

Obstructive Uropathy and Hydronephrosis in Male KK-A^y Mice: A Report of Cases

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ABSTRACT. Uropathy associated with hydronephrosis was observed frequently in our male KK-A^y mouse colony during a long-term study of diabetes. The lesion occurred in 24 of the 31 KK-A^y male mice and accounted for the greatest number of spontaneous deaths among them. It was observed after 4 months of age and involved about hard plugs of altered seminal material resembling the seminal vesicle secretion. The plugs became impacted in the urethral bulb and the bladder. The penile anatomy, with its flexure, pressure on the urethra from the bulbocavernosus muscle, and the characteristic ability of the seminal fluid to easily coagulate to form the vaginal plug may have contributed to the lesion. Correlation between development of the uropathy and diabetes has not been established.—**KEY WORDS:** KK-A^y mouse, obstructive uropathy, urethral plug.

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Obstructive uropathy is an important disease in adult male mice and rats, characterized by extreme urinary retention and hydronephrosis [1, 9, 10]. In some cases, this lesion is the single most common spontaneous cause of death during long-term studies in male mice. The etiology and pathogenesis proposed to account for its occurrence have included formation of urethral plugs derived from the seminal vesicle secretion [1, 9, 10] and trauma-induced inflammation [2, 8]. However, these theories have not been established with certainty. During the past 2 years, a high mortality rate after 4 months of age has been observed in male KK-A^y mice maintained in our mouse colony. A fairly constant observation at necropsy in these animals has been obstruction of the neck of the urinary bladder and the urethra causing extreme urinary retention and hydronephrosis. This lesion occurred in 24 of the 31 KK-A^y mice, and was the apparent cause of death of many of the animals. The KK-A^y mouse carries the yellow obese and diabetes genes. The following is a description of the disorder and of our study of the urinary tract to elucidate its nature.

The mice were housed in plastic cages containing sterilized wood shavings, usually 1 to a cage. The cage size was 23 × 16 × 12 cm. The animals were given pelleted chow CE-2 (14.2 KJ/g, 4.4% crude fat, Clea Japan, Inc., Tokyo) and water *ad libitum*. Room temperature was maintained at 24 ± 2°C. The mice were under lifelong observation for diabetes development through 1997–1998. The viscera, including the deep pelvic structures were examined grossly in 7 males killed at 14 months of age and in 24 dying before this time. Eight macroscopically normal ICR male mice of 10 months of age (Clea Japan, Inc., Tokyo) were examined to compare with KK-A^y mice with urethral lesions.

Histologic study: Examination of pelvic tissues and kidney was made in 10 mice (8 KK-A^y, 2 ICR mice). Four μm-thick serial sections were cut longitudinally or transversely to the penis and stained with hematoxylin and eosin, PAS and Azan Mallory stains.

Resin cast study: Eight mice (2 KK-A^y, 6 ICR mice) were used for this study. The urinary tract was perfused

from the bladder with Ringer's solution before corrosion casts of the urethra were made. Each cast was mounted on an aluminum stub and spattered with gold for scanning electron microscopy. Blood sugar levels were measured at autopsy by means of the enzymatic ultraviolet test with hexokinase (Nippon Roche Co., Ltd.).

Necropsy findings: Extraordinary distension of the bladder, hydroureter and hydronephrosis were seen regularly in male KK-A^y mice dying spontaneously. There was no concomitant infection even in severely abnormal urinary tracts and kidneys. Frequently one or more opaque whitish masses could be seen through the tightly stretched bladder wall (Fig. 1). On opening the bladder such masses were often found lodged in the bladder neck, and histologic sections showed them to extend into the proximal urethra. The obstructive lesions diagnosed as a cause of death were first observed in all of 6 male KK-A^y mice dying at 4 months of age. The number of death associated with obstructive uropathy increased rapidly for next 8 months or so. The urethral lesions were manifest in 12 of 18 males dying between 7 and 14 months. In 6 of 7 males sacrificed at 14 months of age, obstructive uropathy was present as well. In advanced cases, the kidneys were converted into thin-walled sacs with little or no discernible parenchyma.

