SUPERFICIAL KERATECTOMY FOR THE MANAGEMENT OF CORNEAL WOUNDS IN CANINES

By

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THESIS

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DECLARATION

I hereby declare that the thesis entitled **"SUPERFICIAL KERATECTOMY FOR THE MANAGEMENT OF CORNEAL WOUNDS IN CANINES"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Certified that the thesis, entitled **"SUPERFICIAL KERATECTOMY FOR THE MANAGEMENT OF CORNEAL WOUNDS IN CANINES"** is a record of research work done independently by **Sri. J. David Suresh**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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Introduction

INTRODUCTION

Diseases affecting the cornea are commonly encountered in dogs and cats. Usually the vision of the animal is at risk, when the cornea is severely damaged because it is one of the important structures of the refractive media of the eye. In dogs, the common corneal affections are corneal wounds, corneal ulcers, corneal inflammations, corneal erosions and dystrophies. Congenital abnormalities like corneal dermoids and corneal neoplasms are infrequent.

Injury to the cornea often occurs in dogs from cat scratches, contact with sharp objects like thorns, barbed wires, nails and other foreign bodies, chronic irritation from conditions like entropion, trichiasis, distichiasis and from chemical burns (Studdert, 1967 and Startup, 1984). Lesions are mostly in the form of ulcers caused by denudation of epithelium and varying amount of stroma.

Superficial corneal wounds or ulcers heal rapidly, but sometimes it becomes chronic due to irritation or bacterial invasion. The toxins produced locally by the organisms cause rapid degradation of the corneal surface, penetrating ulcers, and threaten the integrity of the cornea as encountered in *Psuedomonas aeruginosa* infection (Studdert, 1967).

Diseases of the cornea in animals and their surgical correction have gained importance during the last few years (Mohanty and Mitra, 1971). Various surgical and medicinal treatments have been tried for the successful management of corneal wound. In many instances, surgical treatments are employed in corneal wound to bring about rapid healing of the cornea.

Superficial keratectomy, corneal transplantation, conjunctival keratoplasty, third eye lid flap and temporary tarsorrhaphy have been reported to promote healing and reduce the healing time.

Superficial keratectomy was advocated to enhance the healing in many types of corneal lesions, (Kirschnar *et al.*, 1989). Nictitans flap has been recommended to protect the cornea and prevent it from further injury (Studdert, 1987). Temporary tarsorrhaphy has been reported to protect the cornea as well as moisten it (Roberts, 1953; Peiffer Jr. *et al.*, 1987a; Williams, 1994). A comparative evaluation of all the three techniques along with medicinal therapy has not been done extensively.

The present study was undertaken with the objective of evaluating the effect of superficial keratectomy for the management of corneal wounds in canines.

Review of Literature

REVIEW OF LITERATURE

Livingston (1950) performed conjunctival flap operation under sodium pentobarbital anaesthesia in a dog suffering from corneal ulcer. The upper bulbar conjunctiva was freed and sutured to the membrana nictitans. The edges of the eye lid were kept in approximation with adhesive tape, and found that this method was excellent in treating perforated corneal ulcer.

Stern (1950) recommended conjunctival flap operations for ulcerative keratitis, deep scratches and gauges as well as through and through lacerations of the cornea. The upper bulbar conjunctiva was used as the flap and it was fixed with lower bulbar conjunctiva using 4/0 catgut. He reported that the operation shortened the healing time and restored normal contour of the cornea.

Roberts (1953) treated 24 dogs and three cats in a span of five years with conditions like corneal ulcers, corneal lacerations, descemetoceles and staphylomas by performing conjunctival flap operation. He used intravenous pentobarbital sodium solution to produce general anaesthesia. The flap was prepared from the bulbar conjunctiva, separated around the limbus and brought over the cornea by purse-string suture. The technique was simple and effective for treating perforated

cornea. Tarsorrhaphy was useful in the treatment of dryness of the cornea, protrusion of the globe and paralysis of the orbicularis occuli muscle. Fluorescein stain was found useful to demonstrate the corneal wound and to assess the rate of healing.

Catcott and Griesemer (1954) studied corneal healing in dog, after experimental induction of injury and evaluated the role of vascularization. They found that the healing of the cornea was delayed when neovascularization of the cornea was interrupted with beta radiation and corticosteroids.

Magrane (1955) reported that the vascularization of the cornea during corneal healing was a defensive mechanism to the cornea against noxious influences. Diseases affecting epithelial structure resulted in superficial vascularization arising from the conjunctiva whereas in diseases affecting the stroma deep vessels originated from the ciliary artery.

Krawitz (1963) reported that the cornea in dog consisted of outer epithelial layer, stroma, descemet's layer and corneal endothelium.

Roberts (1965) described the characteristic appearance of superficial corneal ulcer in twentynine boxer dogs and treated the corneal ulcer with seven per cent alcoholic solution of

iodine as a chemical cautery and found that steroids delayed corneal healing and hence were contra-indicated in superficial corneal ulcers. The healing period varied from 5-35 days. He suggested the use of fluorescein stain to evaluate the healing of corneal ulcer and observed that the denuded corneal surface was stained bright green and newly formed granulation tissue, faint greenish yellow.

Barnett (1966) recommended third eye lid flap for chronic corneal ulceration, deep ulcers and descemetocele and it was preferred over conjunctival keratoplasty.

Belhorn and Henkind (1966) studied the pigmented corneas in dogs and reported that pigmentary keratitis in dog was due to the migration of limbal melanocytes during superficial corneal vascularization.

Hime (1966) used two per cent fluorescein dye to outline the corneal ulcers and noted that the corneal ulcers with greyish floor depressed below the corneal surface was stained bright green.

Knecht *et al.* (1966) performed conjunctival flap operation from the nictitating membrane in a cat suffering from chronic corneal ulcer with a dry black plaque covering the central corneal epithelial surface. After 10 days,

superficial keratectomy was performed along with medicinal treatment and recovery was uncomplicated.

Spreull (1966) reviewed the anatomy and physiology of the cornea in dog and reported that it comprised of five layers: viz. (1) epithelium, (2) Bowman's capsule, (3) Stroma, (4) Descemet's membrane and (5) endothelium. He found that in healing of cornea, the epithelial lesions had healed by epithelialization, the healing completed in four to seven days and stromal injuries by the growth of stromal connective tissue cells and fibroblasts. The endothelial cells were found to migrate over the deep or inner surface of the substantia propria to re-establish the Descemet's layer.

Studdert (1967) reported the absence of well developed Bowman's membrane in canine cornea and suggested that in corneal ulceration, corticosteroids should be avoided as long as the epithelial regeneration was incomplete, as confirmed by fluorescein staining. He recommended conjunctival flap, nictitating membrane flap and tarsorrhaphy to protect the cornea in corneal ulcers.

McEntyre (1968) performed experimental penetrating keratoplasty in dog, and assessed the clarity of the donor cornea by grading it on a 0 to 4+ scale.

Hinton (1969) recommended 0.5 per cent procaine hydrochloride as a suitable local anaesthetic for the eye and the use of fluorescein stain for the detection of small, punctate or superficial ulcers of the cornea.

Shively and Epling (1970) studied the fine structure of canine cornea under electron microscope and stated that the cornea of the dog comprised of all the layers as described in other species but for the absence of complete Bowman's membrane.

Breazile and Howard (1971) observed that the cornea of the dog comprised of five layers, and referred the Bowman's membrane as a basement membrane which was very thin compared to that in the other species.

Howard and Breazile (1971) suggested conjunctival flap operation for deep and chronic corneal injury as it offered a protective covering to the cornea and aided in the healing process. They also recommended the use of fluorescein strips to outline the corneal damage. Systemic administration of antibiotics were suggested along with conjunctival flap operation to prevent infection.

Mohanty and Mitra (1971) carried out conjunctival keratoplasty along with temporary tarsorrhaphy for treatment of descemetocele. They found increased vascularity, marked

improvement in healing of ulcer and return of vision by the eighth day. The cornea regained its normal contour without any inflammatory change, by 15 days.

Startup (1972) noted that the cornea of the dog comprised of four layers and the Bowman's membrane was unrecognizable. Fluorescein dye test demonstrated the superficial ulcers of cornea, degree of the ulceration, and its extent and depth. He found that corticosteroids were effective in controlling unwanted vascularization of cornea but should be used only when the corneal epithelium was restored. Superficial keratectomy was recommended for chronic epithelial erosion and conjunctival flap for deep corneal ulcers. The flap was usually retained for five to six days postoperatively.

Koch *et al.* (1974) performed superficial keratectomy in four dogs having corneal epithelial inclusion cysts and the recovery was uncomplicated.

Rosenthal (1974) recommended superficial keratectomy in the early stages of corneal sequestrum in cat.

Anderson *et al.* (1976) performed modified membrana nictitans flap and retained it for 10 days to treat ulcerative keratitis in cattle. The flap was well tolerated and occasional lateral canthal swelling, inflammation of the membrana nictitans, and conjunctival exudation were observed.

To support the weakened cornea they recommended third eye lid flap technique along with temporary tarsorrhaphy.

Brown (1976) used nictitans flap successfully for the treatment of kerato-conjunctivitis in bovine.

Bistner *et al.* (1977) stated that while performing third eye lid flap scarification of bulbar aspect of the membrana nictitans increased exudation of the serum over the corneal surface and facilitated adhesion between the membrana nictitans and the cornea.

Schmidt (1977) reported that the rate of corneal vessel growth was 0.3 mm to 0.5 mm per day in neovascularization of the cornea during healing process. Third eye lid flap as well as conjunctival flap operation was recommended in corneal ulceration to assist in the healing process.

Slatter *et al.* (1977) studied Uberreiter's syndrome in 463 dogs and suggested superficial keratectomy and partial penetrating keratoplasty as the methods of treatment to be combined with corticosteroids and beta irradiation.

Carter (1981) recommended third eye lid flap to cover the cornea during its healing process as it provided support to the damaged tissue, retained moisture and protected newly formed corneal epithelium. He opined that the free margin of

the third eye lid may get deformed when it is sutured to the upper eye lid.

Slatter (1981) observed that corneal epithelial defects were covered by sliding of the cells sorrounding the margin of the lesion and small stromal defects were filled with epithelial cells forming an epithelial facet. Uncomplicated stromal wounds healed in avascular fashion and destructive lesions healed by vascular healing. Corneal oedema was observed when there was loss of either epithelium or endothelium. Third eye lid flap technique was found to facilitate the healing of the cornea, reduce pain and prevent further injury.

Bromberg (1983) suggested that the third eye lid flaps were useful to protect and support the cornea in corneal ulcers in dogs but the flaps were found to decrease the amount of medication reaching the cornea. He recommended to retain the flap for 14-21 days.

Helper and Blogg (1983) recommended a modified third eye lid flap procedure for corneal damage to facilitate healing. Under general anaesthesia suture was applied on the palpebral surface of the third eye lid without penetrating the bulbar conjunctiva and then taken through the upper fornix on the skin of the upper eye lid with knot placed over a button.

This method allowed the flap to be released whenever needed for examination of the cornea.

Kudva and Deshpande (1983) studied autogenous and homogenous lamellar keratoplasty in bovines and postoperatively graded the clarity of the host cornea and graft cornea on a 0 to 4+ scale.

Kudva *et al.* (1983) histologically studied the healing process of the corneal grafts after autogenous and homogenous lamellar keratoplasty in bovines at different postoperative periods and found that the corneal epithelium and stroma of both graft and host cornea were completely normal by 60th postoperative day.

Rebhun (1983) used fluorescein stain to outline the superficial corneal ulcer in horses. Corticosteroids interfered with normal corneal epithelial mitosis and migration delaying the healing process of the cornea.

Startup (1984) recommended superficial keratectomy for chronic epithelial erosion and conjunctival flap or nictitating membrane flap for corneal ulcers in dogs. He stated that there was a chance of friction between the cornea and the third eye lid flap when the flap was secured to the upper eye lid and adviced to retain the flap upto 14 days. He found that temporary tarsorrhaphy was useful in neurotrophic

ulcerations. Uveitis, panophthalmitis, corneal perforation, vascularization and corneal scarring were the complications of corneal ulceration. He employed two per cent fluorescein stain was employed for routine corneal examination and suggested the use of corticosteroid to control unwanted vascularization when the cornea is fluorescein negative.

Cooley and Wyman (1986) performed superficial keratectomy and conjunctival flap in a mare with bilateral corneal ulcers and the recovery was satisfactory. The healing of cornea consisted of epithelial cell migration within first 24 hours, followed by epithelial cell mitosis and delay in epithelial healing resulted in corneal oedema, vascularization and pain.

Barnett and Sansom (1987) recommended partial tarsorrhaphy in corneal ulceration associated with keratoconjunctivitis sicca. Fornix based conjunctival flap was suggested to prevent corneal rupture and to aid in healing of various types of ulcers and keratocele. They opined that the administration of corticosteroids must be avoided in corneal ulceration with keratoconjunctivitis sicca. Superficial keratectomy was found useful in such cases to remove corneal pigmentation and granulation tissue thus improving the vision.

Bedford (1987) recommended membrana flap in case of penetrating corneal wounds and found that the conjuntival

flaps made from the bulbar conjunctiva in the form of pedicle sutured over the ulcer provided blood supply to the cornea and strengthened it. The conjuntival flap retained for 10 to 15 days minimised the residual scarring and opacity of the cornea.

Latimer *et al.* (1987) excised squamous cell carcinoma of the cornea in a dog by superficial keratectomy combined with cryosurgery using double freeze-thaw cycle and found that the tumour regrowth was not clinically discernible even an year after the treatment. Corneal re-epithelialization was complete in seven days as determined by fluorescein staining.

Peiffer Jr. *et al.* (1987a) stated that nictitans flap or tarsorrhaphy was useful to protect the cornea in the treatment of traumatic proptosis of the globe. The nictitans flap or temporary tarsorrhaphy was retained for seven to ten days.

Peiffer Jr. *et al.* (1987b) reported that tarsorrhaphy was indicated in exophthalmos associated with post proptosis trauma, orbital haemmorrhage or orbital cellulitis. Temporary tarsorrhaphy was performed by placing an interrupted horizontal matress suture over the eyelids, 3 mm from the lid margins and the distance between the bites of a single suture being 5 mm.

Peiffer Jr. *et al.* (1987c) opined that in severely ulcerated cornea conjunctival flap was advantageous over nictitating membrane flap as the former provided protection, support and rich blood supply to the lesion, but the latter acted only as a supportive bandage to the ulcerated cornea.

Peiffer Jr. *et al.* (1987d) stated that in chronic inflammation of the cornea with neovascularization, the superficial stromal vessels invaded the cornea from the conjunctival capillaries at the limbus whereas deeper stromal vessels which were short and less branching originated from the ciliary vessels. The epithelial defects healed rapidly by migration and multiplication of adjacent cells and the stromal defects healed by proliferation and migration of fibroblasts with or without neovascularization. Lamellar keratectomy was suggested in dog and cats in conditions like corneal dermoid, ectopic island of skin, hair follicle and adnexal glands located at the limbus, lymphoid dystrophy, diffuse superficial keratitis, pannus, corneal sequestration, proliferative or eosinophilic granulomatous keratitis and refractory superficial ulcers.

Spiess and Tscharner (1987) recommended superficial keratectomy as a treatment of choice for corneal sequestrum in the cat.

Sansom (1988) identified Staphylococcus, Streptococcus, Corynybacterium, Neiseria and Moraxella species in the normal conjunctival sac of the animals and reported that topical application of framycetin sulphate with a broad spectrum of activity was more effective against psuedomonas.

Stanley (1988) performed superficial keratectomy to remove the excessive pigment and granulation tissue in the treatment of superficial keratitis in dogs.

Startup (1988) suggested superficial keratectomy with conjunctival flap or nictitating flap as a supportive treatment to correct corneal sequestrum in cat. However, keratectomy procedures were contraindicated when there was damage to deeper corneal layers.

Helper (1989) recommended general anaesthesia for ocular surgery. Superficial keratectomy was suggested for the treatment of advanced pannus, superficial corneal ulceration and superficial corneal scarring. Third eye lid flap for 10-14 days after superficial keratectomy was recommended to protect the cornea. Topical steroid-antibiotic therapy was suggested from the sixth postoperative day. Ephiphora was observed after third eye lid flap procedure due to the hypertrophy of third eye lid and scratching and pawing after surgical procedures on the cornea, third eye lid flap and eyelids.

Kirschner *et al.* (1989) performed superficial keratectomy on 14 out of 18 dogs with idiopathic persistent corneal erosions. It was effective in refractive cases of corneal erosions because it caused tight adhesion of epithelium to stroma by scar formation postoperatively. The procedure was reported to be difficult in chronically oedematous corneas and the healing time was prolonged.

Chavkin *et al.* (1990) treated a boxer dog with persistent corneal erosion by performing debridement of the non-adherent corneal epithelium using dry cotton tipped swabs and colibri. Contact lens was applied to support the healing epithelium and lateral tarsorrhaphy was performed to facilitate retention of contact lens. Fluorescein stain was used in routine examination of the cornea to evaluate its healing.

Pandey *et al.* (1990) conducted experiments on 55 dogs with various eye affections like corneal ulcer conjunctivitis and keratitis. Betamethasone sub-conjuctival injection at the dose rate of 4 mg per dog per day was useful in ameliorating the effects of inflammation.

Brooks (1991) observed adhesion between the cornea and the third eye lid flap when the bulbar surface of the nictitans was scarrified.

Christmas (1991) performed third eye lid flap to protect the cornea in two cases of chemical burns in dogs. The flap was retained for three weeks in one animal and 10 days in the other. The ulcers healed with the appearance of neovascularization in both the cases but corneal haziness and pigmentation was noticed only in the case where the flap was retained for three weeks . He opined that the third eye lid flap was indicated in midstromal ulcers of the cornea and the bulbar conjunctival flap for deep stromal ulcers. It provided support, blood supply, products of immune system and serum with anticollagenase effect to the cornea.

