# Comparative Studies on the Validity of Renal Function Tests in the Experimentally-Induced Bovine Glomerulonephritis

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ABSTRACT. To establish the usefulness of the bovine clinical renal function tests, experimental glomerulonephritis was experimentally induced in calves and some renal clearance tests were performed. Two (No. 1, 2) of three calves were injected intravenously with anti-bovine kidney rabbit serum (antiserum) and the other (No. 3) with normal rabbit serum (control serum). The early stage of proliferative glomerulonephritis was observed in the kidneys of Nos. 1 and 2. The degree of lesions in No. 1 was severer than that in No. 2. No remarkable change was observed in the kidneys of No. 3. Endogenous creatinine clearance value ($C_{CRE}$), thiosulfate clearance value ($C_{THIO}$) and maximal tubular secretion of para-amino hippuric acid (TmPAH) of all calves did not show remarkable changes after the injection of antiserum or control serum. In the phenolsulfonphthalein (PSP) test, PSP excretion of Nos. 1 and 2 was disposed to delay after the injection, and in No. 3 there was no significant change after the injection. PAH clearance value ($C_{PAH}$) of No. 1 decreased from 10.24 to 6.96 ml/min/kg (−32%). A small change was noted in the $C_{PAH}$ of Nos. 2 and 3. These results suggest that the simplified method for measuring $C_{PAH}$ performed in this study could assess the lesions formed in No. 1, which was pathologically diagnosed as the early stage of proliferative glomerulonephritis.—KEY WORDS: calf, glomerulonephritis, kidney lesion, renal clearance, renal function test.

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The kidney consists of vasculature, glomeruli, proximal and distal tubules, collecting tubules, pelvis and connective tissue, and each portion has function collectively to achieve its manifold character. The accumulation of systematized data of multiple renal function tests is important to assess the disfunction of each constitution of the nephron and to diagnose renal disease [14].

In the bovine clinic, renal function tests have not yet been advanced for many reasons such as difficulties in urine sampling. However, pathological investigations have revealed kidney lesions not only in cows with clinical renal diseases but also in apparently healthy ones. Therefore, precise renal function tests for bovine species should be established as soon as possible [14].

In this study glomerulonephritis was experimentally induced by injection of anti-bovine kidney serum to calves, and the results of renal clearance tests were compared with the morphological lesions of kidney. Consequently the correlation between the degrees of functional disturbances and the morphological lesions of kidney was examined.

## MATERIALS AND METHODS

*Experimental animals*: Three healthy female Holstein-Friesian calves from 3 to 4 months of age were used in this study. Profiles of the three calves at the beginning of the examination are shown in Table 1. The calves were fed dry hay and commercial calf pellet twice a day and water was supplied ad libitum.

*Anti-bovine kidney rabbit serum (antiserum)*: Glomerular basement membrane (GBM) used as antigen for anti-bovine kidney serum was prepared from perfused kidneys of normal adult Holstein-Friesian cows by the methods of Krisko *et al.* [15] and Spiro [24]. The antigen of 33.3 mg per kg of body weight emulsified with an equal volume of Freund's complete adjuvant was injected intracutaneously into fourteen healthy albino rabbits. After a month, additional immunizations were performed 2 to 4 times by injecting 8 mg of antigen to the rabbits at weekly interval. All rabbits were bled 10 days after the last booster injection. The antiserum was inactivated at 56°C for 30 min and absorbed 3 times with normal bovine erythrocytes [26]. Absorbed antiserum was filtrated through 0.8 and 0.45 μm pore-sized filters (Millipore Corporation, Bedford, Mass., U.S.A.) and stocked at −20°C until utilization. The specificity of antiserum against GBM was checked by the indirect fluorescent antibody technique with FITC-labelled anti-rabbit IgG goat serum.

*Control serum*: Normal rabbit sera were prepared as control serum from five nontreated healthy albino

Table 1. Age and body weight of calves used

| Calf No. | Sex | At the beginning of examination | | At the time of administration of serum | |
|---|---|---|---|---|---|
| | | Age (day) | Body weight (kg) | Age (day) | Body weight (kg) |
| 1 | Female | 80 | 82 | 91 | 84 |
| 2 | Female | 73 | 76 | 78 | 78 |
| 3 | Female | 114 | 90 | 119 | 90 |

Table 2. Kind and dose of serum injected to calves

| Calf No. | Kind of serum | Dose of serum | |
|---|---|---|---|
| | | (m*l*) | (m*l*/kg) |
| 1 | Anti-bovine kidney serum | 158.0 | 1.88 |
| 2 | Anti-bovine kidney serum | 79.6 | 1.02 |
| 3 | Control serum | 162.5 | 1.81 |

rabbits and were treated in the same manner as described above.

*Injection of serum*: Two (Nos. 1 and 2) of the calves were injected intravenously with the antiserum via jugular vein and the other one (No. 3) with control serum as shown in Table 2. The moderate shock was observed temporarily after the injection of the antiserum in Nos. 1 and 2 and they received anti-shock treatments. Ther calves were observed carefully until 8 days after the injection of each serum. Urinalysis was simply performed with using Rabsitix-III (Miles-Sankyo Corporation, Tokyo, Japan).

