Sulfate and galactose metabolism in differentiating ciliary body and iris epithelia: autoradiographic and ultrastructural studies

Lynette Feeney and Robert N. Mixon

Immature and adult rat eyes were bisected and incubated with $^{35}$SO$_4$ and $^3$H-galactose in short-term pulse-chase experiments. Autoradiographs (ARG) of the tissue revealed that very little sulfate is incorporated by the peripheral neural retina, the pigment epithelia of the retina, ciliary body, or iris. The inner, inverted optic cup cells at the ora serrata, i.e., those that are undergoing differentiation into the unpigmented epithelium of the ciliary body, incorporate large amounts of $^{35}$sulfate into “fixable” macromolecules. The sulfate label is chased from the apically located Golgi apparatus to the basal surface of these cells within one hour. Ultrastructurally, these cells are beginning to develop lateral and basal invaginations of the plasma membrane characteristic of the adult secretory epithelial cells. Electron microscopic ARG show label associated with the plasma membranes. The sulfated macromolecules at this site appear to be glycolipids and glycoproteins rather than glycosaminoglycans. The preferential synthesis of these macromolecules and their placement at the cellular site of aqueous humor production suggests a role for these sulfated substances in establishing, and perhaps maintaining, that secretory process. $^3$H-galactose was incorporated into “fixable” macromolecules to some degree by all the neuroepithelial cells. After chase incubation, ARG showed a high concentration of label in differentiated retinal pigment epithelium (RPE), but not in undifferentiated peripheral RPE. Ciliary body unpigmented and pigment epithelium, and iris muscle cells incorporate galactose, but to a lesser degree than either RPE or corneal endothelial cells.

Key words: autoradiography, ciliary body epithelium, differentiation of neuroepithelium, $^3$H-galactose, glycolipids, glycoproteins, glycosaminoglycans, iris, retina, $^{35}$sulfate.

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The neuroepithelial layers of the embryonic optic cup differentiate into specialized adult cells having considerable functional diversity. The posterior optic cup cells differentiate into typical receptor and transmitting neurons and a unique pigment epithelium. The anterior portion of the optic cup develops into secretory epithelia of the ciliary body, or into the smooth muscle and pigment epithelium of the iris. Factors affecting these developmental pro-
cesses are not well understood. Undoubtedly, the differences arise from the expression of various parts of the genome, resulting in differences in the synthetic capabilities of cells in the two sites. This in turn leads to differences in intracellular organelles and other cell components, and ultimately to the striking differences in function of retinal cells versus ciliary body or iris cells.

We have tested the ability of the ocular neuroepithelium in rats of various ages to utilize $^{35}$SO$_4$ and $^3$H-galactose when these cells are undergoing rapid structural and functional maturation to become retina, ciliary body, and iris. Since the retina ends rather abruptly at the ora serrata, and the unpigmented epithelium of the ciliary body differentiates from the same inverted neuroepithelial layer, as the neural retina, it was anticipated that some modulation of synthetic processes would occur in this region of the eye. We found, indeed, that an abrupt change occurred in the utilization of $^{35}$SO$_4$ and $^3$H-galactose in cells differentiating into ciliary body epithelia. At the same time characteristic structural alterations occurred in the unpigmented epithelial cells heralding their conversion to a secretory epithelium.

Methods and materials

The eyes of Sprague-Dawley rats age 10- and 16-days old were used in the sulfate experiments, and age 12- and 18-days old for the galactose experiments. Adult rats were either Sprague-Dawley or Long-Evans breed. Animals were decapitated and the eyes rapidly dissected. The globes were bisected sagittally and most of the lens nucleus was discarded. Half eyes were placed in 15 ml. glass-stoppered Erlenmeyer flasks containing 6 ml. of radioactive incubation medium described below.

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*Adult eyes were incubated for these periods only.