Mishra (1991) successfully treated deep corneal ulcers in a mule by superficial keratectomy and retained third eye lid flap for 31 days. The healing was rapid and the size of the ulcer had reduced considerably.

Mishra and Reddy (1991) performed homogenous lamellar corneal transplantation in mules and protected the cornea for 20 days with third eye lid membranoplasty.

Whitley (1991) reported that the superficial defect of corneal epithelium healed by epithelial sliding and mitosis and the earliest sliding cells were epithelial wing cells.

Christmas (1992) stated that ulcerative and pigmentary keratitis were the corneal lesions frequently seen in Shih

Tzu dogs and recommended superficial keratectomy for the removal of corneal pigment.

Ishizaki *et al.* (1994) studied the role of fibroblasts during corneal wound healing and found that apoptosis played a role in regulating the number of myofibroblasts in the injured cornea. Transmission electron microscopic study revealed the presence of microtendon and fibronexis associated with fibroblastic cells and stress fibres within fibroblastic cells. They concluded that the fibroblasts caused corneal wound contraction which inturn contributed to the formation of opaque scar tissues.

Moore and Jones (1994) performed superficial keratectomy in a cat with corneal stromal abscess and found that the corneal abscess healed completely after two months.

Morgan and Abrams (1994) treated persistent corneal ulcer in 136 dogs and six cats by different surgical procedures, debridement of cornea and topical administration of chloramphenicol and atropine. It was found that healing with multiple punctate keratectomy was 72 per cent, with third eye lid flap 68 per cent and with contact lenses 61.5 per cent. The eyes treated with debridement and medication, application of aprotinin solution, or insertion of the collagen shield had the lowest percentage of healing.

Williams (1994) employed temporary tarsorrhaphy or third eye lid flap techniques in corneal ulcers and stated that third eye lid flap was found superior because the flap moved with the globe during eye movement.

Materials and Methods

MATERIALS AND METHODS

The study was conducted on sixteen apparently healthy adult mongrel dogs of either sex. The dogs were dewormed, screened for blood parasites and tested for normal vision. Animals with visible ocular diseases and disorders were excluded. The selected animals were kept under observation for a period of one week before the experiment on identical conditions of feeding and management. The animals were randomly divided into two groups (Group I and Group II) consisting of eight animals each.

The dogs in each group were numbered serially from 1-8.

In Group I, an injury was created on the ventral half of the cornea in the left eye. After 24 hours, superficial keratectomy of the ventral half of the cornea was performed. The corneal wound was protected by suturing the third eye lid with the upper eye lid and temporary tarsorrhaphy.

In Group II, an injury was created on the ventral half of the cornea in the left eye. After 24 hours, superficial keratectomy of the ventral half of cornea and temporary tarsorrhaphy were performed.

Presurgical preparation of the animal

Framycetin eye drops* was instilled into the eye thrice daily, from three days prior to surgery, and food was withheld for 18 hours before the operation. The eye lashes were trimmed and the area around the eye was shaved and washed with water. The eyes were irrigated with two per cent boric acid solution and framycetin eye drops were instilled, just before surgery.

Induction of injury

Lignocaine** four per cent topical solution was instilled in drops into the left eye of the animals, to effect local anaesthesia. The sensitivity of the cornea was checked by touching it with a wisp of sterile cotton and by watching the blink reflex. Once the blink reflex was abolished, a superficial injury three to five millimetre in diameter was made on the ventral half of the cornea by pricking and scraping with an 18 gauge hypodermic needle (Fig.1).

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* Soframycin - Framycetin sulphate 0.5% ROUSSEL India Ltd., Worli, Bombay.

** Xylocaine - Lignocaine hydrochloride 4% Astra-IDL Limited, Bangalore.

Anaesthesia

All the experimental animals were premedicated with atropine sulphate at the dose rate of 0.04 mg/kg bodyweight administered subcutaneous. After five minutes, xylazine* at the dose rate of 0.5 mg/kg bodyweight was administered intramuscular. Fifteen minutes later, thiopentone sodium** five per cent solution was administered i/v to effect general anaesthesia. The animals were intubated, controlled on right lateral recumbency with its head elevated on a sand bag and the head was draped exposing only the eye to be operated.

Surgical technique

Proptosing the eyeball

A muscle hook was inserted underneath the membrana nictitans, on the lower aspect of the eyeball, in the area of inferior rectus muscle. After retracting the upper eye lid using eye lid retractor and the lower eye lid with the thumb, gentle traction was exerted with the hook to proptose the eyeball sufficiently. The eyeball was fixed in the proptosed position by applying pressure with the hook (Fig.2).

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* Xylaxin - Xylazine hydrochloride 23.22 mg/ml (equivalent to 20 mg of xylazine). Indian Immunologicals, Hyderabad.

** Intraval sodium - Thiopentone sodium injection IP, Rhone-Poulenc (India) Ltd., Bombay

Superficial keratectomy

Superficial keratectomy was performed using No.10 blade with No.3 Bard Parker handle. A horizontal incision was made on the centre of cornea commencing from medial canthus to lateral canthus. It was connected by a semicircular incision on the ventral half of the cornea enclosing the previously made injury and approximately one millimeter away from the limbal border. The depth of the incision was adjusted so that the cornea could be grasped with the rat toothed forceps. Using the corneal tissue forceps, one end of the incised lamella was lifted and a corneal scissors was introduced beneath the lamella. The tips of the scissors were dilated to separate the corneal lamella from its attachments (Fig.3). With the corneal scissors, the separated lamellar layer of the cornea was severed along the line of incision and removed (Fig.4). Throughout the procedure, the corneal surface was kept moist by instilling sterile normal saline solution. After superficial keratectomy, the eye was washed with sterile normal saline, the eyeball was reduced and a few drops of framycetin was instilled.

In Group I, after superficial keratectomy the cornea was covered with third eye lid flap (TEF) using the modified technique recommended by Helper and Blogg (1983). The free edge of the third eye lid was held with a forceps. Using double armed needle with 2/0 braided silk, suture was passed

behind the cartilage, about midway between the free edge and base of the third eye lid, without penetrating the inner conjunctival surface. The double armed needle was passed through the upper fornix and out through the upper eye lid skin dorsal to the lateral canthus at a distance of four millimetre. By traction on the suture thread, the third eye lid was pulled over the cornea until the free edge was in the upper fornix. The suture was passed through a piece of four millimetre polythene tube and knots were applied above the tube. The ends were cut long enough so that by untying the knots the third eye lid could be lowered after untying the knots to facilitate examination of the cornea.

Temporary tarsorrhaphy was performed by placing a single suture, three millimetre from the lid margins (Peiffer Jr. *et al.*, 1987b). A 2/0 braided silk suture was placed subcutaneously taking a horizontal bite from lateral to medial direction on the lower eye lid directing the needle subcutaneously at a distance of five millimetre between the bites. A similar bite was taken on the upper eye lid in a medial to lateral direction. The knot was applied with minimum tension that the eyelids were in apposition without causing inversion of the lid margins.

In animals of Group II, after superficial keratectomy the cornea was covered by performing temporary tarsorrhaphy as mentioned in Group I.

In both the groups, tarsorrhaphy suture was removed on the third postoperative day for observation. The suture was reapplied after examination and retained till the sixth day. The nictitans flap was released and reapplied at three day intervals in Group I to facilitate observation. The sutures fixing the third eye lid was removed on the ninth day.

Venous blood samples were collected for haematological studies prior to surgery, one hour after surgery and at intervals of five days during the period of observation.

Postoperative care

The dogs were kept under observation for a period upto 30 days postoperatively. Benzathine pencillin G 600,000 IU* was administered intramuscular immediately following surgery and was repeated on the fourth day, in all the animals.

Every day the eyelids were cleaned with sterile moist cotton and the eye was irrigated through the lateral canthus with one per cent sterile sodium chloride solution in all the animals. Framycetin eye drops was instilled four times daily through the lateral canthus for a period of five days and dexamethasone-framycetin** eye drops during the rest of the postoperative period upto 30 days.

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* Penidure LA6 - Benzathine penicillin G 600,000 IU, John Wyeth (India) Ltd., Bombay

** Sofracort eye drops - Dexamethazone sodium metasulphobenzoate 0.116% w/v + Framycetin sulphate 1%, Rousel India Ltd., Worli, Bombay

Main items of observation

The following observations were recorded during the study.

1. Physiological parameters such as rectal temperature (°C), pulse rate and respiratory rate prior to surgery, an hour after surgery and on interval of five days till the end of the period of observation.

2. Clinical symptoms

   Symptoms like swelling of the eye lid, lacrimation, corneal edema, vascularization and scratching or pawing.

3. Examination of lacrimal smear

   Lacrimal smear was made by touching the tip of a slide over the inner canthus and making a film over another glass slide. The slides were stained with haematoxylin and eosin and examined for the presence of cellular constituents. The lacrimal smears were examined every third day during the period of observation.

4. At three day intervals, the clarity of the cornea at the operated site was graded on a 0 to 4+ scale as per McEntyre (1968) and corneal healing was evaluated by instilling two per cent fluorescein stain into the eye

and assessing the staining pattern after releasing the sutures.

5. Haematological studies

Blood samples were collected and total and differential leucocyte counts and haemoglobin content (Cyanmethemoglobin method) were estimated (Jain, 1986).

6. Histomorphological study was done with the specimen obtained by enucleating the eyeball under general anaesthesia from two animals in each group on 5th, 10th, 15th and 30th day postoperatively.

(i) Gross examination of the enucleated eyeball was done and changes on the operated surface of the cornea, if any, were recorded. The specimens were preserved in neutral buffered formalin.

(ii) Microscopic examination of the tissue at the site of Keratectomy was done after processing, sectioning and staining. The corneal tissues collected from the specimen were washed under the running tap water for six to eight hours, dehydrated in different grades of alcohol, cleared in chloroform, embedded in paraffin, (Menocal *et al.*, 1980) sections of four to six micron thickness were taken, and stained with haematoxylin and Eosin (H&E) (Luna, 1968) for examination.

Results

RESULTS

The results of the study are presented in Tables 1-16.

The average bodyweight of the animals was 10.06 ± 0.49 kg. In all the animals, atropine sulphate at the dose rate of 0.04 mg/kg bodyweight (s/c), and after five minutes, xylazine at the dose rate of 0.5 mg/kg body weight (i/m) was administered. Fifteen minutes later, general anaesthesia was induced using five per cent solution of thiopentone sodium, administered i/v till the palpebral reflex was abolished and there was sufficient relaxation of muscles of eyeball.

The average dose of atropine sulphate required was 0.40 ± 0.04 mg, xylazine was 5.03 ± 0.24 mg and thiopentone sodium was 168.75 ± 12.39 mg per animal (Table 1).

The induction of anaesthesia was observed in 3.34 ± 0.28 minutes. The duration of anaesthesia was 40.25 ± 2.63 minutes and the time taken for recovery was 121.88 ± 7.34 minutes (Table 2).

Group I

(i) Clinical signs

Clinical signs observed were swelling of eye lid, lacrimation, scratching and pawing, corneal oedema, and vascularization of cornea.

The animals were kept under observation for a maximum of 30 days and eyeballs were enucleated for gross and microscopic examination from animal No.1 and 2 on fifth day, 3 and 4 on 10th day, 5 and 6 on 15th day and 7 and 8 on 30th day.

a. Swelling of eye lid (Table 3)

Swelling of eye lid was noticed in all the animals within 24 hr after surgery and it persisted upto the third postoperative day in six animals and upto the sixth postoperative day in two animals (Dog 5 and 6).

b. Lacrimation (Table 3)

Lacrimation was observed in all the animals from the day of surgery. It was observed upto the third day in two animals (Dog 1 and 2), upto the sixth day in five animals (Dog 4,5,6,7 and 8) and upto the ninth postoperative day in one animal (Dog 3).

c. **Scratching and pawing (Table 3)**

Scratching and pawing was noticed in all the animals from the day of surgery and it persisted upto the third day in seven animals and sixth postoperative day in one animal (Dog 8).

d. **Corneal oedema (Table 4)**

Corneal oedema was observed in all the animals from third day and persisted upto 12th postoperative day in two animals (Dog 7 and 8).

e. **Vascularization** (Table 5)

Six animals (Dogs 3, 4, 5, 6, 7 and 8) in this group showed vascularization of the cornea by the sixth postoperative day. The vessels were seen on the lower half of cornea as a continuation from the adjacent bulbar conjunctiva at the limbus. The vessels were bright red and branched in tree like fashion.

In animal No.5, the vascularization persisted upto the 12th day and in animals 6, 7 and 8 it was noticed upto the 15th day.

f. Other observations

When the third eye lid flap sutures were released in different postoperative periods to examine the cornea, the third eye lid did not return to its original position but was found to cover two-third of the cornea on the third day and the sixth day in all the animals (Fig.5 and 6) and it covered one-third of the cornea on the ninth day. It was found to return to its normal position after the ninth day in animals 5 and 7, but only on the 12th day in dog No.6. When third eye lid was gently retracted with the help of preplaced sutures, no adhesion or bleeding was encountered.

Congestion of the bulbar and palpebral conjunctivae and third eye lid was observed on the third day and it persisted till the sixth postoperative day.

(ii) Physiological parameters (Table 6)

a. Rectal temperature (°C)

The rectal temperature was 39.18 ± 0.13, before surgery, 37.75 ± 0.18 at one hour after surgery, 39.15 ± 0.15 on the fifth day, 39.05 ± 0.17 on the 10th day, 39.13 ± 0.13 on the 15th day, 38.80 ± 0.20 on the 20th day, 38.90 ± 0.10 on the 25th day and 39.20 ± 0.10 on the 30th day.

b. Pulse rate (per minute)

The pulse rate was 118.80 ± 3.61, before surgery, 93.50 ± 2.69 at one hour after surgery, 120.00 ± 6.09 on the fifth day, 120.00 ± 2.30 on the 10th day, 116.50 ± 2.87 on the 15th day, 116.00 ± 2.87 on the 20th day, 128.00 ± 8.00 on the 25th day and 130.00 ± 8.00 on the 30th day.

c. Respiration rate (per minute)

The respiration rate was 30.25 ± 1.44, before surgery, 14.00 ± 1.00 at one hour after surgery, 33.25 ± 1.07 on the fifth day, 28.67 ± 0.84 on the 10th day, 31.00 ± 2.65 on the 15th day, 29.00 ± 1.00 on the 20th day, 33.00 ± 1.00 on the 25th day and 32.00 ± 0.00 on the 30th day.

The rectal temperature, pulse rate and respiration rate did not show marked variation except at one hour after surgery where there was marked reduction in all the three parameters.

(iii) Lacrimal smear

Lacrimal smears that were examined on different postoperative days revealed only denuded epithelium and cellular debris. Inflammatory cells were not present in any of the smears examined.

(iv) Evaluation of corneal healing

a. Fluorescein dye test (Table 7)

3rd postoperative day

All the animals were positive for fluorescein dye test and cornea was seen stained bright green at the keratectomy site (Fig.7).

6th post operative day

All the animals were positive for fluorescein dye test and the keratectomy site was seen stained faint greenish yellow (Fig.8).

9th postoperative day

One animal (dog 8) was positive for fluorescein dye test and the lesion on the cornea was seen stained faint greenish yellow (Fig.9).

Fluorescein dye test was negative in all the animals from 9th day of observation (Fig.10).

b. Clarity at the keratectomy site (Table 8)

3rd postoperative day

The part of cornea where keratectomy was performed showed opacity (4+) in all the animals (Fig.7).

6th postoperative day

The part of cornea where keratectomy was performed showed opacity (4+) in two animals (dogs 3 and 8), (Fig.9) and was very hazy (3+) in the other animals (dogs 4, 5, 6 and 7) (Fig.8).

9th postoperative day

The part of cornea where keratectomy was performed showed opacity (4+) in one animal (dog 8) and in the other animals (dogs 3, 4, 5, 6 and 7) it was very hazy (3+) (Fig.10).

12th postoperative day

The part of the cornea where keratectomy was performed was hazy (2+) in all the animals (Fig.11) except in one animal (dog 8) where it was very hazy (3+).

15th postoperative day

The part of the cornea where keratectomy was performed showed haziness (2+) in all the animals (Fig.12).

The part of cornea where keratectomy performed was clear (1+) from the 18th postoperative day. On 30th postoperative day it was crystal clear (0) in all the animals (Fig.13-17).

(v) **Haemogram (Table 9)**

a. Haemoglobin content (g/dl)

The haemoglobin content was 17.45 ± 0.27, before surgery, 16.51 ± 0.56 at one hour after surgery, 17.31 ± 0.33 on the fifth day, 16.83 ± 0.47 on the 10th day, 17.33 ± 0.31 on the 15th day, 17.21 ± 0.39 on the 20th day, 16.03 ± 1.29 on the 25th day and 17.75 ± 0.75 on the 30th day.

The haemoglobin content did not show marked variation.

b. Total leucocyte count (x 10^3/cu mm)

The total leucocyte count was 10.52 ± 0.27, before surgery, 11.60 ± 0.35 at one hour after surgery, 11.45 ± 0.34 on the fifth day, 11.04 ± 0.26 on the 10th day, 11.43 ± 0.22 on the 15th day, 10.38 ± 0.28 on the 20th day, 10.55 ± 0.45 on the 25th day and 10.78 ± 0.33 on the 30th day.

The variation in total leucocyte count was within the normal range.

c. Differential leucocyte count (per cent)

(1) Neutrophil count (per cent)

The neutrophil count was 72.13 ± 0.95, before surgery, 74.88 ± 0.83 at one hour after surgery, 77.00 ± 0.98 on the fifth day, 73.17 ± 0.70 on the 10th day, 73.50 ± 0.65 on the

15th day, 74.00 ± 0.00 on the 20th day, 73.00 ± 0.10 on the 25th day and 72.50 ± 0.50 on the 30th day.

