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Experimental Posterior Subcapsular Catatacts —with Special Reference to l-Beta-D-Arabinofuranosyl-Cytosine (Ara C)-Induced Cataracts

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Experimental posterior subcapsular cataracts were induced by the action of l-beta-D-arabinofuranosyl-cytosine (ara C) and vincristine sulfate (VCR) in the rat crystalline lens. Differences between these two types of cataracts observed by light microscopy were degenerative necrosis of the anterior capsular epithelial cells (in the ara C-induced cataract), and the transdifferentiation phenomenon of the lens epithelial cells (VCR-induced cataract). Transformation of the epithelial cells and subsequent abnormal cellular differentiation (transdifferentiation) could be the primary trigger in the development of cataracts.

(Key Words: 1-β-D-arabinofuranosyl-cytosine, Vincristine Sulfate, Transdifferentiation, Experimental Posterior Subcapsular Cataract, Rat Lens, Chicken Lens)

INTRODUCTION

An experimental cataract was successfully induced in a rat crystalline lens culture by the actions of 8-methoxypsoralen (hereafter abbreviated to 8MOP) and black light (wave length 298—430nm)(1, 4, 5, 14).

Furthermore, it was discovered that actions of antineoplastic agents extracted from Actinomyces, a fungus (6, 7, 8), a plant alkaloid (vincristine sulfate, hereafter abbreviated as VCR) (9, 10, 11), and synthetic drugs could also cause cataracts of the rat *in vitro*. (12, 13).

In the present study, it was proven that both VCR (a plant alkaloid, a typical inhibitor of microtubules) and 1-beta-D-arabinofuranosyl-cytosine (hereafter abbreviated as ara C, the only known synthetic DNA polymerase alpha — abbreviated as pol-alpha — inhibitor (12, 13) induced cataracts which are grossly similar. However the effects of these two agents on the crystalline epithelial cells markedly differ from each other. Subsequently, it was decided that detailed investigations of the etiological mechanisms of these cataracts will aid in the elucidation of the development of human senile cataracts which morphologically resemble the experimental posterior subcapsular cataracts.

EXPERIMENTAL METHODS

Male white Wistar rats weighing 60 to 70 grams and white Leghorn

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chicks weighing 50 grams were used as the experimental animals. Following decapitation with a guillotine, the crystalline lenses were excised via a posterior approach. The excised lenses were cultured in 4ml of Rat Lens Medium (containing TC199 prepared by the Tokai University Laboratory), to which 5% calf serum (Gibco, U.S.A.) was added, at 37°C in a 5% CO_2 and 95% O_2 atmosphere. Corning petri dishes were used for containers and the culture medium was replaced every 3 days. The crystalline lens from the right eye served as the control. Two μg/ml of VCR (Eli Lilly Company) or ara C (Sigma) was added to the culture medium of the crystalline lens of the left eye of a final concentration of 10^{-5}M. On days 1 and 3, the crystalline lenses were fixed in 10% formalin, dehydrated in alcohol, and embedded in paraffin. With the aid of a Jung type microtome with a Feather S35 knife by Daiwa Koki, Japan, 3μ-microscopic specimens were prepared, stained with hematoxylin-eosin, and observed under microsocpy.

RESULTS

I. Light Microscopic Observations of the Chick Crystalline Lens:

Fig. 1 shows light microscopic findings for uncultured fresh lens. In the anterior capsular epithelial cells, the nuclei were evenly stained and the cells were arranged in a single row in the direction of the bow area. The posterior of the nucleus was generally uniform.

