# *No Aggravation of the Course of Experimental Glomerulonephritis in Spontaneously Hypertensive Rats*

HAROLD D. STEIN, MD, R. BERND STERZEL, MD, JOHN D. HUNT, PhD, REINHARD PABST, MD, and MICHAEL KASHGARIAN, MD

*From the Department of Medicine and Pathology, Veterans Administration Medical Center, West Haven, and Yale University School of Medicine, New Haven, Connecticut; and Department fur Anatomie, Medizinische Hochschule, Hannover, Federal Republic of Germany*

**Functional and morphologic glomerular alterations induced by antiglomerular basement membrane (anti-GBM) nephritis were investigated in spontaneously hypertensive rats (SHR) and normotensive Wistar Kyoto controls (WKY) for assessment of the role of systemic hypertension in immunologically mediated renal injury. Over a 6-week period serial measurements of systolic blood pressure (BP), serum creatinine ($S_{Creat}$), creatinine clearance ($C_{Creat}$), and urinary albumin excretion ($U_{Alb}V$) were obtained with inulin clearances ($C_{Inulin}$) at the end of the study. Renal tissue was examined by light microscopy (LM), electron microscopy, immunofluorescence, flash $^3$H-thymidine autoradiography (AR), and staining for nonspecific esterase (NSE). Immunologic humoral response was evaluated by measurement of rat anti-rabbit IgG antibody production. At all time periods studied, SHR and WKY rats with anti-GBM nephritis demonstrated comparable elevations in $S_{Creat}$ and $U_{Alb}V$ as well as dimunition of $C_{Creat}$ and $C_{Inulin}$ as compared with non-nephritic control rats of each strain. In nephritic WKY rats mild hypertension developed, whereas in nephritic and control SHR rats marked elevations in BP developed. Morphologic injury as assessed by percent glomerular crescents and hypercellularity on LM, numbers of monocyte macrophages by NSE staining, immunofluorescence for IgG, C3, fibrinogen and Ia positivity, and numbers of glomerular $^3$H-thymidine-labeled cells by AR was notably comparable in both nephritic strains. Humoral antibody responses were also shown to be similar in all rats studied. These results demonstrate that the 5-week course of experimental anti-GBM nephritis is not exacerbated by systemic hypertension. Glomerular autoregulatory capacity may be important in determining the extent of immune-mediated renal injury. (Am J Pathol 1986, 122:520–530)**

CLINICAL and experimental studies have suggested that systemic arterial hypertension may aggravate the glomerular damage which is seen with glomerular diseases of immunologic origin,[1] or with diabetes mellitus.[2,3] The pathogenetic mechanisms, however, by which systemic hypertension may add to the glomerular injury are at present incompletely understood. For example, it is unclear whether systemic hypertension, *per se*, is a factor contributing to renal vascular and glomerular damage in glomerulonephritis, or whether transmission of blood pressure elevation to the glomerular capillaries is required to accelerate the glomerular disease.

There is evidence that insufficient preglomerular vasoconstriction, in the presence of systemic hypertension, leads to elevation of the glomerular capillary pressure ($P_{GC}$).[3,4] This may be associated with increased renal blood flow (RBF) and hyperperfusion of glomeruli. Such states of intrarenal hypertension, with or without glomerular hyperperfusion, have been postulated to cause progressive glomerular damage,[4] even in the absence of systemic hypertension. It is therefore of interest that glomerular injury induced by immune-mediated glomerulonephritis was found augmented in hypertensive animals with evidence for intrarenal hypertension and glomerular hyperperfusion, for example, in rats wtih DOCA-salt hypertension[5,6] and in the un-

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Address reprint requests to Michael Kashgarian, MD, Department of Pathology, Yale University School of Medicine, 310 Cedar Street, New Haven, CT 06510.

clipped kidney of rats with two-kidney Goldblatt hypertension.[7]

No renal hyperperfusion or intraglomerular hemodynamic abnormalities have been detected in adult rats with genetic spontaneous hypertension (SHR).[5,8-10] Nevertheless, there are early reports by Masuyama et al[11,12] stating that the course of antiglomerular basement membrane (GBM) nephritis was aggravated in SHRs when compared with their normotensive Wistar Kyoto (WKY) controls. These observations would suggest that systemic hypertension, *per se*, may accelerate glomerulonephritic lesions, regardless of the degree of preglomerular vasoconstriction and independent of glomerular hyperperfusion and hypertension. Unfortunately, these reports failed to provide detailed morphologic and functional data on the course of glomerular damage in the studied animals as well as the nonnephritic SHR controls, which would be required for acceptance of this notion. To reinvestigate this important question, we undertook a time-course study of the functional and morphologic glomerular alterations caused by induction of anti-GBM nephritis in SHR and normotensive WKY rats. Appropriate control groups without anti-GBM nephritis were also examined. The results of this comparative study revealed no differences of the experimental glomerular injury in SHRs with severe hypertension, compared with their normotensive WKY counterparts.

## Materials and Methods

### Design of the Study

An accelerated model of anti-GBM nephritis was induced in spontaneously hypertensive rats of the Okamoto-Aoki strain (SHR) and their normotensive WKY controls. These animals, as well as nonnephritic rats of both strains, were followed over a total time period of 6 weeks. Multiple parameters of renal function and morphology were serially assessed, allowing detailed comparative evaluations of the time course of the glomerular changes in the four study groups.

### Animals

Twenty-eight male SHR and 28 male WKY controls (Taconic Farms, Germantown, NY) entered the study. The initial age of both groups averaged 20 weeks. Rats were allowed free access to standard rat food and drinking water.

### Preparation of Antiserum to Rat GBM

As reported before,[13] GBM was obtained from isolated glomeruli of Sprague–Dawley rats by the Krakower and Greenspan technique of differential sieving and sonification.[14] The antiserum was raised in New Zealand white rabbits by standard immunization procedures. An antiserum dose of 0.5 ml/100 g body weight (wt) had been shown in pilot studies to induce progressive glomerular disease in preimmunized Wistar rats.

### Anti-GBM Nephritis

This accelerated model of immunologic glomerulonephritis was induced by injection of the rabbit antiserum to rat GBM, 5 days after preimmunization of the rats with rabbit IgG, as described before.[13] In brief, rats of either strain received, intracutaneously, 1 mg/100 g body wt rabbit IgG (Cappel Laboratories, Inc., Cochranville, Pa) in 0.5 ml/100 g body wt complete Freund's adjuvant. Five days later, half of each group received intravenously the rabbit anti-rat GBM antiserum; the other half received serum from normal control rabbits (0.5 ml/100 g body wt). Prior to use, the antiserum and the normal rabbit serum were inactivated by heating at 56 C for 30 minutes and preabsorbed with packed normal rat erythrocytes.

