

Corneal Wound Healing

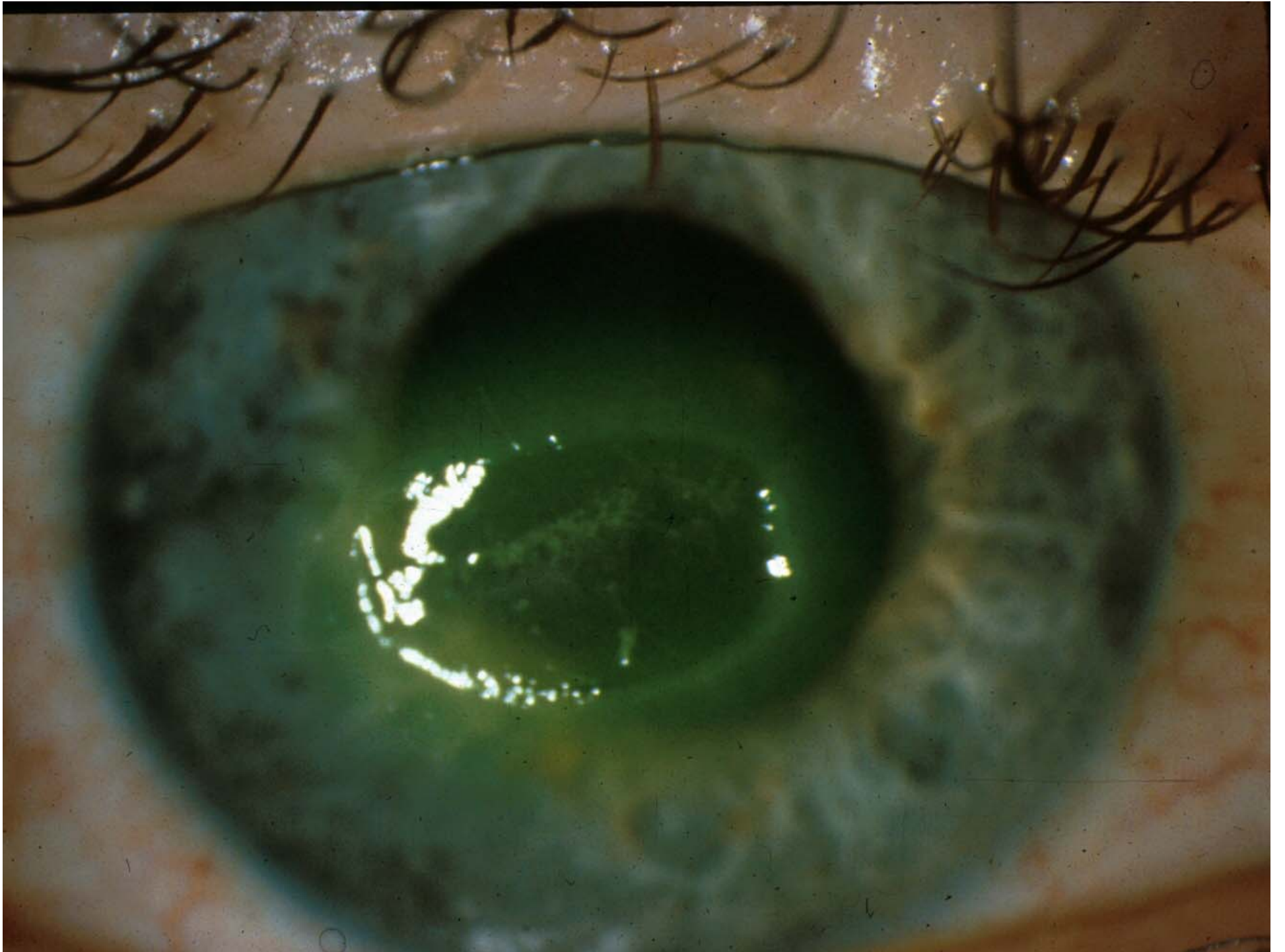
Role of Neuropeptides

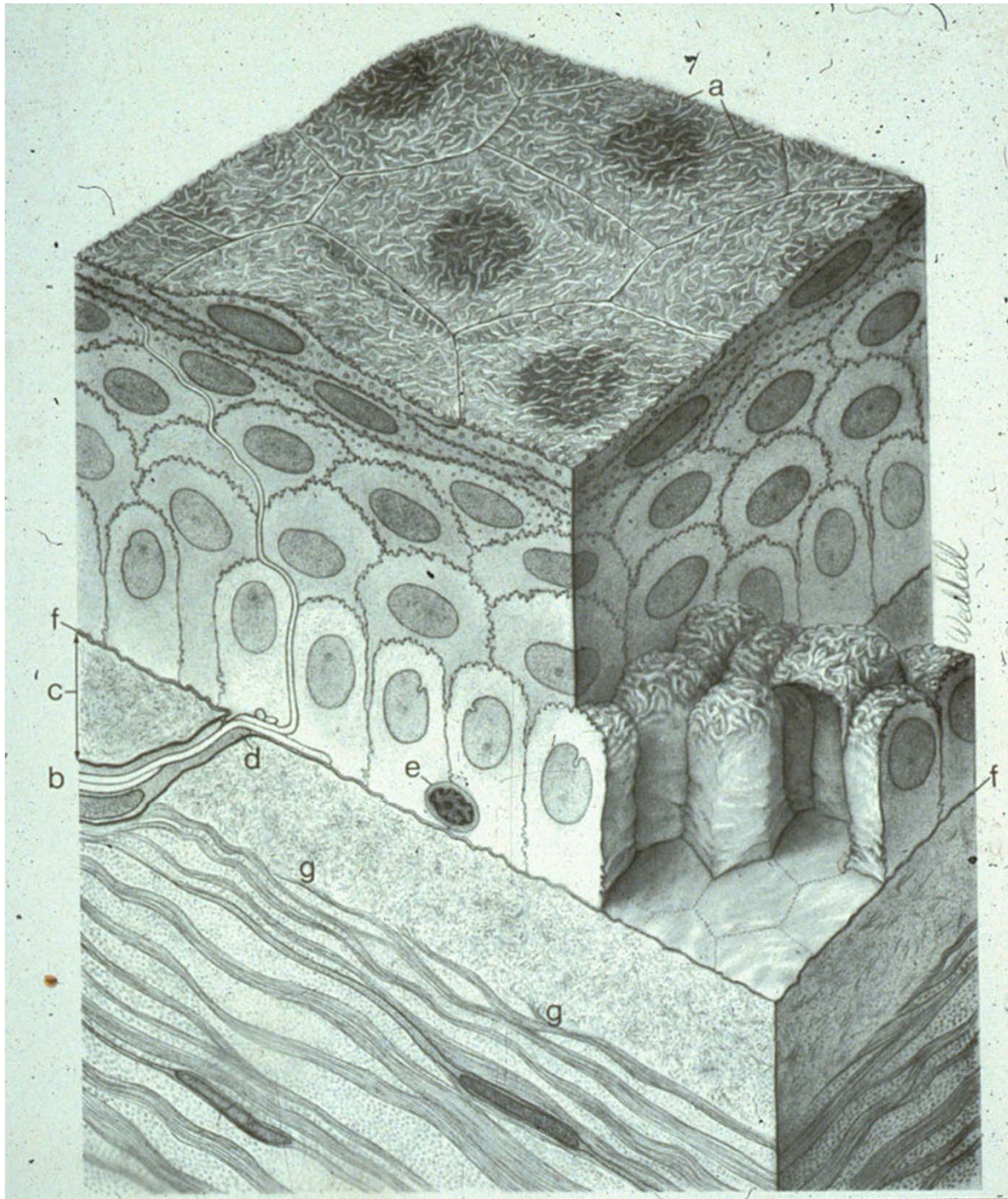
Ted Reid, Ph.D.

