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Spontaneous Pre-Descemet's Membrane Corneal Opacities in Rabbits

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There are few reports of spontaneous ocular changes in laboratory rabbits (1), which when detected may complicate interpretation of ocular results in toxicity testing. The corneal changes that have been described in this species include anterior corneal dystrophy in American Dutch belted rabbits (2) and corneal epithelial dystrophy in New Zealand White (NZW) rabbits (3, 4). In addition, diet-induced corneas lipidosis has been described in NZW rabbits (5). In humans, a number of corneal dystrophies are recognized, including that of pre-Descemet's membrane (6–8). To the authors' knowledge, pre-Descemet's membrane dystrophy has not been reported in laboratory animals. We describe a pre-Descemet's membrane corneal abnormality in rabbits and give some insights into its etiopathogenesis.

Seventy-six albino (43 males and 33 females) NZW (SPF NZW A 1077 INRA) rabbits, 2 to 4 months old, were obtained from a commercial supplier (Centre Lago, Vonnas, France). They were housed individually in stainless steel cages, fed a commercial laboratory diet (UAR 112 C Laboratory Chow; UAR, Villemoisson sur Orge, France), and provided tap water ad libitum. Rabbits were kept at a temperature of 19 ± 1°C and 30 to 70% relative humidity. There were approximately 15 air changes/h and a 12/12-h light/dark cycle. The animal facility has animal care and use programs that are accredited by AAALAC, International; the Animal Care Program operates in accordance with current standards. Eye examinations were performed, using an indirect ophthalmoscope (Welch Allyn, Inc., Skaneateles Falls, N.Y.) with interposition of a 28-diopter Nikon lens and a hand-held slit lamp biomicroscope (Kowa Co., Ltd., Tokyo, Japan). Before examination, pupils were dilated by use of 0.5% tropicamide (Mydriaticum; MSD-Chibret, Paris, France). Ocular examinations were performed after rabbits had been in the laboratory for at least 2 weeks. Eight rabbits were examined for ocular changes every 1 to 2 months for 6 months. After the last ocular examination, rabbits were euthanized by intravenous administration of an overdose of 6% pentobarbital (Pentobarbital Sodique; Sanofi Sante Animale, Libourne, France). Eyes were fixed in Zenker's fixative, processed in routine manner, embedded in paraffin, and cut in 5-μm sections. Sections were stained with hematoxylin and eosin (H&E). Eyes from two rabbits with multifocal and bilateral corneal opacities were immersed in 4F1G (4% formaldehyde + 1% glutaraldehyde) fixative solution. Blocks of approximately 2 x 2 mm^2 were cut in areas determined at ophthalmic examination and embedded in epon. Toluidine blue-stained semi-thin sections were observed by light microscopy to select appropriate areas for ultrastructural study. Uranyl acetate-lead citrate ultra-thin sections were observed, using a Philips C 12 transmission electron microscope at 80 Kev.

Focal or multifocal discrete and/or small deep corneal opacities (Figure 1) were seen in either portion of the cornea (central or peripheral) in 29% of rabbits (11 of 43 males and 11 of 33 females) during ocular examination with the slit-lamp biomicroscope. Six months after the initial examination, in eight rabbits there had been no progression of this change. Corneal opacities were the only ocular abnormality detected. Results of general physical examination of all rabbits were otherwise unremarkable.

Minute pre-Descemet's membrane linear cellular aggregates (Figure 2) were seen by light microscopy. Electron microscopy indicated that these cells were ectopic endothelial cells actively secreting matrix material (Figure 3). This conclusion was based on the following observations: the cells' intracytoplasmic content, presence of a dense and homogenous material associated with the outer cell membrane, location of these cells close to Descemet's membrane, and the cells' linear organization.