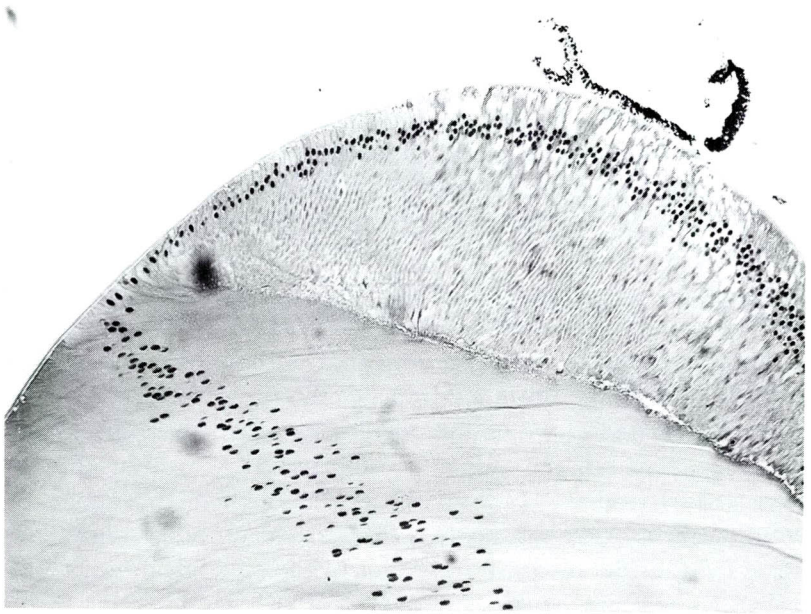

Fig. 1 Fresh lens of a chick weighing 50 grams, hematoxylin-eosin stain, ×150.

Twenty-four hours after the addition of VCR, a reduction of the number of nuclei was seen in the epithelial cells of the anterior capsular region. The epithelial cells were found to have prolapsed in the area closer to the bow area. In the bow area, swelling of the crystalline lens fibers and development of the same bladder cell-like structures were noted (Fig. 2).

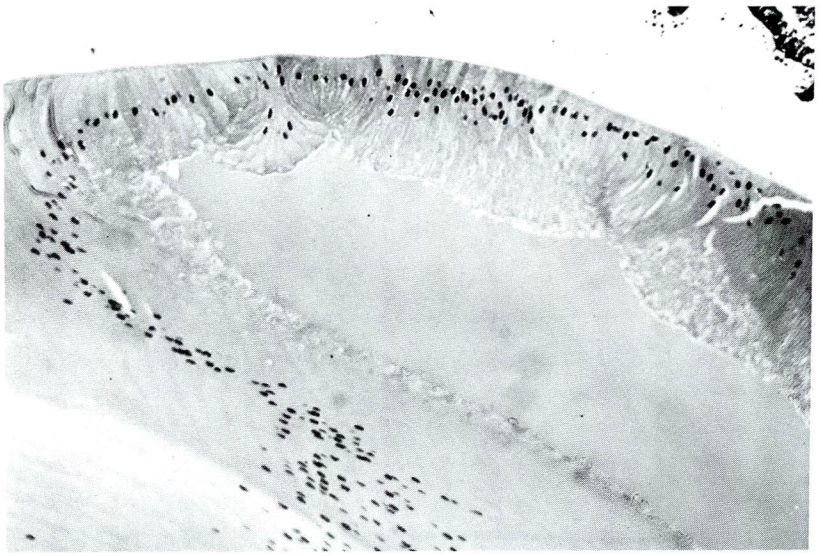

Fig. 2 The crystalline lens of the chick weighing 50 grams. Two μg/ml of VCR has been added to the culture medium. Abnormal differentiation of the bow area epithelial cells has already begun. Hematoxylin-eosin stain, $\times 150$.

Twenty-nine hours after the addition of ara C, the nuclei of the anterior epithelial cells were stained unevenly and some appeared to be undergoing degeneration. The nucleus had prolapsed from the side of the anterior capsule to that of the lens nucleus. In some areas near the anterior capsule at the pupil, a total cellular prolapse was observed (Fig. 3).