2020

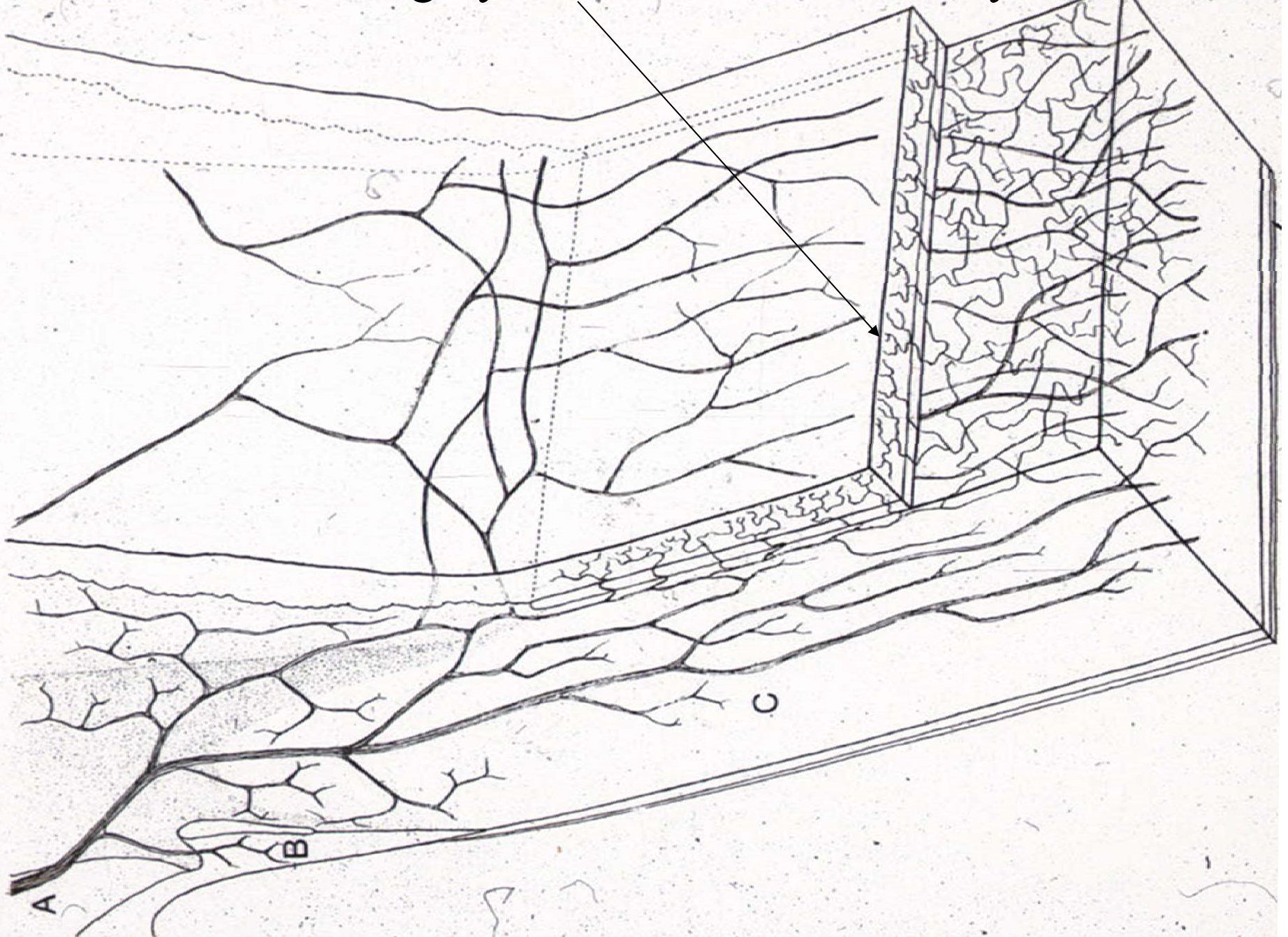
Neurotrophic Ulcer

Magendie (1824) J. Phsiol. 4:176



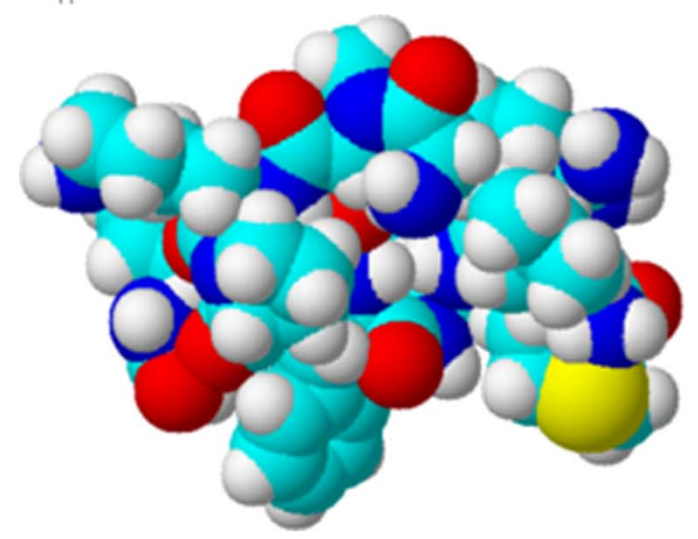
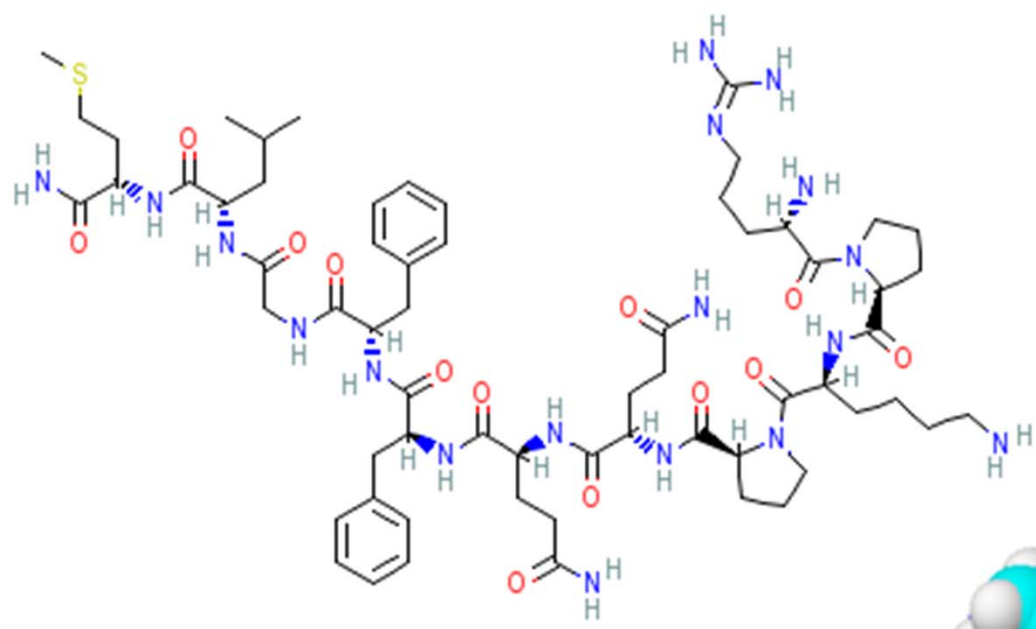


The cornea is highly innervated with sensory nerves



Substance P

Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH₃



SUBSTANCE P

- **Neuropeptide**
- **Tachykinin**
- **Sensory neurotransmitter**
- **Transduction of nociceptive stimuli**

Substance P Effects:

Motor

Constriction of airway, gastrointestinal and selected smooth muscles, enhanced fluid secretion

Sensory

Pain, itch, and cough

Cardiovascular

Increase vascular permeability, vasodilation, ANF regulation

Inflammatory

Release of histamine, leukotrienes and prostaglandins

Substance P Effects (continued):

Immunologic Reactions

Release of IL-1, IL-6 and TNF from mononuclear phagocytes and tissue macrophages

Mitogenic

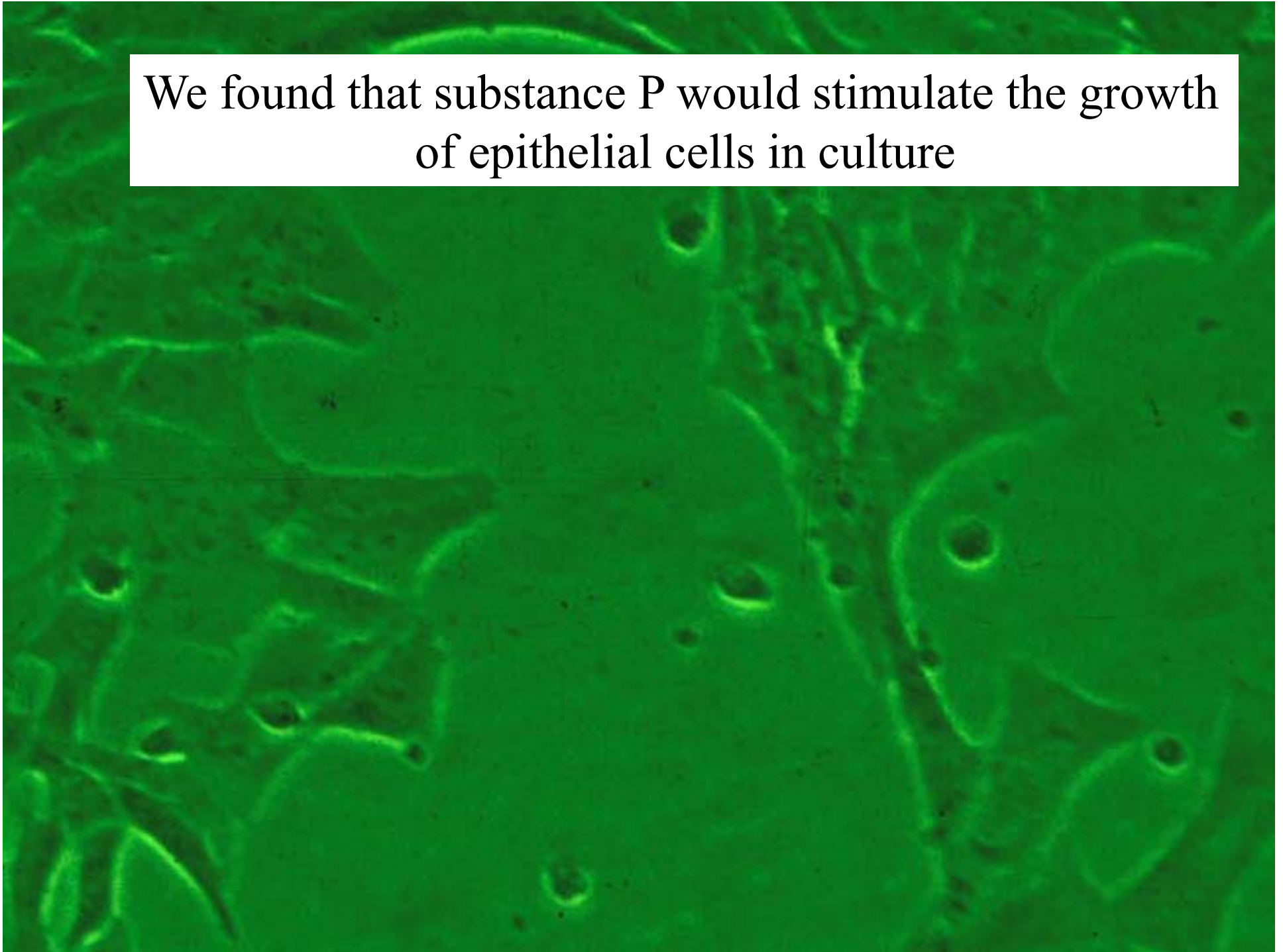
Fibroblast, smooth muscle cells, endothelial cells, lymphocytes