In these experiments we have utilized an in vitro pulse-chase technique. This consists of incubation of eyes in medium containing a radioactive substance (the pulse), followed by incubation in medium containing a large excess of nonradioactive substance (the chase). Cells utilize the labeled material in normal synthetic processes during the brief pulse period. During the chase period maturation, migration and, perhaps, secretion of the newly synthesized labeled molecules takes place. The chase incubation affects a rapid cessation of utilization of the labeled precursor, and at the same time removes most of the nonspecifically bound isotope. Specimens removed and fixed at various times during the chase incubation will show, by autoradiography, the sequential location of labeled "fixable" macromolecules. This technique has two distinct advantages over in vivo injection of label: the higher levels of tissue radioactivity required for electron microscopic autoradiography can be achieved using modest amounts of isotope, and the synthetic activities of human eyes can now be studied using this method.

$^{35}$Sulfate. Pulse medium containing 830 μCi per milliliter of Na$_2$$^{35}$SO$_4$ was prepared as follows: a 2X sterile stock medium was made using 10 ml. of sulfate-free Eagles minimum essential medium (10X), 13.9 mg. of anhydrous calcium chloride, 220 mg. of sodium bicarbonate, 8 mg. of bovine serum albumin, 1 ml. of 200 mM glutamine, and 39 ml. of distilled water. Five millicuries of Na$_2$$^{35}$SO$_4$ (660 mCi per millimole, New England Nuclear) in 3 ml. of water was added to 3 ml. of the stock incubation medium. Chase medium consisted of 1X concentration of stock medium to which 10 mg. per milliliter of Na$_2$SO$_4$ was added.

The following pulse-chase and continuous labeling procedures were carried out on the 10- and 16-day old eyes (Table I).

$^3$H-galactose. Incubation medium containing 250 μCi per milliliter D-galactose-6-$^3$H (150 mCi per millimole, New England Nuclear) was prepared using the stock medium described above. Chase medium contained 10 mg. per milliliter of unlabeled galactose. The following pulse-chase sequences were carried out on 12- and 18-day old animals (Table II).
Fig. 1. (A) Low magnification of aSO\textsubscript{4} autoradiograph, 7' pulse-7' chase; 16-day-old albino rat. Portions of cornea, trabecular meshwork, ciliary body, and detached retina are shown. Silver grains are localized over fibroblasts, perinuclear cytoplasm of corneal endothelial cells, and (arrows) apical cytoplasm of unpigmented epithelial cells of the ciliary body. Sulfate incorporation diminishes as these cells trail off onto the inner surface of the retina. ×170. Inset: Ciliary body epithelial showing high concentration of grains over the apical cytoplasm of unpigmented cells. ×375. (B) Stained serial section of a portion of Fig. 1, A. Intercellular spaces of the unpigmented epithelium is a typical feature of this layer. Vacuolated appearance of the PE is due to amelanotic melanosomes. ×800. BM, basement membrane; C, cornea; CB, ciliary body; E, endothelium; C, Colgi apparatus; I, iris; IS, inner segment; L, lens; Ly, lysosome; M, mitochondria; Me, melanosome(s); MI, microfilaments; Mt, microtubules; N, nucleus; ONL, outer nuclear layer; OS, outer segments; PE, pigment epithelium; R, retina; RER, rough endoplasmic reticulum; T, trabecular meshwork; UPE, unpigmented epithelium; V, vesicle; and Z, zonules.

Table II

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*Adult eyes were incubated for these periods only.

All eyes were incubated at 37° C. in a gently rotating metabolic shaker. Chase incubations were carried out in relatively large volumes, i.e., 10 ml. per eye. Medium was changed at one-hour intervals during long chase periods. Media were gassed with 95 per cent oxygen-5 per cent carbon dioxide for 30 minutes prior to incubation. Flasks were also gassed each time the flask was opened, or at 30-minute intervals in the case of long chase incubations. One or two specimens (half-eyes) were removed after each time period and placed in paraformaldehyde-glutaraldehyde fixative for one hour, postfixed in osmic acid for one hour, dehydrated in a series of acetones and propylene oxide, and embedded in an Epon-Araldite mixture.

