Autoradiographic studies on the growth and development of the lens capsule in the rat

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Growth of the rat lens capsule was analyzed by autoradiography, following injection of $^{35}$S-sulfate or $^3$H-glycine. Although growth was most rapid in young animals, it continued well into adulthood, accompanied by the development of increasing regional variations in thickness. Capsular labeling with both precursors paralleled these dimensional changes, being highest in young animals and greater in regions which thickened most rapidly, with one exception: $^{35}$S-sulfate was heavily incorporated into the capsule at the site of attachment of the suspensory ligament, which was not a site of preferential thickening. A strong staining reaction for acid mucopolysaccharides was obtained in this zone, suggesting that the localized concentration of $^{35}$S may reflect a regional difference in composition of the capsule. The labeled precursors were initially incorporated by the superficial cells of the lens, and subsequently transferred to the capsule, indicating that the capsule was a product of the subjacent cells. Thickening of the capsule gradually displaced the radioactivity outward, away from the cells, due to the deposition of new capsular material on the inner surface. This lamellar mode of apposition may be related to the lamellated fine structure of the capsule.

The mammalian ocular lens, suspended within the aqueous humor, is completely enveloped in an acellular capsule (Fig. 1), which effectively isolates the epithelial population of lens cells from contact with any other cell type. Recent work has strengthened the view that this capsule is a type of basement membrane. Labeled antibodies prepared against basement membranes from kidney or placenta react with the lens capsule. Lens capsule, like other basement membranes, reacts intensely with the periodic acid-Schiff staining technique. Furthermore, there is increasing evidence that basement membranes are epithelial derivatives. As will be shown in this paper, this is also the case with the capsule of the lens. In addition to its importance as a model of basement membranes in general, the development of the lens capsule is of interest in its own right, particularly in view of its significance in the metabolism and function of the lens.

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This work was supported by the United States Public Health Service Grant NB-03807.
A number of early investigators considered the capsule to be fibrous in nature and mesodermal in origin. Some felt that it was a product of the network of capillaries which envelops the lens early in development. One current opinion is that the capsule is a condensation of the collagen-like basement membrane material which initially binds together the intraocular epithelia. Others contend that basement membranes do not participate in the formation of the lens capsule.

Recent writers have tended toward the opinion that the lens capsule is a product of the lens epithelium. The epithelium can repair an injury to the overlying capsule in the adult lens, and can produce capsuleslike material to isolate necrotic debris in the cataractous lens. However, this raises the question of how the lens epithelium, situated anteriorly, can mediate the synthesis of that part of the capsule which coats the posterior surface of the lens. It was once suggested that this portion of the capsule may be synthesized by lens fibers, but no convincing evidence has been presented to support this contention.

Some authors have avoided this problem by simply stating that the capsule is a "secretory product of the lens" produced by "embryonic lenticular cells." It is apparent that the process of formation of the lens capsule, and its expansion about the growing lens, is essentially an unsolved problem.

The autoradiographic studies described below are based upon the observation that $^{35}$S-sulfate is preferentially incorporated into the lens capsule in young rats. In addition, use has been made of $^3$H-glycine, which is also readily incorporated into the growing capsule. To our knowledge, there is no previous experimental work on the normal growth and development of this structure.

**Methods**

In one series of experiments, 71 Long-Evans rats were injected with $^{35}$S-sulfate at 1, 2, 4, 8, or 18 weeks of age. From 8 to 24 animals comprised each of the age groups. The younger rats were unselected as to sex; only females were used in the two oldest groups. The animals were injected intraperitoneally with 4 to 8 $\mu$g per gram of body weight of carrier-free $^{35}$S-sulfate diluted with isotonic saline so that the required dose was contained in a volume of 0.1 to 0.5 ml. The rats were then killed painlessly at intervals from 30 minutes to 3 weeks after injection.

In a second series, 20 Long-Evans rats, 1 week old and unselected as to sex, were injected intraperitoneally with 0.1 ml. of an isotonic saline solution containing 5 to 20 $\mu$g per gram of body weight of $^3$H-glycine. These animals were put to death at intervals between 15 minutes and 9 weeks later. Two additional rats were injected with 20 $\mu$g per gram of tritiated glycine at 9 weeks of age, and sacrificed 1 week later.

