THE PROLIFERATIVE ABILITY OF THE LENS EPITHELIUM IN RAT GALACTOSE CATARACTS —EXAMINATION USING WHOLE-MOUNT PREPARATIONS—

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We gave three-weeks-old rats a diet which included galactose in three different concentrations (15%, 25%, 50%) producing sugar cataracts of three different degrees. Combining the ^3H -thymidine autoradiographic method with the whole-mount preparations of total lens epithelial cells of one lens, we examined the proliferative activity of the lens epithelium in the early stages of cataract crisis (for 14 days). In the lenses of three-weeks-old normal rats, ^3H -thymidine labelled cells were observed mainly in the proliferative zone at the lens equator, and at the same time a few were detected in other parts. On the first day after we started feeding the rats galactose-included diets, the very early stages of cataract formation and an increase in the number of labelled cells were observed in the proliferative zone when compared with the control group on a normal diet. On the fourth day, we found an increase in the number of labelled cells in the proliferative zone and a remarkable ectopic proliferation in other parts. However, in contrast to this, as the cataract progressed from the seventh day to the fourteenth day, we noticed a decrease in the number of labelled cells in the proliferative zone and in the other epithelial layers when compared with the control group. The peak in the number of labelled cells on the fourth day was observed regardless of the galactose concentrations in the diet, and was not proportional to the degree of the cataracts.

In rat galactose cataracts, the cure phenomenon (Diet-Reversal), in which the lens opacity is removed by altering the galactose-including diet to the normal diet, was observed. This Diet-Reversal was proved through light microscopy (17). Moreover, we also reported that aldose reductase inhibitors (ARI) had curative effects and preventive effects on galactose cataracts (1-3, 11, 12). On the basis of the above-mentioned results, we think it is essential that the cataract lens is repaired by the regenerated fibers at the lens equator. That is to say, the examination of the proliferative ability and the kinetics of the regenerated lens epithelium seems to be very important in order to develop the cataract therapeutic agents for the future, though it is impossible at present.

We used the ^3H -thymidine autoradiographic method on thin sections embedded with methacrylate, and proved that, in the developmental process of rat

galactose cataracts, the lens epithelium will repeat the ectopic proliferation even in other parts than the lens equator, and play an important role in the repair of the regenerated lens fibers (13, 14). In particular, on the fourth day after we started feeding rats the 50% galactose-including diet, a remarkable increase in the amount of ectopic proliferation was observed. But to give a conjecture on the whole layer of lens epithelium from only one thin section seemed to be unreasonable. Therefore, in this investigation we used the whole-mount preparations of the lens epithelial layer. In this study, we examined the ability of the rat lens epithelium to proliferate during the critical process of galactose cataracts with time, and investigated the critical mechanism in galactose cataract formation.

MATERIALS AND METHODS

In this experiment, we used Sprague-Dawley strain 39 male rats (each weighing 50 g, three-weeks-old). Three rats were used as normal controls. The other 36 rats were divided into 3 groups of 12, and

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each group was fed the galactose-included diet in a different concentration (15%, 25%, or 50%). We examined each rat on day 1, 4, 7, and 14 after we started feeding the galactose-included diet. For this experiment, we injected 5 μ l of methyl- 3 H-thymidine (81.7 Ci/mmol, New England Nuclear) once into the anterior chamber of each eye. After 2 hr, we used only the anterior segments of the extracted eye balls, incised from the posterior cortex of the lens, and delivered the lens epithelial layer and the capsule of the lens as a lump, from which the whole-mount preparation was made. The preparation was fixed in phosphate buffer (pH 7.4) which included 4% paraformaldehyde.

After a few days fixation, the preparations were applied on the slide, setting the lens capsule downside and the lens epithelium upside. Then using the normal autoradiographic method, these slides were overlaid with the emulsion (Kodak NTB 2), and then the slides were developed, fixed, stained with the contrast stain using toluidine blue. Finally, we observed and recorded the number of labelled silver grains using a light microscopy.

To compare the critical degree of the cataracts in each group given the galactose-included diet at various galactose concentrations, we used other 18 Sprague-Dawley strain male rats. We extracted their eye balls on day 4 and 7 after we started feeding the galactose-included diet, fixed, and embedded them after dehydration using methacrylate, made thin sections and observed them under a light microscopy.

