Acidic glycosaminoglycans and laminin-1 in renal corpuscles of mutant blebs (*my*) and control mice

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Summary. Deposition of glycosaminoglycans and laminin-1 in the renal corpuscles of the kidneys of mylencephalic blebs, 'blebs', (*my*) and normal C57BL/6J mice was compared in embryonic, neonatal (newborn to approximately two days old) and adult animals. Utilization of Alcian Blue 8GX staining, at a pH of 2.5, revealed an increase in acidic glycosaminoglycans in the parietal layer of Bowman's capsule and in general, an increase in the mesangial matrix of the glomerulus of *my* mutant adults. An increase in glycosaminoglycans was also noted in the developing kidney in certain *my* embryos in tissues associated with the glomeruli, but no significant differences were observed between the kidneys of neonatal *my* and control mice. The laminin-1 procedure revealed more deposition of laminin in the basement membrane of the parietal layer of Bowman's capsule in the neonatal and adult mutant *my* mice. Altered deposition of basement membrane and extracellular matrix components may reflect changes in the pattern of development and in the functioning of the kidney. Morphological changes in the human kidney are associated with alteration of function; a similar association may be occurring in the mice homozygous for the *my* gene.

Key words: Blebs, Renal corpuscle, Basement membrane

Introduction

Mylencephalic blebs, known now as 'blebs', *my* mutant mice have exhibited a number of anomalies including kidney agenesis and hydronephrosis (Carter, 1959). In the stock maintained in our colony, the major effect is on the development of the eye (Center and Polizotto, 1992). However, since cystic kidneys are occasionally found in our *my* stock, in these investigations we concentrated on examining and comparing the filtration mechanism of the glomeruli in the kidneys from *my* mutant mice and C57BL/6J normal

control mice. The filtration mechanism of the glomeruli includes fenestrated endothelial cells, podocytes and a basement membrane (Telford and Bridgman, 1990). Our focus has been on the parietal layer and the visceral layer, which is a part of the renal glomerular filtration barrier, of Bowman's capsule and the mesangial matrix of the glomerulus. We examined the basement membrane and the associated extracellular matrix of these tissues. It has been known for some time that basement membranes are composed of a number of components such as glycoproteins, e.g., laminins, proteoglycans and associated glycosaminoglycans and collagens (Yurchenco et al., 1994). The components of the extracellular matrix are essentially the same, being made up of collagens, noncollagenous glycoproteins, and proteoglycans (Kreis and Vale, 1993). Our investigation centered on examination of the components of the renal corpuscle for the presence of acidic glycosaminoglycans and laminin-1 (Burgeson et al., 1994).

Materials and methods

Kidneys from adult, neonatal and embryonic blebs (*my*) and C57BL/6J mice were compared with regard to histochemical changes in the basement membrane and extracellular matrix in the renal corpuscles. Pregnancies were timed by use of the vaginal plug method (plug = day zero) and by correlation with Grüneberg's morphological staging technique (Grüneberg, 1943). Embryos of 14-15 days were removed from pregnant females and fixed in 10% neutral buffered formalin at 5 °C, or occasionally at room temperature. After fixation, the embryos were transferred to 70% ethanol and stored at 5 °C, or rarely at room temperature. Kidneys from neonatal and adult mice were removed, fixed in cold 10% neutral buffered formalin, transferred to 70% ethanol and stored at 5 °C. The tissues were embedded in paraplast, or rarely in parawax, and serially sectioned sagittally at 10 μm; serial cross sections of embryos were also utilized in a few cases.

For acidic glycosaminoglycans, staining with Alcian blue 8GX at a pH of 2.5 with a hematoxylin counter-stain was utilized, according to a modification of a

method used by Steedman (Bancroft, 1975).

