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An Electron Microscopic Study of Early Reversible Hydroxyurea Cataracts *in vitro*

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The crystalline lenses of male white Wistar rats weighing 100 gr. were cultured in a culture fluid containing 5×10^{-2} M hydroxyurea. The epithelial cells forming multiple layers in the equatorial region on the 4th day of incubation and swollen epithelial cells were studied by electron microscopy and the results are summarized as follows;

1. The epithelial cells forming multiple layers retained a desmosome-like structure near the apical region. It was assumed that these cells preserve their epithelial properties.

2. The cellular morphology and subcellular organela had the characteristics of epithelial cells.

3. The scarcity of microtubules in the epithelial multiple layers was interpreted as reduced capacity of these cells for elongation.

(Key Words: Cataract, Hydroxyurea, Microtubules, Elongation)

INTRODUCTION

We have indicated in earlier studies using DNA and RNA inhibitors that early changes in experimental cataracts are represented by the formation of multiple layers and development of bladder cells among the equatorial epithelial cells (7, 8, 9).

It has not yet been clarified, however, whether the cells accumulated in multiple layers in the equatorial region eventually expire and are absorbed by the surrounding tissue or are enclosed as debris, instead of undergoing differentiation into crystalline tissue.

It is also uncertain if these cells which form multi-layers in the equatorial region still possess the potential for eventual differentiation into lens fibers.

Hamai *et al.* (6) observed morphological changes in the intercellular connections of swollen cells in cataracts while Sakuragawa *et al.* (13) conducted a scanning electron microscopic study on swelling of lens cells. However, no electron microscopic observations have been made on the epithelial cells forming multiple layers in the equatorial region.

In the present study, experimental cataracts were created *in vitro* by hydroxyurea (hereafter abbrreviated to HU) and the epithelial cells forming multiple layers in the equatorial region were observed under an electron microscope.

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EXPERIMENTAL METHOD

Male white Wistar rats weighing 100 gr. provided the crystalline lenses. Following sacrifice of the animals by a guillotine, an incision was made from the posterior of the eye and the crystalline lens was removed in Puck's solution under a stereoscopic microscope. Subsequently, the extracted crystalline lens was cultured in 4 ml of Rat Lens Medium (including TC 199 prepared by the Pharmaceutical Department of Tokai University) which also contained 5% calf serum (GIBCO, U.S.A.), in an atmosphere of 5% CO_2 and 95% air at 37°C.

For cultivation, Corning Petri dishes were used and the culture fluid was renewed every 4 days. The right crystalline lens was cultured as an untreated control while HU (Sigma) was added to the left crystalline lens in such quantities that the final concentration was 5×10^{-2} M. In the exchange of the culture fluid on the 4th day of cultivation, no HU was added to the left crystalline lens which was cultured until the 6th day.

The crystalline lenses on the 4th and 6th days of incubation were cut into blocks $2.5 \times 2.5 \times 2.5$ mm in size and fixed overnight in a 2.5% glutaraldehyde solution containing 0.025 M phosphate buffer (pH 7.4). The tissue was then washed 3 times (at intervals of one hour each) using 0.1 M phosphate buffer (pH 7.4) and fixed by 1% osmic acid containing the same buffer for 2 hours. Following dehydration through a series of alcohol solutions, the tissue was imbedded in Epon 812. Ultra-thin sections were prepared by an LKB Ultrotome 8800 using a diamond knife, stained by uranyl acetate, examined under a JEM 100 C electron microscope, and photographed.

RESULTS

I. Electron microscopic observations of the epithelial cells in the bow (equatorial) area of the control crystalline lens on the 4th day of incubation (Fig. 1)

In the section of the bow area where epithelial cells were forming multiple layers, there was a band-like region parallel to the cortex where cells showed high electron density. Electron density was found to be particularly high in the apical portion (abbreviated as AP) farthest from the bow area. These cells with a high electron density were also noted to have a large population of polysomes and many vesicles and mitochondria. Microtubules were found both in the epithelial cells and the cortex. The nucleus of the epithelial cells had a normal appearance. Intercellular connections were unremarkable. The epithelial cells were arranged parallel to the cortex, forming a thin and elongated line.

II. Electron microscopic observations of the epithelial cells at the bow area of the HU-treated crystalline lens on the 4th day of incubation (Fig.2, 3 and 4).

