Retinal Capillaries: Basement Membrane Thickening by Galactosemia Prevented with Aldose Reductase Inhibitor

Abstract. *A twofold thickening of capillary basement membranes of rat retinas resulting from dietary galactose was prevented by sorbinil, an inhibitor of aldose reductase. Since the basement membrane thickening was ultrastructurally similar to that typical of diabetic retinopathy, it may indicate changes in vessel permeability and susceptibility to hemorrhage. Galactosemic rats should be useful models for studying basement membrane–related complications of diabetes and for examining the potential biochemical regulation of basement membrane synthesis by aldose reductase inhibitors.*

Aldose reductase, which has been implicated in sugar cataracts (*1–5*), certain corneal healing defects (*6–7*), and peripheral neuropathy (*8*) of diabetic and galactosemic animals, now appears to be involved in other diabetic-like pathologies. While the normal physiological role of this enzyme in most tissues remains unknown, under the conditions of high plasma sugar concentrations encountered in diabetes and galactosemia, aldose reductase converts these sugars to their respective sugar alcohols (polyols). These polyols are not readily metabolized, nor do they penetrate cell membranes easily. Thus, once formed at significant rates, they may accumulate to very high levels in cells, leading to hypertonicity, alteration of ion permeability, and eventual cell death with consequent tissue changes such as cataract formation. Treatment of diabetic or galactosemic rats with potent aldose reductase inhibitors such as sorbinil decreases the accumulation of polyols, which in turn appears to prevent the formation of cataracts in lenses (*3–5*), defective healing in scraped corneas (*6–7*), and decreased conduction velocity in motor nerves (*8*).

Recently, aldose reductase was implicated in the formation of the thicker yet apparently more porous basement membranes found throughout the vasculature of chronic diabetics (*9*). Such vascular changes could contribute to several of the complications of diabetes, including retinopathy, nephropathy, and peripheral microangiopathy. If aldose reductase is indeed involved in such complications of diabetes, then similar diabetic-like pathological conditions should occur in the galactosemic state (*10*). The present study was initiated to determine whether early microangiopathic changes occur in the retinal capillaries of galactosemic rats and, if so, whether they can be prevented by oral administration of the aldose reductase inhibitor sorbinil.

Male Sprague-Dawley rats were separated into three groups at weaning and fed a control diet of NIH-07 laboratory feed (Zeigler Bros.), a galactose diet having a 1:1 ratio by weight of galactose to NIH-07, or a galactose and sorbinil diet consisting of the galactose diet with 2 g of sorbinil (Pfizer) added per 5 kg. After 28 and 44 weeks the right eyes of three or four rats from each group were enucleated and fixed by immersion in 2.5 percent glutaraldehyde buffered to *p*H 7.2 with 50 m*M* sodium cacodylate. Narrow portions (0.2 by 1.0 mm) oriented radially from near the optic nerve were taken from the superior temporal sector of the central retina and postfixed in O_sO_4. This was followed by standard preparation for electron microscopy. To minimize variability for the quantative

Table 1. Basement membrane thickness in retinal capillaries. Values are means ± standard deviations.

Diet	Number of animals	Number of sections analyzed	BMA (μm^2 per 1000 μm)
	28 weeks on diet		
Control	3	47	96.7 ± 14.1
Galactose	3	31	151.9 ± 18.7
Galactose + sorbinil	3	35	99.1 ± 14.1
	44 weeks on diet		
Control	4	37	93.9 ± 12.3
Galactose	4	40	194.4 ± 40.4
Galactose + sorbinil	4	40	105.8 ± 17.2

