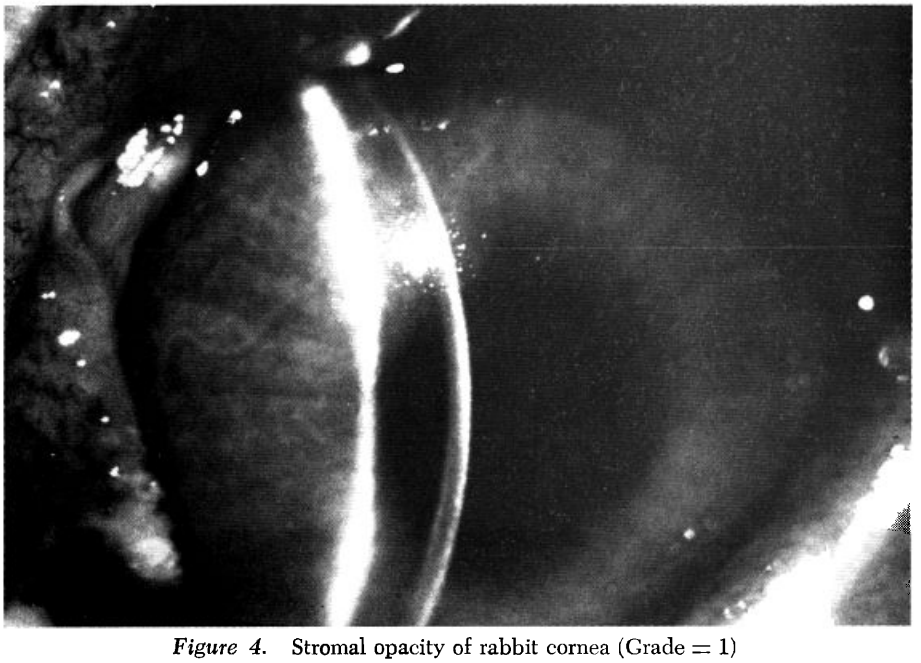

Figure 4. Stromal opacity of rabbit cornea (Grade = 1)

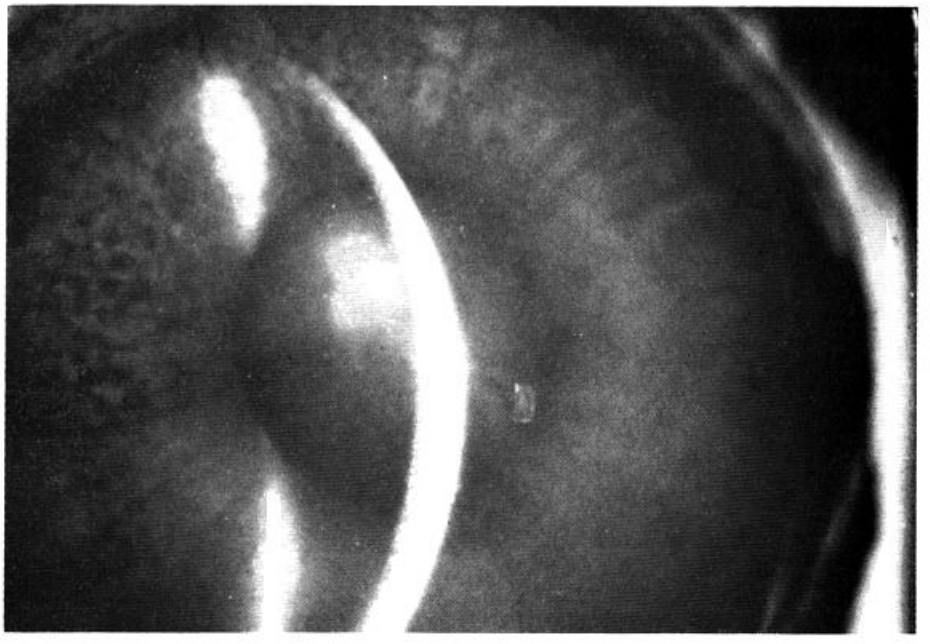

Figure 5. Stromal opacity of rabbit cornea (Grade = 2)

