Clinic, Karolinska Hospital, S-104 01, Stockholm 60, Sweden. °The present address of G. Lennerstrand is: The Eye Clinic, Karolinska Hospital, S-104 01, Stockholm 60, Sweden.

Key words: extraocular muscle, cat, threshold nerve stimulation, units, electrical and mechanical properties, recruitment of motor units in eye movements.

REFERENCES

1. Lennerstrand, G.: Electrical activity and isometric tension in motor units of the cat's inferior oblique muscle, Acta Physiol. Scand. **91**: 458, 1974.
2. Bach-y-Rita, P., and Ito, F.: In vivo studies on fast and slow muscle fibers in cat extraocular muscles, J. Gen. Physiol. **49**: 1177, 1966.
3. Hess, A., and Pilar, G.: Slow fibers in the extraocular muscles of the cat, J. Physiol. **169**: 780, 1963.
4. Henneman, E., and Olson, C. B.: Relations between structure and functions in the design of skeletal muscles, J. Neurophysiol. **28**: 581, 1965.
5. Burke, R. E.: Motor unit types of cat triceps surae muscle, J. Physiol. **193**: 141, 1967.
6. Goldberg, S., and Lennerstrand, G.: Motor effects of intracellular stimulation of abducens neurons. To be published.
7. Vilis, T.: Mechanical properties of the extraocular muscles of the cat. Ph. D. Thesis, McGill University, Montreal, 1973, pp. 1-167.
8. Granit, R., and Burke, R. E.: The control of movement and posture, Brain Res. **53**: 1, 1973.
9. Yamanaka, Y., and Bach-y-Rita, P.: Conduction velocities in the abducens nerve correlated with vestibular nystagmus in cats, Exp. Neurol. **20**: 143, 1968.
10. Lennerstrand, G.: Mechanical studies on the retractor bulbi muscle and its motor units in the cat, J. Physiol. **236**: 43, 1974.

Localization of ^{35}sulfated macromolecules at the site of active transport in the ciliary processes.

LYNETTE FEENEY AND ROBERT MIXON.

Autoradiography shows that the unpigmented epithelium of the ciliary body incorporates ^{35} sulfate into macromolecules that move to the basal surface of the cell. The labeled macromolecules appear to be in the cell membrane and may be sulfatides, glycolipids known to be associated with the Na^+-K^+-dependent ATPase responsible for active transport of sodium in other types of cells. In the ciliary processes, sulfatides would be involved in the secretion of aqueous humor. Lens epithelium appears to synthesize similar sulfated macromolecules that move to the apical (lens fiber) surface of the cell.

This paper concerns hitherto unreported sulfated macromolecules in the ciliary processes of the rat eye. The sulfated compounds appear to differ from other sulfated macromolecules such as chondroitin sulfate or keratosulfate and are not found in adjacent neuroepithelially derived tissues of the iris and retina, although they may be present in lens epithelium. These macromolecules may be involved in the secretion of aqueous humor, since sulfated membrane components found in kidney and other salt-transporting tissues, such as salt glands of marine birds, are known to be associated with the Na^+-K^+-activated ATPase responsible for active transport of sodium.[1] The technique of ^{35}sulfate autoradiography which reveals these membrane-associated sulfated macromolecules, may provide a new tool for locating the exact portion of a cell membrane carrying out active transport functions.

Methods and materials. Eighteen eyes of albino rats (postnatal age 10 and 16 days, and adults) were bisected and incubated in gassed (95 per cent O_2: 5 per cent CO_2) Eagle's medium containing 800 μCi per milliliter of $Na_2{}^{35}SO_4$, for seven or 30 minutes ("pulse" incubation), and then transferred to a medium containing 10 mM unlabeled Na_2SO_4 for seven, 30, 45, or 60 minutes ("chase" incubation). Both the pulse and the chase incubations were conducted in glass-stoppered Erlenmeyer flasks rotating in an environmental shaker at 37° C. The halved eyes were fixed and prepared for light and electron microscopic autoradiography as described elsewhere.[2]

