NOTE *Laboratory Animal Science*

# Microvasculature of Hydronephrotic Kidneys in KK-A$^y$ Mice

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ABSTRACT. Spontaneous hydronephrosis in KK-A$^y$ mice was studied using light and electron microscopy and scanning electron microscopy of resin casts to evaluate micro vascular changes in the kidney. The renal parenchyma was extremely thin as a result of tubular atrophy. Histologically, varying degrees of glomerulosclerosis were observed. Ultrastracturally, marked thickenings of the glomerular basal lamina, an increase in mesangial cells and matrix, and marked effacement of foot processes were observed. In resin casts, a marked reduction in number of glomeruli was evident. The capillaries were thin, strangulated and torn-off to varying degrees in severely affected glomeruli. In the medulla, the three-dimensional capillary network running along the tubules was lost and changed to a two-dimensional vascular bed. Despite severe hydronephrosis, the glomerular capillary network was relatively well preserved, being either slightly or moderately injured in approximately 60% of surviving glomeruli.

KEY WORDS: corrosion cast, glomerulus, hydronephrosis.

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Hydronephrosis is a well-known disease in laboratory mice and rats. Its etiology and pathogenesis have not been established with certainty. However, theories proposed to account for its occurrence have included urinary back-pressure caused by proteinaceous urethral plugs [10], trauma-induced inflammation of the urethra [2], vesico-ureteral reflux [6] and urogenital abnormalities [5]. Although much attention has been devoted to studying the pathogenesis of the disease, few studies have dealt with the changes occurring in the renal microvasculature. Schwartz *et al.* [9] reported that renal blood flow declines to 41% of normal in hydronephrotic kidneys and suggested that the blood flow reduction might be caused by preglomerular vasoconstriction. A clear understanding of disturbances in renal vessels morphology in hydronephrosis is essential for the proper hemodynamic evaluation of the hydronephrotic kidney.

Renal vascular changes in this disease have been studied in Wistar rats by the corrosion casting scanning electron microscopic technique [7]. The aim of the present study was to obtain more detailed information on the microvascular changes in the hydronephrotic kidney as seen in KK-A$^y$ mice.

During developing hydronephrosis the renal parenchyma becomes so thin due to tubular atrophy that it appears transparent and sheet-like. In this thin sheet, the renal microvasculature, i.e. afferent and efferent arterioles and glomeruli can be seen easily. This permits studies of function of the renal vessels such as direct microscopical observation of glomerular renal vascular function *in vivo*, or intracellular microelectrode recordings from juxtaglomerular granulated and vascular smooth muscle cells *in vitro* [3]. Detailed information concerning the microcirculation of the hydronephrotic kidney could be useful for such investigations as well.

The KK-A$^y$ mouse carries the yellow obese and diabetes genes and is characterized by severe hyperinsulinemia and hyperglycemia. Severe hydronephrosis accompanies diabetes in these mice with high frequency and it has been suggested that the initial stages of hydronephrosis related to the metabolic perturbations of diabetes [4]. We previously reported a case of obstructive uropathy with hydronephrosis caused by altered seminal material plugging the urethra in the KK-A$^y$ mice (8).

Ten diabetic KK-A$^y$ mice (male, 6 months of age, Nippon Clea Co., Ltd.) were used for this study. The mice were under lifelong observation for diabetes development through 1997–1998. Eight of the ten died naturally and in each case the body was found in the morning (presumably within 12 hours after death). All eight were necropsied and diagnosed as having obstructive uropathy and hydronephrosis. The kidneys were prepared for histological examination. The two remaining mice each had a distended abdomen and after being diagnosed clinically as having hydronephrosis each was killed by an overdose of anesthetic ether. The euthanised mice were prepared for blood tests, and histologic and scanning electron microscopic observations. Blood sugar levels were measured at necropsy in both the euthanized animals by means of the enzymatic ultraviolet test with hexokinase (Nippon Roche Co., Ltd.). Four additional normal KK mice (male, 6 months of age, Nippon Clea Co., Ltd.) were sacrificed to serve as controls.

