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Effects of Maternal Renal Dysfunction on Fetal Development

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1. Introduction

Maternal conditions affect the growth of the fetal kidney, which begins to secrete urine during late gestation (Bakala et al., 1985; Schaeverbeke & Cheignon, 1980). For example, during pregnancy, the maternal kidney undergoes various changes such as an increase in glomerular filtration rate (Baylis, 1994; Atherton & Pirie, 1981) and an alteration in tubular function (Dafnis & Sabatini, 1992). Furthermore, maternal undernutrition by the restriction of protein intake induces low fetal birth and leads to renal morphological and physiological changes (Mesquita et al., 2010). Maternal bilateral ligation of the uterine artery, protein restriction, smoking, nephrotoxic medication as well as salt loading cause intrauterine growth retardation (IUGR) to fetuses (Bentz & Amann, 2010), inducing fetal programing of renal function. Previously, we investigated the development of the fetal kidney during maternal renal dysfunction induced by bilateral ureteral ligation (Okada et al., 1997) and uninephrectomy (Okada et al., 2000, 2006), and we found that the development of the fetal kidney is accelerated by the operations. Furthermore, we found that maternal bilateral ureteral ligation (Okada & Morikawa, 1988) and uninephrectomy (Okada et al., 1998) decrease fetal body weight. In this chapter, we present a summary of the changes that occur in the fetal kidney after maternal bilateral ureteral ligation, uninephrectomy, and subtotal (5/6) nephrectomy, in addition to a summary of the changes that occur in the remaining kidney after uninephrectomy and 5/6 nephrectomy. Although 5/6 nephrectomy produces a bigger functional demand to maternal and fetal kidneys than the other two operations, little information is available on fetal development of the kidney when the maternal kidney is 5/6th removed. Therefore, we focused on studying the remaining kidney of unilateral and 5/6 nephrectomy and the fetal kidney under maternal renal dysfunction.

2. Effects of nehrectomy on remaining kidney

Nephrectomy can be used for research on renal growth and renal failure. Specifically uninephrectomy can be performed for research on the biology of compensatory renal growth (Fine, 1986) and subtotal nephrectomy for renal failure. In studies of subtotal nephrectomy, 3/4 nephrectomy (Friedman & Pityer, 1986) and 5/6 nephrectomy

(Manotham et al., 2004) have been performed. In this section, effects of uninephrectomy and 5/6 nephrectomy on the remaining kidney are discussed.

2.1 Effects of uninephrectomy on the cell proliferation and EGF in the remaining kidney

Compensatory renal growth after unilateral nephrectomy has been intensely investigated (Dicker & Shirley, 1971; Johnson & Vera Roman, 1966; Mok et al., 2003) and includes cellular hypertrophy, hyperplasia, and apoptosis (Wang et al., 1997). In the adult kidney, differentiated nephrons are relatively quiescent, with few cells undergoing mitosis (Girardi et al., 2002). Compensatory response after renal mass ablation shows increase in both cell size and cellular protein content without any increase in cell number (Girardi et al., 2002). The compensatory renal growth largely occurs by hypertrophy rather than hyperplasia of the remaining nephrons (Chen et al., 2005). Kanda et al. (1993) have observed that 24 h after uninephrectomy of an adult mouse kidney, there was an initiation of the proliferation of cortical tubular cells in the remaining kidney stained with anti-BrdU antibody. On the other hand, it has been seen that the compensatory response to uninephrectomy is stronger in immature animals than in adult ones (Fine, 1986) and the removal of the contralateral kidney induces an evident increase in cell proliferation, especially in proximal tubules, in young uninephrectomized rats (Girardi et al., 2002). Furthermore, we have recently reported that in the remaining kidney of uninephrectomized immature (3-week-old) rats few proliferatiomg cell nuclear antigen (PCNA)-positive cells in distal tubules were little observed and that a significant increase in the PCNA-positive cell ratios was observed in the proximal convoluted and straight tubules (Okada et al., 2010). Moreover, we have reported that in the immature rats 1 and 3 days after uninephrectomy there were significant increases in renal weight and the PCNA-positive cell ratios of the glomerulus and proximal convoluted and straight tubules (Okada et al., 2010). Kanda et al. (1993) have also reported that in immature rats, the increase in kidney mass immediately after nephrectomy is mainly due to hyperplasia, whereas 2 weeks after nephrectomy both hyperplasia and hypertrophy processes equally participate. Therefore, we concluded that there is an increase in proliferative activity in the proximal tubules during the early stage of compensatory renal growth in immature rats. Thus, the compensatory reaction of immature animals is different from that of adult animals, in terms of proliferative activity but is similar with regard to an increase in proliferating cells during the 24 h after uninephrectomy.

Considering the relationship of epidermal growth factor (EGF) to the elevated proliferating activity of nephrectomized immature animals, we examined the immunolocalization of EGF (Okada et al., 2010). Although Toubeau et al. (1994) and Jung et al. (2005) have observed the immunolocalizations of EGF in distal convoluted tubules and the thick ascending limb of Henle's loop of adult rat kidneys, our results revealed that EGF-positive cells localized to the proximal and the distal tubules in the kidney of immature rats indicating the difference of immunolocalization of EGF in the kidney between adult and immature animals (Okada et al., 2010). There are controversial reports on the involvement of EGF on the proliferative activity of the adult kidney. For example, Pugh et al. (1995) have found that the addition of EGF to renal organ culture medium increases uptake of BrdU while Toubeau et al. (1994) have found that cell proliferation occurs immediately after the decrease of renal EGF and its receptor (EGFR) in the rat experimentally subjected to acute renal failure. Further, in the unilaterally ureter obstructed rat model, the administration of EGF causes a decrease in

proliferative activity in the contralateral kidney (Chevalier, 1999). Moreover, EGF expression and the role of EGF in the developing kidney is also controversial as follows: in the developing kidney, EGF expression appears as nephrons mature (Nouwen et al., 1994); exogenous EGF delays the development of the loop of Henle by reducing both apoptosis and cell proliferation (Lee et al., 2004); robust EGF synthesis is clearly a characteristic of the mature kidney rather than that of a rapidly-growing fetal organ (Goodyer et al., 1991a); renal EGF is undetectable in the human fetus (Goodyer, et al., 1991b) and renal EGF content is increased from days 6 to 21 after birth in mice (Gattone et al., 1992).

We have recently observed that on the first post-operative day, the proximal tubular cells showed a weaker reaction to EGF antibody in uninephrectomized rats than in sham-operated rats and that the degree of reactivity to EGF was the same in both groups on the third post-operative day and that the level of expression of preproEGF mRNA was significantly lower in uninephrectomized rats than in sham-operated rats at the first post-operative day (Okada et al., 2010). These findings reveal that unilateral nephrectomy in immature rats causes an increased proliferative activity and a decreased expression of EGF in the remaining kidney during the early period of compensatory renal growth.

2.2 Effects of subtotal (5/6) nephrectomy on the remaining kidney: Growth factor and protein restriction

Subtotal (5/6) nephrectomy has been performed on laboratory animals to achieve a model for chronic renal failure (Manotham et al., 2004; Zhang et al., 1999). After the reduction of renal mass, remnant renal tissue was less able to maintain stability of blood flow and filtration rate during variations in renal perfusion pressure (Brown et al., 1995), and various pathological changes are observed. In the remaining kidney, glomerular sclerosis is observed following increased proliferative activity in glomeruli (Floege et al., 1992), and renal fibrosis is accompanied with apoptotic cells (Li et al., 2004). The remaining kidney of 5/6 nephrectomized animals was utilized as a model for renal fibrosis (Johnson et al., 1997; Nangaku et al., 2002; Yang et al., 2001) and a model for glomerular sclerosis (Floege et al., 1992; Griffin et al., 1994). Since in immature rats as well as adult animals, 5/6 nephrectomy induces significant increases in BUN, glomerular sclerosis index (GSI), and interstitial fibrosis score (IFS), compared with sham-oprated ones (Mino et al., 2007), 5/6 nephrectomized immature animals are useful as animal model for renal failure.

In adult animals, it has been reported that a low protein diet reduces uremic toxicity in experimentally-induced uremia (Sterner et al, 1994) and that in the 5/6 nephrectomized renal failure model, the restriction of protein intake improves renal failure (Heller et al., 1994). It has been reported that a low protein intake improves survival over normal protein intake in 3/4 nephrectomized immature rats (Friedman & Pityer, 1986). Further, Mino et al. (2007) have reported that a low protein diet induces a significant decrease in BUN, GSI, and IFS in 5/6 nephrectomized immature rats. These findings suggest that protein intake restriction is effective in preventing renal failure in immature animals, which is in accord with the results in adult rats.