2. Lymphocyte count (per cent)

The lymphocyte count was 20.13 ± 0.72, before surgery, 20.38 ± 0.91 at one hour after surgery, 20.75 ± 1.22 on the fifth day, 21.17 ± 0.95 on the 10th day, 19.75 ± 0.85 on the 15th day, 18.00 ± 0.00 on the 20th day, 19.50 ± 1.50 on the 25th day and 20.00 ± 1.00 on the 30th day.

3. Eosinophil count (per cent)

The eosinophil count was 5.88 ± 0.44, before surgery, 3.63 ± 0.57 at one hour after surgery, 4.00 ± 0.50 on the fifth day, 4.00 ± 0.52 on the 10th day, 4.75 ± 0.75 on the 15th day, 5.00 ± 1.00 on the 20th day, 5.50 ± 0.50 on the 25th day and 6.00 ± 1.00 on the 30th day.

4. Monocyte count (per cent)

The monocyte count was 1.88 ± 0.30, before surgery, 1.25 ± 0.45 at one hour after surgery, 1.00 ± 0.19 on the fifth day, 1.33 ± 0.42 on the 10th day, 2.00 ± 0.00 on the 15th day, 2.50 ± 0.50 on the 20th day, 2.00 ± 0.00 on the 25th day and 1.50 ± 0.50 on the 30th day.

5. **Basophil count (per cent)**

The basophil count was 0.125 ± 0.13, before surgery, 0 at one hour after surgery, 0 on the fifth day, 0.033 ± 0.21 on the 10th day, 0 on the 15th day, 0.50 ± 0.50 on the 20th day, 0 on the 25th day and 0 on the 30th day.

The differential leucocyte count did not show any significant variation.

(vi) Gross examination of the enucleated eyeballs 5t postoperative day

In dogs 1 and 2 the keratectomy site was opaque, irregular and lustreless. The adjoining ventral portion of the cornea appeared a little raised above the surface due to corneal edema. Vascularization of the cornea was absent in both the specimens.

10th postoperative day

In dogs 3 and 4, the keratectomy site was very hazy and lustreless. Corneal oedema was present in dog 3 and vascularization was observed on the ventral part of cornea of both the specimens (Fig.18).

15th postoperative day

In dogs 5 and 6, the keratectomy site was hazy and lustreless. Corneal oedema was not observed but vascularization of the cornea was observed in the eye of dog 6 but was absent in the other.

30th post operative day

In dog 7 and 8, the keratectomy site was crystal clear and lustrous.

(vii) Histopathology

Microscopical examination of the keratectomy site on the fifth postoperative day revealed necrosis of the epithelial cells at the border of the wound and inflammatory oedema in the corneal epithelium and stroma. There were scattered inflammatory cells in the stroma (Fig.19). Epithelial facet formation with fibroplasia of the stroma and mild haemmorrhage at the keratectomy site were observed in the specimens of 10th postoperative day (Fig.20). Necrosis of the epithelial cells at the keratectomy site subepithelial and stromal oedema, markedly swollen basal cells with degeneration and necrotic changes and active proliferation of epithelial cells were also observed (Fig.21).

Mild necrosis and degeneration of the lining epithelium, mild stromal oedema (Fig.22) as well as the new fibrovascular tissue in the stroma were observed in the specimens of 15th postoperative day (Fig.23). Active proliferation of epithelial cells, thickened epithelium and fibroplasia in the stroma were observed on the 30th postoperative day (Fig.24).

Table 1. Bodyweight of dogs, dose of atropine sulphate, xylazine and quantity of thiopentone sodium

Number of animal*	Body weight (kg)	Atropine sulphate (mg)	Xylazine (mg)	Thiopentone sodium (mg)
1.	11	0.44	5.5	175
2.	10	0.40	5.0	175
3.	9	0.36	4.5	175
4.	9	0.36	4.5	125
5.	9	0.36	4.5	175
6.	8	0.32	4.0	200
7.	12	0.48	6.0	150
8.	5	0.20	2.5	100
9.	10	0.40	5.0	225
10.	10	0.40	5.0	150
11.	12	0.48	6.0	200
12.	13	0.52	0.5	200
13.	9	0.36	4.5	150
14.	12	0.48	6.0	175
15.	11	0.44	5.5	175
16.	11	0.44	5.5	150
Mean± S.E.	10.06±0.49	0.40±0.04	5.03±0.24	168.75±12.39

* Animals 1-8 in Gr.I and animals 9-16 in Gr.II

Table 2. Induction time, duration and recovery from anaesthesia in dogs administered with atropine sulphate-xylazine-thiopentone

Number of animal*	Induction time (min)	Duration (min)	Recovery (to standing) (min)
1.	3	43	167
2.	5	50	185
3.	2	60	135
4.	5	40	158
5.	3	40	77
6.	3	37	134
7.	1.5	12	85
8.	3	38	108
9.	5	40	105
10.	3	46	110
11.	3	32	108
12.	4	46	136
13.	3	42	107
14.	5	35	115
15.	3	32	97
16.	2	51	123
Mean± S.E.	3.34±0.28	40.25±2.63	121.88±7.34

* Animals 1-8 in Gr.I and animals 9-16 in Gr.II

Table 3. Day of disappearance of clinical signs observed following superficial keratectomy, third eye lid flap and temporary tarsorrhaphy in dogs (Group I)

Animal No.	Swelling of eye lid	Lacrimation	Scratching and pawing
1.	3	3	3
2.	3	3	3
3.	3	9	3
4.	3	6	3
5.	6	6	3
6.	6	6	3
7.	3	6	3
8.	3	6	6

Table 4. Corneal oedema after superficial keratectomy, third eye lid flap and temporary tarsorrhaphy in dogs (Group I)

Animal No.	Interval (Days)									
	3	6	9	12	15	18	21	24	27	30
1	+									
2	+									
3	+	+	+							
4	+	+	-							
5	+	+	+	-	-					
6	+	+	-	-	-					
7	+	+	+	+	-	-	-	-	-	-
8	+	+	+	+	-	-	-	-	-	-

\+ Present
\- Absent

Table 5. Vascularization of the cornea after superficial keratectomy, third eye lid flap and temporary tarsorrhaphy in dogs (Group I)

Animal No.	Interval (Days)									
	3	6	9	12	15	18	21	24	27	30
1	-									
2	-									
3	-	+	+							
4	-	+	+							
5	-	+	+	+	-					
6	-	+	+	+	+					
7	-	+	+	+	+	-	-	-	-	-
8	-	+	+	+	+	-	-	-	-	-

\+ Present
\- Absent

Table 6. Rectal temperature (°C), pulse rate (per min) and respiration rate (per min) before surgery, one hour after surgery and on different postoperative periods in dogs (Group I)

Parameters and units	Intervals							
	0 min	1 hour	5th day	10th day	15th day	20th day	25th day	30th day
Rectal temperature (°C)	39.18± 0.13	37.75± 0.18	39.15± 0.15	39.05± 0.17	39.13± 0.13	38.80± 0.20	38.90± 0.10	39.20± 0.10
Pulse rate (per minute)	118.80± 3.61	93.50± 2.69	120.00± 6.09	120.00± 2.30	116.50± 2.87	116.00± 2.87	128.00± 8.00	130.00± 8.00
Respiration rate (per minute)	30.25± 1.44	14.00± 1.00	33.25± 1.07	28.67± 0.84	31.00± 2.65	29.00± 1.00	33.00± 1.00	32.00± 0.00

Table 7. Fluorescein dye test of the eye after superficial keratectomy in dogs (Group I)

Animal No.	Interval (Days)									
	3	6	9	12	15	18	21	24	27	30
1	++									
2	++									
3	++	+	-							
4	++	+	-							
5	++	+	-	-	-					
6	++	+	-	-	-					
7	++	+	-	-	-	-	-	-	-	-
8	++	+	+	-	-	-	-	-	-	-

++ Positive - bright green staining
+ Positive - faint greenish yellow staining
- Negative - not stained

Table 8. Clarity of the corneal surface after superficial keratectomy in dogs (Group I)

Animal No.	Interval (Days)									
	3	6	9	12	15	18	21	24	27	30
1	4+									
2	4+									
3	4+	4+	3+							
4	4+	3+	3+							
5	4+	3+	3+	2+	2+					
6	4+	3+	3+	2+	2+					
7	4+	3+	3+	2+	2+	1+	1+	1+	1+	0
8	4+	4+	4+	3+	2+	1+	1+	1+	1+	0

Clarity grading = 0 = crystal clear
1+ = clear
2+ = hazy
3+ = very hazy
4+ = opaque

Table 9. Haemogram before surgery, one hour after surgery and on different postoperative periods in dogs (Group I)

Parameters and units	Interval (days)							
	0 min	1 hour	5th day	10th day	15th day	20th day	25th day	30th day
Haemoglobin content (g/dl)	17.45± 0.27	16.51± 0.56	17.31± 0.33	16.83± 0.47	17.33± 0.31	17.21± 0.39	16.03± 1.29	17.75± 0.75
Total leucocyte count (10^3/cu mm)	10.52± 0.27	11.60± 0.35	11.45± 0.34	11.04± 0.26	11.43± 0.22	10.38± 0.28	10.55± 0.45	10.78± 0.33
Neutrophil count (%)	72.13± 0.95	74.88± 0.83	77.00± 0.98	73.17± 0.70	73.50± 0.65	74.00± 0.00	73.00± 1.00	72.50± 0.50
Lymphocyte count (%)	20.13± 0.72	20.38± 0.91	20.75± 1.22	21.17± 0.95	19.75± 0.85	18.00± 0.00	19.50± 1.50	20.00± 1.00
Eosinophil count (%)	5.88± 0.44	3.63± 0.57	4.00± 0.50	4.00± 0.52	4.75± 0.75	5.00± 1.00	5.50± 0.50	6.00± 1.00
Monocyte count (%)	1.88± 0.30	1.25± 0.45	1.00± 0.19	1.33± 0.42	2.00± 0.00	2.50± 0.50	2.00± 0.00	1.50± 0.50
Basophil count (%)	0.125± 0.13	0	0	0.33± 0.21	0	0.50± 0.50	0	0

Fig.1. Photograph of the eye showing experimentally induced corneal wound outlined with fluorescein staining

Fig.2. Photograph of the eye showing the proptosed eye ball prior to superficial keratectomy

Fig.3. Photograph of the eye showing superficial layer of cornea being removed during superficial keratectomy

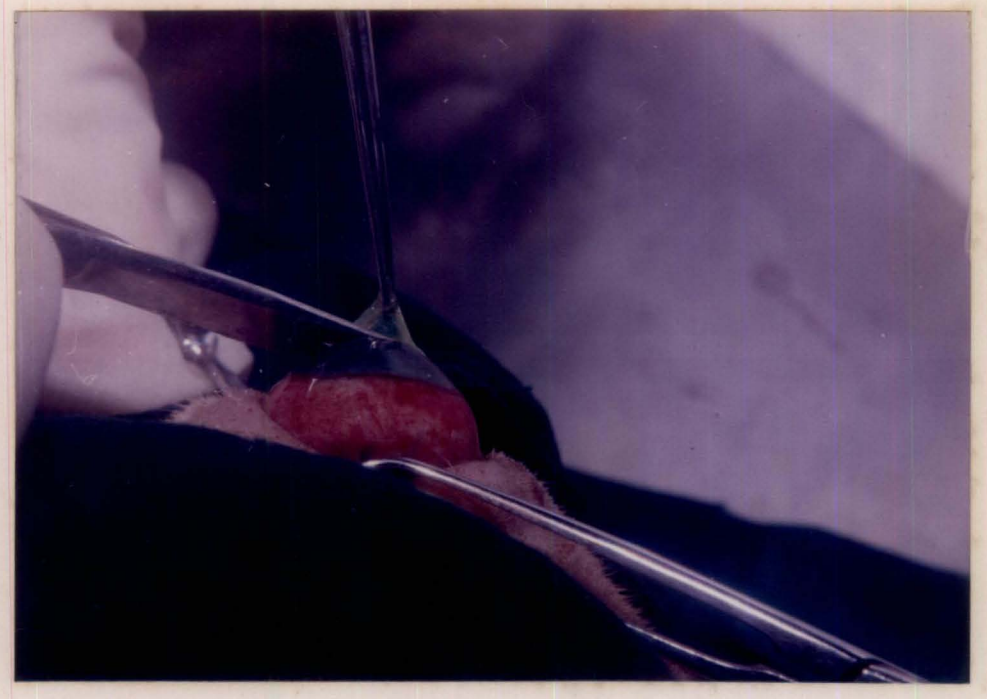


Fig.4. Photograph of the eye and the corneal surface stained with fluorescein dye immediately after superficial keratectomy

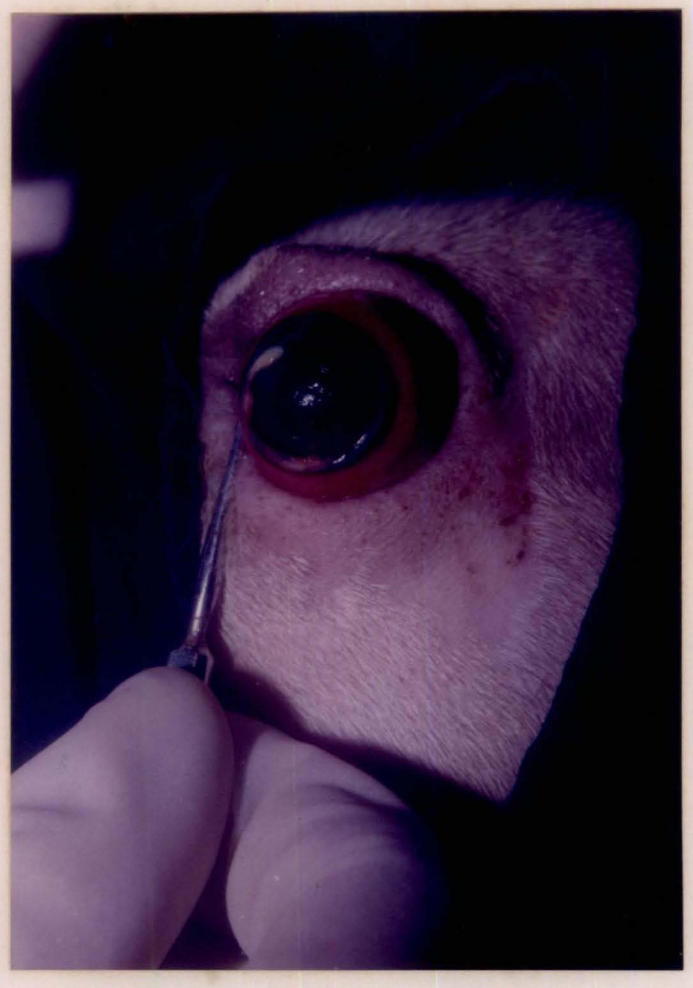
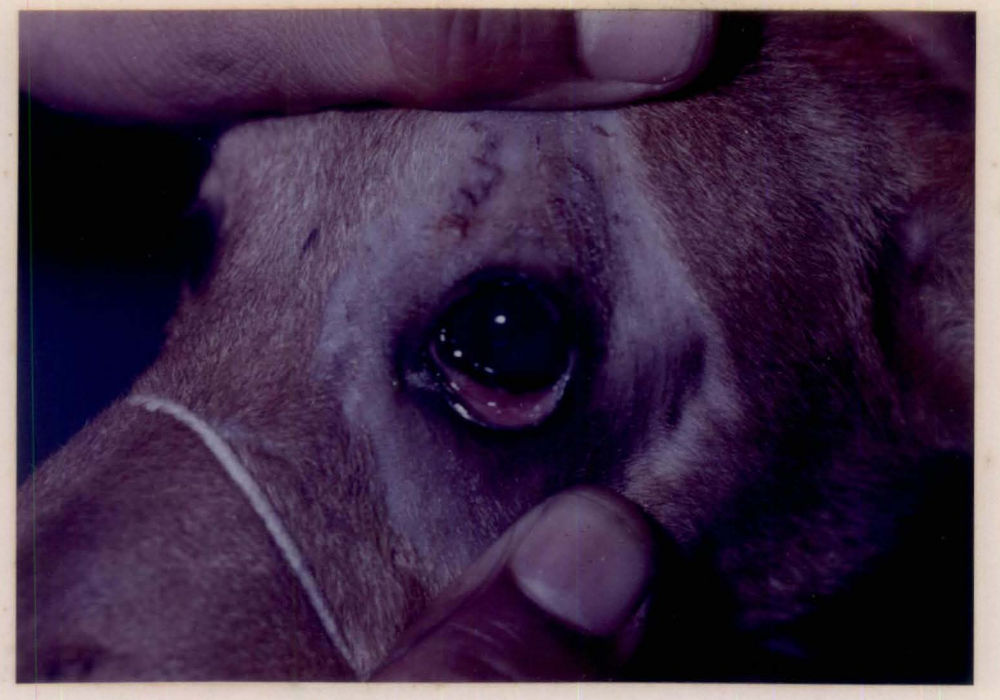


Fig.5. Photograph of the eye on 3rd postoperative day in Group I showing the third eye lid covering two-third of the eye ball after releasing the third eye lid flap

Fig.6. Photograph of the eye on the 6th postoperative day in Group I, showing the third eye lid covering two-third of the eye ball after releasing the third eye lid flap

Fig.7. Photograph of the eye on 3rd postoperative day in Group I, showing bright green staining after flourescein dye test. Opacity at the keratectomy site (4+)

Fig.8. Photograph of the eye on the 6th postoperative day in Group I, showing faint greenish yellow staining after fluorescein dye test. Haziness at the keratectomy site (3+)