Each left kidney was removed from each mouse after tying of the renal artery. For light and electron microscopy, tissue samples were fixed in 10% formalin and 2.5% glutaraldehyde, post-fixed in 1% osmium respectively. Thin sections were stained with haematoxylin and eosin and periodic acid Schiff reagent for light microscopy. Ultrathin sections were conventionally stained with uranyl acetate and lead citrate for electron microscopy.

For corrosion cast scanning electron microscopy, the right kidneys were perfused with Ringer's solution at 37°C via the abdominal aorta. The renal arterial vasculature was filled with acrylic resin. After polymerization, the kidney was immersed in a 20% NaOH solution, and then samples were rinsed in distilled water. Resin casts were then dissected

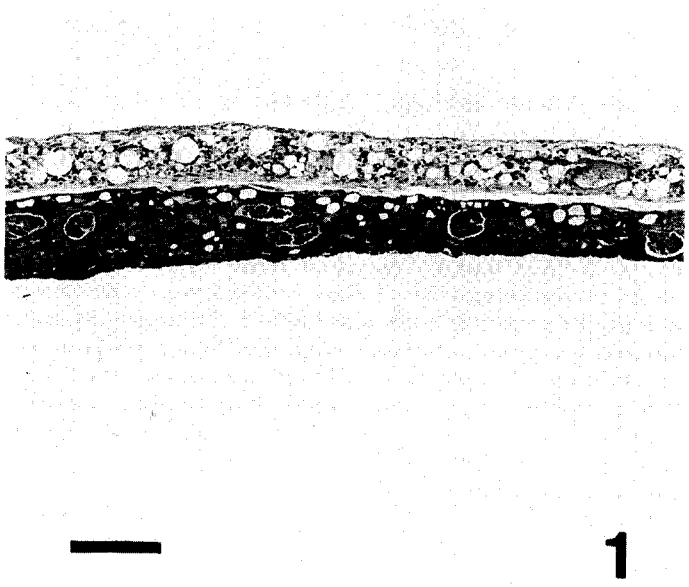

Fig.1. Histology of the KK-A$^y$ mouse kidney with severe hydronephrosis. Note the thinned atrophied parenchyma. Haematoxylin-eosin stain. Bar=100 $\mu$m.

under a binocular microscope and each piece of casts was mounted on an aluminum stub and spattered with gold for scanning electron microscopy.

Extraordinary distension of the bladder, ureter and kidney was seen grossly in each KK-A$^y$ mouse. There was no concomitant infection even in severely affected urinary tracts and kidneys. Frequently one or more opaque whitish masses could be seen through the tightly stretched bladder wall. The bilateral kidneys were converted into thin-walled sacs with little or no discernible parenchyma. Blood sugar levels of the animals ranged from 113 to 258 mg/dl.

An extremely dilated renal pelvis and considerably narrow cortex were observed. The medulla and the papilla were severely atrophied, forming a sheet of 100 to 500 $\mu$m in thickness (Fig. 1). Varying degrees of glomerular atrophy, tubular distension, hydopic degeneration of tubular epithelium and interstitial fibrosis were apparent. In severely affected glomeruli, capillary had narrow, slit-like lumens or had entirely disappeared and their position being occupied by mesangial cells (Fig. 2). The arcuate arteries and veins were compressed and distorted, their diameter was reduced. The inner medullary vasculature showed no evidence of thrombosis or endothelial swelling.

Observations by electron microscopy largely confirmed the nature of the glomerular atrophy seen by light microscopy. Marked thickenings of the glomerular basal lamina, an increase in mesangial cells and matrix and prominent thickenings and effacement of foot processes were observed.