Trophic Factors

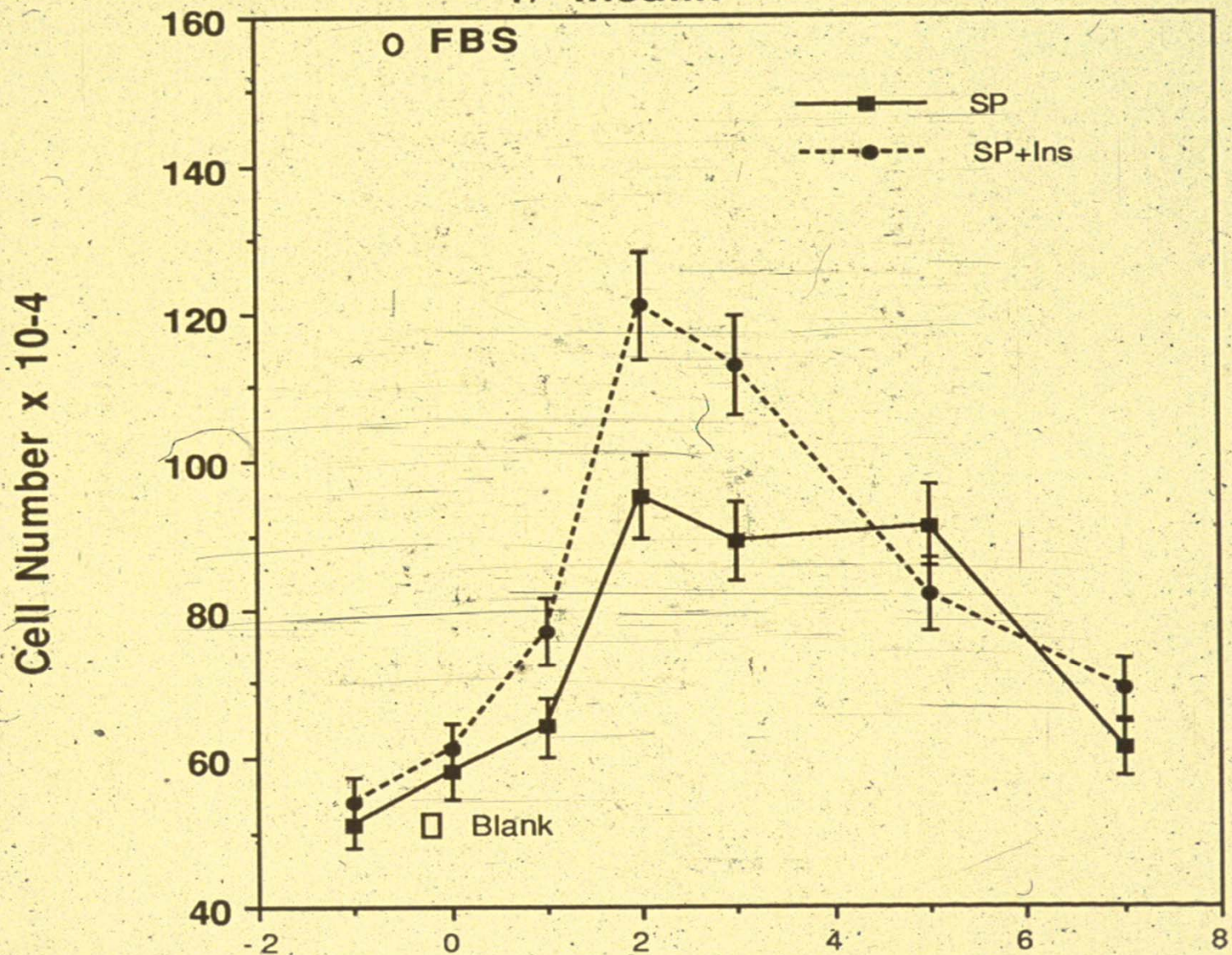
Leukocytes

Substance P as a Growth Factor

We found that substance P would stimulate the growth of epithelial cells in culture