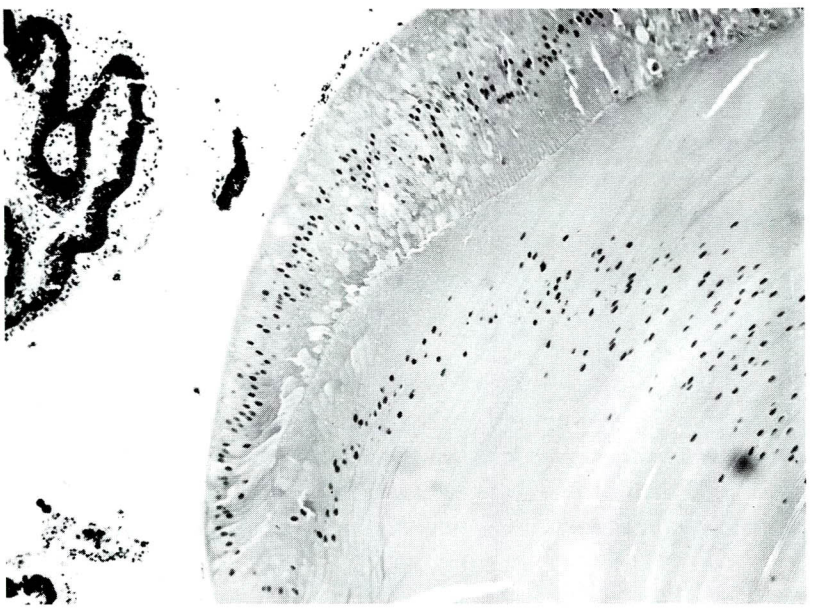

Fig. 3 The crystalline lens of a chick weighing 50 grams. Twenty-nine hours following the addition of 10^{-5}M ara C to the culture medium. Degeneration and necrosis of the epithelial cells have already begun. Hematoxylin-eosin stain, $\times 150$.

II. Light Microscopic Observations of the Rat Crystalline Lens:

A few anterior epithelial cells of the control lens appeared in multiple layers near the bow area but the others remained identical in appearance to the fresh lens (Fig. 4).

Fig. 4 The crystalline lens of a white Wistar rat weighing 60-70 grams. Control on day 3. The anterior capsular epithelial cells have formed multiple layers in the bow area. Hematoxylin-eosin stain, ×150.

On the third day of VCR treatment, the epithelial cells of the lens anterior capsular region still appeared normal. In the bow area, the epithelial cells formed multiple layers. The area was characterized by some cells which had prolapsed and those which had dissociated from the basement membrane of the secondary lens fibers and gravitated toward the posterior capsule. Newly developed bladder cells were noted adjacent to the multiple cell layers. The secondary lens fibers immediately adjacent to these new bladder cells were characterized by a somewhat swollen or shrunken appearance. Macroscopically, formation of vesicles and vacuoles of the anterior cortex was proportional to the extent of turbidity in the region (Fig. 5).

On the 6th day, the anterior capsule epithelial cells of the lens in the ara C-containing medium had undergone necrotic degeneration and lost their integrity, leaving only a little nuclear debris. In the bow area, epithelial cells remained in multiple layers. Vesicle and vacuole formation were noted in proportion to the extent of the posterior cortex turbidity (Fig. 6).

DISCUSSION

Cell death associated with transformation due to inhibition of cellular DNA metabolism has been considered as one of the aging phenomena (15, 16). Cell transformation and disturbances of cell differentiation (transdifferentiation) are believed to be involved in carcinogenesis (17).