The eyes from one rat 92 weeks of age were obtained for histological examination only. The eyes from each animal were fixed in Bouin-Hollande solution, double-embedded in nitrocellulose-paraffin, and sectioned sagittally at 5 $\mu$. Sections were prepared for autoradiography by coating with Kodak NTB2 liquid emulsion by the dipping technique, followed by exposure at 4°C under low humidity for 1 to 12 weeks. They were then developed in Kodak Dektol for 2 minutes at 17°C, and fixed in Kodak acid fixer. Sections were stained with hematoxylin after development. Many of the sections were also stained with periodic acid–Schiff (P.A.S.) prior to autoradiography. For histological examination only, addi-
 Autoradiographic studies of lens capsules

Volume 5
Number 6

585
tional sections were stained with Mowry's modification of the colloidal iron technique for acid mucopolysaccharides, or P.A.S.-hematoxylin.

The thickness of the lens capsule at 1, 2, 4, 8, and 18 weeks of age was measured with an ocular micrometer at 100 µ intervals throughout the capsular circumference in sections taken at or near the midline. The distribution of capsular radioactivity was estimated in autoradiograms by similarly spaced silver grain counts (obtained with an ocular disc calibrated in squares) in each of the several age groups.

Results

The network of capillaries which surrounds the lens in 1-week-old rats became progressively attenuated in older animals, and had disappeared by 4 weeks of age. In the equatorial zone of lens fiber formation, the cells of the lens epithelium were columnar in form. Anteriorly, in the younger animals, they were cuboidal, except near the anterior pole, where they were further reduced in height. With age, the epithelial cells became increasingly flattened, and were distinctly squamous in the 92 week rat (Figs. 3-14).

Growth of the lens capsule was most rapid in young animals, but continued throughout the period studied. The increase in size included growth in area—reflected in the progressively greater distance from anterior to posterior pole—as well as an increase in thickness (Fig. 2). The rate of thickening varied regionally. In 1-week-old rats, the capsule was slightly thicker anteriorly, but localized differences were not prominent. During succeeding weeks, the increase in thickness was appreciably greater on the anterior surface. At 4 weeks of age and later, capsular thickness increased sharply, moving anteriorly from the equator, then was fairly constant, except for a minor thinning near the anterior pole (Fig. 2). In the oldest animals, a slight decrease in thickness at the equator and posterior pole was superimposed over the anterior-posterior differences evident in the younger rats. Continued growth of the lens capsule during adult life is indicated by the fact that at 92 weeks the capsule was significantly thicker anterior to the equator than at 18 weeks (Figs. 3-14).

Autoradiographic analysis of lenses from animals injected with 35 S-sulfate revealed that incorporation of inorganic sulfate was negligible in the lens in all age groups,
Fig. 3 to 14. For legends see opposite page.
except in the epithelium and the superficial lens fibers which were in contact with the lens capsule. Thus, the initial reaction was essentially restricted to the lens epithelium, anteriorly, and the posterior extremities of the immature and growing lens fibers posteriorly (Figs. 15, 16, 23). Within 1 hour after injection, some capsular labeling was evident. By 2 hours, radioactivity could be detected in the lens capsule throughout its extent, posteriorly as well as anteriorly (Figs. 21-23). By 3 to 4 hours, practically all of the initial cellular labeling had been displaced into the lens capsule (Fig. 17).

In the two youngest age groups, in which remnants of the capillary network were present, a reaction over the endothelial cells was observed, but there was no indication that the radioactive material was transferred to the capsule. The nearby ciliary epithelium also showed significant $^{3}$H labeling, which declined markedly during the first day after injection.

The intensity of the autoradiographic reaction varied with age, as well as regionally within the capsule in each age group. Labeling was very intense in the youngest animals, and progressively declined in older rats (Figs. 18-29). In the 1- through 8-week-old rats, the reaction was more intense anteriorly than posteriorly. In these age and regionally related respects, then, labeling intensity corresponded with growth in thickness. However, one striking exception was observed. In a zone slightly anterior to the equator, there was a marked concentration of radioactivity. (In the 18-week-old series, this was the only region in which significant labeling was detected at the dosage and exposures used.) Peak labeling occurred some 150 to 200 $\mu$ anterior to the equator, and extended posteriorly past the equator, and anteriorly through and slightly beyond the epithelial cell proliferative zone (Fig. 2). This regional concentration of labeling was apparently stable. It was still evident without noticeable change 3 weeks after injection (Figs. 18-20, 24-26). In fact, there appeared to be little or no significant loss of capsular labeling in any region in any of the age groups within the period studied.