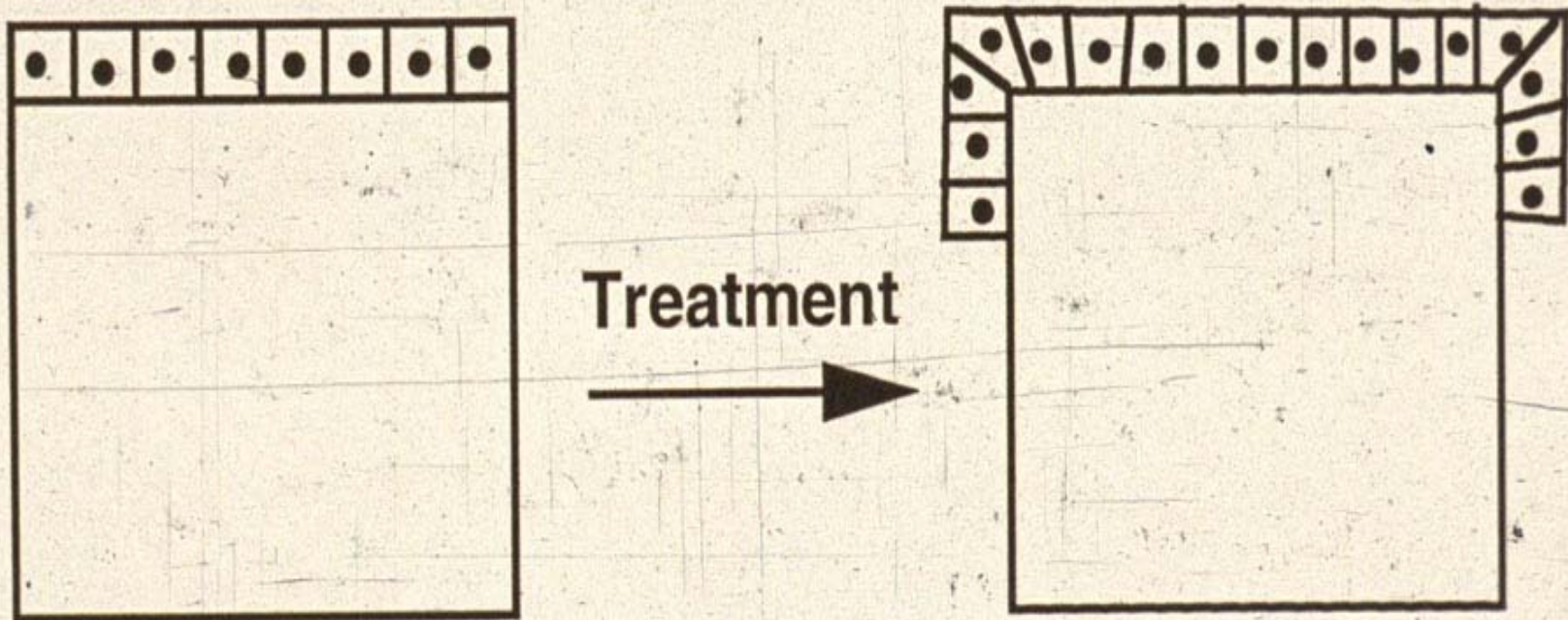


Cell Growth - SP dose response Lens Epithelial Cells +/- Insulin

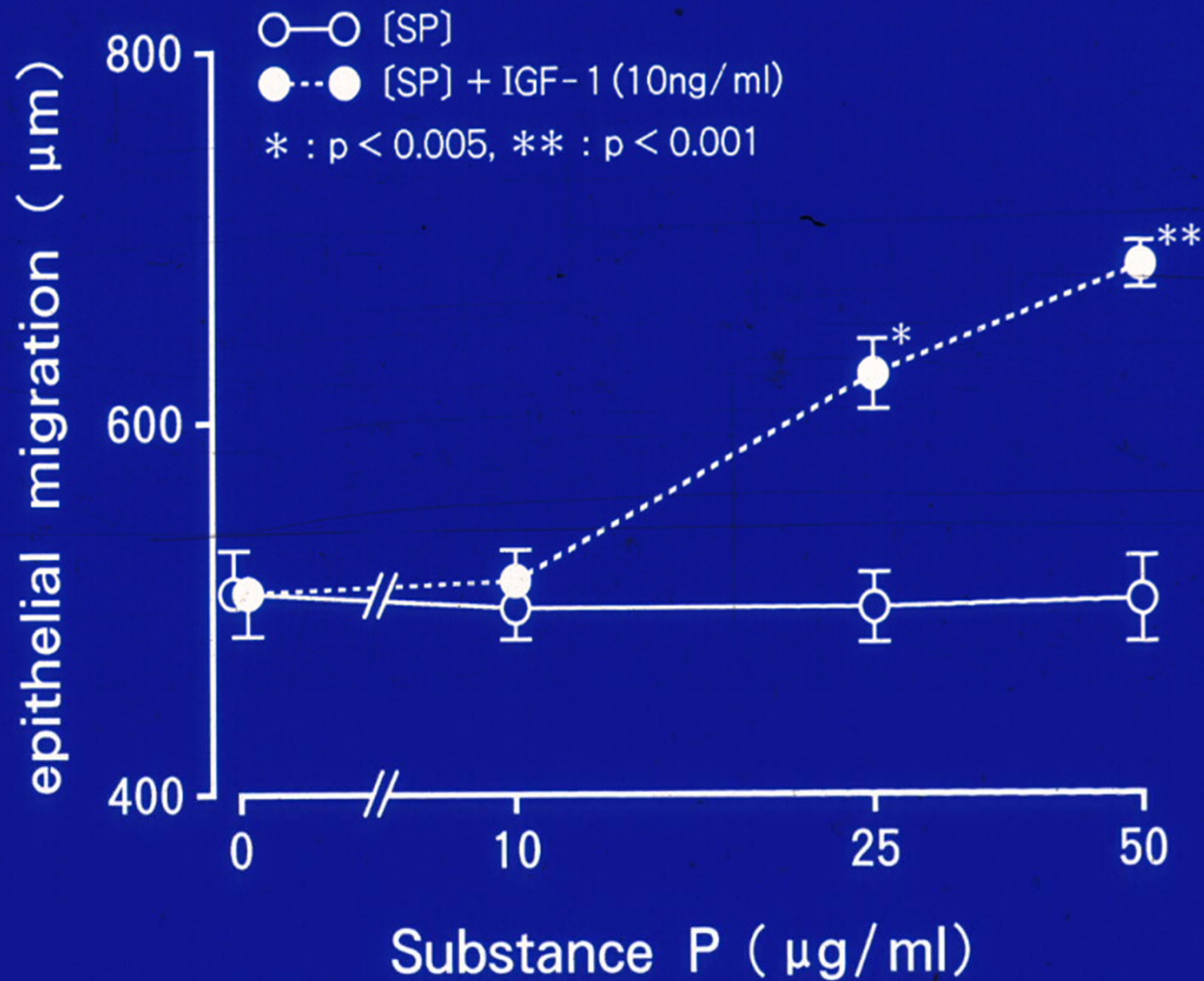


SP Effects on Corneal Epithelial Cell Migration

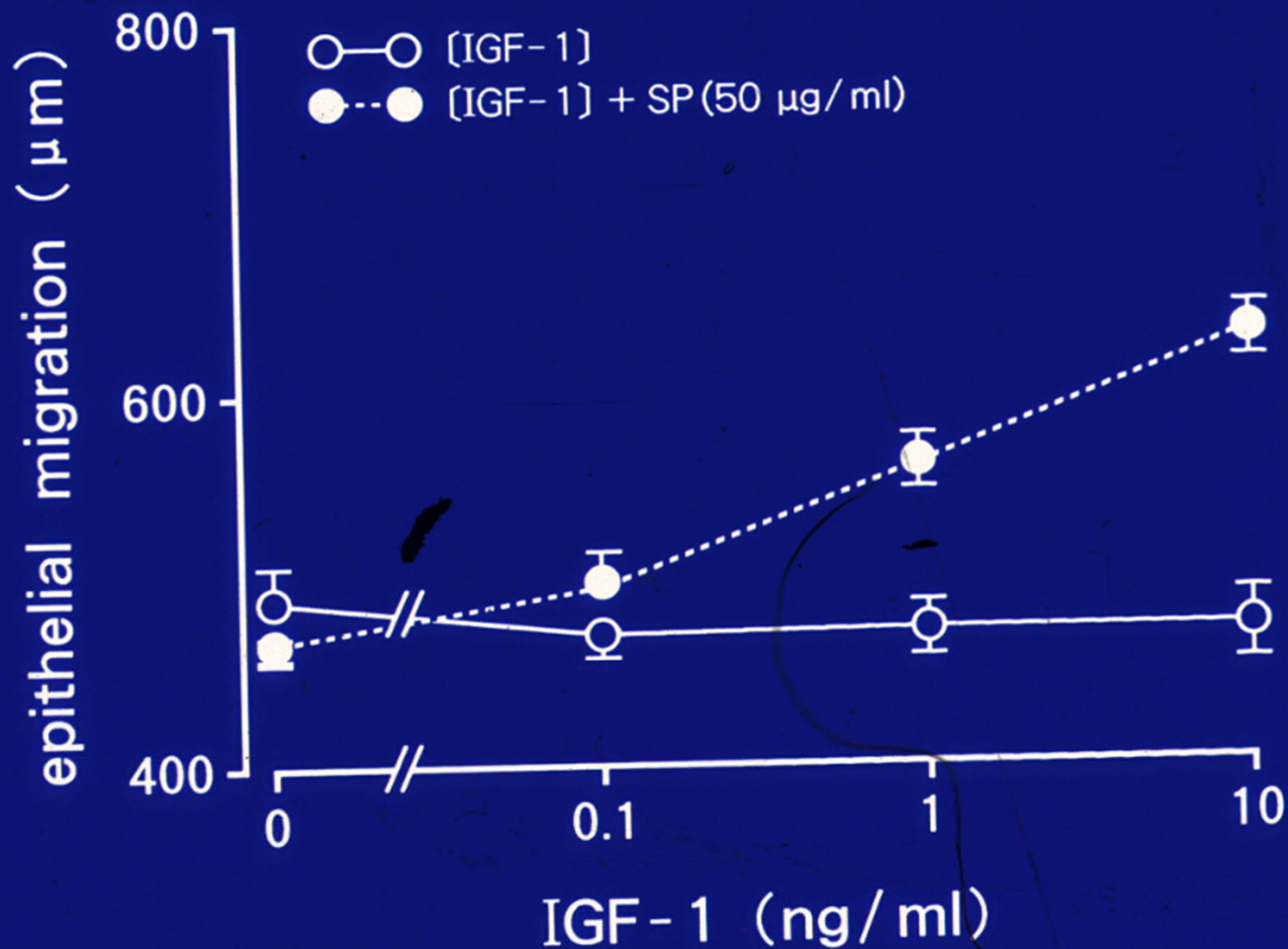
Tissue block taken from a rabbit cornea
and allowed to re-epithelialize



Synergistic Effects of Substance P with IGF-1 on Corneal Epithelial Migration



Synergistic Effect of Substance P with IGF-1 on Corneal Epithelial Migration



Cornea block study

- Only substance P showed enhancement of re-epithelialization of cornea block
- Substance P was synergistic with IGF-1

CAPSAICIN

- **Sensory denervation decreases corneal epithelial mitoses (Mishima 1957)**
- **Sensory denervation impairs corneal epithelial wound healing (Beverman and Schimmelpfennig 1980)**
- **Capsaicin induces corneal lesions in mice and rats (Shimizv et al 1984)**
- **Sensory denervation induces corneal lesions in rabbits (Gilbard and Rossi 1990)**

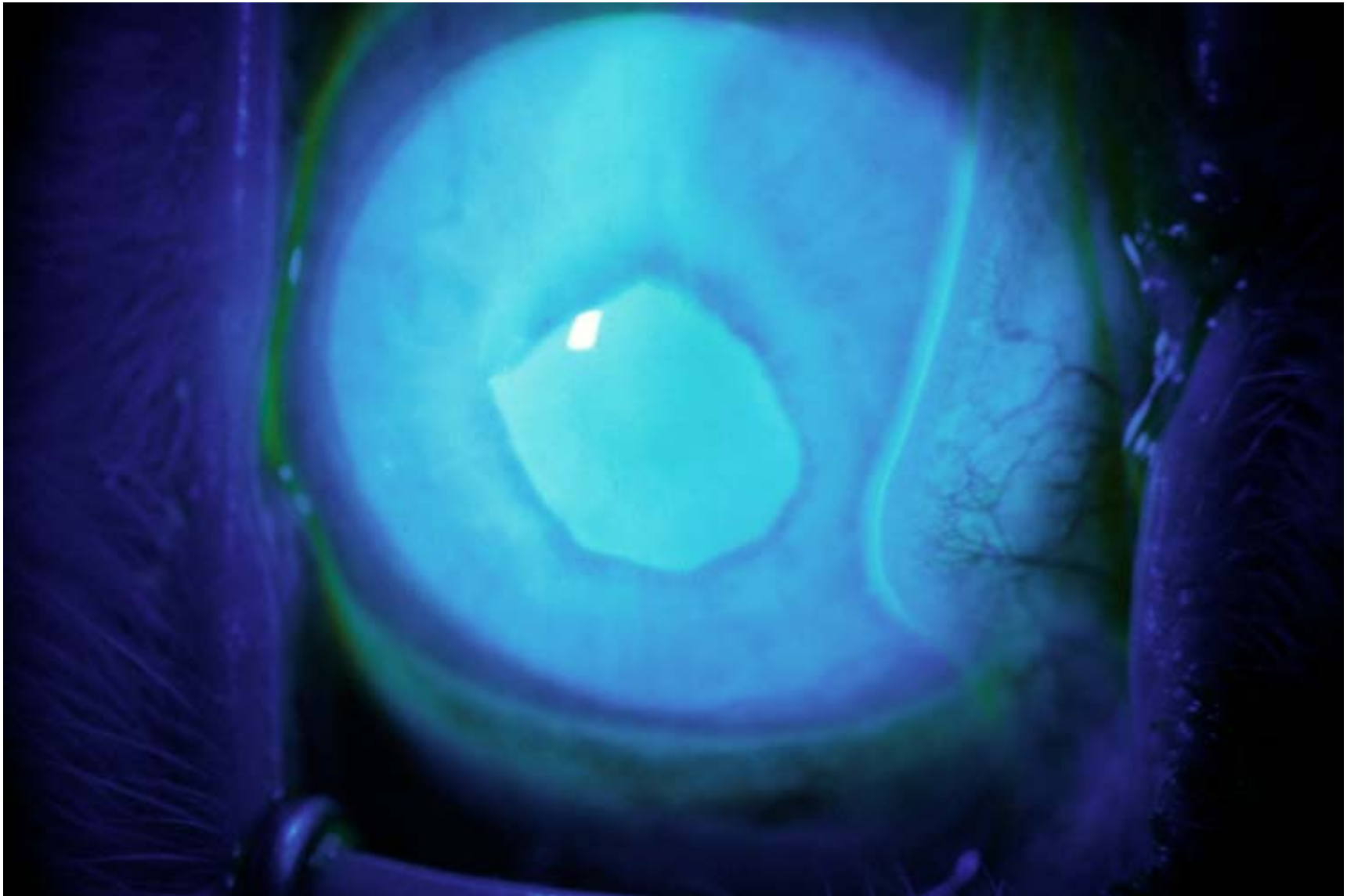
CORNEA

- **Nerve fibers containing SP immunoreactivity**
- **SP reduced in the cornea by capsaicin**

Corneal wound healing experiment

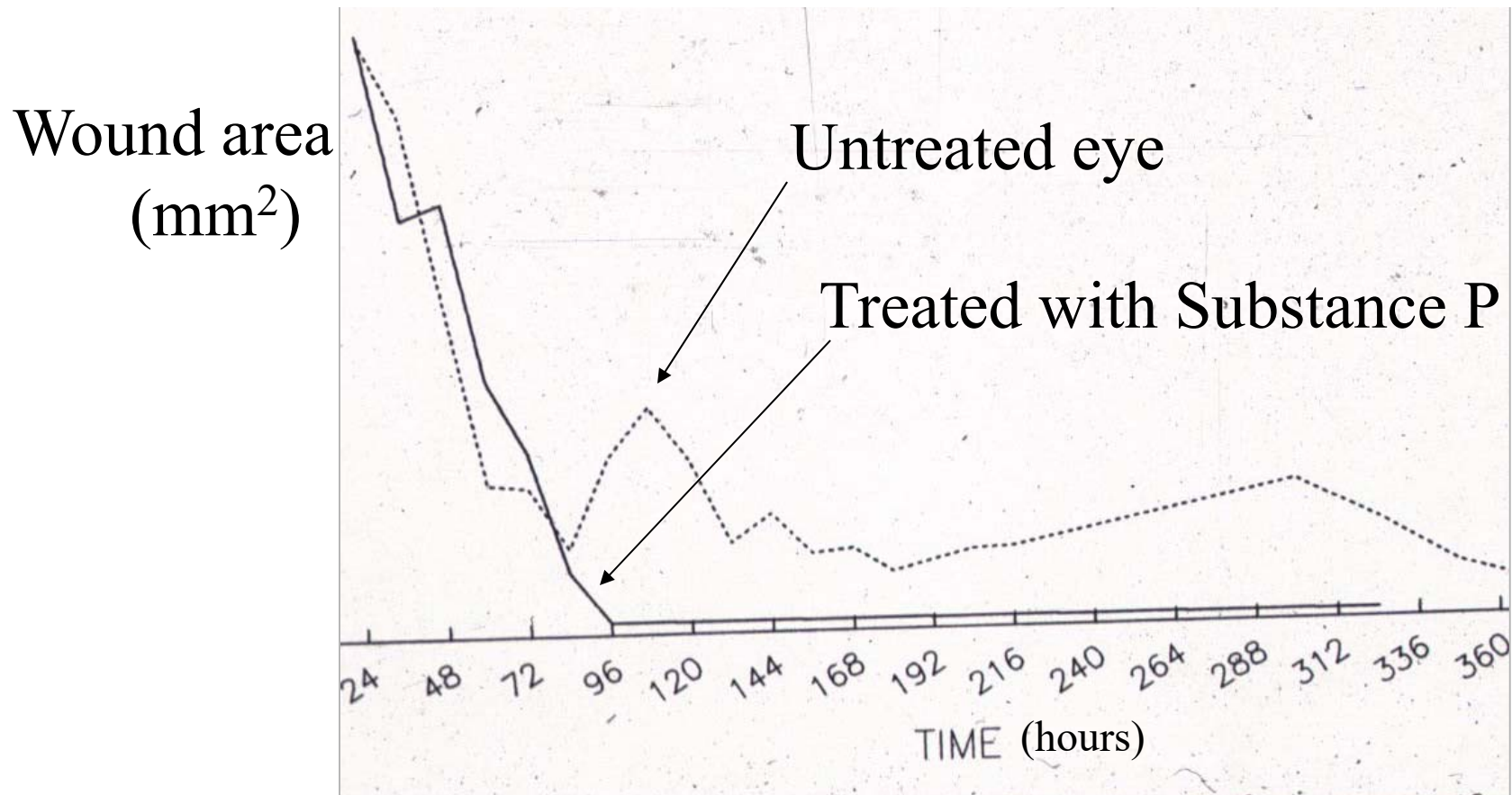
- Rabbits treated with capsaicin to deplete neuropeptides in sensory nerves
- Rabbits had corneal epithelium removed three weeks later
- One eye treated with substance P the other eye not treated
- Healing documented photographically

