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IMMUNOHISTOCHEMICAL DEMONSTRATION OF
JUXTAGLOMERULAR CELLS
IN THE KIDNEYS OF
DOMESTIC MAMMALS AND FOWLS

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Intrarenal distribution of renin-containing cells in 13 mammals and 2 fowls was immunohistochemically demonstrated. The immunoreactive cells were found as swollen epithelioid cells localized in regions of glomerular vascular poles. An overwhelming number of renin-containing cells were demonstrated mainly in the tunica media of the afferent glomerular arterioles. In 7 mammals and 1 fowl, however, a few quantities of immunoreactive cells were also notable in the same regions as the efferent glomerular arterioles, and especially in the sheep and goat, a remarkable number of immunoreactive cells were localized not only in the walls of the afferent glomerular arterioles but also in the interlobular arteries. These cells were highly variable in size and figure and were located away from the vascular poles, and they were demonstrable even in the tunica adventitia in arterioles or arteries. In the kidneys of fowls, positive cells were observed frequently in the mesangial regions along the glomerular capillaries.

The present results and the relative histoplanimetric quantification of renin-containing cells suggest the possibility that the cells, which have been regarded as the only derivative of smooth muscle cells from the tunica media of the afferent glomerular arterioles, might also be derived from cells in the tunica adventitia of the glomerular associated vessels and also the mesangial cells in the glomerulus.

Key words : renin, juxtaglomerular cell, kidney, domestic animal

INTRODUCTION

The juxtaglomerular apparatus, which has been known as an important regulatory apparatus for blood pressure, is composed of the following vascular elements : afferent and efferent glomerular arterioles, extraglomerular mesangial (lakis) cells, juxtaglom-

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erular (JG) cells, and the tubular element : macula densa.²⁾ More recently, intrarenal localizations of renin and the substances of the angiotensin series were histochemically demonstrated in JG cells of human,^{1,5)} rat,^{17,19)} and mouse.^{15,16)} However, no detailed studies on the intrarenal distributions and the cellular origin of the renin-containing cells have been performed in domestic mammals and fowls.

The present study provides evidence for the localization of renin-containing cells in the kidneys of domestic and laboratory mammals and in fowls, which was found by applying an anti-renin serum. The features of the intrarenal distribution and the possible cellular origin of the cells obtained by relative histoplanimetry were compared and discussed.

MATERIALS AND METHODS

Materials : Kidney tissues were taken from horse, cow, pig, goat, sheep, dog, cat, rabbit, hamster, guinea pig, rat, mouse, field-vole (*Microtus montebelli*), chicken and duck. The tissues were fixed by Bouin's solution, then embedded in paraffin and cut into 4 μ m sections.

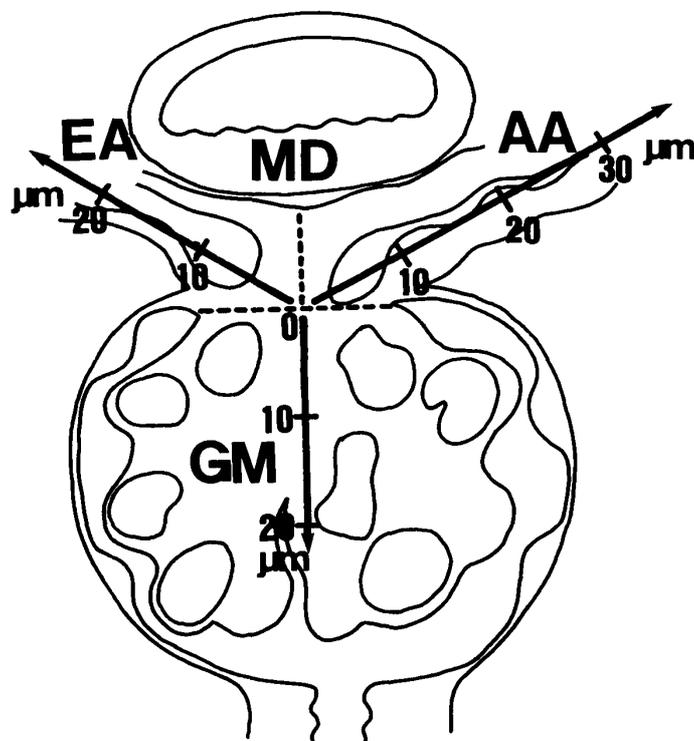
Antiserum : Antiserum against the purified renin from the mouse submandibular gland were raised in rabbits.⁶⁾

