Differential vital staining of the canine cornea and conjunctiva with fluorescein-rose bengal and alcian blue

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ABSTRACT

The literature on the use of vital stains in the clinical investigation of corneal lesions is reviewed. The application of a combination of fluorescein-rose bengal mixture and alcian blue was investigated in fifty-eight canine eyes prior to the experimental production of keratoconjunctivitis sicca. The results are presented and it was concluded that the fluorescein-rose bengal mixture was a convenient method of utilizing the staining properties of both agents, that the repeated application of rose bengal is unwise and that alcian blue is a useful adjunct for demonstrating the mucous thread and for differentiating mucus and cellular debris.

INTRODUCTION

Vital staining is staining of a living tissue by a dye in dissolved state when it comes in contact with the tissue, and does not refer to staining of individual cells (Norn, 1962, 1972). In human ophthalmology, many stains have been used to demonstrate the appearance, progression and regression of specific ocular lesions. In veterinary ophthalmology fluorescein is widely used to demonstrate defects in the corneal epithelium. More recently, rose bengal has been used, especially in the diagnosis of keratoconjunctivitis sicca (Gelatt, 1972; Slatter, 1973). This report describes the use of a fluorescein-rose bengal mixture, followed in some cases by alcian blue solution, in evaluating the conjunctiva and cornea of the dog. The use of a similar solution in man has been reported (Norn, 1962, 1964a, b, c, 1967). The stainings of normal eyes in this study were obtained prior to experimental induction of keratoconjunctivitis sicca by administration of phenazopyridine hydrochloride (Slatter, 1973; Slatter & Davis, 1973; Bryan &
Slatter, 1973). In addition, the mucous thread was found to be a constant feature of the normal canine conjunctival sac as it is in man.

**LITERATURE SUMMARY**

(1) *Fluorescein*

Staining of the cornea with fluorescein, either as a sterile solution or in impregnated paper strips, is a routine procedure in the dog and man (Duke-Elder, 1965; Startup, 1969; Magrane, 1971). Various authors draw different conclusions about the mechanism of fluorescein staining. In a recent review Norn (1972) stated that fluorescein does not stain cells, mucin, fat or other normal superficial conjunctival components but that it enters defects in the corneal epithelium and stains the tissue fluid surrounding the epithelial cells and in the corneal stroma. Unlike other ophthalmic vital stains (e.g. rose bengal, iodonitrotetrazolium) fluorescein does not enter the cells (Norn, 1972). Fluorescein does not stain Descemet’s membrane and consequently deep corneal lesions penetrating to this level stain around the edges only, where the dye penetrates the superficial epithelium and corneal stroma. Thorough staining of the cornea can be performed with fluorescein impregnated strips only if the strip has been previously wetted with a suitable vehicle (Norn, 1967a, b). The danger of contamination of this vehicle is just as great as that for a multidose vial of fluorescein solution. Ideally, individual vials of fluorescein should be used.

(2) *Rose bengal*

Rose bengal is used to stain lesions of the conjunctiva and cornea and is a significant aid in the diagnosis of keratoconjunctivitis sicca, especially early in the disease, in both man and the dog. The dye enters and stains cells in the early stages of degeneration, indicating the beginning of pathological alterations (Norn, 1967a, b). Rose bengal also stains normal cells initially, but these cells soon lose the stain whereas degenerate cells retain it. Degenerate cells of cornea and conjunctiva, purulent exudates and mucin are all stained a red colour. Instillation of rose bengal into the conjunctival sac of the dog may be quite painful, manifested by blepharospasm and conjunctival hyperaemia, followed by an increase in mucoid discharge on the following day. An increased number of neutrophils was also observed in these trials in conjunctival scrapings on the day following fluorescein-rose bengal. Fluorescein itself is not irritant. Thorough washing of the conjunctival sac with physiological saline will minimize, although not eliminate, painful adverse reactions.