### Blood Pressure Measurement

Systolic blood pressure of awake rats was determined weekly by tail cuff measurements using a Doppler flow meter (Model 802A, Parks Electronics Lab, Beaverton, Ore).

### Renal Function

Urinary albumin excretion was assessed in 24-hour urine collection samples, obtained by keeping the rats in metabolic cages at various time points of the study. Rat albumin concentration was determined by single radial immunodiffusion using the Mancini technique with a specific purified rabbit antibody to rat IgG (Cappel Laboratories), as previously described.[15] In addition, 24-hour urine collections and serum were examined for the content of creatinine by the Folin-Wu method.[16] The values for serum creatinine and creatinine clearance were used to monitor the time course of gross renal excretory function in awake rats. The glomerular filtration rate (GFR) was determined at the end of the study by measurements of inulin clearance in anesthetized rats. Anesthesia was induced with Inactin (Byk Gulden, Konstanz, West Germany) at a dose of 0.01 g/100 g body wt, as described.[15] Constant body temperature of 37 C was maintained by placing rats on a regulated heating pad (Watlow, St. Louis, Mo). A tracheostomy was performed, and the trachea was intubated with a 25-mm polyethylene catheter (PE 200).

The external jugular vein (PE 10), carotid artery (PE 50), and bladder (PE 50) were subsequently cannulated. Surgical fluid losses were replaced with 0.15 M NaCl (saline), equal to 1 ml plus 1% body wt, followed by a priming dose of 20 $\mu$Ci $^3$H-methoxy inulin (New England Nuclear, Boston, Mass) from a stock solution containing 100 $\mu$Ci/ml saline. These boluses were followed by a maintenance infusion of saline containing $^3$H-inulin, thus administering saline at a rate of 1% body wt/hr and 13 $\mu$Ci of $^3$H-inulin per hour. Rats were allowed to equilibrate for 1 hour. During four subsequent 20-minute study periods, urine was collected under oil. Serum was obtained from arterial blood at the midpoint of each period. The clearances for inulin were calculated, pooled, and used as described before.[15] The arterial blood pressure of the studied rats was monitored throughout via the carotid artery (Gould-Statham, transducer model P236B, Gould recorder 2200, Gould Inc., Cleveland, Ohio).

### Renal Morphology

Renal tissue was obtained when the rats were sacrificed, either at 1 week or at 5 weeks after serum injection. The kidneys were removed from the anesthetized rats as follows: after ligation of the abdominal aorta below the renal arteries and transsection of the inferior vena cava, the kidneys were perfused for 5 minutes with saline via a carotid artery catheter, which led to uniform kidney blanching, and then removed and processed.

### Light Microscopy

Half of one kidney was fixed in Bouin's solution and processed for light-microscopic examination, as described before.[17] Four-micron-thick sections were stained with Masson's trichrome stain. Hypercellularity and mesangial widening or sclerosis were scored on a scale of 0 to 4+.[18] The presence and morphologic features of crescents were evaluated in at least 50 glomeruli and the frequency expressed in percent affected glomeruli. The results on coded sections were obtained blindly by three observers, and the data were pooled.

### Histochemistry

Portions of renal tissue were fixed in buffered formaldehyde acetone, pH 6.6, washed in sucrose-gum acacia buffer for 24 hours, and then snap-frozen. Cryostat sections were stained for nonspecific $\alpha$-naphthylbutyrate esterase, as described before.[13,17] Using this method, one can readily discern dark-red–stained monocyte-macrophages in glomerular tufts and crescents as well as in interstitial spaces. Cells of normal glomeruli do not stain except for rare cells in glomerular tufts.

### Autoradiography

At week 5 of the study, 3–5 rats of each group received a single intravenous injection of $^3$H-thymidine 2 hours before sacrifice. The dose was 1 $\mu$Ci/g body wt (sp. act. 5 Ci/mM, New England Nuclear). Pole tissue of 1 kidney was fixed in Schaffer's solution and processed for autoradiography as described previously.[13] AR 10 stripping film (Kodak, Stuttgart, West Germany) was used and developed after 70–120 days of exposure. The 5-$\mu$-thick sections were stained with hematoxylin and eosin. A minimum of 100 glomeruli on 2 or 3 sections was evaluated for the presence and number of labeled cells (ie, with 5 or more grains over their nucleus). The results were expressed as the mean number of labeled cells per glomerular cross-section which included tuft and crescent, if present.

### Immunofluorescence Microscopy (IF)

Renal tissue was snap-frozen in liquid nitrogen. Parallel cryostat sections were cut at 4 $\mu$ and stained with fluoresceinated antisera to rabbit IgG, rat IgG, IgM, C3 fibrin (Cappel Laboratories), and rat Ia-antigen (Accurate Biochemicals, Hicksville, NY). Appropriate positive and negative tissues served as controls. The intensity of IF staining on coded sections was scored independently by three observers in a blinded evaluation on a scale of 0–4+. The overall IF evaluation for a given antigen was expressed as the observed range; in addition, the arithmetical means of the IF scores from all observers for all examined rats were calculated.

### Electron Microscopy

One-millimeter blocks of cortical tissue were fixed for 4 hours at 4 C in 4% formaldehyde, 5% glutaraldehyde fixative in 0.2 M cacodylate buffer, pH 7.2. After washing in buffer, the tissue was processed for electron microscopy as previously described.[17] Thin sections were cut on an LKB III ultramicrotome and examined with a Zeiss EM 10B electron microscope.