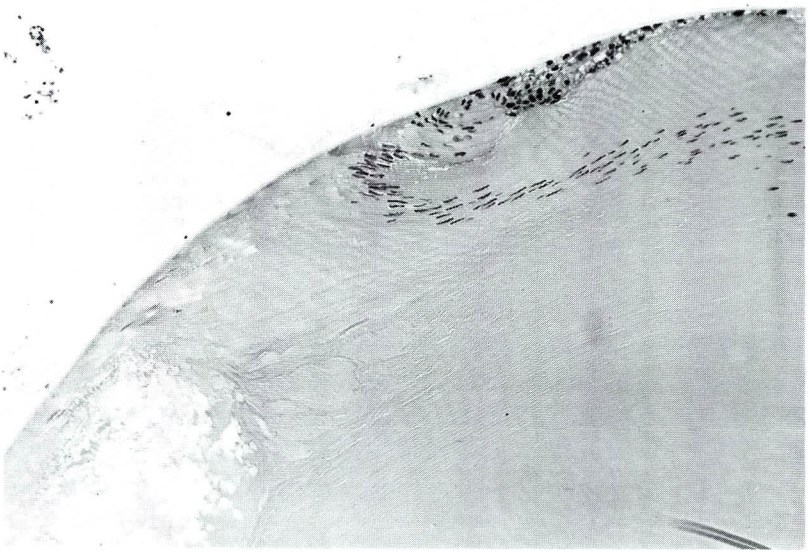

Fig. 5 The rat crystalline lens in the culture medium with 2μg/ml of VCR added. Day 3. Bladder cells are seen in the bow area. Vesicle formation is seen in the posterior subcapsular region. Hematoxylin-eosin stain, × 150.

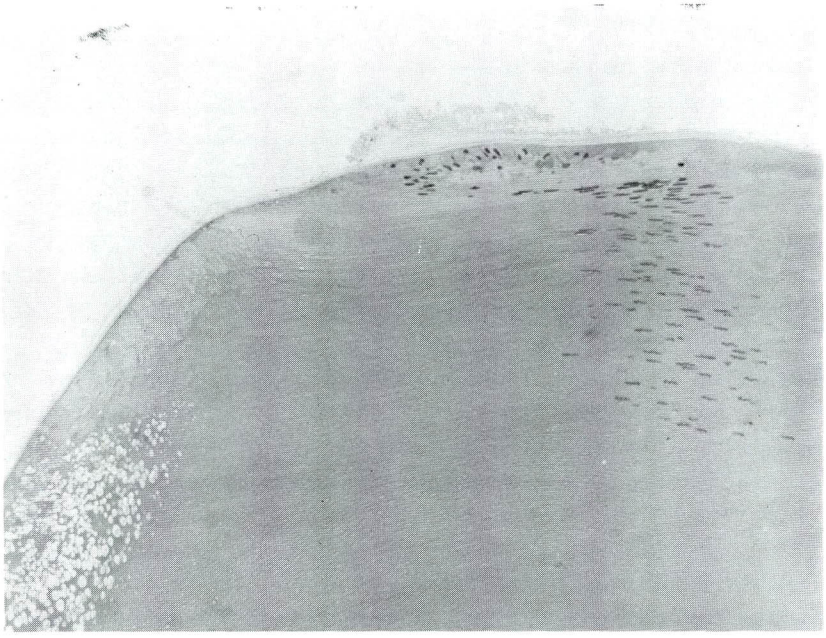

Fig. 6 The rat crystalline lens in the culture medium containing 10^{-5}M ara C. Day 6. Degenerative necrosis of the anterior capsular epithelial cells and formation of vesicles in the anterior subcpsular region are noted. Hematoxylin-eosin stain, × 150.

In the postnatal growth of a normal crystalline lens, the normal differentiation process might participate in the acquisition of lens clarity.

Abnormal cell differentiation has been assumed to be induced by actions of such factors as antineoplastic agents, other synthetic chemicals, and environmental pollutants derived from nature. It is conceivable that the crystalline lens (part of the transparent tissue of the eye) becomes progressively turbid as a result of this disturbance of the cell differentiation process. Consequently, the age-related changes of DNA in the lens epithelial cells and derangement of differentiation of these cells must emerge as a focal point in the elucidation of the mechanism of the development of cataracts.

We have already established that experimental cataracts can be induced by DNA and RNA inhibitors (6, 7, 8). Among these experimental conditions, the cataract induced by ara C indicated involvement of lens epithelial cell DNA polymerase alpha (hereafter abbreviated as pol. alpha) inhibition (12, 13). With the addition of ara C of the cultured cells, chromosonal abbreviation is known to take place (unpublished data). It is most probable, therefore, that cataracts develop as a result of transformation of the lens epithelial cell DNA, subsequent abnormal cell differentiation, and transdifferentiation which are limited to the cells replicable in the serial cultures. Uneven stainability, reduction of the number of nuclei and irregular arrangement of the cells (Fig. 3) of the chick lens in the present study are believed to substantiate this hypothesis. Furthermore, necrosis of the epithelial cells seen in the rat crystalline lens culture seems to illustrate an extreme case of the above-mentioned process. A relatively small change in the bow area, on the other hand, is believed to be due to the less exaggerated effect of ara C (in comparison with VCR) on transdifferentiation into the secondary lens fiber formation.

