In vivo biomicroscopy and photography of meibomian glands in a rabbit model of meibomian gland dysfunction

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The usefulness of transillumination and biomicroscopy of the lid margin in assessing meibomian gland dysfunction was studied in a rabbit model. Transillumination of rabbit lids treated with topical epinephrine for 2 to 3 months revealed plugging of the meibomian gland orifice. Plugging appeared to be correlated histopathologically with increased thickness and hyperkeratinization of the ductal epithelium at the orifice. Continued treatment resulted in microcystic changes within the duct, which were not easily discernible by routine examination without the aid of a transilluminator. Cystic changes were correlated with dilation of the duct by retained desquamated cornified cells. Biomicroscopy proved valuable in identifying and documenting early lesions associated with epinephrine-induced meibomian gland dysfunction. (INVEST OPHTHALMOL VIS SCI 22:660-667, 1982.)

Key words: meibomian gland dysfunction, biomicroscopy, chalazia, acne rosacea, histopathology

Meibomian gland dysfunction is believed to be an important factor in various types of lid-margin disease, including those associated with acne rosacea and seborrhea dermatitis. These cases frequently present with such morphologic changes as microchalazia and chalazia. To date, methods to clinically document microchalazia formation have been of equivocal value. Tapie presented a biomicroscopic technique in 1977 for the identification and documentation of changes in meibomian-gland morphology; however, discussion of this technique pointed to the difficulty in distinguishing differences in meibomian gland morphology in most patients. A rabbit model of meibomian gland dysfunction has recently been described that uses topical epinephrine. To document (in vivo) the progressive anatomic changes in the rabbit meibomian glands, we adapted biomicroscopic and photographic techniques. The usefulness of these techniques in identifying subclinical changes will be demonstrated in this article. In addition, insights into the histopathologic correlation of meibomian gland dysfunction as identified by these techniques are presented.

Materials and methods

Ten adult New Zealand white rabbits weighing from 2 to 4 kg were divided into two experimental groups of five animals each. Two additional animals were used as a normal control group. The lids of each rabbit were evaluated by gross
Fig. 1. Transillumination of the rabbit lid was performed by applying slight pressure from the illuminator probe (arrow) underneath the lid, resulting in eversion of the lid. Glands were clinically examined with a Nikon 20-diopter lens.

examination with an OS-3001 transilluminator (Medical Instrument Research Associates, Inc., Boston, Mass.) (Fig. 1). Slight pressure from the transilluminator probe resulted in eversion of the lid, which facilitated examination of the meibomian glands through the palpebral conjunctiva. A Nikon 20-diopter indirect lens was also used to aid visualization. All glands appeared normal prior to drug treatment.

Experimental group A received one drop of topical 0.5% epinephrine hydrochloride in each eye, and group B received 2% epinephrine hydrochloride in each eye. Control animals did not receive drugs. Drops were administered twice daily at 8:00 A.M. and 5:00 P.M. for 5 consecutive days each week. Drops were not administered over the weekend. Animals were continued on this regimen for 6 months. Every 2 weeks lids were examined with the aid of a transilluminator and indirect lens. Morphologic changes in the gland were documented by transilluminated slit-lamp photography with a Zeiss Photo-slit lamp with high-speed infrared Kodak film (HIE 135-20) at 1/60 sec and a setting of f/32.

At 6 months, animals were euthanized with sodium pentobarbital (Euthanol) administered intravenously through an ear vein. Lids were removed, fixed in phosphate-buffered formalin, pH 7.4, embedded in paraffin, and serially sectioned to identify specific lesions. Selected sections were stained for neutral lipid (oil red O) and keratin according to the method of Ayoub-Shklar.7

Results

Animals receiving no drops or 0.5 epinephrine did not develop changes in meibomian glands clinically, by transillumination, or by histopathologic study. Animals in group B (2% epinephrine) slowly developed mild erythema of the conjunctiva associated with clinical engorgement or dilation of individual meibomian glands, especially those located on the lower tarsal plate near the inferior punctum. In advanced cases, expression of engorged glands resulted in large amounts of cheesy material.

Histologically, meibomian glands from “normal” control rabbit eyes demonstrated simple branched acinar glands containing a single long central duct with an orifice located just anterior to the mucocutaneous junction. Acini were connected along the length of the main duct by means of short ductules (Fig. 2, A). Although the ductal epithelium has been characterized as being of a keratinizing nature,8 the presence of desquamating cells within the duct lumen was not a normal finding. Large globular masses were occasionally observed in the lumen, which appeared to be composed of cellular debris or condensed secretory material that was positive to periodic acid–Schiff and neg-
Fig. 2. A, Light micrograph of normal meibomian gland. Note central duct (D) extends throughout length of gland and connects to single acini by means of short ductules (arrow). Inset, Orifice of the gland is located anterior to the mucocutaneous junction. Arrowhead, Meibomian gland orifice; C, conjunctiva. (hematoxylin-eosin; ×36; inset, ×88.) B, Light micrograph of orifice from 2% epinephrine-treated lid exhibiting ductal plugging. Overall gland structure appears normal; however, the central duct does contain an increased accumulation of cellular debris (D). The orifice of the gland was markedly narrowed (arrowhead) by the presence of a well-developed stratum corneum in addition to the thickened keratinized epithelium (H & E; ×36; inset, ×88.)
ative to keratin. By transillumination, individual "normal" meibomian glands appeared as a single "bunch of grapes," with acini represented as small grapelike clusters of hypoilluminescent areas (Fig. 3, A). The duct and orifice of the gland appeared to transmit light and therefore their presence was only suggested by the outline of acini surrounding these structures (Fig. 3, A, arrowhead).