Microscopic findings: The seminal material in the urethra was completely amorphous and stained a pale pink in hematoxylin-eosin stained sections, thus resembling seminal vesicle fluid. The bladder and urethral lumen of the male mice contained a white, rubbery mass that conformed to the contours of the passage (Figs. 2 and 3). They usually extended proximally to the flexura penis (in the mouse and rat, the middle part of the penis turns caudally at an acute angle in the nonerect state) and sometimes through the bladder neck into the bladder. The urethral plugs were not found distal to the flexura penis in any male mice. In severely afflicted mice, the seminal material adhered to the urethral epithelium to form an urethral plug occluding the urethral cavity completely. The urethral plugs also caused enlargement or compression of the cavernous spaces around the urethra. The cavernous spaces lost their configuration



Fig. 1. KK-A^y mouse with extremely distended urinary bladder due to blockage of urethra with coagula of seminal proteins. A white intravesicular mass is visible through bladder wall (arrow). On both sides the kidneys are affected with severe hydronephrosis. Bar=1 cm.

and were replaced with fibrous tissue in some cases. In normal ICR mice, though seminal vesicle fluid was usually found in the urethral cavity, adhesion of seminal material to the urethral wall was not observed. Little or no inflammatory infiltration or fighting trauma were observed in any portion of the genitalia in mice afflicted with the uropathy. Little or no differences in the protein content of the urethral plugs and the seminal vesicle epithelium between KK-A^y and ICR mice were disclosed by observations of PAS and/or Azan-Mallory stained sections.

In hydronephrotic kidneys, an extremely dilated pelvis and considerably narrow cortex and medulla were observed. Shrunken glomerulae, tubular distension, degeneration of tubular epithelium and interstitial fibrosis were also present (Fig. 4).

Resin cast findings: In normal ICR male mice, several folds were observed to run longitudinally through the whole length of the urethra. Diameter of the urethra averaged 300 μ m. The urethra at the penile flexure was reduced in diameter to 234 μ m on average. The urethra at the urethral bulb where the bulbocavernosus muscle attached was strongly compressed dorso-ventrally by constriction of the muscle (Fig. 5-1). In KK-A^y male mice with the bladder greatly distended with urine, the penile flexure and several folds as seen in normal ICR mice were lost. A longitudinal concave representing a urethral plug was observed along the ventral side of the urethra (Fig. 5-2. arrow). In one of the two mice, the distal urethra to the penile flexure was twisted.

Obstructive uropathy causing extreme urinary retention and hydronephrosis has been observed rather frequently in