The procedure for laminin-1 was performed by utilizing a modification of avidin-biotin peroxidase labelling (D'Errico et al., 1986). In order to avoid tryptic digestion of the adhesive, the slides were treated first with Vectabond Reagent (Vector Laboratories, Burlingame, CA). A 0.3% H_2O_2 solution in PBS, pH 7.2, was used to block endogenous peroxidase. Incubation in 0.1% trypsin/0.1% $CaCl_2$ in Tris buffer was employed to digest any inhibitors produced by the formalin fixation. The non-specific staining was blocked with 10% goat serum (Vector Laboratories) in PBS. The slides were incubated in laminin-1 antiserum which was rabbit anti-mouse laminin against Engelbreth-Holm-Swarm mouse sarcoma tissue (Sigma Laboratories). This was followed by incubation in biotinylated antirabbit IgG (Vector Laboratories) and incubation in the avidin-biotin complex (Vector Laboratories). The slides were subsequently stained with Hanker-Yates Reagent (Polysciences, Inc., Warrington, PA) and counterstained with hematoxylin. All observations were carried out by means of light microscopy.

Results

As noted in 1992 (Center and Polizotto), preliminary results indicated alterations in the deposition of the glycosaminoglycan components of the basement membranes in the kidneys of mylencephalic blebs (*my/my*) mice. We have confirmed this observation and carried out expanded research based on this earlier observation. Alcian blue staining of fifteen kidneys from adult blebs (*my/my*) and C57BL/6J control mice revealed more glycosaminoglycans in the basement membrane of the parietal layer of Bowman's capsule in the kidneys from *my* mice (Fig. 1). In addition, Figure 1 shows that the glycosaminoglycan deposition in the mesangial matrix of the glomerulus in the kidney from a *my* mutant was greater than that in the control kidney tissue. However, the amount of glycosaminoglycans does appear to vary in the mesangial cells in the glomeruli found in different *my* mutants. There was generally greater deposition in the glomeruli of the kidneys from the *my* mutants than in the corresponding region within the kidneys of the control mice. The visceral layer of Bowman's capsule exhibited varying amounts of glycosaminoglycans in both the *my* mutants and the control mice.

In an attempt to determine the developmental sequence of the deposition of glycosaminoglycans in the renal corpuscles of the kidneys of both mutant and control mice, kidneys from embryonic and neonatal mice were studied. Staining of seven embryos of 14-15 days gestation with Alcian blue revealed that, in general, the developing kidneys in the *my/my* embryos showed an increase in the amount of glycosaminoglycans in the tissues associated with the glomeruli, particularly in the developing parietal layer of Bowman's capsule (Fig. 2).

In the eight kidneys from neonatal mice (newborn to approximately two days old) which were also stained with Alcian blue, there was some evidence of glycosaminoglycans in the renal corpuscles of the kidneys of both the *my* and control mice. Indeed, there appeared to be slightly more evidence of glycosaminoglycans in the parietal layer of the capsule in the controls than in the *my/my* embryos in some cases.

In an attempt to study further the basement membrane components of the renal corpuscle, nine kidneys from adult blebs mice and C57BL/6J mice were subjected to the laminin procedure outlined above. Different sections of some specimens were utilized for either Alcian blue or laminin-1 procedures. In general, an increase in the amount of laminin and more uniform deposition were evident in the basement membrane of the parietal layer of Bowman's capsule in the kidneys from mutant *my* mice. In addition, slightly more laminin was seen in the visceral layer of Bowman's capsule and in the mesangial cells of the glomerulus in the kidneys from the *my* mutants (Fig. 3).

Embryonic and neonatal mutant and control mice were also subjected to the laminin procedure. In a limited study of 14 1/2 to 15 day old embryos, no evidence of laminin deposition was found in the glomerular or Bowman's capsular tissue in either mutant or control embryos in the two embryos in which kidney tissue was evident. Ten kidneys from neonatal mice (newborn to approximately two days old) were also subjected to the laminin procedure. In general, more laminin deposition was noted in the basement membrane of the parietal layer of Bowman's capsule in the kidneys from *my/my* mice than in the corresponding tissue in the control neonatal mice (Fig. 4). Occasionally, hints of laminin deposition were seen in the visceral layer and mesangial matrix of the *my* mutants; no laminin deposition was evident in these tissues in the neonatal C57BL/6J mice.