For a quantitative analysis of labelled cells, the

density of these cells was measured using a color image processor, SpiccaII (Avio). A scale with an area of $0.54169 \times 10^{-3} \text{ mm}^2$ was scanned from one end of the equatorial zone to the other. In each group, at least six locations on at least three whole-mount preparations of lenses were examined. Mean values of the labelled cell count (cell count/ $0.54169 \times 10^{-3} \text{ mm}^2$) were plotted graphically against the distance from a fixed end of the equatorial zone.

RESULTS

(1) Three-weeks-old normal rats

The labelled cells were mainly observed locally in the proliferative zone at the lens equator, but were also detected in the other parts evenly and we recognized a few labelled cells in the anterior subcapsular central zone (Fig. 1).

(2) Groups fed the galactose-included diet at different galactose concentrations

On the first day after we started feeding the galactose-included diet in all groups, we observed an increase in the number of the labelled cells only in the proliferative zone (Fig. 2). The degree of ectopic proliferation in the anterior subcapsular central zone was the same in every group.

On the fourth day after feeding, in all groups, we observed a remarkable increase of labelled cells in the anterior subcapsular central zone (Figs. 3, 6). For all galactose concentrations, the increase in the number of labelled cells at the proliferative zone was also observed. We compared the critical degrees of the

FIG. 1. Localization of labelled cells on the whole-mount preparation of lenses of normal three-weeks-old rats. The silver grains are associated with the nuclear area. C: center, E: equator. Bar=1 mm.

cataracts, using the light microscopic sections, fiber swelling and liquefaction spread from the anterior subcapsular area to the posterior subcapsular area and at the lens equator in the 50% group (Fig. 4A). In the 25% group (Fig. 4B), liquefaction was observed only

in a local region from the lens equator to the anterior subcapsular area. In the 15% group (Fig. 4C), cataracts were difficult to observe.

On the seventh day after feeding, in all groups, we recognized a remarkable decrease in the number of

FIG. 2. Localization of labelled cells on the whole-mount preparation of rat lenses first day after the start of a diet including 50%(A), 25%(B), 15%(C) galactose. C: center, E: equator. Bar=1 mm.

FIG. 3. Localization of labelled cells on the whole-mount preparation of rat lenses fourth day after the start of a diet including 50%(A), 25%(B), 15%(C) galactose. C: center, E: equator. Bar=1 mm.

the labelled cells both in the proliferative zone and in the central zone (Figs. 5, 6). The decrease was significant compared with the normal control group. On the 14th day after feeding, we also recognized a decrease in the total number of labelled cells.

DISCUSSION

The fact is that the lens epithelium in the equatorial proliferative zone continues DNA synthesis and mitosis even after its maturation has been investigated in rats, mice and rabbits using ^3H -thymidine autoradiography under a light microscopy

(7-9, 10, 18). In our previous study (13), using similar methods and lenses of three-weeks-old and seven-weeks-old rats, we found a few thymidine-labelled cells (labelled cells), but only in the lens epithelial proliferative zone. In addition, there was no evidence of ectopic proliferation in the anterior subcapsular central zone. Harding *et al.* (9), however, who used whole-mount preparations of the lens epithelium, recognized labelled cells also in the centroanterior subcapsular region. Their results were consistent with the ones reported here which showed considerable ectopic proliferation in the anterior subcapsular epithelium of three-weeks-old rats using whole-mount preparations. However, their methods differed from ours in the way the whole-mount preparations were made and in that they soaked the lenses in the medium containing ^3H -thymidine. Thus, in order to discuss the presence of labelled cells, it may be necessary to examine the whole-mount preparations of the lens epithelium.

For the neonatal rat lenses, however, it has been proved that DNA synthesis and mitosis are taking place in all areas of the anterior subcapsular epithelium as well as in the equatorial proliferative zone by ^3H -thymidine radioautography under a light microscopy (7, 10). For example, Hanna *et al.* (7) revealed the presence of active ectopic proliferation in the lenses of new-born rats between one day and two weeks after their birth, demonstrating DNA synthesis in both the proliferative zone and the anterior subcap-

sular central zone. We obtained similar results using sections of the lenses of new-born rats removed on the day of birth or on the 3rd day after birth. In addition, we observed active ectopic proliferation on the whole-mount preparations of lenses from three-days-old rats (15). Proliferative ability, which all lens epithelial cells share when they are first created, may be lost during maturation, except in the proliferative zone where it is retained all throughout their lives.