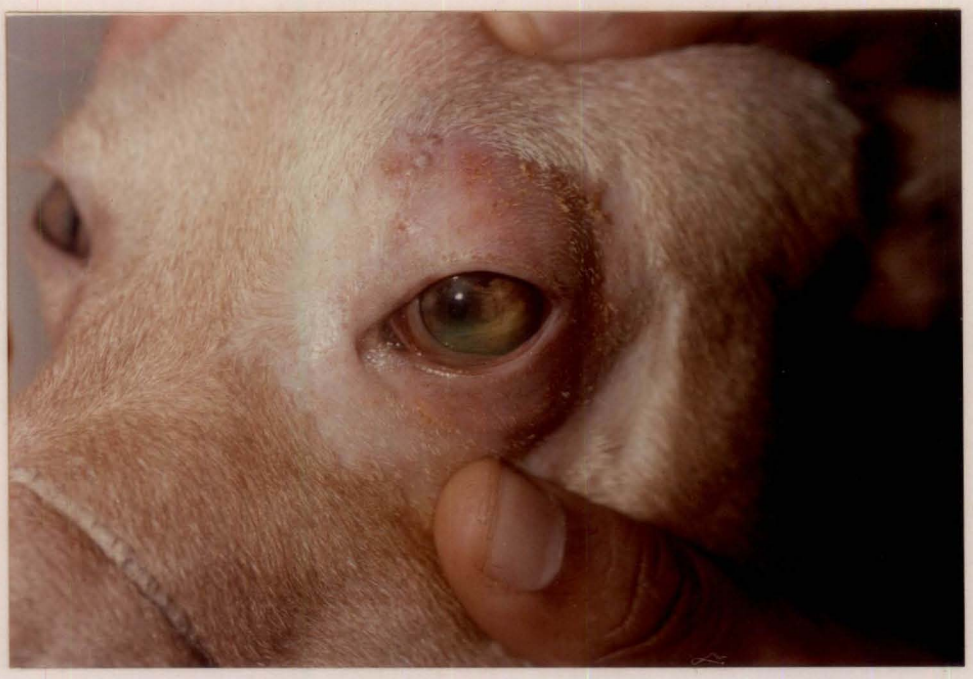
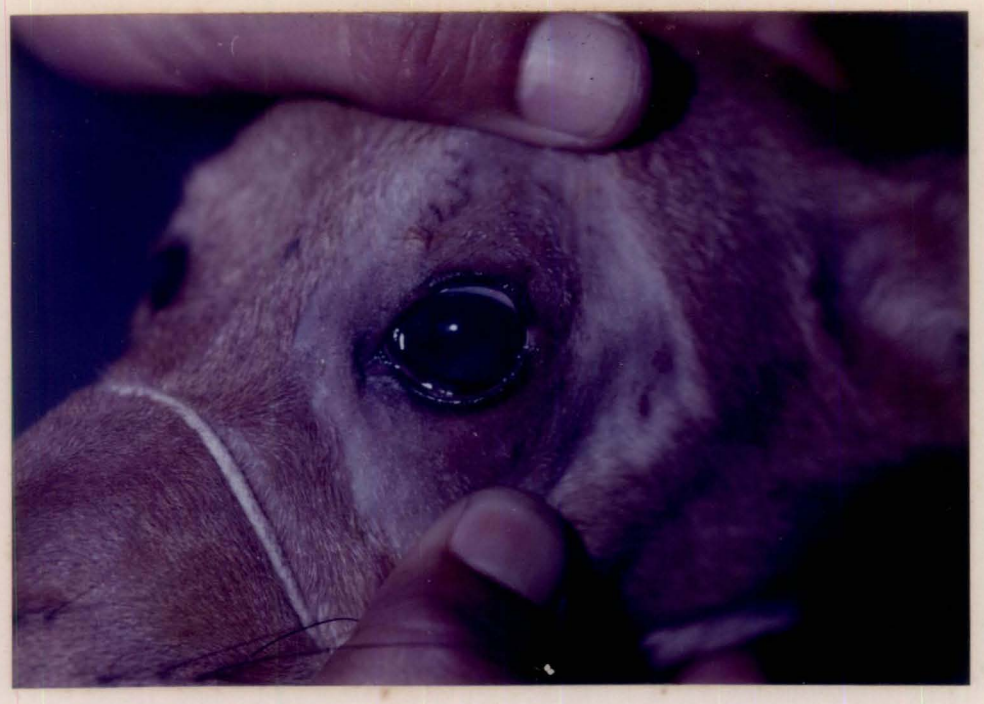


Fig.9. Photograph of the eye on the 9th postoperative day (Dog 8) in group I, showing faint greenish yellow staining after fluorescein dye test. Opacity at the keratectomy site (4+)

Fig.10. Photograph of the eye on the 9th postoperative day in group I, showing the third eye lid covering one-third of the globe and absence of staining after fluorescein dye test. Haziness at the keratectomy site (3+)



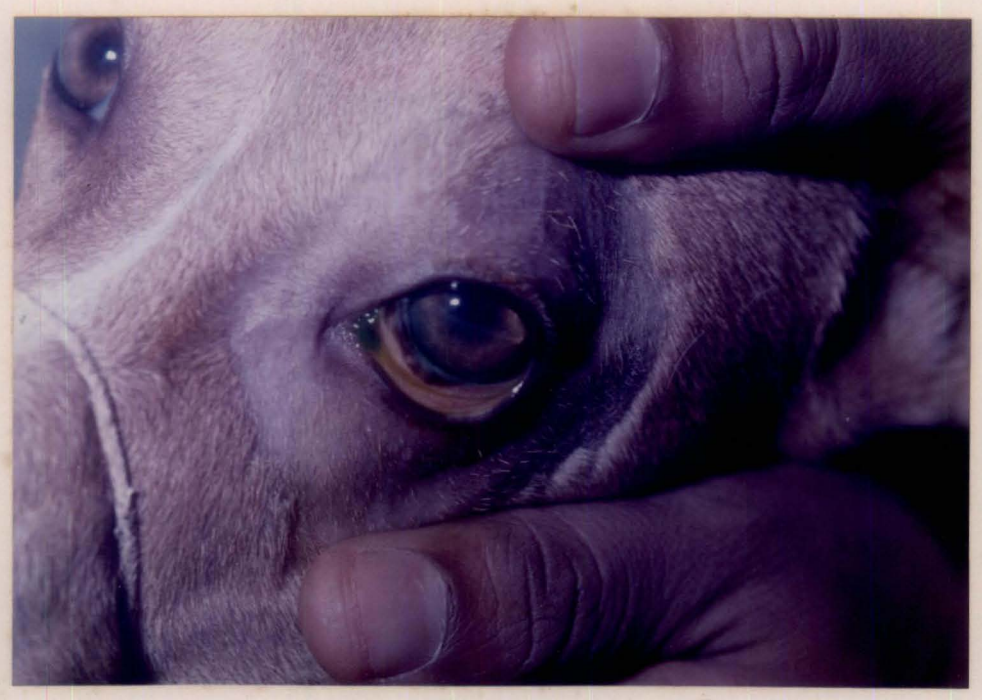


Fig.11. Photograph of the eye on 12th postoperative day in Group I, showing absence of staining with fluorescein dye. Haziness at the keratectomy site (2+)

Fig.12. Photograph of the eye on the 15th postoperative day in group I. Haziness at the keratectomy site (2+)

Fig.13. Photograph of the eye on the 18th postoperative day in group I, showing clear corneal surface (1+ clarity) at the keratectomy site

Fig.14. Photograph of the eye on the 21st postoperative day in Group I, showing clear corneal surface (1+ clarity) at the keratectomy site

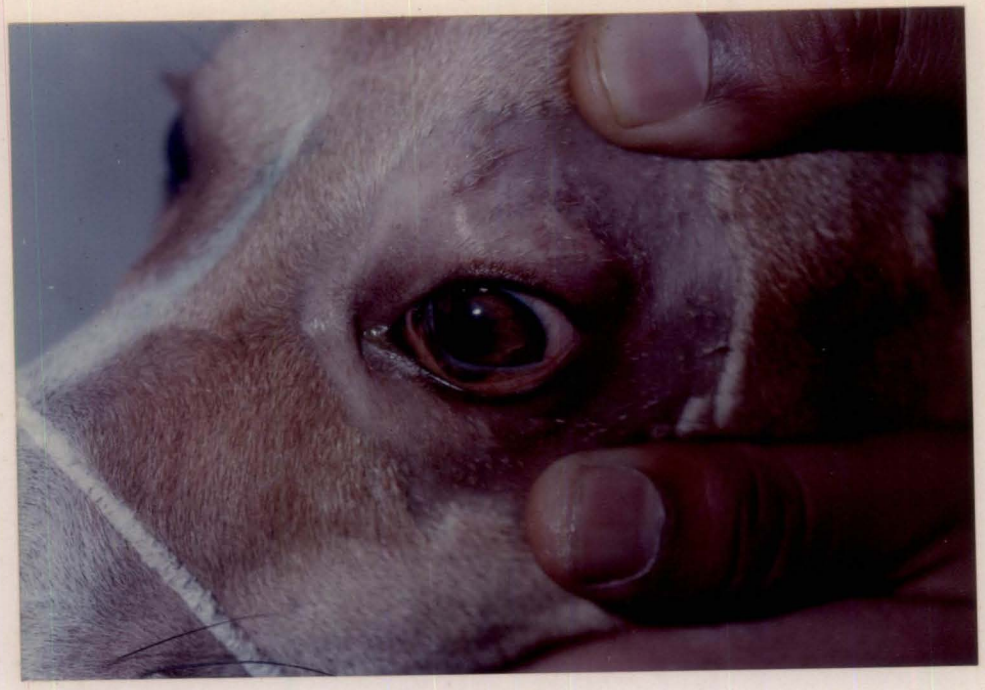


Fig.15. Photograph of the eye on the 24th postoperative day in Group I, showing clear corneal surface (1+ clarity) at the keratectomy site

Fig.16. Photograph of the eye on 27th postoperative day in group I, showing clear corneal surface (1+ clarity) at the keratectomy site

Fig.17. Photograph of the eye on 30th postoperative day in group I, showing crystal clear corneal surface (0 clarity) at the keratectomy site

Fig.18. Photograph of the eye on 10th postoperative day in group I (dog 3) showing vascularization of the cornea

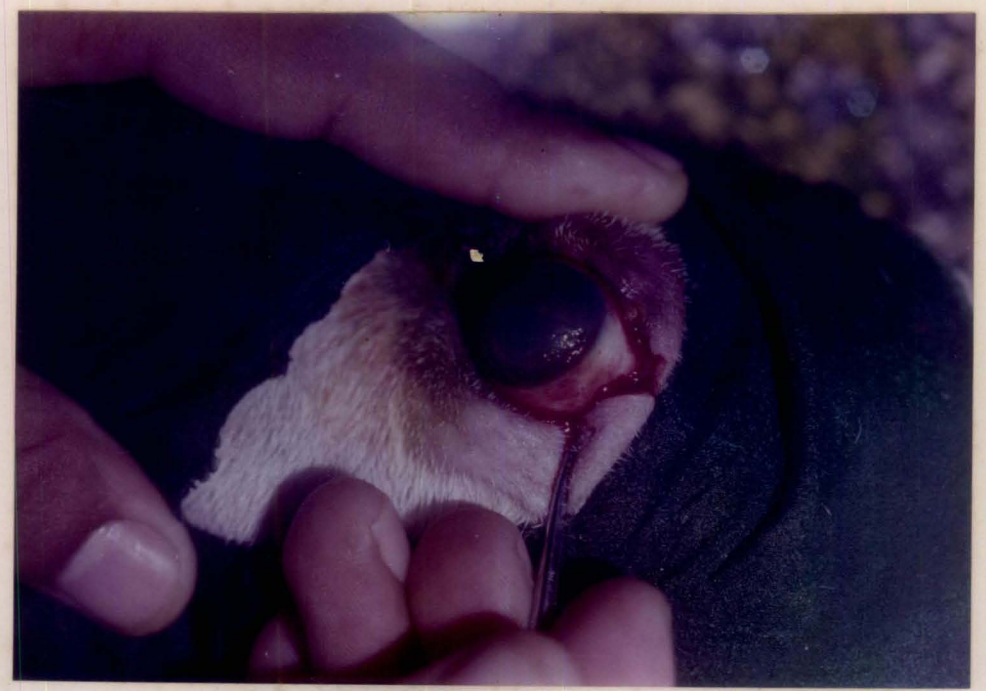


Fig.19. Photomicrograph of the cornea on fifth postoperative day in Group I, showing necrosis of the epithelial cells, inflammatory oedema in the corneal epithelium and stroma and scattered inflammatory cells in the stroma at the keratectomy site (H&E x 320)

Fig.20. Photomicrograph of the cornea on the 10th postoperative day in group I, showing epithelial facet formation, mild haemmorhage and fibroplasia of the stroma at the keratectomy site (H&E x 320)

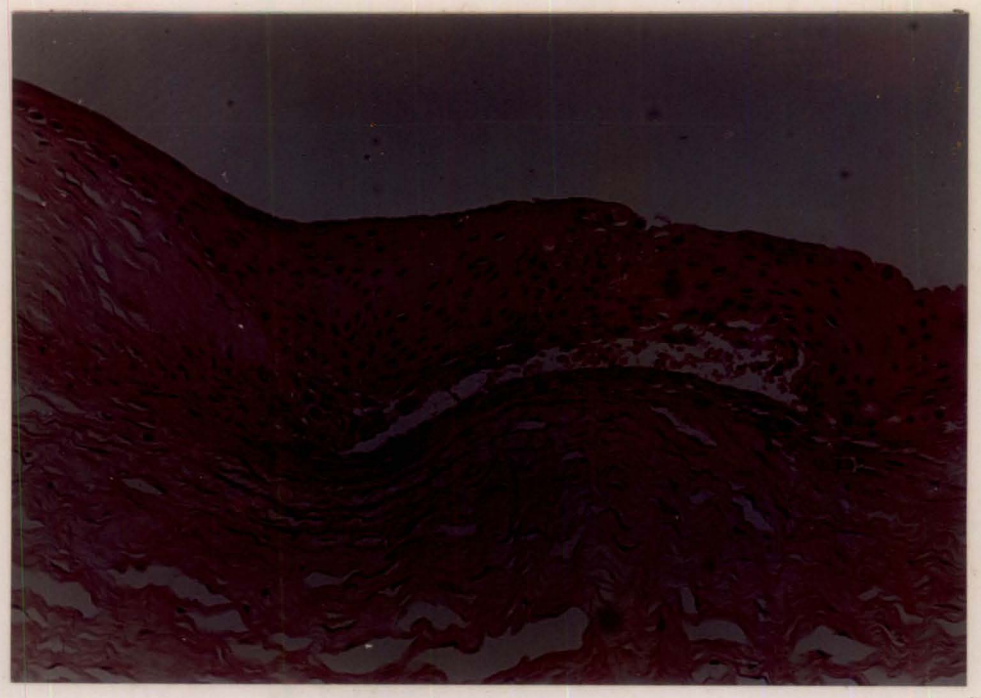


Fig.21. Photomicrograph of the cornea on 10th postoperative day in group I, showing subepithelial and stromal oedema and markedly swollen basal cells at the keratectomy site (H&E x 320)

Fig.22. Photomicrograph of the cornea on 15th postoperative day in group I, showing mild necrosis and degeneration of lining epithelium and mild stromal oedema at the keratectomy site (H&E x 320)

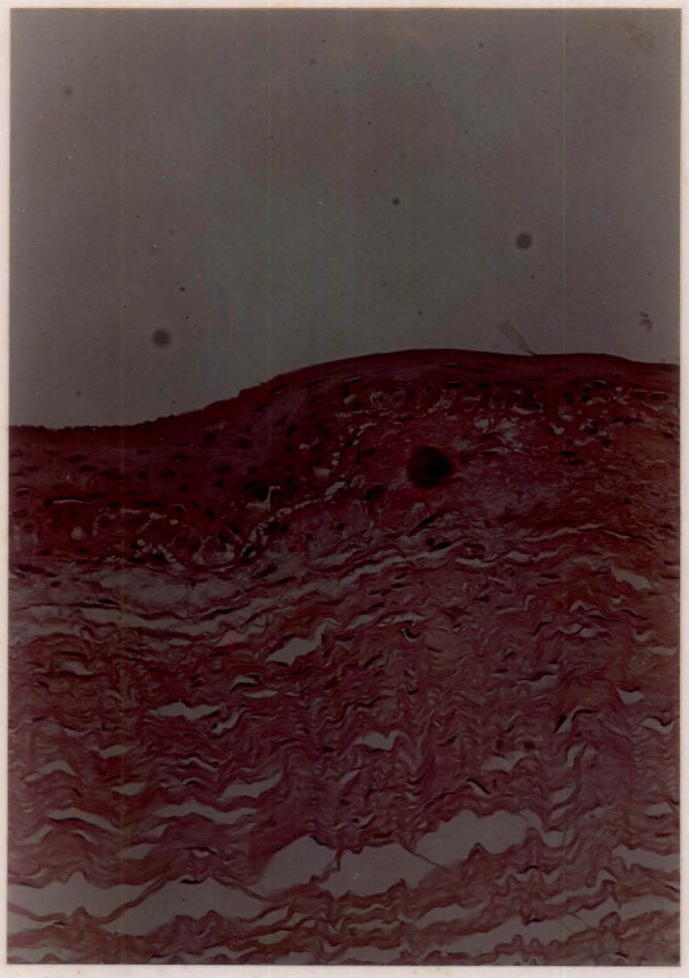


Fig.23. Photomicrograph of the cornea on the 15th postoperative day in group I, showing the presence of fibrovascular tissue in the stroma (H&E x 320)