*Renal function test*: Endogenous creatinine (CRE) clearance test was carried out on all calves following the method described by Sanjo [21]. CRE clearance value ($C_{CRE}$) was determined by repeating the procedure two times.

Phenolsulfonphthalein (PSP) test was performed according to Kawamura *et al.* [14]. In this test 6 mg/head of PSP was injected into the jugular vein of calves. Fractionated urine samples were collected 4 times at 15 min intervals during 60 min after the injection and PSP excretion in each fractional urine sample was expressed as U15, U30, U45 and U60 value (urinary excretion percentage). A 0.6% PSP solution (Daiichiseiyaku Corporation, Tokyo, Japan) was used for the injection. These two tests were performed before and 6 days after the injection.

Para-amino hippuric acid (PAH) / thiosulfate (THIO) clearance test was employed depending on Nihei [20] and performed by the simplified method of simultaneous dripping injection of PAH and THIO. In this test 20 mg per kg of body weight of PAH (20% PAH solution, Daiichiseiyaku Corporation, Tokyo, Japan) and 100 mg per kg of body weight of THIO were injected into the jugular vein.

Determination of maximal tubular secretion of PAH (TmPAH) was followed using 20% PAH solution (Daiichiseiyaku Corporation, Tokyo, Japan) based on the method described by Nihei [20]. These two tests were performed before and 7 days after the injection.

To obtain enough volume of urine samples, all calves were given orally 3 *l* of warm water added a small amount of milk, 30–60 min prior to the beginning of each test. Urine was collected through a 10, 12, or 14 Fr. balloon catheter (Terumo Corporation, Tokyo, Japan) inserted into the bladder. The bladder was rinsed by sterilized physiological saline prior to the beginning of each test and just before the finish of each test. Blood samples were taken from the jugular vein and centrifuged at 3,000 rpm for 15 min at 4°C. The separated serum were stocked at −20°C until determination. The clearance value (C) was calculated as follows:

$$C\ (ml/min) = Uc \times Uf / Sc \text{ or } Pc$$

Sc: Serum concentration (mg/d*l*)
Pc: Plasma concentration (mg/d*l*)
Uc: Urine concentration (mg/d*l*)
Uf: Volume of urine (m*l*/min)

PAH clearance value ($C_{PAH}$) shows the effective renal plasma flow, which must be rectified by the exclusion rate in order to obtain renal plasma flow (RPF). However, there have been no studies on the PAH exclusion rate of bovine. In this study $C_{PAH}$ was regarded as RPF. Renal blood flow (RBF) was also calculated as follows:

$$RBF\ (ml/min) = C_{PAH} \times 100 / (100\text{-}Ht)$$

Ht: Hematocrit value (%)

Body surface area (BS) of calves was calculated as follows:

$$BS\ (m^2) = 0.15 \times BW^{0.65}\ [5]$$

BW: body weight

TmPAH was calculated as follows:

TmPAH (mg/m*l*) = Uc × Uf − 0.83Pc × GFR [20]
GFR: Glomerular filtration rate

*Autopsy and histopathology*: All the calves were sacrificed 8 days after the injection. Kidneys and the other main organs were fixed in 10% formalin, embedded in paraffin. The sections of the organs of 4 $\mu$m thick were made and stained with hematoxylin-eosin (HE) and periodic acid-Schiff (PAS).

*Immunofluorescence technique*: Fluorescent antibody test was performed by the indirect method with FITC-labelled anti-rabbit IgG goat serum [13].

## RESULTS

In the indirect fluorescent antibody test, antiserum against GBM of normal cow reacted with normal bovine kidney. Brilliant specific fluorescence was observed along glomeruli and Bowman's capsule (Fig. 1). Control serum did not react with normal bovine kidney.

Nos. 1 and 2 were injected intravenously with the antiserum and No. 3 with the control serum in the same manner. Nos. 1 and 2 temporarily fell in a shock immediately after the injection but recovered within 1–3 hr. After that, no clinical change was observed. In No. 3 no clinical change was also observed either before or after the injection.

Urinalysis of Nos. 1 and 2 showed a positive for protein and slight increase in epithelial cells, erythrocytes and leucocytes, but that of No. 3 indicated no abnormality in the urine.

In the CRE clearance test of each calf, there was a slight difference in $C_{CRE}$ between before and after the injection of the antiserum or control serum (Table 3).

The results of the PSP test in No. 1 revealed that the value of U15, U30 and U60 were 13, 15 and 6% lower than those obtained before the injection, respectively (Table 4). Excretion of PSP in No. 1 checked after the injection showed a delaying tendency as compared to that before the injection. Of No. 2 the value of U15, U30 and U60 were 16, 12 and 9% lower than those obtained before the injection, respectively. PSP excretion of No. 2 also showed a delaying tendency compared with that before the injection (Table 4). U15 and U30 value of No. 3 were negligibly lower than those obtained before injection, but U45 and U60 value were almost the same as those checked before the injection.