Capsaicin Treated Rabbit with Corneal Epithelial Defect



Experimental Data

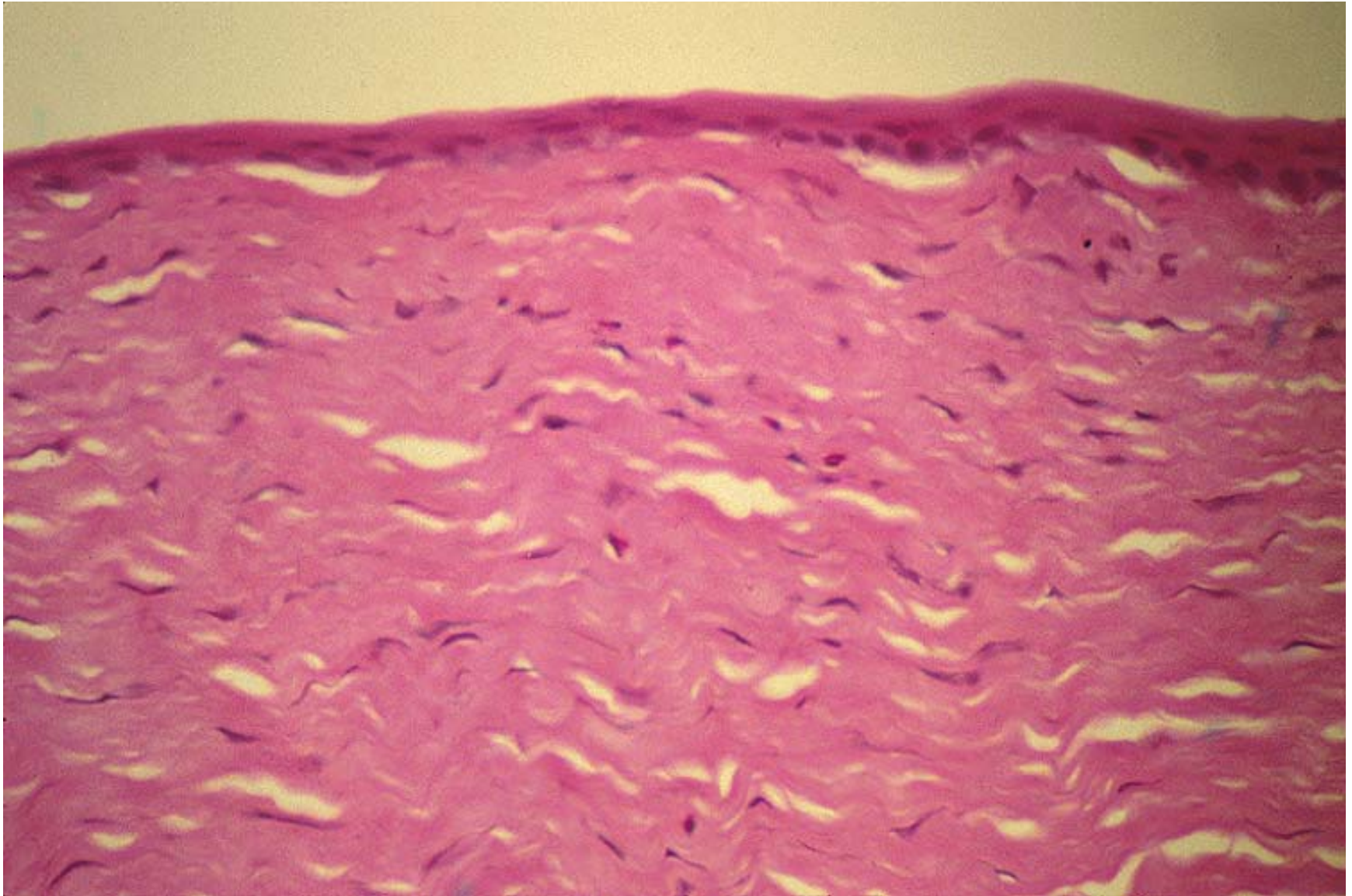
Rabbit Corneal wound size vs time in hours



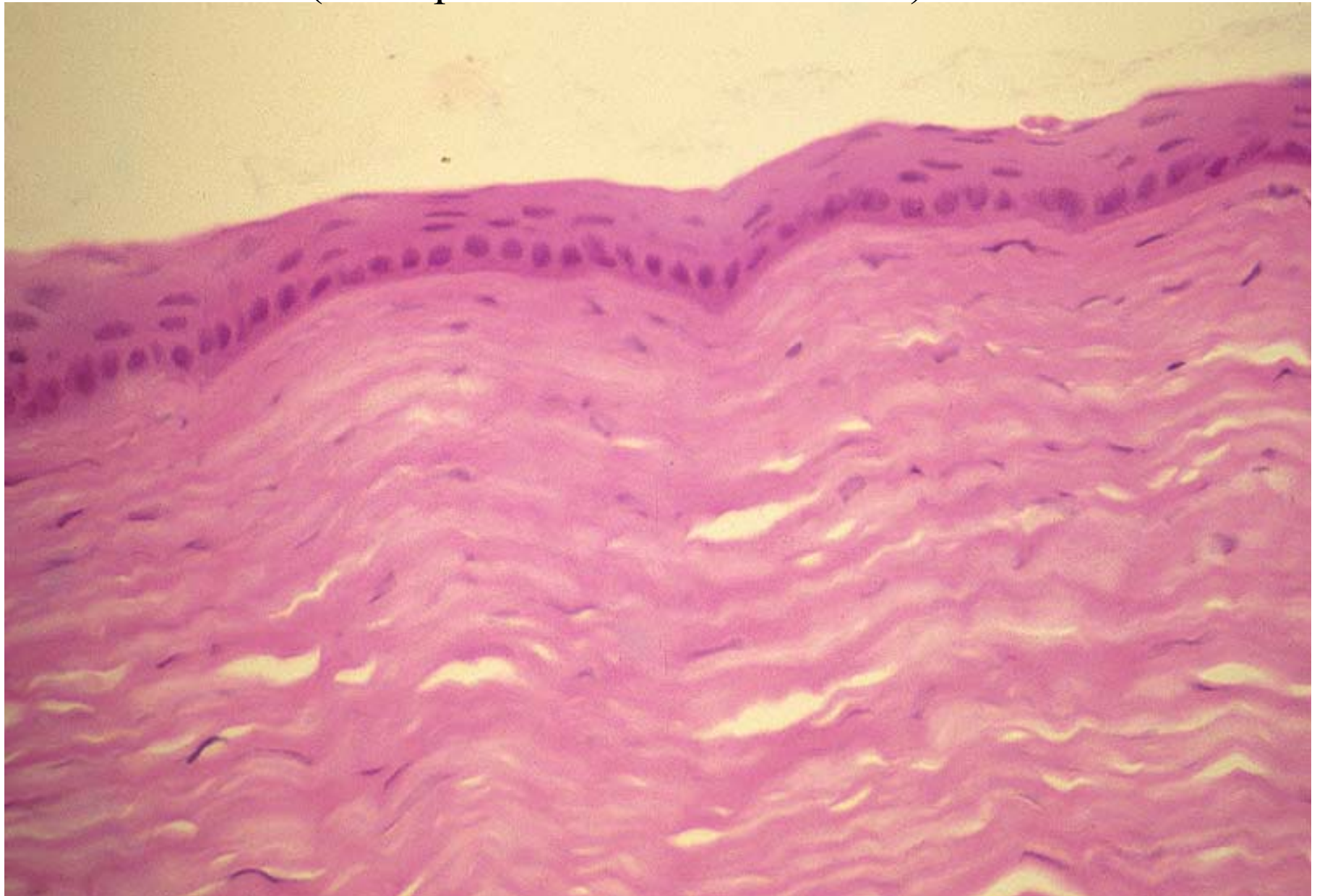
Rabbit Wounding Experiment Data

- The substance P treated eye healed in 96 hours
- The untreated eye appeared to heal at the same rate as the treated eye, but the wound could not close properly
- The epithelium of the untreated eye appeared to be loosely attached

Cornea of capsaicin rabbit after healing - not treated with Substance P
(note epithelium is not well attached)