Immunohistochemistry : Deparaffinized sections were exposed to absolute methanol, 0.1% H₂O₂ in 0.01M PBS and 1% normal goat serum for 1 hr at room temperature, respectively, for the removal of endogeneous peroxidase activity. The sections were reacted with 1 : 3000 dilutions of anti-mouse renin serum for 48 hr at 4°C. The sections were treated subsequently with the respective antisera following the PAP method. Sections for controls were incubated with non-immunized rabbit serum or PBS instead of anti-renin serum. Counterstain was made with hematoxylin.

Histoplanimetry : For the evaluation of relative locations and frequencies of the renin-containing cells by histoplanimetry, a standard point for measurement of the distances from the point to the positive cells in afferent or efferent glomerular vessels or intraglomerular mesangial regions was tentatively settled at the vascular poles of the glomerulus (text-fig.1).

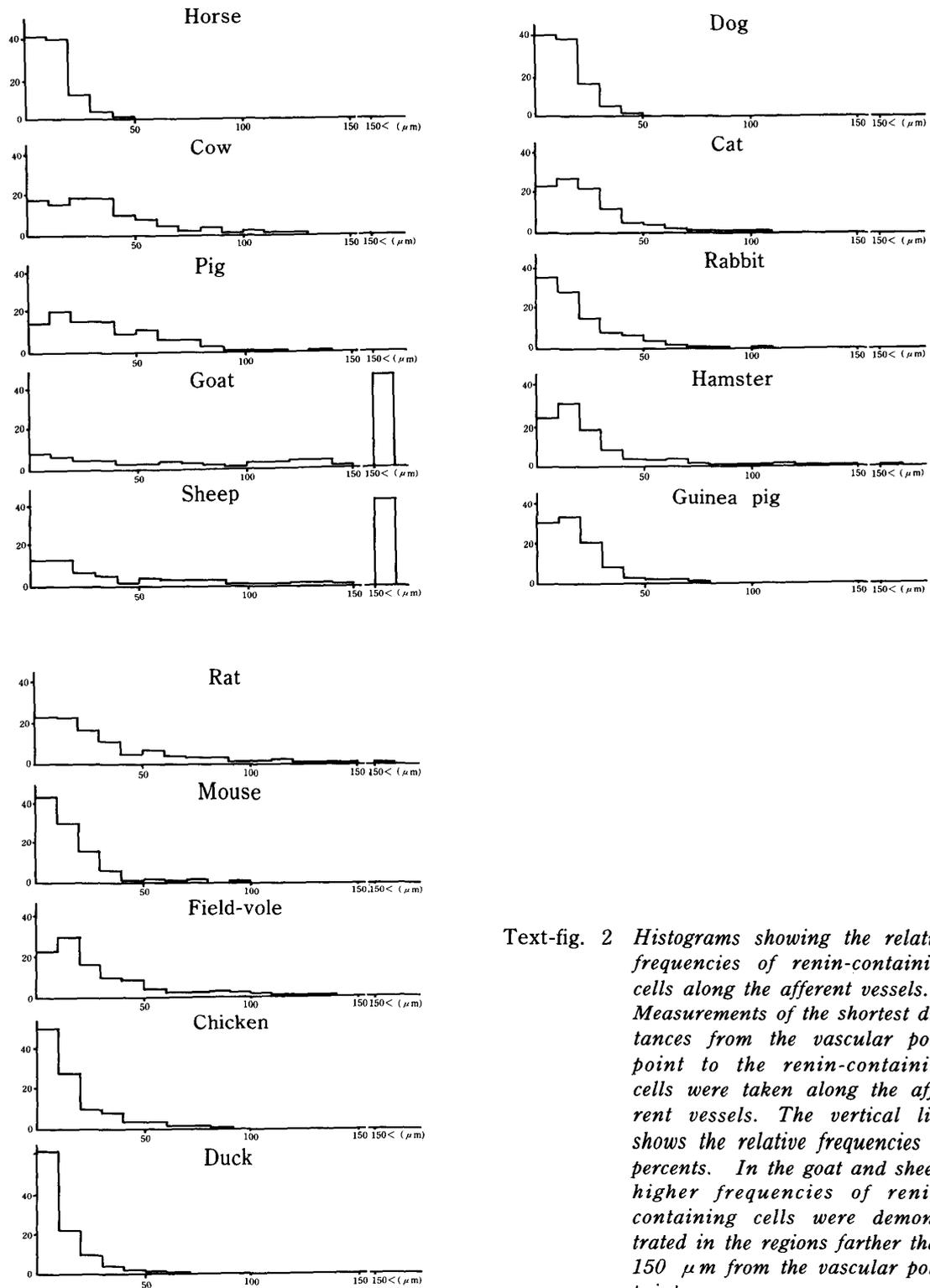
RESULTS

The renin-containing cells were predominantly demonstrated in the regions of tunica media of the afferent glomerular arterioles in all animals examined in this study (figs. 1-12). Histoplanimetrical results showing their relative locations were summarized in table 1. The relative distributions of renin-containing cells localized along the afferent vessels were shown in text-figure 2. In the kidneys of horse, pig, cow, goat, sheep, cat, rabbit, hamster, guinea pig, rat, mouse and field-vole, over 90% of the renin-containing cells were localized in the regions of the tunica media in the afferent glomerular arterioles just adjacent to the vascular pole (figs. 3-6). The rest



TEXT-FIG. 1 Schematic presentation of the method for measurement of the locality of renin-containing cells by histoplanimetry. AA: afferent glomerular arteriole. EA: efferent glomerular arteriole. GM: glomerular mesangium. MD: macula densa.

were found in the tunica media of the efferent glomerular arterioles or in the glomerular mesangial regions, and over 60% of these positive cells located in the afferent arterioles were crowded within narrow encircled regions of $40\ \mu\text{m}$ in radius from the vascular pole-point. However, in the kidneys of goat and sheep, the renin-containing cells, which were highly variable in size and figure, were found scattered in broad regions of the tunica media or in the tunica adventitia of interlobular arteries (figs. 7 & 8, text-fig. 2). Only in the case of dog kidney, it was remarkably noted that more than 15% of the renin-containing cells were localized in the glomerular mesangial regions, while a very few cells were demonstrated