$C_{PAH}$ in No. 1, which was 10.24 m*l*/min/kg before the injection, decreased to 6.96 m*l*/min/kg after the

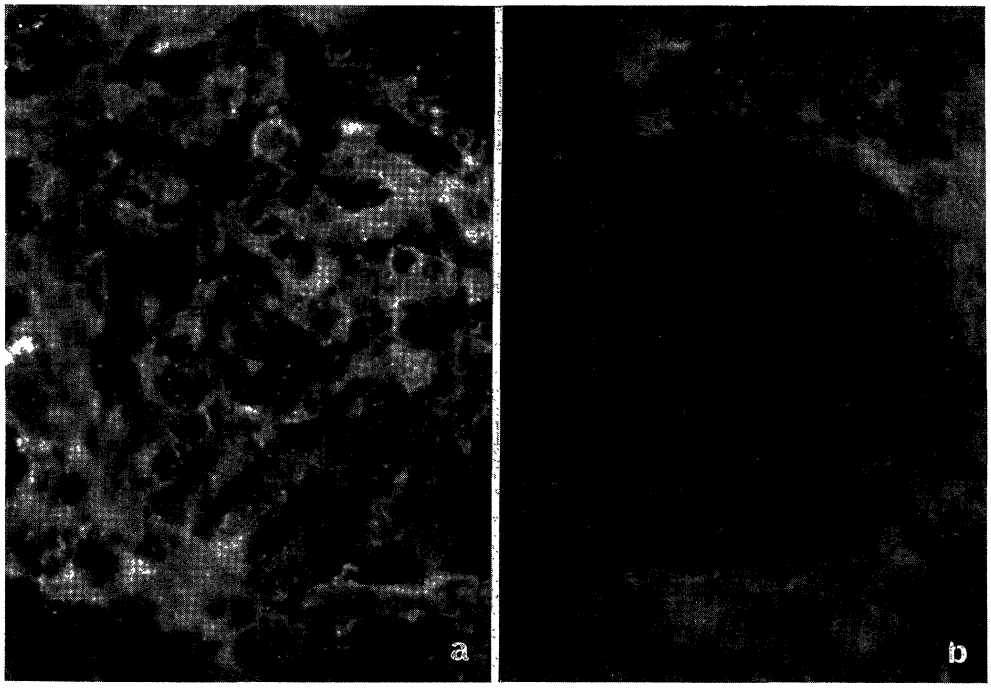


Fig. 1. Indirect immunofluorescent staining of frozen sections of normal bovine kidney. a: Antiserum used in this study causing brilliant specific fluorescence along the glomeruli and Bowman's capsule, ×430. b: Control serum inducing no specific fluorescence, ×430.

Table 3. $C_{CRE}$ determined at pre-and post-injection of each serum in calves

| Calf No | Body weight (kg) | Body surface area ($m^2$) | Serum CRE(mg/dl) (First) | Serum CRE(mg/dl) (Second) | Urine CRE(mg/dl) (First) | Urine CRE(mg/dl) (Second) | Urine volume(ml/min) (First) | Urine volume(ml/min) (Second) | $C_{CRE}$ (ml/min) | $C_{CRE}$ (ml/min/kg) | $C_{CRE}$ (ml/min/$m^2$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 (pre)[a] | 82 | 2.63 | 1.00 | 1.00 | 9.00 | 9.13 | 15.93 | 15.87 | 144.09 | 1.76 | 54.79 |
| 1 (post)[b] | 86 | 2.71 | 1.28 | 1.29 | 14.63 | 13.00 | 12.63 | 13.47 | 138.51 | 1.61 (91.5)[c] | 51.11 (93.3)[c] |
| 2 (pre)[a] | 76 | 2.50 | 1.35 | 1.32 | 16.73 | 10.70 | 19.48 | 29.60 | 240.68 | 3.17 | 96.27 |
| 2 (post)[b] | 80 | 2.59 | 1.13 | 1.10 | 11.48 | 12.04 | 27.72 | 24.32 | 273.91 | 3.42 (107.8)[c] | 105.76 (109.9)[c] |
| 3 (pre)[a] | 90 | 2.80 | 1.10 | 1.10 | 45.50 | 15.80 | 4 10 | 10.01 | 156.69 | 1.74 | 56.06 |
| 3 (post)[b] | 93 | 2.85 | 1.10 | 0.95 | 21.50 | 27.25 | 7 68 | 5.85 | 158.96 | 1.71 (98.3)[c] | 55.78 (99.5)[c] |

a) Pre-injection of each serum.
b) Post-injection of each serum.
c) Percentage to pre-injection value.

Table 4. PSP excretion rate in urine determined at pre- and post-injection of each serum in calves

| Calf No. | $U_{15}$ (%) | $U_{30}$ (%) | $U_{45}$ (%) | $U_{60}$ (%) |
|---|---|---|---|---|
| 1 (pre)[a] | 31.0(31.0)[c] | 16.0(47.0) | 7.0(53.0) | 3.8(56.8) |
| 1 (post)[b] | 27.0(27.0) <87.0>[d] | 13.0(40.0) <85.1> | 7.4(47.4) <89.4> | 6.0(53.4) <94.0> |
| 2 (pre)[a] | 39.6(39.6) | 13.0(52.6) | 6.9(59.5) | 4.3(63.8) |
| 2 (post)[b] | 33.3(33.3) <84.0> | 12.8(46.1) <87.6> | 7.5(53.6) <90.0> | 4.7(58.3) <91.4> |
| 3 (pre)[a] | 40.5(40.5) | 12.9(53.4) | 5.8(59.2) | 4.4(63.6) |
| 3 (post)[b] | 38.3(38.3) <94.6> | 12.1(50.4) <94.4> | 9.1(59.5) <100.5> | 6.1(65.6) <103.1> |

a) Pre-injection of each serum.
b) Post-injection of each serum.
c) Total percentage of PSP.
d) Percentage to pre-injection value (total).
$U_{15}$ Urinary excretion percentage of PSP 15 min after PSP injection.

injection, which was 31.1% decrease. RBF of No. 1 decreased from 16.00 to 9.66 ml/min/kg (Table 5), 38.7% decrease. $C_{PAH}$ and RBF in No. 2 were almost the same as those obtained before the injection. $C_{PAH}$ of No. 3 increased 16.1% after the injection.