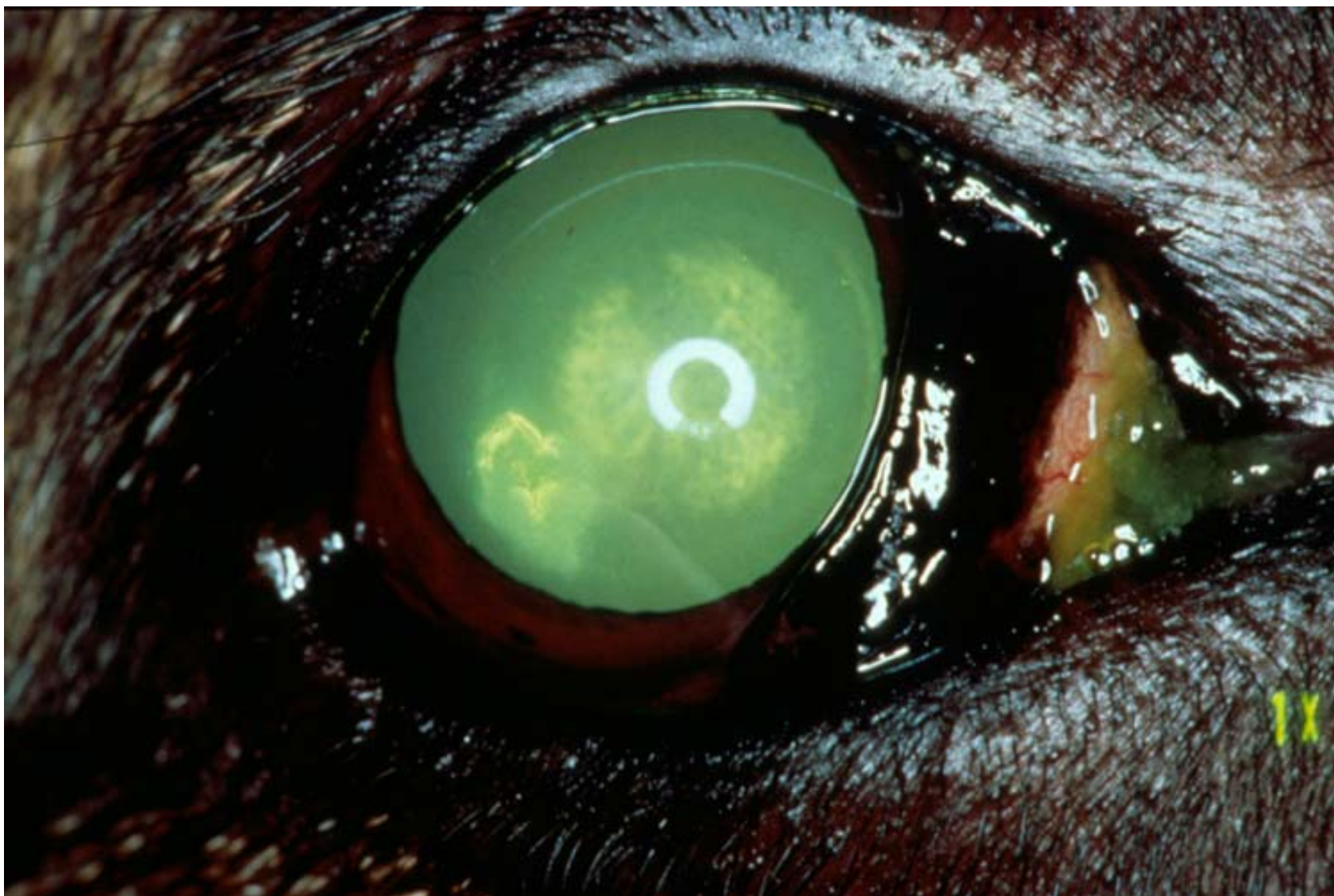
Cornea of capsaicin rabbit after healing - treated with Substance P
(note epithelium is well attached)



Neurotrophic Ulcers in Dogs

- A clinical trial using normal dogs that presented to a ophthalmic veterinarian with neurotrophic ulcers, was carried out.
- All dogs in the trial had the ulcers for a minimum of one month
- 15/16 dogs with neurotrophic ulcers healed in one week after receiving substance P eye drops twice daily (the one dog never healed)

Dog eye with corneal ulcer in the lower left corner
(haze in the background is a cataract)



Dog cornea after one week treatment
with substance P - no ulcer





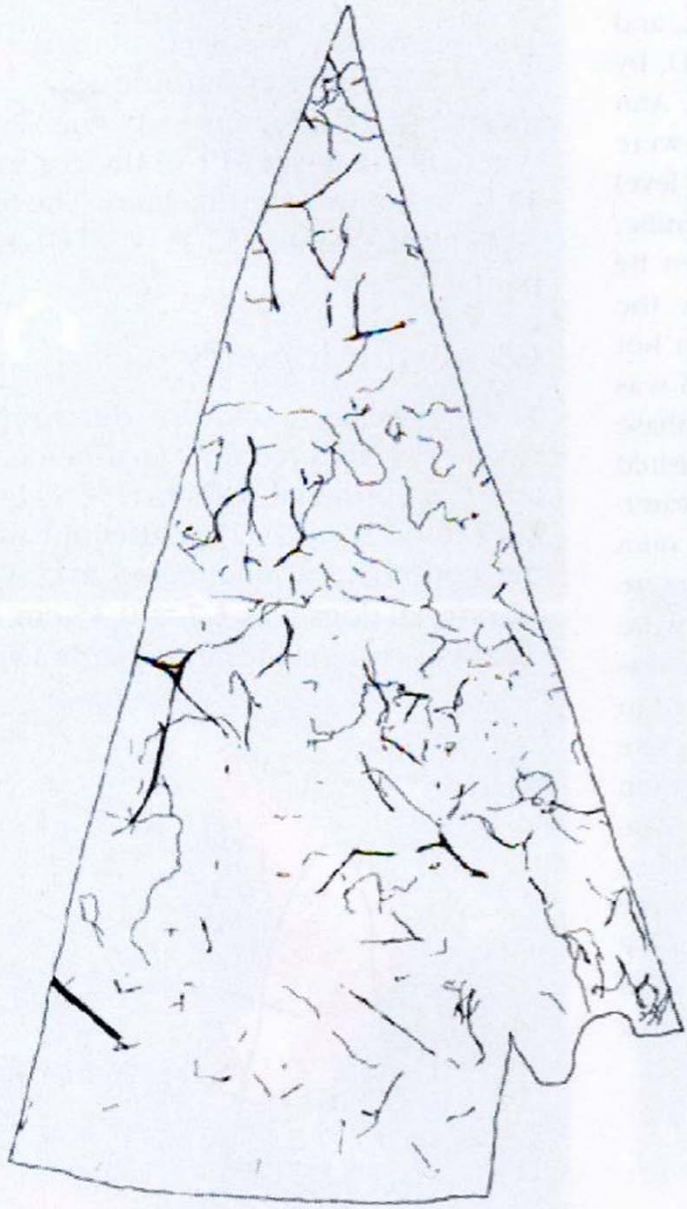


CONTROL

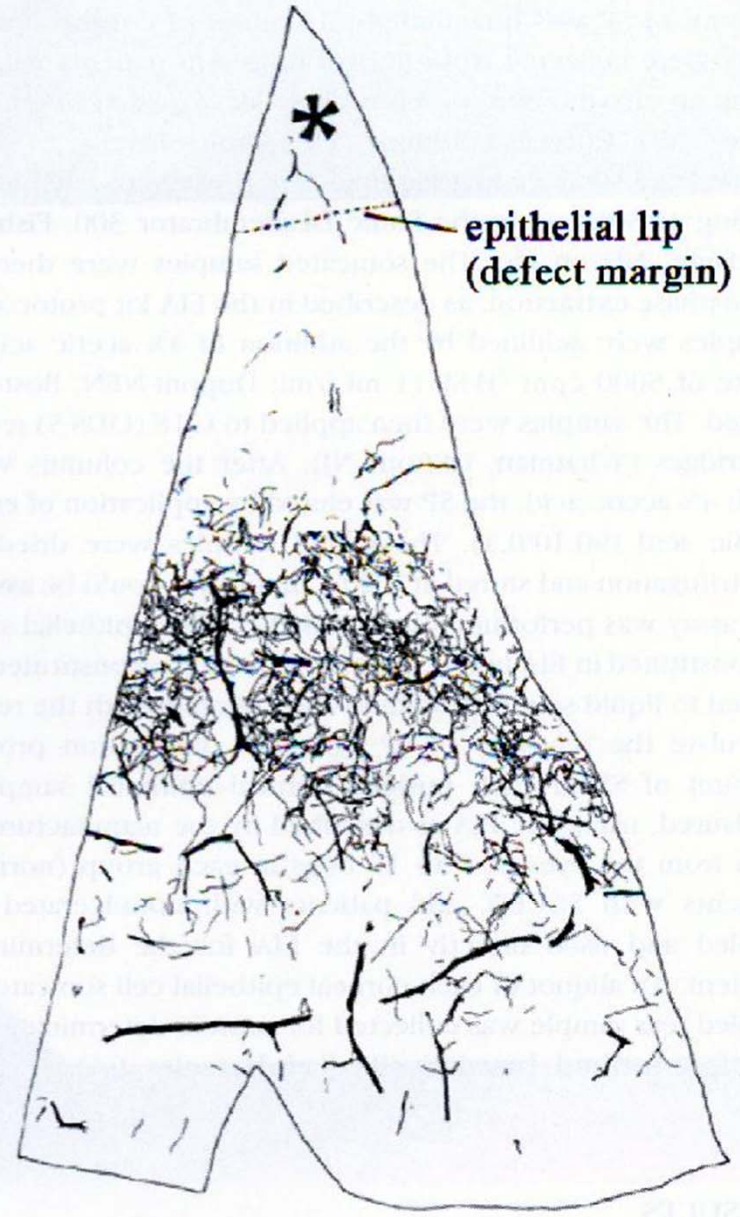
CHRONIC EPITHELIAL DEFECT

SP

a.



b.



Two year old girl with corneal ulcers

1. Patient born with no corneal sensory nerves
2. Patient had a severe corneal ulcer in right eye - about to perforate -skin flap was pulled to cover it
3. Patient later developed a corneal ulcer in left eye. Ulcer was about to perforate

Two year old patient with skin flap
over ulcer in right eye



Same patient with large corneal ulcer in left eye



Protocol

- Patient was treated with substance P plus IGF-1 in an eye drop twice daily (one drop every 15 minutes for two hours in the morning and at night - the drop contained normal saline)
- Substance P added over two hours since it has to be present in the wound for that time period
- The patient showed complete healing of the ulcer in two weeks.