The vasculature in hydronephrotic kidneys had lost its three dimensional nature and become two-dimensional. In particular, the medullary vascular system with its striated peritubular capillary bed had disappeared. The arcuate artery and vein were distorted and flat (Fig. 3). Each interlobular artery, afferent and efferent arteriole and glomerulus could be seen easily through the atrophied medullary vascular bed and the hierarchy of these vessels was well preserved in spite of the drastic atrophy of the renal parenchyma. Varying degrees of damage were evident in affected glomeruli. In severely damaged glomeruli, the glomerular capillary arrangement was lost for the most part and the capillaries were reduced in size and strangulated and/or torn off to varying degrees (Figs. 6, 7). Approximately 60 of 100 randomly selected surviving glomeruli showed slightly or moderately damaged configuration and their capillary networks were relatively well preserved [Fig. 5]. Interestingly, the atrophy of the glomeruli seemed to occur in pairs or in layers from the juxtamedullar to the surface regions (Fig. 4). As previously noted by Schwartz *et al.* [9] and Bührle *et al.* [3], not all glomeruli were damaged to an equal extent so that sometimes nearly intact glomeruli were found in the vicinity of severely damaged ones (Fig. 4).

Glomerular lesions observed in the present study comprised glomerular sclerosis characterized by thickened basal membrane, effacement of food processes and an increase in mesangial cells in the affected glomeruli. Although such lesions are also a feature of diabetic glomeruli, we concluded that these glomerular lesions found in the KK-A$^y$ mice in the present study could unequivocally be attributed to hydronephrosis, since the KK-A$^y$ mouse kidney also showed an extremely dilated pelvis and thinned atrophied parenchyma. In addition, the nodular changes in the capillary loops that can be frequently observed in the diabetic glomeruli were not

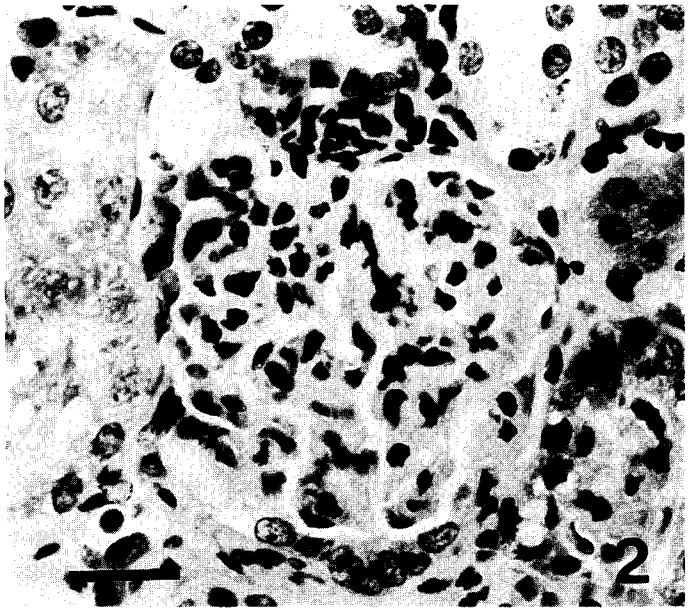

Fig. 2. A severely affected hydronephrotic glomerulus. The glomerulus shows a marked shrinkage and its capillaries are slit-like or disappeared. Haematoxylin-eosin stain. Bar= 20 μm.

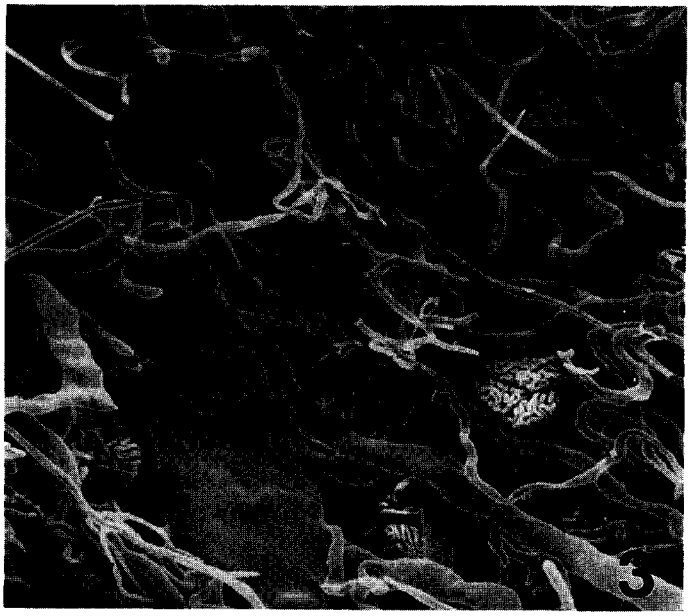

Fig. 3. A survey scanning electron micrograph of the resin cast blood vascular beds of the hydronephrotic kidney (viewed from the renal pelvis). Each afferent and efferent arteriole and glomerulus can be seen easily through the atrophied medullary vasculature. v: arcuate vein. Bar= 200 μm.