In animals injected with $^{3}$H-glycine at 1 week of age, a heavy initial incorporation in all the lens cells was observed, although the reaction was most intense in the growing fibers near the surface. Some radioactivity could subsequently be detected throughout the capsule, posteriorly as well

Fig. 3 to 14. Photomicrographs. (P.A.S.-hematoxylin stain. $\times 1,150$.)
Fig. 3. One-week-old rat, lens capsule, region near anterior pole. Note the small blood vessel on the outer surface of the capsule. The lens epithelium is low cuboidal.
Fig. 4. One-week-old rat, lens capsule, region between equator and proliferative zone (cf. Fig. 1). Note the blood vessel on the outer surface of the capsule, and the mitotic figures in the epithelium. The epithelium is columnar.
Fig. 5. One-week-old rat, lens capsule, region near posterior pole. Note the blood vessel, and the absence of an epithelial cell layer.
Fig. 6. Ten-week-old rat, lens capsule, region near anterior pole.
Fig. 7. Ten-week-old rat, lens capsule, region between equator and proliferative zone.
Fig. 8. Ten-week-old rat, lens capsule, region near posterior pole.
Fig. 9. Eighteen-week-old rat, lens capsule, region near anterior pole.
Fig. 10. Eighteen-week-old rat, lens capsule, region between equator and proliferative zone.
Fig. 11. Eighteen-week-old rat, lens capsule, region near anterior pole.
Fig. 12. Ninety-two-week-old rat, lens capsule, region near anterior pole. Note that in this region the capsule is thicker than it is in the comparable region of the 18-week-old adult capsule (Fig. 9). The epithelium is squamous.
Fig. 13. Ninety-two-week-old rat, lens capsule, region between equator and proliferative zone. The capsule has continued to thicken in this region also (cf. Fig. 10). The epithelium is squamous.
Fig. 14. Ninety-two-week-old rat, lens capsule, region near posterior pole. No additional thickening of the capsule has occurred in this region.
Figs. 15 to 26. For legends see opposite page.
as anteriorly, within 2 hours after injection. Labeling of the walls of the extracapsular capillary network was observed at early intervals, and gradually declined as the vessels were resorbed over the succeeding 3 weeks. The ciliary epithelium was also heavily reactive, but became depleted of most of this labeling within 24 hours after injection.

Capsular labeling was somewhat higher anteriorly, where thickening of the capsule was greater (Fig. 2). In this respect, the distribution of radioactivity was similar to that obtained with 35S-sulfate. However, with 3H-glycine as precursor, there was no regional intensification of capsular labeling in the vicinity of the equator (Fig. 2).

As early as 1 week after injection of tritiated glycine, there was an indication that the autoradiographic reaction over the anterior portion of the capsule was heavier near its outer, free surface. In subsequent weeks, the labeled portion of the capsule was progressively displaced outward, away from the subjacent cells, by the apposition of additional capsular material from within. In rats injected with 3H-glycine 5 to 9 weeks before sacrifice, the reaction in the thicker parts of the capsule occurred as a thin, discrete band on the outer surface of an otherwise essentially unreactive membrane (Fig. 30). The same process was discernible in the 35S-sulfate experiments (Fig. 24), but was less striking due to the lower autoradiographic resolution obtained with this radioisotope.

In the rats injected with 3H-glycine at 9 weeks of age, and killed 1 week later, capsular labeling was most intense in a narrow band near the inner (lenticular) surface of the anterior part of the capsule (Fig. 31). No heightened labeling near the equator was observed.

The capsule stained uniformly in its entirety with the periodic acid-Schiff procedure. The colloidal iron sequence for

Figs. 15 to 26. Autoradiograms. (35S-sulfate injection. P.A.S.-hematoxylin stain. x1,150.)
Fig. 15. Two-week-old rat, killed one-half hour after injection, region near anterior pole. The radioactivity is localized in the lens epithelium.
Fig. 16. Two-week-old rat, killed 1 hour after injection, region slightly posterior to the equator. The labeled material is concentrated in the extremities of the growing lens fibers, in contact with the capsule. Some radioactivity appears to be present in the capsule.
Fig. 17. Two-week-old rat, killed 3 hours after injection, region near anterior pole. Labeled material, present initially in the epithelium (cf. Fig. 15), has been transferred to the lens capsule (partially detached).
Fig. 18. Four-week-old rat, killed 3 weeks after injection, region near anterior pole. The capsule is radioactive.
Fig. 19. Four-week-old rat, killed 3 weeks after injection, region between the equator and proliferative zone. This is the region of most intense capsular labeling after injection of 35S-sulfate.
Fig. 20. Four-week-old rat, killed 3 weeks after injection, region near the posterior pole. The capsule, which is thinner in this region, is less heavily labeled.
Fig. 21. Two-week-old rat, killed 2 hours after injection, region near anterior pole. The transfer of radioactive material from epithelium to capsule is partially completed.
Fig. 22. Two-week-old rat, killed 2 hours after injection, region between equator and proliferative zone. Radioactivity is present in the capsule and in the outer portion of the epithelial cells.
Fig. 23. Two-week-old rat, killed 2 hours after injection, region near posterior pole. Radioactivity is present in the capsule and in the immediately adjacent portion of the underlying lens fibers.
Fig. 24. Five-week-old rat, killed 3 weeks after injection, region near anterior pole. The outer part of the capsule is labeled more intensely than the inner part.
Fig. 25. Five-week-old rat, killed 3 weeks after injection, region between equator and proliferative zone. The capsule continues to be most heavily labeled in this region.
Fig. 26. Five-week-old rat, killed 3 weeks after injection, region near posterior pole. The capsule is discretely labeled.
Figs. 27-33. For legends see opposite page.
acid mucopolysaccharides also stained the capsule, but only in the general region of the equator was it intense. Here, in all preparations, the capsule was stained a distinct blue color, which was heaviest slightly anterior to the equator, and decreased in intensity anteriorly and posteriorly (Figs. 2, 32). The colloidal iron-positive zone corresponded with the zone of regionally heightened ³⁵S-sulfate accumulation. In some preparations staining appeared to be strongest in the outer, zonular lamella into which the fibers of the suspensory ligament insert, and in the inner layers, in direct contact with the underlying epithelium (Fig. 33).