Results. The unpigmented epithelium of the ciliary body was found to have silver grains over the apical cytoplasm where the Golgi apparatus is located, after a seven-minute pulse-seven-minute chase incubation. Specimens chased 30 to 60 minutes showed the labeled macromolecules at the basal surface of the unpigmented epithelial cells (Figs. 1 and 2). By electron microscopy the autoradiographic grains are associated with the convoluted basal and lateral plasma membranes of the unpigmented epithelial cells (Fig. 3). Results are most prominent in 10-day-old specimens, but 16-day-old and adult specimens show similar utilization of sulfate by the ciliary body. Similar incorporation of ^{35}sulfate into macromolecules was not apparent in the pigment epithelium of the ciliary body nor in the neuroepithelial layers of the iris. Retinal neurons, glia, pigment epithelium, and vascular cells all incorporate ^{35}sulfate into macromolecules, but neither the quantity nor the pattern of movement of the labeled macromolecules is comparable to that observed in the unpigmented epithelium of the ciliary processes. The observations on the retina and further details on the utilization of sulfate by immature and adult ciliary body and iris are described elsewhere.[3, 4]

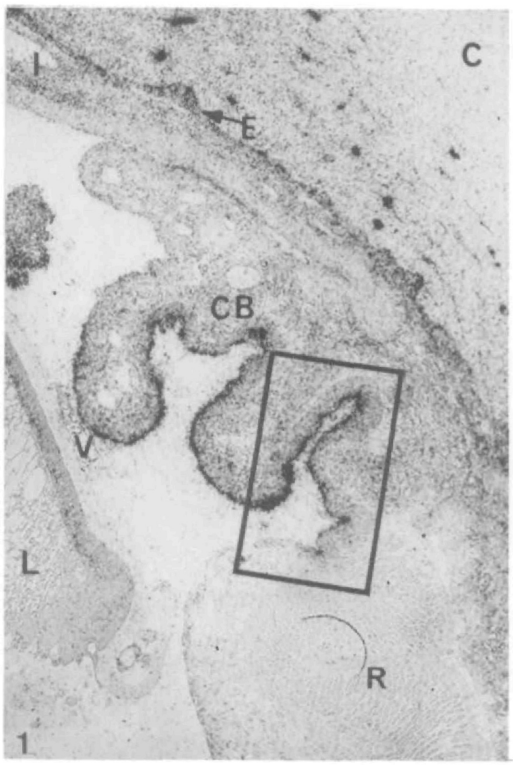

Fig. 1. Autoradiograph of 10-day-old rat eye, pulsed 30 minutes in ^{35}sulfate and chased 45 minutes. Silver grains overlie the basal surface of the unpigmented epithelial cells of the ciliary body. Lens epithelial cells have grains at their apical (lens fiber) pole. (Lens fibers are degenerated owing to bisection of the lens.) Corneal keratocytes and endothelial cells are heavily labeled. Retina and iris are relatively free of grains. Six-week exposure. ×95. ***Abbreviations used in figures:*** C, cornea; CB, ciliary body; E, endothelium; I, iris; L, lens; PE, pigment epithelium; R, retina; UPE, unpigmented epithelium; and V, vessel of tunica vasculosa lentis.

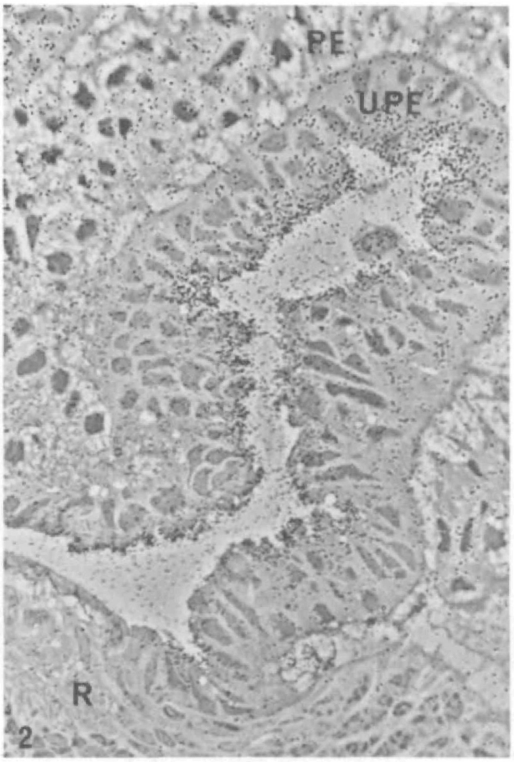

Fig. 2. Serial section of area outlined in Fig. 1, showing basal location of grains in the unpigmented epithelium of the ciliary body and paucity of grains elsewhere. Phase contrast. Three-week exposure. ×350.