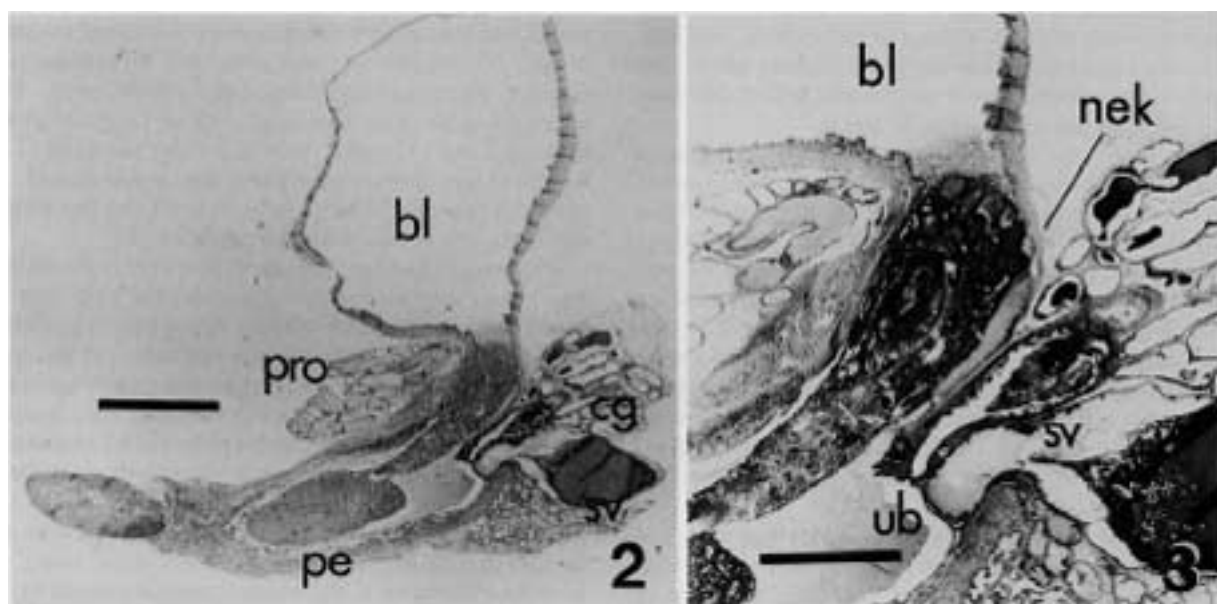


Fig. 2. Longitudinal section of the bladder and genitalia of a KK-A^y mouse showing plug of seminal material. bl: bladder; pro: prostate; sv: seminal vesicle; cg: coagulating gland; pe: penis. Bar = 2 mm.

Fig. 3. A closer view of Fig. 2 showing seminal material obstructing the urethra. Note that the seminal material morphologically resembles seminal vesicle secretion. ub: urethral bulb; nek: bladder neck. Bar=1 mm.

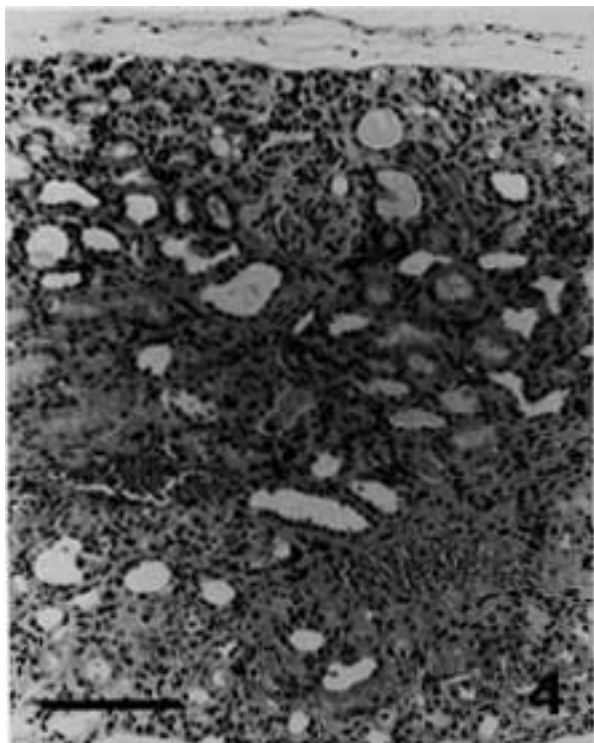


Fig. 4. KK-A^y mouse kidney with hydronephrosis. HE stain. Bar=100 μ m.

male mice of various strains [9, 10]. In some cases the lesion is the single most common spontaneous cause of death in long-term studies and mortality may be as high as 40% in B6C3F1 or ICR mice [2]. There are some mechanisms that might explain the urinary tract lesions [9]: (a) obstruction by urethral plug or calculus, or by keratotic epithelium; (b) compression by pelvic adipose tissue, fat necrosis nodules or disease of the pubic symphysis; (c) loss of mural motility of the bladder resulting from neuropathic or inflammatory alterations; or (d) fighting and fighting related lesions of the prepuce/penis [2, 8]. In the present study results indicate that the first possibility is the most likely one. Histological observation revealed that proteinaceous plugs resembling vesicular gland secretions were present in the bladder, the bladder neck, and the proximal urethra. No other significant pathologic findings such as neoplasms or infection were evident in any of the KK-A^y mice observed. Fighting is common in mice housed in groups and may include injuries to the prepuce/penis that result in inflammation/infection and scar formation leading to blockage of the urethral passage. This, however, seems not to have been the case in the present study, as our KK-A^y mice were caged individually. No fighting or fighting related wounds in the prepuce/penis were seen. Although the etiology of the white coagulum in the urethra of the mice is uncertain, metabolic perturbations may be a significant contributory factor. This contention is enforced by the finding that a low caloric diet