Discussion

The rat lens capsule accommodates the growing population of cells which it encloses by increasing in size well into adult life, although its rate of growth (like that of the lens) decelerates with age. In 1-week-old rats the capsule is slightly thicker over the anterior surface of the lens. In succeeding weeks, thickening is disproportionately greater in this region, so that in the adult animal it is more than ten times thicker anteriorly than it is near the posterior pole.

Capsular growth was analyzed by tracing the fate of two capsular precursors, "H-glycine and ³⁵S-sulfate. Since the capsule consists of a collagen-like protein, part of which is mucopolysaccharide and sulfated, the "H-glycine was probably incorporated mainly into capsular protein, and the ³⁵S-sulfate into sulfated mucopolysaccharides. Labeling of the capsule with both precursors was highest in young animals, and more intense anteriorly, corresponding to the regional and age-related differences in growth rates. The retention of capsular labeling at later intervals, after injection in young animals, and the greatly diminished labeling in older rats, suggests that turnover of capsular constituents is low or negligible during the period studied. Consequently, the evidence indicates that capsular labeling reflects capsular growth.
An apparent exception is the relatively intense labeling near the equator which follows administration of $^{35}$S-sulfate. This localized, heightened reaction was temporally stable, but did not correspond to a site of accentuated thickening, nor did it occur in rats injected with $^3$H-glycine. Instead, it corresponded with the site of major attachment of the suspensory ligament, and was characterized by preferential staining with a procedure designed to detect acid mucopolysaccharides. Accordingly, labeling in this zone may also be a result of growth—a process of growth, however, upon which is superimposed a regional difference in composition of the capsule (an increase in the proportion of sulfated mucopolysaccharides). This, in turn, may be related to structural requirements arising from insertion of the suspensory ligament.

The concept that polysaccharides may be unevenly distributed in different regions of the capsule is supported by biochemical studies. Current evidence suggests that there may also be age-related differences in the proportions of protein and carbohydrate in the capsule.

The autoradiographic preparations, which depict an initial cellular incorporation of sulfate or glycine in the superficial cells of the lens, followed by the subsequent transfer of labeled material to the capsule, indicate that the capsule is a product of the cells of the lens. Although the perilenticular vascular network and the ciliary epithelium incorporated both metabolites, there was no evidence that either transferred labeled material to the capsule. The capillary net had vanished by 4 weeks, but the capsule continued to grow. Furthermore, it is apparent that the capsule grows from within. Thickening of the capsule after labeling with $^3$H in young animals gradually displaced the radioactivity outward, away from the cells, indicating that new capsular material was being deposited on the inner surface. This was confirmed by injecting older animals, in which the capsule was thicker. In this case the radioactive activity occurred at the inner surface 1 week after injection. This lamellar mode of apposition may be related to the lamellated fine structure of the capsule, visualized with the electron microscope. Perhaps the older capsular layers on the outer surface compensate for the gradually increasing volume of the enclosed lens by stretching or thinning.

All of the cells which contribute to the formation of the lens capsule are either intermittently or continuously engaged in other functional activities. The relative thinness of the posterior portion of the capsule may in part be due to the fact that the growing lens fibers which produce it have already devoted the major part of their metabolic machinery to the synthesis and intracellular accumulation of lens protein.

The authors gratefully acknowledge the valuable technical assistance of Mrs. Mirdza Berzins.

REFERENCES


Autoradiographic studies of lens capsules