in the efferent glomerular arterioles (figs. 9 & 10). In the kidneys of chicken and duck, the renin-containing cells were demonstrated frequently both in the tunica media of the afferent glomerular arterioles and in the glomerular mesangial region (figs. 11 & 12). Histoplanimetrically, the relative values of the positive cells in the mesangial region of chicken and duck were extremely high as compared with those of mammalia, and attained around 30%, respectively. Only a few or no renin-containing cells were localized in the efferent glomerular regions (tab. 1).



Text-fig. 2 *Histograms showing the relative frequencies of renin-containing cells along the afferent vessels. Measurements of the shortest distances from the vascular pole-point to the renin-containing cells were taken along the afferent vessels. The vertical line shows the relative frequencies in percents. In the goat and sheep, higher frequencies of renin-containing cells were demonstrated in the regions farther than 150 μm from the vascular pole-point.*

TABLE 1 *Histoplanimetric evaluation of relative locations among the afferent or efferent vessels or glomerular mesangial regions.*

ANIMALS	AFFERENT	EFFERENT	MESANGIAL(%)
horse	100	0	0
cow	100	0	0
sheep	99.8	0	0.2
goat	99.8	0.2	0
pig	98.3	0.3	1.4
dog	84.0	0.7	15.2
cat	97.6	0.6	1.8
rabbit	98.6	0.4	0.6
guinea pig	100	0	0
hamster	99.8	0	0.2
rat	98.6	0.4	1.0
mouse	94.9	0.4	4.7
field-vole	99.7	0	0.3
chicken	61.0	0.2	37.8
duck	70.5	0	29.5

DISCUSSION

Immunohistochemical study of the renin-containing cells was performed firstly by EDELMAN & HARTROFT,⁴⁾ who demonstrated the JG cells of rabbit and dog kidneys using the fluorescent-antibody technique. More recently, the technique of renin purification has been established by MURAKAMI & INAGAMI using hog kidneys.¹¹⁾ It has been confirmed that the immunoreactivity of the anti-renin serum used in the present study was specific for the renin purified from the mouse submandibular gland.⁶⁾

In the mammalian kidneys of this study, nearly all the renin-containing cells of mouse and rat kidneys were exclusively demonstrated at the vascular poles of the glomerulus as reported by the previous authors,^{15,16,17,19)} and this was also the case in the ungulata kidneys. While in the carnivora, a number of renin-containing cells could be detected both in the efferent glomerular arterioles and in the mesangial regions, and this feature was more remarkable in fowl kidneys. In the kidneys of chickens, numerous JG cells were localized in the mesangial region with Bowie's stain,⁸⁾ and this feature was clearly confirmed in the present immunohistochemical study.