(10.0 KJ/g) reduced urethral obstruction and death to one half that of mice receiving a high caloric diet (16.3 KJ/g) [10]. Diani *et al.* [4] reported urinary bladder distension and a high rate of mortality in male KK-A^y mice fed with breeding chow (11% crude fat) and they suggested that the urinary lesions might have interacted with the metabolic perturbations of diabetes. We fed our male KK-A^y mice long-term (4–14 months) with this high caloric diet (16.3 KJ/g, 4.4% crude fat) and blood sugar levels of the mice ranged from 145 to 258 m/dl. This may have caused the severe uropathy associated with a high rate of mortality, though male and female KK-A^y mice are short-lived and 50% of the animals dies at 70 weeks of age (personal communication, Clea Japan, Inc., Tokyo). It is therefore conceivable that intake of a high caloric diet leads to metabolic disturbance in the seminal vesicle and coagulating gland, causing the seminal secretions to change into a material easy to coagulate and become impacted in the urethra. This contention may be supported by the fact that the secretions from the seminal vesicle and coagulating gland physiologically have a characteristic propensity to coagulate soon in the vagina to form a vaginal plug after copulation. In addition, testosterone is known to be an important factor in obstructive uropathy as the lesion does not occur in females (or rarely if at all) and it can be prevented by castration in males [10]. Testosterone administration has been reported to result in vesical hypertrophy [6]. Furthermore, male mice used for breeding have a higher rate of mortality due to uropathy than in nonbreeder males [10]. The present corrosion cast studies of the urethral passage showed a narrowing at the penile flexure and compression at the penile bulb by the bulbocavernosus muscle. It may thus be difficult for the viscous seminal material to run through the flexure during ejaculation. The bulbocavernosus muscle plays an important role in penile erection by compressing the urethral bulb to move blood in the cavernous spaces toward the penile glans. It may sound paradoxical, but frequent contraction of the muscle during breeding activity can disturb the seminal materials to be excreted. This may explain why a seminal plug is found frequently in the proximal urethra and the neck of the bladder in many cases and why breeder male mice have a higher rate of uropathy than nonbreeder males.

In severe uropathy, where the urethral plug completely occluded the urethral cavity and destroyed the cavernous spaces around the urethra, disturbance of reproduction involving impotence together with difficulty in excreting urine and/or semen could be expected.

In human diabetes, stasis of urine in the bladder is known to occur as a result of neuropathic bladder dysfunction, bladder decompensation, or sphincter involvement [3]. The high incidence of uropathy in the present case of KK-A^y mice may be attributed to metabolic disorder caused by long-term feeding of a high-calorie diet. The anatomical structure of the penis and the urethra and the chemical content of the seminal secretions may have provided an additional stimulus to harden the seminal materials plugging