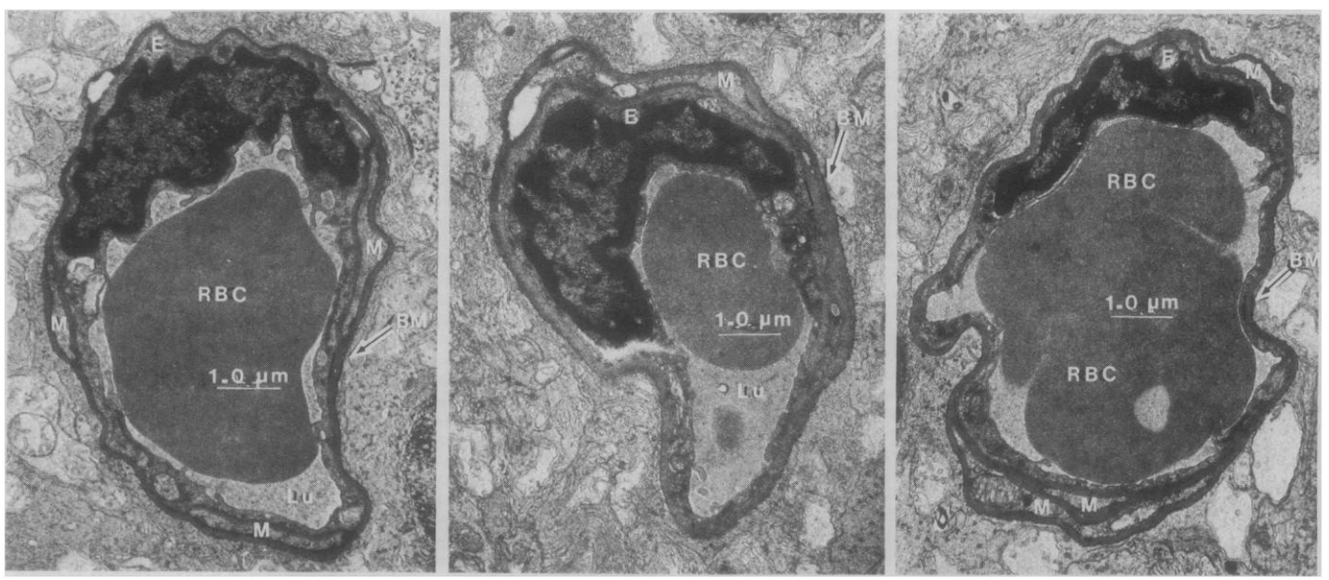

Fig. 1. Basement membrane thickness in retinal capillaries of the outer plexiform layer in rats on a normal diet (left), galactose diet (center), and galactose and sorbinil diet (right) for 44 weeks. Abbreviations: *BM*, basement membrane; *E*, endothelial cell area; *Lu*, lumen area; *M*, mural cell area; and *RBC*, red blood cell (×8500).

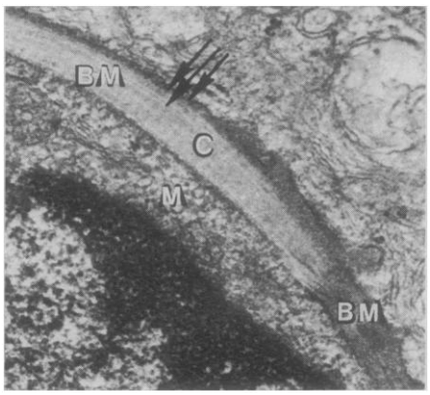

Fig. 2. Collagen (*C*) with 640-Å banding period (arrows) in basement membrane (*BM*) surrounding the mural cell (*M*) of a retinal capillary from a rat maintained on the galactose diet for 44 weeks (×26,000).

analyses, the ultrastructural examination was limited to the outer plexiform layer of the retina. Micrographs were taken of all the capillaries encountered in that region in at least four sections representing slightly different areas of the superior temporal sector of the central retina.

Retinal capillaries of the outer plexiform layer in all three groups had a similar general appearance in ultrathin cross sections (Fig. 1). That is, the total cross-sectional area (*T*) of each capillary contained regions that could be defined as mural (pericyte) cell area (*M*), endothelial cell area (*E*), lumen area (*Lu*), and basement membrane area (*BMA*). Some lumina contained portions of red blood cells. Only the basement membranes of the retinal capillaries were consistently different among the three groups. They appeared to be thicker in rats fed the galactose diets for 28 or 44 weeks than in rats given the control diet or the galactose and sorbinil diet. Also, extensive regions of basement membrane containing collagen with a banding periodicity of 640 Å (Fig. 2) were frequently found in rats on the galactose diet, while only small areas were occasionally found in rats on the control or the galactose and sorbinil diets. The regions of basement membrane bordering mural cells alone, endothelial cells alone, or both were similar in appearance and therefore were not distinguished in any of the quantitative analyses.

To measure the differences in basement membrane thickness, a quantitative method based on computer planimetry was developed. This method avoids some of the limitations inherent in most published methods for determining the thickness of such a complex structure as the basement membrane (*11*, *12*). The analyses were performed on electron micrographs of approximately ten capillaries from each rat, which were printed at a final magnification of ×25,000 and selected by using as the only two criteria the closeness to a 90° transverse cut and the sharpness of boundary distinctions. Quantitative planimetry was done with a Tetronix 4010 terminal equipped with a 4953 graphics tablet and 4932 recorder (*7*, *13*). The boundaries of the different regions found in the cross section of a retinal capillary (Fig. 1) were carefully outlined with inks of different colors. Then the perimeter of each region was traced over the graphics tablet by means of the available NIH DIGSET digitizing program. A coding system was employed and the tracings were done by individuals having no knowledge of the experimental groups involved. All coded tracings were then analyzed by using the available NIH MLAB program, and the analyzed curves were plotted and compared to the original micrographs to eliminate possible errors (Fig. 3). The final data were entered into the NIH PROPHET computer system and analyzed with its regression and statistical procedures. Basement membrane area and basement membrane length (*BML*) per capillary cross section were determined with the following formulas:

$$BMA = T - (M + E + Lu)$$

$$BML = \frac{\text{length of lines delimiting } BMA}{2}$$

From these calculations, the relative thickness could be expressed in terms of basement membrane area per unit length (*BMA*/*BML*) (*12*).

As shown in Fig. 4 and Table 1, at 28 weeks the rats on galactose had capillary basement membranes that were approximately 57 percent thicker than those of rats on the control or galactose and sorbinil diets ($P < 0.01$, *t*-test). By 44 weeks, the area per unit length of capillary basement membranes of galactose-fed rats increased to approximately twice that of rats fed the control or galactose and sorbinil diets, while no significant increases in the latter two groups were observed. Moreover, there was no significant difference between the rats fed the control diet and the rats fed the galactose and sorbinil diet for either time period. All the rats fed galactose or galactose and sorbinil were galactosemic, having blood galactose levels greater than 100 mg/dl, as measured by gas-liquid chromatography.

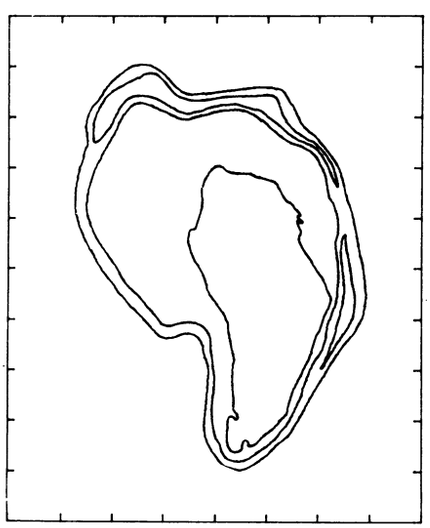

Fig. 3. Computer tracing of the retinal capillary shown in the center panel of Fig. 1.

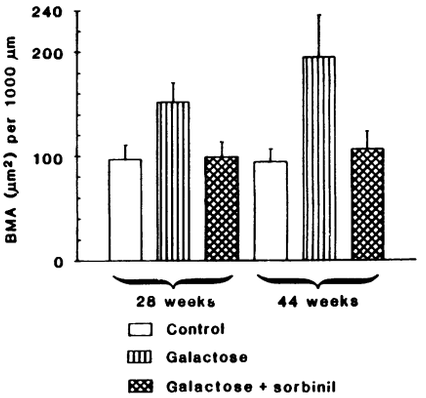

Fig. 4. Basement membrane thickness in retinal capillaries (outer plexiform layer). Values are means ± standard deviations.

Diet-induced galactosemia therefore resulted in a time-related thickening of basement membranes in retinal capillaries that could be prevented by the aldose reductase inhibitor sorbinil. This suggests that aldose reductase may be involved in the thickening of basement membranes.

Similar studies on the basement membranes in kidneys and other tissues should reveal how useful galactosemia might be as a model for diabetes and to what extent sorbinil might be effective in retarding or preventing other complications of diabetes.

W. GERALD ROBISON, JR.*
PETER F. KADOR
JIN H. KINOSHITA
Laboratory of Vision Research, National Eye Institute, Bethesda, Maryland 20205

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* To whom requests for reprints should be sent.

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Increased Brain Size and Cellular Content in Infant Rats Treated with an Opiate Antagonist

Abstract. *From birth to day 21, rat offspring received daily injections of naltrexone at a dosage that blocked morphine-induced analgesia 24 hours a day. At 21 days, body, brain, and cerebellar weights of naltrexone-injected animals were 18, 11, and 5 percent greater than corresponding control weights. In addition, morphometric analysis of the cerebrum revealed a somatosensory cortex that was 18 percent thicker than that of the controls. The cerebellum of naltrexone-treated rats was 41 percent larger in total area and contained at least 70 percent more glial cells and 30 percent more granule neurons. Neurons derived prenatally were unaffected by drug treatment. These results show that naltrexone can stimulate body and brain growth in rats and suggest a role for the endorphin and opiate receptor system in development.*