In the kidneys of sheep, so-called peripolar cells encircling the vascular pole within Bowman's space were demonstrated in the glomerular tuft by RYAN et al.¹³⁾ They discussed the ontogenetical relationship between the peripolar cells and JG cells.¹⁰⁾ In this study, however, these cells could not be identified because of their locality and immunoreactivity.

Concerning the morphogenesis of JG cells, which have been regarded as a modification of smooth muscle cells,¹⁴⁾ detailed results in the pig kidneys were reported by KAZIMIERCZAK, and he suggested that JG cells were of the same origin as intra- and extramesangial cells,⁷⁾ Moreover, in the ontogenetical view of the chicken kidney, JG cells have also appeared in the mesangial region with age after hatching,⁸⁾ and in the cytoplasm of mesangial cells, numerous myofilaments and attachment bodies were noted as one of the typical characteristics of smooth muscle cells.⁹⁾ From the present results, therefore, the facts that the renin-containing cells were immunohistochemically demonstrated not only in the tunica media of arterioles but also in the tunica adventitia or the mesangial regions suggest the following possibilities: that the adventitial cells of the arterioles and/or arteries and the mesangial cells may differentiate into renin-containing cells, or that the cells change their locality from the tunica media of the arterioles during their development. This characteristic locality of renin-containing cells may support the hypothesis that the secretion of renin is performed at a site beside the adventitia or via the glomerular capillaries, rather than through the endothelium of arterioles.¹²⁾

Recently the intrarenal renin-angiotensin system has attracted the attention of many investigators,^{3,18)} and the system has been considered to be closely associated with the locality of renin-containing cells. The results of the present study provide a basis for the classification of the possible roles and processes in the intrarenal renin-angiotensin system.

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PLATE I

- Fig. 1 Immunohistochemical demonstration of renin-containing cells in the mouse kidney. Renin-containing cells are clearly notable in the cortical area (arrows). $\times 68$
- Fig. 2 Renin immunoreactivity in a rabbit kidney. Note the remarkable reactivity in the afferent arteriole (arrow). G : glomerulus. $\times 340$
- Fig. 3 Renin-positive vascular pole in a horse kidney. Immunoreactivities are observed in the extraglomerular mesangial region attached to the macula densa (arrow). G : glomerulus $\times 340$
- Fig. 4 Renin-containing cells observed in the tunica media of the afferent arteriole in a pig kidney (arrows). G : glomerulus $\times 170$