### ELISA for Antibody to Rabbit IgG

Rat anti-rabbit IgG was measured in serum with the use of an enzyme-linked immunoabsorbent assay (ELISA), with the use of polyvinyl chloride (PVC) microtiter plates coated with rabbit IgG. Briefly, 96-well PVC plates (Falcon 3911; VWR Scientific, Boston, Mass) were coated with chromatographically purified rabbit

IgG (Cappel), 200 μl/well of 1 ng/ml phosphate-buffered saline, pH 7.4 (PBS), by incubation for 2 hours at 37 C. After plates were emptied by inversion, nonspecific binding was blocked by incubation for 1 hour at 22 C with human serum albumin (HSA) (Sigma Chemical Company, St. Louis, Mo), 200 μl/well of 1% HSA in PBS. After washing three times by filling and emptying wells with PBS containing 0.05% Tween 20 and once with distilled water, plates were dried and stored covered with parafilm at 4 C until used. Serial dilutions of 100 μl rat serum in PBS-Tween containing 1% HSA (PBS-Tween HSA) were incubated for 1 hour at 37 C in duplicate wells of rabbit IgG-coated plates. After washing three times in PBS-Tween, bound antibody was detected with the use of horseradish peroxidase-conjugated rabbit anti-rat IgG (Cappel), 100 μl/well using a 1/800 dilution in PBS-Tween HSA, with an incubation for 1 hour at 37 C. After final washings three times with PBS-Tween, color was developed with orthophenylenediamine as substrate (100 μl/well of 3.7 mM in 160 mM disodium hydrogen phosphate/30 mM citric acid buffer, pH 6.0, containing $H_2O_2$ 5 ml of 30% $H_2O_2$/25 ml buffer) with incubation for 20 minutes in the dark. After stopping the reaction with 4N $H_2SO_4$ (100 μl/well), absorbence was read at 500 nm by spectrophotometry.

### Statistical Analysis

The unpaired Student *t* test or Wilcoxon's rank sum test was used for comparison of the data for the studied groups. A probability of 0.05 or less was considered to be statistically significant. All data in text, figures, and tables are given as means ± SEM.

## Results

### General

All rats of the SHR and WKY groups tolerated well the preimmunization procedure and the injection of anti-GBM serum or normal rabbit serum. Initial body weights were comparable in the 4 groups (SHR control, 348 ± 7 g; SHR-nephritis, 336 ± 12 g; WKY control, 339 ± 12 g; WKY-nephritis, 350 ± 10 g). All experimental animals gained less weight than the nonnephritic rats. By Week 3, the majority of nephritic rats appeared ill, as manifested by ruffled fur, tearing eyes, and listlessness. During Week 5, 2 rats of the SHR-nephritis group and 3 rats of the WKY-nephritis group died. At the end of the study (end of Week 5), nephritic rats of both strains tolerated the acute clearance experiment rather poorly, compared with rats of the control groups. During anesthesia, their blood pressure was less stable, and 2 SHRs with nephritis and 3 WKY rats with nephritis died during the clearance study.

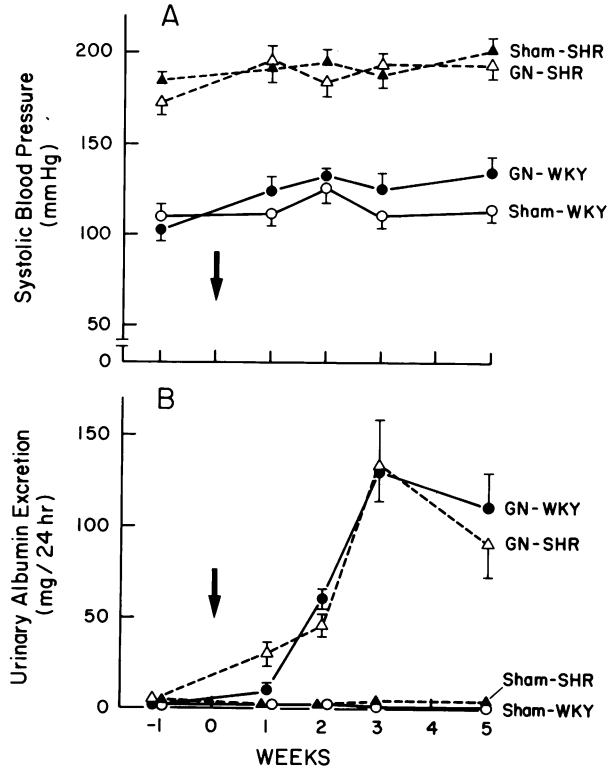


**Figure 1A**—Time course of systolic BP in awake SHR and WKY rats. At the last day of Week −1 (time point 0; *arrow*), rats received either rabbit antiserum to rat GBM (GN groups) or normal rabbit serum (sham groups). **B**—Time course of urinary albumin excretion. Data are given as means ± SEM from 5–9 rats per group. See text for statistical evaluation.

### Functional Studies

#### *Blood Pressure*

Figure 1A illustrates the time course of mean systolic blood pressure (BP) of unanesthetized rats in all groups. Mean baseline BP was significantly elevated in SHR versus WKY rats (180 ± 7 versus 107 ± 6 mmHg; $P < 0.05$). After induction of anti-GBM nephritis, a slight increment in mean BP was noted in WKY rats, and BP became significantly higher than in WKY controls by the fifth week of nephritis (137 ± 11 versus 112 ± 10 mmHg; $P < 0.05$). Nevertheless, it remained far below the BP range of the nephritic SHR group (194 ± 10 mmHg), which was not significantly different from SHR controls (201 ± 11 mmHg).

#### *Albumin Excretion ($U_{Alb}V$)*

Non-nephritic SHR excreted more albumin than nonnephritic WKY at all times (at baseline, Week −1; SHR, 0.574 ± 0.30 mg/24 hours; WKY, 0.146 ± 0.020 mg/24

Table 1—Time Course of Renal Excretory Function Before and After Serum Injection*

| | Week −1 | | Week +1 | | Week +3 | | Week +5 | |
|---|---|---|---|---|---|---|---|---|
| | WKY | SHR | WKY | SHR | WKY | SHR | WKY | SHR |
| $S_{Creat}$ (mg/dl) | | | | | | | | |
| Sham | | | | | | | | |
| $\bar{x}$ | 0.88 | 0.94 | 0.87 | 1.03 | 0.82 | 1.14 | 0.95 | 1.15 |
| ± | 0.05 | 0.10 | 0.04 | 0.03 | 0.06 | 0.03 | 0.06 | 0.05 |
| GN | | | | | | | | |
| $\bar{x}$ | 0.92 | 1.01 | 0.86 | 0.97 | 1.18† | 1.19 | 1.55† | 1.51† |
| ± | 0.04 | 0.05 | 0.03 | 0.04 | 0.09 | 0.07 | 0.11 | 0.04 |
| $C_{Creat}$ (ml/min/100 g) | | | | | | | | |
| Sham | | | | | | | | |
| $\bar{x}$ | 0.19 | 0.14 | 0.19 | 0.18 | 0.24 | 0.19 | 0.25 | 0.17 |
| ± | 0.02 | 0.02 | 0.02 | 0.01 | 0.03 | 0.02 | 0.05 | 0.01 |
| GN | | | | | | | | |
| $\bar{x}$ | 0.18 | 0.16 | 0.18 | 0.18 | 0.14‡ | 0.15‡ | 0.10‡ | 0.12† |
| ± | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 | 0.00 |
| $C_{Inulin}$ (ml/min/100 g) | | | | | | | | |
| Sham | | | | | | | | |
| $\bar{x}$ | | | | | | | 0.40 | 0.43 |
| ± | | | | | | | 0.10 | 0.11 |
| GN | | | | | | | | |
| $\bar{x}$ | | | | | | | 0.15† | 0.21† |
| ± | | | | | | | 0.12 | 0.14 |

* Sham groups received normal rabbit serum; rats with GN (glomerulonephritis) received rabbit anti-rat-GBM serum at the first day of Week 1. Data points for $S_{Creat}$ and $C_{Creat}$ are means ± SEM for 6–12 awake rats; data for $C_{Inulin}$ are means ± SEM for 4 to 5 anesthetized rats.