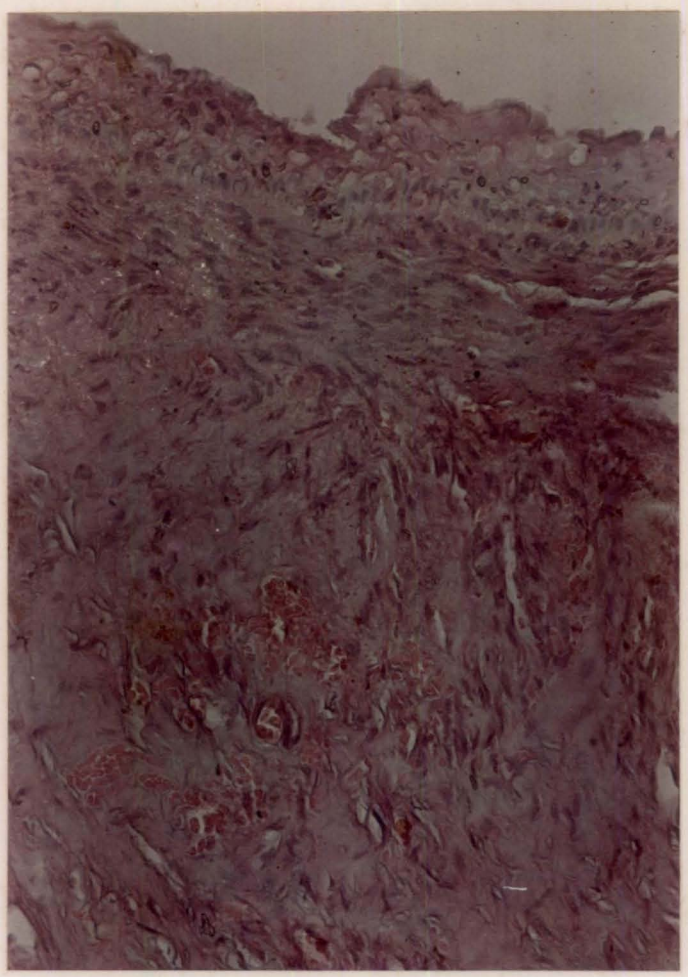
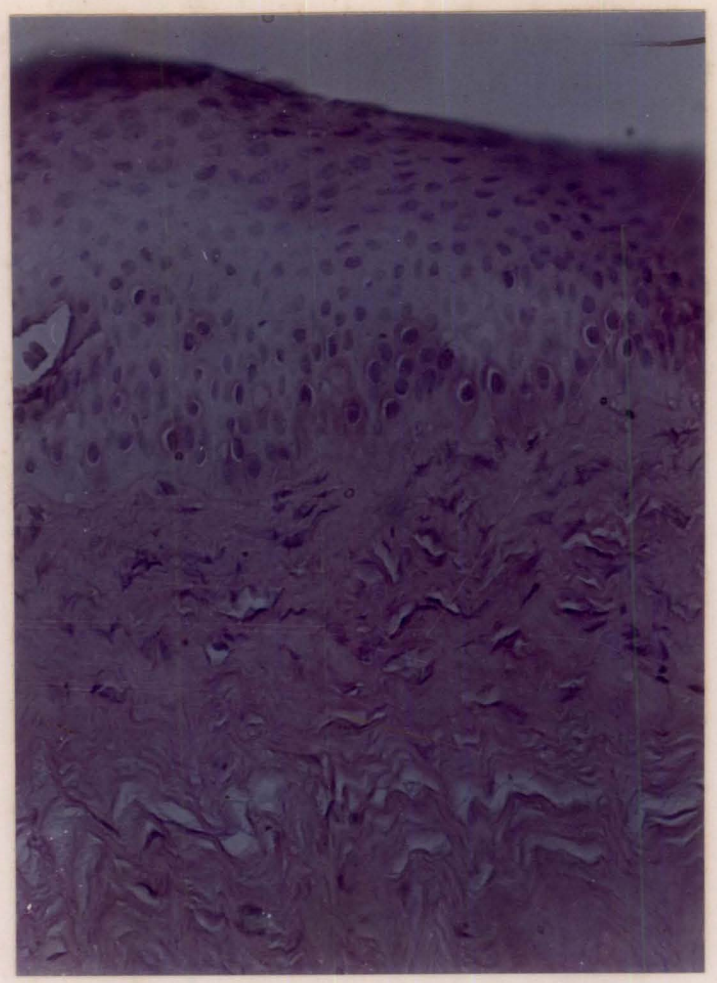


Fig.24. Photomicrograph of the cornea on the 30th postoperative day in group I, showing active proliferation of epithelial cells, thickened epithelium and fibroplasia of the stroma (H&E x 400)



Group II

(i) Clinical signs

Clinical signs observed were swelling of eye lid, lacrimation, scratching and pawing, corneal oedema, and vascularization of cornea.

The animals were kept under observation for a maximum of 30 days and eyeballs were enucleated for gross and microscopic examination from animal No.1 and 2 on the fifth day, 3 and 4 on 10th day, 5 and 6 on 15th day and 7 and 8 on 30th day.

a. Swelling of eye lid (Table 10)

Swelling of eye lid was noticed in all the animals within 24 hrs after surgery. It persisted upto the third day in five animals (Dog 1,2,4,5 and 7) and in three animals it persisted upto the sixth postoperative day (Dog 3,6,8).

b. Lacrimation (Table 10)

Lacrimation was observed in all the animals in this group from the day of surgery. It persisted upto the third day in two animals (Dog 1&2) and in other animals it persisted upto the sixth postoperative day.

c. **Scratching and pawing** (Table 10)

Scratching and pawing was exhibited by all the animals from the day of surgery. It persisted upto the third postoperative day in two animals (Dog 1 and 2) and in other animals it persisted upto the sixth postoperative day.

d. **Corneal oedema (Table 11)**

Corneal oedema was observed in five animals (dogs 1, 2, 5 6 and 8) from the third day and persisted upto the ninth postoperative day in one animal (dog 8).

e. **Vascularization (Table 12)**

Vascularization of the cornea was observed in dogs 3 and 4 on the third day and the sixth day respectively whereas it was not observed in the other animals. The vessels were seen on the ventral portion of the cornea as continuation from the adjacent bulbar conjunctiva at the limbus. The vessels were bright red and branched in tree like fashion. In both the animals, the vascularization persisted upto the ninth postoperative day.

f. **Other observations**

Congestion of the bulbar and palpebral conjunctivae and third eye lid was observed on the third day and it persisted upto the sixth postoperative day.

Self mutilation of sutures was observed in two animals - dog No.4 on the first day and in dog No.7 on the eighth day and sutures were reapplied.

(ii) Physiological parameters (Table 13)

a. Rectal temperature (°C)

The rectal temperature was 39.20 ± 0.11 before surgery, 37.64 ± 0.16 at one hour after surgery, 39.11 ± 0.11 on the fifth day, 39.13 ± 0.12 on the 10th day, 39.08 ± 0.12 on the 15th day, 38.90 ± 0.10 on the 20th day, 38.90 ± 0.10 on the 25th day and 39.15 ± 0.15 on the 30th day.

b. Pulse rate (per minute)

The pulse rate was 119.75 ± 1.53 before surgery, 94.25 ± 2.71 at one hour after surgery, 118.00 ± 3.57 on the fifth day, 117.67 ± 3.12 on the 10th day, 110.00 ± 4.55 on the 15th day, 103.00 ± 5.00 on the 20th day, 105.00 ± 7.00 on the 25th day and 116.00 ± 4.00 on the 30th day.

c. Respiration rate (per minute)

The respiration rate was 31.75 ± 1.91 before surgery, 12.50 ± 0.91 at one hour after surgery, 31.00 ± 1.73 on the fifth day, 29.67 ± 2.02 on the 10th day, 29.50 ± 1.50 on the 15th day, 27.00 ± 1.00 on the 20th day, 25.00 ± 1.00 on the 25th day and 27.00 ± 1.00 on the 30th day.

The rectal temperature, pulse rate and respiration rate did not show marked variation except at one hour after surgery where it showed marked reduction in all the three parameters.

(iii) Lacrimal smear

Lacrimal smears that were examined on different postoperative days revealed only denuded epithelium and cellular debris. Inflammatory cells were not observed in any of the smears.

(iv) Evaluation of corneal healing

a. Fluorescein dye test (Table 14)

3rd postoperative day

All the animals were positive for fluorescein dye test and cornea was seen stained bright green at the keratectomy site (Fig.25).

6th postoperative day

All the animals were positive for fluorescein dye test and cornea was seen stained faint greenish yellow at the keratectomy site (Fig.26).

9th postoperative day

All the animals were negative for fluorescein dye test from the ninth postoperative day (Fig.27).

b. Clarity at the keratectomy site (Table 15)

3rd postoperative day

The part of cornea where keratectomy was performed showed opacity (4+) in all the animals (Fig.25).

6th postoperative day

The part of cornea where keratectomy performed was very hazy (3+) in all the animals (Fig.26).

9th postoperative day

The part of cornea where keratectomy performed was very hazy (3+) in animals 3, 4 and 5 (Fig.27) and it was hazy (2+) in animals 6, 7 and 8.

12th postoperative day

The part of cornea where keratectomy was performed was hazy (2+) in all the animals (Fig.28).

15th postoperative day

The part of cornea where keratectomy performed was hazy (2+) in all the animals (Fig.29).

The part of cornea where keratectomy performed was clear (1+) from the 18th postoperative day and it was found crystal clear (0) from 27th postoperative day (Fig.30-34).

(v) Haemogram (Table 16)

a. Haemoglobin content (g/dl)

The haemoglobin content was 16.51 ± 0.50 before surgery, 15.26 ± 0.83 at one hour after surgery, 15.39 ± 0.74 on the fifth day, 16.27 ± 0.63 on the 10th day, 15.75 ± 1.18 on the 15th day, 14.21 ± 0.49 on the 20th day, 16.06 ± 1.01 on the 25th day and 16.50 ± 0.50 on the 30th day.

The variations in haemoglobin content was not marked.

b. Total leucocyte count (x 10^3/cu mm)

The total leucocyte count was 10.87 ± 0.26 before surgery, 11.53 ± 0.63 at one hour after surgery, 11.30 ± 0.49 on the fifth day, 10.82 ± 0.85 on the 10th day, 11.11 ± 0.76 on the 15th day, 10.17 ± 1.28 on the 20th day, 10.40 ± 1.00 on the 25th day and 10.14 ± 0.05 on the 30th day.

The variation in total leucocyte count was within normal range.

c. Differential leucocyte count

(1) Neutrophil count (per cent)

The neutrophil count was 69.13 ± 1.88 before surgery, 71.00 ± 2.19 at one hour after surgery, 71.00 ± 1.38 on the fifth day, 71.66 ± 1.61 on the 10th day, 70.00 ± 2.04 on the 15th day, 64.50 ± 0.50 on the 20th day, 68.50 ± 2.50 on the 25th day and 69.00 ± 0.00 on the 30th day.

2. Lymphocyte count (per cent)

The lymphocyte count was 25.25 ± 1.99 before surgery, 24.50 ± 2.17 at one hour after surgery, 23.50 ± 1.70 on the fifth day, 22.67 ± 1.36 on the 10th day, 22.50 ± 1.85 on the 15th day, 30.00 ± 2.00 on the 20th day, 23.50 ± 0.50 on the 25th day and 23.00 ± 2.00 on the 30th day.

3. Eosinophil count (per cent)

The eosinophil count was 4.00 ± 0.38 before surgery, 3.63 ± 0.32 at one hour after surgery, 3.88 ± 0.67 on the fifth day, 3.83 ± 0.70 on the 10th day, 5.00 ± 1.00 on the 15th day, 4.00 ± 1.00 on the 20th day, 4.50 ± 1.50 on the 25th day and 6.00 ± 2.00 on the 30th day.

4. Monocyte count (per cent)

The total monocyte count was 1.63 ± 0.32 before surgery, 0.88 ± 0.30 at one hour after surgery, 1.25 ± 0.37 on the fifth day, 1.67 ± 0.42 on the 10th day, 2.00 ± 0.41 on the 15th day, 1.50 ± 1.50 on the 20th day, 3.00 ± 1.00 on the 25th day and 1.00 ± 1.00 on the 30th day.

5. Basophil count (per cent)

The basophil count was 0.13 ± 0.13, before surgery, 0 at one hour after surgery, 0 on the fifth day, 0.16 ± 0.16 on the 10th day, 0 on the 15th day, 0 on the 20th day, 0.50 ± 0.50 on the 25th day and 0 on the 30th day.

The variation in differential leucocyte count were within normal range.

(vi) Gross examination of the enucleated eyeballs

5th postoperative day

In dogs 1 and 2, the keractectomy site was opaque, irregular and lustreless. The ventral portion of cornea was found raised a little above the surface due to corneal oedema. Vascularization was absent.

10th postoperative day

In dogs 3 and 4, the keratectomy site was hazy and lustreless. Corneal oedema was absent but vascularization was present in both the specimens.

15th postoperative day

In dogs 5 and 6, the keratectomy site was hazy and lustreless. Corneal oedema and vascularization were absent in both the specimens.

30th postoperative day

In dogs 7 and 8, the keratectomy site was crystal clear and lustrous. Corneal oedema and vascularization were absent in both the specimens.

(vii) Histopathology

The keratectomy site was found to be covered by wing cells sliding from the margin of the wound on the fifth postoperative day. Increased cellularity of the stroma was noticed (Fig.35), epithelial facets, nuclear debri, pigmentation and fibroplasia was observed in the stroma (Fig.36). In the specimens of 10th day, epithelial facets and increased fibroplasia of the stroma was noticed (Fig.37). In the specimens of 15th day hyperplasia

of the epithelial surface at the keratectomy site with completely replaced corneal epithelium was observed (Fig.38). In 30th day specimens thickened epithelial layer and active proliferation of epithelium was noticed. Fibroplasia of the stroma close to the epithelial layer was also apparent (Fig.39).

Table 10. Day of disappearance of clinical signs following superficial keratectomy and temporary tarsorrhaphy in dogs (Group II)

Animal No.	Swelling of eye lid	Lacrimation	Scratching and pawing
1.	3	3	3
2.	3	3	3
3.	6	6	6
4.	3	6	6
5.	3	6	6
6.	6	6	6
7.	3	6	6
8.	6	6	6

Table 11. Corneal oedema after superficial keratectomy and temporary tarsorrhaphy in dogs (Group II)

Animal No.	Interval (Days)									
	3	6	9	12	15	18	21	24	27	30
1	+									
2	+									
3	-	-	-							
4	-	-	-							
5	+	+	-	-	-					
6	+	-	-	-	-					
7	-	-	-	-	-	-	-	-	-	-
8	+	+	+	-	-	-	-	-	-	-

\+ Present
\- Absent

Table 12. Vascularization of the cornea after superficial keratectomy and temporary tarsorrhaphy in dogs (Group II)

Animal No.	Interval (Days)									
	3	6	9	12	15	18	21	24	27	30
1	-									
2	-									
3	+	+	+							
4	-	+	+							
5	-	-	-	-	-					
6	-	-	-	-	-					
7	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-

\+ Present
\- Absent

Table 13. Rectal temperature (°C), pulse rate (per min) and respiration rate (per min) before surgery, one hour after surgery and on different postoperative periods in dogs (Group II)

Parameters and units	Intervals							
	0 min	1 hour	5th day	10th day	15th day	20th day	25th day	30th day
Rectal temperature (°C)	39.20± 0.11	37.64± 0.16	39.11± 0.11	39.13± 0.12	39.08± 0.12	38.90± 0.10	38.90± 0.10	39.15± 0.15
Pulse rate (per minute)	119.75± 1.53	94.25± 2.71	118.00± 3.57	117.67± 3.12	110.00± 4.55	103.00± 5.00	105.00± 7.00	116.00± 4.00
Respiration rate (per minute)	31.75± 1.91	12.50± 0.91	31.00± 1.73	29.67± 2.02	29.50± 1.50	27.00± 1.00	25.00± 1.00	27.00± 1.00

Table 14. Fluorescein dye test of the eye after superficial keratectomy in dogs (Group II)

Animal No.	Interval (Days)									
	3	6	9	12	15	18	21	24	27	30
1	++									
2	++									
3	++	+	-							
4	++	+	-							
5	++	+	-	-						
6	++	+	-	-						
7	++	+	-	-	-	-	-	-	-	-
8	++	+	-	-	-	-	-	-	-	-

++ Positive - bright green staining
+ Positive - faint greenish yellow staining
- Negative - not stained

Table 15. Evaluation of clarity of the corneal surface after superficial keratectomy in dogs (Group II)

Animal No.	Interval (Days)									
	3	6	9	12	15	18	21	24	27	30
1	4+	-								
2	4+	-								
3	4+	3+	3+							
4	4+	3+	3+							
5	4+	3+	3+	2+	2+					
6	4+	3+	2+	2+	2+					
7	4+	3+	2+	2+	2+	1+	1+	1+	0	0
8	4+	3+	2+	2+	2+	1+	1+	1+	0	0

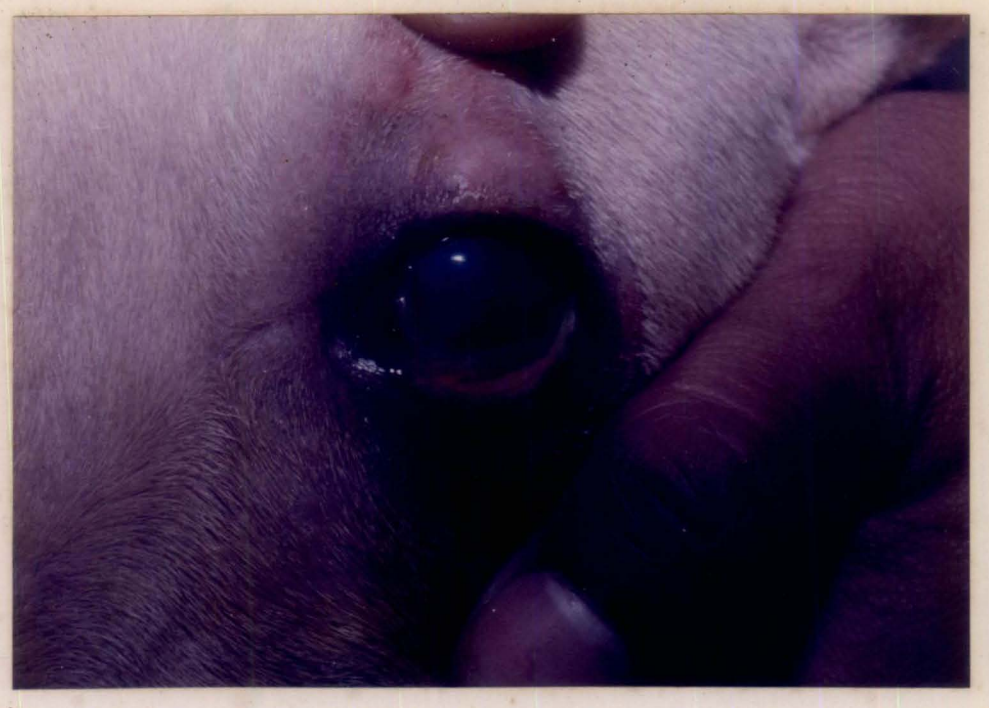
Clarity grading = 0 = crystal clear
1+ = clear
2+ = hazy
3+ = very hazy
4+ = opaque