Discussion

It appears that changes in the make up of the basement membrane tissues of different regions of the mutant mice are due to the expression of the *my* gene. A linkage between kidney abnormalities and eye defects has been noted occasionally in humans (McKusick, 1994). An association of eye and kidney abnormalities is evident in the *my* mutant mice. It is known that extracellular matrix components influence the functioning of tissues (Slater, 1996). One glycosaminoglycan, heparan sulphate, which is found on the glomerular basement membrane, is thought to have a role in regulating the filtration of molecules in the kidney (Kreis and Vale, 1993). Glomerular basement membranes in the rat kidney were changed in cases of induced diabetes mellitus and this appeared to involve biosynthesis or turnover of abnormal extracellular matrix proteins (McCarthy et al., 1994). It is known that the glomerular basement membrane (GBM) is the principle glomerular filter which allows passage of small proteins

(40,000MW or less) but it does not allow larger proteins to pass through (Telford and Bridgman, 1990). The functioning of the adult kidney in *my* stock animals may be impaired by excess deposition of acidic glycosamino-glycans in association with the basement membrane of Bowman's capsule and in the mesangial matrix of the
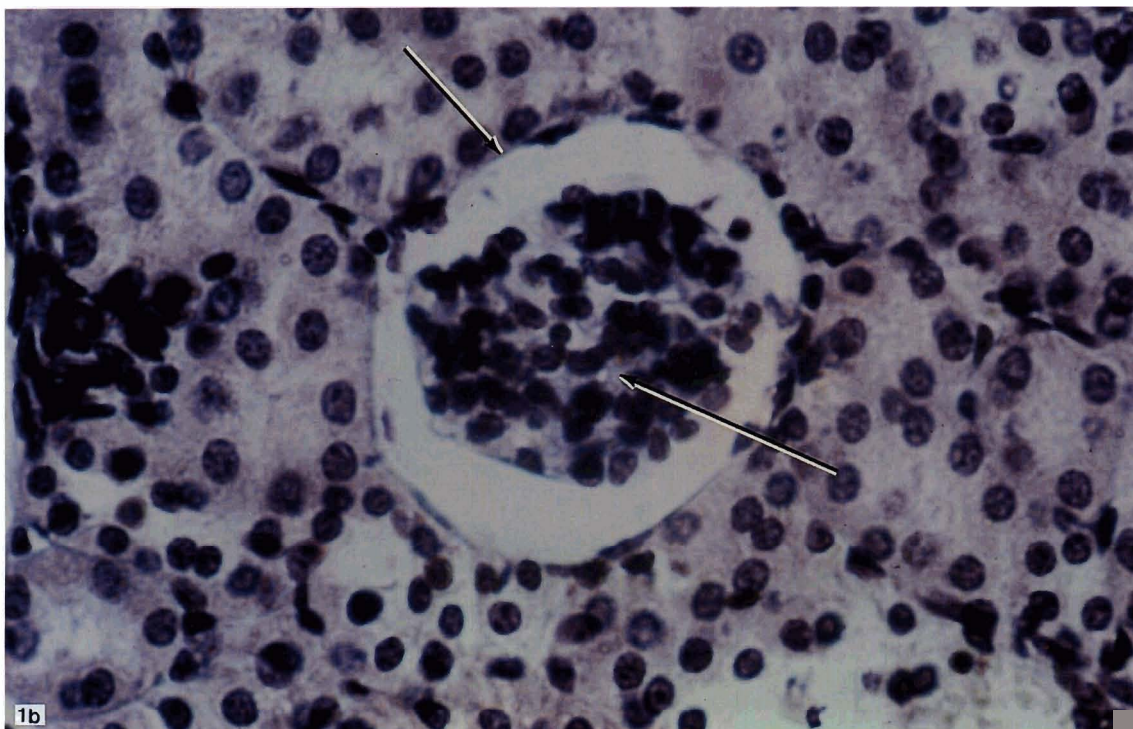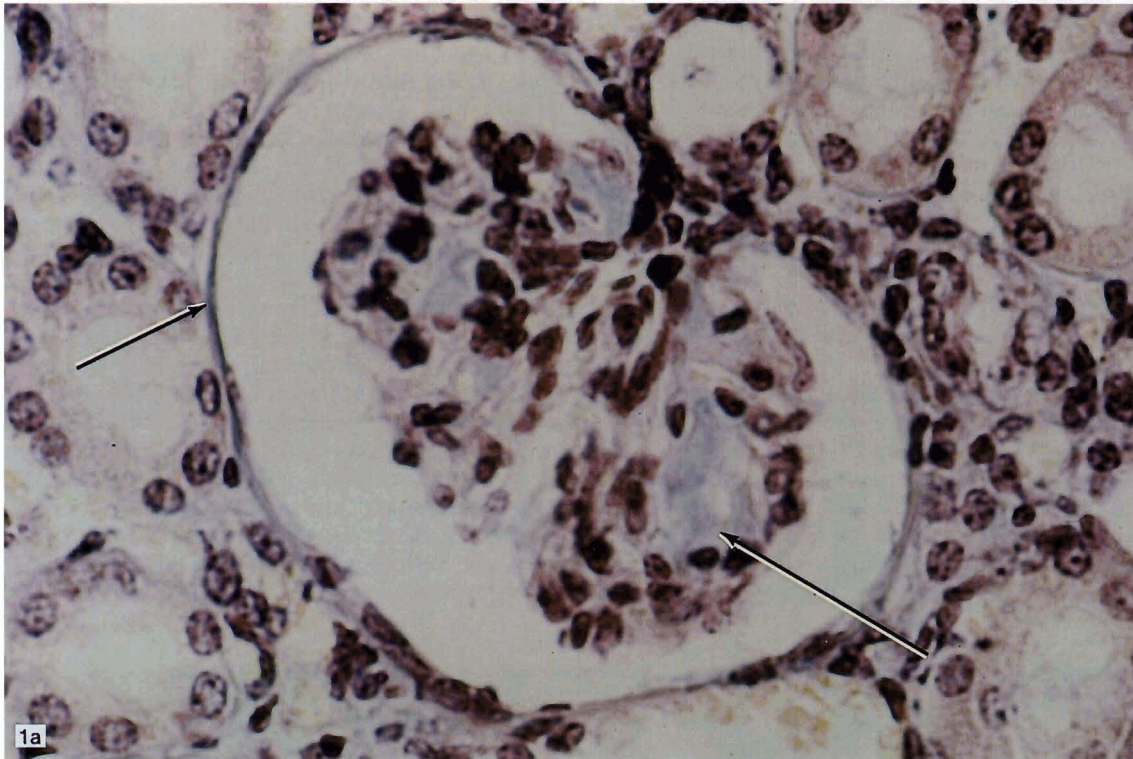
Fig.1. a. Section of a kidney from an adult blebs (*my/my*) mouse showing a single renal corpuscle. Short arrow indicates parietal layer of Bowman's capsule, long arrow indicates mesangial matrix of glomerulus. **b.** Section of a kidney from an adult C57BL/6J mouse showing a single renal corpuscle. Short arrow indicates parietal layer of Bowman's capsule. Long arrow indicates mesangial matrix of glomerulus. More deposition of glycosaminoglycans is seen in these areas of the renal corpuscle in the kidney of the *my/my* adult. Alcian blue and hematoxylin staining. x 1,000