As shown in Fig. 2. a group of epithelial cells with a high electron density formed a band below the degenerated epithelial cells with a low electron density immediately under the anterior capsule. At the end of this band farthest from the bow area, the cells were elongated in a vertical direction. Some of the cells forming this band had large nuclei with irregular oval shapes

and indentations at the periphery. The populations of polysomes appeared somewhat reduced in these cells. The vesicles and mitochondria were swollen but their numbers were not reduced. Only a few microtubules were noted. Among these epithelial cells, those close to the apical portion showed systematic arrangement in a desmosome-like structure. Accumulation of a homogenous substance was seen in the intercellular spaces (Fig. 3).

The group of epithelial cells near the bow area showed vacuole formation and the presence of subcellular organella or what appeared to be fragments of cell membranes in their cytoplasm.

A homogenous substance had accumulated in the intercellular spaces. The interdigitation of epithelial cells with a low electron density under the anterior capsule was atrophic and the intercellular spaces formed vacuoles. Only a few microtubules were noted (Fig. 4).

Compared with the cortex and the degenerated epithelial cells under the anterior capsule, the number of microtubules in the epithelial cells which had a high electron density and formed multiple layers, was extremely small.

III. Electron microscopic observations of the bow area epithelial cells of the control crystalline lens on the 6th day of incubation.

In the area of multiple layer formation in the bow area, the epithelial cells with a high electron density formed a band-like area parallel to the cortex. In the apical portion of this band-like area, the electron density was particularly high. In the same area, the population of polysomes was also large, and vesicles and mitochondria were abundant. The nuclei were normal. The epithelial cells were elongated, paralleling the cortex. No special findings were seen in the intercellular junctions.

IV. Electron microscopic observations of epithelial cells in the bow area of the HU-treated crystalline lens on the 6th day of incubation (the tissue was exposed to HU for the first 4 days and kept unexposed for the last 2 days) (Fig. 5).

In the region where the epithelial cells formed multiple layers in the bow area, a group of cells with high electron density were arranged in a band around the cortex. The arrangement of these cells was identical to those seen in the control on the 4th and 6th days of incubation. Microtubules were abundant in the epithelial cells and the cortex. Compared with the tissue which had been treated with HU and examined on the 4th day of incubation, the intercellular junctions appeared more dense (Figs. 6 and 7).

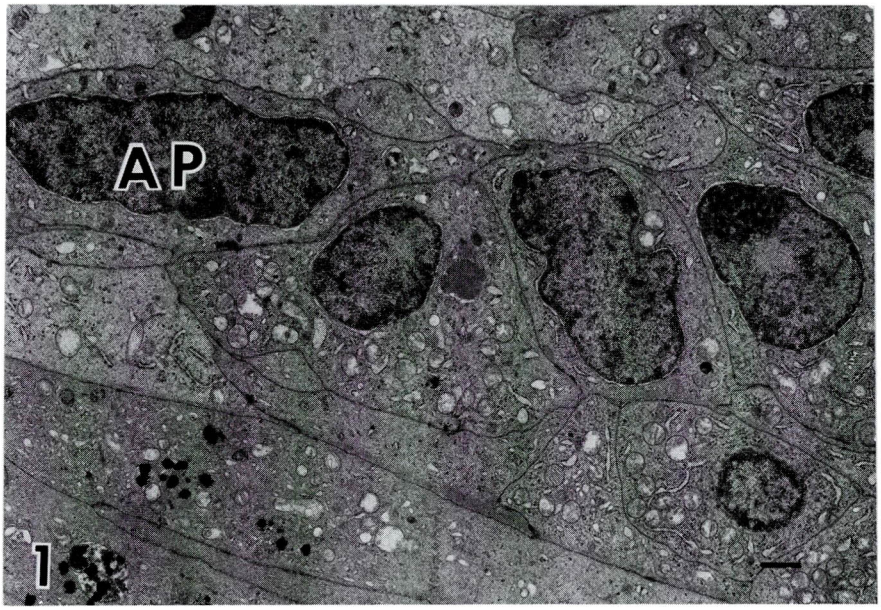

Fig. 1 Electronmicrographs of the control crystalline lens on the 4th day of the culture. AP indicates apical portion. The bar indicates a length of 1 micron. ×7, 500.