Table 16. Haemogram before surgery, one hour after surgery and on different postoperative periods in dogs (Group II)

Parameters and units	Interval							
	0 min	1 hour	5th day	10th day	15th day	20th day	25th day	30th day
Haemoglobin content (g/dl)	16.51± 0.50	15.26± 0.83	15.39± 0.74	16.27± 0.63	15.75± 1.18	14.21± 0.49	16.06± 1.01	16.50± 0.50
Total leucocyte count (10^3/cu mm)	10.87± 0.26	11.53± 0.63	11.30± 0.49	10.82± 0.85	11.11± 0.76	10.17± 1.28	10.40± 1.00	10.14± 0.05
Neutrophil count (%)	69.13± 1.88	71.00± 2.19	71.00± 1.38	71.66± 1.61	70.00± 2.04	64.50± 0.50	68.50± 2.50	69.00± 0.00
Lymphocyte count (%)	25.25± 1.99	24.50± 2.17	23.50± 1.70	22.67± 1.36	22.50± 1.85	30.00± 2.00	23.50± 0.50	23.00± 2.00
Eosinophil count (%)	4.00± 0.38	3.63± 0.32	3.88± 0.67	3.83± 0.70	5.00± 1.00	4.00± 1.00	4.50± 1.50	6.00± 2.00
Monocyte count (%)	1.63± 0.32	0.88± 0.30	1.25± 0.37	1.67± 0.42	2.00± 0.41	1.50± 1.50	3.00± 1.00	1.00± 1.00
Basophil count (%)	0.13± 0.13	0	0	0.16± 0.16	0	0	0.50± 0.50	0

Fig.25. Photograph of the eye on the 3rd postoperative day in group II, showing bright green staining after fluorescein dye test. Opacity at the keratectomy site (4+)

Fig.26. Photograph of the eye on the 6th postoperative day in group II, showing faint greenish yellow staining after fluorescein dye test. Haziness at the keratectomy site (3+)



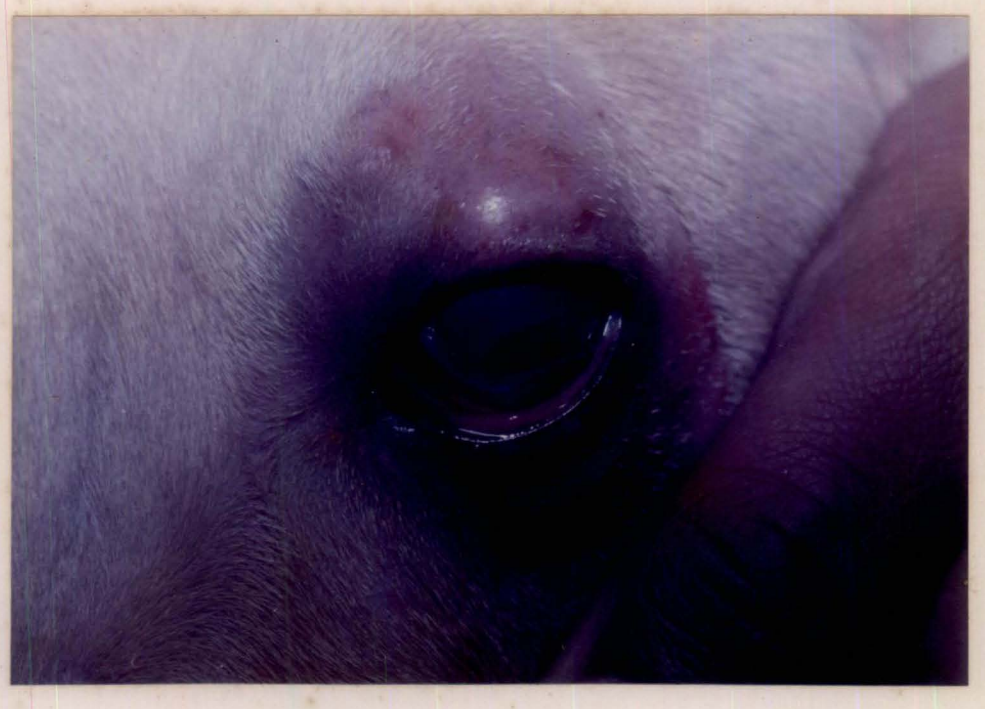
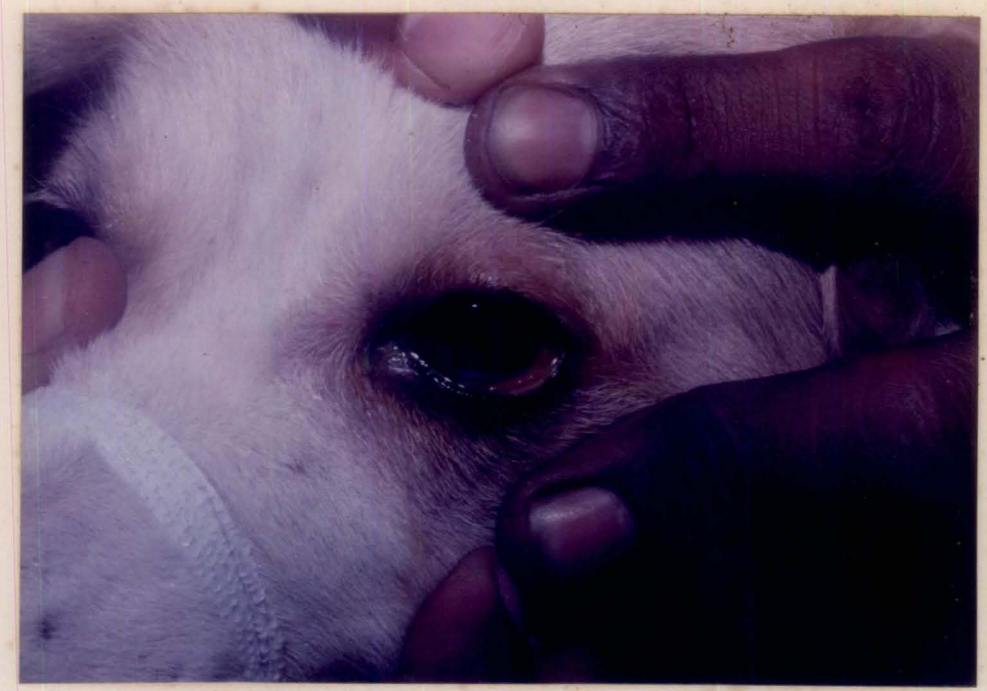


Fig.27. Photograph of the eye on the 9th postoperative day in group II, showing absence of staining after fluorescein dye test. Haziness at the keratectomy site (3+)

Fig.28. Photograph of the eye on the 12th postoperative day in group II. Haziness at the keratectomy site (2+)

Fig.29. Photograph of the eye on the 15th postoperative day in group II. Haziness at the keratectomy site (2+)

Fig.30. Photograph of the eye on the 18th postoperative day in group II, showing clear corneal surface (1+ clarity) at the keratectomy site



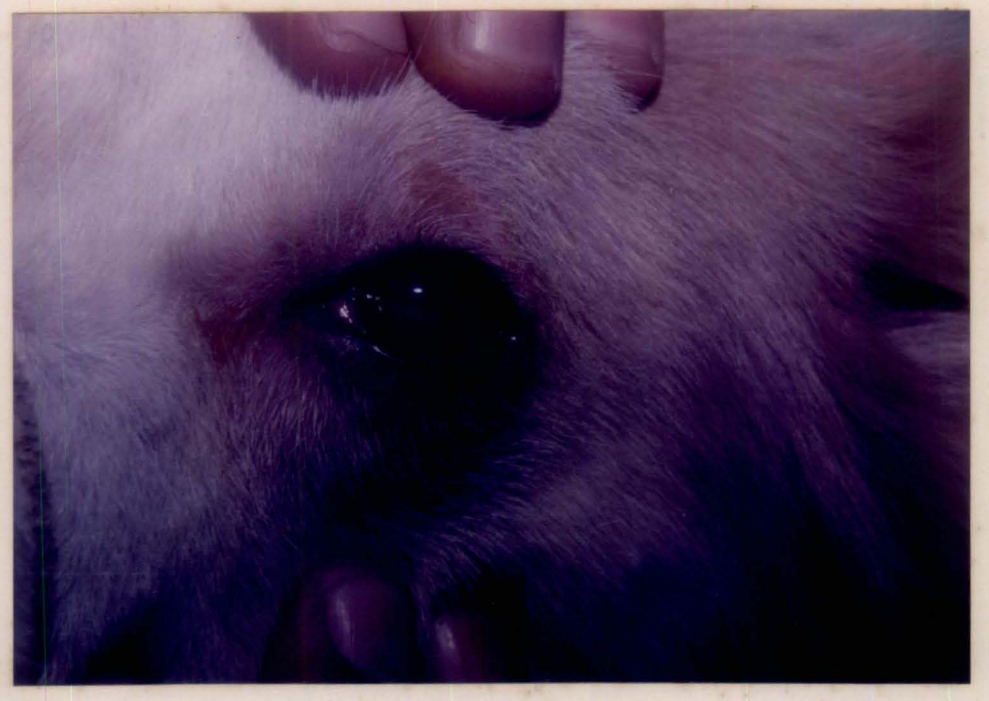
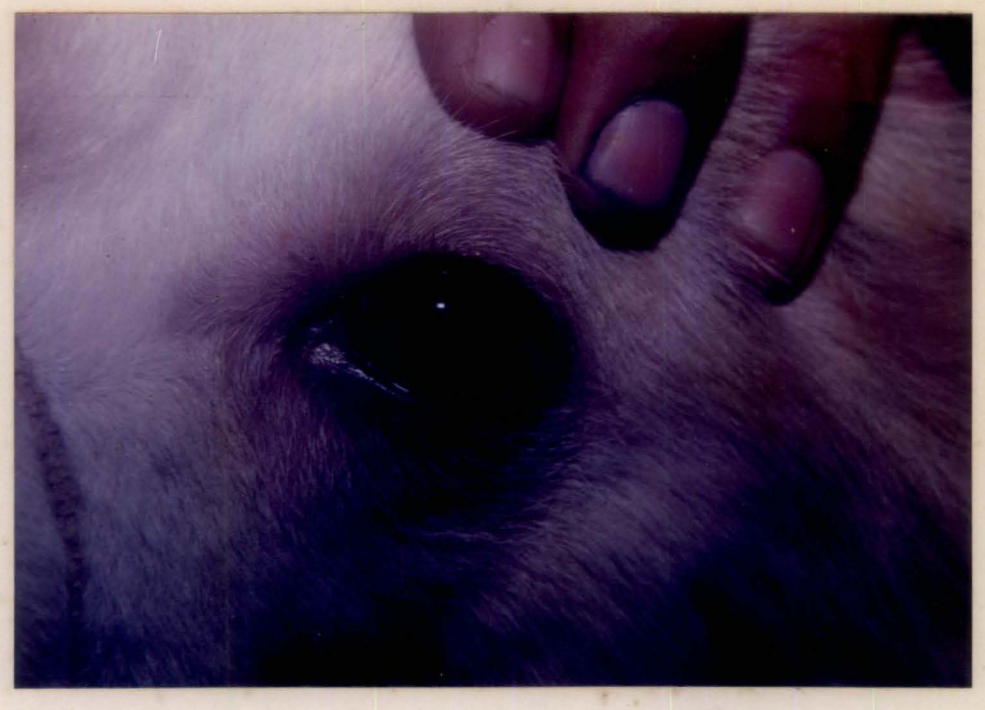


Fig.31. Photograph of the eye on 21st postoperative day in group II, showing clear corneal surface at the keratectomy site

Fig.32. Photograph of the eye on 24th postoperative day in group II, showing clear corneal surface (1+ clarity) at the keratectomy site



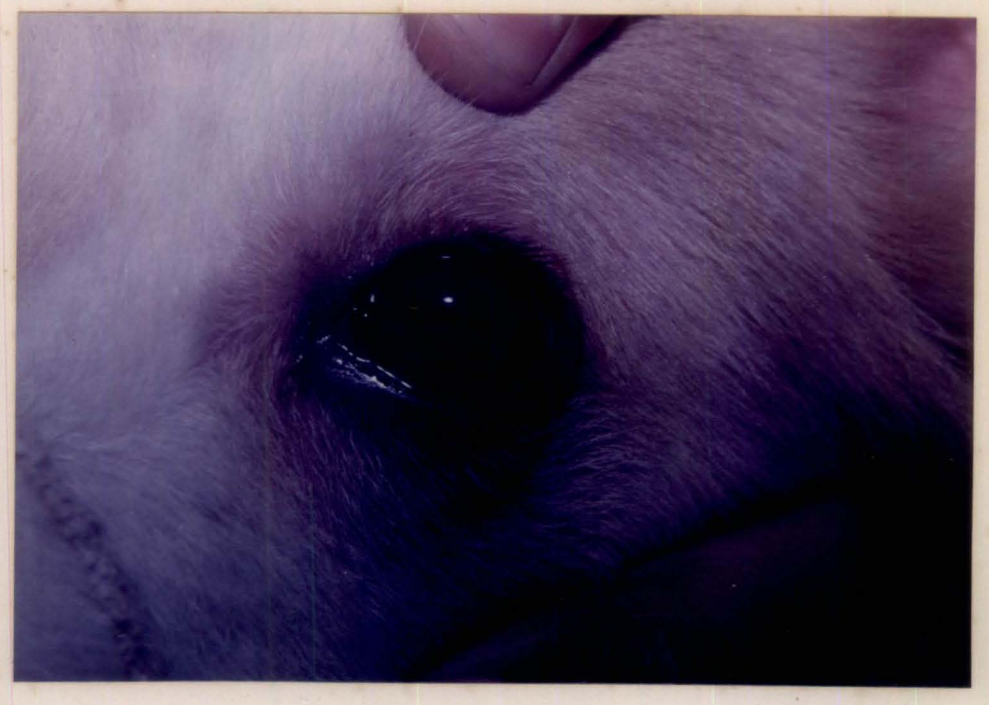


Fig.33. Photograph of the eye on 27th postoperative day in group II, showing crystal clear corneal surface (0 clarity) at the keratectomy site

Fig.34. Photograph of the eye on 30th postoperative day in group II, showing crystal clear corneal surface (0 clarity) at the keratectomy site

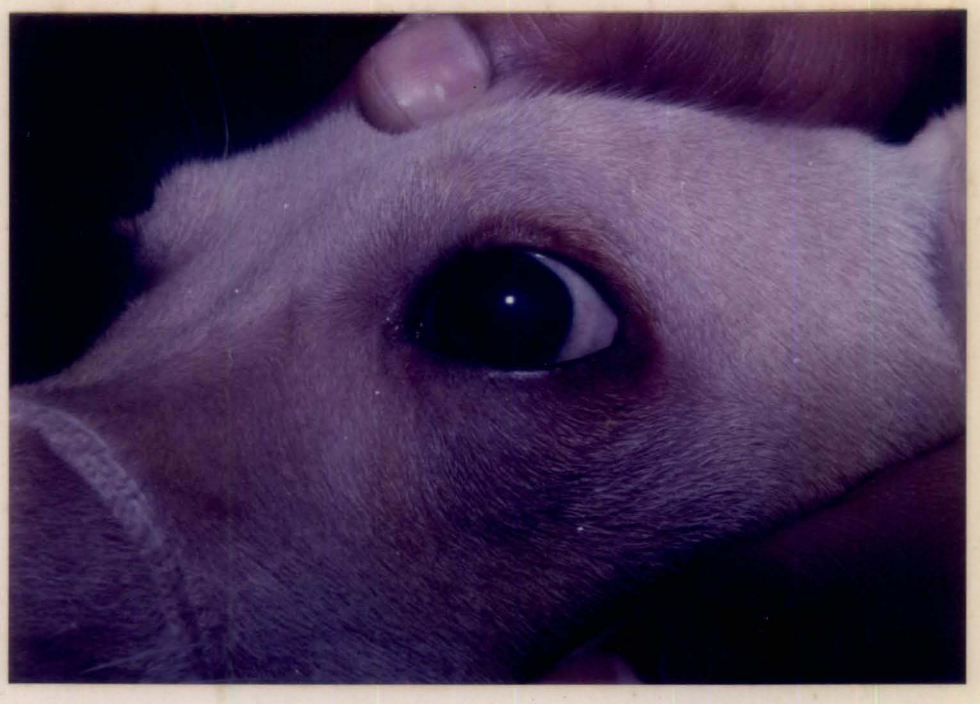


Fig.35. Photomicrograph of the cornea on the 5th postoperative day in group II, showing sliding of wing cells from the margin of the wound and increased cellularity of the stroma at the keratectomy site (H&E x 320)

