DNA synthesis and cell division in the cultured ocular lens

C. V. Harding and Mildred Newman Thayer

Adult New Zealand white rabbit lenses were isolated and perfused in culture chambers for periods up to 70 hours. Perfusion with tissue culture medium 199 containing 23 per cent rabbit serum for 46 or 70 hours resulted in the stimulation of many cells in the central area of the epithelial layer to undergo thymidine incorporation and mitosis. There was disorganization of some of the epithelial cells in these cultured lenses. The location of the area of cells in which this disorganization occurred corresponded to the area in which there were thymidine incorporation and mitosis. Lenses perfused for 46 or 70 hours in tissue culture medium 199 containing 23 per cent rabbit serum dialysate tended to show thymidine incorporation in the peripheral region of the epithelial layer, but not in the central area. Disorganization of the cells was not evident in these lenses. Occasionally, small areas of probable injury (most likely sustained at the time of isolation) were present in the lenses. Each such area was surrounded by cells which had incorporated thymidine. The results are discussed in terms of the possible nature of the stimulus to cell division in this system.

In the normal lens of an adult rabbit, cellular proliferation is confined mostly to a band of cells (the germinative zone) which is situated near the equator of the lens. This distribution of dividing cells can be altered following mechanical injury, or following isolation and maintenance of the lens in organ culture. This deliberate alteration of the normal pattern of proliferation might provide information which could be useful in analyzing the underlying mechanism of control of cell division in the normal as well as the injured lens.

It has been found that isolation and maintenance of the rabbit lens in organ culture can result in an activation of some of the cells (those in the central area of the epithelial layer) to undergo proliferation. In the present study, it has been found that this pattern of activated cells can, under certain conditions, be correlated with a structural alteration in the cellular layer. This structural alteration is described, and its possible significance in terms of the activation of cell division is discussed.

Methods and materials

Young American Dutch rabbits (0.7 to 0.9 kilograms) and young New Zealand white rabbits (1.6 to 2.4 kilograms) were used. The lenses were isolated by a procedure employed in earlier studies from this laboratory, or by the method of Bakker. The isolated lenses were then placed in culture chambers and perfused with tissue culture media. A rate of 38 ml per minute was used in all experiments. The perfusion media used were as follows: (1) tissue culture medium 199, (2) 199
Fig. 1. Autoradiogram of epithelium from rabbit lens perfused 46 hours in serum-199. The entire preparation is shown at very low magnification. Note mottled appearance of entire central area of preparation because of uneven distribution of cells. Fig. 3 shows portion of this preparation under higher magnification. (Harris' hematoxylin.)

containing 23 per cent rabbit serum (serum-199), and (3) 199 containing 23 per cent rabbit serum dialysate (serum-dialysate-199). All solutions contained penicillin (200 units per milliliter). The serum and serum dialysate were prepared by the Colorado Serum Co.° For each experiment, the serum and serum dialysate were obtained from the same lot of pooled serum. At the end of the period of perfusion, the lenses were allowed to remain in the chambers. Some of the perfusion medium was removed and replaced with a similar solution containing tritiated thymidine (final concentration in chamber = 5 μc per milliliter; specific activity, 3 curie per millimole). After two hours’ incubation in the radioactive solution, the lenses were fixed in alcohol-acetic acid, 3:1, and whole-mounts of the lens epithelium were subsequently prepared for autoradiography.10,11,12 It was found that under the experimental conditions described there was a certain tendency for cell loss to occur during the preparation of the whole-mounts from American Dutch rabbits, whereas whole-mounts from the larger New Zealand white rabbits showed very little or no cell loss. Also, in these experiments, the larger New Zealand white rabbit lenses remained more transparent than lenses from American Dutch rabbits of the weight range used. In some experiments, the lens epithelium was treated with Feulgen stain prior to autoradiography. In other experiments, it was stained with Harris' hematoxylin after autoradiography.