To gain some insight into this change's development with age and its transmission, an ocular survey using the slit lamp was performed at the breeder facility. Twenty-one 1-year-old breeders (3 males and 18 multiparous females) were examined, as well as five 2-week-old littermates, seven 6-week-old littermates, and one 2-year-old female breeder. Similar corneas opacities were observed in 30% of 1-year-old, 20% of 2-week-old, and 71% of 6-week-old rabbits. The 2-year-old rabbit also was affected. The corneal opacities were discrete regardless of the age of rabbits.

The inheritance of this change was investigated. From the mating between a normal male and an affected female, the ratio of affected to total offspring was 1 in 5. From the mating between an affected male and an affected female, the ratio of affected to total offspring was 5 in 7. These results would suggest that this change is hereditary. Further matings would be necessary to determine the specific mode of inheritance. The change was assumed to be present at birth because it was seen in 2-week-old rabbits.

Pre-Descemet's membrane corneal opacities have been reported in humans and are usually bilateral, symmetric, and likely to occur past the age of 30 to 40 years; they consist of discrete, linear, or punctate opacities in the deep

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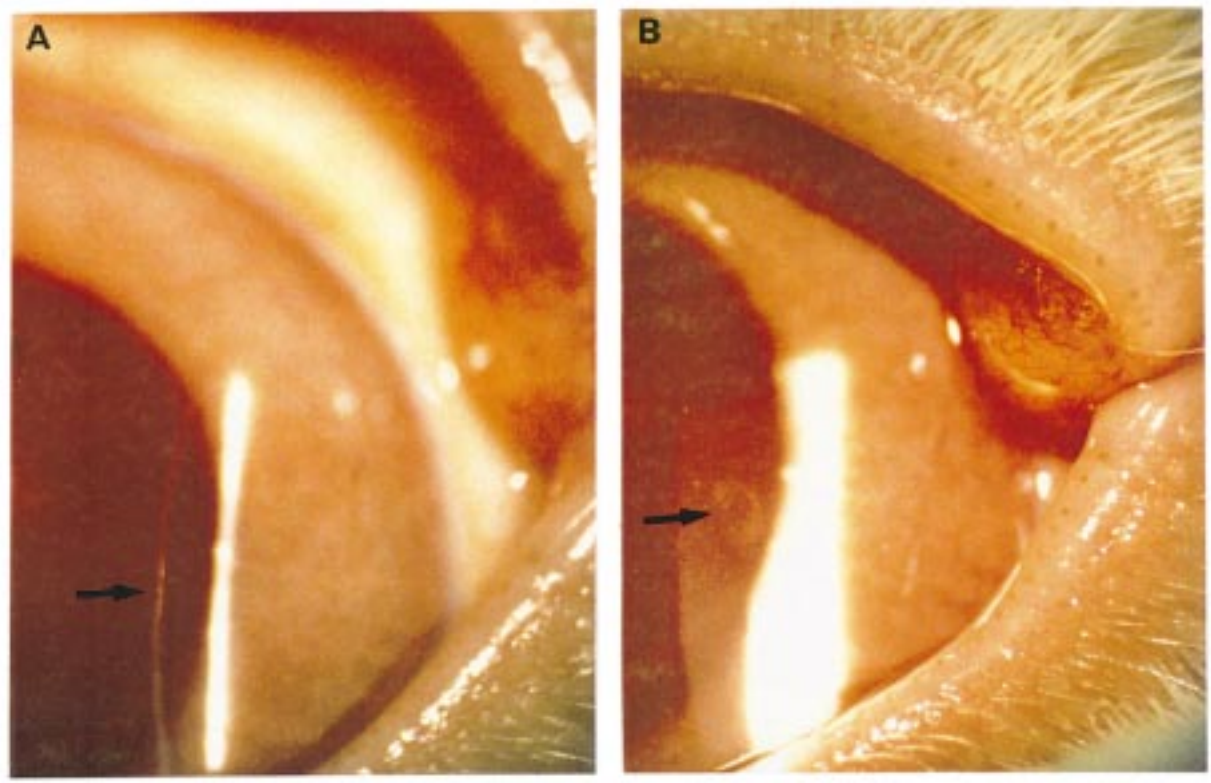

Figure 1. Discrete focal deep corneal opacities. **(A)** narrow slit and **(B)** large slit. Magnification, x25.