(3) *Fluorescein-rose bengal mixture*

Norn (1964b, c) reported the use of a fluorescein-rose bengal mixture in man. After washing the cornea and conjunctiva with sterile saline, this mixture was followed by a few drops of 1% alcian blue. This method was used because alcian...
blue stains mucus which has been previously stained with the rose bengal but does not stain corneal and conjunctival lesions. This mucus which is stained by both the rose bengal and alcian blue is coloured green. The use of these three stains allows corneal epithelial defects, degenerate cells and mucus to be differentiated. Alcian blue may cause permanent tattooing of the corneal stroma if applied to defects in the corneal epithelium and consequently should not be used if such a defect is suspected. The use of a mixture of the three stains would simplify application but alcian blue is chemically incompatible with rose bengal so it must be applied as a separate solution.

(4) Iodonitrotetrazolium

More recently Norn (1972) has recommended the use of 1% iodonitrotetrazolium and 0·25% alcian blue for conjunctival lesions. Unlike rose bengal which stains both dead and degenerating cells, iodonitrotetrazolium stains only degenerating cells. The colourless dye enters only degenerating cells and is reduced to the red dye formazan by intracellular enzymes. This reduction does not occur in dead cells because of cessation of necessary enzymatic activity. This technique has the advantage that it does not stain normal cells as often happens with rose bengal. The staining properties of fluorescein, rose bengal, alcian blue and iodonitrotetrazolium are summarized in Table 1. (Iodonitrotetrazolium was not used in this series.)

<table>
<thead>
<tr>
<th>Stain</th>
<th>Structures Stained</th>
<th>Indication</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescein</td>
<td>Tissue fluid surrounding corneal epithelial cells and in corneal stroma</td>
<td>Epithelial defects</td>
<td>Green</td>
</tr>
<tr>
<td>Rose bengal</td>
<td>Dead and degenerate cells of conjunctiva and cornea Mucus</td>
<td>Degenerative lesions of conjunctiva and cornea Presence of increased mucus and conjunctival discharges</td>
<td>Pinkish-red</td>
</tr>
<tr>
<td>Alcian blue</td>
<td>Mucus</td>
<td></td>
<td>Blue (green in mucus previously stained red with rose bengal)</td>
</tr>
<tr>
<td>Iodonitrotetrazolium</td>
<td>Degenerate cells</td>
<td>Degenerate conjunctival lesions</td>
<td>Red</td>
</tr>
</tbody>
</table>

Detailed descriptions of the human mucous thread have been made (Norn, 1963, 1966, 1967a, b, 1971, 1972). Conjunctival mucus is produced by the goblet cells of the conjunctiva and extruded into the conjunctival sac as fibrils which join together and form a thread in the ventral fornix. This thread migrates, with the blinking action of the eyelids, to the medial canthus where it may accumulate on the skin. A small mucous thread is also present in the dorsal fornix. This accounts for the normal accumulation of mucus at the medial canthus in both
man and dog. The mucous thread, which is not normally visible unless stained, cleans the conjunctival sac and accumulates dust and desquamated cells.

**MATERIALS AND METHODS**

Fifty-eight normal canine eyes were stained with fluorescein-rose bengal mixture and alcian blue prior to experimental production of keratoconjunctivitis sicca. Tear flow values for these dogs were normal at this stage as measured by the Schirmer tear test. The following solutions were prepared aseptically (Norn, 1972):

(1) Fluorescein sodium 50 mg  
Rose bengal 50 mg  
Sodium chloride 0.05 mg  
Aq. ad. 5 g  
(2) Alcian blue 50 mg  
Aq. ad. 5 g

Sodium chloride is not added to alcian blue because precipitation will occur.