Opioid compounds, in addition to having analgesic and behavioral effects, are known to alter cell function and growth, particularly in developing neural systems (*1–3*). Clinical observations of infants and children exposed in early life to opiates such as heroin and methadone reveal a retardation in somatic and neurobiological development (*4*). Similar perturbations in growth have been reported in laboratory animals subjected to opioids perinatally (*1–3*, *5*) and in cells in culture treated with exogenous and endogenous opioids (*6*). This interference in growth is stereospecific and is blocked by coadministration of narcotic antagonists (*2*, *5*), with the locus of opioid action postulated to reside at the opiate receptor (*2*, *7*). We administered naltrexone, a potent narcotic antagonist, to infant rats at a dosage that continuously blocks the opiate receptor from interaction with endogenous opioid peptides. The prolonged naltrexone exposure stimulated brain development, indicating that the opiate receptor is related to mechanisms of neurobiological growth and that endogenous opioids serve in the regulation of nervous system development.

Newborn Sprague-Dawley rats, reared in litters of eight pups per mother, were given daily subcutaneous injections of naltrexone (50 mg/kg) or sterile water until 21 days of age (weaning). By that time, the naltrexone-treated offspring had body, brain, and cerebellar weights that were 18, 11, and 5 percent greater than the control weights (Table 1). Macroscopic dimensions of the brain and cerebellum in the naltrexone-treated animals were 2 to 11 percent larger than those of the control animals (Table 1). Morphometric analysis of histological sections from the somatosensory cortex and cerebellum showed an enlargement of both regions in naltrexone-treated offspring (Table 2). In particular, areas of cerebellum analyzed were 41 to 45 percent larger than in controls. Further analysis of the cerebellum revealed increases in cellular content in sections of the pyramidal lobe (Table 3). The number of internal granule neurons per section was increased 30 percent and the number of glial cells (oligodendrocytes and astrocytes) in the medullary layer was increased 70 percent. The total population of glial, basket, and stellate cells in the molecular layer was increased 169 percent (Fig. 1). Furthermore, the effect of naltrexone on cell number in the cerebellum appeared to be directed solely toward cell populations derived during the treatment interval, since Purkinje cells, which are generated prenatally, did not change in number with perinatal naltrexone exposure (Table 3).

These results demonstrate that naltrexone can markedly stimulate the course of somatic and neurobiological development in the rat. The dose of naltrexone mediating these effects (50 mg/kg) represented 2 to 3 percent of the median lethal dose for adult rats (*8*). This low dose antagonized morphine-induced analgesia completely and effectively. Measurement of nociceptive responses (Analgesia Meter, Technilabs) 30 minutes after challenge with morphine sulfate (0.2 mg/kg) showed that the naltrexone blocked opiate receptors 24 hours a day.

Opiate receptors have been identified in brain and body tissues during ontogeny (*9*), endorphin immunoreactivity has been recorded in fetal brain and spinal cord cells (*10*), and endorphins have been found in the plasma and brain tissues of developing organisms (*11*). Our experiments show that when opiate receptors are continuously blocked, presumably preventing the interaction of these receptors with endogenous opioid peptides, larger animals with correspondingly bigger brains develop. This effect occurred in both sexes. Enlarged brain size was accompanied by an increase in the number of neurons and glia, particularly those arising postnatally. The number of neurons derived prenatally did not appear to be affected by exposure to naltrexone. The larger animals also showed an acceleration in neurobehavioral ontogeny and in the appearance

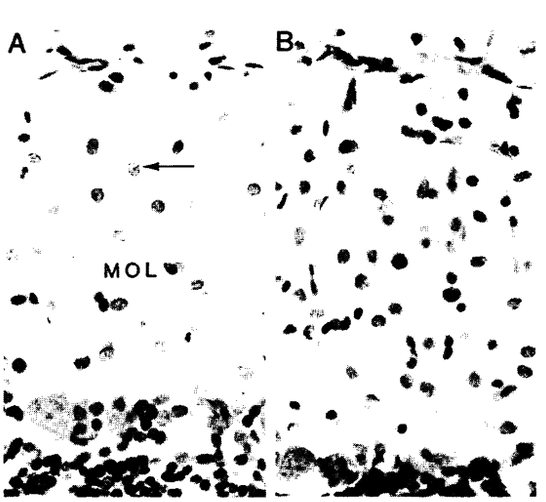

Fig. 1. Molecular layer (*MOL*) from the cerebellar pyramidal lobe of 21-day-old control (A) and naltrexone-treated (B) rats. The number of neural cell nuclei (arrow) is greater in the naltrexone-treated animals. Rats were fixed by cardiac perfusion with 10 percent neutral buffered Formalin and processed in polyester wax, and tissue was stained with hematoxylin and eosin (×830).