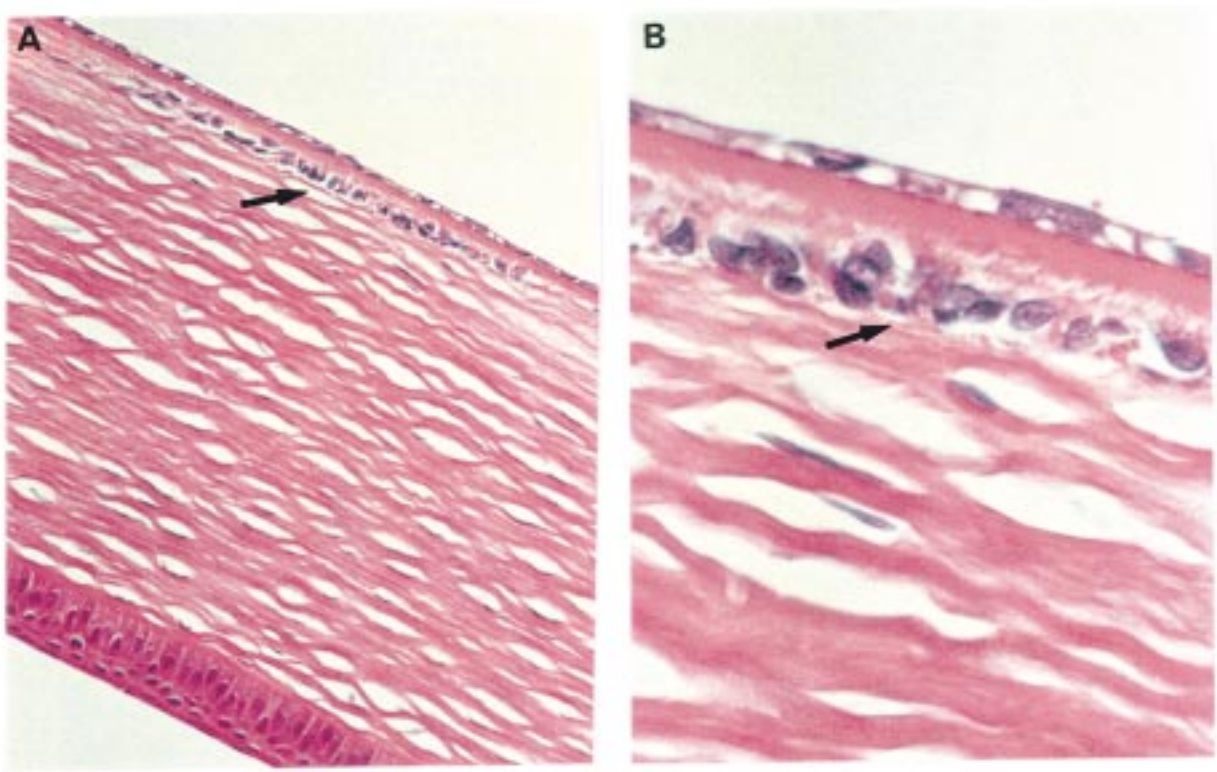

Figure 2. Photomicrographs of sections of pre-Descemet's membrane. Notice linear cellular aggregates. H&E stain; **(A)** magnification, x100 and **(B)** magnification, x400.