Results
The distribution of radioactive nuclei in lenses perfused 46 hours with serum-199 differed consistently from those perfused for the same time in serum-dialysate-199. For example, Fig. 1 shows, under very low magnification, the entire epithelial preparation of a lens perfused 46 hours in serum-199. The central area of the same preparation under higher magnification (Fig. 3) shows many radioactive nuclei in this region. The tendency for radioactive nuclei to be distributed within the central area

°The serum dialysate was prepared by dialyzing serum against an equivalent volume of glass-distilled water for 24 to 30 hours. The dialysate thus represents the dialyzable components of serum, present at approximately half their normal concentration in serum. These experiments do not, therefore, give us information on the comparative effectiveness of whole serum and the dialyzable fraction of serum.
Fig. 2. Autoradiogram of epithelium from rabbit lens perfused 46 hours in serum-dialysate-199. The entire preparation is shown at very low magnification. Note presence of radioactive nuclei distributed in the form of a wide ring. No radioactive nuclei appear in the center. Fig. 4 shows portion of epithelium from center of this same preparation. (Harris' hematoxylin.)

Fig. 3. Autoradiogram of epithelium from central region of lens perfused 46 hours in serum-199 (same preparation shown in Fig. 1). Note presence of radioactive nuclei and uneven distribution of the cells. (Harris' hematoxylin.)
Fig. 4. Autoradiogram of portion of epithelium from center of preparation from lens perfused 46 hours in serum-dialysate-199 (same preparation shown in Fig. 2). Note absence of radioactive nuclei and mitotic figures and the relatively uniform distribution of the cells compared with Fig. 3. (Harris' hematoxylin.)

Fig. 5. Autoradiogram of epithelium from lens perfused 46 hours in serum-199. The entire preparation is shown under very low magnification. Radioactive nuclei are present in the central area. This can be verified in Fig. 7, which shows this preparation under high magnification. Note mottled appearance of a portion of the central area because of uneven distribution of the cells. (Harris' hematoxylin.)
Table I. Summary of results of perfusion of New Zealand white rabbit lenses for 46 and 70 hours with serum-199 and serum-199-dialysate

<table>
<thead>
<tr>
<th>Perfusion time</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum-199</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>46 hours</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>70 hours</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Serum-199-dialysate*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>46 hours</td>
<td>0</td>
<td>1</td>
<td></td>
<td>1</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>70 hours</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Classification criteria for serum-199-dialysate lenses:
0—No detectable radioactive nuclei (r.n.).
1—Very small number r.n. in periphery.
2—Wide ring, with relatively small number r.n. (essentially nothing centrally).
3—Wide ring, with intermediate number r.n. (essentially nothing centrally).
4—Wide ring, with very large number r.n. (essentially nothing centrally).
5—No activation in center of preparation.

†No activation in center of preparation.
§Show mottling.

was characteristic of the serum-199 46 hour lenses. Fig. 2 shows the other lens from the same rabbit, perfused for 46 hours in serum-dialysate-199. This preparation has essentially no radioactive nuclei in the center (as can be seen under higher magnification in Fig. 4). It does, however, have a large number of radioactive nuclei in the peripheral region. The tendency for the radioactive nuclei to be distributed in the form of a peripheral band was characteristic of the serum-dialysate-199 46 hour lenses. The intensity of the reaction, however, varied somewhat from experiment to experiment (Table I). Figs. 5 and 7, for example, show under low and high powers, respectively, the preparation from another serum 46 hour lens; and, Figs. 6 and 8, the preparation from a serum-dialysate-199 46 hours lens. Again the two lenses were from the same animal. The peripheral band shown in Fig. 6 consisted of a relatively

Fig. 6. Autoradiogram of epithelium from lens perfused 46 hours in serum-dialysate-199. Entire preparation is shown under very low magnification. Note distribution of radioactive nuclei in the form of a wide ring. This ring is more intense than in the one shown in Fig. 2. Compare with Fig. 8, which shows center of this preparation under higher magnification. (Harris' hematoxylin.)
Fig. 7. Autoradiogram of epithelium from central region of lens perfused 46 hours in serum-199 (same preparation shown in Fig. 5). Note presence of radioactive nuclei and uneven distribution of the cells. Mitotic figures are evident. (Harris' hematoxylin.)

Fig. 8. Autoradiogram of portion of epithelium from center of preparation from lens perfused 46 hours in serum-dialysate-199 (same preparation shown in Fig. 6). Note absence of radioactive nuclei and mitotic figures and the relatively uniform distribution of the cells compared with Fig. 7. (Harris' hematoxylin.)
Fig. 9. Autoradiogram of epithelium from lens perfused for 70 hours in serum-199. Entire preparation is shown under low magnification. Note persistence of mottled appearance. Radioactive nuclei and mitotic figures are present within the mottled area. (Harris' hematoxylin.)