Increased glomerular hydraulic pressure and the ultrafiltration plasma proteins contribute to the onset and progression of chronic renal damage (Remuzzi et al., 2005). Inhibitor of angiotensin converting enzyme (ACE) and angiotensin type II receptor antagonists can be

used in combination to maximize renin-angiotensin-aldosterone system (RAAS) inhibition and more effectively reduce proteinuria and GFR decline in diabetic and non-diabetic renal disease (Remuzzi et al., 2005; Klein et al., 2003). Since activation of RAAS induces decreased level of endothelial nitric oxide synthase (eNOS) expression (Zhao et al., 2005), it is thought that NO also plays an important role in the progression of renal failure. Therefore, in 5/6 nephrectomized animals, factors, including RAAS and NO, relating to the progression of renal failure and effects of protein restriction are discussed.

We have previously reported that in the normal developing kidney in perinatal rats, EGF plays an important role in proliferative activity (Okada et al., 2001) and apoptosis (Okada et al., 2003). Further, chronic renal failure caused by 5/6 nephrectomy is improved by extrinsic EGF (Moskowitz et al., 1992), and EGF accelerates the regeneration of tubular cells and ameliorates renal failure (Humes et al., 1989). Furthermore, in 5/6 nephrectomized immature rats, the incidence of TUNEL positive cells in distal tubules was lowered and more EGF-positive cells in the segments were observed after protein restriction (Mino et al., 2007). These findings reveal that protein restriction is effective in preventing renal tubular scarring in immature rats and that EGF is involved in the process of this prevention.

Transforming growth factor-β (TGF-β) suppresses proliferative activity of the cells (Cheng & Grande, 2002) and converts renal tubular epithelial cells to fibroblasts (Stahl & Felsen, 2001). The fibroblast cells induce fibrosis of the renal tubular interstitium (Blobe et al., 2002; Iwano et al., 2002). TGF-β is also related to the infiltration of macrophages (Wahl et al., 1987). Feeding of a low protein (6% protein) diet to 5/6 nephrectomized immature rats induces decreased immunoreactivity of TGF-β (Fig. 1) and ED1 (Fig. 2). The increased expression of TGF-β in the distal tubules of the kidney is involved in the damage of the remaining kidney of 5/6 nephrectomized animals and protein restriction suppresses the elevation of TGF-β expression and the progression of renal failure.

The expression of NO is increased in the kidney of animals with renal failure, and increased expression of NO is involved in the process of renal failure (Matubara et al., 2000). In the developing kidney, NO plays an important role in maintaining normal physiological function (Solhaug et al., 1996) and in regulating renal homodynamics (Han et al., 2005). Maintenance of adequate NO is an additional mechanism for the preservation of vasculature in progressive renal diseases (Kang et al., 2002). Inhibition of inducible NO synthase (iNOS) provides a mechanism against the developing ischemic renal injury and inflammation in the adult rat kidney (Mark et al., 2005). Nuclear factor-kappa B (NF-κB) is known to be involved in the induction of the human iNOS gene (Taylor et al., 1998). Furthermore, elevation in renin-angiotensin system (RAS) activity and DNA-binding activity of NF-κB are involved in the progression of renal failure in the remnant kidney of 5/6 nephrectomized rats (Ots et al., 1998; Fujihara et al., 2007). On the other hand, decreased expression levels of eNOS in the glomerulus are often seen in rats with renal disease (Bremer et al., 1997). In the remnant kidney model, inhibition of NO synthesis with L-NAME resulted in a decline of renal function and severe glomerulosclerosis (Kang, et al., 2002). In the current model for endotoxin-induced thrombotic microangiopathy, which accompanies glomerular injury, endothelial injury is associated with decreased eNOS expression levels (Shao et al., 2001). NO produced by eNOS confers antioxidant properties on vascular cells (Walford & Loscalzo, 2003), and the endogenous low steady state levels of NO produced by eNOS

dynamically regulates mitochondrial respiration. This regulation thus provides protection against H_2O_2 mediated injury and death (Paxinou et al., 2001).

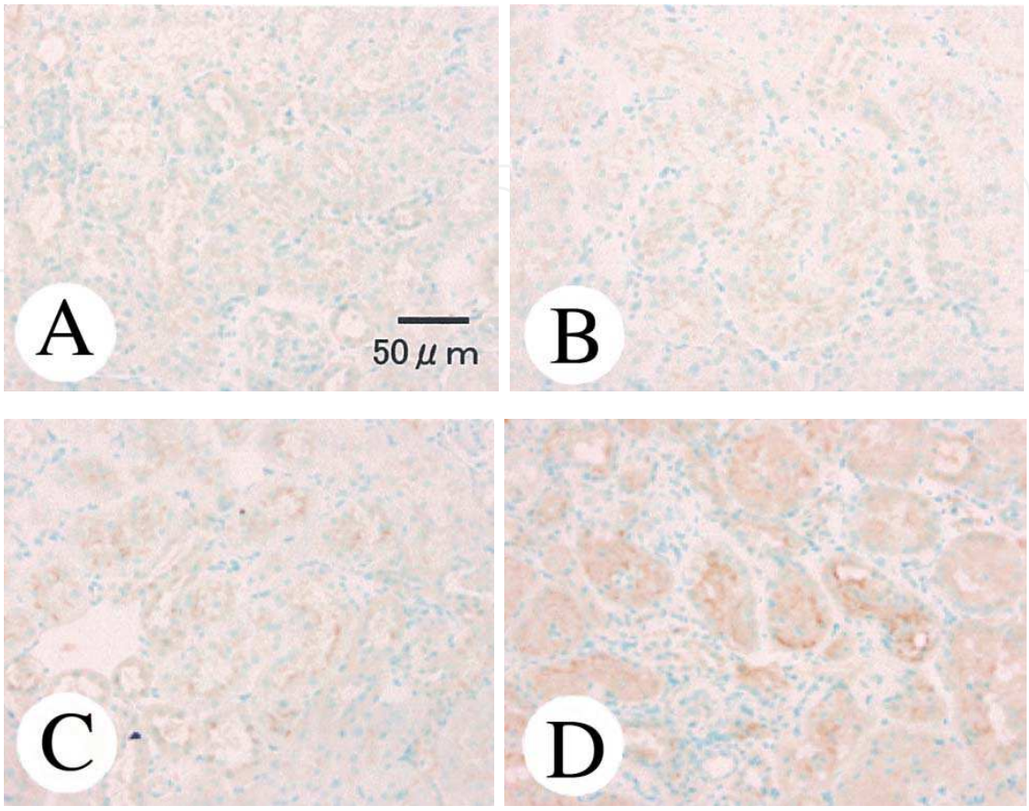

Fig. 1. Kidneys of 5/6 nephrectomized immature rats stained with an anti-TGF-β antibody. bar = 50 μm. **A**, low protein group 4 weeks after the operation. TGF-β positive cells are seen in the distal tubules. **B**, normal protein group 4 weeks after the operation. TGF-β positive cells are seen in the distal tubules and the reaction activity to TGF-β antibody is stronger compared to that in the age-matched low protein group in figure 1A. **C**, low protein group 8 weeks after the operation. The reaction activity to TGF-β antibody is stronger when compared with those in the low protein group 4 weeks after the operation in figure 1A. **D**, normal protein group 8 weeks after the operation. The reaction activity to TGF-β is stronger compared to those in the age-matched low protein group in figure 1C and is stronger compared to that in the normal protein group 4 weeks after the operation in figure 1B.

Thus, eNOS has a protective role in glomerulonephritis (Shao et al., 2001; Heering et al., 2002), and RAS is involved in changes in eNOS expression (Zhao et al., 2005; Varziri et al., 2002). As shown in figure 3, the eNOS positive cells were observed in the glomerulus in the rats of every group at 4 and 8 weeks after the operation in 5/6 nephrectomized immature rats (Fig. 3B and D). The positive reaction to the anti-eNOS antibody was stronger in the 4-week postoperative rats than in the 8-week postoperative rats, and the reaction was stronger in the protein-restricted rats than in the control rats 4 weeks after the operation (Fig. 3).

