# Renal Function in Dogs with Hypertension Induced by Immunologic Nephritis[1] (37577)

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Although in acute glomerulonephritis elevation of systemic arterial pressure is common, the mechanism of development of this type of hypertension remains obscure. While there are specific differences between various forms of human glomerulonephritis and Masugi nephritis in the dog, there are also similarities which warrant using this model for the study of the mechanism of the related hypertension. Previous studies in dogs (1–5) have used dosages of anti-canine kidney serum which have resulted in acute renal failure of relatively short duration, accompanied by marked decrements in glomerular filtration rate and renal plasma flow and the rapid development of azotemia and complete renal failure.

In the present investigation, a schedule of dosages of rabbit anticanine kidney antibody was employed which we anticipated would result in chronic hypertension without acute renal failure. This was attempted in three trained female dogs in which renal function studies were carried out for periods of time varying from 7.5 to 9 mo. A fourth male dog was prepared with catheters and flowmeter implantation, in order to supply additional hemodynamic data.

*Materials and Methods.* Following a control period of about 2 wk, three female mongrel dogs received injections of canine kidney glomerular membrane antibody (IgG). In two of these (M and H) which developed hypertension, the sequence and dosage of IgG injection are detailed in the respective figure legends (Figs. 2 and 3). The third female (S), although treated comparably and followed for a similar period of time, never developed hypertension, although albuminuria and hematuria were noted during the course of treatment.

During the control period, a minimum of three measurements of physiological parameters were obtained. For the first 2 mo following the initial injection procedure in dogs H, S, and M, biweekly observations were made, then once or twice a week during the third and fourth months. During the fifth and sixth months, weekly observations only were made. One dog (M) was continued through 9 mo with measurements at 2-wk intervals. The male dog (B) was followed until Day 26 after starting the IgG treatment, when he died suddenly (for dosage sequence, see legend to Fig. 4). Autopsy revealed a massive pleural effusion, a severe membranous glomerulonephritis with extensive tubular necrosis (Fig. 1C), pulmonary edema, and dilatation of the heart, consistent with cardiac failure.

*Hemodynamic and renal function measurements.* Two of the animals (H and S) had a carotid loop procedure as previously described (6) prior to the injection of antiserum, in order to simplify arterial blood pressure measurements and blood sampling. In dog M, femoral arterial pressure readings were made. Pressure was measured by direct needle puncture. In dog B, pressures were recorded by means of indwelling microtransducers in the left ventricle and aorta. A sine wave electromagnetic flow probe was installed around the ascending aorta (7). Both mean and pulsatile pressures were recorded on a Beckman (Offner) type S dynograph recorder; heart rates were obtained from the pres-

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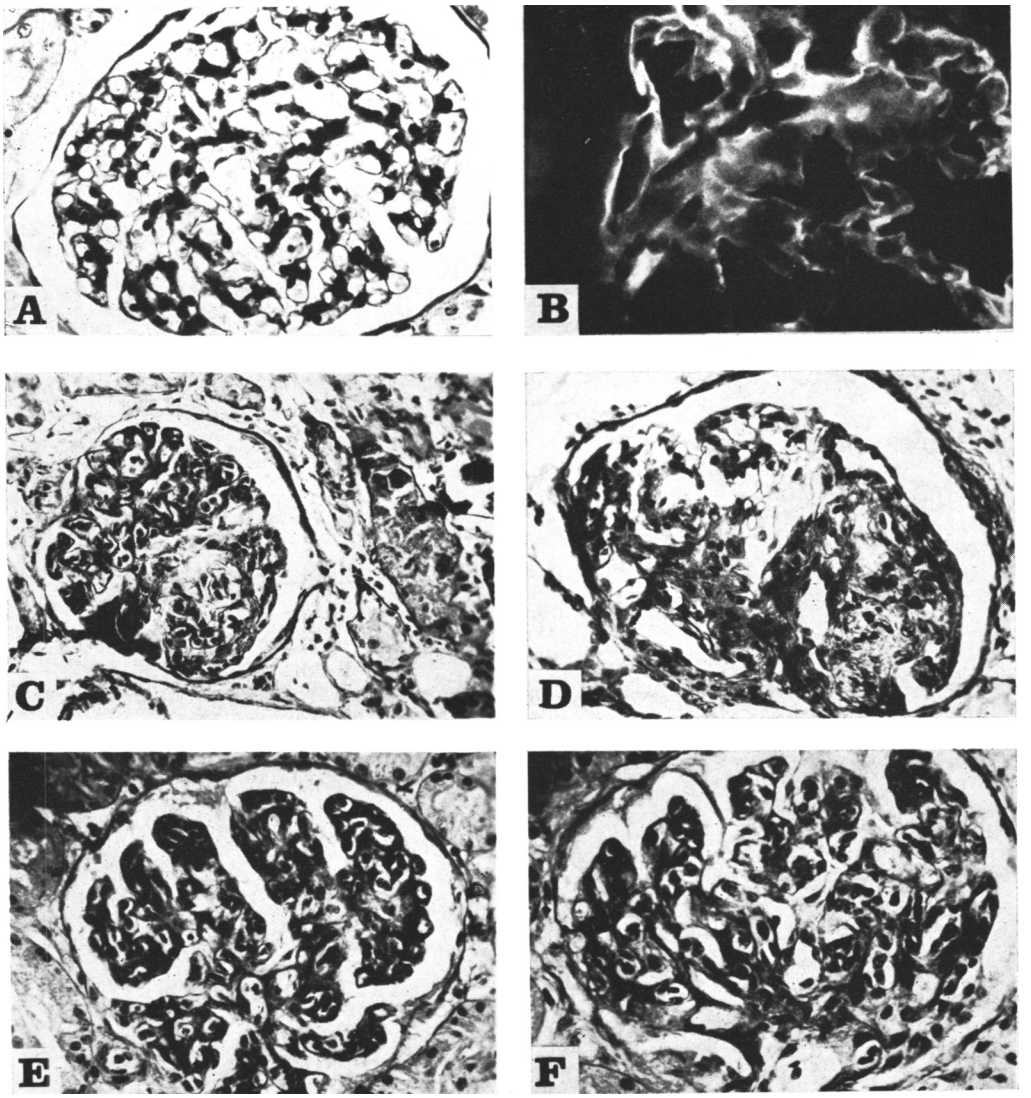