Left eye after two weeks treatment with substance P
No ulcer remaining



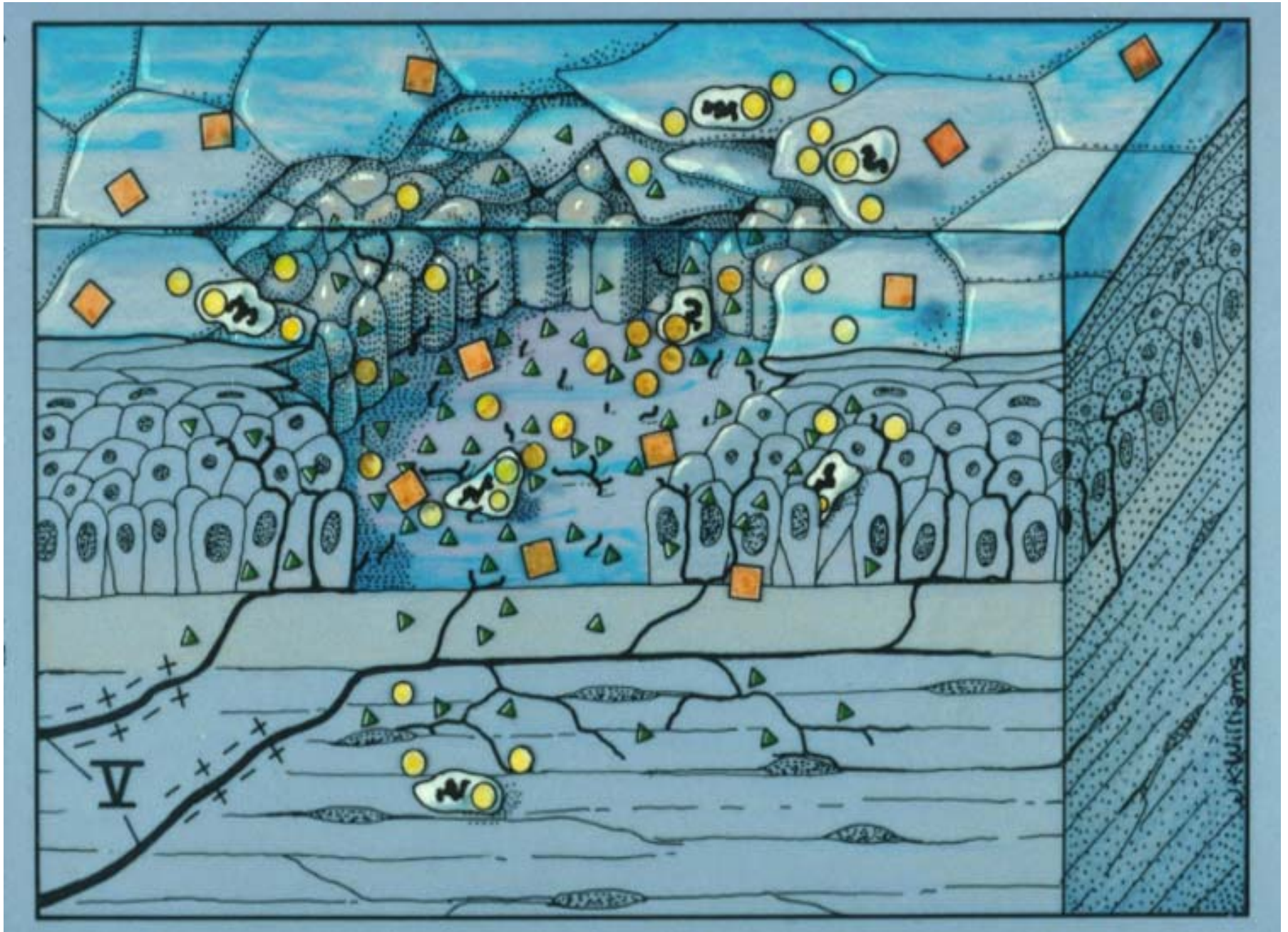
Cornea transplant

- After the corneal ulcer had healed the patient was given a corneal transplant in both eye.
- The transplant was treated with substance P plus IGF-1 to re-epithelialize the cornea.
- Both eyes re-epithelialized normally

Right eye, six months after cornea transplant



Role of nerves in wound healing



Overall Picture

1. Blood borne
 - a. Free factors
 - b. Blood cells
2. Nerve mediation
3. Matrix

Substance P and Wound Healing

- Nerves play a role in wound healing
- If the nerves are compromised you have defective wound healing
 - Diabetics
 - Burns
 - Bed sores

**The Effect of Topical Substance-P
and Insulin-like Growth Factor (IGF-1)
in Epithelial Healing after
Photorefractive Keratectomy (PRK) in
Rabbits**

Corneal Photorefractive Surgery - PRK

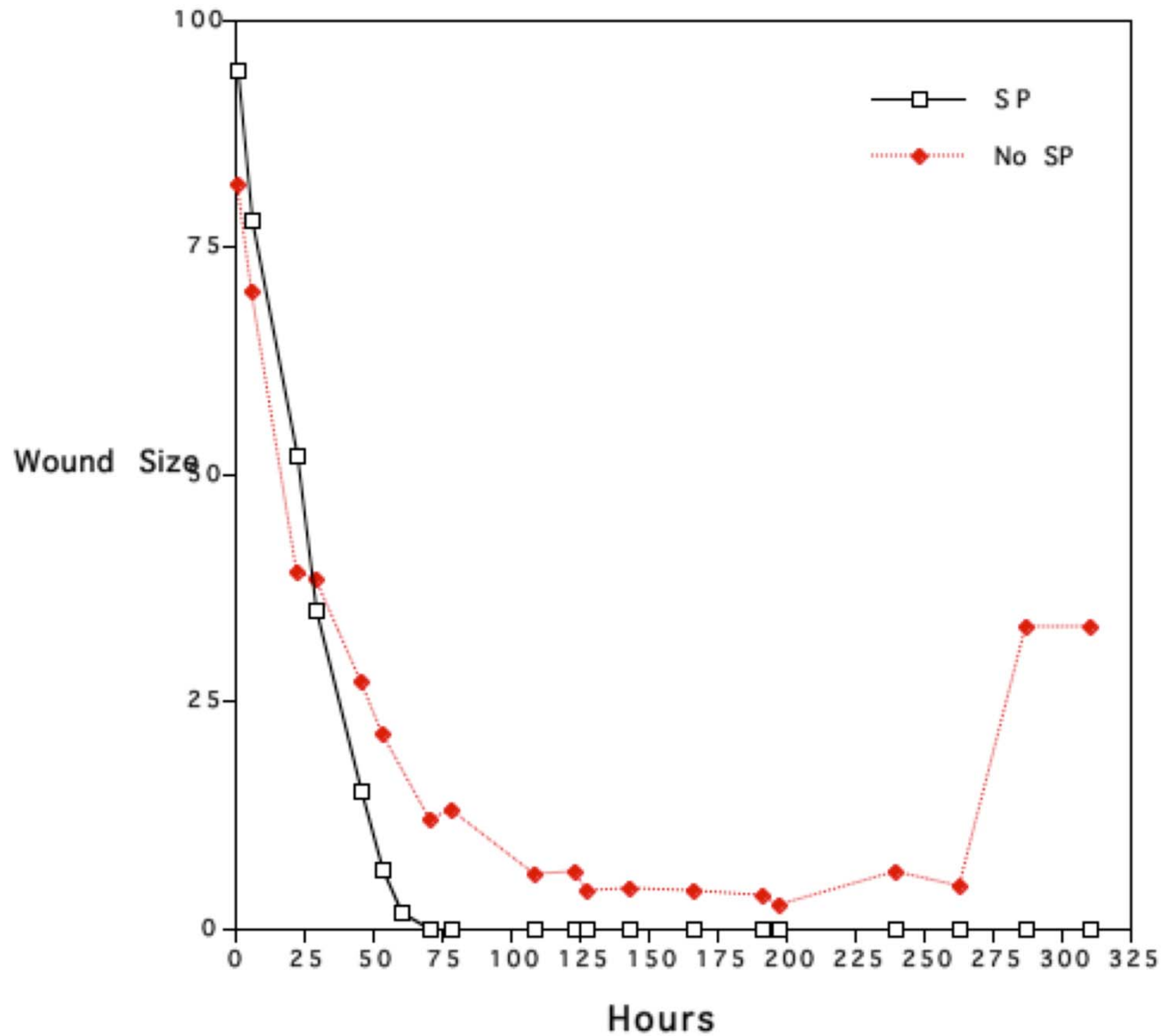
- The preferred method of surgery since it can repair astigmatism
- However it is only used in severe myopia cases due to the longer healing time which causes a transitory haze and longer pain
- Several million cornea refractive surgery cases in the U.S. each year

Experimental Protocol

- Rabbit corneas were de-epithelialized (9.0 mm) using a combined chemical (18% alcohol) and mechanical technique.
- Excimer photo ablation was performed bilaterally in 6 rabbits (12 eyes) with an 8.0 mm ablation zone and 70 μ m depth using ladder vision laser.
- The right eye was chosen for treatment with Substance-P (250 μ g/ml) and IGF-1 (25ng/ml) in haluronic acid, one drop twice a day, and the other eye served as the control.
- Epithelial healing process evaluated and documented twice a day until healing completion.