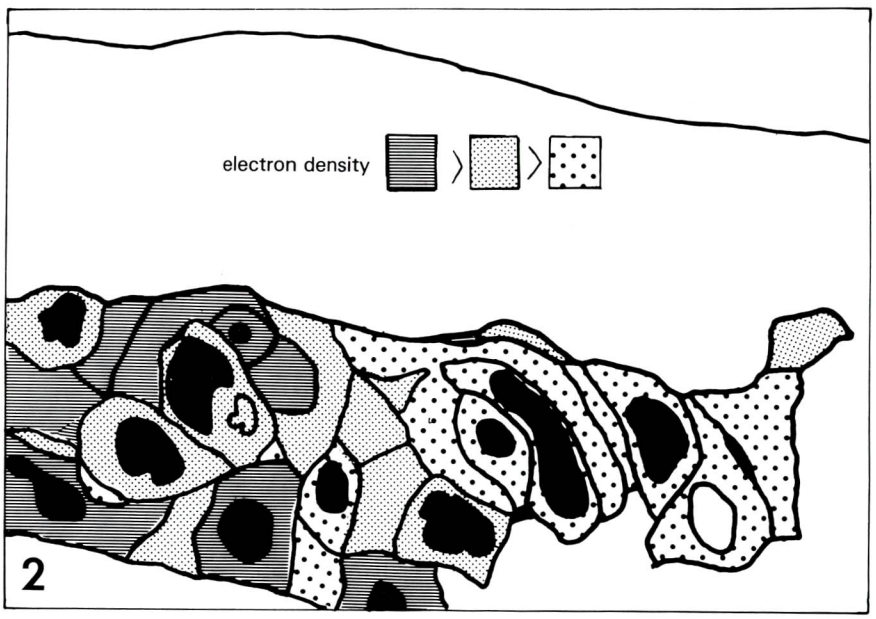

Fig. 2 Distribution of electron dense epithelial cells forming multiple layers in the HU-treated crystalline lens on the 4th day of culture.

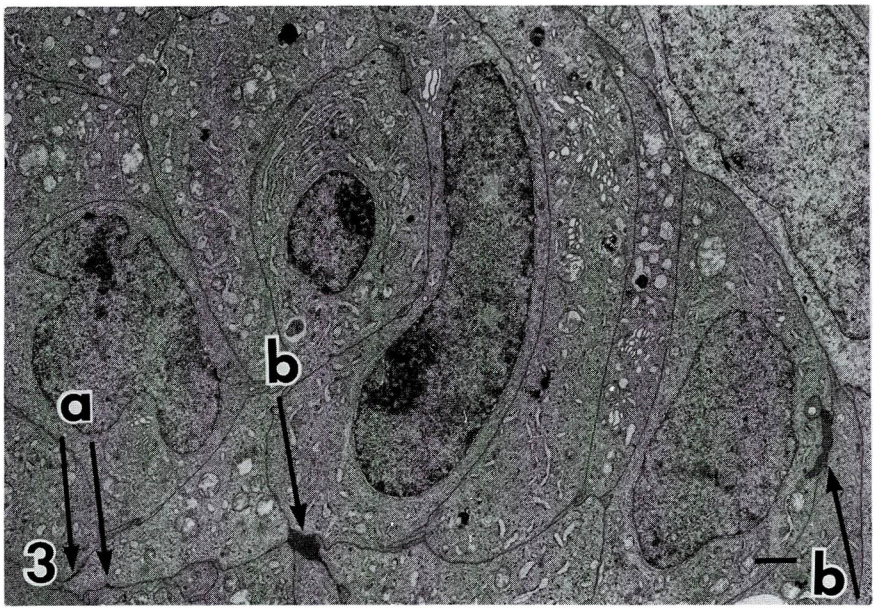

Fig. 3 A group of multiple-layer forming epithelial cells seen in the HU-treated crystalline lens on the 4th day of incubation; the cells are those farthest from the bow area. Elongated cells and systematic arrangement of a desmosome-like structure near the apical portion are seen. A homogenous substance is accumulated in the intercellular spaces. Arrow a (desmosome-like structure), Arrow b (homogenous substance). ×7,500.

