Capillary basement membrane thickening is typical of the diabetic retina, and aldose reductase appears to be involved since a diabetic-like thickening can be induced by galactose feeding and prevented with aldose reductase inhibitors. Because aldose reductase is present in the Mueller's cells, studies were undertaken to determine if thickening of the retinal inner limiting membrane, which is the basement membrane of these cells, can be induced by long-term galactose feeding and be prevented with an aldose reductase inhibitor. Weanling male, Sprague-Dawley rats were given a 50% galactose diet with or without an aldose reductase inhibitor (0.04% tolrestat, Ayerst). Quantitative computer planimetry on electron micrographs demonstrated a significant galactose-induced thickening of the inner limiting membrane which was prevented by the aldose reductase inhibitor. The results were consistent with the notion that basement membrane thickening is involved in diabetic retinopathy and can be delayed or prevented with aldose reductase inhibitors.

Materials and Methods. Weanling male, Sprague-Dawley rats were given lab feed (NIH-07 laboratory feed, Zeigler Bros., Gardners, PA), lab feed with galactose (50%), or galactose feed with a potent aldose reductase inhibitor (tolrestat, 0.04% by weight, Ayerst AY-27, 773, courtesy of Ayerst Laboratories Research, Inc., Princeton, NJ), administered as described elsewhere. All animals were maintained under a 12 hr on/12 hr off diurnal light cycle with cage illuminations of 15 to 30 foot candles. After 88 weeks on the diets, the rats were killed by an overdose of sodium pentobarbital, the eyes were enucleated, and the retinas were prepared for electron microscopy as described previously, assuring precise sampling of the same region of the central retina in all animals. Animal care and treatment conformed to the ARVO Resolution on the Use of Animals in Research.

Quantification was done on at least ten electron micrographs from each animal enlarged to ×50,000. These were analyzed by computer planimetry using a Bioquant II digitizing tablet and morphometric analysis system (R & M Biometrics, Nashville TN). The measurements were made on three animals in each group, and the results were expressed in nanometers.

Results. The inner limiting membrane of the retina was visibly thicker in the rats fed the galactose diet than it was in rats fed the control diet or the galactose diet plus tolrestat (Fig. 1). The average thickness of the inner limiting membrane was 37.3 ± 2.0 nm in the rats fed the control diet for 88 weeks (Fig. 2). The thickness was increased (P < 0.01) to 46.3 ± 2.2 nm in the galactose-fed rats, but remained like the controls in rats fed the aldose reductase inhibitor along with the galactose feed (40.5 ± 2.2 nm). The inner limiting membrane was not only thicker, but had a very rough appearance in the galactose-fed rats.
Fig. 1. Aldose reductase-related thickening of the inner limiting membrane (ILM) and the capillary basement membranes (CBM) of the retina. The micrographs were taken from representative transections of the central retina of rats fed: control diet (A), 50% galactose diet (B), or 50% galactose diet with tolrestat added (C). Note the granularity (arrowheads) of the inner limiting membrane along its vitreal surface in galactose-fed rats not treated with tolrestat (B). Calibration bars = 1.0 and 0.5 μm for all the micrographs and insets, respectively.

Transections viewed at high magnification revealed that the roughness resulted from the presence of many particles of various electron opacities and diameters attached mainly to its vitreal surface (Fig. 1B). Most of the particles were more electron opaque than the inner limiting membrane. They ranged in...
diameter from approximately one-half to one and one-half times the thickness of the inner limiting membrane itself. No such particles were present on the inner limiting membranes of the controls or the galactose-fed rats that were treated with tolrestat. As reported in detail elsewhere, there was marked thickening of the basement membranes of retinal capillaries only in the rats fed the galactose diet without an aldose reductase inhibitor. Figure 1 demonstrates this thickening in capillaries adjacent to the inner limiting membrane.

Discussion. Long-term galactose feeding of normal rats induced a thickening of the inner limiting membrane similar to the thickening reported in long-term streptozotocin diabetic rats. However, this basement membrane thickening was not nearly so striking as the two-fold thickening which was reported for rat retinal capillaries after only 44 weeks of galactose feeding, nor as the marked basement membrane thickening in the retinal capillaries of the rats in this study.

The thickening of the inner limiting membrane in galactose-fed rats and its prevention with an aldose reductase inhibitor is consistent with the presence of aldose reductase in the Mueller's cells, and with the apparent role of aldose reductase in several diabetic complications. Because the galactose-induced particles were limited mainly to the vitreal surface of the inner limiting membrane, they may represent products of some ocular reaction to the long-term presence of mature sugar cataracts which also occurred only in the galactose-fed rats which were not treated with tolrestat. Further studies are needed to determine a possible role of aldose reductase in alterations of the inner limiting membrane and other changes in the vitreoretinal juncture where neovascularization often originates in diabetes.

Key words: inner limiting membrane, capillary basement membrane, aldose reductase, galactose, model of diabetes

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