The results of $C_{THIO}$ and TmPAH are shown in Tables 6 and 7. The difference between before and after the injection of the antiserum or control serum was just a little in each calf.

At autopsy, pin point-size hemorrhagic foci were scattered on the cortex of both kidneys of Nos. 1 and 2 and petechiae were more prominent in No. 1 than in No. 2. There were no distinguishable lesions in both kidneys of No. 3. Slight serous bronchopneumonia was present in the right lung of No. 2 and scattered miliary hemorrhagic foci were observed in the mucous membrane of the urinary bladder of Nos. 2 and 3. There were no gross lesions in the other organs.

Histopathologically, slight swelling and proliferation of endothelial and mesangial cells of glomeruli

Table 5. $C_{PAH}$ determined at pre- and post-injection of each serum in calves

| Calf No. | Body weight (kg) | Body surface area ($m^2$) | Serum PAH (mg/dl) | Urine PAH (mg/dl) | Urine volume (ml/min) | $C_{PAH}$ (ml/min) | $C_{PAH}$ (ml/min/kg) | $C_{PAH}$ (ml/min/$m^2$) | Ht (%) | RBF (ml/min/kg) | RBF (ml/min/$m^2$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 (pre)[a] | 81 | 2.61 | 1.20 | 51.50 | 19.33 | 829.58 | 10.24 | 317.85 | 36 | 16.00 | 496.64 |
| 1 (post)[b] | 84 | 2.67 | 1.40 | 111.00 | 7.37 | 584.39 | 6.96 (68.9)[c] | 218.85 (68.9)[c] | 28 | 9.66 (61.3)[c] | 303.96 (61.2)[c] |
| 2 (pre)[a] | 78 | 2.55 | 0.97 | 25.94 | 29.03 | 776.33 | 9.95 | 304.44 | 34 | 15.08 | 461.27 |
| 2 (post)[b] | 80 | 2.59 | 1 45 | 36.95 | 31.24 | 796.08 | 9.95 (100.0)[c] | 307.37 (101.0)[c] | 32 | 14.63 (97.0) | 452.01 (98.0) |
| 3 (pre)[a] | 92 | 2.84 | 1 52 | 60.50 | 19.40 | 772.17 | 8.39 | 271.89 | 31 | 12.16 | 394.04 |
| 3 (post)[b] | 97 | 2.93 | 1.13 | 41.50 | 25.73 | 944.95 | 9.74 (116.1)[c] | 322.51 (118.6)[c] | 30 | 13.91 (114.4)[c] | 460.73 (116.9)[c] |

a) Pre-injection of each serum.
b) Post-injection of each serum.
c) Percentage to pre-injection value.

Table 6. $C_{THIO}$ determined at pre-and post-injection of each serum in calves

| Calf No. | Body weight (kg) | Body surface area ($m^2$) | Serum THIO (mg/dl) | Urine THIO (mg/dl) | Urine volume (ml/min) | $C_{THIO}$ (ml/min) | $C_{THIO}$ (ml/min/kg) | $C_{THIO}$ (ml/min/$m^2$) |
|---|---|---|---|---|---|---|---|---|
| 1 (pre)[a] | 81 | 2.61 | 12.50 | 113.29 | 19.33 | 175.19 | 2.16 | 67.12 |
| 1 (post)[b] | 84 | 2.67 | 40.00 | 905.16 | 7.37 | 166.78 | 1.99 (92.8)[c] | 62.46 (93.1)[c] |
| 2 (pre)[a] | 78 | 2.55 | 34.00 | 297.72 | 29.03 | 254.20 | 3.26 | 99.69 |
| 2 (post)[b] | 80 | 2.59 | 29.00 | 256.37 | 31.24 | 276.17 | 3.45 (105.8)[c] | 106.33 (106.9)[c] |
| 3 (pre)[a] | 92 | 2.84 | 49.80 | 367.42 | 19.40 | 143.13 | 1.56 | 50.40 |
| 3 (post)[b] | 97 | 2.93 | 61.00 | 399.98 | 25.73 | 168.71 | 1.74 (111.5)[c] | 57.58 (114.2)[c] |

a) Pre-injection of each serum.
b) Post-injection of each serum.
c) Percentage to pre-injection value.