Fig. 10. Autoradiogram of epithelium from lens perfused for 70 hours in serum-dialysate-199. Entire preparation is shown under low magnification. Note absence of mottled appearance. Radioactive nuclei are present in peripheral band which encircles preparation. A portion of this peripheral band is shown in Fig. 11. (Harris' hematoxylin.)
Fig. 11. Portion of peripheral band of radioactive nuclei from lens perfused 70 hours in serum-dialysate-199 (same preparation shown in Fig. 10). (Harris’ hematoxylin.)

large number of radioactive nuclei compared with Fig. 2.

Radioactive nuclei were still evident in the central region of serum-199 lenses perfused 70 hours (Fig. 9), and the peripheral ring of radioactive nuclei were also still evident in serum-dialysate-199 lenses perfused for 70 hours (Figs. 10 and 11).

Another difference between the serum-199 and the serum-dialysate-199 lenses was the distribution of cells within the area of intense DNA-synthetic and mitotic activity. Typically, within the highly activated areas of the New Zealand white rabbit lenses perfused with serum-199, there were many small, roughly circular areas in which the cells appeared relatively large or spread out. The remaining cells were present as a more dense population. This is evident in Figs. 7, 12, and 13. This distribution gave a “mottled” appearance to the preparation under low power (Figs. 1, 5, and 9). Other experiments carried out under similar conditions, but with small American Dutch rabbits (approximately 1 kilogram), showed central activation of both DNA synthesis and mitosis, but did not have this mottled appearance.

The major experimental results obtained with the New Zealand white rabbits are summarized in Table I. The autoradiograms are classified according to the relative number and distribution of radioactive nuclei. The classification was done by visual estimation. It can be seen that all of the serum-199 46 hour lenses had some degree of activation within the central area. Sixteen out of the 28 lenses (57 per cent) showed an intense central activation. It will be noticed that all of the lenses showed some degree of mottling. On the other hand, none of the serum-dialysate-199 46 hour lenses showed a significant activation in the very center of the epithelial layer. These lenses tended to show a ring of radioactive nuclei of variable thickness and intensity. The autoradiograms from these lenses were classified according to the width and intensity of this ring. Even the most intense autoradiograms from these lenses, however, showed no evidence of mottling.
In order to demonstrate the over-all distribution of mitotic figures within the epithelial layer, counts were made of mitotic figures within each of a series of microscopic fields across the entire preparation from a serum-199 lens which showed a pronounced activation. The results in Table II indicate that the mitotic figures

Fig. 12. Feulgen preparation of portion of central region of lens perfused 46 hours in serum-199. Note regions of high cell density, which surround areas of low-cell density. Many mitotic figures are evident. Compare with Fig. 13. (Feulgen stain.)

Fig. 13. Higher magnification of portion of preparation from lens perfused 46 hours in serum-199, shown in Fig. 12. Note the mitotic figures. (Feulgen stain.)
Table II. Number of mitotic figures in each of a series of fields across the diameter of epithelial preparation from serum-199 46 hour lens

| Field No. | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 |
|-----------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| No. of mitotic figures | 0  | 4  | 18 | 35 | 37 | 42 | 25 | 22 | 25 | 50 | 35 | 37 | 42 | 25 | 22 | 25 | 50 | 35 | 37 | 42 |

The preparation had been treated with Feulgen stain. Each field is 420 μ square.

are present in abundance in the central area, but not in the periphery. In the present experiments, the lenses were not removed from the perfusion chambers in order to incubate in thymidine. The perfusion medium was removed, either in whole or in part, and replaced with the radioactive solution. This involved no manipulation of the lenses and required very little time. The majority of serum-199 46 hour lenses from these experiments had large numbers of mitotic figures. The relatively large numbers found, as compared with previous experiments in which the lenses were removed to another vessel for incubation in the radioactive solution, may be because of this change in procedure.