† $P < 0.05$, comparison of data of GN rats versus sham rats.

‡ $P < 0.01$; comparison of data of GN rats versus sham rats.

hours; $P < 0.01$). As seen in Figure 1B, significant increases of $U_{Alb}V$ over baseline levels became evident in all nephritic rats at the end of the first week of nephritis. While mean $U_{Alb}V$ in the nephritic SHR group was higher than in WKY rats at week 1 ($P < 0.05$), nephritic SHR and WKY rats excreted very similar amounts of albumin for the rest of the 5-week study period, independent of the BP.

### *Renal Excretory Function*

As shown in Table 1, serum creatinine ($S_{Creat}$) and creatinine clearance ($C_{Creat}$) were largely comparable at baseline (Week −1) and at the end of the first week in all 4 study groups, although SHR values for $S_{Creat}$ tended to be higher and for $C_{Creat}$ lower than those of WKY rats. However, after 3 and 5 weeks of nephritis, WKY rats had lower $C_{Creat}$ than untreated WKY. The nephritic SHR group showed a similar, albeit somewhat less pronounced, fall of $C_{Creat}$, compared with age-matched SHR controls. Corresponding with the decline of $C_{Creat}$, values for $S_{Creat}$ increased in both nephritic groups in Weeks 3 and 5.

Studies for the clearance of inulin were performed in anesthetized rats at the end of the study (Table 1). The results were obtained from a smaller number of animals (n = 4 or 5) which survived the induced nephritis and tolerated the anesthesia. The absolute values for $C_{Inulin}$ were uniformly higher than for $C_{Creat}$. However, both parameters of renal function revealed similar proportional declines in nephritic animals, compared with the age-matched control groups.

### *Analysis of Rat Anti-Rabbit IgG Antibody Formation*

Figure 2 shows the results of ELISA tests for the two strains of rats that had received rabbit IgG followed by serum of normal rabbits (Figure 2A) or of rabbits immunized with rat GBM (Figure 2B). Almost all animals followed the same general trend with antibody levels reaching maximum values after 3 weeks; there was then a decline in all groups, except for nephritic SHR animals. With the exception of the nephritic WKY group after the first week, the mean values of SHR and WKY groups were quite similar at each time point and showed no significant differences. In both strains, the normal rabbit serum appeared to elicit a higher antibody response than the corresponding anti-GBM serum. These observations were apparent with serum diluted 1/10,000 as well as 1/50,000 (data not shown).

## Morphologic Studies

### *Light Microscopy*

Semi-quantitative evaluation of sections of paraffin-embedded kidney tissue obtained after 1 and 5 weeks and examined with Masson's stain revealed comparable degrees of glomerular hypercellularity, crescent for-

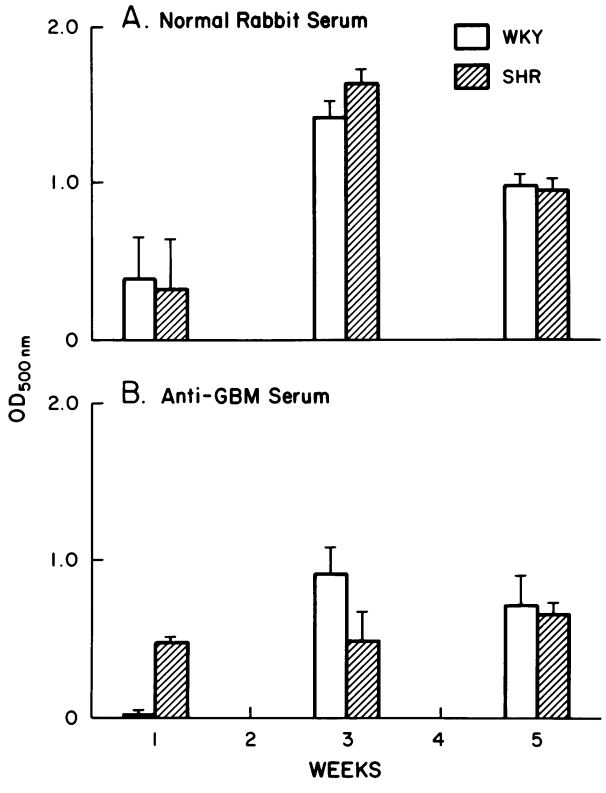


**Figure 2**—Time course of results from ELISA tests for antibody to rabbit IgG in serum of SHR (□) and WKY rats (□), expressed as absorbance at 500 nm. **A**—Sham rats given normal rabbit serum; **B**—Nephritic rats given rabbit antiserum to rat GBM. Data are means ± SEM from 4–6 rats per group. Differences between SHR and WKY groups were not statistically significant, except after 1 week in rats given antiserum to GBM ($P < 0.01$).

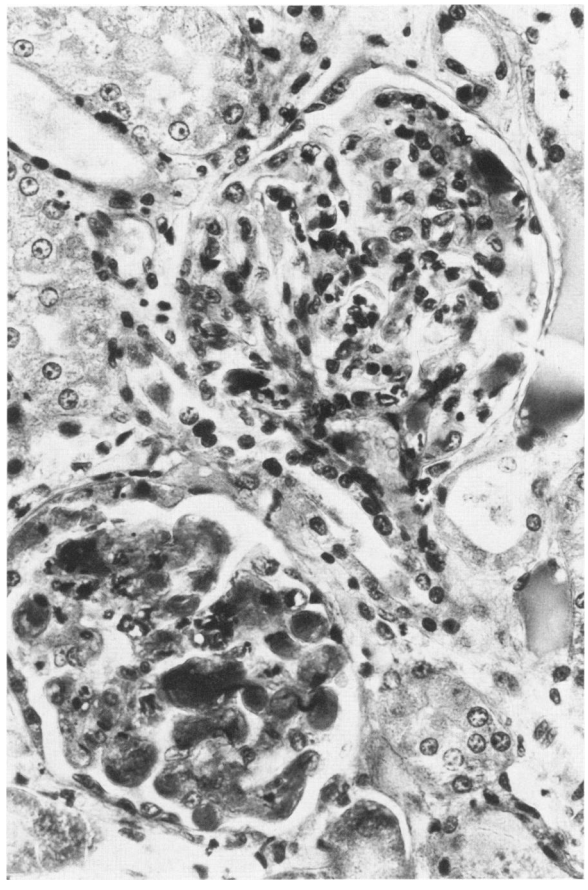

**Figure 3**—Photomicrograph of glomeruli from SHR rat 1 week after injection with rabbit antiserum to rat GBM. There is diffuse hypercellularity, focal necrosis, and crescent formation. There is interstitial edema associated with leukocytic infiltration. No differences were seen between SHR hypertensive rats and WKY normotensive controls. (Masson, ×250)

