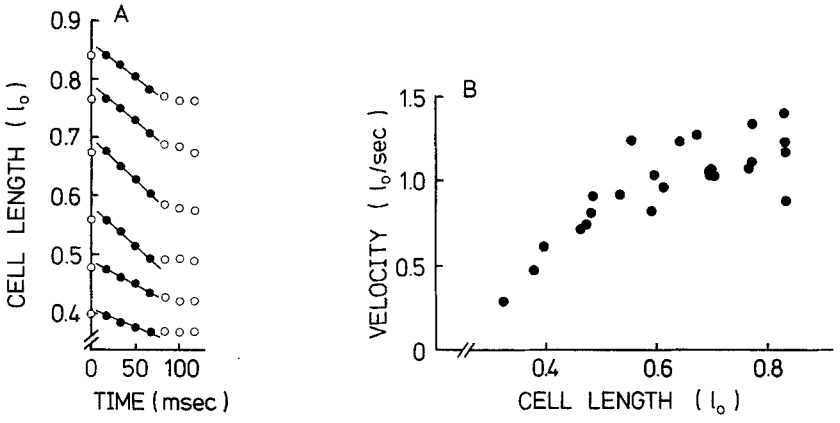

Figure 3. A. Twitches elicited in an isolated cell at various cell lengths. Single electric stimulation was repeatedly applied to the cell at intervals of 10 s so that the initial length from which the cell contracted decreased progressively (shown from top downwards) due to the residual contractions. Straight lines are the least-squares regressions of the data shown by filled circles. l_s, 840 μm. B. Relation between the shortening velocity and the initial cell length obtained from repeatedly induced twitches (as shown in A) in four isolated cells. The lengths and the shortening velocities were normalized to l_0 and l_0/s, respectively.

study because passive tension appeared transiently when the cells were stretched beyond this length[6]. This result shows a marked contrast with the relation between V_{max} and fiber length in striated muscle, in which V_{max} decreases sharply below 0.8 l_0[10]. It also suggests that the intracellular resistance to the contractile force, e.g. collisions between the thick filaments and the dense bodies[7] or increases in the intracellular pressure which might occur as the cells shorten, is negligible at lengths over 0.5 l_0, and the ascending limb of the length-tension relation of the isolated PRM cells depends largely on the amount of overlap between the thick and the thin filaments[6,7].

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Intercellular junctions of hyperplastic retinal pigment epithelium[1]

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Summary. In rats with retinopathies induced by excess fluorescent light or injections of urethane, the retinal pigment epithelium (RPE) undergoes focal hyperplasia. Neither intravascularly injected horseradish peroxidase or lanthanum nitrate penetrated the sensory retina at these hyperplastic sites. Electron microscopy revealed that this was due to the persistence of intact tight junctions among a single layer of hyperplastic cells facing the sensory retina. These junctions prevented intraocularly injected microperoxidase from passing as well. Cells within the hyperplastic foci were connected only by adherent junctions that presented no permeability barrier.
Key words. Retinal pigment epithelium; epithelium; permeability; hyperplasia; pathology.

Epithelia form barriers by means of their intercellular tight junctions. Given the physiologic importance of such barriers, we must consider changes in intercellular junctions and epithelial permeability when diseases affect them. In the eye, for example, a blood-retinal barrier exerted in part by the intercellular tight junctions of the retinal pigment epithelium (RPE) is disrupted in some retinal diseases[4]. These tight junctions and transport systems in the RPE plasma membrane control the passage of molecules and ions into and out of the sensory retina, and are probably important for maintaining photoreceptor function. Thus, we were interested when we noticed that in rats with experimentally induced hyperplasia of the RPE, a condition seen in human retinal disease[5], the RPE still prevented intravascularly injected tracers from entering the sensory retina. This report describes this phenomenon and the reason for its persistence in the face of extensive changes in the RPE sheet: a single layer of tight junctions remained where the hyperplastic focus faced the sensory retina.