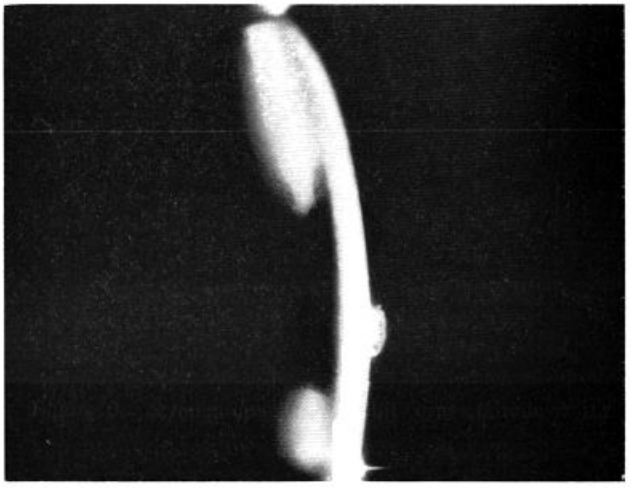

Figure 6. Stromal opacity of rabbit cornea (Grade = 3)

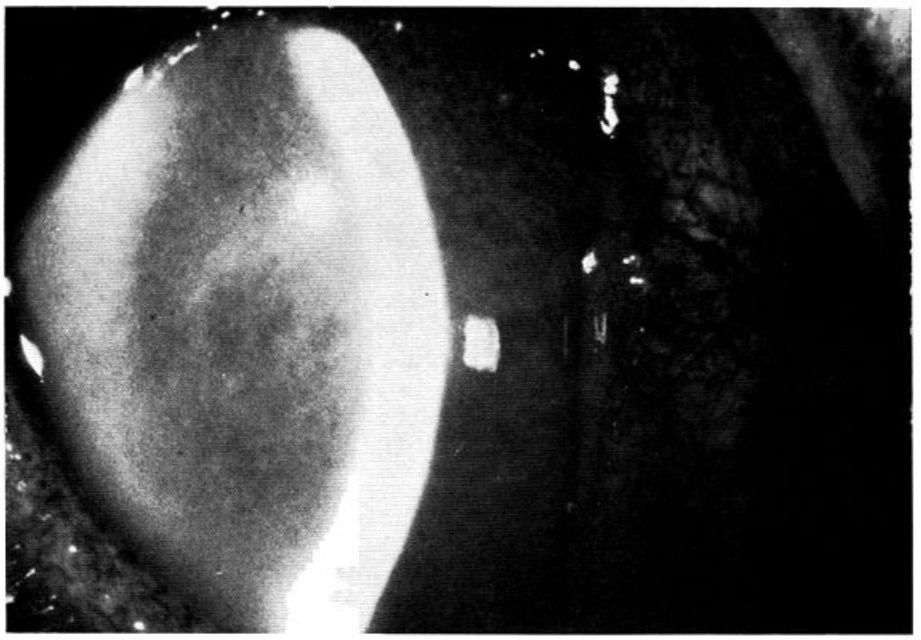

Figure 7. Stromal opacity of rabbit cornea (Grade = 4)

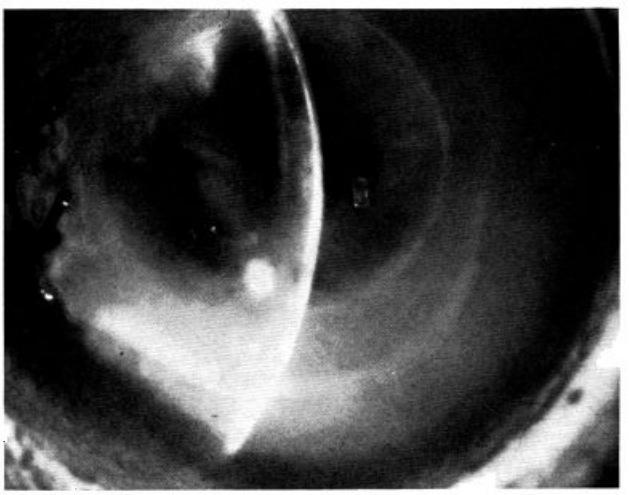

Figure 8. Panus [neovascularization—invasion of blood vessels (at lower part of figure) into rabbit cornea] (Grade = 1). (Note: With most ocular pathology, other changes, such as corneal opacity of Grade 3 for this eye, commonly occur)