Lens epithelial cells have autoradiographic grains over the cytoplasm at early pulse-chase intervals. After 60 minutes of chase incubation some labeled macromolecules are seen in the lens capsule, but greater numbers are found at the apical pole of the cell (Fig. 1). The quantity of grains in the lens epithelium is much less than that in the unpigmented epithelium of the ciliary body.

Discussion. The initial localization of silver grains over the Colgi apparatus in the apical cytoplasm of the unpigmented epithelial cells of the ciliary body indicates attachment of inorganic sulfate to macromolecules that subsequently are fixed by aldehyde-osmic acid fixatives. Within an hour after pulse-labeling the sulfate-labeled molecules move to the basal surface of the cell, i.e., the surface bordering the posterior chamber. By electron microscopic autoradiography the labeled macromolecules were found associated with the plasma membrane, and unlike chondroitin sulfate, they did not appear to be extruded from the cell (for instance, label was not found in zonular fibers, basement membranes of the ciliary epithelium, nor in the vitreous body). The most dense autoradiographs are obtained from 10-day-old animals when the neuroepithelial cells are undergoing rapid expansion of their basal and lateral membranes to become differentiated secretory cells.[4] The anterior chamber is expanding and filled with aqueous humor at this time. The diminished incorporation of ^{35}sulfate in the 16-day-old eyes probably reflects slower growth at this age, whereas in adult eyes, the autoradiographs depict mainly the turnover of the ^{35}sulfated macromolecules.

Karlsson, Samuelsson, and Steen[1] have demonstrated an association between sulfated glycolipids (also called sulfatides or sulfated cerebrosides) and Na^+-K^+-dependent ATPase in kidney medulla, salt gland of marine birds, and rectal glands of elasmobranchs. Salt-loading affects a conjoint rise