0.2 ml fluorescein-rose bengal was placed in each eye. After 1 minute the conjunctival sac was carefully flushed with 2–3 drops of sterile saline to remove excess dye. The eye was then examined with a slit lamp, binocular loupe, and focal light source. Alcian blue (0.2 ml) was then instilled and staining of mucus or exudates recorded. In the later stages of production of keratoconjunctivitis sicca some stainings were performed, but not on a regular basis as it was considered that the irritation produced would affect subsequent tear flow values and conjunctival cytology (Slatter, 1973).

**RESULTS AND DISCUSSION**

Retention of fluorescein was not observed in any of the normal eyes. Many normal eyes showed rose bengal retention in various locations (Table 2).

These figures are similar to those found by Gelatt (1972) when 1% rose bengal was used alone. The minute punctate areas were not restricted completely to the

| Table 2. Rose bengal retention by conjunctiva and cornea in fifty-eight normal canine eyes |
|---------------------------------|----------------|----------------|
| Location                        | Rose bengal retention |
| Dorsolateral cornea (minute punctate areas) | No. | %  |
| Dorsal palpebral conjunctiva     | 27  | 47 |
| Dorsal bulbar conjunctiva       | 14  | 24 |
| Ventral palpebral conjunctiva   | 55  | 95 |
| Nictitating membrane (external surface) | 53  | 91 |
dorsolateral cornea but were found there in greater numbers. The mucous thread was found to be present in almost all eyes after staining with 1% alcian blue (Fig. 1) but in some cases the thread was very small and trapped within folds of conjunctiva in the ventral fornix.

As tear flow was decreased by the experimental treatment, the minute punctate areas in the cornea were found in a greater number of the dogs, the areas were more scattered over the cornea and staining was a more intense red. Staining of all areas of the conjunctiva was much more intense and widely distributed in animals with decreased tear flow. Clinical signs of keratoconjunctivitis sicca were present in these animals (pain, photophobia, conjunctival hyperaemia and slight mucopurulent discharge) but histopathological lesions were not observed in the cornea or conjunctiva, indicating that rose bengal was distinguishing early cellular alterations (severe keratoconjunctivitis sicca was not produced with phenazopyridine hydrochloride). The use of alcian blue facilitated the differentiation of normal mucus from mucopurulent exudates containing cellular debris.

The significant conclusions from these experiments are:

1) The mixture of fluorescein and rose bengal is a more convenient method of administering the dyes than the use of each alone. Staining properties are not affected by this mixing.

2) Increase in frequency and intensity of staining of both cornea and conjunctiva with rose bengal was correlated with decreased tear flow when severe histopathological lesions were not present. For this reason, it is believed that rose bengal is valuable in the early detection of keratoconjunctivitis sicca.
(3) The repeated use of rose bengal in the same eye is unwise and likely to result in inflammation of the conjunctiva. (Increased numbers of neutrophils were noted in conjunctival scrapings taken 24 hr after the use of rose bengal-fluorescein mixture.)

(4) The mucous thread is present in the dog as in man and probably has the same functions.

(5) Alcian blue (1%) is a useful adjunct in the examination of the eye for
   (a) Visualization of the normal mucous thread.
   (b) Differentiation of mucous and cellular debris after rose bengal staining.

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References
Norn, M.S. (1964a) Acta ophthal. 42, 84.
Norn, M.S. (1967b) Am. J. Ophthal. 64, 1078.

Résumé. Les publications sur l'usage de colorants vitaux dans l'investigation clinique des lésions de la cornée, sont sous revue. Avant la production expérimentale de Keratoconjunctivite sèche, on a étudié, dans cinquante-huit yeux canins, l'application d'un mélange de fluorescéine-rose bengale et de bleu alcian. On a présenté les résultats et on a conclu que le mélange de fluorescéine-rose bengale a été une méthode pratique pour utiliser les propriétés colorantes des deux agents, que l'application répétée de rose bengale n'est pas à recommander et que le bleu alcian est un complément utile pour démontrer les filaments muqueux et pour différencier le mucus et les déchets cellulaires.