Table III
Slit-Lamp Examination of the Anterior Chamber

Flare	
Grade 1	Slight change in optical density from normal; barely detectable flare
Grade 2	Easily detectable turbidity within the anterior chamber; moderate change in optical density
Grade 3	Marked clouding of aqueous humor; underlying structures plainly visible

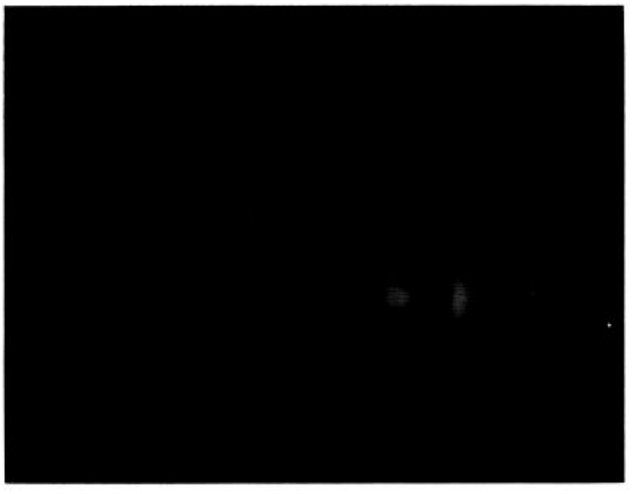

Figure 9. Normal anterior chamber of rabbit eye (Grade = 0)

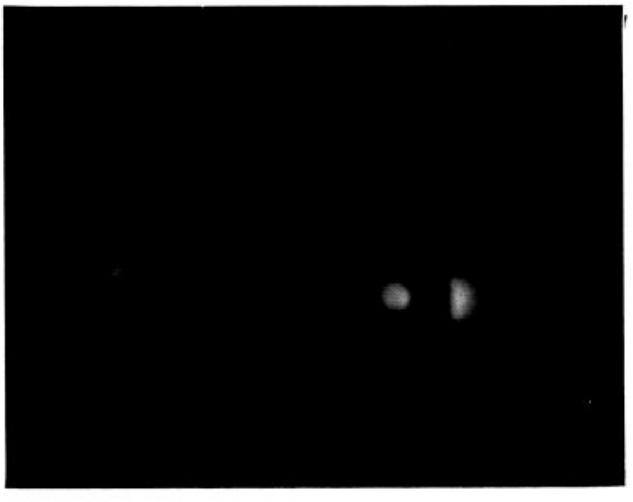

Figure 10. **Aqueous flare (indicative of the presence of cells and/or protein material) in the anterior chamber of rabbit eye (Grade = 1). (Note: Flare is a Tyndall phenomenon)**

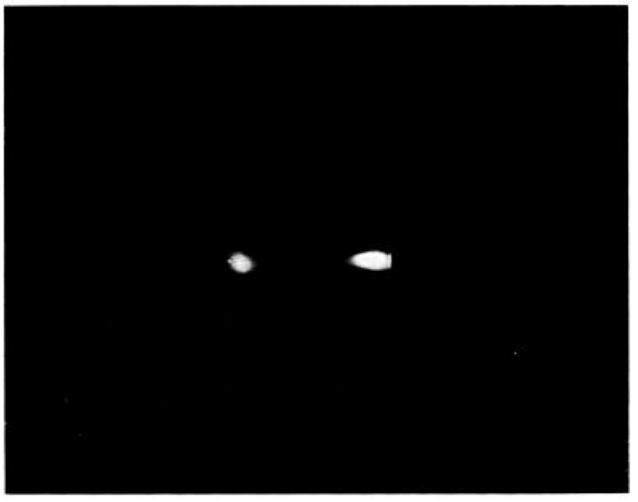

Figure 11. Aqueous flare of the anterior chamber of rabbit eye (Grade = 2)