Tissue from all experiments was prepared for light and electron microscopic autoradiography. For light microscopy sections 0.5, 1.0, and 2.0 μ thickness were placed on gelatin-subbed slides and coated with Ilford L4 emulsion. Slides were stored in light-tight boxes containing Drierite.
Fig. 2. (A) Adult albino eye; 7' pulse-7' chase specimen. Sulfate incorporation is less, but the distribution is similar to that of the immature eye. The posterior half of the ciliary body shows greater concentrations of grains in the unpigmented epithelial cells than does the anterior half. Incorporation ceases abruptly at the ora serrata. Note heavy labeling of fibroblasts. x175. Inset: Enlargement of boxed area. x295. (B) Stained serial section of a portion of Fig. 2, A. x420. For abbreviations, see legend to Fig. 1.

Complete sets of slides were developed after 3, 6, and 10 weeks in D-19 for 5 minutes at 20°C. One section in each set was stained with toluidine blue in 1 per cent borax. All sections were mounted in oil and photographed using either bright field or phase optics. For electron microscopic autoradiography, gold sections were filmed with Ilford L4 emulsion by the loop technique. Contiguous thin sections were examined and photographed using an RCA EMU 3F or a Philips 300 electron microscope.

Results

Autoradiography.

$^{35}$Sulfate. Specimens fixed immediately after a seven-minute (7') pulse showed some nonspecific binding of radioactivity despite the numerous rinses employed during preparation of the tissue for embedding. Nonetheless, the 7' incubation showed that sulfate was rapidly incorporated into macromolecules by corneal fibroblasts that are synthesizing large amounts of keratosulfate and chondroitin sulfate at this time. A 7' incubation in chase medium reduced the number of grains over the corneal stroma, without altering the dense concentration of grains over the keratocytes (Figs. 1 and 2). The grains in chased specimens are interpreted, therefore, to indicate the location of $^{35}$sulfate at synthetic or postsynthetic sites.

In the ciliary body epithelium initial incorporation, 7' pulse, 7' chase, was seen at the apical pole of the unpigmented epithelial cells in both immature (10- and 16-day old) (Fig. 1) and adult (Fig. 2) eyes. Cells in the posterior half of the ciliary body epithelium showed greater concentrations of grains than in the anterior or iridial portion. In immature eyes, the unpigmented epithelial cells trail off onto the inner surface of the retina at the ora serrata, and these cells showed a gradual de-
crease in sulfate incorporation (Fig. 1). In adult eyes punctate clusters of grains occurred at the apical pole of the unpigmented epithelial cells of the ciliary body (Fig. 2). This pattern ceased abruptly at the ora serrata (Fig. 2, inset).

After a 45' chase incubation the sulfate label had moved to the basal (inner) surface of the unpigmented epithelial cells (Fig. 3, inset). Electron microscopic autoradiography shows that the grains are associated with the basal and lateral plasma membranes of the unpigmented epithelial cells (Fig. 3) and that they follow the course of the elaborate invaginations of these membranes. They do not appear to be associated with the basement membrane except incidentally where plasma membrane and basement membrane are contiguous. Grains were rarely found over zonular fibers or the vitreous face.

Incubation of the halved eyes in the presence of $^{35}$SO, for several hours loads all the sulfate-metabolizing cells (Figs. 4, 5, and 6). Autoradiographs from these long pulse incubations demonstrate more clearly than short pulse-chase incubations those cells which synthesize relatively small amounts of sulfated macromolecules, such as the neuroepithelial layers of the iris, the epithelia of the iridial portion of the ciliary body, and the pigment epithelium of the ciliary body. The heavier concentration of grains at the basal surface of the unpigmented epithelial cells of the ciliary body is quite distinct in immature eyes (Fig. 4).
Fig. 4. \(^{35}\)Sulfate; 4 hour pulse-7' chase; 10-day-old albino rat. (A) Autoradiograph showing relative utilization of sulfate in cornea, iris, and ciliary body, and developing trabecular meshwork. Both layers of iris neuroepithelium are notable for their relative paucity of silver grains. The plicae of the cornea ciliaris are cut obliquely so that successively deeper, or more internal, portions are viewed. The most dense grain concentrations are at the basal surface of the unpigmented epithelial cells. \(\times 120.\) (B) Phase photomicrograph of a portion of Fig. 4, A showing the major concentration of grains over the basal region of the unpigmented epithelial cells. Vacuolated appearance of the PE due to amelanotic melanosomes. \(\times 850.\) For abbreviations, see legend to Fig. 1.