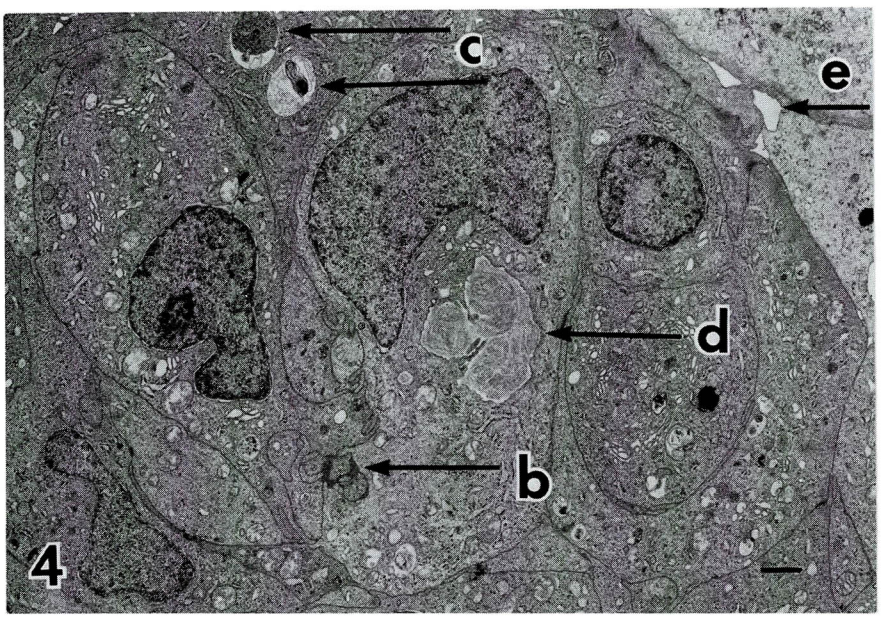

Fig. 4 A group of epithelial cells forming multiple layers near the bow area in the HU-treated crystalline lens on the 4th day of incubation. Among the swollen cells, some are characterized by necrotic debris and vacuole formation. Vesicles or vacuoles are formed in the intercellular spaces. Arrow c (necrotic debris), Arrow d (vesicles), Arrow e (vacuole). ×7,500.

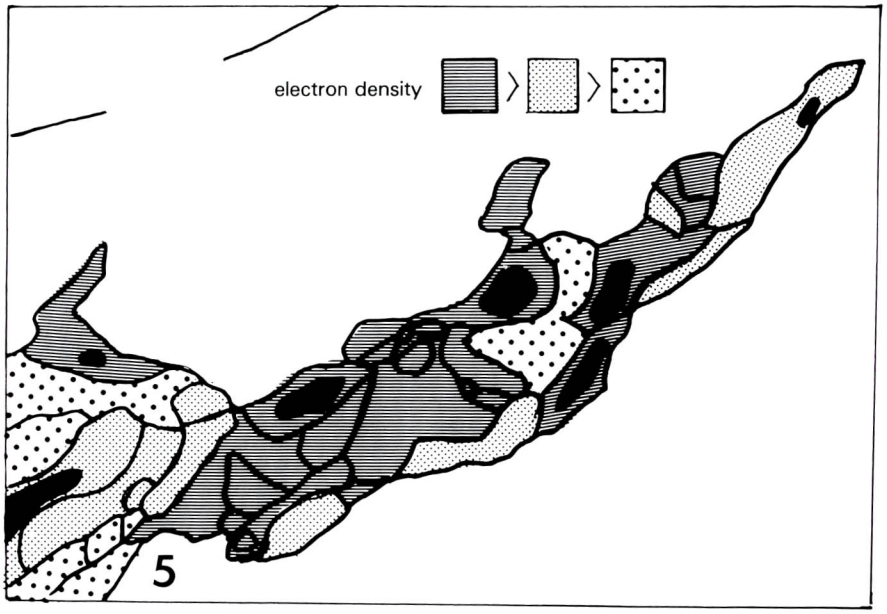

Fig. 5 Schematic view of six-day HU-treated crystalline lens epithelial cells in the bow area (the tissue was exposed to HU for the first 4 days but not for the last 2 days). (See text)