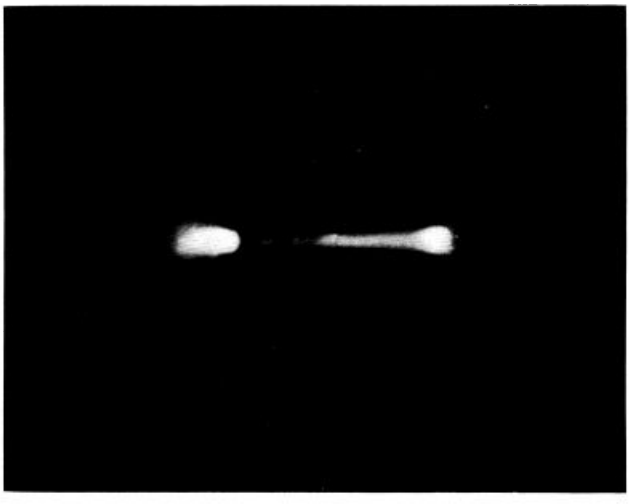

Figure 12. Aqueous flare of the anterior chamber of rabbit eye (Grade = 3)

Iris

The iris is an easily visualized structure and is extremely sensitive to chemical irritation and immunogenic reactions. As in the Draize method, we have found that iris hyperemia may be easily quantitated with the aid of the slit lamp (Table IV) (Figs. 13–15). In addition, however, iris edema may also be evaluated, either as a separate grading system or in combination with the hyperemia scores. Occasionally, pathology anterior to the iris, such as severe anterior chamber hypopyon and corneal opacities, obscures the iris from view.

Table IV
Slit-Lamp Examination of the Iris

Iritis	
Grade 1	Slight congestion of iris vessels; usually only part of iris involved
Grade 2	Mild congestion of iris vessels, all vessels of the iris involved
Grade 3	All vessels of the iris moderately congested
Grade 4	All vessels of the iris congested and dilated, with very little normal tissue observable

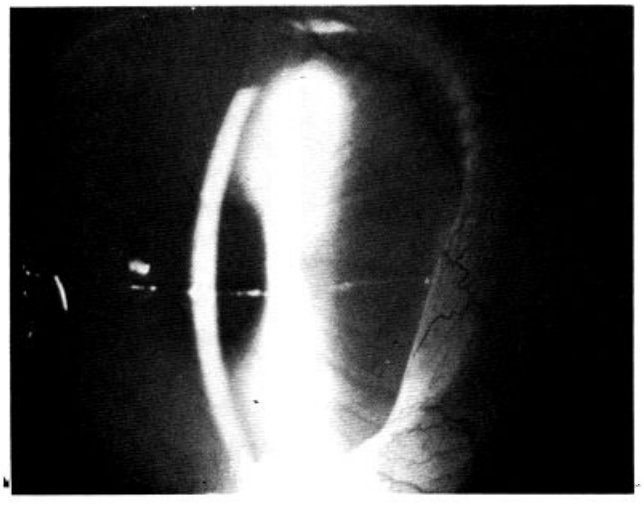

Figure 13. Normal iris of rabbit eye (Grade = 0)

Lens

The lens represents a critical structure for evaluation since any drug-related pathological change in the lens represents a substantial toxic phenomenon. Lens pathology also was not covered in the original Draize method, but is usually observed as either opacities of the anterior or posterior capsule, cortex, and/or some combination of all (Fig. 16). Separate grading scales may be established for each type of observed opacity. As an example of a grading system for lens opacities, Table V shows changes in anterior capsule integrity.