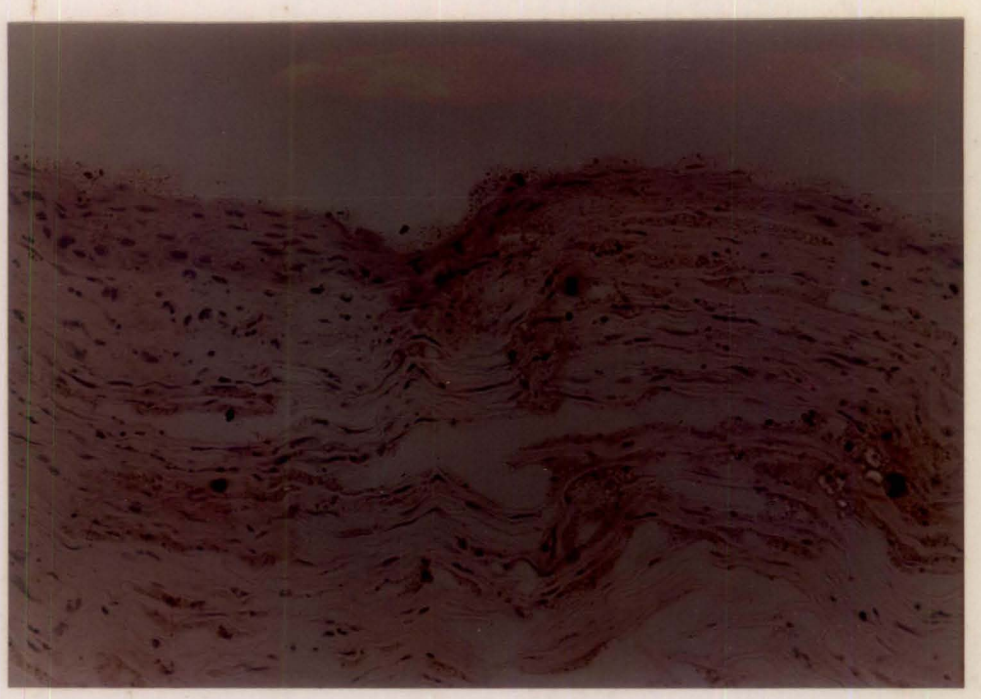
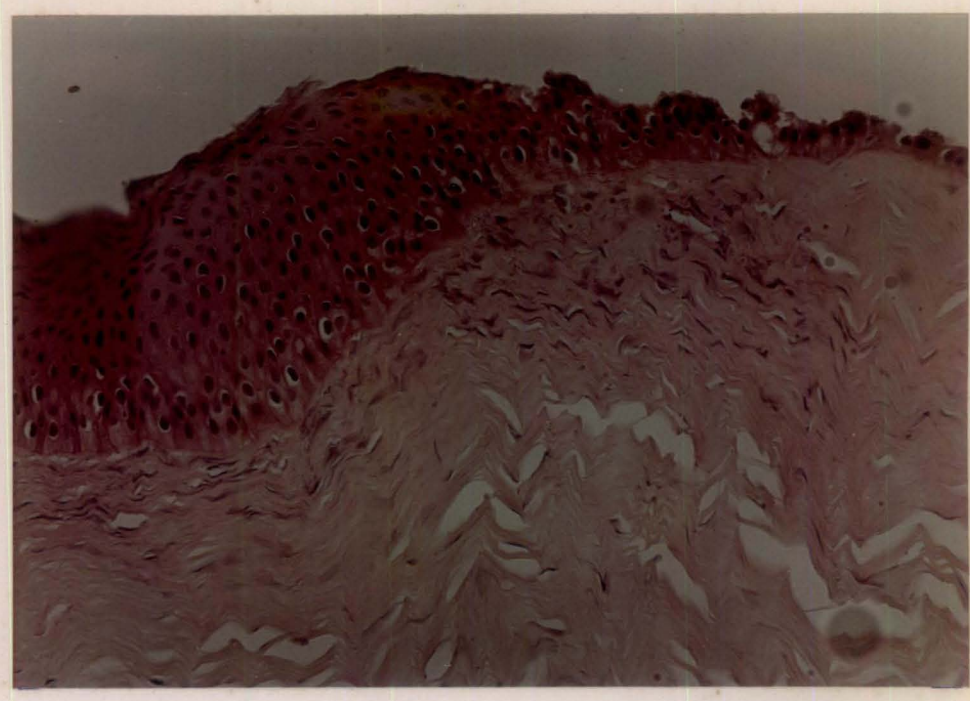


Fig.36. Photomicrograph of the cornea on the 5th postoperative day in group II, showing epithelial facets, nuclear debris, pigmentation and fibroplasia of the stroma at the keratectomy site (H&E x 320)

Fig.37. Photomicrograph of the cornea on the 10th postoperative day in group II, showing epithelial facet and increased fibroplasia of the stroma at the keratectomy site (H&E x 320)

Fig.38. Photomicrograph of the cornea on the 15th postoperative day in group II, showing hyperplasia of the epithelial surface at the keratectomy site (H&E x 320)



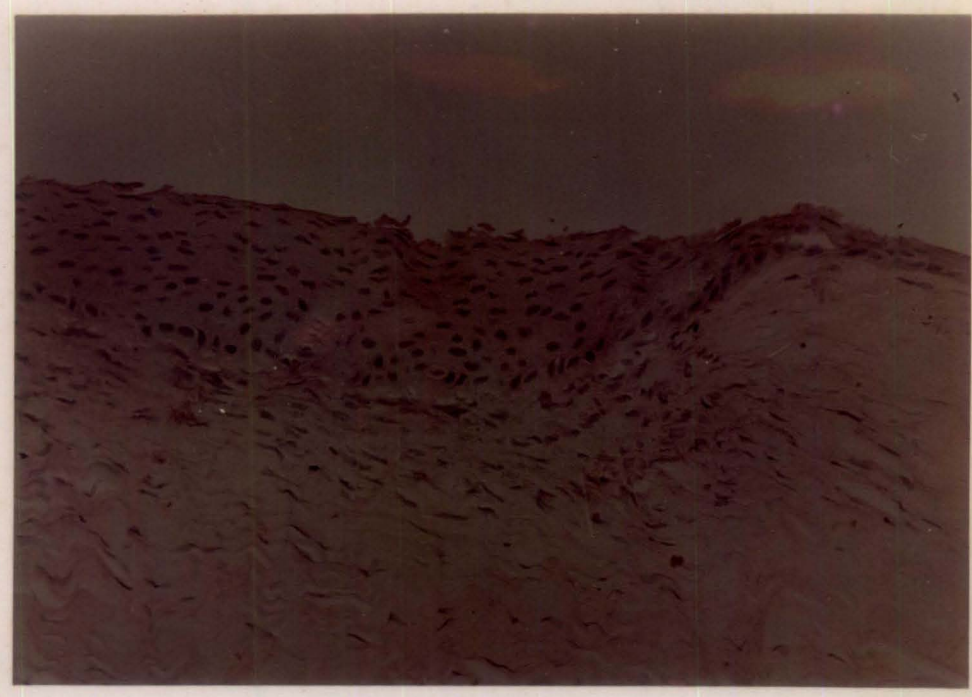
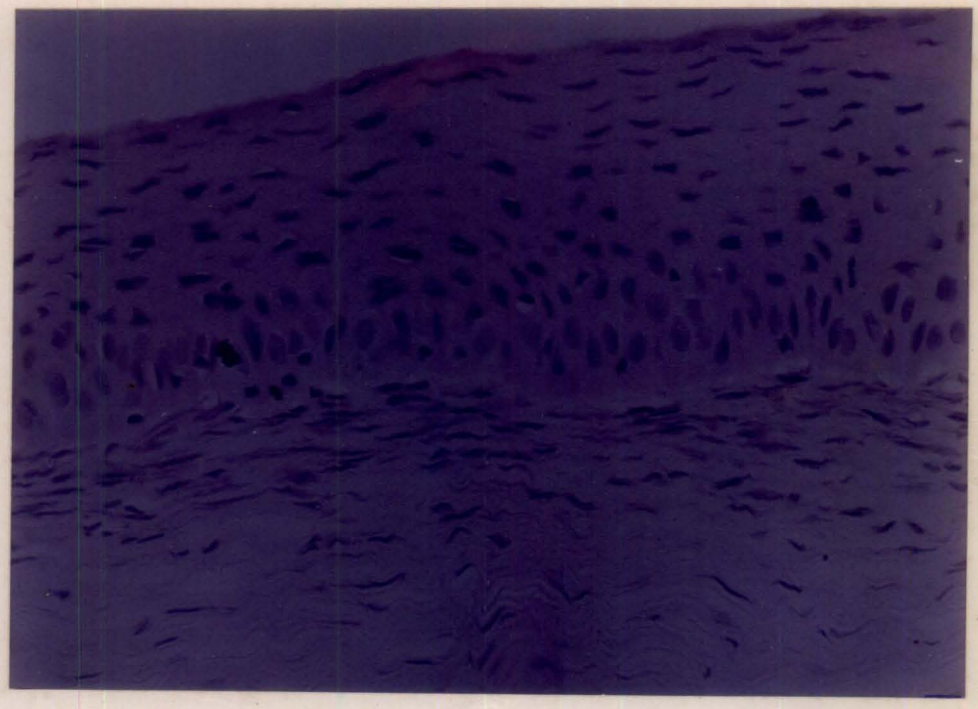


Fig.39. Photomicrograph of the cornea on 30th postoperative day in group II, showing active proliferation of the epithelium, thickened epithelial layer and fibroplasia of the stroma at the keratectomy site (H&E x 400)



Discussion

DISCUSSION

Many corneal diseases and disorders impair the vision of the animal and may predispose to permanent damage if the conditions are not corrected by either medical or surgical means. Different types of surgical interventions are employed to restore vision whenever corneal affections do not respond to medical treatment and also to enhance the healing when treatment is prolonged.

In the present study, superficial keratectomy was performed as a treatment for experimentally created corneal wounds in canines. On the ventral half of the cornea, experimental injury was created and 24 hours later superficial keratectomy was performed. Third eye lid flap combined with temporary tarsorrhaphy was performed to cover the cornea in one group of eight animals and temporary tarsorrhaphy alone was done in a second group of eight animals.

As microorganisms which are normally present (Sansom, 1988) in the conjunctival sac can be pathogenic, the eye was irrigated from three days prior to surgery with two per cent boric acid solution and framycetin drops was instilled (broad spectrum antibiotic) thrice daily.

General anaesthesia is preferred in most extraocular procedures and in all intraocular surgery (Helper, 1989). In

the present study, superficial keratectomy was done under premedication with atropine sulphate (subcutaneous) and xylazine (intramuscular) followed with thiopentone sodium five per cent solution intravenous for general anaesthesia to effect.

The average dose of thiopentone sodium required for surgical anaesthesia was 16.77 mg/kg bodyweight, the time taken for induction was 3.34 ± 0.28 minutes, the duration of anaesthesia was 40.25 ± 2.63 minutes and the time taken for recovery was 121.88 ± 7.34 minutes. Satisfactory plane of surgical anaesthesia, analgesia, relaxation of muscles of eyeball and sufficient duration of anaesthesia were achieved with xylazine-thiopentone sodium combination. The induction and recovery were smooth and anaesthesia was satisfactory for proptosing the eyeball for surgery. The use of barbiturates for anaesthesia in ophthalmic surgical procedures in dogs have been reported by Livingston (1950) and Roberts (1953).

In the present study, after superficial keratectomy, to cover the cornea, modified third eye lid flap and temporary tarsorrhaphy were performed in Group I and temporary tarsorrhaphy alone in Group II.

The modified third eye lid flap procedure involved drawing the third eye lid over the cornea and securing it by passing a double armed suture from the palpebral surface of the third eye

lid to the upper eye lid. For temporary tarsorrhaphy, a single suture was applied subcutaneously through the skin of each lid. Temporary tarsorrhaphy was maintained till the sixth postoperative day in both the groups and third eye lid flap in Group I was retained till the ninth day.

At three day intervals, third eye lid flap and temporary tarsorrhaphy were relieved by loosening the sutures for examination of the cornea and the sutures were reapplied. For the treatment of corneal lesions, superficial keratectomy (Peiffer Jr. *et al.*, 1987c and Spiess and Tscharner, 1987), superficial keratectomy combined with third eye lid flap and/or temporary tarsorrhaphy (Studdert, 1967; Anderson *et al.*, 1976; Slatter, 1981 and Mishra, 1991), third eye lid flap alone (Barnett, 1966; Knecht, 1966; Howard and Breazile, 1971; Stanley, 1988 and Christmas, 1991) were recommended. Mohanty and Mitra (1971) employed temporary tarsorrhaphy to cover the cornea after conjunctival keratoplasty.

Third eye lid may get deformed when free margin is secured to the upper eye lid with several sutures (Carter, 1981) and there is a chance of friction between the flap and the cornea (Startup, 1984). When the third eye lid is fixed to the episcleral tissue, the flap may fail if the sutures are placed deep or the globe may get penetrated by misdirected needle. However, this procedure minimises the friction between the flap

and the cornea as it allows the flap to move along with the globe (Slatter, 1981). The flap reduces pain, protects the weakened cornea (Peiffer Jr. *et al.*, 1987a), prevents further injury (Anderson *et al.*, 1976 and Studdert, 1987), and promotes healing (Schmidt, 1977 and Startup, 1988). Various authors have recommended retension of the third eye lid flap for 10-14 days (Slatter, 1981; Helper and Blogg, 1983 and Startup, 1984) or seven to ten days (Anderson *et al.*, 1976) and the temporary tarsorrhaphy for seven to ten days (Mohanty and Mitra, 1971 and Peiffer Jr. *et al.*, 1987a). In the present study the third eye lid flap was retained for nine days and the temporary tarsorrhaphy for six days. The duration of retention was found satisfactory for promoting healing after superficial keratectomy.

Swelling of eye lids was noticed within 24 hours after surgery in both the groups. In Group I, it persisted upto the third day in six animals and upto the ninth day in two animals. In Group II, it persisted upto the third day in five animals and upto the sixth day in three animals. Lacrimation was noticed in all the animals of both the groups immediately after surgery and in Group I, it persisted upto the third day in two animals, upto the sixth day in five animals and upto the ninth day in one animal. In Group II, lacrimation persisted upto the third day in two animals and upto the sixth day in the remaining animals. The longer period of observation of

swelling of eye lid and lacrimation in Group I, is probably due to the inflammation and hypertrophy of the third eye lid. Similar observators like swelling of the upper eye lid during third eye lid flap procedure which disappeared after three to four days was reported by Slatter (1981). Roberts (1965), Hime (1966) and Studdert (1967) observed pain, lacrimation and eye lid spasm as the usual clinical signs during the early stages of corneal damage. Hypertrophy of the third eye lid was suggested as one of the causes for ephiphora (Helper, 1989).

Scratching and pawing was noticed in all the animals of both the groups from the day of surgery. It persisted upto the third day in all the animals of Group I except in one animal in which it persisted upto the sixth day. In Group II, two animals had scratching and pawing on the third day and in the remaining animals, it was noted upto the sixth day. However, manipulation resulting in self mutilation of sutures was observed only in Group II in two animals. The pain and irritation of the surgical procedures on the cornea, eye lid and third eye lid probably caused the animals to manipulate the region of the eye as observed by Helper (1989).

Corneal oedema in the present study was encountered in both the groups. In Group I, corneal oedema was observed in all the animals from the third day and it persisted upto the 12th day in two animals. In Group II, corneal oedema was

noticed in five animals on the third day and it persisted upto the ninth day in one animal. Corneal oedema may develop whenever the corneal epithelium is lost and would result in loss of corneal transparency, since epithelium and endothelium control the water content of the cornea (Hime, 1966; Startup, 1972; Slatter, 1981; Startup, 1984 and Peiffer Jr. *et al.*, 1987d).

In all the animals, the eye was irrigated every day with one per cent sterile sodium chloride solution during the postoperative period upto 30 days. Longer duration of corneal oedema, in Group I when compared to Group II, may be due to the presence of vascularization and the reduced efficacy of irrigation with sodium chloride because it could not reach the corneal surface in sufficient volume owing to the presence of third eye lid flap. Helper (1989) opined that temporary clearance of corneal oedema could be achieved by irrigating cornea with three per cent sodium chloride solution. Rosenthal (1974) and Rebhun (1983), recommended osmotic agents like five per cent sodium chloride to alleviate the corneal oedema whereas Slatter (1981) suggested both three per cent and five per cent. Bromberg (1983) stated that third eye lid flap was found to decrease the quantity of medication reaching the cornea.

Vascularization of the cornea was observed in both the groups in the present study. In Group I, it was noticed within the sixth postoperative day and it persisted in three animals upto the 15th day. Clinical disappearance of the vessels were noticed after the 15th postoperative day. In Group II, the vascularization was noticed within third day in one animal and by ninth day in two animals. Clinical disappearance of the vessels were noticed after ninth postoperative day. The vessels were bright red and branched in tree like fashion and hence were considered as superficial vessels.

Longer duration of vascularization and higher incidence in Group I, may be due to the irritation caused by the friction between the cornea and the third eye lid flap or the inflammation. Decreased quantity of framycetin-dexamethosone reaching the corneal surface due to the presence of third eye lid flap may also have contributed to it.

Invasion of cornea with new vessels during its healing process was reported by Catcott and Griesemer (1954), Magrane (1955), Krawitz (1963) and Slatter (1981). Peiffer Jr. *et al.* (1987d) observed two type of vessels - superficial and deep - where the superficial vessels were bright red and branched like tree. Though vascularization is a beneficial response in corneal healing, (Magrane, 1955 and Krawitz, 1963), the vessels

caused decreased transparency, in growth of pigments and scar tissue formation (Catcott and Griesemer, 1954; Schmidit, 1977).

Since corticosteroids suppress vascularization and bring about beneficial effects (Roberts, 1954; Studdert, 1967; Helper, 1989 and Pandey *et al.*, 1990), topical administration of framycetin-dexamethasone solution was adopted in this study. Corticosteroids had caused the clinical disappearance of vascularization after 15th day in Group I and the ninth day in Group II. Slatter (1981) opined that the use of corticosteroid during corneal healing inhibited epithelial regeneration, infiltration of inflammatory cells, fibroblastic activity and endothelial regeneration resulting in delayed healing. Rebhun (1983) found corticosteroids delayed the normal mitosis and migration of corneal epithelial cells during healing process. Hence use of corticosteroid is recommended during corneal healing only after the epithelialization is complete as evidenced by fluorescein-negative staining (Catcott and Griesemer, 1954; Howard and Breazile, 1971; Startup, 1972; Startup, 1984 and Barnett and Sansom, 1987). However, healing in the present study was not affected by the use of corticosteroid.