Occasionally lenses were noted which showed evidence of injury, probably sustained at the time of dissection. Such probable areas of injury were usually at or near the periphery, and were characterized by a local disorganization of the cells. The cells in this area stained more deeply with Harris' hematoxylin and there were radioactive nuclei and mitotic figures present. Fig. 14 shows an area of epithelium, from a serum-dialysate-199 46 hour lens, which contains two probable injury sites. In this case, each site is surrounded by a ring of radioactive nuclei. Such injury reactions have also been seen in lenses maintained in the culture system of Bito. They are of particular interest in relation to the in vivo reaction to mechanical injury, in which case a wave of stimulation of DNA synthesis and mitosis moves slowly outward from the site of injury.16

Fig. 14. Portion of epithelium from lens perfused 46 hours in serum-dialysate-199. Note two areas of probable injury (most likely sustained at time of isolation). Each is surrounded by a number of radioactive nuclei. (Harris' hematoxylin.)
Discussion

Interesting features of the highly activated lenses perfused with serum-199 for 46 hours are the distribution and general appearance of the cells as compared with those perfused with serum-dialysate-199. As the low-power photograph of an autoradiogram in Fig. 5 indicates, the central portion of the epithelial layer appears relatively dark compared with the periphery. Examination under higher magnification (Fig. 7) shows that this is due to the presence of radioactive nuclei and also to the presence of dense areas of stained nuclei. Mitotic figures are also evident in abundance. Roughly circular areas in which the cells appear relatively large or spread out are surrounded by dense areas of cells (more cells per unit area.) This distribution lends a mottled appearance to the preparation under low magnification. In fact, in most experiments it has been possible to detect this motting at the time the layer is removed from the lens during preparation of the whole-mounts (before staining and autoradiography). The serum-199 46 hour lenses can be distinguished from the serum-dialysate-199 46 hour lenses even at this time.

Wherever there is motting in the serum-199 46 hour New Zealand white rabbit lenses, there are radioactive nuclei and mitotic figures. The mottled pattern and associated central activation of thymidine incorporation and mitosis may possibly, therefore, be interrelated. Recent studies have indicated that the pattern, in at least an abbreviated form, can appear after 22 hours of perfusion with serum-199, in which case a small number of associated radioactive nuclei are evident. The possibility that such structural changes represent a primary stimulus to cell division or are in some way related to the primary stimulus remains to be determined.

It has also been observed by Bito\textsuperscript{14} that under certain conditions rabbit lenses, maintained for long periods of time in a special culture system devised by this worker, have circular areas of nonproliferating cells, each such area surrounded by a relatively high cell density containing cells undergoing thymidine incorporation. His recent dissertation\textsuperscript{15} should be consulted for a discussion of these experiments, in which the role of physical factors in regulating cell division in the lens has been emphasized.

Cogan\textsuperscript{16} has emphasized the importance of wrinkling of the capsule as a common stimulus for cellular proliferation. Evidence of proliferation has been seen subadjacent to small folds in the lens capsule. The epithelial cells thus fill in the space between the capsule and the fibers. Cogan's evidence that a lifting of the capsule (with adhering cells) from the lens fibers is a stimulus for cell proliferation could explain the results obtained in the present experiments. The marked rearrangement of the epithelial cells seen in Figs. 1, 3, 12, and 13 (motting) might well involve a separation of the capsule-cell layer from the fibers. It is also conceivable that such a separation could occur in the absence of a motting pattern, and thus explain the activation seen in the American Dutch serum-199 46 hour lenses, as well as some of the serum-dialysate-199 46 hour lenses described (Fig. 6). Such possibilities might be tested by examination of the histology of suitably prepared lenses.

Although structural alterations may play a role in the activation, it seems that the chemical composition of the perfusion medium can alter the nature of the response. The pattern of distribution of DNA-synthesizing cells and mitotic figures can be affected by the composition of the perfusion medium. As reported, rabbit lenses can be perfused for 46 or 70 hours with serum-dialysate-199 without extensive stimulation of the central area, even though the cells in this region appear normal and many cells in the periphery may be undergoing DNA synthesis and mitosis. On the other hand, the central area of lenses perfused for 46 or 70 hours with serum-199 can show an extensive activation.

The relative contributions of physical
and chemical factors to the activation remain unresolved. If the primary stimulus to cell division involves a physical alteration, it may be characterized by structural changes which might be detected with slit-lamp examination, or in suitably prepared cross-sections for light and electron microscopy. Such investigations are in progress.

REFERENCES