The experience in this laboratory has been that the investigator should not integrate numerical values for each parameter into an average score. The data are best represented by calculating the mean scores for each parameter separately and indicating the incidence. An overall average score as one can calculate by the Draize method does not allow the investigator to illustrate or tabulate what ocular pathological change is more prominent. We no longer calculate average scores but present the data as in Table VI.

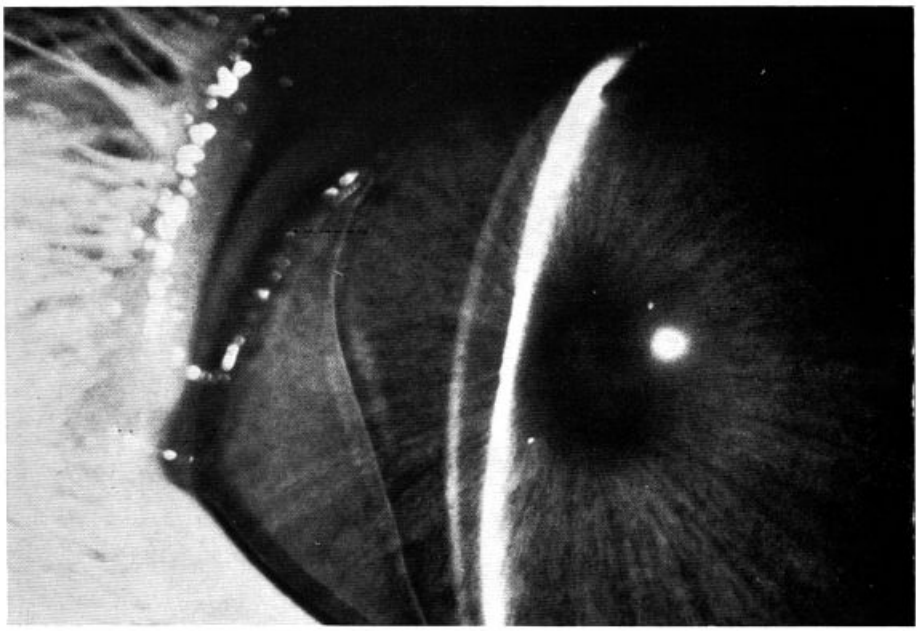

Figure 14. Iritis (congestion of iridal vessels) of rabbit eye **(Grade = 2)**

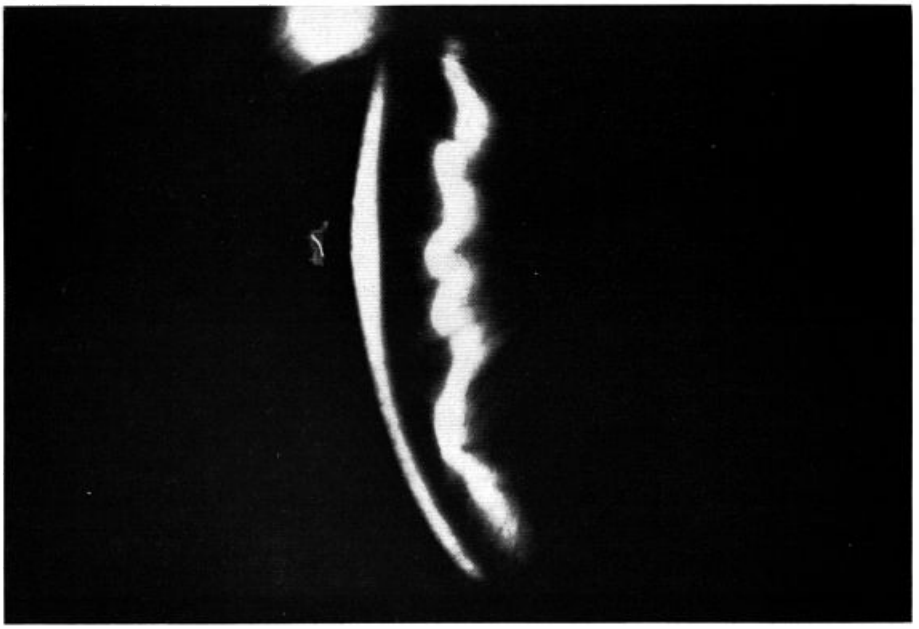

Figure 15. Iritis of rabbit eye (Grade = 4). (Note: **Hyperfolding–edema)**

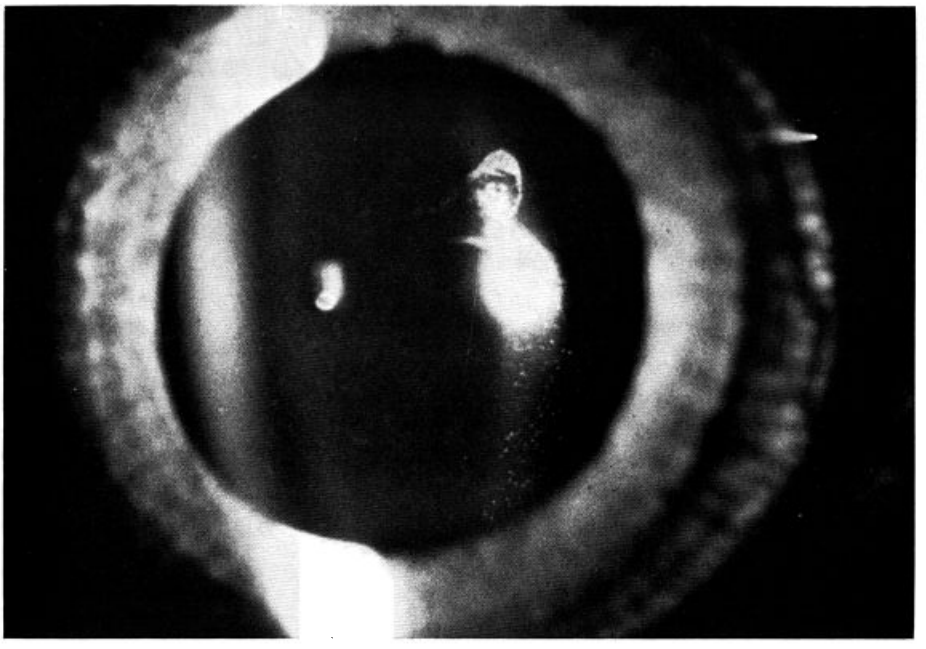

Figure 16. Posterior capsular and subcapsular cataract, rabbit

Table V
Slit-Lamp Examination of the Lens

Anterior capsule	
Grade 1	Faint precipitates on the anterior lens capsule, usually localized in 1 or 2 areas
Grade 2	Precipitates around the entire circumference of the anterior capsule; no vacuoles present
Grade 3	Precipitates around the entire circumference of the anterior lens capsule; localization of precipitates in the central portion of the capsule forming ring or semiring deposits (corresponds to the size of the normal pupil); vacuoles present
Grade 4	Complete clouding of the anterior capsule by precipitates; numerous vacuoles present; the lens cortex and posterior capsule usually may not be observed due to the clouding

Conclusion

In conclusion, it has been found that slit-lamp examination of experimental animal eyes is a valuable tool for the ophthalmic investigator. Various agents, administered either topically to the animal eye, or even systemic compound administration, can cause significant ocular pathology. Since many of these changes are too subtle to be detected by macroscopic examination, slit-lamp

Table VI
Ocular Changes Graded with Aid of Slit Lamp during Topical Ocular Exposure to Various Shampoo Formulations