Materials and methods. Five albino (Sprague-Dawley) and 9 pigmented (Long-Evans) rats were respectively exposed to fluorescent light or received s.c. injections of urethane by procedures that have been described[6,7]. Phototoxic rats were examined 9–12 months later, and urethane rats 8-12 weeks later. Observations in normal rats were drawn from those used in previous studies[8,9]. Tissue was obtained for electron microscopy by anesthetizing the rats (sodium pentobarbital, 40 mg/kg b.wt, i.p.) and removing the eyes into 2% formaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2--7.4; or by perfusing this fixative

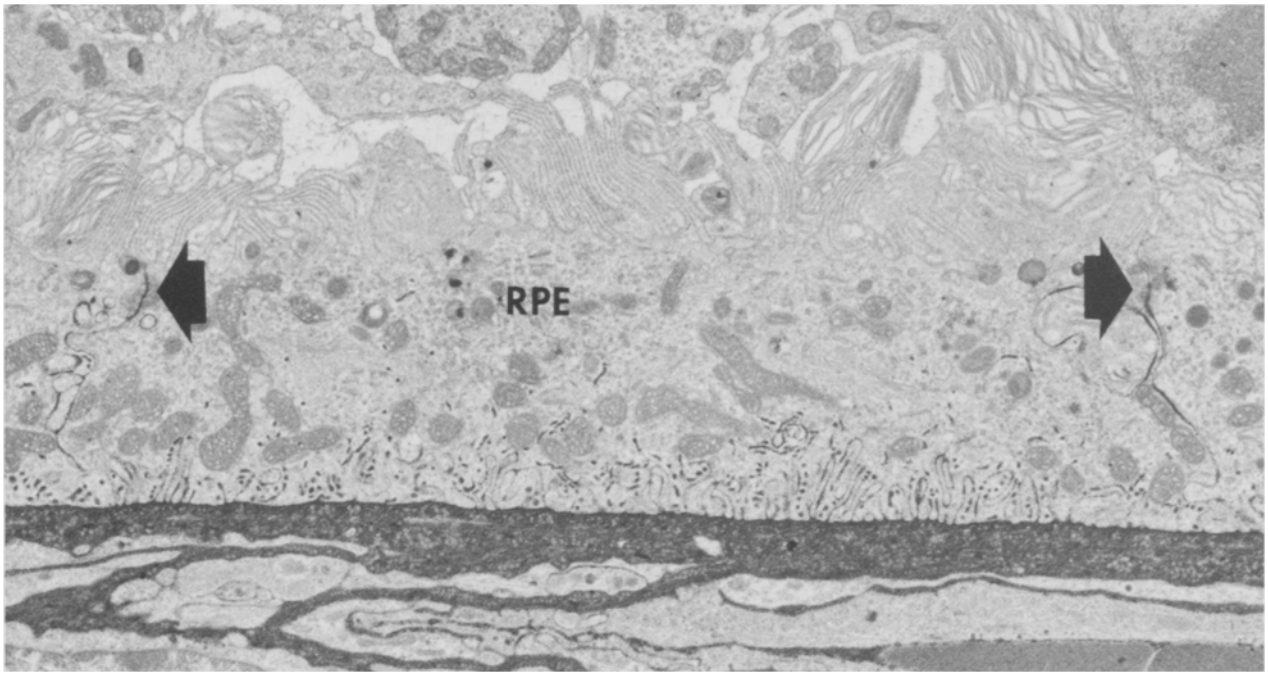

Figure 1. Normal, non-hyperplastic RPE in a phototoxic rat. HRP (black deposits) has leaked out of the choriocapillaris at bottom of picture and between RPE cells. Junctional complexes (arrows) prevent tracer entry into sensory retina. × 8300.

through the heart and then immersing the eyes in it overnight. Slices of tissue were treated with osmium tetroxide, dehydrated and embedded in plastic resin for thin sectioning. Some pieces of tissue were stained with tannic acid by including 0.5% tannic acid in the aldehyde fixative.

RPE permeability was gauged by injecting horseradish peroxidase (HRP; 0.3 mg/g b.wt in 0.5 ml saline; Sigma Chemical Corp., St. Louis, MO) via a femoral vein or 1 ml of 4% lanthanum nitrate in saline via a common carotid artery 1–15 min prior to fixation; and by injecting 20 µl of 2.5% microperoxidase (Sigma, Type MP-11) in saline into the vitreous cavity 2 h prior to euthanasia. HRP and microperoxidase were localized by the deposition of a diaminobenzidine reaction product[10]. Tissue for localization of lanthanum deposits was processed as described above.