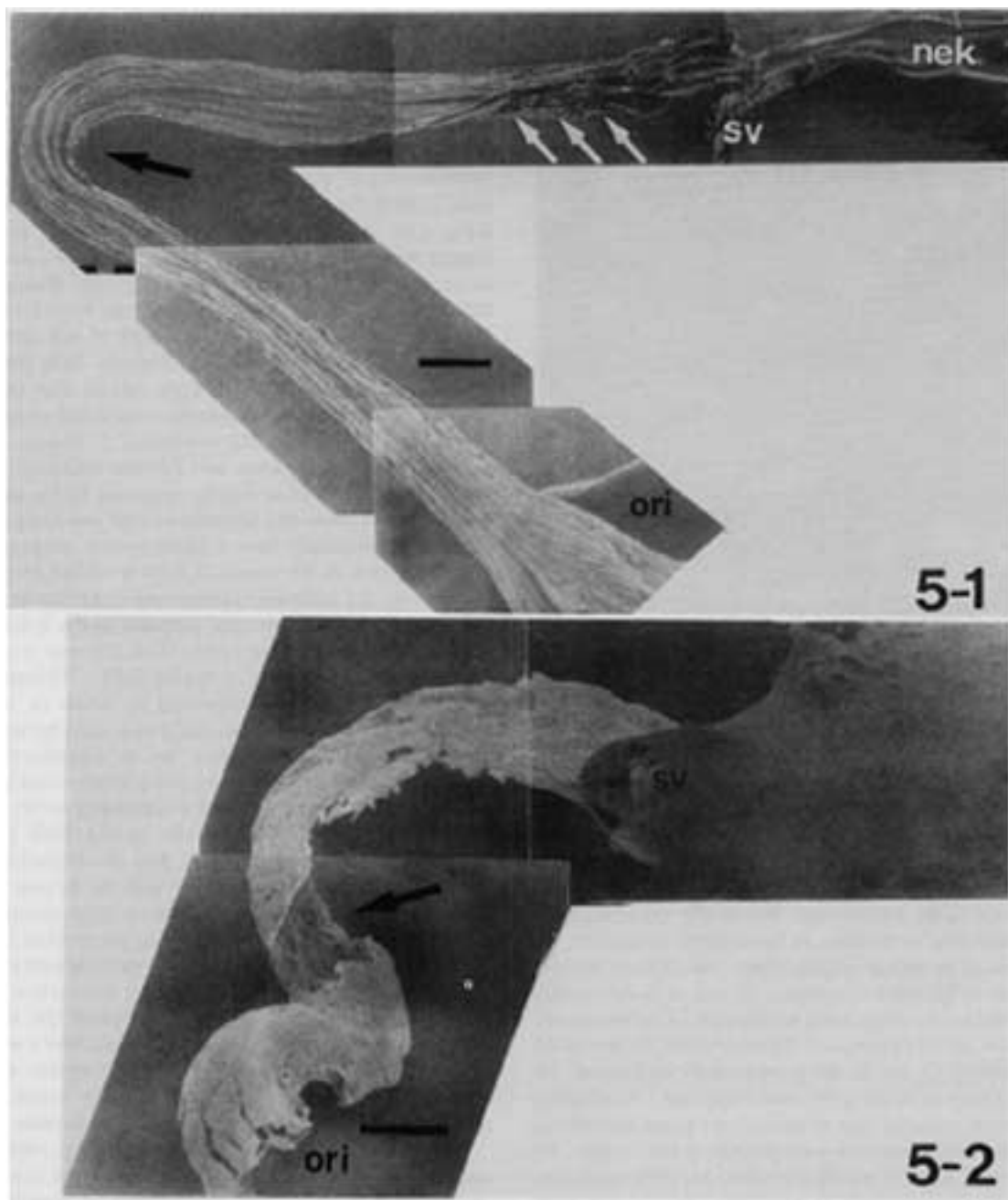


Fig. 5. Scanning electron micrographs of resin cast.

Fig. 5-1. The normal mouse urethra showing the penile flexura (arrow) and the squeezed urethra caused by contraction of the bulbocavernosus muscle (white arrows). ori: external urinary orifice. Bar=300 μ m.

Fig. 5-2. The urethra of KK-A^y mouse with obstructive uropathy. Note the twisted urethra with concave representing a urethral plug (arrow). Bar = 300 μ m.

the urethra. However, any attempt to correlate these with the development of the urethral plug would be purely speculative.

Although there may be various explanations for hydronephrosis [5, 7, 11], in the present case it is highly likely that urethral plugs were the cause of hydronephrosis

in the KK-A^y mice.

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