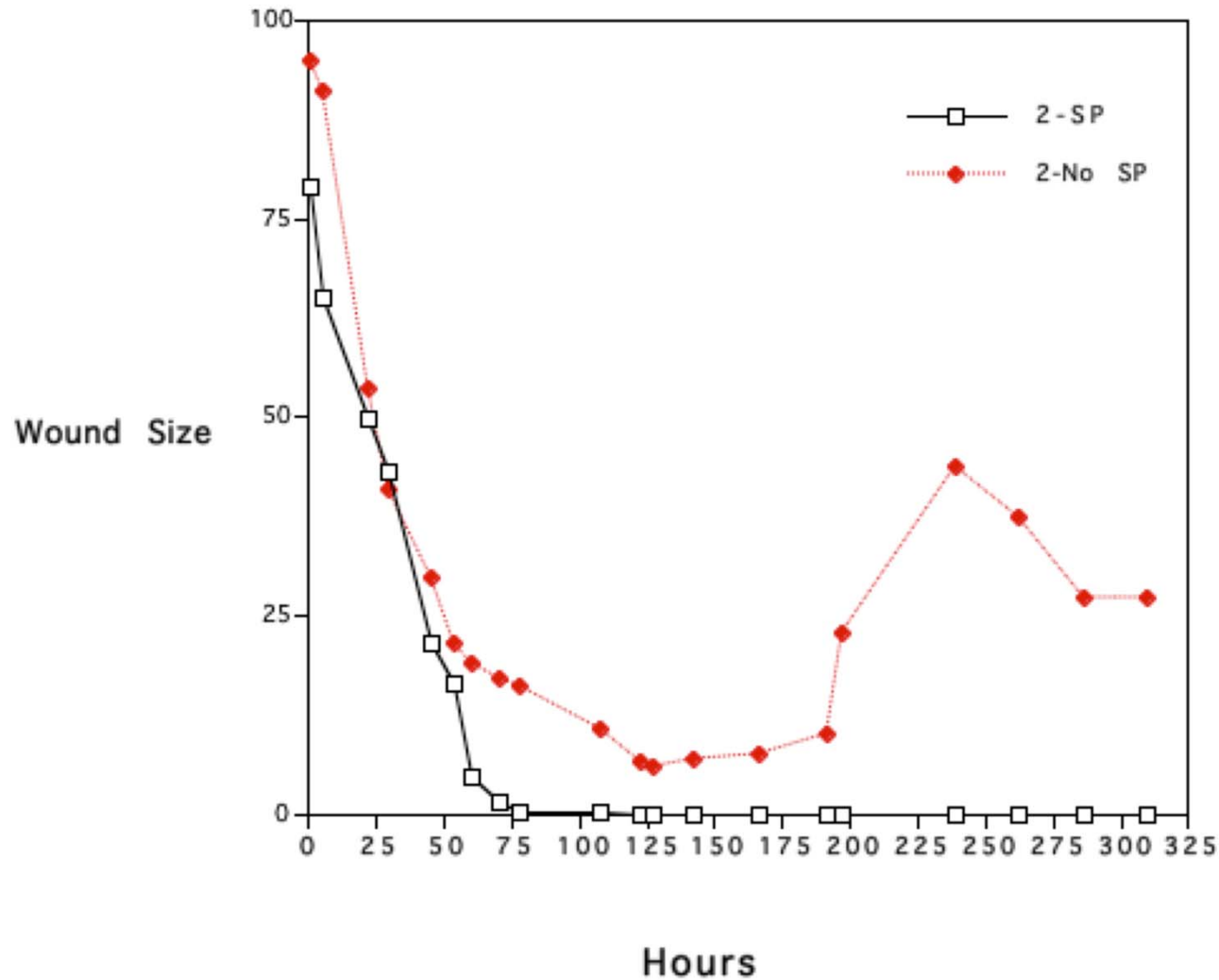
Treatment of Rabbit eyes after PRK with and without substance P and IGF-1 in Haluronic acid

Rabbit #1

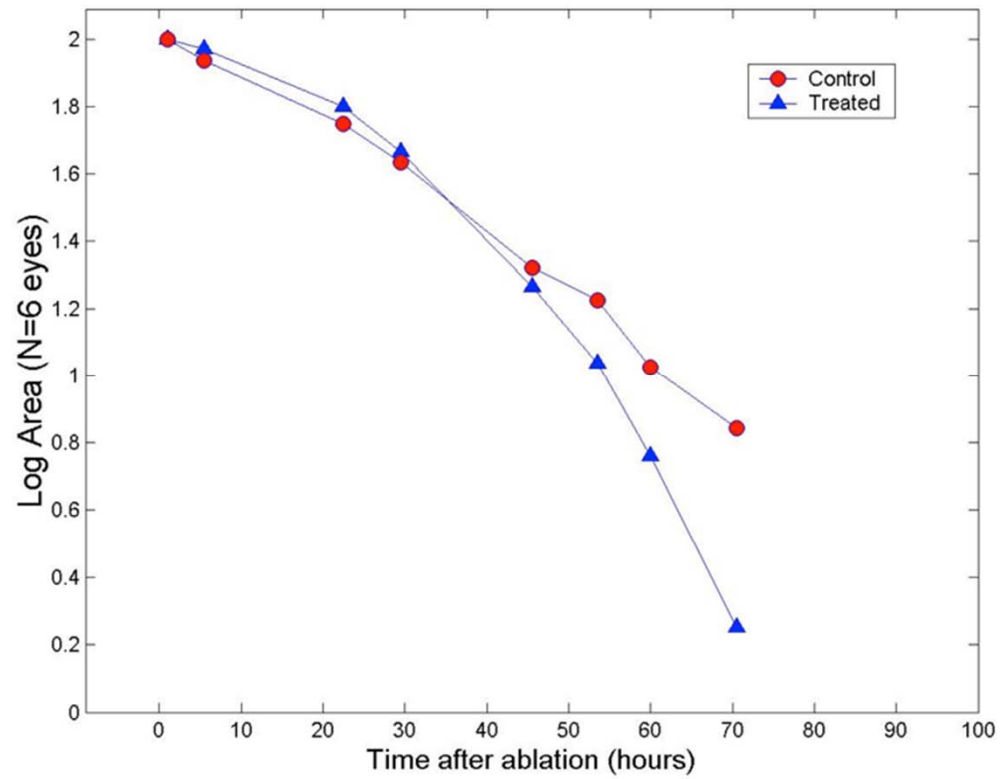


Treatment of Rabbit eyes after PRK with and without substance P and IGF-1 in Haluronic acid

Rabbit #2



Log unhealed corneal area (median of 6 eyes)



Substance P treatment after PRK

- Substance P shortened the healing time with an average healing time of 85.5 hrs.
- The average healing time for a non-treated eye was 126.4 hrs
- Two of the non-treated eyes developed persistent epithelial defects and were not used in calculating the average non-treated healing time

Diabetic Ulcers

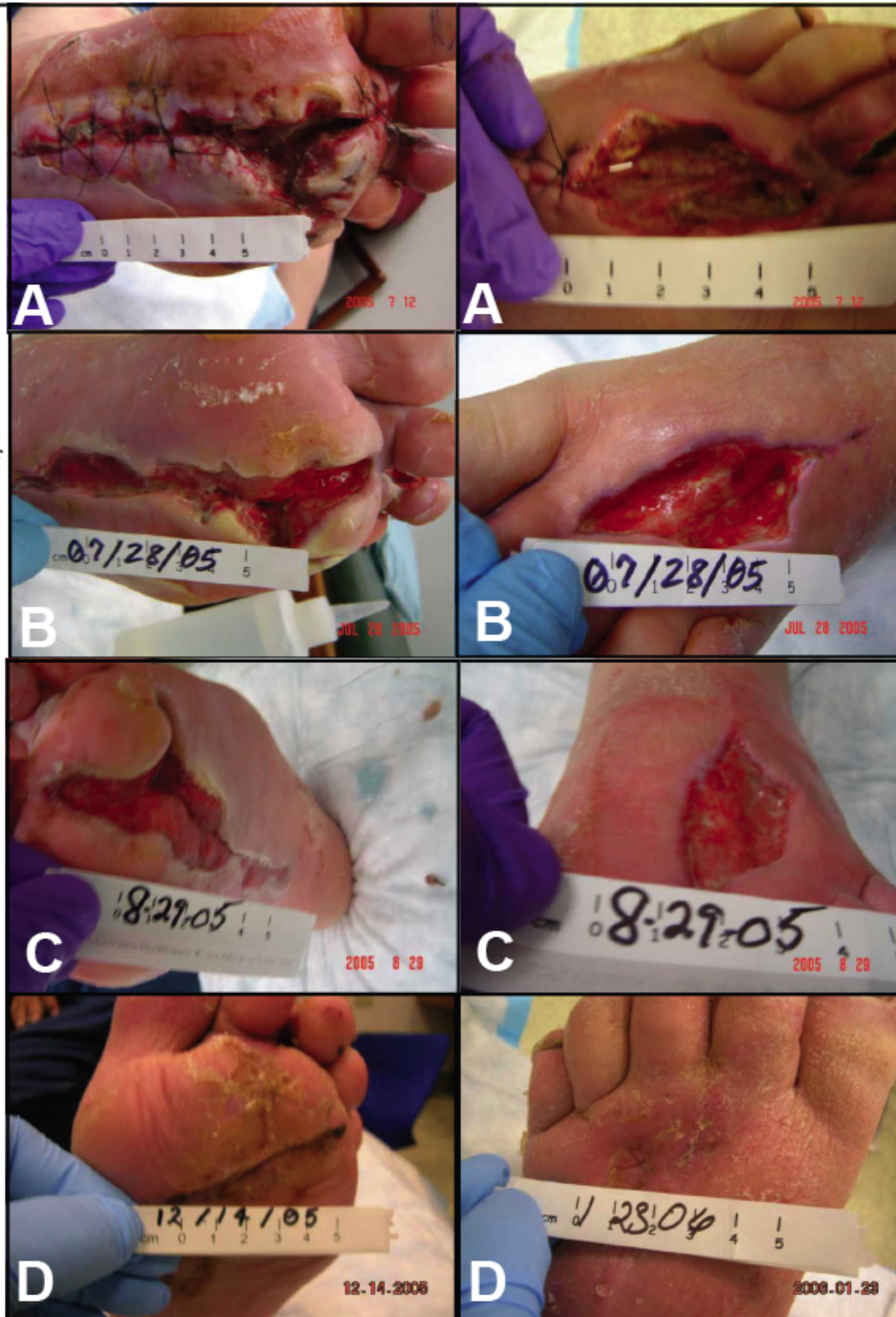
Randy Wolcott

CASE HISTORY:

NAME: C.G.

ACCT#: 11279

The patient is a very pleasant 50-year-old white male with diabetes mellitus with severe peripheral neuropathy. Decreased perfusion of the foot and difficult to manage diabetes. The patient sustained severe trauma to his foot and underwent operative repair. The surgery site has dehisced. The patient had quite a bit of local infection with swelling of the foot. The patient was started on aggressive wound care management including weekly debridement, IV antibiotics and use of Substance P. The patient has shown better than expected healing. In fact, he had a group of physicians demand that he pursue major amputation of the limb. He declined. The patient has reasonable medical probability that he will salvage his limb.



Healing time - diabetic ulcers