Mino et al. (2010) have observed that the remaining kidney of 5/6 nephrectomized and protein-restricted immature rats exhibits an elevated levels of the endothelial eNOS protein

expression and a decrement in iNOS positive cells in the distal tubules and in the expression of renin mRNA. They also concluded that protein-restriction is effective in preventing renal failure of immature rats and that the changes in the expression levels of renin, eNOS, and iNOS are involved in the process of this prevention.

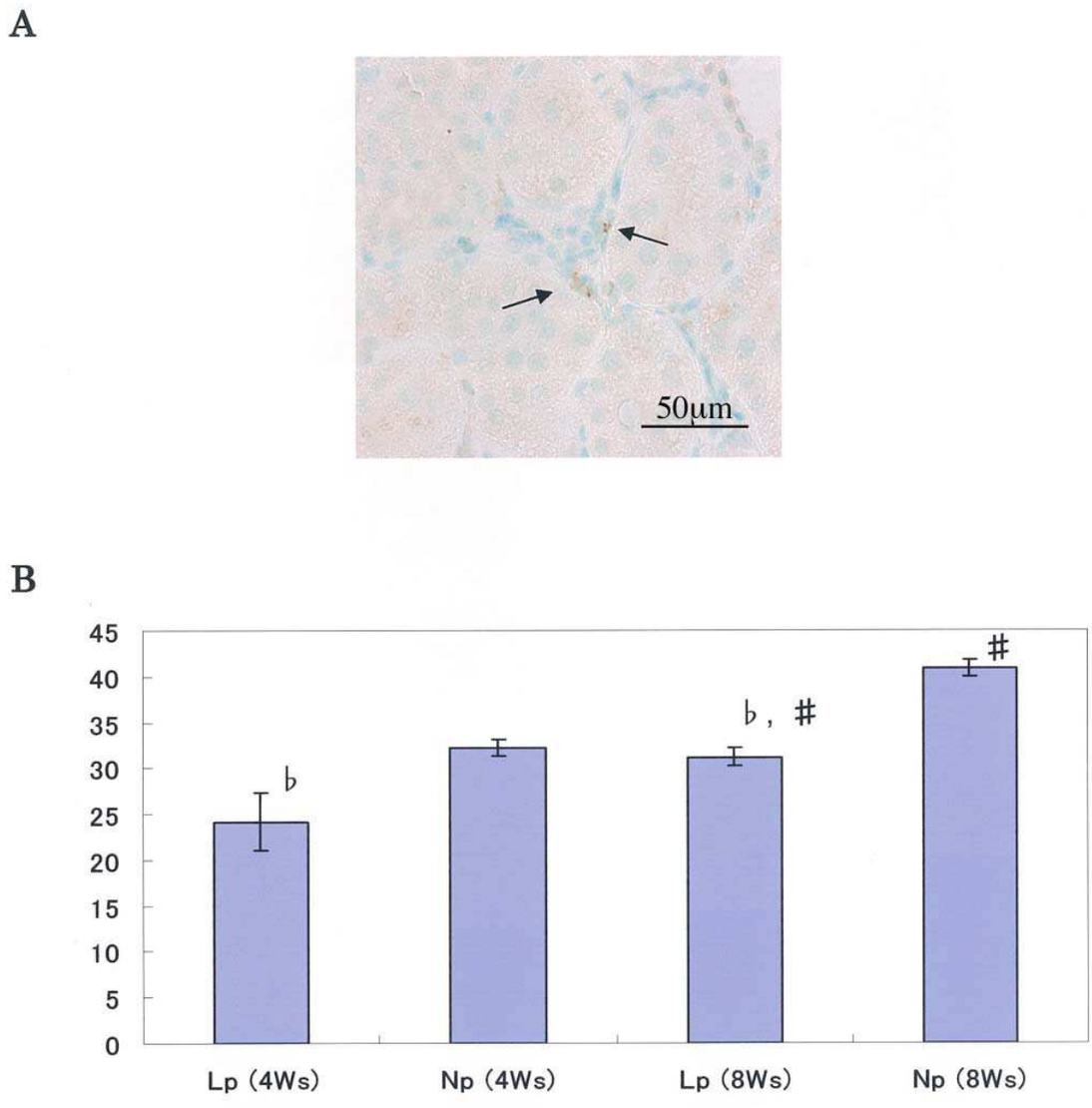

Fig. 2. **A**, Kidney stained with anti-ED1 antibody. ED1 positive cells (arrows) are observed in the interstitium. **B**, Changes in number of ED1 positive cells per area (300 x 400 μm) (Means±SEM) in the kidney of 5/6 nephrectomized rats.
Lp (4Ws), low protein group 4 weeks after the operation.
Np (4Ws), normal protein group 4 weeks after the operation.
Lp (8Ws), low protein group 8 weeks after the operation.
Np (8Ws), normal protein group 8 weeks after the operation.
b, Significantly different from age-matched normal protein group ($p<0.05$).
#, Significantly different from same group 4weeks after the operation ($p<0.05$)

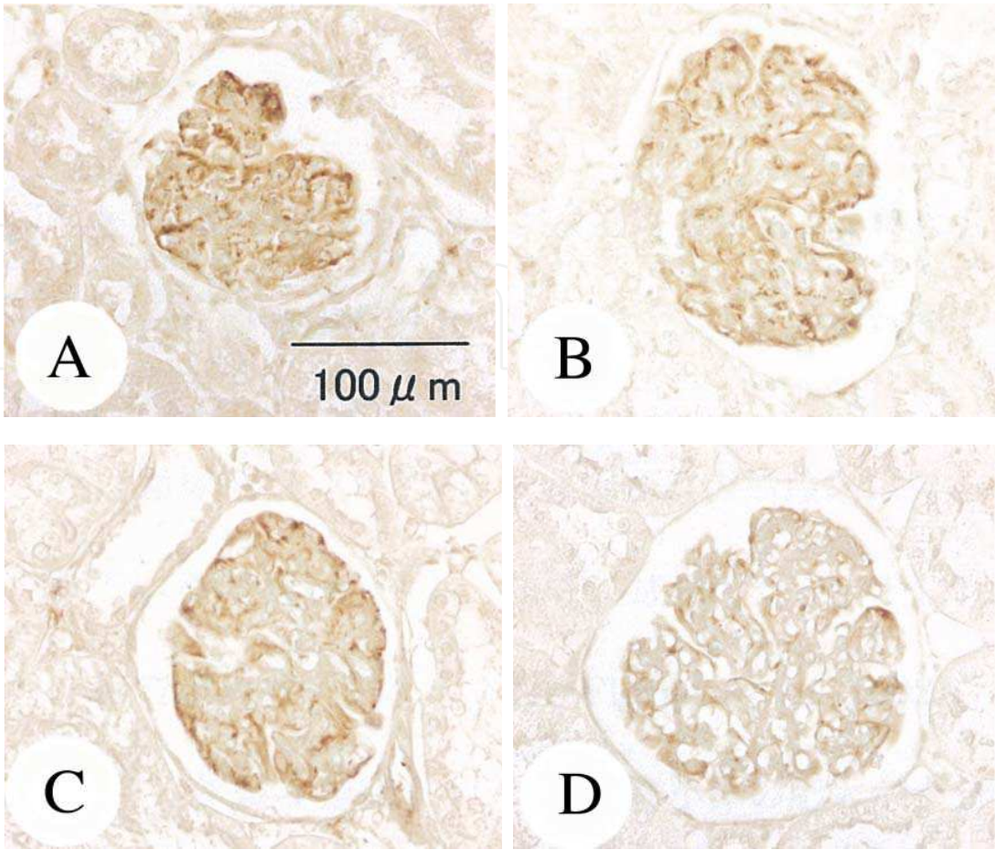

Fig. 3. Kidneys of 5/6 nephrectomized immature rats stained with an anti-eNOS antibody. bar = 100 μm. **A**, Low protein diet fed rats 4weeks after the operation. The eNOS positive cells are seen in the glomerulus. **B**, Normal protein diet fed rats 4 weeks after the operation. The reaction activity to eNOS antibody is weaker compared to that in age-matched low protein diet fed rats in figure 1A. **C**, Low protein diet fed rats 8 weeks after the operation. The reaction activity to the eNOS antibody is weaker when compared with those in the low protein diet fed rats 4 weeks after the operation in figure 1A. **D**, Normal protein diet fed rats 8 weeks after the operation. The reaction activity to the eNOS antibody is slightly weaker when compared with that in the normal protein diet fed rats 4 weeks after the operation in figure 1B and is slightly weaker compared to that in the age-matched low protein diet fed rats in figure 1C.