seen in the glomeruli of the KK-$A^y$ mice in the present study. However, the possibility could not be ruled out the possibility that diabetes may play a part in the formation of these glomerular lesions. Further studies are needed to clarify the pathogenesis involved, including pathophysiological analysis of the hydronephrotic glomerulus in animals diagnosed as having concurrent hydronephrosis and diabetes.

The drastic changes in renal cortical and medullary vasculature including atrophy and/or disappearance of glomeruli observed in the present study of the hydronephrotic mouse kidney correspond to those reported in rats [7]. Schwartz *et al.* [9] reported that the events that lead to these vascular

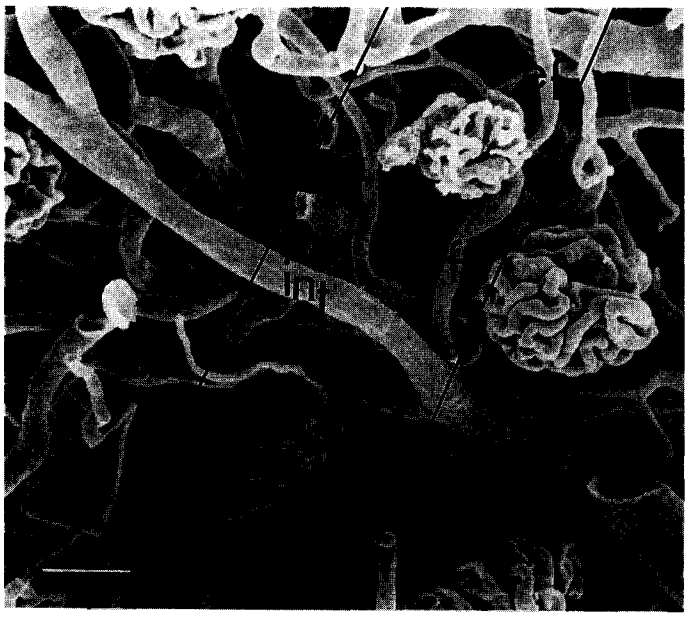

Fig. 4. An interlobular artery with afferent arterioles supplying glomeruli. Note that the fundamental pattern of the glomerular vascular tree is well preserved and that glomerular atrophy occurs in pair and/or in layers. int: interlobular artery, af: afferent arteriole, ef: efferent arteriole. Bar= 100 $\mu$m.

Fig. 5. A nearly normal hydronephrotic glomerulus. Bar= 50 $\mu$m.

lesions may include pressure atrophy and arterial and venous obstruction due to the increased intrapelvic pressure. Another prominent feature of the hydronephrotic kidney is that the glomerular atrophy occurs in the form of laminar and/or focal changes, rather than total degenerative changes. The reason why lesions occur in adjacent pairs of glomeruli or layers of glomeruli from the juxtamedullary area to the cortex is unknown, but the lesions might be attributed to increased inner pressure caused by the distended renal pelvis.