mation, and mesangial matrix increase in nephritic SHR versus nephritic WKY rats. After 1 week, the predominant alterations consisted of pronounced hypercellularity of glomerular tufts and presence of cellular epithelial crescents. In both SHR and WKY rats with nephritis, the tufts of all glomeruli were affected quite uniformly. The percentage of crescents was 16% ± 9% in SHR (n = 4) (Figure 3) and 21 ± 10 in WKY rats (n = 5). At this time, no sclerotic changes were noted to accompany the hypercellularity found in tufts and crescents. The interstitium showed patchy infiltrates of mononuclear cells without fibrosis. Results of a more detailed histologic evaluation of tissue obtained at the end of the fifth week are summarized in Table 2. Again, the glomerular changes of nephritic rats of the SHR and WKY strains appeared rather similar. At this time, the hypercellularity of tufts and crescents was associated with pronounced increases of extracellular material and sclerosis of the mesangium as well as of the crescents which now appeared in greater frequency than after 1 week (Figures 4 and 5). Tubulointerstitial disease was noted in all nephritic rats and included tubular dilation, interstitial hypercellularity, and fibrosis. Changes of renal arterioles were only noted in SHR. They were limited to mild intimal proliferation, without conspicuous changes of the internal elastic lamellae or medial smooth muscle cells. Vascular abnormalities were seen more frequently in the inner cortical zones. They were not noticeably increased in nephritic SHR, compared with sham SHR controls.

### *Histochemistry*

Nonspecific α-naphthylbutyrate esterase (NSE) staining for monocyte-macrophages revealed frequent NSE-positive cells in glomeruli of all nephritic rats examined after 1 and 5 weeks. The ranges of NSE-positive cells counted per glomerular cross-section were similar in nephritic rats of either strain (Table 2). In all cases, the majority of NSE-positive cells were noted in epithelial crescents (Figure 6). There were more NSE-positive cells in the renal interstitium of nephritic rats, particularly after 5 weeks, than in untreated controls of the WKY and SHR groups.

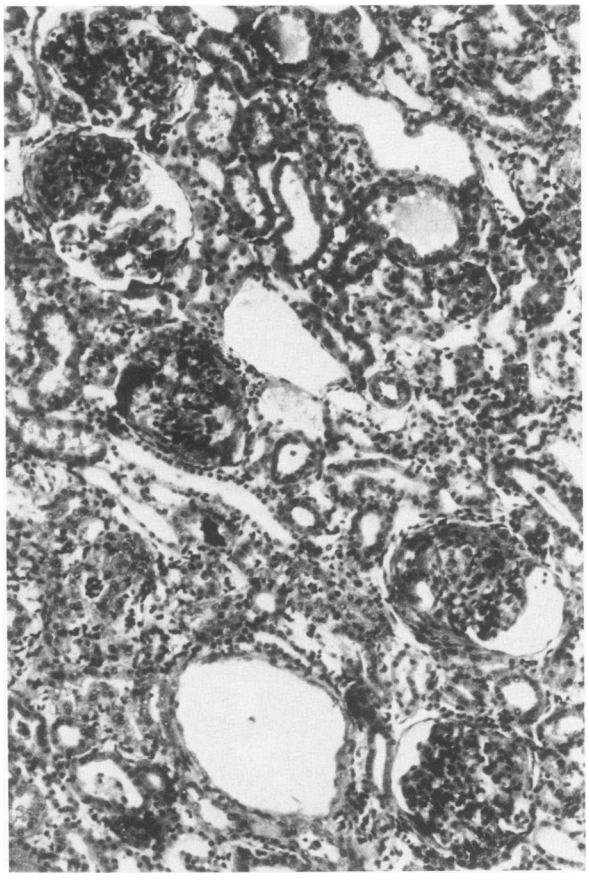

**Figure 4**—Low-power photomicrograph of an SHR rat, 5 weeks after injection with rabbit antiserum to rat GBM. All of the glomeruli are hypercellular, and the majority have crescents. The tubules are dilated, and proteinaceous casts are present. SHR and WKY rats both had a similar degree of involvement. (Masson, ×150)

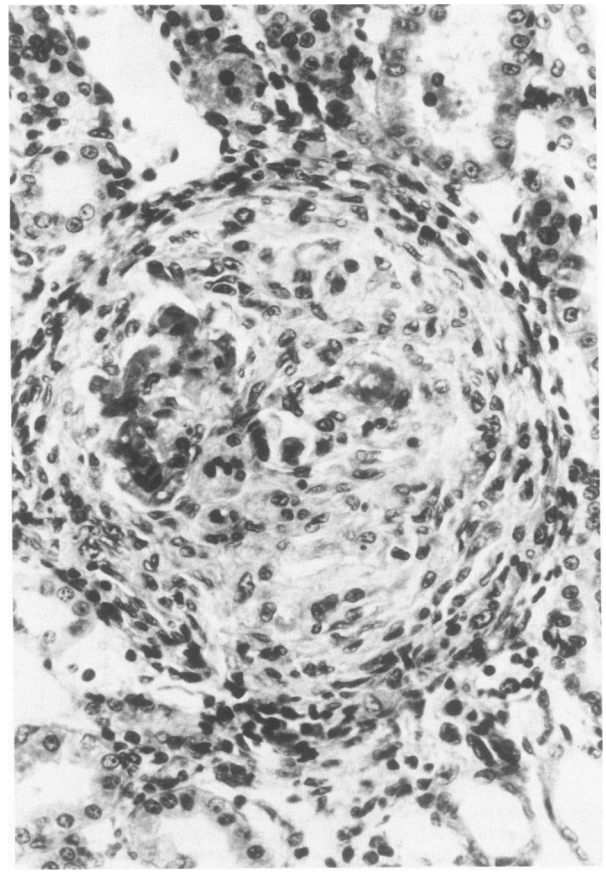

**Figure 5**—Photomicrograph of a glomerulus of a WKY rat 5 weeks after injection with rabbit antiserum. The architecture of the glomerulus is distorted by persistent hypercellularity, focal sclerosis, and the presence of a crescent undergoing fibrosis. There is periglomerular interstitial inflammation and fibrosis. The changes at 4 weeks were identical in SHR and WKY rats. (Masson, ×400)

