The effects of aging and inflammation on corneal endothelial wound healing in rabbits.

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After transcorneal freezing, the rates and patterns of corneal endothelial wound healing were compared in mature and young rabbits by autoradiographic analysis of ^{3}H-thymidine incorporation and scanning electron microscopy. Healing was slower and less extensive in mature corneas than in young ones. Regardless of animal age, however, healing occurred by cell division and the migration of newly divided cells onto the wound surface. Spontaneously occurring severe inflammation appeared to reduce the ability of corneal endothelial cells to replicate their DNA.

Previous studies of corneal endothelial wound healing have used materials of relatively fixed ages related to the species studied: rabbits were young,[1] cats were mature,[1, 2] and human material was from mature and often elderly patients[3] or donors.[4] The purpose of this study was to examine the effects of aging on the rates and patterns of corneal endothelial regeneration after transcorneal freezing in rabbits. Our data indicate that in mature rabbits the rate of corneal endothelial wound healing is slower and less extensive than in young rabbits but that regardless of animal age, healing is accomplished by the migration of newly divided cells onto the wound area. Spontaneously occurring inflammation also reduces the rate of endothelial regeneration.

Methods. For all experiments, the central 30% of both corneas of 1-year-old and 6- to 8-week-old rabbits were injured by transcorneal freezing.[2] In the first of two autoradiographic experiments, six young and four mature animals were injected intracamerally at 19 hr and again at 22 hr after injury with 2 μCi of ^{3}H-thymidine (27 Ci/mmol; New England Nuclear). At 24 hr after freezing, the animals were sacrificed to provide a 5 hr pulse of label early in the healing process. In the second experiment, 10 young and three mature rabbits were given single intracameral injections of 5 μCi of ^{3}H-thymidine 24 hr after injury and then allowed to regenerate their corneal endothelia for 12 days before sacrifice. All animals were sacrificed by an overdose of Na-pentobarbital, and the eyes were immediately removed and fixed in 10% neutral formalin. Autoradiography was performed on flat mounts of corneal endothelium with an exposure time of 3 to 4 weeks. For scanning electron microscopy (SEM) studies, three mature and four young animals were sacrificed on the fifth day after transcorneal freezing, and six mature and five young animals were sacrificed on the ninth or tenth day. The corneas were excised, fixed, and prepared as described previously[5] and then examined in an AMR 1000 scanning electron microscope.

Table I. Effects of age on endothelial cell density 12 days after transcorneal freezing

	*Cells/mm²**		
	Center of corneal wound	*Peripheral cornea*	*% regeneration*
Young	3075 ± 337	3220 ± 352	95.5
Mature	1160 ± 294	2350 ± 113	49.4

All cells were counted in each of 10 fields of 183 μm diameter with a Zeiss microscope and a 63× objective and Optovar setting of 1.6× (26,302 mm²).

*Means ± S.D. of counts from two animals of each age group.

Results. Autoradiographic analysis of flat mounts of Descemet's membrane and corneal endothelium showed that between 19 and 24 hr after transcorneal freezing, a band of cells encircling the wound edge incorporated ^{3}H-thymidine into their nuclear material. The width of this band of thymidine incorporation was as much as three times wider in young rabbits than in mature ones (Fig. 1, *A* and *B*). Labeled nuclei in both age groups appeared to be oval, whereas nuclei well outside the area of injury had a more stellate shape.

When ^{3}H-thymidine was injected 24 hr after injury and the wounds were allowed to heal for 12 days, all the endothelial cells in the healed wound area, regardless of animal age, contained some radioactive material in their nuclei (Fig. 1, *C* and *D*). The variation in grain density over different nuclei implies that not all cells divided an equal amount of times after being labeled. However, the presence of silver grains over all nuclei indicates that each cell underwent a limited number of divisions after labeling, since more than a few divisions would dilute the radioactivity to below detectable levels. In young animals, the cell density in the center of the wounded area 12 days after transcorneal freezing was almost equal (95%) to that of the normal cells in the periphery (Table I). In contrast, the cell density in the center of the wound was 50% of that in the periphery in the mature animals, indicating that cell division was less extensive in older animals. Note that in young animals the density of endothelial cells in the peripheral cornea was 3200 cells/mm² whereas in mature animals it was 2300 cells/mm².