Results. After light or urethane treatment similar retinopathies developed: the retinal photoreceptors were destroyed and retinal capillaries grew into the RPE, as described[6-9]. At some of these sites, as well as areas where intraepithelial capillaries did not occur (confirmed by serial 2-micron sections examined light microscopically) the RPE cells underwent hyperplasia (figs 1, 2). They formed clusters or strata of cells in varying states of differentiation along Bruch's membrane, the thick basement membrane that separates the RPE from the choriocapillaris, a dense choroidal capillary plexus (fig. 2). These hyperplastic foci always contained a layer of mature cells directly facing the sensory, or neural, retina (fig. 2). They were connected by junctional complexes identical to those connecting RPE cells in normal rats of where hyperplasia had not occurred in experimental rats[8,9]. The junctional complexes consisted of tight junctions, gap junctions and adherent junctions in close proximity to each other, situated where the apices of adjacent cells met (figs 2, 3). Only small adherent junctions, particularly noticable in tissue treated with tannic acid, connected the cells beneath this surface layer, regardless of their maturity (fig. 4).

Up to 15 min after injection, HRP or lanthanum penetrated the choriocapillaris and permeated the extracellular space about the cells within the hyperplastic foci (fig. 2). The tight junctions connecting the layer of RPE cells facing the sensory retina prevented tracer entry into it (fig. 2, 5). Microperoxidase did not penetrate the junctions, either. By 2 h after intraocular injection the tracer had diffused across the sensory retina to the RPE junctional complexes. They retarded further tracer passage both

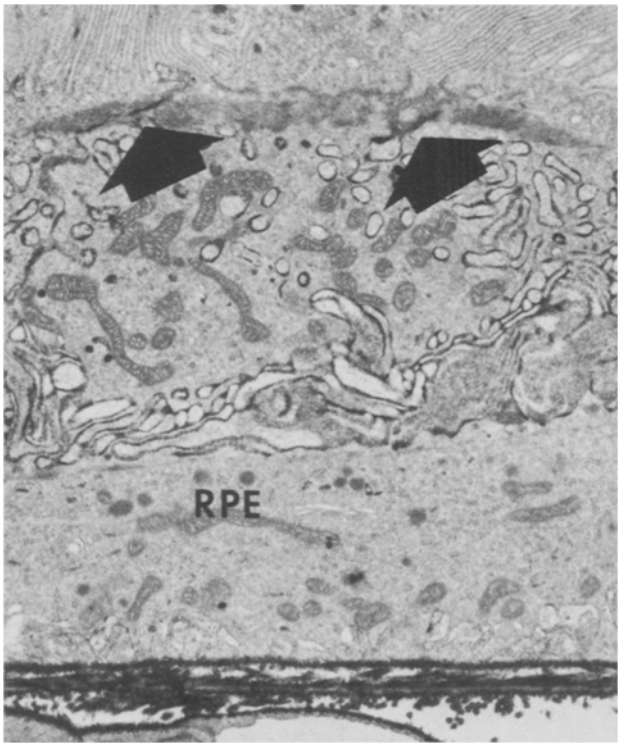

Figure 2. Hyperplastic RPE cells are outlined by HRP reaction product in a phototoxic rat. Junctional complexes (arrows) at cells facing sensory retina retard tracer. Choriocapillaris is at bottom of picture. × 8300.

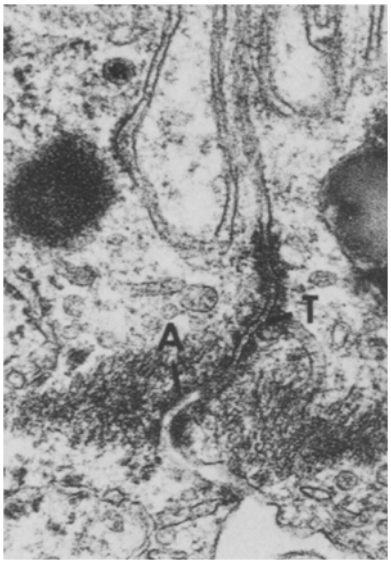

Figure 3. Junctional complex of hyperplastic RPE cells facing sensory retina. Tight junctions (T) and adherent junctions (A) are seen. × 54,000.

at hyperplasia sites and where non-hyperplastic RPE occurred (fig. 6).