### *Autoradiography for $^3$H-Thymidine*

Two hours after flash-labeling, nonnephritic sham rats of the SHR and WKY strains, sacrificed at week 1, showed comparable means of labeled cells per 100 glomerular cross sections (26.5 ± 7.0 versus 20.3 ± 4.0). At this time, the results for nephritic SHR and WKY rats were 63.8 ± 11.9 and 71.3 ± 16.1, respectively ($P < 0.05$). At the end of Week 5, nonnephritic rats of both strains had the identical mean of 10.8 labeled cells per 100 glomeruli (Table 2). Glomeruli of age-matched nephritic SHR and WKY rats had approximately 20 times as many labeled cells. They were located in tufts and crescents. The insufficient resolution in the 5-$\mu$-thick sections precluded more precise identification of the labeled cell types. However, blinded evaluation of tissue from SHR and WKY rats did not reveal significant differences of the autoradiographic findings within glomeruli of the two strains.

### *Immunofluorescence*

At 1 and 5 weeks of nephritis, IF staining with goat anti-rabbit IgG revealed linear GBM staining (scale 0 to 4+) in all nephritic animals of either strain, with controls being negative. The IF scores for rabbit IgG ranged between 3+ and 4+ in both SHR and WKY (mean scores at 1 week: SHR, 3.8; WKY, 3.7; at 5 weeks: SHR, 3.5; WKY, 3.3). After 5 weeks of nephritis, the results for rabbit ant-rat IgG were similar (mean scores: SHR, 3.1; WKY, 3.2). In addition, there was trace to 1+ granular mesangial staining in nephritic rats and in age-matched controls. At 1 week, nephritic rats showed 1–2+ linear IF for rat IgG (mean scores: SHR, 1.4; WKY, 1.2). Linear glomerular tuft staining for C3 (scored 1–2+) was found in all nephritic rats, again showing no difference between nephritic SHR and WKY rats; the glomerular crescents were negative for C3. Fibrinogen IF stain was strongly positive (2–3+) in all

Table 2—Histologic Evaluation of Glomeruli*

| | Sham | | GN | |
|---|---|---|---|---|
| | WKY | SHR | WKY | SHR |
| Hypercellularity of glomerular tuft† | 0 | 0 | 3+ | 3+ |
| Increase of mesangial matrix/ glomerular sclerosis† | 0 | 0 | 3+ | 3+ |
| Glomerular crescents (% of glomeruli) | 0 | 0 | 78 ± 28 | 69 ± 12 |
| Esterase-positive cells/glomerulus‡ | 0–3 | 0–2 | 11–28 | 5–26 |
| $^3$H-TdR labeled cells/100 glomeruli§ | 10.8 ± 1.3 | 10.8 ± 2.1 | 188.6 ± 10.0 | 191.5 ± 13.3 |

* Kidneys were obtained in the 5th week after serum injection (Sham, normal rabbit serum; GN, rabbit antiserum to rat GBM); 5–7 rats per group.
† Scale of 0–4.
‡ Range.
§ Mean ± SEM.

crescents at Weeks 1 and 5 and essentially negative in glomerular tufts. No differences between the two nephritic groups could be demonstrated. IF staining for Ia antigen revealed greatly increased numbers of Ia-positive cells in the renal interstitium of nephritic rats of the SHR and WKY strains, more so after Week 5 than Week 1. These animals had also increased numbers of Ia-positive cells in glomeruli (Figure 7), predominantly located in crescents and relatively scarcely in glomerular tufts. No differences of renal Ia staining were noted between SHR and WKY animals.

### *Electron Microscopy*

At 1 week, the glomerular architecture was distorted by a generalized increase in cellularity. Numerous mononuclear cells were present in the capillary lumens.