SEM of the central portion of the wound in young animals revealed that normal cell shape was attained within 10 days (Fig. 2, *B*). In contrast, in old animals, many cells appeared to be larger than

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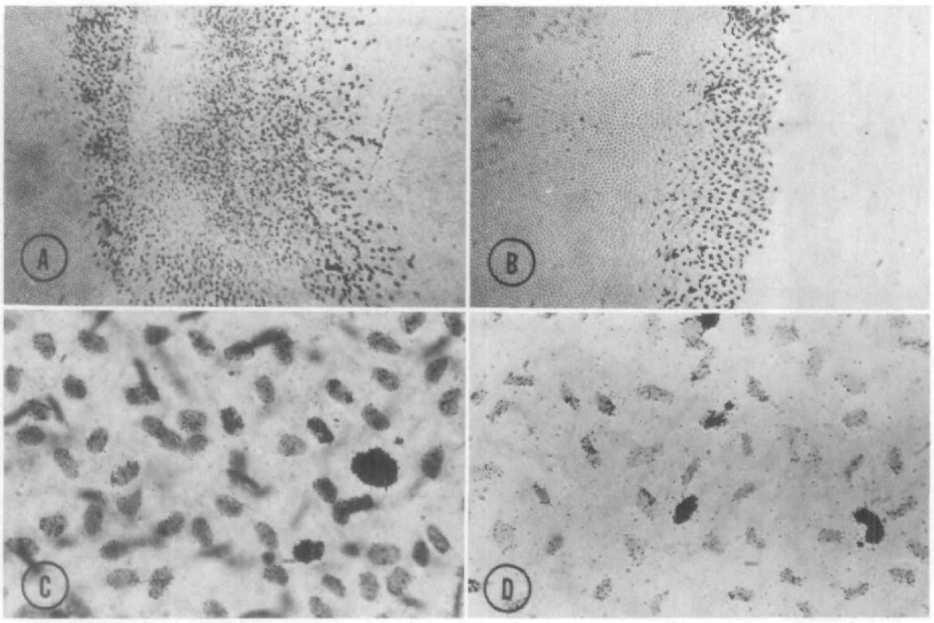

Fig. 1. Autoradiographic analysis of ^{3}H-thymidine incorporation by the corneal endothelia of young (**A** and **C**) and mature (**B** and **D**) rabbits after transcorneal freezing. Twenty-four hours after freezing, the band of labeled nuclei surrounding the wound was up to three times wider in young animals (**A**) than in mature ones (**B**). Twelve days after freezing, all endothelial nuclei in the center of the former wound areas were labeled, regardless of animal age (**C** and **D**). (**A** and **B** ×140; **C** and **D** ×756.)