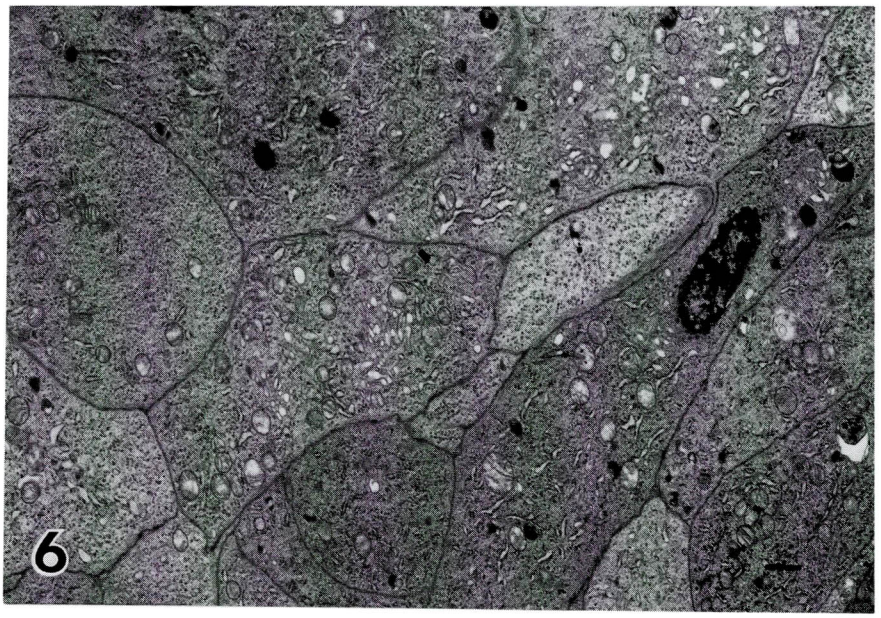

Fig. 6 A group of epithelial cells with high electron density are arranged in a band around the cortex just under the necrotic epithelial cells.

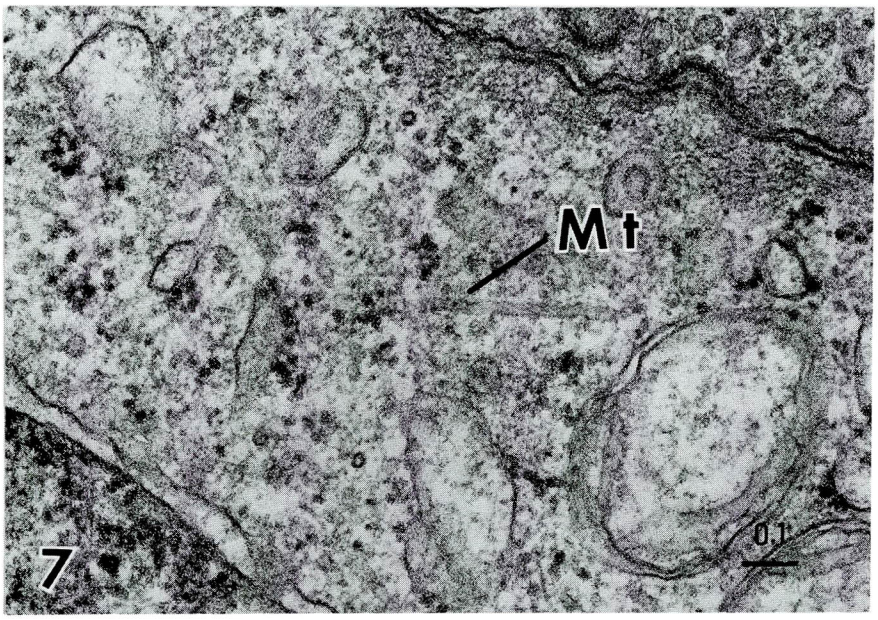

Fig. 7 Band-like epithelial cells have relatively abundant microtubules compared with band-like epithelial cells on the 4th day of HU-treated lens epithelial cells. Mt indicates microtubule.

DISCUSSION

The initial change in cataracts is said to be the development of cells which absorb water and become swollen (hydropic cells) (3, 2). It is well-known that cellular swelling takes place in the equatorial epithelial cells in X-ray induced cataracts (10, 11).

Cogan *et al.* (1) attributed such a cellular phenomenon to a loss of capacity for differentiation by bladder cells and their prolapse toward the posterior capsule. Tokunaga (14) also described accumulation of epithelial cells without differentiating capacity in the equatorial region and their inclusion under the posterior capsule.

The present authors produced experimental cataracts *in vitro* using nucleic acid inhibitors (7, 8, 9). In these studies, accumulation of epithelial cells at the equatorial region and appearance of the swollen cells which are the initial signs of cataracts were also noted as the initial changes in the cataracts.