glomerulus. Glycosaminoglycans contribute to the dynamic role of the basement membrane in rates of molecular migration, and regulation of cell growth (Slater, 1996). The role of the glycosaminoglycans associated with the parietal layer of Bowman's capsule in kidney function remains unclear. The parietal region of Bowman's capsule showed the most consistent changes in the renal corpuscles of the mutant *my* mice.

The pattern of glycosaminoglycan deposition differs in the kidneys of embryonic *my* mutant mice from that found in the kidneys of control C57BL/6J embryos. However, little difference is evident between the kidneys from neonatal control animals and those from neonatal mutant mice. It seems that the effects of the *my* gene are not uniform throughout development as has been previously noted in the investigation of the etiology of defective eyes in *my* stock animals (Center and Polizotto, 1992).

Basement membranes also function as sites of interaction among cells. Laminins are viewed as an important part of basement membranes (Yurchenco et al., 1994). Our investigations indicate that the laminin associated with the mesangial matrix and basement membrane tissues of the parietal layer of Bowman's capsule exhibits an increase in adult *my/my* mice in comparison with adult control animals. Kidneys of

neonatal *my/my* mice also show more deposition of laminin in the parietal layer of Bowman's capsule in comparison with the controls. Changes in the pattern of expression of the isoforms of laminin throughout development of the human kidney have been observed. Different isoforms may play a role in differences in adhesion of the basement membrane (Virtanen et al, 1995). The various isoforms of laminin may play a role in the glomerular basement membrane 'fusion and splicing' noted by Abrahamson and St. John (1992) in the developing mouse kidney. The laminin isoforms (laminin-1 being the best described) may help provide for the various functions of basement membranes (Aumailley and Kreig, 1996). Increased laminin in the basement membrane tissues in the kidneys of *my* mutant mice may alter the permeability of tissues in the kidney and interfere with normal kidney function. It has been found that a thickened, disrupted glomerular basement membrane alters the filtration barrier and allows proteins to be lost into the urine (Bergman et al, 1996). One can relate morphological changes in the kidney to changes in function in humans. Thickening of the glomerular basement membrane and mesangial matrix are found in cases of human diabetes, Type I (Yurchenco et al., 1994). The role of the basement membrane and extracellular matrix components in the functioning of the renal