were observed in both kidneys of Nos. 1 and 2 (Fig. 2). The kidney sections stained with PAS revealed slightly thickening of GBM and Bowman's capsule basement membrane in Nos. 1 and 2 (Fig. 3). Slight hemorrhage and proteinaceous fluid were noted in Bowman's space. These lesions were more marked in No. 1 than in No. 2. Granular and hyaline droplet degeneration was detected in some of the epithelial cells of uriniferous tubules, and casts were seen in the lumen of renal tubules of No. 2. Slight lymphocyte and plasma cell infiltration and hemorrhage were observed in the interstitium of Nos. 1 and 2 (Fig. 4). The interstitial lesions were more prominent in No. 2 than in No. 1. Proliferation of mesenchymal cells was observed around the vascular pole side of Bowman's capsule in No. 1 (Fig. 2). On the other hand, no lesions were detected in both kidneys of No. 3. Slight edema was seen in the left ventricular myocardium of No. 1 and slight bronchopneumonia and small necrotic foci of the liver in No. 2. There were slight hemorrhagic inflammatory changes in the mucous membrane of the urinary bladder of Nos. 2 and 3. In the other organs, there were no significant histopathological lesions.

Kidney sections stained with FITC-labelled anti-rabbit IgG goat serum showed brilliant linear

Table 7. TmPAH determined at pre-and post-injection of each serum in calves

| Calf No. | Body weight (kg) | Body surface area ($m^2$) | Serum PAH (mg/dl) | Urine PAH (mg/dl) | Urine volume (ml/min) | $C_{THIO}$ (ml/min) | $Tm_{PAH}$ (ml/min) | (ml/min/kg) | (ml/min/$m^2$) |
|---|---|---|---|---|---|---|---|---|---|
| 1 (pre)[a] | 81 | 2.61 | 17.50 | 1550.0 | 8.00 | 175.19 | 98.55 | 1.22 | 37.76 |
| 1 (post)[b] | 84 | 2.67 | 29.50 | 1250.0 | 12.67 | 166.78 | 117.54 | 1.40 (114.8)[c] | 44.02 (116.6)[c] |
| 2 (pre)[a] | 78 | 2.55 | 23.49 | 964.2 | 17.77 | 254.20 | 121.72 | 1.56 | 47.73 |
| 2 (post)[b] | 80 | 2.59 | 23.10 | 660.0 | 28.34 | 276.17 | 134.10 | 1.68 (107.7)[c] | 51.78 (108.5)[c] |
| 3 (pre)[a] | 92 | 2.84 | 32.50 | 1620.0 | 10.22 | 143.13 | 126.95 | 1.38 | 44.70 |
| 3 (post)[b] | 97 | 2.93 | 20.50 | 800.0 | 20.19 | 168.71 | 132.81 | 1.37 (99.3)[c] | 45.33 (101.4)[c] |

a) Pre-injection of each serum.
b) Post-injection of each serum.
c) Percentage to pre-injection value.

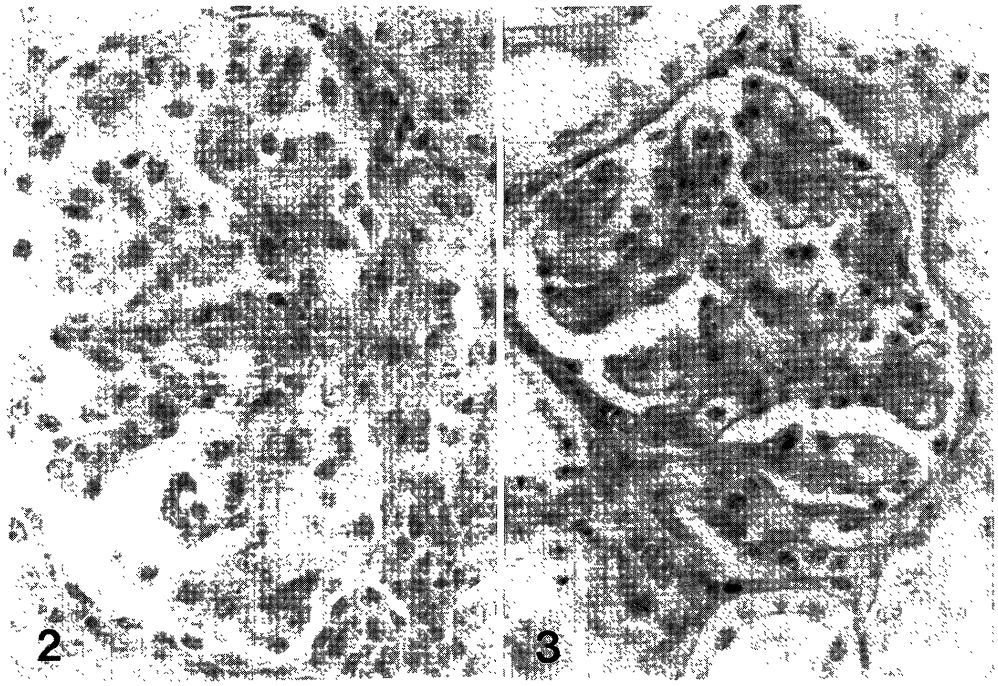


Fig. 2. Renal lesions in No. 1, which was injected with antiserum. Swelling and proliferation of endothelial and mesangial cells of glomeruli and infiltration of mesenchymal cell around the vascular pole. HE-stain, ×320.