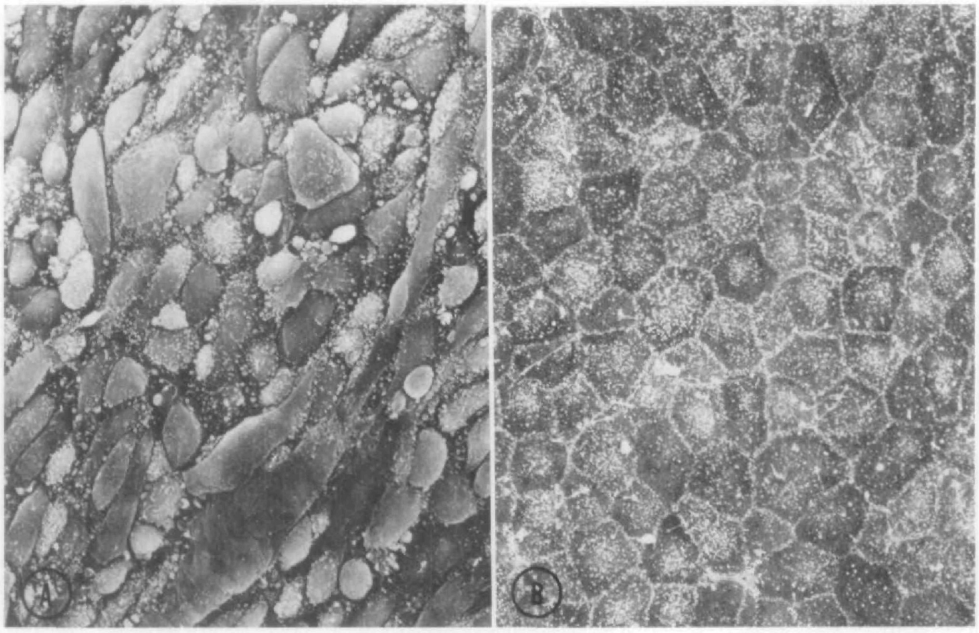

Fig. 2. Scanning electron micrographs of central regenerated rabbit corneal endothelia from young rabbits 5 days (**A**) and 10 days (**B**) after transcorneal freezing. The wounds were covered with cells by 5 days, and normal morphology was attained by 10 days after freezing. (Both ×500.)

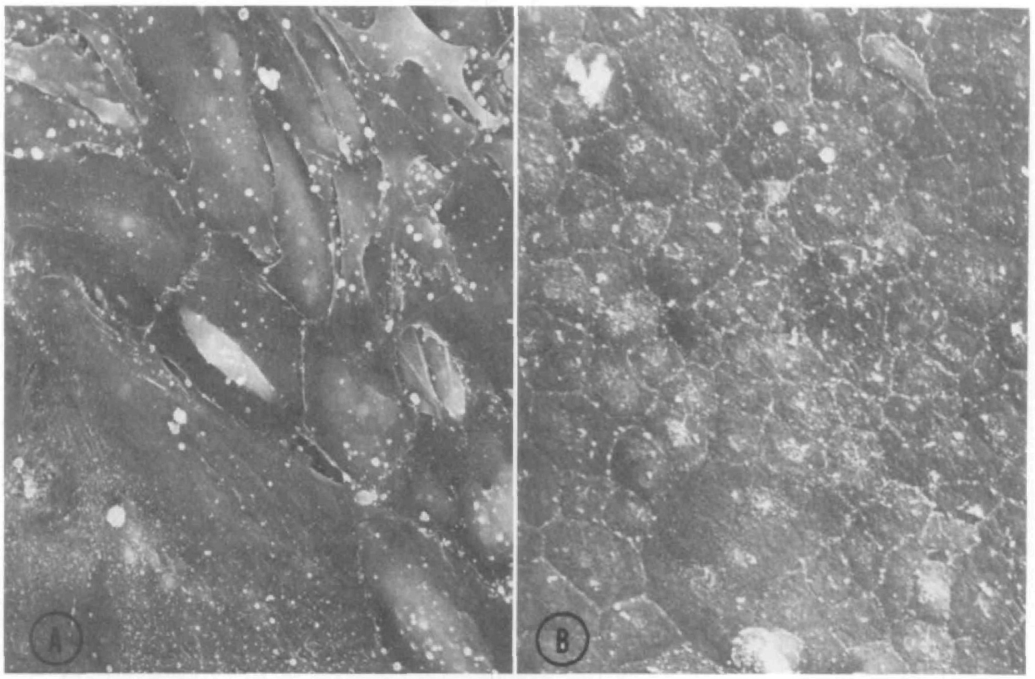

Fig. 3. Scanning electron micrographs of central regenerated corneal endothelia from mature rabbits 5 days (A) and 10 days (B) after transcorneal freezing. By 5 days after injury the central wound areas contained only a few flattened cells (A). By 10 days the cells in the central wound were still heterodisperse in size (B). (Both ×500.)

normal at 10 days (Fig. 3, *B*). At 5 days, cells were already densely packed in the exact center of the wound in young animals (Fig. 2, *A*), whereas in mature animals, there were only a few large, flat cells in the center of the wound at 5 days (Fig. 3,*A*).