3. Effects of maternal renal dysfunction on the development of fetal kidney

The concentrations of many substances are known to change physiologically in pregnancy relative to the length of gestation (Kelly et al., 1978). The study of renal physiology during pregnancy has been mainly related to glomerular function (Studd, 1971; MacLean et al., 1972). In humans, glomerular filtration rate (GFR) reaches a peak of 40-50% higher than nonpregnant values between 9 and 11 weeks of pregnancy and is sustained until at least the 36th week (Dafnis & Sabatini, 1992). Atherton & Pirie (1981) have reported that GFR and salt and water reabsorption significantly elevate in early pregnancy. Furthermore, maternal renal function elevates during pregnancy in rats (Churchill et al., 1982). Throughout

gestation, fetal waste products are primarily excreted by maternal kidneys through placental circulation (Liggins, 1972) and fetal kidneys start secreting urine during the final days of gestation (Bakala et al., 1985). This implies that an increase of fetal waste products in circulation, indicative of the latter gestational period, stimulates fetal kidneys to start urine production. Therefore, development of the fetal kidney is thought to be stimulated by functional demands associated with maternal renal dysfunction.

3.1 Effects of ligation of maternal ureters on the development of the fetal kidney

When pregnant rats are subjected to bilateral ureteral ligation for one day, the BUN level is elevated 5 to 8 times of control values, and BUN passes through the placenta into the fetal circulation to stimulate fetal kidney urine production (Matsuo et al., 1986). Fetal urine production is thereby stimulated by maternal ureteral ligation (Wells, 1946). We observed that in the kidney of fetuses of fetal days 20 and 22 from bilaterally ureter ligated mothers apical vacuoles in the proximal tubular cells are increased by the ligation (Okada & Morikawa, 1990). Furthermore, the surface area of the glomerular basement membrane and the length of the glomerular capillary per unit volume of glomerulus are increased (Okada & Morikawa, 1993) and shortening of the time for filtration of HRP through the glomerular basement membrane is observed in the fetuses from the ligated mothers (Okada et al., 1997). Therefore, the maternal bilateral ligation causes an elevation of BUN concentration, acceleration of the growth and differentiation of the proximal tubules, accelerated formation of fetal glomerular basement membrane, and stimulated glomerular function in the filtration in the fetal rat kidney when the fetal kidney is functional in urine production.

3.2 Effects of maternal uninephrectomy on the development of fetal kidney

In uninephrectomized pregnant rats on day 5 of gestation, BUN concentration is significantly increased 1 day after the operation and remained high at term (Okada et al., 1998). Previously, we have reported that glomerular volume (Okada et al., 1994) and proximal tubular length (Okada et al., 1995) are larger in pups from uninephrectomized mothers than in those from sham-operated ones, suggesting the accelerated renal development was influenced by maternal uninephrectomy. By the electron microscopic observation on distribution of cationized ferritin (CF) in fetal glomerulus after CF injection, we found that the formation of anionic sites in the glomerular basement membrane of fetuses is accelerated by uninephrectomy (Okada et al., 1998). Furthermore, we previously reported that maternal uninephrectomy induces lowered proliferative activity in mature glomerulus and enhanced positive reactions to both EGF and EGFR antibodies in proximal tubular cells in the fetal kidney (Okada et al., 2000). Recently, we have revealed that an increase in apoptosis in the collecting ducts of fetal kidney is induced by maternal uninephrectomy and that the increase is related to the decreased expression of *bcl-2*, an apoptotic suppressor gene (Okada et al., 2006).

3.3 Effects of maternal subtotal nephrectomy on the development of fetal kidney

3.3.1 Aim of study

Fetal waste products are primarily excreted by maternal kidneys through placental circulation (Liggins, 1972). During the final days of gestation, fetal kidneys start secreting

urine (Bakala et al., 1985). This implies that an increase of fetal waste products in circulation, indicative of the latter gestational period, stimulates fetal kidneys to start urine production. Therefore, development of the fetal kidney is thought to be stimulated by functional demands associated with maternal renal dysfunction. Bilateral ureteral ligation for 1 day (Matsuo et al., 1986) and uninephrectomy (Okada et al., 1998) induce an elevation in BUN, 5 to 8 times of control values and 1.5 times of control values, respectively. In a preliminary study, rats died in the third day after bilateral ureteral ligation; we therefore concluded that bilateral ureteral ligation cannot be used as a model for chronic renal failure. Similarly, uninephrectomy cannot be used as a model of renal failure because it does not cause pathological changes in the remaining kidney. Therefore, 5/6 nephrectomy, which induces renal fibrosis (Nangaku et al., 2002; Yang et al., 2001) and glomerular sclerosis (Griffin et al., 1994), is used for to model chronic renal failure (Manotham et al., 2004). There have been several reports on the development of the fetus from 5/6 nephrectomized mothers. Gibson et al. (2007) have examined changes in growth and urine volume of fetal sheep with maternal 5/6 nephrectomy. Salas et al. (2003) have examined fetal body weight and placental weight with maternal 5/6 nephrectomy. However, neither groups studied the kidney from the 5/6 nephrectomized mothers. Brandon et al. (2009) have determined the plasma renin level of offspring from 5/6 nephrectomized mothers and reported that in the neonates there is an impaired ability to regulate glomerular filtration independent of arterial pressure. In this section, experiments were designed to investigate the development of the fetal kidney under maternal renal dysfunction by 5/6 nephrectomy.

3.3.2 Materials & methods

Animals and tissue processing: Wistar strain rats were reared under ordinary conditions (24 ± 1˚C, 14 hrs light and 10 hrs dark) and were given both a commercial diet (CE-2, Clea, Osaka, Japan) and water *ad libitum*. The day following an overnight mating was determined as day 1 of gestation. To make maternal renal dysfunction conditions, 5/6 nephrectomy was performed. Under isoflurane anesthesia, on day 5 of gestation 2/3 of the left kidney were excised and on day 12 of gestation the right kidney was removed. The body weights of 5/6 nephrectomized or sham-operated mothers were measured on days 3, 5, 7, 10, 12, 14, 18, 20, and 22 of gestation. BUN levels of 5/6 nephrectomized or sham-operated mothers were measured. Under isoflurane anesthesia, blood was drawn from the plexus ophthalamicus with a capillary glass tube at days 3, 7, 10, 14, 18, 20, and 22 of gestation. BUN concentration was determined with an automatic dry chemistry analyzer system (Spotchem SP-4410, Kyoto Daiichi-Kagaku Kyoto, Japan). On day 22 of gestation, fetuses were removed from the uterus under isoflurane anesthesia. Under the anesthesia, fetal kidneys were removed and fixed in methanol-Carnoy's solution [a mixture of methanol, chloroform, acetic acid (6:3:1)] or 10 % neutral buffered formalin. The kidneys were dehydrated in a graded series of alcohol, embedded in Tissue Prep (Fisher Scientific, Fair Lawn, NJ, USA), and sectioned at 6 μm. The sections from methanol-Carnoy fixed material were treated with PCNA, TGF-β, and TGF-β receptors (TGF-βRI and TGF-βRII) antibodies. The sections from formalin fixed material were treated with TUNEL method.

Detection of apoptotic cells and immunohistochemical procedures: After deparaffinization with xylene, the sections were transferred to distilled water through a degraded series of ethanol and were rinsed in phosphate buffered saline. The apoptotic cells were detected by TUNEL

methods, using an in situ apoptosis detection kit (Takara, Kyoto, Japan). Briefly, the sections were incubated with the TdT enzyme and FITC-labeled dUTP at 37 °C for 90 min, further incubated with an anti-FITC HRP conjugate at 37 °C for 30 min, and finally incubated with DAB for 5 min. Negative controls were produced by omitting the TdT enzyme. Immunostaining for PCNA was performed by incubating with mouse anti-human PCNA antibody (19A2, Coulter Immunology, Hialeah, FL, USA, 1:160) at 4 °C overnight, after which the sections were incubated with biotinylated rabbit anti-mouse immunoglobulins antibody (BioGenex Laboratories, San Roman, CA, USA, 1:50) and streptavidin conjugated peroxidase (Zymed Laboratories, South San Francisco, CA, USA, 1:50) for 30 min, respectively. The TGF-β and the TGF-βRI immunostainings were performed as follows: The sections were incubated with rabbit anti-human TGF-βRI antibody (Santa Cruz Biotech, Santa Cruz, CA, USA, 1:100) at 4 °C overnight or rabbit anti-porcine TGF-β antibody (R&D Systems, Minneapolis, MN, USA, 1:100) at 4 °C for 3 nights. Then, the sections were incubated with biotinylated goat anti-rabbit IgG antibody (1:200) and avidin-biotin peroxidase complex (1:200) for 30 minutes, respectively. Last, the sections were incubated with DAB for 5 min. Negative controls were produced by omitting the primary antibody in immunohistochemical procedure. No positive immunoreactivity was recognized when antibody was preincubated with an excess of antigen (25μg/ml human TGF-βRI peptide, Santa Cruz Biotech, Santa Cruz, CA, USA; TGFβ antibody, 1mg/ml human TGFβ King Brewing, Kakogawa, Japan).