In the application of VCR, the necrotic process of the lens epithelial cells — both in rat and chick cultures — was less pronounced in comparison to that treated with ara C. In the bow area, however, the abnormal elongation was marked and the development of bladder cells accentuated this abnormality. VCR, together with colchicine and Colcemid® , are known inhibitors of microtubules and responsible for the development of experimental posterior subcapsular cataracts in rats (Table 1) (9, 10, 11). A colchicine-induced experimental cataract is shown in Fig. 7. Its histopathological features are identical to those induced by VCR. Colcemid® has also been found to induce cataracts with similar pathological manifestations (unpublished data). An investigation of the relationship between disturbances of the microtubules and the development of bladder cells is currently being planned. Furthermore, we have already presented data indicating that the development of bladder cells is a result of derangement of the microtubules (9, 10, 11).

Granted that injuries of the lens epithelial cells result in induction of experimental cataracts, three agents — stimulators of lens transdifferentiation (antineoplastic agents, for example), viruses, and radiation — are considered to be the causative factors in these celluar injuries. Among these three agents, the role of the stimulators of lens transdifferentiation will un-

fortunately become greater in proportion to the increase of both natural and man-made environmental pollutants. On the other hand, efforts toward reduction of environmental pollutants may lead to the prevention of senile cataracts.

Table 1. Experimental cataracts and the mechanism of their development.

Type of cataract	Anterior cortical cataract	Posterior subcapsular cataract
Causative agents	8—MOP + ultraviolet ray Mitomycin C Daunomycin	Vincristine sulfate Ara C, Colchicine, Colcemid®
Mode of action	Epithelial cell damage due to abnormal replication	Inhibition of microtubules

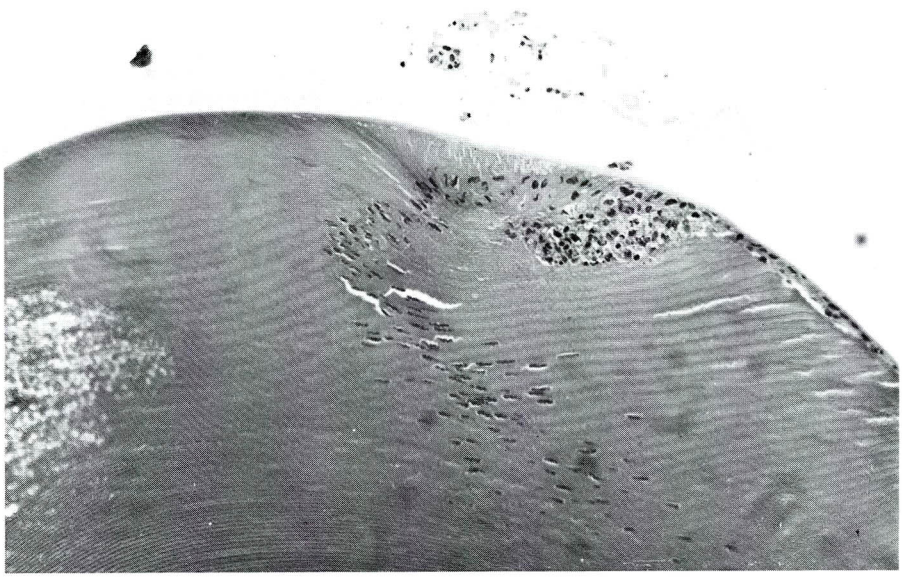

Fig. 7 The rat crystalline lens in the culture medium containing 10^{-5}M colchicine. Day 2. Bladder cells are seen in the bow area. Hematoxylin-eosin stain, ×150.

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