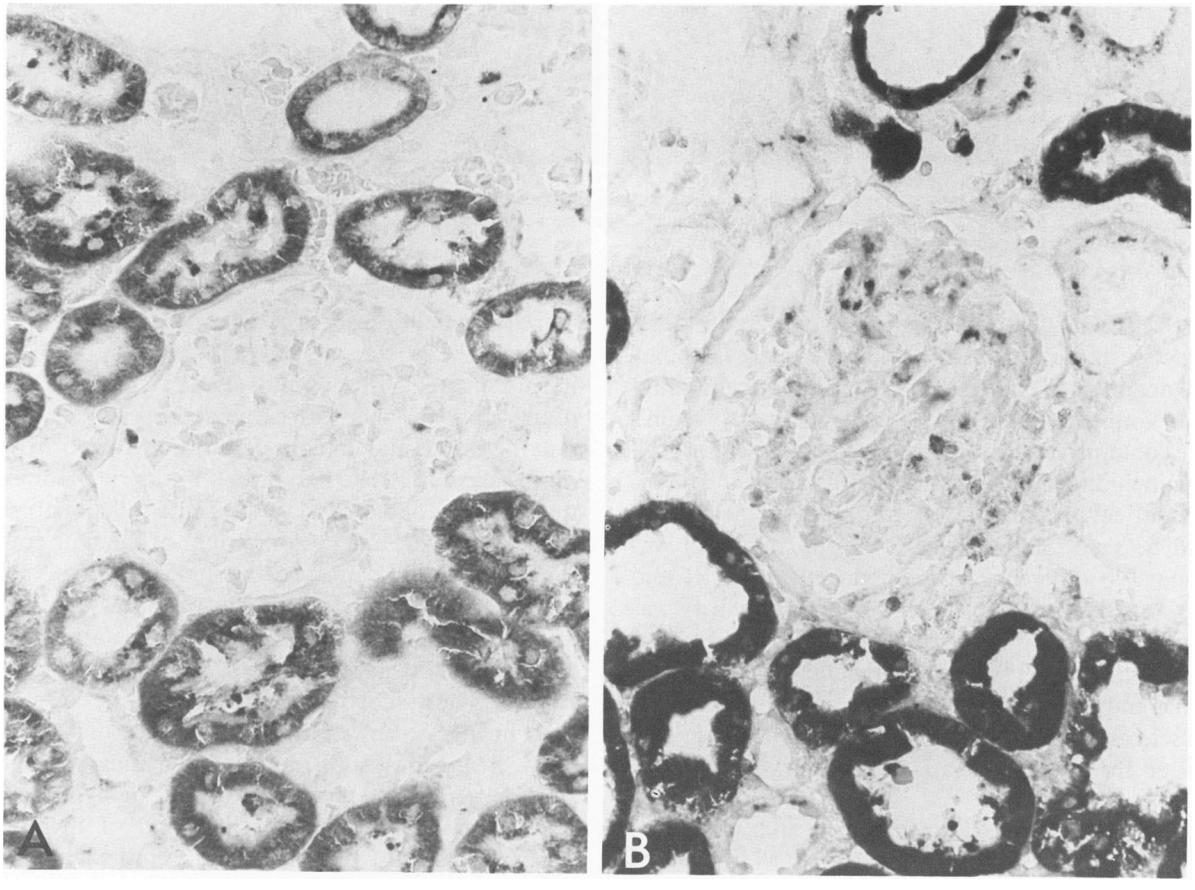


**Figure 6A**—Glomerulus from an untreated SHR rat stained for nonspecific α-naphthylbutyrate esterase. Only rare esterase positive cells are present in the glomerulus. (×250) **B**—Glomerulus from an SHR rat with anti-GBM nephritis stained for nonspecific esterase. Numerous esterase-positive cells are present in the glomeruli. (×250)

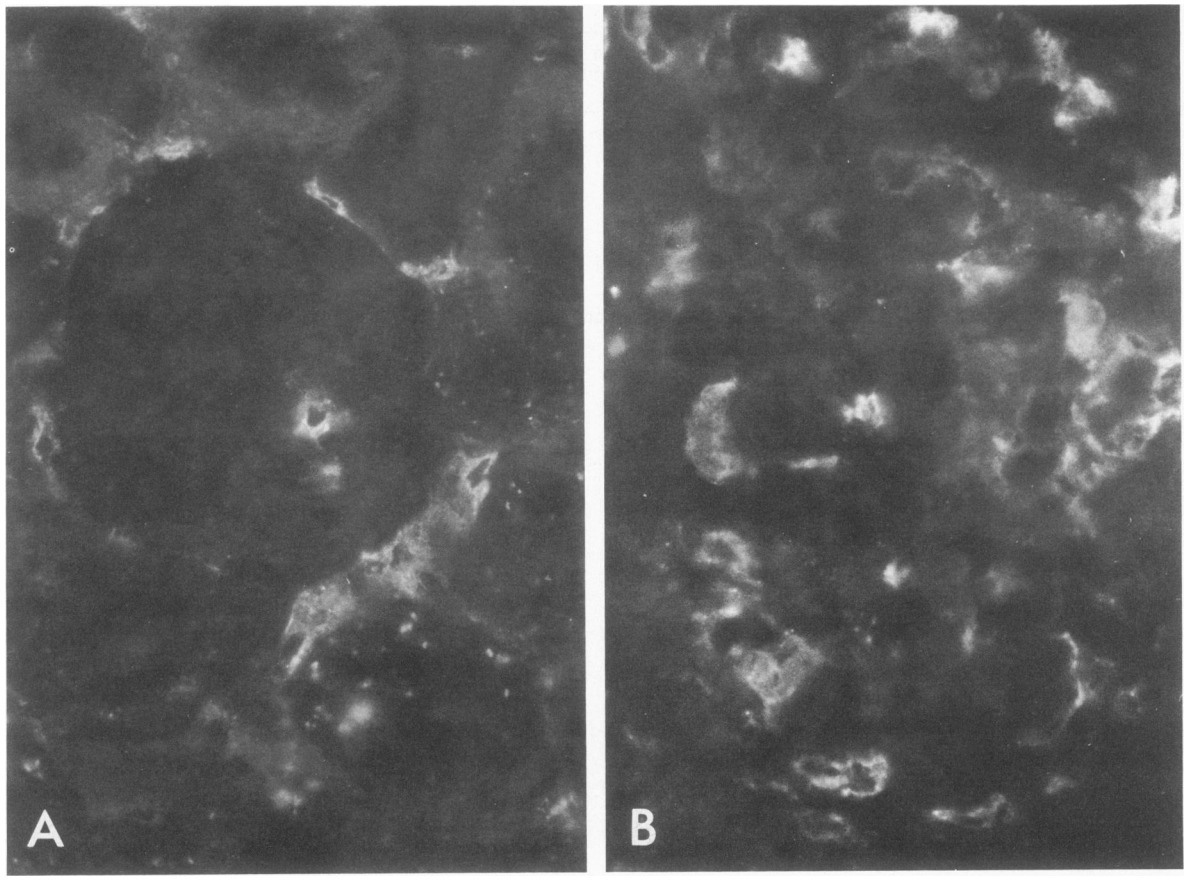


**Figure 7A**—Kidney of an untreated WKY rat stained with fluorescent antiserum to Ia antigen. Rare Ia-positive cells are present in the glomerulus. Interstitial staining for Ia is present. (×250) **B**—Kidney of a nephritic WKY rat stained for the presence of Ia antigen. There is an increased number of Ia-positive cells in the glomerulus and in the interstitium. (×400)

Some cells with features of monocytes were in direct contact with the basement membrane in areas apparently denuded of endothelial cells. Similar cells could also be found in widened mesangial regions. Bowman's space contained crescents formed from a mixture of cell types, including monocytes and epithelial cells. The endothelial cells were swollen, and occasional mitoses could be seen. Subendothelial fibrillar material was present. No difference was noted between SHR and WKY rats. At 5 weeks, the increase in cellularity was less prominent, and glomerular capillaries were generally patent. There was a marked increase in mesangial matrix, and many tufts demonstrated capillary collapse with sclerosis. Again, no significant difference was noted between the changes seen in SHR and WKY strains.