Fig. 3. Slight thickening of GBM and Bowman's capsule seen in No. 1, which was injected with antiserum. PAS-stain, ×360.

specific fluorescence along GBM and Bowman's capsule of Nos. 1 and 2 (Fig. 5). Linear specific fluorescence was also seen around a part of the uriniferous tubules. However, no specific fluorescence was detected in the kidneys of No. 3 (Fig. 5).

## DISCUSSION

In glomerulonephritis caused by renal-toxic serum, changes appear first in mesangial cells, followed by proliferation of epithelial and endothelial cells of glomeruli [3, 27]. Thickening of GBM

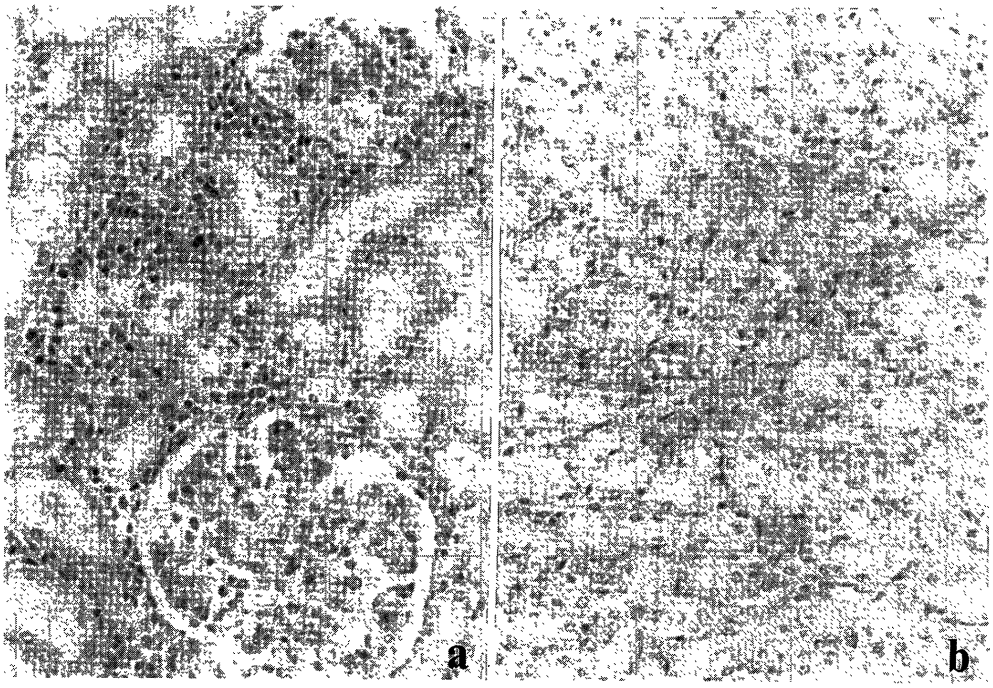


Fig. 4. Interstitial changes in No. 2, which was injected with antiserum. HE-stain, ×180. a: Infiltration of lymphocytes and plasma cells.
b: Hemorrhage in interstitium.

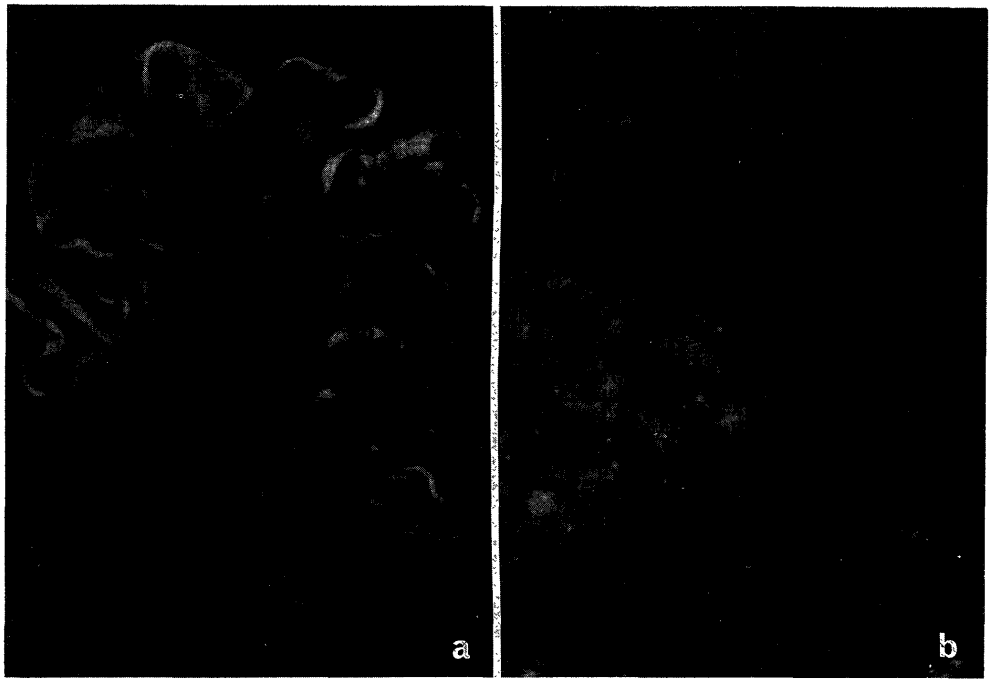


Fig. 5. a: Immunofluorescent staining of calves with FITC-labelled anti-rabbit IgG goat serum. Brilliant linear specific fluorescence along GBM and Bowman's capsule in No. 1, which was injected with antiserum, ×430.
b: No specific fluorescence in the kidney of No. 3, which was injected with control serum, ×430.