Discussion. When the retinal pigment epithelium undergoes hyperplasia, transforming from a monotonous sheet of one cell type into a mound of cells in varying states of differentiation, the epithelium as a whole retains its permeability barrier properties. We suggest that as RPE cells undergo mitosis some daughter cells retain their position facing the sensory retina and keep their junctional complexes intact, while other daughter cells come to occupy a space beneath them. The continued mitosis of these cells would form clusters of cells in varying states of maturity. Similar observations have been made in cats whose RPE proliferated after retinal detachment[11]. Daughter cells lost their apical-basal polarity and organelles associated with RPE maturity, such as abundant smooth endoplasmic reticulum.

The intercellular tight junctions of RPE of normal rats and that of phototoxic rats and urethane rats at nonhyperplastic sites do not permit HRP, ionic lanthanum or microperoxidase to pass[8, 9, 12, 13]. The inability of these tracers to penetrate the RPE tight junctions at hyperplastic sites suggests that normal RPE permeability is maintained here as well, in spite of extensive reorganization of the epithelial sheet and the presence of cells in varying states of maturity. It is probable, however, that during mitosis the cells facing the sensory retina do experience a brief lapse in their junctional impermeability, as in thyroid epithelial cells undergoing mitosis[14].

The hyperplasia we have studied may be a regenerative response or the result of other factors, such as the loss of photoreceptors, which have been suggested to suppress the mitosis of RPE cells[11]. Whatever the cause, it would seem that rat RPE cells can maintain their tight junctions and their associated barrier functions in the face of insults calling forth hyperplasia like that seen in retinal disease. This probably reflects an inherent ability by RPE cells to form and maintain tight junctions independent of external stimuli.

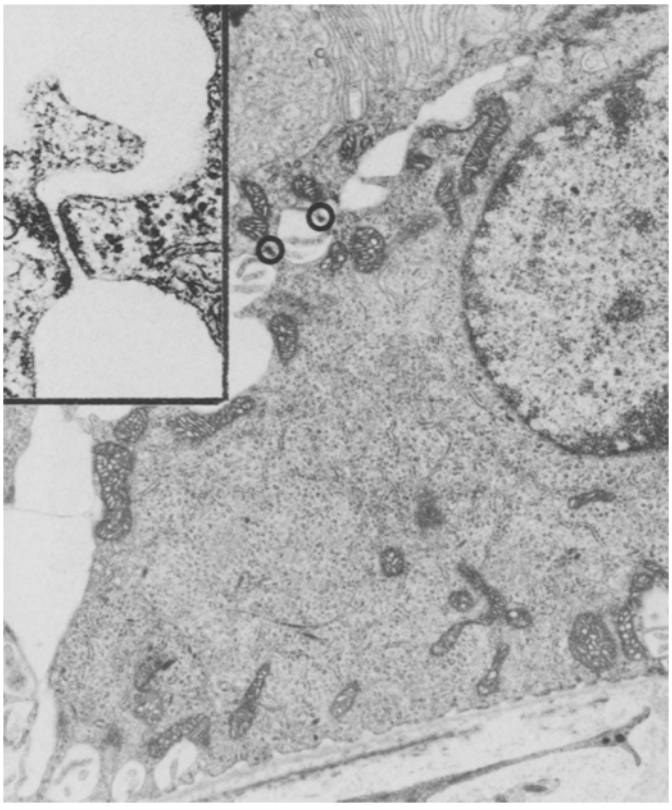

Figure 4. Undifferentiated cell in hyperplastic cluster. Punctate adherent junctions are circled, and seen at higher magnification in inset. × 9800. Inset × 54,000.