In adult eyes (Figs. 5 and 6), localization of grains at the periphery of the unpigmented epithelial cells is also apparent, but there is also much more scatter than in immature eyes. Corneal tissue is also more heavily labeled in the immature specimens than in the adult specimens (compare Figs. 4 and 5). Incorporation of sulfate by the ciliary body in the adult eye was also much greater than the incorporation in the retina (Fig. 5, inset).

SULFATE LABELING OF OTHER TISSUES. A number of other structures observed in this study also incorporate \(^{35}\)sulfate into “fixable” macromolecules. These include the lens epithelium, the corneal endothelium, and the walls of hyaloid vessels. In the lens epithelium some label was chased to the basal (lens capsule) surface of the cell, but a greater proportion was chased to the apical (lens fiber) pole of the cell. Label in the corneal endothelium was chased toward Descemet’s membrane. Label in the hyaloid vessels appeared to be unaffected by chase.

\(^{3}H\)-galactose. In the 12-day old animals after 7' pulse-7' chase grain concentrations were quite dense over the neuroepithelial layers of the ciliary body and iris and the pigment epithelium of the retina, but not over the neural retina (Fig. 7, A and B). When time was allowed for the in vivo maturation of the newly synthesized molecules (i.e., following 0.5 to 2.0 hours chase incubation), the concentration of grains in the retinal pigment epithelium increased (Fig. 8, A and C) while that in the neuroepithelial layers of the ciliary body and iris remained the same or declined (Fig. 8, A and B).

In 18-day old animals, the most outstanding difference in the utilization of galactose occurred in the corneal endothelium. At this age the corneal endothelium-utilized galactose and grain concentrations were as great as those in the retinal pigment epithelium (Fig. 9, A and C). In the ciliary body both the unpigmented and the pigment epithelium showed heavy diffuse labeling in the 14' and 30' specimens (Fig. 9). At the ora...
Fig. 5. $^{35}$sulfate; 2 hour pulse-7' chase; adult albino rat. Low-power micrograph showing how adult sulfate ARG differs from immature animal (Fig. 4, A and B). The cornea stroma is only slightly labeled, but there is heavy labeling of trabecular meshwork. Ciliary body labeling is similar to that in young animals, although less intense. Retina is detached from the ora serrata and lies at lower right. x90. Inset: Enlargement of outlined area to show the greater incorporation of sulfate by ciliary body epithelium compared to equatorial retina. Retinal labeling occurs as a hand in the inner segments, the outer plexiform layer, and a diffuse zone that extends from the inner plexiform layer to the inner limiting membrane. x225. For abbreviations, see legend to Fig. 1.

serrata some cells along the inner surface of the retina had grain concentrations equal to that in the ciliary body epithelium. In the iris grains occurred in nearly equal concentration in the smooth muscle and the pigment epithelial layers (Fig. 9, C). With chase incubations of 0.5 to 2.0 hours the overall grain concentration in the epithelial layers of the ciliary body and iris was the same or somewhat reduced, whereas that in the corneal endothelium and the pig-

Fig. 6. Phase photomicrograph of adult ciliary processes from specimen similar to Fig. 5. Some basal localization of grains in the unpigmented epithelium is apparent but is much less intense than in immature specimens. x380. For abbreviations, see legend to Fig. 1.

ment epithelium of the retina was increased.

The labeling pattern in the adult animal was less intense that in the 18-day old animal, but the general distribution and the shifts in concentration following chase incubations was the same.