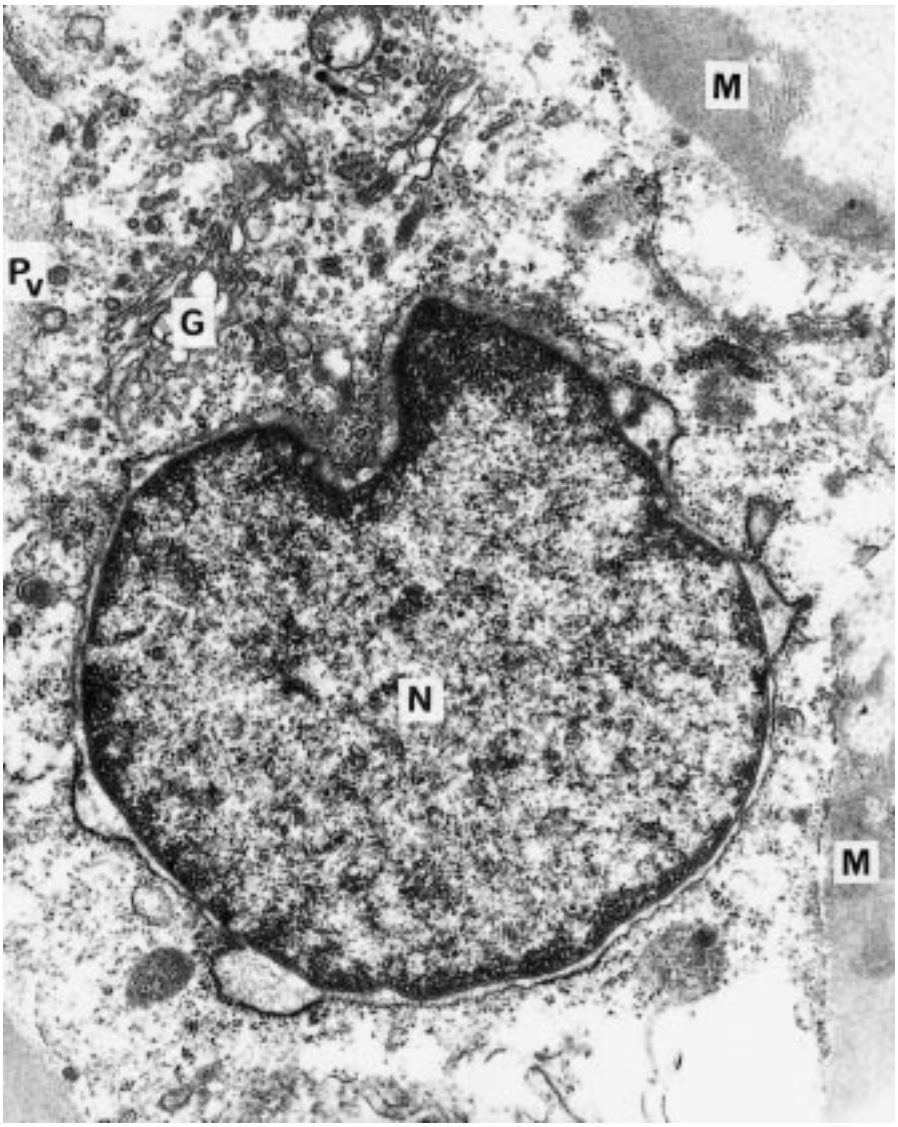

Figure 3. Electron micrograph of deep corneal stroma. The cell in the center has external homogenous material (M) associated with the outer membrane, a round nucleus (N), and a Golgi system (G) with numerous vesicles and pinocytic vacuoles (Pv). Uranyl acetate and lead citrate; magnification, x40,000.

stroma. Pathologic involvement is limited to posterior stromal keratocytes, with vacuolation and enlargement of affected cells with lipid-like material. These findings support the hypothesis of degeneration associated with aging (9) and are different from those in rabbits in which there was no lipid-like storage.

In conclusion, the pre-Descemet's membrane corneal changes seen in rabbits consisted of ectopic active endothelial cells and were different from the pre-Descemet's membrane corneal changes in humans. To our knowledge, the changes seen in theses rabbits have not been reported in other animals. This discrete lesion, present throughout the life of rabbits, had no sex predilection and no clinical progression with age. It appeared to be hereditary; however, the exact pattern of inheritance was not determined. This lesion should be considered a spontaneous ocular change when performing safety assessment in NZW rabbits.

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