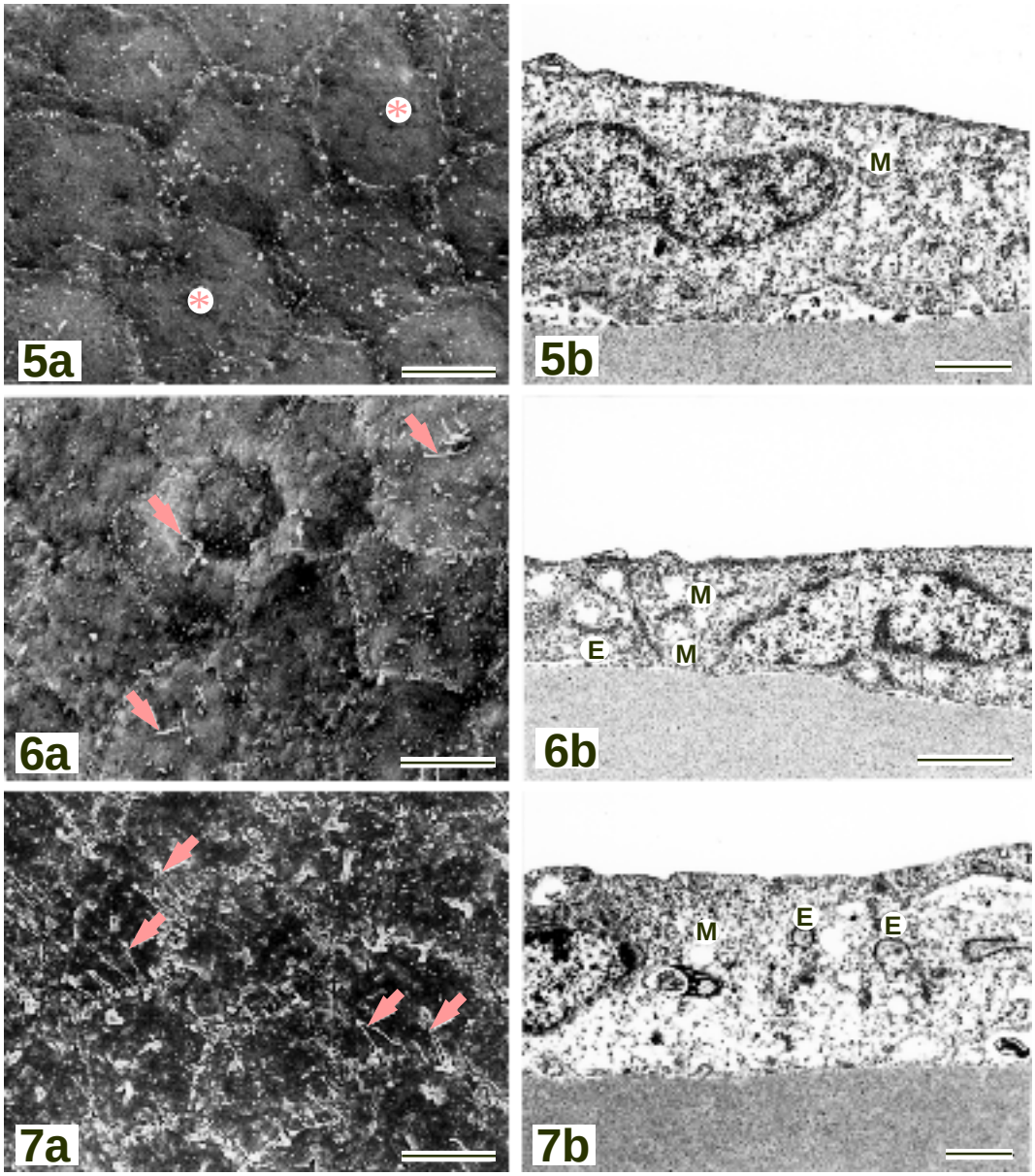

Fig. 5. Scanning (**a**) and transmission (**b**) electron micrographs of a rat cornea preserved at 0˚C for 1 day.
a: The cell surface protrudes slightly (*) and the cell boundaries are clearly visible (bar = 10 μm).
b: Mitochondria show slight swelling (M) and other intracellular organellae are relatively well preserved (bar = 1 μm).

Fig. 6. Scanning (**a**) and transmission (**b**) electron micrographs of a rat cornea preserved at 0˚C for 2 days.
a: The cell surface is irregular and the cell boundaries are just recognizable. Some elongated microvilli (arrows) are observed (bar = 10 μm).
b: The mitochondria (M) become swollen, but their cristae are partly preserved. The rough-surfaced endoplasmic reticulum (E) is relatively well preserved (bar = 2 μm).

Fig. 7. Scanning (**a**) and transmission (**b**) electron micrographs of a rat cornea preserved at 0˚C for 7 days.
a: The cell surface is almost intact. The microvilli on the peripheral margin of the endothelium are markedly elongated (arrows) (bar = 10 μm).
b: The mitochondria are vacuolated and no cristae are visible (M). The rough-surfaced endoplasmic reticulum is swollen (E). The density of the cytoplasmic matrix decreases in the basal region of the cells (bar = 1 μm).