Ultrastructure. Cells in the transition zone between retina and ciliary body were examined by electron microscopy for structural features that might distinguish those cells that incorporate $^{35}$SO$_4$ and $^1$H-galactose, from neighboring cells that incorporate little or no isotope. The unpigmented epithelial cells on the anterior side of the ora serrata that incorporate both isotopes were beginning to develop the lateral and basal invaginations of the plasma membrane characteristic of the adult cell (Figs. 10 and 11). Their basal
surface, but not the invaginations, was covered by a thin basement membrane with which the fibrils of the zonules were associated (Fig. 10). The unpigmented cells differed in degree of differentiation, probably owing to the stage of the cell cycle each cell was in when this actively mitotic layer was fixed for microscopy. Mitochondria were not predominately associated with the basal plasma membrane invaginations; rather they were dispersed throughout the cytoplasm. Some cells contained only free ribosomes whereas neighboring cells contained an elaborate network of RER (Fig. 11). The relatively small Golgi apparatus was located apically in the cell. The Golgi cisternae were quite flat, usually three to four in number [and show few dilations] (Fig. 12, lower inset). Many small, and no large, vesicles surrounded the cisternal stacks. Coated vesicles in the vicinity were small, i.e., 50 m\(\mu\) in diameter. A few lysosomes, identified by their dense membrane, the lucent space between membrane and contents, and homogeneous dense contents, were found in most cells (Fig. 10). Microtubules occurred in the apical cytoplasm, in the perinuclear region, and near plasma membranes. Bundles of microfilaments were frequently oriented parallel to lateral plasma membrane invaginations (Fig. 11).

The basal cytoplasm of the unpigmented ciliary body cells was scrutinized for structural features that might have a bearing on the movement of sulfate from the apical pole of the cell to the basal pole. Several different kinds of vesicles were found in close proximity to the basal surface. These include: (1) large, 100 m\(\mu\) in diameter, coated vesicles; (2) elements of the RER; and (3) small, 40 to 50 m\(\mu\) in diameter, vesicles. Only the large coated vesicles were seen in direct continuity with the basal and lateral plasma membranes (Fig. 11, upper inset).

Retinal pigment epithelial cells were examined to see if there were structural modifications that might account for the hiatus in galactose incorporation in cells underlying the ora serrata region (i.e., cells at arrow in Figs. 8, A and 9, A). Retinal pigment epithelial cells that incorporated galactose faced an expanding interphoto-
Fig. 8. (A) 3H-galactose; 12-day-old albino rat; 15' pulse-2-hour chase. The heaviest concentrations of grains occur over the pigment epithelium of the retina, and this pattern ceases near the ora serrata (arrow). The dilator muscle of the iris, unpigmented epithelium of the ciliary body, and corneal endothelium have lesser concentrations. x100. (B) Enlargement of cornea, iris, and ciliary body from Fig. 8, A. x270. (C) Enlargement of retinal pigment epithelium from Fig. 8, A. x1,300. For abbreviations, see legend to Fig. 1.

receptor space, had numerous long apical microvilli, and contained many lysosomes. The cells that did not incorporate galactose faced an undifferentiated neural retina and contained fewer lysosomes and more RER than the posterior cells, features reflecting their relatively undifferentiated condition. Pigment epithelial cells of the ciliary body, where galactose incorporation resumed, differed from those underlying the ora serrata in having a highly convoluted basal plasma membrane and practically no RER. The cytoplasm was filled with polyribosomes and amelanotic melanosomes. A fairly large Golgi apparatus and a few mitochondria were also present.

Discussion

Sulfate. Immature eyes give denser autoradiographs with labeled sulfate than adult eyes. This reflects the utilization of sulfate by the growing cells in the assembly of
Fig. 9. 3H-galactose; 18-day-old albino rat. (A) 15' pulse-15' chase (30' specimen). Low magnification showing general pattern of incorporation in peripheral retina, ciliary body, sclera, and cornea. The heaviest concentration of grains is seen over retinal pigment epithelium and corneal endothelium. Densely labeled cells at ora serrata are in continuity with unpigmented epithelium of the ciliary body. The heavy label in the pigment epithelium of the peripheral retina disappears near the ora serrata, then resumes in the ciliary body. Arrow marks region from which electron micrograph in Fig. 12 was made. ×80. (B) Phase photomicrograph of area near arrow in Fig. 9, A. Grains are diffusely scattered over both layers of epithelia in the ciliary body. Cells at the junction of retina and ciliary body show the vigorous incorporation of galactose characteristic of ciliary body epithelia. ×450. (C) Phase photograph of anterior portion of Fig. 9, A. Corneal endothelium shows greater concentration of grains than in 12-day-old animals. ×550. For abbreviations, see legend to Fig. 1.

new structures. This is seen most dramatically when the epithelium of the ciliary processes is undergoing differentiation for its adult secretory function.