In group B, transillumination revealed several changes that were not immediately apparent with gross clinical examination. In three animals, several orifices of the inferior glands became hypoilluminescent between 2 to 3 months after initiation of epinephrine treatment (Fig. 3, B, arrowhead). Clinical histopathologic correlation of these subclinical lesions indicates that the hypoilluminescent orifice corresponded to an increased thickness of the keratinized epithelium at the orifice, with a marked presence of a well-developed stratum corneum not typically observed in normal glands (Fig. 2, B, inset). These changes may have resulted in occlusion of the duct; however, the degree of desquamated cornified cells and the increased thickness of the epithelium were not as remarkable as had been expected by the marked changes in transillumination.

In two rabbits, hypoilluminescent of the orifice was followed by a progressive loss of acini combined with development of hypoilluminescent microcysts occurring over the next 3 to 4 months (Fig. 4). These cysts corresponded to dilations of the duct and contained predominantly desquamated cornified cells lined by hyperkeratinized epithelium (Fig. 4, B, inset). In one case, continued production of cornified cells resulted in dilatation of the entire duct with almost complete loss of acinar tissue (Fig. 5).

**Discussion**

A role for the meibomian gland in disorders of the lid margin has been postulated by some investigators. Analyses of lipid components from meibomian-gland secretions of normal subjects and from those with lid-margin disorders have been undertaken by us and others, with equivocal results. Nevertheless, there appears to be a definite progression of clinically observable morphologic changes in the meibomian glands during the course of lid margin disease, e.g., acne rosacea and chalazia. In such patients it has been the clinical observation of one of us (R. E. S.) that the meibomian glands seem to become irregularly dilated, resembling swollen piano keys. This may progress over several months to form apparent local obstructions, microchlaazia, and in some cases, frank chalazia. The following associated clinical features may be found: inspissation of material in meibomian glands, thickening of lid margins, vascularization across the posterior lid margin, and generalized secondary conjunctivitis with foamy tears.

In the rabbit model, the clinical course is characterized by an initial mild erythema of the conjunctiva followed by swelling or dilatation of the meibomian glands. Although in the late stages of this disease the glands were markedly dilated and contained large amounts of cheesy material, no inflammatory component similar to chalazia was identified. Through the use of transillumination biomicroscopy, the earliest changes noted were plugging of the ductal orifice, followed by dilation and hypoilluminescent of the deep portions of the duct.

These experimental lesions are similar in clinical appearance to the lipid concretions described by Tapie using similar biomicroscopic techniques on patients with chalazia and blepharitis. Although the exact nature of the material contained within these concretions is unknown, it is possible that their presence clinically indicates areas of ductal plugging or plugging of the ductless leading to individual acini and therefore may parallel the changes seen in the rabbit model. Biomicroscopic evaluation and follow-up of such lesions may give important insights into the pathogenesis of meibomian gland dysfunction states. Certainly the use of biomicroscopy in the present experimental model proved invaluable in identifying the early subclinical change of orifice plugging. Progressive loss of
Fig. 3. A, Biomicroscopic photograph of lid from untreated rabbit. Individual meibomian glands (large arrow) are seen as "grapelike" clusters of hypoilluminescent spots corresponding to individual acini (small arrow). The orifice and duct are illuminescent and their presence is suggested only by illuminescent areas surrounded by the hypoilluminescent spots of acini (arrowheads). (×24.) B, Biomicroscopic photograph of 2% epinephrine-treated lid 2 months after initiation of treatment. Note dark opacities or hypoilluminescence of gland orifice (arrowhead). (×24.)
Fig. 4. A, Biomicroscopic photograph of 2% epinephrine-treated lid 4 months after initiation of treatment. Note presence of hypoilluminescent microcyst deep within gland (arrow). Orifice also exhibits hypoilluminescence. (×24.) B, Micrograph showing prominent cystic dilations of duct deep within gland (arrow). This cyst contained predominately desquamated cornified cells and was lined by hyperkeratinized ductal epithelium. In this gland, the ductal orifice is also narrowed by hyperkeratinized ductal epithelium (arrowhead). (H & E; ×42; inset, ×103.)
Fig. 5. A, Biomicroscopic photograph of 2% epinephrine-induced cystic change of meibomian gland. Note complete loss of normal gland structure. (×10.) B, Light micrograph of lesions identified in A. There is extensive dilation of duct, with loss of normal acini. Material within duct was composed of cornified desquamated cellular debris. (H & E; ×42.)
acini and microcystic changes preceding the replacement of the meibomian gland by the keratic cysts were also easily identified and documented by biomicroscopy.

From our studies, it would appear that hyperkeratinization of the ductal epithelium resulted in plugging of the meibomian gland orifice. It is possible that blockage may have resulted from the pharmacologic effects of epinephrine on the muscles of Riolan, which when contracted may inhibit the flow of meibomian secretions (meibum) through the orifice in rabbits. However, it is not generally believed that such mechanical blockage results in hyperkeratinization and keratic cyst formation. Therefore epinephrine may be involved in the hyperkeratinization of the deeper ductal epithelium. Hyperkeratinization and keratic cyst formation in the meibomian gland has also been associated with polychlorinate biphenyl (PCB) poisoning in primates and humans.

The similarity between the histopathologic changes induced by epinephrine and PCB suggest that epinephrine has a primary effect on the keratinization of the ductal epithelium. Little is known, however, regarding cystic degeneration of the rabbit meibomian gland; therefore, extensive studies are indicated to clarify further the temporal sequence, histopathologic and ultrastructural changes, and alteration in lipid (meibum) constituents associated with this model of meibomian gland dysfunction.

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REFERENCES