These phenomena have also been noted in reversible cataracts using HU (9). In the HU-induced cataracts, however, the epithelial cells which accumulated in the equatorial region were elongated below the epithelial cells of the anterior capsule toward the pupillary region. An electron microscopic study was thus conducted to ascertain whether these cells are devoid of differentiating capacity as described by Cogan (1) and Tokunaga (14).

In crystalline lenses which were incubated with HU for 4 days, the epithelial cells forming multiple layers had a desmosome-like structure near

the apical region. Such a finding suggested retention of the epithelial properties by these cells (6). Abundance of polysomes within the cytoplasm indicated that the cells retain the capacity for protein synthesis. Debris of subcellular organella or what appeared to be fragments of cell membranes were noted within the cells, as in the findings for the Fraser cataract described by Hamai (4). Microtubules were abundant in the cortex but were scarce in the epithelial cells in multiple layers. Such a finding may indicate a reduction in the capacity for cellular differentiation (12).

Homogenous substances which accumulated in the intercellular spaces may be components of the culture fluid. This finding resembles the appearance to epithelial cells of the crystalline lens in galactose-induced cataracts in Hamai's study using a tracer (5). It is highly probable that HU participates in the changes occurring in the epithelial cells.

From the above findings, it was ascertained that the epithelial cells in multiple layers, while being forced toward the cortex in the equatorial region, retain a desmosome-like structure for the intercellular junction, continue protein synthesis, process intracellular debris, and achieve gradual reduction in electron density. Ultrastructural morphology and characteristics of subcellular organella indicate retention of the epithelial cellular properties, but the capacity for differentiation is presumed to be impaired. In the crystalline lens which had been exposed to HU only for the first 4 days but incubated without HU for an additional 2 days, the epithelial cells forming multiple layers were elongated and microtubules were abundant. This finding suggested that, at certain times during the pathological process, these epithelial cells are capable of reverting to the original normal state.

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REFERENCES

1) Cogan DG, Donaldson DD and Reese AB: Clinical and pathological characteristics of radiation cataract. AMA Arch Ophthalmol 47: 55—70, 1952.
2) Cogan DG: Anatomy of lens and pathology of cataract. Exp Eye Res 1: 291—295, 1962.
3) Friedenwald JS and Rytel D: Contribution to the histopathology of cataract. Arch Ophthalmol 53: 825—831, 1955.
4) Hamai Y, Kuwabara T: Early cytologic changes of Fraser cataract. Invest Ophthalmol 14: 517—527, 1975.
5) Hamai Y: The permeability of the lanthanum nitrate in the rat lens. Folia Ophthalmol Jpn 28: 1487—1493, 1977.
6) Hamai Y, Takahashi S and Chikuda F: Intracellular communication in the lens. Acta Soc Ophthalmol Jpn 82: 561—571, 1978.
7) Mikuni I, Fujiwara T and Obazawa H: Experimental cortical subcapsular cataract induced by vincristine sulfate *in vitro*. Acta Soc Ophthalmol Jpn 83: 1241—1251, 1980.

8) Mikuni I, Fujiwara T and Obazawa H: Induction of experimental posterior subcapsular cataracts by vincristine sulfate and 1-β-D-arabinofuranosyl-cytosine (ara C). Acta Soc Ophthalmol Jpn 84: 1532—1540, 1980.
9) Mikuni I and Fujiwara T: Histopathology of reversible cataract produced by hydroxyurea *in vitro*. Folia Ophthalmol Jpn 32: 903—907, 1981.
10) Okusawa T: Die experimetelle Untersuchung über die durch Rontgenstrahlen versachten Veranderungen des Linsenepithels. Acta Soc Ophthalmol Jpn 37: 814—835, 1933.
11) Okuda K and Sugimoto S: Histological findings of atomic bomb radiation cataract more than thirty years after irradiation. Folia Ophthalmol Jpn 30: 874—881, 1979.
12) Piatigorsky J, Webster HF and Wollberg M: Cell elongation in the cultured embryonic chick lens epithelium with and without protein synthesis. J Cell Biol 55: 82—92, 1972.
13) Sakuragawa M, Kuwabara T, Kinoshita J and Fukui HN: Swelling of the lens fibers. Exp Eye Res 21: 381—394, 1975.
14) Tokunaga T: Histopathological findings of radiation cataract. Jap J Clin Ophthalmol 17: 587—592, 1938.