Radioactive sulfate in incorporated initially into the apical cytoplasm of the unpigmented epithelial cells of the ciliary body. Inorganic sulfate is known to be incorporated into organic molecules in the Golgi apparatus of various cell types where a high-energy intermediate, phosphoadenosine phosphosulfate, transfers the sulfate to sugar moieties. The Golgi apparatus of the unpigmented epithelial cells
Fig. 10. Twelve-day-old animal. Electron micrograph of cells anterior (left) and posterior (right) to ora serrata cleft. The unpigmented epithelial cells show numerous basal and lateral plasma membrane invaginations (arrows). A basement membrane covers the basal surface. Pigment epithelial cells contain numerous amelanotic melanosomes. Zonular bundles lie in the gap. Cells on the retinal side are Müller cells and elements of a vessel wall. ×2,960. For abbreviations, see legend to Fig. 1.

is located in the apical portion of the cytoplasm. The autoradiographic grains localized at the apical pole of these cells in specimens fixed 14' after initial exposure to $^{35}SO_4$, probably represent incorporation of labeled sulfate into macromolecules by the Golgi apparatus. Within an hour the newly synthesized sulfated macromolecules move from the region of the Golgi apparatus to the basal surface of these cells.

Three classes of sulfated macromolecules occur in mammalian tissues: glycosaminoglycans (GAG), glycoproteins, and glycolipids. GAG are components of ground substance and their synthesis and secretion has been studied most extensively in connective tissue cells. Newly synthesized GAG is secreted from the cell one-half to two hours after addition of labeled sulfate or galactose. The sulfated GAG found in homogenates of nerve tissue may be somewhat different from those in connective tissues. They have shorter turnover times and their exact location in neural tissue has not been established. Sulfated glycoproteins have not been well characterized, but they occur in mucous secretions of the stomach, salivary gland, etc., and in the brain. Sulfated lipids (sulfatides, or sulfated cerebrosides) are characteristically structural components of membranes. Sulfatides are found in high concentration in kidney medulla, avian salt gland, elasmobranch rectal gland, and other tissues specialized for the active transport of sodium. Salt-loading experiments show that an increase in the sulfatide concentration in the duck salt gland is accompanied by an increase in the Na-K-activated ATPase activity. These data suggest a role for
Fig. 11. Twelve-day-old animal; 7' pulse-7' chase specimen. Unpigmented epithelial cell of ciliary body. The Golgi apparatus is located in the apical cytoplasm, as is most of the RER. Basal and lateral membrane invaginations are evident (arrows), and large coated vesicles also occur in these regions. x16,200. Upper insets: Large coated vesicles in close associations with (V₁, V₃) and in continuity with (V₂) the plasma membrane. x39,000. Lower inset: Golgi region of an adjacent cell showing the narrow cisternae and associated small vesicles. x18,600. For abbreviations, see legend to Fig. 1.
Fig. 12. Pigment epithelium of an 18-day-old rat retina in the peripheral area where $^3$H-galactose incorporation is greatly reduced. The cytoplasm contains mainly RER, rather than SER characteristic of differentiated PE cells. Apical microvilli are filled with amelanotic melanosomes. Few lysosomes are present. The overlying interphotoreceptor space is shallow and apical microvilli are shorter than in more posterior regions. ×32,500. For abbreviations, see legend to Fig. 1.

this class of anionic lipids in Na translocation.