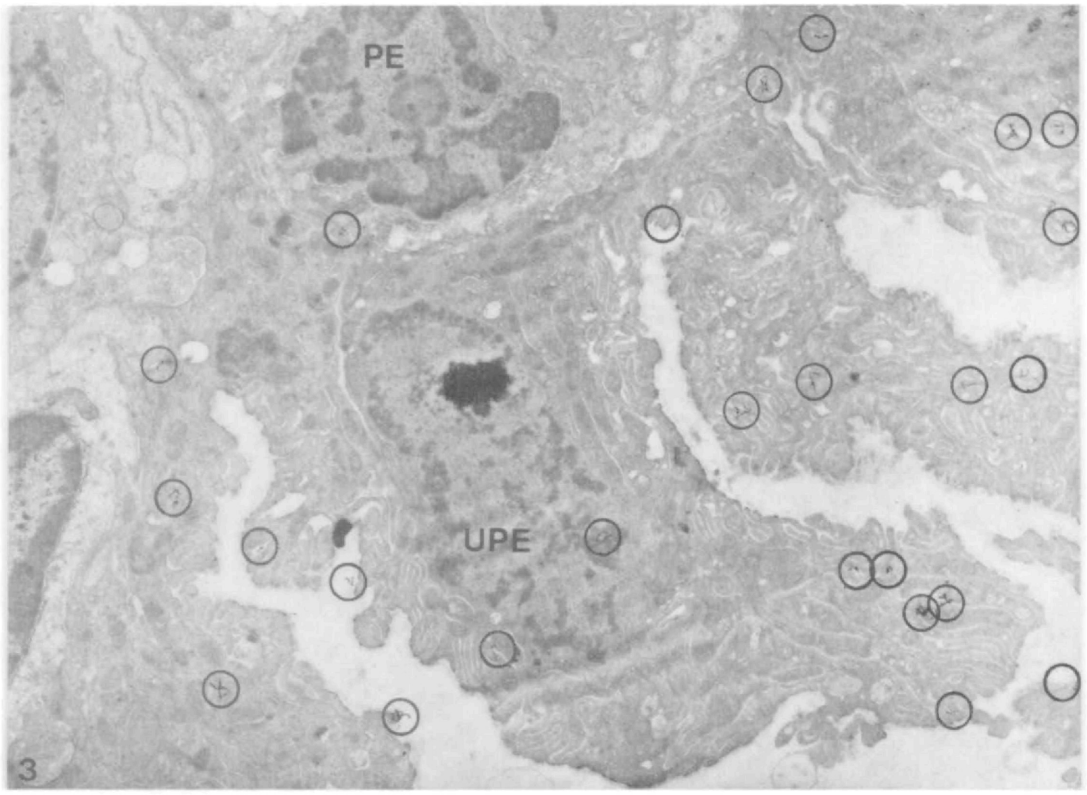

Fig. 3. Low-power electron microscopic autoradiograph showing the localization of labeled sulfate on membrane invaginations of the UPE. Grains are circled for easier visualization. Adult rat. Two-hour pulse, seven-minute chase. ×6,500.

in sulfatide concentration and Na^+-K^+-activated ATPase activity. Since ATPase is a membrane-bound enzyme,[5] their data suggest that sulfated lipids and ATPase reside together in membranes actively transporting sodium. In the ciliary processes ouabain-sensitive ATPase has been demonstrated histochemically on the plasma membrane of the unpigmented epithelium of adult eyes, principally at the lateral interdigited surfaces and at the apical (i.e., pigment epithelial) surface.[6, 7] The cellular site of ^{35}sulfate-labeled macromolecules in the present study correlates with the location of the histochemically demonstrable ATPase.

In the lens epithelium, ^{35}sulfate is incorporated into macromolecules that move to surface membranes. The labeled macromolecules were chased to the apical (i.e., toward the lens fibers) pole of the cell, whereas in the unpigmented epithelium of the ciliary processes it was chased to the basal pole of the cell. Such differences are consistent with the direction of sodium transport in each of the epithelial layers.

Biochemical studies are in progress to identify the sulfated macromolecules in the ciliary processes. Preliminary data show that ^{35}sulfated glycosaminoglycans are not present in homogenates of ciliary processes prepared by the pulse-chase technique that yielded the autoradiograph in Fig. 1.[8] If the sulfated macromolecules described above prove to be sulfatides, the technique of ^{35}sulfate autoradiography will provide a morphologic tool for locating, with improved precision and resolution, exact sites of active transport in many different kinds of cells.

From the John E. Weeks Memorial Laboratory of Ophthalmology, Department of Ophthalmology, University of Oregon Medical School, Portland, Ore. 97201. This investigation was supported in part by Grant No. 00715 from the National Eye Institute. Submitted for publication May 16, 1974.
Reprint requests: Dr. Lynette Feeney, Department of Ophthalmology, University of Oregon Medical School, 3181 S.W. Sam Jackson Park Rd., Portland, Ore. 97201.

Key words: aqueous humor secretion of ciliary body epithelium, lens epithelium, sodium transport, sulfatides, ^{35}sulfate.

REFERENCES

1. Karlsson, K.-A., Samuelsson, B. E., and Steen, G. O.: Lipid pattern and Na^+-K^+-dependent adenosine triphosphatase activity in the salt

gland of duck before and after adaptation to hypertonic saline, J. Mem. Biol. 5: 169, 1971.
2. Feeney, L.: Synthesis of interphotoreceptor matrix. I. Autoradiography of ^{3}H-fucose incorporation, INVEST. OPHTHALMOL. 12: 739, 1973.
3. Feeney, L., and Mixon, R. N.: Synthesis of interphotoreceptor matrix. II. Autoradiography of ^{35}sulfate and ^{3}H-galactose incorporation in immature and adult rat eyes, In preparation.
4. Feeney, L., and Mixon, R. N.: Sulfate and galactose metabolism in differentiating ciliary body and iris epithelia: autoradiographic and ultrastructural studies, In preparation.
5. Bonting, S. L.: Sodium-potassium-activated adenosinetriphosphatase and cation transport, *in:* Membranes and Ion Transport, Bittar, E. E., editor. New York, 1970, Wiley-Interscience.
6. Kaye, G. I., and Pappas, G. D.: Studies on the ciliary epithelium and zonule. III. The fine structure of the rabbit ciliary epithelium in relation to the localization of ATPase activity, J. Microscopie 4: 497, 1965.
7. Shiose, Y., and Sears, M.: Fine structural localization of nucleoside phosphatase activity in the ciliary epithelium of albino rabbits, INVEST. OPHTHALMOL. 5: 152, 1966.
8. Bentley, J. P., Feeney, L., and Mixon, R. N.: Unpublished data.