Areas with mild infiltrations of polymorphonuclear leukocytes were noted near the wound margins in all corneas collected 24 hr after freezing. In all areas where infiltrations were extensive, ^{3}H-thymidine incorporation by endothelial cells was greatly reduced (Fig. 4). The inflammatory cells showed no signs of labeling.

Discussion. We have shown that aging reduces the rate of corneal endothelial regeneration by 50% in mature rabbits compared to young rabbits. Regardless of age, however, endothelial healing occurred by the division and migration of newly divided cells onto the wound surface. Similar age-related decreases have been reported for a variety of physiological parameters[6] and may be caused by changes in t-RNA which can produce slower and less accurate protein synthesis in aged animals.[7]

We have also presented data that cellular inflammation may reduce ^{3}H-thymidine incorporation. During later stages of healing, inflammation may also cause a loss of contact inhibition in endothelial cells, leading to the production of multilayered endothelia and retrocorneal membranes (Staatz, unpublished). Multilayered endothelial regeneration appears to be fairly common when inflammation is extensive in both the cornea[8, 9] and the vascular system.[10]

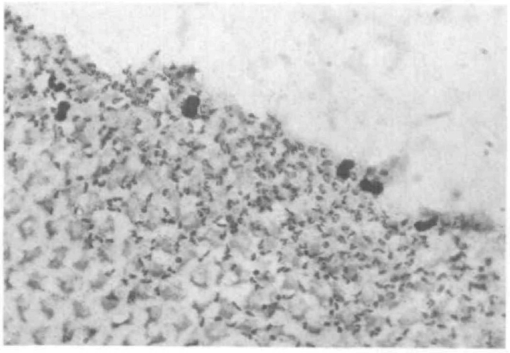

Fig. 4. Autoradiograph illustrating inhibitory effect of inflammatory cells on ^{3}H-thymidine incorporation by regenerating rabbit corneal endothelium. Many small, dark-staining polymorphonuclear leukocytes are seen in this field with endothelial nuclei. Compare to Fig. 1, A and B. (×350.)

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Head rotation trajectories compared with eye saccades by main sequence relationships.

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A helmet apparatus permitted duration, peak velocity, and peak acceleration measurements as functions of magnitude of horizontal head rotation; these "main sequence" data give evidence for multipulse-step neurological signals appropriate for time optimal control of head rotation similar to those of saccadic eye movements.

Head movements play an essential role in gaze and closely interact with eye movements, in part via the vestibular ocular reflex.[1-4] The very rapid saccadic eye movement trajectory has been studied by many researchers since the time of Dodge; parameterization of these trajectories has shown important and consistent relationships between the magnitude of the saccade and its peak velocity, peak acceleration, and duration—called the main sequence relationships.[5] These qualitative behavioral studies have helped to define normal and abnormal saccades in a variety of experimental and clinical situations. Together with considerations of time optimal control theory,[6, 7] these behavioral relationships led to the finding of dynamic overshoots in most normal saccades; this implies multiple pulse-step controller signals, a prediction confirmed by a recent electromyographic study in man (Kenyon RV, Stark L, and Scott AB: Electromyography of saccades with dynamic overshoots (submitted); Society for Neurosciences Abstract, 1977) and by single unit studies in animals.[8]

The present brief research report on head rotation provides a set of main sequence behavioral data for comparison and contrast with eye rotations.

Methods. The experimental apparatus was designed to measure rotational head movement in the horizontal plane.[3, 4] A metal flange was bolted to the center of rotation on top of a plastic bicycle helmet. An acrylic rod, flexible enough to act as a universal joint, was fixed vertically to the helmet flange, and the other end was clamped to a low-torque, low-backlash potentiometer which was fixed in a parallelogram linkage frame so that the center of rotation need not be ascertained exactly. Thus torsional movement of the helmet varied the resulting electrical signal directly. The subject's head was strapped into the helmet and positioned at the center of a horizontal arc perimeter. A 37 cm rigid metal rod, attached to the front of the helmet, radially projected to the subject's midsagittal fixation plane with a small red fixation target at the end. This, together with a fiduciary

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