has been observed in this type of glomerulonephritis [1, 4, 6, 17, 23]. In the present study the kidney lesions of Nos. 1 and 2, which were injected with anti-bovine kidney serum (antiserum), were mainly composed of swelling and proliferation of mesangial and endothelial cells of the glomeruli. The kidney sections stained with PAS demonstrated the thickening of GBM in Nos. 1 and 2, but the degree of the thickening was slight. Therefore the primary importance of the glomerular damage detected in Nos. 1 and 2 was the proliferation of mesangial and endothelial cells. Also, the lesions were in the early stage of proliferative glomerulonephritis, and the degree of the lesions was severer in No. 1 than in No. 2.

On the other hand, partial degeneration of epithelial cells of the uriniferous tubules was observed in No. 2. Tubular lesions were slight but not diffuse, and were caused by the presence of a common antigen shared with GBM and the tubular basement membrane [23]. The interstitial hemorrhage and cellular infiltration may have been due to the injection of antiserum, as reported by Shirota and Fujiwara [23]. Many workers have suggested that immunoreaction by the injection of anti-kidney serum [1, 9, 18, 19, 25] also occurred in many organs other than the kidneys. It was speculated that the hemorrhage of mucous membrane of the urinary bladder resulted from catheterization, which also accounted for the increase of urinary sediment of Nos. 1 and 2. Circulation has an effect on the renal function tests; in the present study the histopathological change of heart was only slight edema in the left ventricular myocardium of No. 1.

The measurement of $C_{CRE}$ and $C_{THIO}$ [16] are both available for assessment of GFR. GFR generally decreases at an early stage of acute glomerulonephritis, improves with recovery of the disease and decreases in proportion to the damage in chronic glomerulonephritis [22]. However there is a small change in GFR in nephrosclerosis [22]. In the present study, the changes of $C_{CRE}$ and $C_{THIO}$ of Nos. 1 and 2 were slight, which was consistent with histopathological findings; the damage of GBM mainly characterized by glomerular filtration was comparatively slight.

PAH injected intravenously is mainly excreted from proximal renal tubules and partially from glomeruli. If the plasma concentration is lower than 5 mg/d*l*, about 90% of PAH will be excreted into the urine during one blood circulation through the kidney in human beings [8]. Therefore $C_{PAH}$ reflects RPF and measurement of $C_{PAH}$ seems to be useful for the assessment of renal circulatory function [8]. Kaufman and Bergman [12] reported that RPF and RBF in sheep suffering from hypoglycemia and ketosis caused by starvation were decreased by 25–30%. Johnson [11] reported that $C_{PAH}$ decreased under the condition of dehydration in bovine. Bieri [2] reported that RBF in cat with moderate renal damage decreased to 12–15 m*l*/min/kg and further decreased to less than 12 m*l*/min/kg in cases of severe renal damage, though the average of RBF in normal cat was 18 m*l*/min/kg. In the present study, RPF of No. 1 obtained after the injection of antiserum decreased about 30%, but in No. 2 RPF was almost the same as that obtained before the injection. These results consisted with histopathological findings that were mainly composed of renal circulatory damage such as swelling and proliferation of mesangial and endothelial cells of glomeruli and cellular infiltration around the vascular pole of Bowman's capsule. These results suggest that the simplified method for measurement of $C_{PAH}$ performed in this study could diagnose the early stage of proliferative glomerulonephritis such as lesions in No. 1; however, it is difficult to assess the slight lesions which were seen in No. 2 and characterized by slight proliferation of mesangial and endothelial cells of the glomeruli.

PSP test is performed clinically as a valid screening test for the assessment of renal function in human beings [10]. PSP injected intravenously binds with albumin and is mainly excreted from proximal renal tubules, making it a good indicator for RPF. U15 value of this test changes in proportion of RPF, so it is useful as a screening test for the renal circulatory system [7]. U15 value of Nos. 1 and 2 showed a tendency to decrease after the injection of antiserum. It was considered that this test also reflected damages to the renal circulation system of Nos. 1 and 2.

Measurement of TmPAH was reported to be useful to estimate the proximal renal tubular function [8]. TmPAH of No. 2, which developed partial degeneration of renal tubules, showed little change after the injection of antiserum; therefore, this method was not able to assess the slight lesions such as observed in the uriniferous tubules of No. 2.