Determination of PCNA positive cell ratio: To determine the PCNA positive ratio in the glomerulus, 10 glomeruli were used. To determine the ratio in the proximal tubules, more than 500 nuclei were used in the proximal convoluted and straight tubules respectively. The nuclei positive and negative to PCNA were counted and the ratio of positive nuclei to total nuclei was expressed as a percentage.

3.3.3 Results & discussion

The result that a 5/6 nephrectomy operation on pregnant rats induced a significant decrease in body weight on day 14 of gestation and thereafter (Fig. 4) indicates that the operation has applied a burden to the mother. A slightly but significant increment in BUN concentration was induced after removing 2/3 of the left kidney, an intense increase in BUN was induced after removing the right kidney, and gradual decrease in BUN was observed on day 14 and thereafter (Fig. 5). The elevation in BUN concentration of nephrectomized mothers implies the elevation in functional demand to remaining kidney. Since the fetal kidney becomes functional during the late gestation period (Bakala et al., 1985), the decrease in BUN from day 14 to 22 of gestation reflects the instigation of fetal urine production. On day 22 of gestation, the concentration of urea nitrogen (UN) in maternal blood, fetal blood, and amniotic fluid were significantly higher in 5/6 nephrectomized pregnant rats than in sham-operated ones (Fig. 6). This finding suggests that increased UN in 5/6 nephrectomized mother passes through placenta to fetal blood circulation and that fetal kidney secretes the UN to the amniotic fluid. This notion is well in-line with the reports by Matsuo et al. (1986) which suggest that increased maternal BUN induces the elevation in fetal BUN and by Garcia et al. (1988) that found that an increase in fetal renal function induces the elevation in urea nitrogen levels of the amniotic fluid.

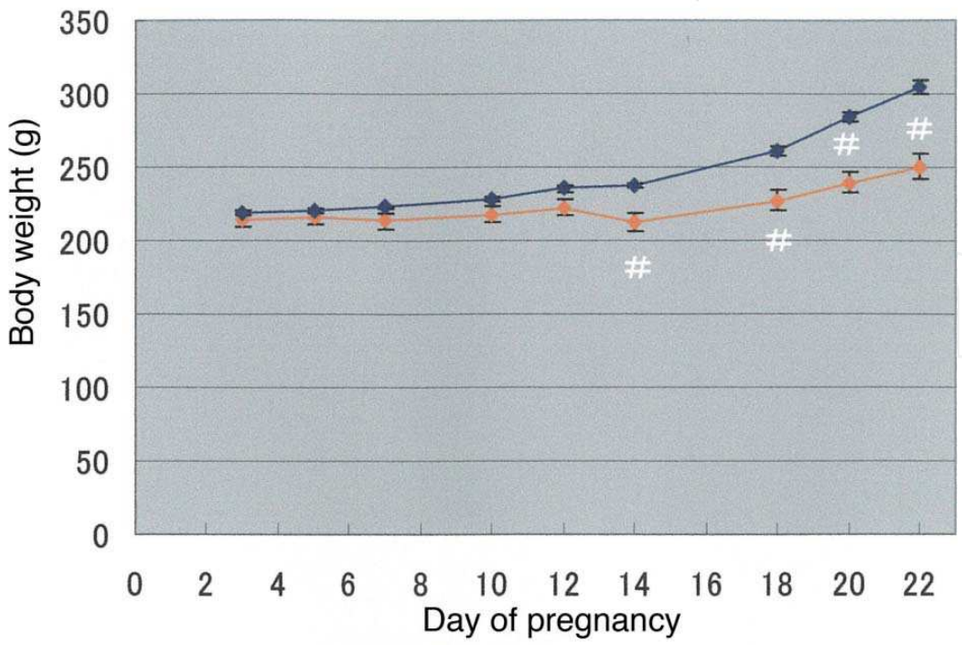

Fig. 4. Changes in body weights (Means±SEM) of 5/6 nephrectomized ( ) and sham-operated ( ) mothers
#, Significantly different from age-matched sham-operated mothers ($p<0.05$)

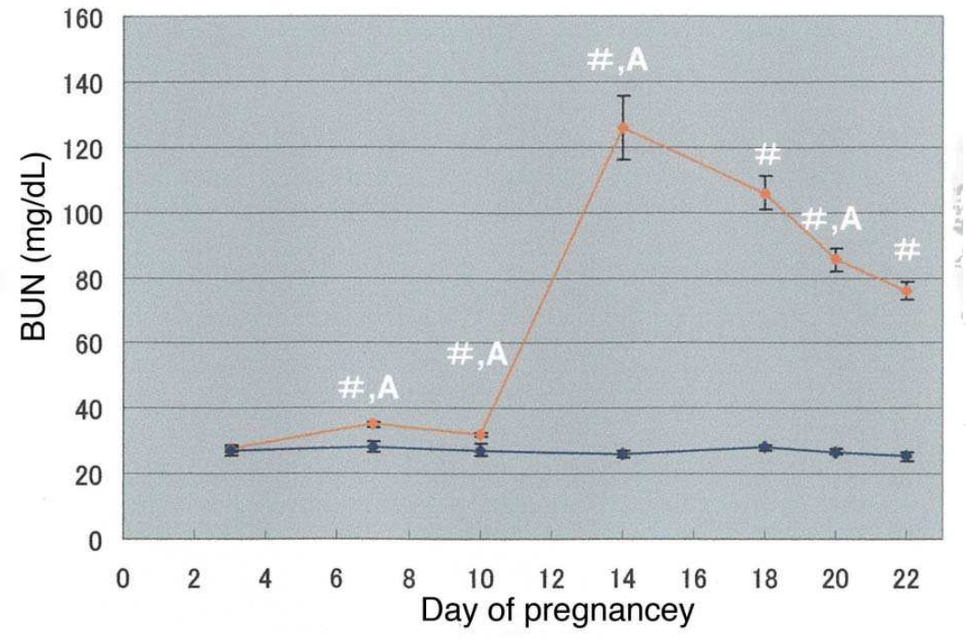

Fig. 5. Changes in BUN concentration (Means±SEM) of 5/6 nephrectomized ( ) and sham-operated ( ) mothers
#, Significantly different from age-matched sham-operated mothers ($p<0.05$)
A, Significantly different from preceding age group ($p<0.05$)

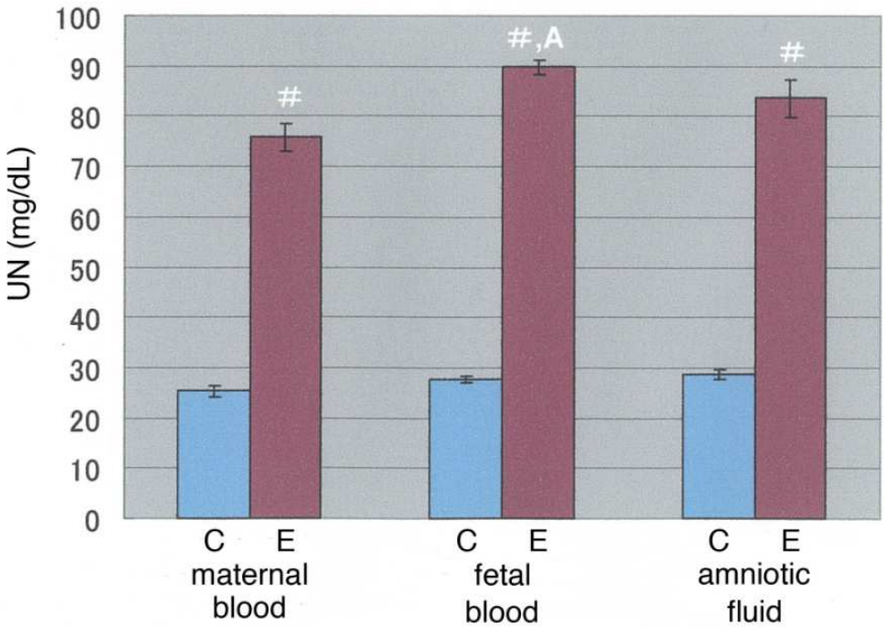

Fig. 6. The concentration of urea nitrogen in maternal blood, fetal blood, and amniotic fluid (Means±SEM) of 5/6 nephrectomized (E) and sham-operated (C) pregnant rats on day 22 of gestation.
#, Significantly different from sham-operated group ($p<0.05$)
A, Significantly different from maternal blood, in the same group ($p<0.05$)