Several developmental processes are taking place in the vicinity of the differentiating ciliary body, any one, or combination of which, could require sulfated macromolecules. In addition to the growth and differentiation of the neuroepithelial cells themselves, elaboration of basement membranes, assembly and growth of the zonules, and expansion of the vitreous body are also in progress. To our knowledge, sulfated
macromolecules have not been detected biochemically in either the zonular fibers or the vitreous body, and we found little or no autoradiographic reaction associated with these structures. Although lens capsule, the basement membrane of lens epithelium, contains sulfated substances of undetermined composition, our autoradiographs show that in the unpigmented epithelium of the ciliary body most grains overlie the elaborate infoldings of the cell membrane rather than the smooth contour of the basement membrane (Fig. 3). Preliminary experiments in our laboratory indicate that the labeled substance(s) in pronase-digested homogenates of the ciliary processes is not precipitable by either ethanol or cetylpyridinium chloride, and therefore is not a GAG. Also, chromatographic analysis of chloroform:methanol extracts show the presence of significant amounts of labeled sulfatides. These data suggest that the most likely developmental phenomenon requiring large amounts of sulfate is the synthesis of sulfated glycolipids or glycoproteins for the rapidly expanding basal plasma membranes. Synthesis of specialized membrane components required for the active transport of sodium may be the critical step that transforms undifferentiated anterior neuroepithelial cells into an epithelium that secretes aqueous humor.

Cytoplasmic structures that might transfer the sulfate from its early location in the apical region to the basal surface of the cell were not readily apparent. There were no organelles invariably present in the vicinity of the Golgi apparatus that were also found at the basal surface. The basal cytoplasm did contain numerous large coated vesicles, many of which were in continuity with the plasma membrane. Other studies have shown, however, that these kinds of vesicles are involved in uptake of exogenous substances. Therefore, despite their prominence at the site of membrane expansion, we tentatively assume that they are involved in endocytosis rather than exocytosis, and that transfer of sulfated macromolecules takes place via more subtle mechanisms than translocation of membranous vesicles.

In human eyes an Alcian blue- and colloidal iron-positive material is found between adjacent cells of the unpigmented epithelium of the pars plana of the ciliary body. The substance was thought to be hyaluronic acid owing to its staining, its removal by hyaluronidase, and its anatomic relationship to the hyaluronic acid-containing vitreous body and aqueous humor. The present study raises the possibility that some of this extracellular polyanionic material might be the sulfated macromolecules demonstrated in this study. Histochemical and enzymatic procedures are being employed to clarify this question and to further elucidate the role of polyanionic macromolecules in the function of the ciliary body epithelium.

**Galactose.** Galactose is utilized in more restrictive synthetic pathways than is glucose. It is not a precursor of the galactosamine of GAG when glucose is available, as it was in our incubation medium. Thus, the autoradiographs in this study most likely represent synthesis of glycoproteins and glycolipids.

Initially there is rapid incorporation of galactose into "fixable" molecules by all of the neuroepithelial layers. However, when the periods of chase are lengthened, only the retinal pigment epithelium shows a marked increase in label. This suggests, first of all, that the tritiated precursor is utilized in a different way by the retinal pigment epithelium than by the anterior neuroepithelial layers. Second, it implies that 45' to 120' is required to build some of the 3H-galactose into a more stable molecular form that can be fixed by aldehyde-osmic acid.

Studies on amphibian pigment epithelium in vivo showed that 3H-galactose was initially distributed throughout the cytoplasm and then became concentrated in the oil droplet after eight hours. Since mammalian pigment epithelium has no oil droplet, and our studies were short-term,
we cannot compare galactose utilization in the two studies.

The ability to utilize galactose for the synthesis of "fixable" macromolecules is poor in undifferentiated retinal pigment epithelial cells near the ora serrata (Fig. 7, A) and in immature corneal endothelial cells (Fig. 8, A). This synthetic capability is acquired later and reflects the further differentiation of these cells. In the pigment epithelium the cells that utilize galactose have many lysosomes, organelles that contain glycoproteins. Also, these cells face an expanding interphotoreceptor space containing glycoproteins and GAGs that may be secreted by the pigment epithelial cells and/or be part of the cell coat of their apical microvilli. The undifferentiated cells, on the contrary, lack lysosomes, and the interphotoreceptor space has not yet formed in this peripheral region of the retina. Thus, the requirement for galactose for synthesis of some intracellular and extracellular components may not be expressed in the relatively undifferentiated cells.

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REFERENCES