It was Harding *et al.* (9) who first described the method to evaluate the proliferative ability of the lens epithelium using ^3H -thymidine autoradiography on the whole-mount preparations of normal lenses from rats, rabbits and frogs. Our report may be the first to apply this method to the study of the pathogenesis of rat galactose cataract as a model for sugar cataracts.

A paper which reported the proliferative ability of the lens epithelium during the development of the rat galactose cataracts using ^3H -thymidine autoradiography on sections has previously been published (6). The writers created the galactose cataracts by feeding six-months-old rats with a diet containing 70% galactose and evaluated the DNA synthesizing ability of the lens epithelium. According to this report, the number of labelled cells peaked at twelve times more than the normal value, on the fourth to sixth day after the start of the diet containing 70% galactose. In addition, they observed these labelled cells equally in every part of the lens epithelium, which indicated the presence of ectopic proliferation in the

FIG. 4. Light microscopic images of sections of rat lenses fourth day after the start of a diet including 50%(A), 25%(B) and 15%(C) galactose. Bar = 500 μm .

FIG. 5. Localization of labelled cells on the whole-mount preparation of rat lenses seventh and fourteenth day after the start of a diet including 50%(A), 25%(B, fourteenth day), 15%(C) galactose. C: center, E: equator. Bar=1 mm.

anterior subcapsular central zone. We, too, performed similar experiments (13, 14), and our results showed that the number of labelled cells in the equatorial proliferative zone and the anterior subcapsular central zone peaked a little earlier than the fourth day after we began to feed the rats the food containing 50% galactose. It was considered that this earlier peak was main-

ly due to the fact that the rats used in this investigation were relatively young three-week-old ones. It is very interesting to note that the peak was reached on the fourth day after the start of feeding, regardless of which concentration of galactose was used.

Moreover, some previous reports had shown the mitotic cycle of rat lens epithelium in the process of

FIG. 6. Image analyzed data of labelled cells on 50% galactose-fed rat lens. Vertical axis indicates the total number of labelled cells. Horizontal axis indicates the number of scanned areas, corresponding to the lens diameter. a: 1st day after feeding galactose diet, b: 4th day, c: 7th day, d: control.

galactose cataract and diabetic cataract. These reports counted the number of mitosis cells on the whole-mount preparations. Cotlier (4) reported that an increase of the average number of mitotic figures of 320% was observed after 3 days of galactose feeding, of 460% after 4 days, and that a decrease of 28% after 5 days was found. Grimes & von Sallmann (5) also mentioned that the wave of increased mitotic activity was rapid on onset, reached a peak at 4 days, and subsided by 7 days under both alloxan-diabetic and galactose-fed rats. It was very interesting to note that their results of 4 days peak of mitotic cells on sugar cataracts corresponded to our data in this study. They also could not give the reasons for these phenomena.

A new finding, which could not be obtained using ^3H -thymidine autoradiography on sections, is a conspicuous decrease in the number of labelled cells in the proliferative zone and the anterior subcapsular central zone when measured seven or fourteen days after the start of galactose feeding. The number is smaller than that found in normal rat lenses of the same age. This is an opposite phenomenon in the development of the cataract, which is interesting in understanding what triggers ectopic proliferation. The density of total lens epithelial cells of one lens on the seventh day was higher than that of the fourth day, using image analyzed data (16). This is the possibility for these decreased number on the seventh day.

We determined the degree of cataract on light microscopic sections as the first step in finding the reason for the peak in the number of the labelled cells four days after the start of the diet and the reason for the conspicuous decrease from the seventh to the fourteenth day. Four days after the start of feeding, a conspicuous fiber swelling and liquefaction extending to the subcapsular regions was observed in the 50% group, whereas cataracts were difficult to observe in the 25% and 15% groups. Therefore, it is unlikely that swelling and liquefaction of the lens fibers on microscopic sections which are indicators of the cataract, are triggers of cell proliferation. In addition, as the cataract progressed, the number of labelled cells decreased below the level in normal rat lenses, when measured seven or fourteen days after the start of feeding. These facts along with above-mentioned evidence suggest that the change in the number of proliferating cells does not correlate with the degree of the cataract. The mechanism of these phenomena awaits further investigation.

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