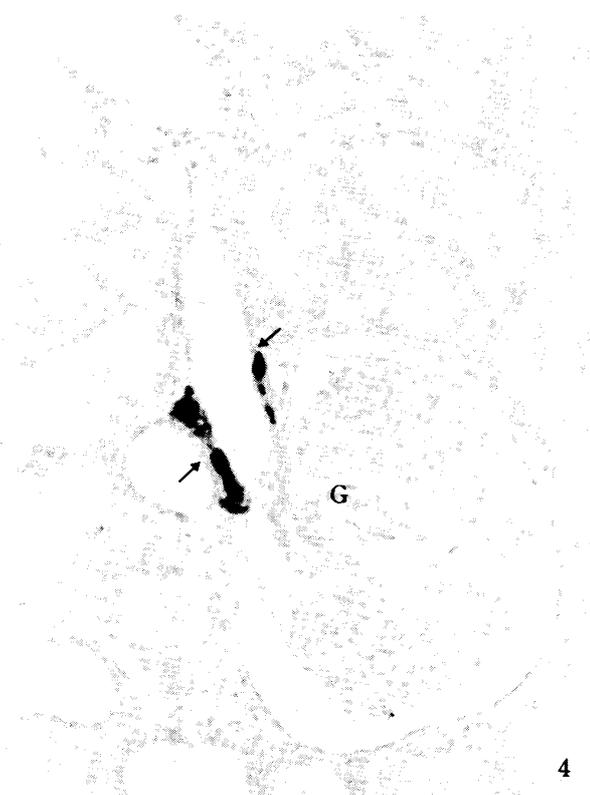
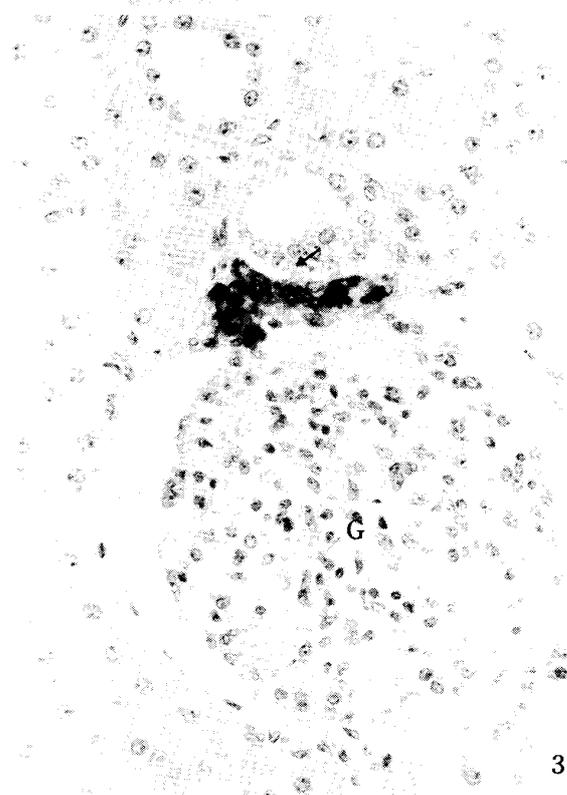
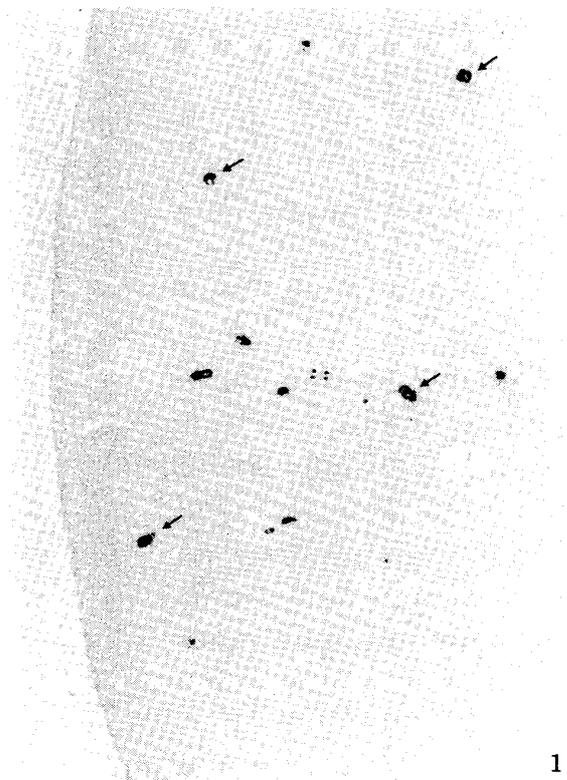


PLATE II

- Fig. 5 Renin-containing cells demonstrated at the vascular pole in a cow kidney (arrow). G : glomerulus $\times 170$
- Fig. 6 Renin positive afferent arteriole in a rat kidney (arrows). G : glomerulus $\times 340$
- Fig. 7 Immunocytochemical demonstration of renin-containing cells in a goat kidney. Positive cells were found sparsely in the tunica media or the adventitia of the afferent arteriole (small arrow) and the interlobular artery (large arrows). G : glomerulus $\times 170$
- Fig. 8 Immunocytochemical demonstration of renin-containing cells in a sheep kidney. Positive cells existed sparsely along the afferent arteriole (small arrow) and the interlobular artery (large arrow). G : glomerulus. $\times 170$