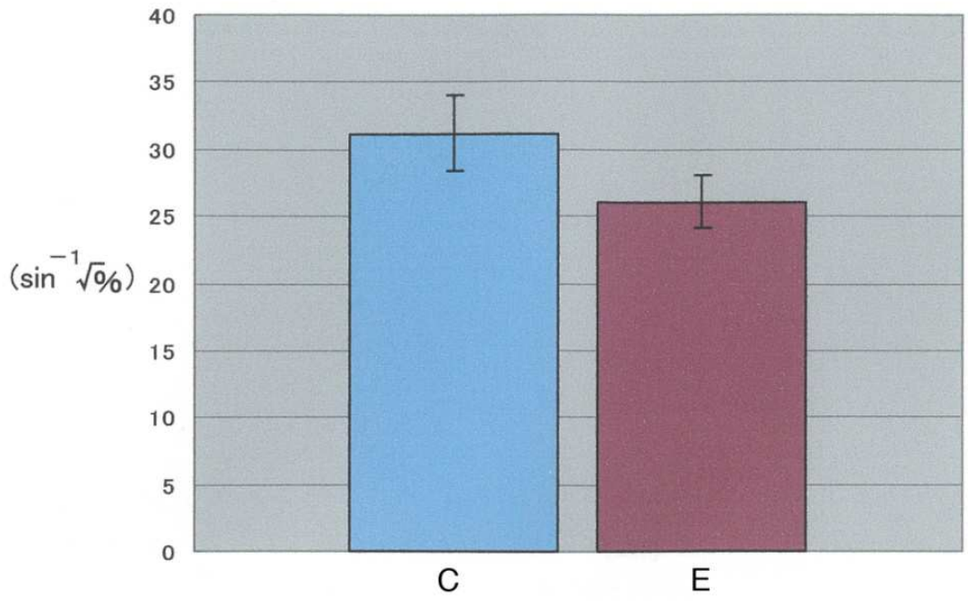

Fig. 7. PCNA positive cell ratio (Means±SEM) in the kidneys of fetuses from 5/6 nephrectomized (E) and sham-operated (C) mothers.
An insiginficant difference is observed between the 2 groups.

The PCNA positive cell ratio in the kidney of fetuses from 5/6 nephrectomized mothers was slightly lower than that of fetuses from sham-operated mothers but not significant (Fig. 7). We previously reported that the more the glomerulus develops, the lower the PCNA positive cell ratio in the kidney of perinatal rats (Okada et al., 2001); therefore, the slight decrease in the ratio of fetal glomerulus with maternal 5/6 nephrectomy reflects nonsuppressive effect on renal development by the operation.

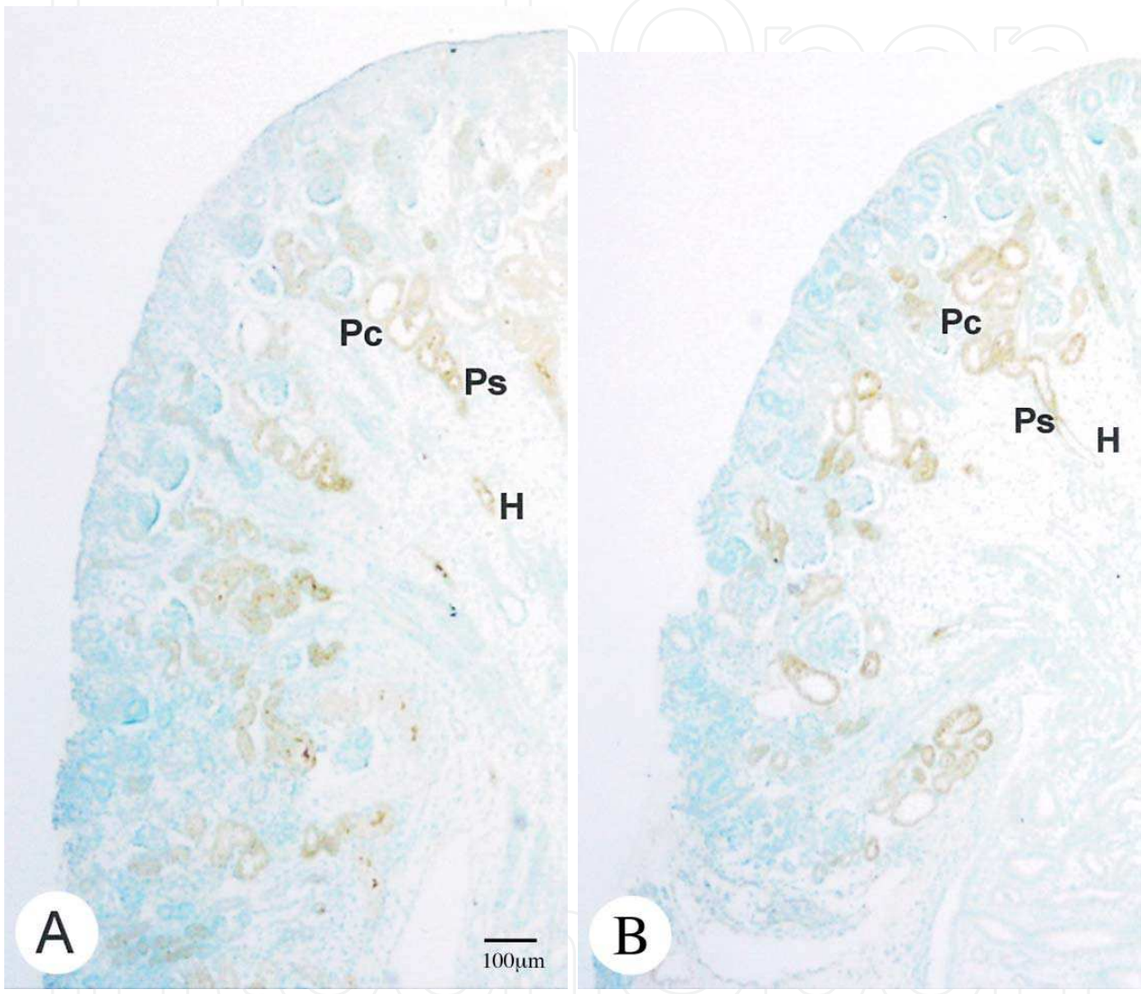

Fig. 8. Fetal kidneys stained with an anti-TGF-β antibody. bar = 100μm. **A**, fetus from sham-operated mothers. **B**, fetus from 5/6 nephrectomized mothers. In both fetuses, positive reactions are observed in the proximal tubules and the loop of Henle and no remarkable difference is observed between the fetuses. Ps, proximal straight tubules; Pc, proximal convoluted tubules; H. loop of Henle.