In early hydronephrotic kidney, increased intrapelvic pressure causes kinking, stretching and/or obstruction of the major vessels such as arcuate and interlobular arteries and

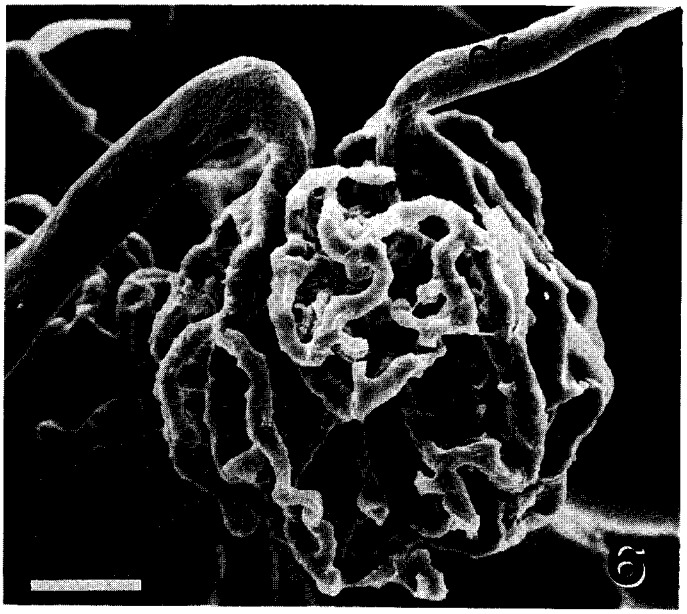

Fig. 6. A moderately damaged hydronephrotic glomerulus. Note narrowed and strangulated capillaries. Bar= 50 $\mu$m.

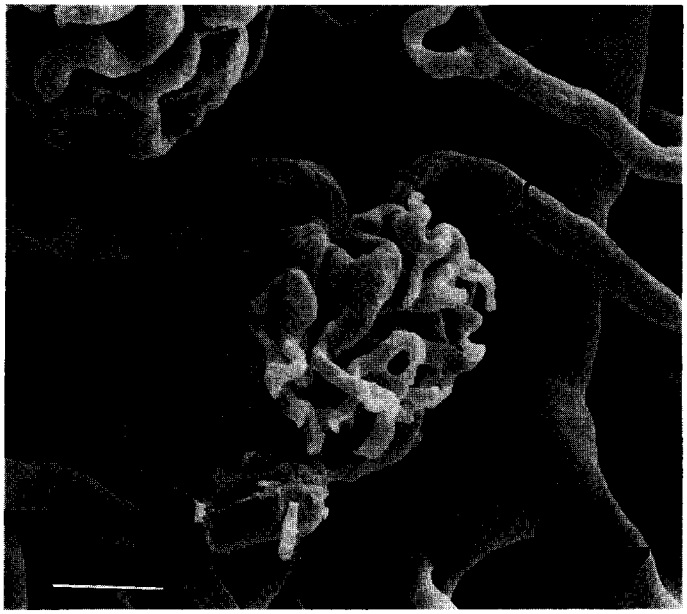

Fig. 7. A severely damaged hydronephrotic glomerulus. Note that capillaries are reduced in number and are torn off. This glomerulus may represent that in Fig. 2. Bar= 50 $\mu$m.

veins. Schwartz *et al.* [9] reported that these vascular changes induce the renal blood flow to reduce to 41% of normal and they suggested that reduction of renal blood flow might be caused by preglomerular vasoconstriction. Vascular constriction, which is caused by a precapillary sphincter, has been reported in the afferent and efferent arterioles [1]. However, no evidence was found to demonstrate the preglomerular vasoconstriction in hydronephrotic kidneys in the present study. Obstruction and/or narrowing of the major arteries and veins and destruction of the microvascular system including the glomerular capillary network might be involved in the drastic reduction of renal blood flow in this disease.

The present study showed a drastic decrease in the number

of glomeruli in the hydronephrotic kidney. Approximately 60% of the remaining glomeruli, however, were relatively well preserved. Bührle *et al.* [3], who investigated glomerular microcirculation as well as electrophysiological and biochemical properties in experimental hydronephrosis in mice, reported that the functions of the surviving glomeruli, the rennin-containing juxtaglomerular and smooth muscle cells of the afferent arteriole remained intact. This may help to explain why animals with such severe hydronephrosis as seen in the present study can survive for long periods. In addition, it was surprising to learn that marked atrophy of the renal parenchyma involving reduction in blood flow of up to 41% of normal does not usually impair viability [9].

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