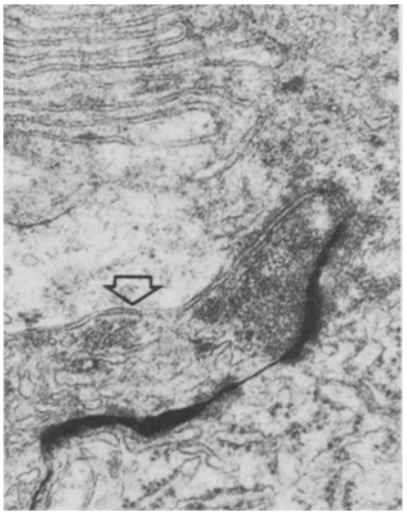

Figure 5. Black deposits of lanthanum fill extracellular space between hyperplastic RPE cells. Junctional complex prevents tracer from entering extracellular space of sensory retina (arrow). × 42,000.

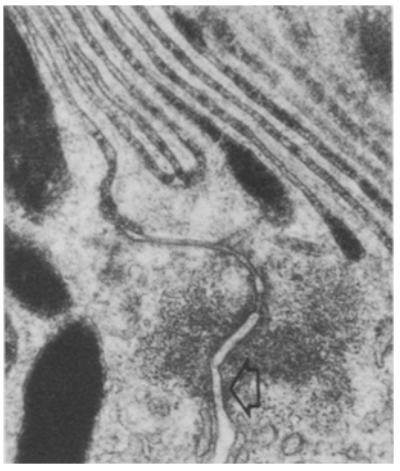

Figure 6. Black reaction product of intraocularly injected microperoxidase does not penetrate tight junctions of hyperplastic RPE; extracellular space on choroidal side of RPE (arrow) does not contain tracer. × 42,000.

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The effects of time of equilibration with cryoprotectants at 0 °C prior to freezing on the survival of mouse embryos frozen by the two-step method

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Summary. Mouse embryos were frozen by the two-step method after equilibration for 0.1–60 min with cryoprotectants at 0 °C. No survival or a very low survival was obtained after equilibration for only 0.1 min. The morulae showed the highest survival rates when equilibration time was 5–30 min with 2 M DMSO, 20–30 min with 2 M glycerol, 5–10 min with 2 M ethylene glycol and 20–30 min with 2 M propylene glycol, respectively.
Key words. Mouse embryos; two-step freezing; cryoprotectant; equilibration time; permeation.

Mammalian embryos have been preserved at low temperatures only if the cryoprotectant is present in the suspending solution during cooling[1]. The cryoprotectants such as dimethyl sulfoxide (DMSO) or glycerol used for embryo freezing are able to penetrate cells and the amount of permeation is reflected to some extent by the duration of exposure prior to freezing. Although most workers have exposed embryos to cryoprotectants for about 10–20 min prior to initiating freezing of embryos, the mechanisms by which these compounds protect embryos against freezing damage remain obscure[1]. It is suggested that DMSO probably need not permeate 8-cell mouse embryos to protect them against freezing damage[2–4].

A two-step cooling procedure is useful in the study of various factors influencing the recovery of cells following freezing and thawing[5]. The present experiments were designed to examine the effect of equilibration time with various cryoprotectants, prior to freezing, on the survival of mouse embryos frozen by the two-step method.

Materials and methods. Female ICR mice, aged 4–6 weeks, were induced to superovulate by the i.p. injection of 5 IU of pregnant mare serum gonadotropin followed 48 h later by 5 IU of human chorionic gonadotropin (hCG). They were mated with males of the same strain. The 8-cell and morula stage embryos were flushed from the reproductive tracts with a modified Dulbecco's phosphate-buffered saline (PBS)[6] at 67–70 and 77–79 h after the injection of hCG, respectively.

The 10–25 embryos were pipetted into 10 × 100 mm glass tubes containing 0.1 ml PBS and cooled to 0 °C. The cryoprotectant in 0.15 ml PBS was added to samples at 0 °C in a single addition. The cryoprotectants used were DMSO, glycerol, ethylene glycol and propylene glycol and the concentration of cryoprotectant was usually 2 M. The samples were equilibrated for 0.1–60 min at 0 °C after the addition of cryoprotectant and then transferred without ice-seeding from 0 °C into constant temperature ethanol baths at either −20 °C (for embryos for which DMSO was the cryoprotectant) or −40 °C (for embryos with other cryoprotec-