Treatment[a]	Observation Period (Hr)	Flare	Iritis	Cornea Opacity Area		Fluorescein Staining Intensity Area	
Shampoo A	0	0[b] (0/6)[c]	0 (0/6)	0 (0/6)	0 (0/6)	0 (0/6)	0 (0/6)
	1	0.1 (1/6)	0.5 (3/6)	1.6 (6/6)	2.1 (6/6)	1.3 (6/6)	2.1 (6/6)
	24	0 (0/6)	0 (0/6)	0.5 (3/6)	1.0 (3/6)	0.3 (2/6)	0.3 (2/6)
Shampoo B	0	0 (0/6)	0 (0/6)	0 (0/6)	0 (0/6)	0 (0/6)	0 (0/6)
	1	0 (0/6)	0.1 (1/6)	0.3 (2/6)	0.5 (2/6)	0.8 (4/6)	1.0 (4/6)
	24	0 (0/6)	0 (0/6)	0 (0/6)	0 (0/6)	0 (0/6)	0 (0/6)
Shampoo C	0	0 (0/6)	0 (0/6)	0 (0/6)	0 (0/6)	0 (0/6)	0 (0/6)
	1	0.5 (3/6)	1.0 (6/6)	3.2 (6/6)	4.0 (6/6)	1.8 (6/6)	2.4 (6/6)
	24	0.1 (1/6)	0 (0/6)	2.1 (5/6)	3.1 (5/6)	1.3 (4/6)	1.5 (4/6)
Control	0	0 (0/6)	0 (0/6)	0 (0/6)	0 (0/6)	0 (0/6)	0 (0/6)
	1	0 (0/6)	0 (0/6)	0 (0/6)	0 (0/6)	0 (0/6)	0 (0/6)
	24	0 (0/6)	0 (0/6)	0 (0/6)	0 (0/6)	0 (0/6)	0 (0/6)

[a] 1:2 dilutions of three commercially available shampoos were instilled into 6 rabbit eyes. The eyes were observed at 1 and 24 hours after treatment.
[b] Mean score. The score or each parameter was graded by scales as indicated in this monograph. For each time period, the scores were totaled and divided by the number of eyes for the mean score.
[c] Incidence. Number of eyes demonstrating the ocular change/number of eyes tested.

examinations are now used as an integral, routine part of the evaluation of new drug entities in animals which are ultimately designed for ocular use. Since many cosmetics and other formulations are now also evaluated for ocular irritation, we feel that slit-lamp examinations may be useful for the study of the toxicity potential of these periocular applied agents.

Although photographic recordings of ocular changes are useful tools to the investigator, we have found that simple reproducible numerical grading scales may be easily established to document slit-lamp examinations. However, we have found that stereophotographs are an essential aid for teaching purposes wherein several investigators are called upon to grade pathologic changes in experimental animals. Without stereophotographs, the developer of a subjective grading scale must spend many hours with each investigator to enable combinability of results. Compared to this alternative, a collection of stereophotographs representative of the various grades of a specific condition may be projected for a group and subsequently viewed individually as necessary, even during actual examination procedures. These techniques of teaching have greatly reduced investigator-to-investigator variabilities.

Table VII
Evaluation of Slit Lamp

Disadvantages
Cost of equipment
Greater time expenditure for examination
Advantages
Detection and scoring of minute pathologies not possible with macroscopic examination
Examination of anterior chamber, lens, vitreous, and retina
Greater flexibility in determining degrees of pathologic changes, i.e., easier to develop concentration response relationships and/or differences between formulations
Reduced investigator-to-investigator variability
Photographic recording of observations possible during examination

An example of the type of data derived with use of the grading scales and slit-lamp examination is presented in Table VI. Three commercially available shampoos were evaluated for ocular irritation potential in rabbit eyes. The data were tabulated to demonstrate the severity (mean scores) and frequency (incidence) of each ocular parameter. Based on these data, the investigator indicated that Shampoo B had the lowest potential for ocular irritation while Shampoo C had a high potential for ocular damage.

In summary (Table VII), although there are disadvantages to the routine use of the slit lamp for examination of experimental animal eyes, we have found the techniques described in this text superior, in most instances, to macroscopic methods.

(Received June 20, 1972)

References

(1) Draize, J. H., Woodard, G., and Calvery, H. O., Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes, *J. Pharmacol. Exp. Therap.*, **82**, 377–90 (1944).