TGF-β was mainly localized in the distal tubule and insignificant differences in immunoreactivity of TGF-β between fetuses from 5/6 nephrectomized and sham-operated mothers were observed (Fig. 8). The stronger immunoreactivity of the TGF-βRI in collecting tubules was noted in fetuses from 5/6 nephrectomized mothers (Fig. 9). Addition of TGF-β to the culture medium induces an inhibition of the differentiation from the metanephric

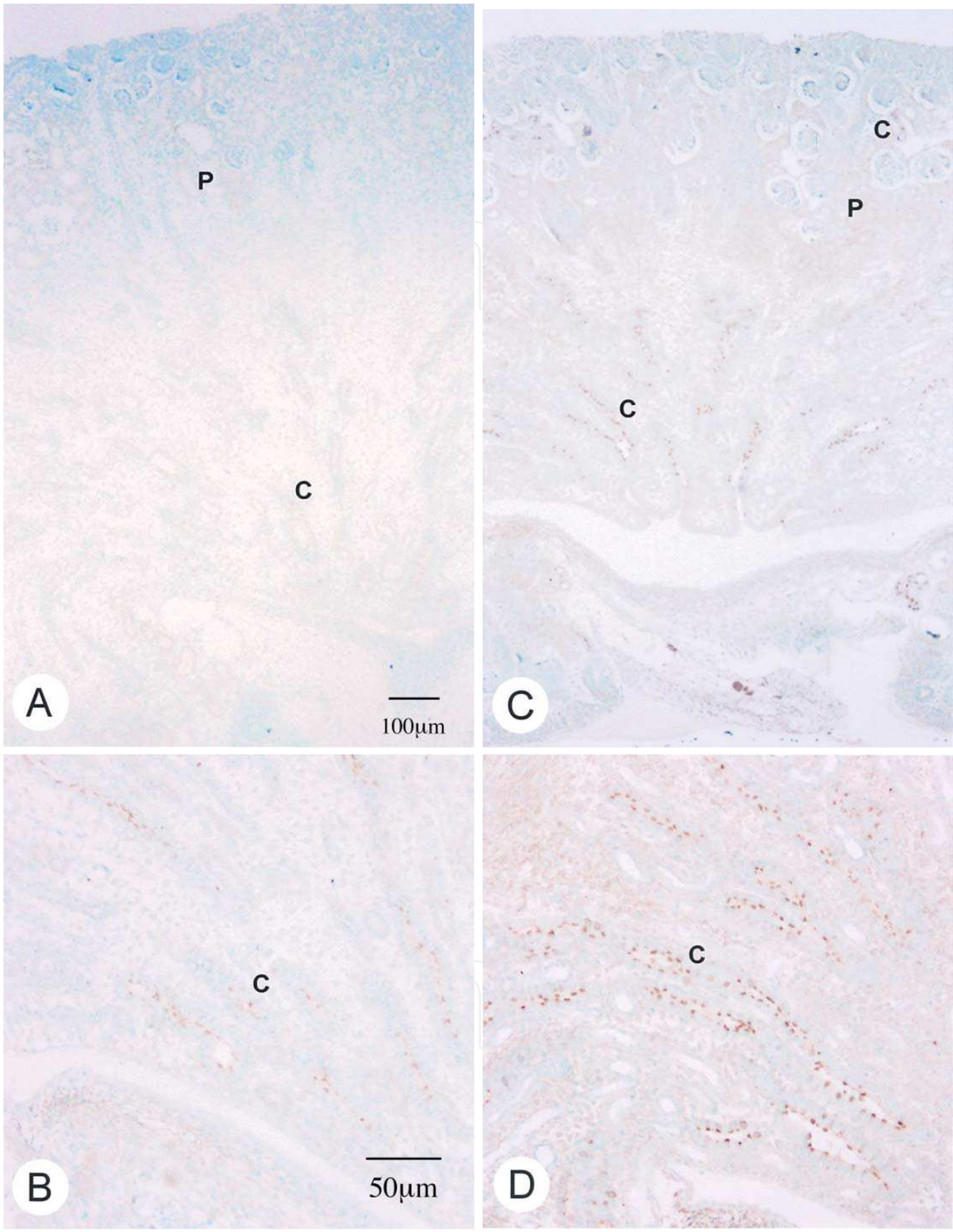

Fig. 9. Fetal kidneys stained with an anti-TGF-βRI antibody. **A** and **B**, fetus from sham-operated mothers. **C** and **D**, fetus of from 5/6 nephrectomized mothers. More TGF-βRI positive cells of the collecting ducts in the medullary zone are seen in the fetus from 5/6 nephrectomized mothers than in sham-operated ones. P, proximal tubules, C, collecting ducts.

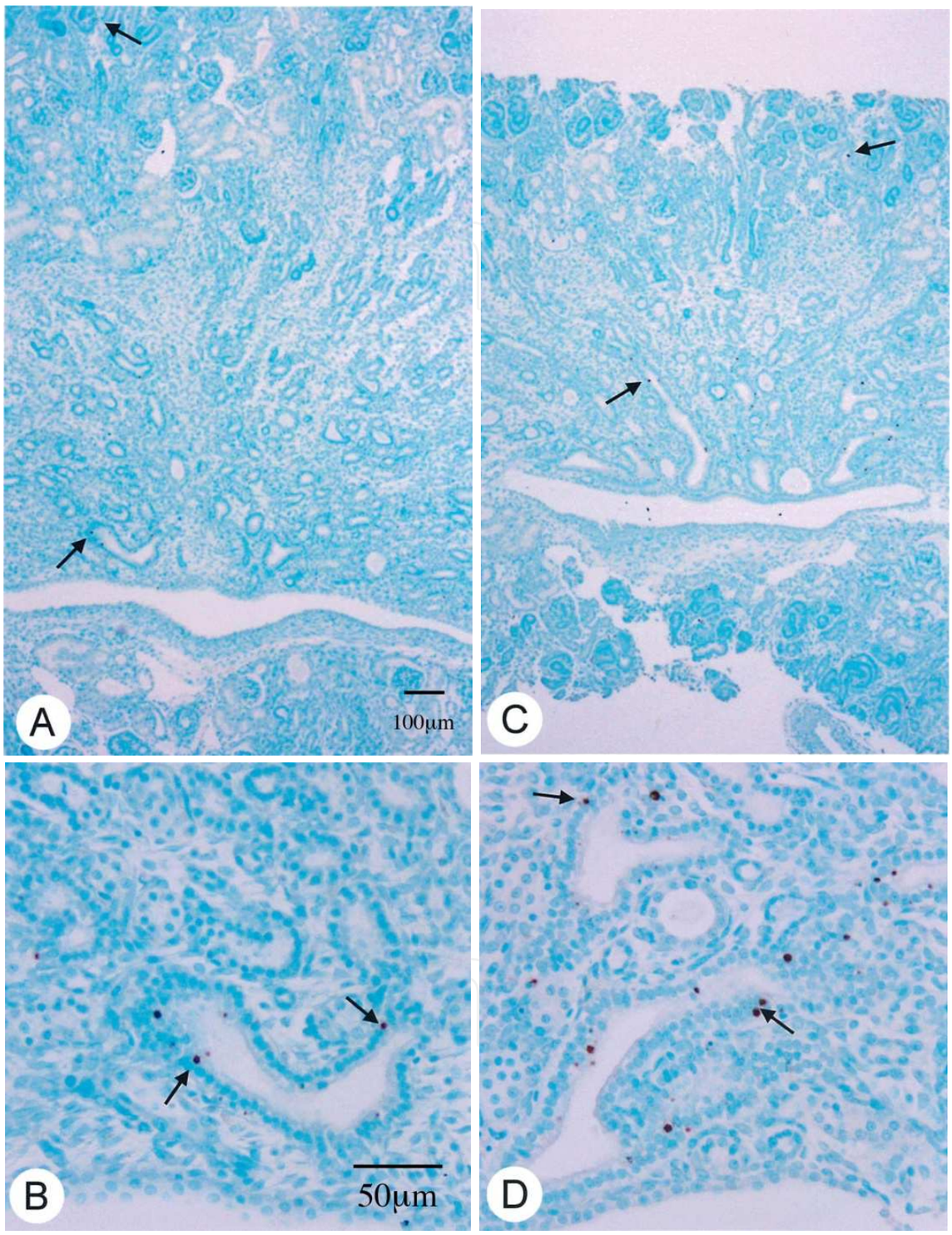

Fig. 10. Fetal kidneys stained with TUNEL methods from sham-operated mothers (**A** and **B**), and 5/6 nephrectomized mothers (**C** and **D**). More TUNEL positive cells (arrows) are seen in the fetus from 5/6 nephrectomized mothers than in that from sham-operated mothers, especially in the collecting ducts in the medulla.

blastema to the nephron and addition of neutral antibody to TGF-β causes the acceleration in nephron formation (Rogers et al., 1993). The changes in TGF-βRI of the fetal kidney by maternal 5/6 nephrectomy may be related to the differentiation of collecting tubules, especially apoptosis in the tubules, because more TUNEL positive cells were observed in the kidney of fetuses from 5/6 nephrectomized mothers than in the kidney of fetuses from sham-operated ones (Fig. 10). Kim et al. (1996) has reported on the developing kidney of the rat and found that intercalated cells show an apoptotic feature and are removed by neighboring principal cells or inner medullary collecting duct (IMCD) cells, resulting in the differentiation of the collecting ducts. Lee et al. (2004) have observed a delayed elimination of type A intercalated cells in the medullary collecting duct with a decreased apoptotic index in the collecting duct of the renal medulla and concluded that apoptosis plays an important role in the morphogenesis of the renal papilla during kidney development. Therefore, the result that the number of TUNEL positive cells in the collecting ducts per unit area (1mm^2) of the kidney was significantly larger in fetuses from 5/6 nephrectomized mothers than in fetuses from sham-operated ones (Fig. 11) indicates that 5/6 nephrectomy of pregnant rats causes the acceleration of the differentiation of the collecting ducts in the fetal kidney. These results suggest that maternal renal dysfunction induces apoptosis in the fetal kidney and that the development of collecting ducts is largely involved in the elevated expression of TGF-β receptor.