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REFERENCES

1. Bergstein, J. and Litman, N. 1975. Interstitial nephritis with anti-tubular-basement-membrane antibody. *New Engl. J. Med.* 292: 875–878.
2. Bieri, P. 1976. Laboratory diagnosis of kidney diseases in the cat with special reference to para-amino-hippuric acid (PAH) clearance. *Kleintierprax.* 21: 189–196.
3. Cochrane, C. G., Unaue, E. R., and Dixon, F. J. 1965. A role of polymorphonuclear leukocytes and complement in nephrotoxic nephritis. *J. Exp. Med.* 122: 99–119.
4. Couser, W. G., Steinmuller, D. R., Stilmant, M. M., Salant, D. J., and Lowenstein, L. M. 1978. Experimental glomerulonephritis in the isolated perfused rat kidney. *J. Clin. Invest.* 62: 1275–1287.
5. Dalton, R. G. 1968. Renal function in neonatal calves: Urea clearance. *Br. Vet. J.* 124: 451–459.
6. Feenstra, K., Lee, R., Greben, H. A., Arends, A., and Hoedmaker, P. J. 1985. Experimental glomerulonephritis in the rat induced by antibodies directed against tubular antigens. *Lab. Invest.* 32: 235–242.
7. Filippich, L. J., English, P. B., and Ainscow, J. 1985. Phenol-sulfonphthalein test in healthy sheep and in sheep with reductions in functional renal mass. *Am. J. Vet. Res.* 46: 733–738.
8. Gillenwater, J. Y. 1986. Chapter 9. The pathophysiology of urinary obstruction. pp. 542–578. *In*: Urology, 5th ed. (Walsh, P., Gitters, R. F., Perlmutter, A. D., and Stamey, T. A. eds.), W. B. Saunders Company, Philadelphia.
9. Hirota, S. and Shimizu, F. 1980. Kinetics of heterologous antirat tubular antigen antibody in rat kidney. *Jpn. J. Exp. Med.* 50: 351–358.
10. Honda, N. 1982. Chapter 3. Diagnosis of renal disease. pp. 48–65. *In*: Naikagakusho, vol. 3 (Oda, T., Omae, T., Toyokura, Y., and Murao, M. eds.), Nakayama-shoten, Tokyo (in Japanese).
11. Johnson, K. G. 1971. Renal function in *Bos taurus* and *Bos indicus-crossbred* cows under conditions of normal hydration and mild dehydration. *Res. Vet. Sci.* 12: 438–447.
12. Kaufman, C. F. and Bergman, E. N. 1978. Renal function studies in normal and toxemic pregnant sheep. *Cornell Vet.* 68: 124–137.
13. Kawamura, A. J. 1977. Fluorescent antibody techniques and their applications, 2nd ed., University of Tokyo Press, Tokyo.
14. Kawamura, S., Saito, N., Higuti, S., and Yasuda, S. 1983. The study of renal functional test in bovine. *Tohoku J. Vet. Clinic* 5: 30–49 (in Japanese).
15. Krisko, I., Bernardo, E., and Sato, C. S. 1977. Isolation and characterization of rat tubular basement membrane. *Kidney Int.* 12: 238–243.
16. Leslie, B. R. and Voughan, E. D. Jr. 1986. Chapter 2. Normal renal physiology. pp. 75–93. *In*: Urology, 5th ed. (Walsh, P., Gitters, R. F., Perlmutter, A. D., and Stamey, T. A. eds.), W. B. Saunders Company, Philadelphia.
17. Mauer, S. M., Sutherland, D. E. R., Howard, R. J., Fish, A. J., Najarian, J. S., and Michael, A. F. 1973. The glomerular mesangium. *J. Exp. Med.* 137: 553–570.
18. Nagasawa, T. and Shibata, S. 1969. Immunofluorescence studies on the anatomic distribution of the soluble antigen responsible for the production of nephrotoxic serum nephritis. *J. Immunol.* 103: 736–740.
19. Neale, T. J., Woodroffe, A. J., and Wilson, C. B. 1984. Spontaneous glomerulonephritis in rabbits: Role of a glomerular capillary antigen. *Kidney Int.* 26: 701–711.
20. Nihei, T. 1983. Chapter 14. pp. 793–844. *In*: Rinshoukensa-houteiyo (Kanai, I. and Kanai, M. eds.), Kinbara-Shuppan, Tokyo (in Japanese).
21. Sanjo, T. 1974. Renal clearance tests. pp. 15–33. *In*: Renal Functional Tests (Ikegami, K. ed.), Nanzando, Tokyo (in Japanese).
22. Schaeffer, A. J. and DelGreco, F. 1986. Chapter 60. Other renal disease of urologic significance. pp. 2342–2360. *In*: Urology, 5th ed. (Walsh, P., Gitter, R. F., Perlmutter, A. D., and Stamey, T. A. eds.), W. B. Saunders Company, Philadelphia.
23. Shirota, K. and Fujiwara, K. 1982. Nephropathy in dogs induced by treatment with antiserum against renal basement membrane. *Jpn. J. Vet. Sci.* 44: 767–776.
24. Spiro, R. G. 1967. Studies on the renal glomerular basement membrane. *J. Biol. Chem.* 242: 1915–1922.
25. Sternberg, M., Grandpre, P. D., Pelletier, V., and Carter, M. 1979. Excretion and synthesis of basement membrane disaccharide units in Masugi nephritis. *Kidney Int.* 16: 329–336.
26. Usui, M., Seinai, H., and Matuhasi, T. 1972. The method of producing of antibody against human IgG. pp. 51–63. *In*: Immunochemistry (Migita, T. ed.), Nakayama-shoten, Tokyo (in Japanese).
27. Yamanaka, Y., Sugisaki, H., and Masugi, Y. 1981. The mesangial response in glomerulonephritis. *Igaku-no-ayumi* 119: 380–387 (in Japanese).