## Discussion

The main finding of this study is that systemic hypertension, *per se*, does not aggravate the course of glomerular injury induced by immune-mediated glomerulonephritis. The functional and morphologic glomerular abnormalities were not different in spontaneously hypertensive Wistar rats (SHR) when compared with normotensive Wistar Kyoto (WKY) controls. These results are at variance with earlier reports which claimed that the glomerular damage of anti-GBM-nephritis was more severe in SHR than in WKY rats.[11,12] However, the communications of Masuyama and collaborators[11,12] failed to provide a detailed account of the time course of the glomerular damage in the studied rats. This problem was compounded by the lack of follow-up data on nonnephritic SHR, necessary for appreciation of inherent glomerular abnormalities that may occur in these rats.[19,20] The model of immunologic glomerular injury used in the present study was an accelerated form of anti-GBM nephritis. By morphology, similarly progressive glomerular damage occurred in the hypertensive and normotensive groups, consisting of increasing hypercellularity of glomerular tufts, epithelial crescent formation, and widening of mesangial matrix. In rats of both strains, the cell increase was due, in part, to hyperplasia of intrinsic glomerular cells, as documented by the autoradiographic studies after pulse labeling and

by electron microscopy. In addition, all nephritic rats showed similar degrees of glomerular infiltration of esterase-positive and Ia-surface-antigen-positive monocyte-macrophages. This indicates that, in both strains, there is a comparable involvement of these scavenger and mediator cells.

Analyzing the 5-week course of functional parameters, our findings do not show any evidence for aggravated glomerular injury in SHR in spite of very marked blood pressure elevation in these animals. In fact, several parameters of the glomerular disease, such as clearances of creatinine and inulin and urinary albumin excretion indicated transiently more pronounced renal injury in nephritic WKY rats than in SHRs, but these differences did not reach statistical significance.

It is unlikely that a difference in glomerular damage between SHR and WKY animals was missed because of a weaker immune response in the SHR strain, possibly compensating for any hypertension-dependent glomerular changes. As shown by the ELISA data on serum samples, the hypertensive rats mounted as strong a response in antibody formation to injected rabbit IgG as the WKY controls. Also, the immunofluorescence findings in glomeruli of tissue sections from both rat strains revealed nearly identical semiquantitative scores for deposition of heterologous IgG and for autologous IgG and C3. While no unequivocal proof, these IF results argue against a discrepancy in binding of antibody to the GBM of SHR versus WKY. These observations are in accordance with findings of Okuda et al,[21] who reported that the induction of active autologous immune-complex-mediated glomerulonephritis was comparable in SHR rats to that in WKY controls. It is of interest that these workers did not observe substantial worsening of the glomerulonephritic lesions in SHR rats, compared with WKY rats, although in hypertensive rats, proteinuria was higher after 3 months and renal vascular abnormalities were more pronounced after 7 months of nephritis. The excretory renal function was comparable in nephritic rats with and without hypertension.[21]

The present results exclude a major abnormality of the cellular immune system involved in the regulation of antibody production. However, we did not assess direct parameters of cellular immunity, some of which have been observed to be subnormal in certain SHR strains.[22] At present, there is little evidence that T-cell-mediated effector mechanisms play a critical role in the pathogenesis of progressive glomerulonephritis.[23,24] It would, therefore appear unlikely that a defect of cell-mediated immunity in the SHR group may have completely offset and obscured any hypertension-mediated contribution to the glomerular damage. This assumption is in agreement with our finding of very similar glomerular infiltrations of monocyte-macrophages in nephritic rats of both strains. If the response of immunospecific T cells had been impaired, one might expect to find diminished attraction of monocytes to the glomeruli.

In contrast to the present results obtained in rats with genetic hypertension, recent detailed reports have demonstrated that the course of glomerulonephritis-induced glomerular damage is more severe in other types of experimental hypertension. For example, Neugarten et al have reported worsened pathologic findings of anti-GBM nephritis in the unclipped kidney of rats with the 2-kidney model of Goldblatt hypertension.[7] This experimental arrangement renders the unclipped kidney in a state of hyperperfusion and hyperfiltration.[5] Interpretation of the obtained results of this study, however, is complicated by the possibility that hyperperfusion of the unclipped kidney may have led to increased glomerular delivery and deposition of rat antibody to the foreign anti-GBM IgG, when compared with normal control kidneys, and that this difference may have contributed to the augmented injury in the kidney exposed to systemic hypertension. Iversen and Ofstad observed that the course of heterologous immune complex glomerulonephritis (passive Heymann nephritis) is aggravated in rats with unilateral nephrectomy and DOCA-salt hypertension.[6] This model of hypertension is also characterized by renal hyperperfusion.[5] As in the study of Neugarten et al,[7] it is difficult to say whether the augmented glomerulonephritic lesions in the hypertensive rats were due to increased glomerular deposition of immunoglobulins or directly due to "physical factors" of glomerular hyperperfusion and hypertension, or whether both mechanisms were contributory.

In contrast to the situation in Goldblatt hypertension and in DOCA-salt hypertension, adult SHR have been demonstrated to have high preglomerular resistance and normal renal autoregulation, affording maintenance of RBF, $P_{GC}$, and GFR in the normal range.[5,8-10] Additionally, SHR show no or mild increases of urinary excretion of protein and albumin during the first 7-8 months of their life,[19,20] a finding confirmed by the present study. By contrast, rats with Goldblatt hypertension[7] or with DOCA-salt hypertension[6] develop much greater degrees of proteinuria. The present observation of an unaltered course of anti-GBM nephritis in hypertensive rats of the SHR strain is consistent with the following interpretation: since adult SHR and WKY controls do not differ greatly in respect to intraglomerular microcirculatory forces, no additional "physical factors" are active in glomeruli of SHR that would affect the nephritic lesions in the hypertensive animals; moreover, RBF has been shown to be com-

parable in SHRs and WKY rats, so that glomerular delivery and deposition of pathogenetically relevant immunoglobulins should be similar.

The present study does not address the question whether long-term renal vascular changes in SHRs may cause glomerular changes in addition to those due to glomerulonephritis. They consist primarily of sclerotic alterations of renal arterioles and, secondarily, of glomeruli.[19] Moreover, it has been reported that such lesions may develop in SHRs as late as after 1 year, even when systemic blood pressure has been maintained in the normal range by administration of antihypertensive agents.[20] Such confounding aspects of the renal pathologic finding in SHRs were avoided in this investigation by limiting the study period to 5 weeks of glomerulonephritis, at an age when renal vascular abnormalities of the rats are absent or slight.

In summary, our observations show that the 5-week course of experimental glomerulonephritis in Wistar rats of the Kyoto strain is not worsened by the presence of spontaneous systemic hypertension. On the basis of these results and by inference from the work of other investigators,[1,6,7] the available information favors the assumption that systemic hypertension augments the injury of glomerulonephritis only when it is associated with glomerular hyperfusion and/or glomerular hypertension. Glomerular autoregulatory capacity may be an important determinant of the degree of potential morphologic alteration in states of experimental glomerulonephritis. Studies utilizing agents that modify preglomerular and postglomerular arteriolar resistances should be further undertaken in these models.

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