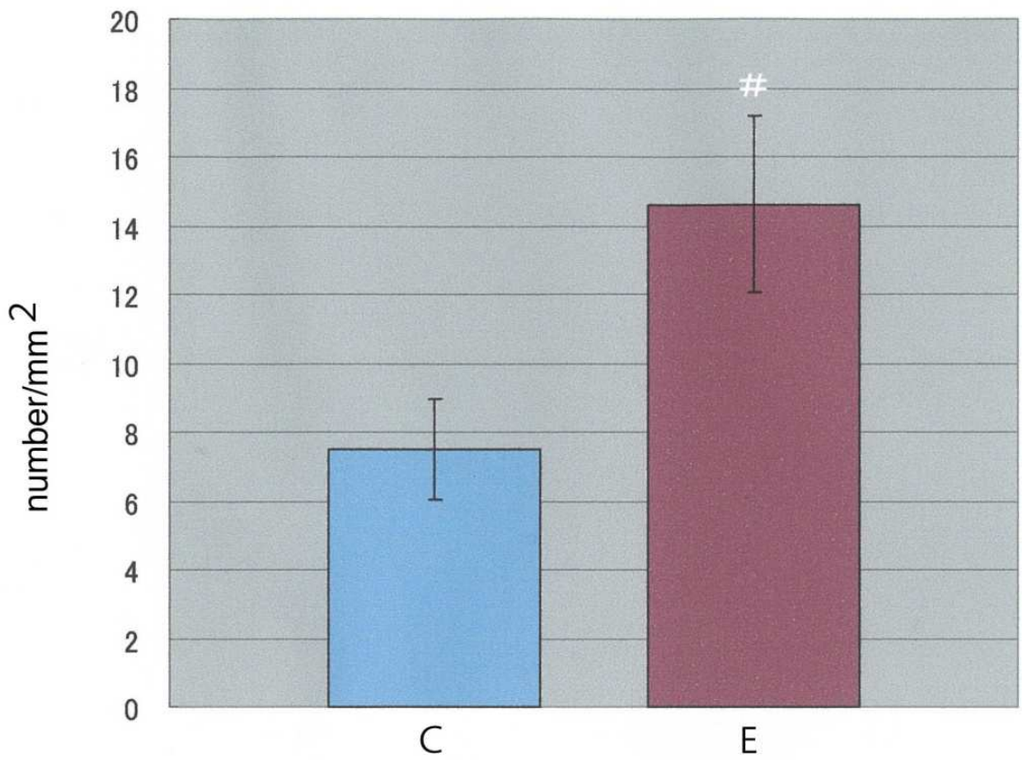

Fig. 11. The number of TUNEL positive cells per unit area (1mm^2) (Means±SEM) of the kidney of fetuses from 5/6 nephrectomeized (E) and sham-operated (C) mothers.
#, Significantly different from fetuses from sham-operated mothers.

4. Effects of the maternal renal dysfunction on the fetus

Infants who are born small have been reported to have higher blood pressure in adulthood (Woods & Weeks, 2004). Furthermore, a clear relation between low birth weight and adverse renal outcome is evident as early as during childhood (Dötsch et al., 2011). The Intrauterine Growth Retardation (IUGR) is very important health problem with a prevalence estimated at ~10% in the general population (Gardosi, 2011). The IUGR model can be produced by bilateral ligation of uterine artery, protein restriction, smoking, nephrotoxic medication as well as salt loading (Bentz & Amann, 2010). Exposure to maternal low protein diet in utero induces IUGR and increases expression of glomerular AT1 receptors and reduces AT2 receptor expression in young rat (Battista et al., 2002; Sahajpal & Ashton, 2005). A low protein diet of the mother is associated with lower birth weight and higher blood pressure in offspring, due to the suppression of renin mRNA and the decrease in the angiotensin II levels in the newborn rat kidney (Woods et al., 2001; Zimmerman & Dunham, 1997).

As described in the above sections, the development of the fetal kidney is accelerated by maternal bilateral ureteral ligation and maternal uninephrectomy. However, the operations induce the suppression of fetal growth in terms of the body and renal weights in Table 1. Maternal 5/6 nephrectomy also induces low body and renal weight in fetuses. Therefore, maternal 5/6 nephrectomy may be one of the means for producing an IUGR model. Thus, to clarify whether maternal 5/6 nephrectomy is a good model for IUGR, further studies including investigation of changes renin-angiotensin system will be need.

Groups	Body weight (g)	Renal weight (mg)	Reference
Bilateral ureterar ligated group	5.00±0.11*	27.17±0.84	Okada and Morikawa, 1988
Sham-operated group	5.56±0.14	30.31±1.40	
Uninephrectomized group	5.29±0.10*	25.42±0.79	Okada et al., 1998
Sham-operated group	5.73±0.12	26.23±0.85	
5/6 nephrectomized group	4.36±0.14*	18.74±0.62*	The present study
Sham-operated group	4.90±0.14	20.59±0.91	

Table 1. Body weight and renal weight (Means±SEM) of fetuses on fetal day 22 from bilateral ureter ligated, uninephrectomized, 5/6 nephrectomized, and sham-operated mothers.
*, significantly different from sham-operated group ($p<0.05$).

5. Conclusion

Detrimental maternal conditions such as renal failure, have various influences on the offspring. Maternal uninephrectomy induces changes in fetal renal development, including an increase in the glomerular volume and proximal tubular length, suggesting the

accelerated renal development is influenced by maternal uninephrectomy. Acceleration of development of the fetal kidney is also seen 24 hours after maternal bilateral ureteral ligation, which is a model of acute renal failure. 5/6 nephrectomy to immature and adult animals induces renal fibrosis and glomerular sclerosis and leads to renal failure, and the progression of renal failure is suppressed by protein intake restriction. The 5/6 nephrectomy to pregnant rats (as a model of pregnant renal failure) induced a significant decrease in body weight and a significant increase in BUN concentration. By maternal 5/6 nephrectomy, however, the immunoreactivity of TGF-βRI is strengthened and TUNEL positive cells are increased in the collecting ducts of the kidney of fetuses, suggesting the acceleration of fetal development of the kidney. Based upon these findings, maternal renal dysfunction induces various desirable effects on fetuses, while a phenomenon similar to IUGR is also seen.

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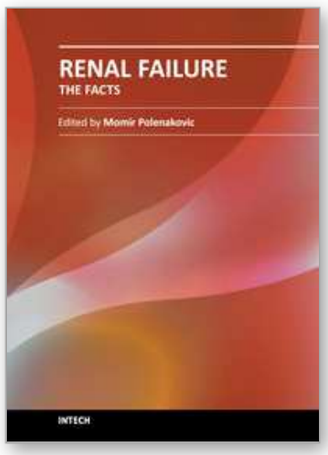

Renal Failure - The Facts

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The book "Renal Failure - The Facts" consists of some facts about diagnosis, etiopathogenis and treatment of acute and chronic renal failure. Acute, as well as chronic renal failure is great medical problems and their treatment is a burden for the budget of each government. The purpose of the chapters is to present some important issues of diagnosis and causes of AKI, as well as caused by snakes and arthropods, after cardiac surgery, as well as some therapeutic achievements in AKI. Well presented are the psychological condition in patients on haemodialysis, as well as the treatment of diabetic uremics. The book is aimed at clinicians with a special interest in nephrology, but it should also prove to be a valuable resource for any generalists who encounter a nephrological problems in their day-to-day practice.

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