Rod and cone densities in the Rhesus.

CALVIN K. ADAMS, JOSE M. PEREZ, AND MINNIE N. HAWTHORNE.

Rod and cone densities were determined at 25 locations along the temporal horizontal meridian of the right eye of an 8.7 kilogram male Rhesus, using an unstained retinal whole-mount preparation and viewed with interference contrast optics. Results indicate systematic differences between the Rhesus and previously reported data for man, particularly in rod densities.

The spatial distributions of the rods and cones in the retina determines, in a very basic way, many of the capabilities and characteristics of the entire visual system. Significant advances in our understanding of the structure and function of the retina and visual pathways have occurred in the recent past. However, one area of investigation—the quantification and specification of the spatial distribution of rods and cones—has not been adequately investigated. For the human retina, there has been only one systematic investigation, and it was conducted 39 years ago on one retina.[1] There is no comparable study for any other primate species: only in cat has there been a comparable study.[2] The objective of this report is to describe the rod and cone densities along the temporal horizontal meridian of the Rhesus retina using a new technique.

Methods. An 8.7 kilogram adult male Rhesus was anesthetized with sodium pentabarbital (45 mg. per kilogram, intravenously). The eyes were enucleated, opened at the ora, and placed in 10 per cent buffered formalin. Immediately thereafter, the front of the eye was removed, and the sclera, choroid, and pigment epithelium very carefully peeled from the neural retina. To flatten the retina, five meridional cuts, 10 to 15 mm. long were made from the margin toward the fovea. The retina was then mounted on a thin coverglass and covered with nonadhering lens paper. The retina and paper were held in place with rubber bands. After fixation for four days, the tissue was dehydrated through a standard series of alcohols, cleared with xylene, and mounted, unstained, in Permount between two thin coverglasses.

For quantification, the retina was viewed with a Reichert inverted microscope using interference contrast (Nomarski) optics. Position of the retina was read from the vernier scales on the x and y controls of the mechanical stage. The horizontal meridian was defined as a band 0.2 mm. wide running through the fovea and the center of the optic disc. The receptor mosaic at the level of the inner segments was photographed at 25 locations along the temporal horizontal meridian on high-resolution 35 mm. film with a 63/0.65 NP objective. A calibration slide (10 μm intervals) was photographed at the same magnification. The photographs were projected on a tangent screen at a final magnification of 4,000 for counting. At each retinal location, an area 50 by 50 μm (2,500 μm^2) was counted for both rods and cones. Each location was counted five times, with repeat counts done on independently selected areas. Criteria for counting an area, and identifying rods and cones were the same as those used by previous investigators.[1, 2, 4] Raw receptor counts were converted to densities per square millimeter of fresh tissue using Osterberg's formula.[1] Linear shrinkage was estimated using the method of Rolls and Cowey.[3] The foveal-optic disc distance was measured in five adult Rhesus eyes processed as described above. The average linear shrinkage was 24.1 per cent. To convert millimeters of whole-mount retina to degrees of visual angle in the living retina, the procedure of Rolls and Cowey[3] was used. Using their value of 0.246 mm. per degree of living tissue and our average shrinkage value of 24.1 per cent, the value of 0.187 mm. per degree for our fixed tissue was obtained.

Results. Fig. 1 provides two illustrations of the receptor inner segment mosaic in the Rhesus. In Fig. 1, *A*, the foveal cone inner segment mosaic is illustrated. The receptors are arrayed in a honeycomb-like mosaic. The diameter of the cone inner segments corresponds closely with those given by Polyak[5] for Rhesus. Fig. 1, *B* shows the peripheral mosaic. The rods and cones can be clearly dif-