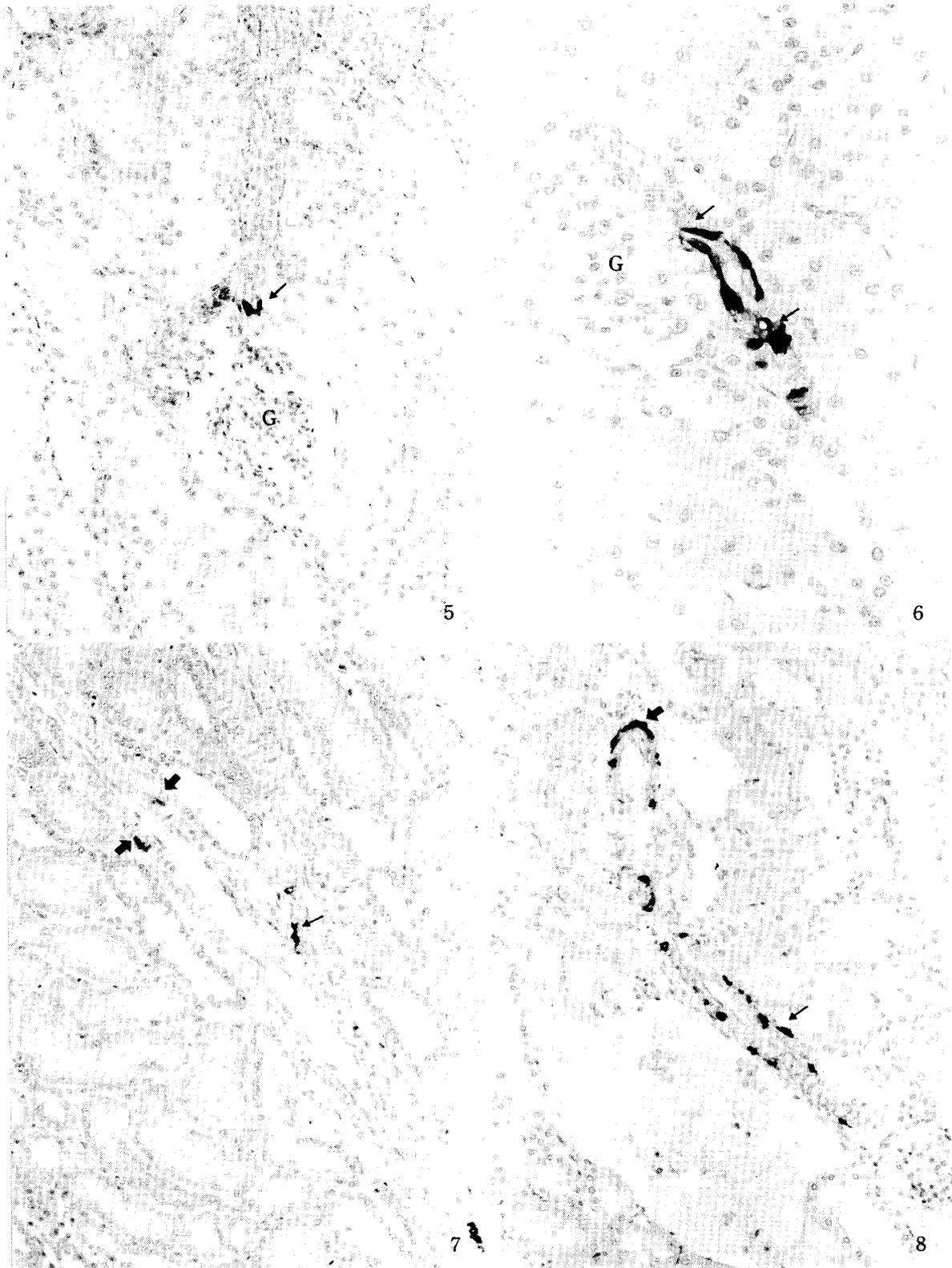


PLATE III

- Fig. 9 Renin immunoreactivities in the efferent arterioles (small arrow) of a dog kidney. The activities in the efferent arteriole were weaker than those in the afferent one (large arrow). G : glomerulus $\times 340$
- Fig. 10 Renin-containing cells in the efferent arteriole (small arrow) of a cat kidney. The immunoreactivities in the efferent arteriole were weaker than those in the afferent one (large arrow). G : glomerulus $\times 340$
- Fig. 11 Renin immunoreactivities in the glomerular mesangial region of a chicken kidney. Renin-positive cells are observed along the glomerular capillaries (arrows). G : glomerulus $\times 340$
- Fig. 12 Renin immunoreactivities observed in the afferent arteriole (large arrow) and in the glomerular mesangial region (small arrow) of a duck kidney. G : glomerulus $\times 680$