–196˚C made it possible to prolong storage periods for up to a year or longer, freezing and thawing cause structural damage to the endothelium (Capella et al., 1965; Van Horn et al., 1972; Doughman, 1988). Thus, it is logically assumed that organs should be stored at the lowest temperature possible without freezing (Yoshida et al., 1999).

All organic matter has its own specific subzero freezing temperature: –1.0˚C in the rat liver (Yoshida et al., 1999), –0.6˚C in the rat heart (Wicomb and Cooper, 1984) and –0.8˚C in human plasma (Storey and Storey, 1990). Since the rat corneal endothelium is very thin, it is impossible to measure its freezing point with a thermometer. In this study, we tried to preserve corneas at 0˚C, assuming that corneas do not freeze at this temperature.

Previously, Taylor et al. (1989a,1989b) stored rabbit isolated corneas at 0˚C. They reported that the endothelial ultrastructure was maintained during storage for 3 and 5 days in a hyperkalemic solution, CPTES*. According to their transmission electron microscopic findings, the mitochondria showed moderate swelling with a diffuse pallid matrix, as well as condensed and beaded forms. The smooth endoplasmic reticulum was swollen and vesiculated. Their ultrastructural findings were almost identical to the present study. According to their method, polypropylene vials containing a preservation medium were placed in an evacuated Dewar flask containing ice in a 4˚C refrigerator. In this study, we used a special refrigerator that can precisely control the temperature from room temperature to –5.0˚C at 0.1˚C intervals (Yoshida et al., 1999).

Nowadays, Optisol (Steinemann et al., 1993) and Dexsol (Skelnik et al., 1988; Lass et al., 1990) (Chiron Co., Irvine, CA) are used by most eye banks in the United States as solutions for preserving isolated corneas at 4˚C. These solutions have enabled their preservation for up to 1 week (Bourne, 1991). Although the corneoscleral preservation has increased in Japan, it has not been widely used all over the world (Shimazaki et al., 1993). In this study, we preserved rat whole globes at 0˚C in EP-II, which has been generally used for whole globe preservation in Japan.

Fluid movement in the corneal endothelium is thought to be related to a pump-leak transport mechanism in which fluid from the anterior chamber leaks into the stroma across a leaky apical junction, while fluid is actively pumped from the stroma into the anterior chamber (Barry et al., 1995). The Na^+-K^+ ATPase pump of the corneal endothelium has been described by many investigators: the pump is located in the lateral membrane and actively transports sodium and bicarbonate ions into the anterior chamber; along this osmotic gradient, water moves from the stroma to anterior chamber (Klyce and Beuerman, 1988; Dohlman, 1994; Edelhauser et al., 1994; Barry et al., 1995). Hodson (1971) demonstrated that perfusion with a bicarbonate free medium caused reversible stromal swelling. Thus, it was presumed that the pump was partly inactivated by the removal of bicarbonate (Hodson, 1971). Since EP-II does not contain bicarbonate ions, it extends the lifetime of the corneal endothelium by controlling pump function consumption.

The present scanning electron microscopic study showed no significant ultrastructural differences in the endothelial surfaces of corneas preserved at either 4˚C or 0˚C for up to 2 days. After 7 days' storage at 4˚C, the cells showed marked destruction (Fig. 4a), but were fairly-well preserved at 0˚C. Furthermore, transmission electron microscopic findings indicated that the corneal endothelium preserved at 4˚C showed more distinct mitochondrial swelling than that preserved at 0˚C. These changes became evident after 7 days' storage (Figs. 4b and 7b). Ultrastructural changes were also noted in the corneas preserved at 0˚C: the presence of vacuoles, condensation of the mitochondrial matrix, a swollen rough-surfaced endoplasmic reticulum and a decrease in the cytoplasmic density in the basal region. However, most of the surface cell membrane and cell organellae were retained (Fig. 7b). The density of the cytoplasmic matrix decreased in the basal re-

*CPTES: corneal-potassium-TES, a potassium-rich balanced salt solution containing the impermeant biological buffer compound N-Tris(hydroxymethyl)methyl-2-amino ethane sulphonate.

gion of the endothelia preserved for 1 and 2 days at 4˚C or 7 days at 0˚C (Figs. 2b, 3b and 7b). This may have been due to the leakage of fluid into the paracellular space across the leaky apical junction.

There were fewer ultrastructural changes in the corneal endothelia preserved at 0˚C than those at 4˚C, which are thought to be reversible. Since the endothelial metabolism below 0˚C is reduced in comparison with that at 4˚C, consumption of ATP and activity of the Na^+-K^+ ATPase pump are minimized in an endothelium preserved at 0˚C. Recently, Yoshida (1999) reported that the concentration of ATP in the rat liver preserved at –0.8˚C was higher than that at 4˚C. Thus, the lifetime of the endothelial cells is extended if they are preserved at 0˚C.

The utilization of corneas preserved as whole globes at 4˚C is restricted to within 48 h, because the endothelium is exposed to stagnant aqueous humor which has metabolic waste products and tissue necrosis (Bito and Salvador, 1970; McCarey and Kaufman, 1974). The present findings indicate the possibility for longer preservation of whole globes if they are stored at 0˚C.

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