The third eye lid flap and temporary tarsorrhaphy were relieved at three day interval for routine postoperative observations of the cornea. The third eye lid was found to

cover two-third to one-third of the cornea between third day and ninth day, even after the sutures were released. This may probably be due to the stretching of third eye lid, hyperemia and inflammation of the third eye lid, and temporary loss of elasticity. On ninth day third eye lid flap was released and it was found that the flap returned to its normal position between ninth and 12th postoperative days. There was no bleeding while the third eye lid was gently separated from the cornea for routine examination during the postoperative periods. This indicated that there was no adhesion between the cornea and the third eye lid flap and thereby no growth of blood vessels from the flap to the cornea during the healing process. However, suturing the third eye lid to the upper lid without penetrating the bulbar surface of the third eye lid prevented the adhesion of the flap with the cornea. Slatter (1981) found that adhesion between the third eye lid flap and the cornea was rare when compared to that of bulbar conjunctiva. However, adhesion between the third eye lid flap and the cornea was reported (Bistner *et al.*, 1977 and Brooks, 1991) when the bulbar surface of the nictitans was sacrified prior to fixing the flap.

Other symptoms such as congestion of bulbar and palpebral conjunctivae as well as that of the third eye lid were observed within the sixth day. This may be due to the inflammatory response to the experimental injury and surgical trauma.

Similar observations were reported by Slatter (1981) during surgical manipulation of the third eye lid, eye lids and cornea.

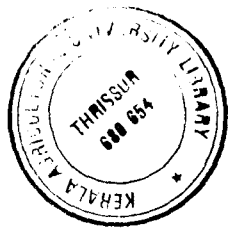
Physiological parameters like rectal temperature, pulse rate and respiratory rate did not show any marked variation except at one hour after surgery wherein the reduction observed was probably due to the effect of anaesthesia.

The haemoglobin content, total leucocyte count and differential leucocyte count did not show any marked variation during the different postoperative periods in both the groups. Examination of lacrimal smear at three day intervals during the postoperative periods revealed the presence of denuded epithelium and cellular debris throughout the period of study. But inflammatory cells were not observed. These observations suggest that there was no systemic response in the surgical procedure adopted.

Evaluation of the progress of corneal healing was carried out at three day intervals during the postoperative periods using fluorescein dye test. The fluorescein dye test was positive in both the groups on the third day and the cornea at the keratectomy site was seen stained bright green. On sixth day, the cornea at the keratectomy site was fluorescein-negative in all the animals of both the groups except in one animal (Dog no.8) in Group I, which was stained faint greenish

yellow on ninth day and was fluorescein-negative by the 12th day. The reduction in the degree of staining progressively indicated that the epithelialization of cornea after superficial keratectomy started within six days, was progressive and complete by ninth postoperative day in all the animals of both the groups except in one dog in Group I (Dog No.8) where the epithelialization was complete by only on the 12th day. Hime (1966) and Howard and Breazile (1971) observed that the cornea stained bright green after fluorescein dye test when it was devoid of epithelium. Roberts (1965), Hime (1966), Howard and Breazile (1971) and Startup (1972) found that the newly laid down granulation tissue of the cornea during its healing stained faint greenish yellow and became fluorescein negative after the corneal epithelium became intact or once the corneal epithelialization is complete.

The observations in the present study is in agrement with the observations of Spreull (1966), Studdert (1967), Slatter (1981) and Helper (1989). Use of fluorescein solution for evaluating the corneal healing in corneal ulcers was reported by Roberts (1953), Roberts (1965), Hime (1966), Studdert (1967) and Hinton (1969) and fluorescein strips were reported by Howard and Breazile (1971); Startup (1972); Rebhun (1983) and Startup (1984).



Corneal clarity after keratoplasty was graded on a 0 to 4+ scale in dogs (McEntyre., 1968) and bovines (Kudva *et al.*, 1983). On evaluation of the clarity of the cornea at the keratectomy site using this scale, all the animals in both the groups had 4+ opacity or clouding on the third day. On the sixth day, four animals in Group-I were having 3+ haziness and two animals had 4+ opacity at the keratectomy site. In Group II, all the animals had 3+ haziness on the sixth day. On the ninth day five animals had 3+ haziness and one animal had 4+ opacity in Group I. In Group II on the ninth day, three animals had 3+ haziness while the remaining animals had 2+ haziness. In both the groups on 12th postoperative day, 2+ haziness was observed at the keratectomy site in all the animals. On the 18th day, the keratectomy site was found clear (1+) in all the animals in both the groups and was found to persist upto the 27th day in Group I and 24th day in Group II. The keratectomy site became crystal clear on the 30th day in group I and 27th day in Group II. Roberts (1965) observed the healing period of cornea after superficial keratectomy as 5 to 35 days in dogs. The complete clinical healing of the cornea was found delayed by three days in Group I than in Group II. Longer duration of corneal oedema, vascularization, and manipulation of the flap during routine examination of the cornea during different postoperative periods may have delayed in corneal healing in Group I.

In the present study, in Group I in which superficial keratectomy and third eye lid flap with temporary tarsorrhaphy were performed, the duration required for corneal healing was 18-30 days, whereas in Group II, where superficial keratectomy and temporary tarsorrhaphy was performed, corneal healing required 18-27 days. This is in agreement with the observation of Helper (1989).

Gross examination of the enucleated eyeballs on the fifth day in Group I, demonstrated opaque, irregular and lustreless surface at the keratectomy site and presence of corneal oedema. The keratectomy site was hazy on the 10th day and in one specimen (dog 3) corneal oedema was present. Neovascularization at the keratectomy site was noticed on the 10th day (dog 3,4) and on the 15th day (dog 6) with the vessels extending from the limbal margins. Corneal surface was crystal clear in the 30th day specimens.

In Group II, gross examination of the eyeballs enucleated from dog 1 and 2 (fifth day) revealed that the keratectomy site was irregular, lustreless and opaque with corneal oedema. The specimens from dog 4 and 5 (10th day) revealed that the site was hazy and lustreless with superficial neovascularization. The keratectomy site was hazy and lustreless in the specimens of 15th day. Corneal surface was crystal clear in the specimens of 30th day.

Progressive healing was apparent in the specimens studied and it indicated that the healing of cornea at the keratectomy site started as early as third day and completed within 30 days.

Microscopical examination of the cornea at the keratectomy site in the specimens of fifth postoperative day in Group I, revealed necrosis of the epithelial cells at the border of the wound, inflammatory odema in the corneal epithelium and stroma, scattered inflammatory cells in the stroma whereas those from Group II on the same interval showed the presence of wing cells sliding from the margin of the wound, increased cellularity of the stroma, epithelial facet formation, nuclear debri, pigmentation and fibroplasia.

In the specimens of 10th day in Group I, epithelial facet formation with fibroplasia of the stroma, mild haemmorrhage, necrosis of the epithelial cells, subepithelial and stromal oedema, swollen basal cells and active proliferation of epithelial cells were observed at the keratectomy site. In Group II, the 10th day specimens showed epithelial facet formation and increased fibroplasia. Epithelial facet formation and fibroplasia of the stroma were observed as early as fifth day in Group II but this was evident only on 10th postoperative day in the specimens of Group I.

Whitley (1991) observed that epithelial defects healed by epithelial sliding and mitosis and the epithelial wing cells were the earliest sliding cells during epithelial sliding. Slatter (1981) found the basal cells which were forced toward the surface and and become flattened as wing cells. Spreull (1966) and Studdert (1967) mentioned the healing of the superficial corneal defects by a process of epithelialization.

In the specimens of 15th day in Group I, mild necrosis and degeneration of the lining epithelium, mild stromal oedema and new fibrovascular tissue in the stroma were observed. In Group II, the specimens on the 15th day revealed hyperplasia of the epithelial surface at the keratectomy site with completely replaced epithelium. In the specimens of 30th day, active proliferation of epithelial cells, thickened epithelium and fibroplasia in the stroma were observed in both the groups. Whitley (1991) observed that the corneal epithelium would be completely replaced in about 14 days.

Histological observation showed that the epithelial regeneration has started as early as fifth postoperative day (dog 1 and 2 in Group II) and was almost complete by the 15th day (dog 3 and 4 in Group II). The corneal healing was completed within 30 days.

Summary

SUMMARY

The experiment was conducted on sixteen, apparently healthy adult mongrel dogs of either sex, randomly divided into two groups viz., Group I and II, each consisting of eight animals.

Under topical anaesthesia using four per cent lignocaine hydrochloride solution, superficial injury on the ventral half of cornea of the left eye was created and after 24 hours, superficial keratectomy was performed on the ventral cornea under general anaesthesia. In Group I, the cornea was protected with the third eye lid flap and temporary tarsorrhaphy whereas in Group II, only tarsorrhaphy was performed to protect the cornea.

The animals were premedicated with atropine sulphate (0.04 mg/kg bodyweight) s/c and after five minutes xylazine (0.5 mg/kg bodyweight) i/m. Anaesthesia was induced with five per cent solution of thiopentone sodium i/v. Induction of anaesthesia was complete by 3.34 $\pm$ 0.28 minutes, duration of surgical anaesthesia was 40.25 $\pm$ 2.63 minutes and the time taken for recovery was 121.88 $\pm$ 7.34 minutes. The animals were kept under observation for 30 days.

Clinical symptoms exhibited by the animals of both the groups in different postoperative periods were recorded. In

Group I, swelling of eye lid, scratching and pawing were observed from the day of surgery upto the sixth postoperative day. Lacrimation was observed upto the ninth postoperative day. In Group II, swelling of eye lid,lacrimation, scratching and pawing were observed upto the sixth postoperative day.

In Group I, corneal oedema was present in all the animals from the third postoperative day and in two animals it persisted upto the 12th day. In Group II, five animals had corneal oedema on the third day which persisted upto the sixth day in two animals and in one animal it was seen upto the ninth day.

Vascularization in Group I was observed in all the animals on the sixth postoperative day and it persisted upto the 15th postoperative day in three animals. In Group II, vascularization was observed only in two animals between the third and sixth postoperative day.

In Group I, third eye lid was found to cover 2/3rd the eyeball on the third day when the sutures were relieved for examination of the cornea and it was found to return to its normal position between ninth day to twelfth day. Congestion of the bulbar and palpebral conjunctivae and third eye lid were noticed between third day and sixth day in all the animals of both the groups.

During the postoperative period, the rectal temperature (°C), pulse rate and respiratory rate did not show any marked variation in both the groups. Lacrimal smear examination on different postoperative period revealed denuded epithelium and cellular debris.

Fluorescein dye staining test during postoperative period in both the groups revealed signs of progressive healing of the keratectomy site in all the animals. The keratectomy site in Group I was bright green on the third postoperative day and became fluorescein negative on the ninth postoperative day except in one animal. In Group II, all the animals became fluorescein negative on the ninth postoperative day.

The clarity of the cornea at the keratectomy site showed progressive clearing of the cornea during the postoperative period. In Group I, the keratectomy site became crystal clear on 27th day whereas in Group II it became crystal clear on 30th postoperative day.

Haemogram during the postoperative period did not show significant variation in the haemoglobin content. The variation in the total leucocyte count and differential leucocyte count were within the normal range in both the groups.

The eye balls were enucleated from two animals each on the fifth, 10th, 15th and 30th day for gross and histopathological evaluation.

Gross examination of the enucleated eyeballs in Group I on the fifth day showed opacity, on the 10th day mild haziness and corneal oedema and on the 15th day, haziness at the keratectomy site. Vascularization of the cornea was noticed in both the specimens collected on the 10th day and in one specimen collected on the 15th day. The keratectomy site appeared crystal clear on the 30th postoperative day. In Group II, the keratectomy site showed opacity on the fifth day, mild haziness on the 10th day and 15th day. Vascularization of the cornea was noted in both the specimen collected on the 10th day. The keratectomy site appeared crystal clear and lustrous on the 30th day.

Microscopical examination of the corneal specimens in Group I, revealed necrosis of epithelial cells and inflammatory oedema in the epithelium and stroma on the fifth day, epithelial facet formation and fibroplasia on the 10th day, presence of fibrovascular tissue in the stroma on the 15th day and active proliferation of epithelial cells, thickened epithelium and fibroplasia of the stroma on the 30th day.

In Group II, sliding of the wing cells into the wound from its margin were noticed with epithelial facet formation and

fibroplasia of stroma on the fifth day, and increased fibroplasia on the 10th day. The corneal epithelium was completely replaced on the 15th day and thickened epithelium with active proliferation of epithelial cells and fibroplasia of stroma was observed on the 30th day specimens.

Based on these findings in the study, the following conclusions were drawn,

1. Anaesthesia using atropine sulphate-xylazine-thiopentone sodium combination was satisfactory for the superficial keratectomy procedure in dogs.

2. The incidence of neovascularization and corneal oedema are higher after superficial keratectomy with third eye lid flap and temporary tarsorrhaphy, though complete healing of the cornea occurred within 30 days.

3. Superficial keratectomy followed by temporary tarsorrhaphy is associated with minimal postoperative complications and complete healing occurred within 27 days.

4. The epithelialization of the cornea was complete clinically between sixth and ninth day and can be correctly assessed by fluorescein dye testing.

5. Topical application of Dexamethasone in the eye was found effective to suppress the vascularization of cornea after superficial keratectomy

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SUPERFICIAL KERATECTOMY FOR THE MANAGEMENT OF CORNEAL WOUNDS IN CANINES

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ABSTRACT OF A THESIS

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ABSTRACT

The present study was undertaken to evaluate superficial keratectomy in the management of experimentally created corneal wounds in canines.

The experiment was conducted on sixteen, apparently healthy adult mongrel dogs of either sex, randomly divided into two groups viz., Group I and II, each consisting of eight animals.

Under topical anaesthesia using four per cent lignocaine solution, superficial injury on the ventral half of cornea of the left eye was created and after 24 hours, superficial keratectomy was performed on the ventral cornea under general anaesthesia. In Group I, the cornea was protected with the third eye lid flap and temporary tarsorrhaphy whereas in Group II, only tarsorrhaphy was performed to protect the cornea.

The animals were premedicated with atropine sulphate (0.04 mg/kg bodyweight) s/c and after five minutes xylazine (0.5 mg/kg bodyweight) i/m. Anaesthesia was induced with five per cent solution of thiopentone sodium i/v. Induction of anaesthesia was complete by 3.34 $\pm$ 0.28 minutes, duration of surgical anaesthesia was 40.25 $\pm$ 2.63 minutes and the time

taken for recovery was 121.88 ± 7.34 minutes. The animals were kept under observation for 30 days.

Clinical symptoms exhibited by the animals of both the groups in different postoperative periods were recorded. In Group I, swelling of eye lid, scratching and pawing were observed from the day of surgery upto the sixth postoperative day. Lacrimation was observed upto the ninth postoperative day. In Group II, swelling of eye lid, lacrimation, scratching and pawing were observed upto the sixth postoperative day.

In Group I, corneal oedema was present in all the animals from the third postoperative day and in two animals it persisted upto the 12th day. In Group II, five animals had corneal oedema on the third day which persisted upto the sixth day in two animals and in one animal it was seen upto the ninth day.

Vascularization in Group I was observed in all the animals on the sixth postoperative day and it persisted upto the 15th postoperative day in three animals. In Group II, vascularization was observed only in two animals between the third and sixth postoperative day.

In Group I, third eye lid was found to cover 2/3rd the eyeball on the third day when the sutures were relieved for examination of the cornea and it was found to return to its normal position between ninth day to twelfth day. Congestion

of the bulbar and palpebral conjunctivae and third eye lid were noticed between third day and sixth day in all the animals of both the groups.

During the postoperative period, the rectal temperature (°C), pulse rate and respiratory rate did not show any marked variation in both the groups. Lacrimal smear examination on different postoperative period revealed denuded epithelium and cellular debris.

Fluorescein dye staining test during postoperative period in both the groups revealed signs of progressive healing of the keratectomy site in all the animals. The keratectomy site in Group I was bright green on the third postoperative day and became fluorescein negative on the ninth postoperative day except in one animal. In Group II, all the animals became fluorescein negative on the ninth postoperative day.

The clarity of the cornea at the keratectomy site showed progressive clearing of the cornea during the postoperative period. In Group I, the keratectomy site became crystal clear on 27th day whereas in Group II it became crystal clear on 30th postoperative day.

Haemogram during the postoperative period did not show significant variation in the haemoglobin content. The variation in the total leucocyte count and differential

leucocyte count were within the normal range in both the groups.

The eyeballs were enucleated from two animals each on the fifth, 10th, 15th and 30th day for gross and histopathological evaluation.

Gross examination of the enucleated eyeballs in Group I on the fifth day showed opacity, on the 10th day mild haziness and corneal oedema and on the 15th day, haziness at the keratectomy site. Vascularization of the cornea was noticed in both the specimens collected on the 10th day and in one specimen collected on the 15th day. The keratectomy site appeared crystal clear on the 30th postoperative day. In Group II, the keratectomy site showed opacity on the fifth day, mild haziness on the 10th day and 15th day. Vascularization of the cornea was noted in both the specimen collected on the 10th day. The keratectomy site appeared crystal clear and lustrous on the 30th day.

Microscopical examination of the corneal specimens in Group I, revealed necrosis of epithelial cells and inflammatory oedema in the epithelium and stroma on the fifth day, epithelial facet formation and fibroplasia on the 10th day, presence of fibrovascular tissue in the stroma on the 15th day and active proliferation of epithelial cells, thickened epithelium and fibroplasia of the stroma on the 30th day.

In Group II, sliding of the wing cells into the wound from its margin were noticed with epithelial facet formation and fibroplasia of stroma on the fifth day, and increased fibroplasia on the 10th day. The corneal epithelium was completely replaced on the 15th day and thickened epithelium with active proliferation of epithelial cells and fibroplasia of stroma was observed on the 30th day specimens.