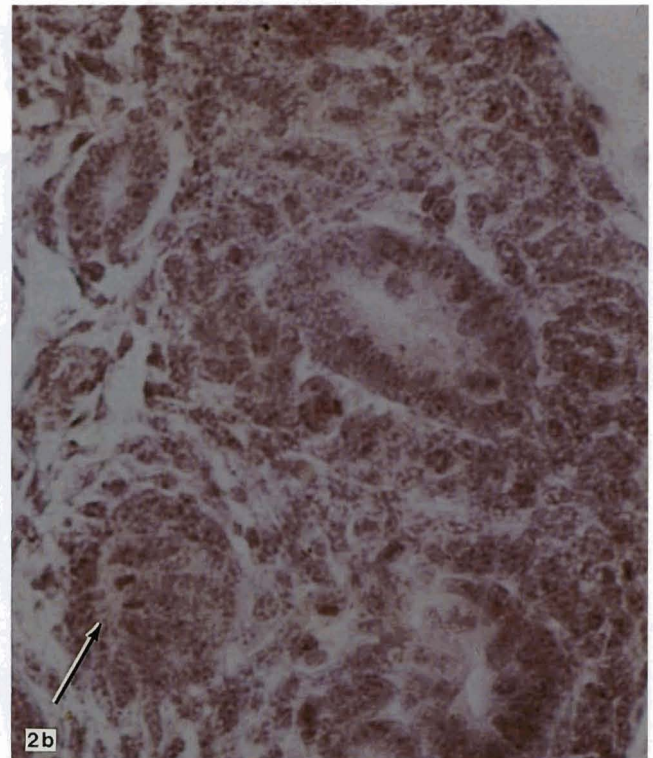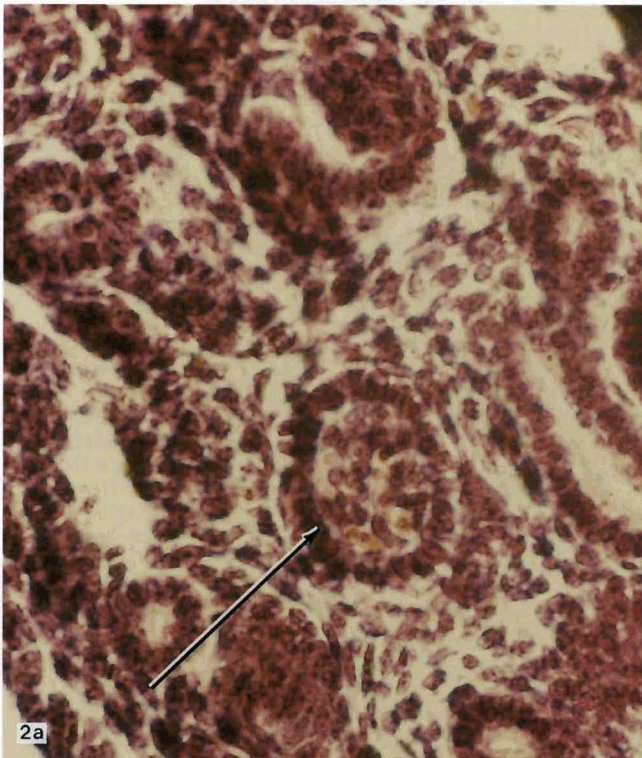
Fig. 2. a. Section of a 15 day *my/my* embryo showing developing kidney tissue. Arrow indicates developing parietal layer of Bowman's capsule. b. Section of a 14 1/2 day C57BL/6J embryo showing developing kidney tissue. Arrow indicates region of parietal layer in developing Bowman's capsule. Slightly more glycosaminoglycans is evident in the developing parietal layer (arrow) of Bowman's capsule in the *my/my* embryo. Alcian blue and hematoxylin staining. x 1,000
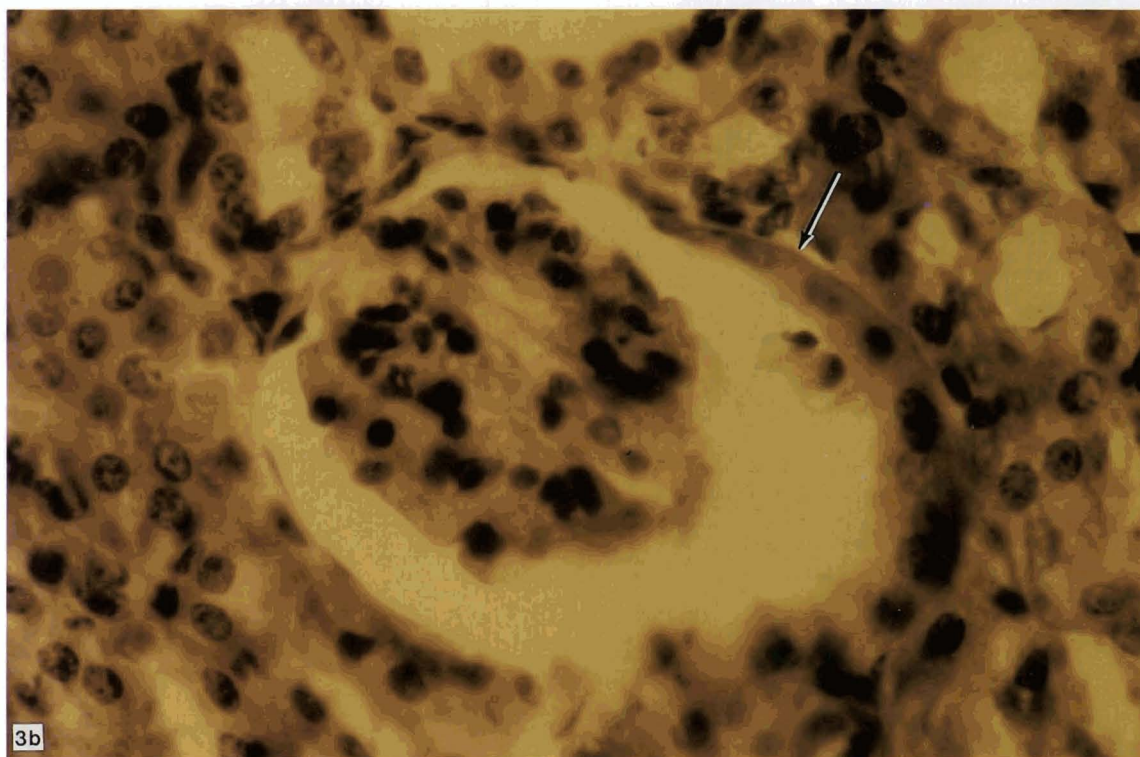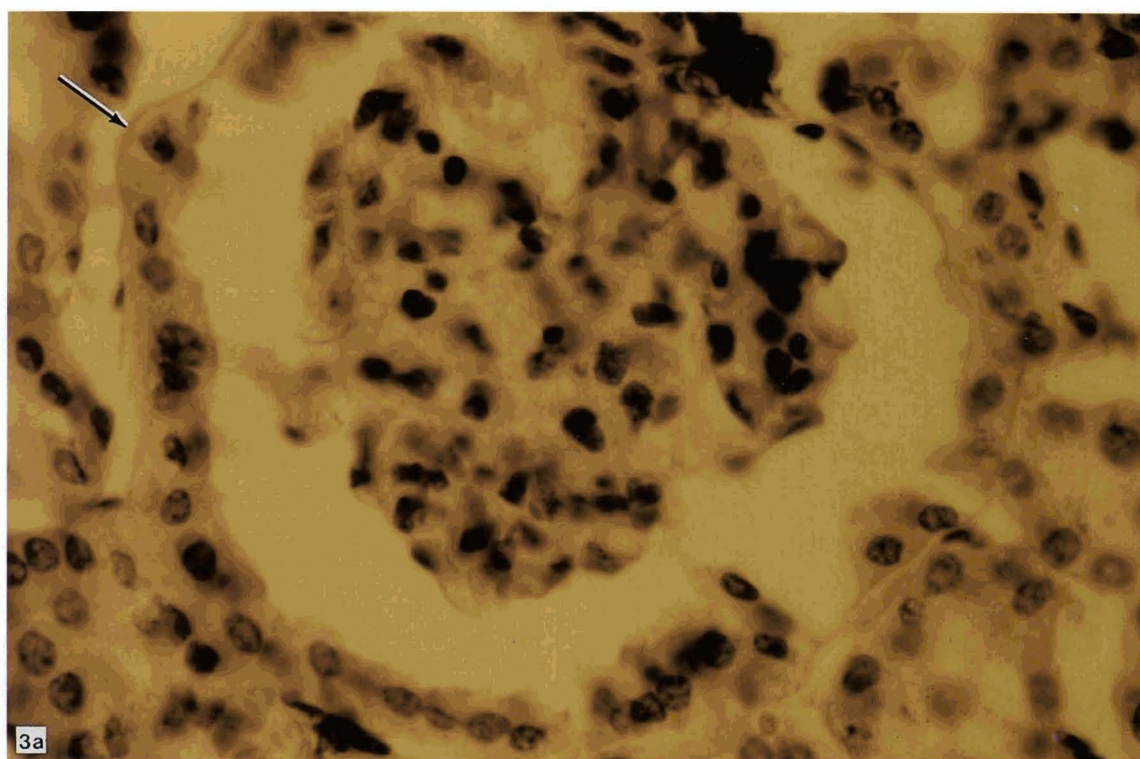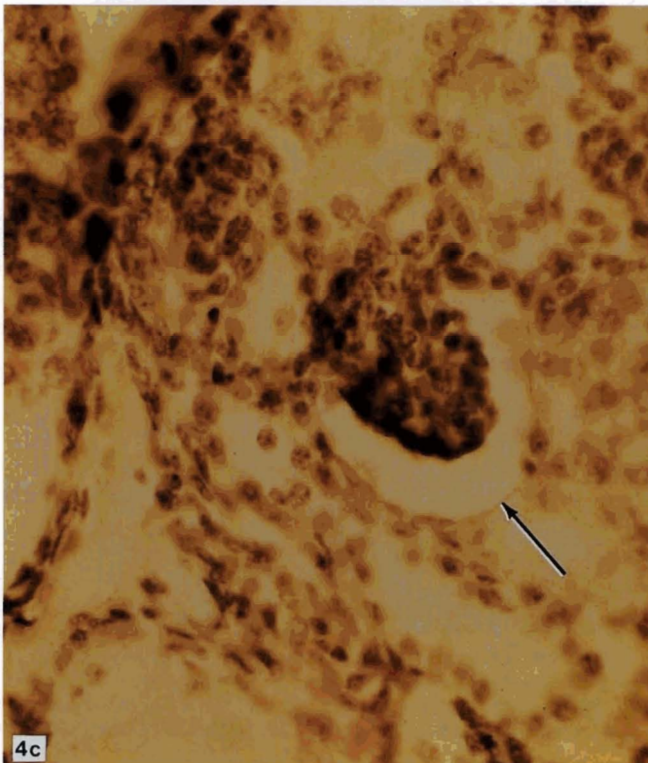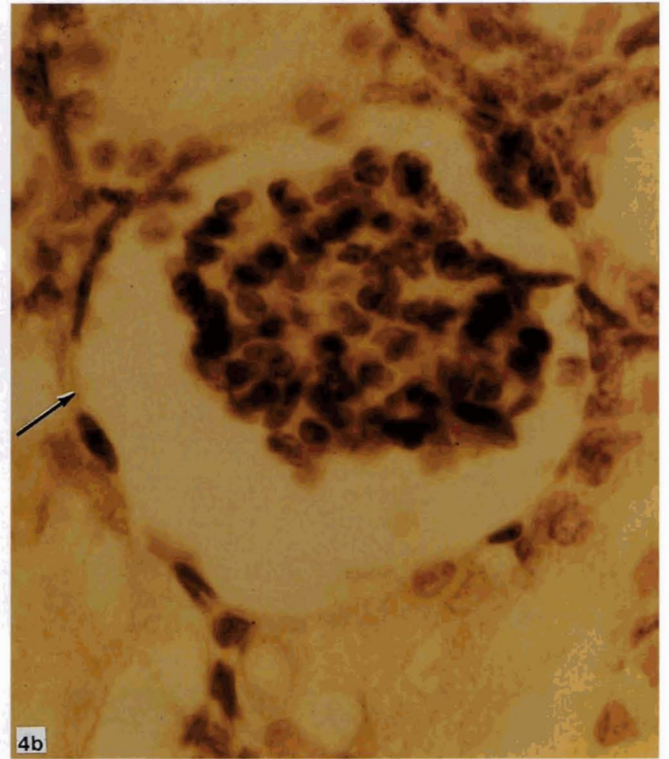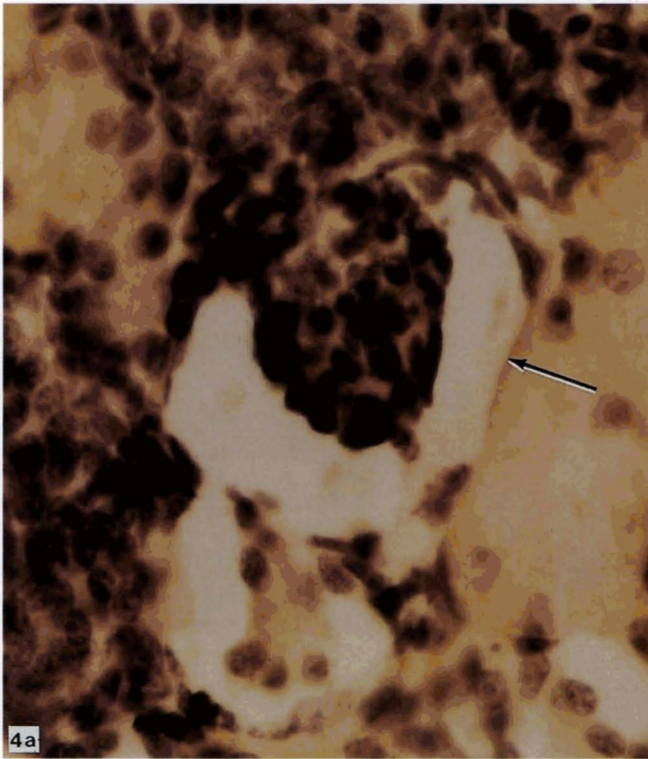
Fig. 3. a. Section of a kidney from an adult *my/my* mouse showing a single renal corpuscle. Arrow indicates basement membrane of parietal layer of Bowman's capsule. **b.** Section of a kidney from an adult C57BL/6J mouse showing a single renal corpuscle. Arrow indicates basement membrane of parietal layer of Bowman's capsule. More uniform deposition of laminin-1 is seen in the basement membrane of the parietal layer of Bowman's capsule in the *my/my* mouse. Avidin-biotin-peroxidase labeling of laminin, Hanker-Yates and hematoxylin staining. Background staining modified from purple to tan in photographs so that the basement membrane is more visible. x 1,000

Fig. 4. a. Section of a kidney from an approximately two day old *my/my* mouse showing a single renal corpuscle. Arrow indicates basement membrane of the parietal layer of Bowman's capsule, x 1,000. b. Section of a kidney from an approximately two day old C57BL/6J mouse showing a single renal corpuscle. Arrow indicates basement membrane of the parietal layer of Bowman's capsule. Phosphate Buffered Saline (PBS) was substituted for anti-laminin-1 antisera. Nuclei are stained with hematoxylin but no laminin-1 staining is evident in this control. x 1,000. c. Section of a kidney from an approximately two day old C57BL/6J mouse showing a single renal corpuscle. Arrow indicates basement membrane of the parietal layer of Bowman's capsule. x 600. More laminin-1 is seen in the basement membrane of the *my/my* mouse (Figure 4a). Avidin-biotin-peroxidase labeling of laminin. Hanker-Yates and hematoxylin staining. Background staining modified from purple to tan in photograph so that the basement membrane is more visible.

corpuscle needs further investigation in both humans and mice, especially with regard to laminin deposition and its possible role in kidney function.

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