FIG. 1A. Glomerulus of normal dog. No pathologic changes. PAS stain. Mag. 500 d. (B) Glomerulus of Dog H, demonstrating a linear distribution of immunofluorescence of the rabbit anti-dog kidney antibody used to induce the glomerular lesions. This pattern was present in all animals. Mag. 700 d. (C) Glomerulus of Dog B that died in renal and cardiac failure, 26 days after injections were begun. Note the hypercellularity and narrowing of the capillary lumina, irregular thickening of the basement membrane, fibrin deposition in the glomerulus, and focal necrosis and acute inflammation of the surrounding tubules. PAS stain. Mag. 230 d. (D) Glomerulus from Dog B showing scarring of the glomerular tuft, thickening of basement membranes and Bowman's capsule and focal mesangial cell proliferation. PAS stain. Mag. 350 d. (E) and (F) These are the characteristic healed lesions of Dog M & S respectively, showing hypercellularity of the glomerulus, irregular thickening of the basement membrane, hypersegmentation of lobules and deposits of protein in Bowman's space. Mag. 350 d. (E and F).

sure tracings.

Conventional bladder urinary collections were made for measurement of renal clearances. Venous blood samples were drawn at the midpoint of the urine collection periods for routine analysis of endogenous creatinine,

urea, osmolality, sodium and potassium clearance. When $C_{PAH}$ was measured, a subcutaneous dose was given with an iv priming dose; this gave reasonably constant plasma levels of PAH which ranged between 1.24 to 2.5 mg% in different animals. PAH was measured by the method of Smith *et al.* (8) on diluted urine and 3% TCA plasma filtrates.

Chemical procedures for the other renal clearance analyses included endogenous creatinine in plasma from 5% sodium tungstate filtrates and urine (9), urea nitrogen in blood and urine with the Hyland UN-test (Div. Travenol Laboratories, Inc., Los Angeles), electrolytes with a Beckman atomic absorption spectrophotometer, and plasma and urine osmolality with an Advanced Instrument, Inc., freezing point depression apparatus.

*Body fluid compartment measurements.* Extracellular space volumes were approximated by inulin space measurements made at 77 and 123 days after the initial antiserum injection series, done in the unanesthetized animal by a modification of the single injection method of Cardoza and Edelman (10) and terminally at 238 and 284 days by a constant infusion technique (11). Inulin was measured by the anthrone method of Davidson and Sackner (12). Blood volumes were conventionally determined at the time of inulin space determination by injection of Cardiogreen (Hynson, Westcott and Dunning, Inc.), 0.5 mg/kg, iv, and withdrawing venous samples at 5, 10, 15 and 20 min. Plasma concentrations were corrected back to zero time for calculating plasma volumes by assuming an exponential loss rate based on the decrease in concentration found for each 5 min interval. Correction for hematocrit ratios gave total blood volumes.

*Miscellaneous measurements.* Blood plasma proteins were measured by methods described by Bullock (13). Plasma and urine renin and aldosterone were measured by radioimmunologic techniques of Haber *et al.* (14), and Mays *et al.* (15), respectively. Circulating catecholamines were assayed from venous blood samples using the method of Weil-Malherbe (16). Plasma cholesterol was measured with a Stanbio SR cholesterol test kit (Stanbio Lab., Ind., San Antonio, TX).

*Preparation of antigens for production of anti-kidney antibodies.* The homogenate of dog kidney cortex was sonicated and frozen in aliquots until ready for use. For initial injections of antigen, the homogenate was emulsified with equal volumes of complete Freund's adjuvant. For subsequent injections, the sonicated kidney homogenate alone was injected. Rabbits were injected in the hind foot pads and two other subcutaneous sites with the kidney antigen in Freund's adjuvant once, and then subsequently without Freund's adjuvant in two sc sites 3×/wk for at least 4 wk. Ten days following the last injection of antigen the animals were bled (approx 50 ml) and subsequently subjected to a second and third series of antigen injections before bleeding for the second and third time.

After collection of serum, it was pooled and absorbed against dog erythrocytes. Immunoglobulins were precipitated by half saturation with ammonium sulfate at room temperature. The IgG fraction was recovered from DEAE columns and concentrated by dialysis filtration, filtered through Millipore filters with a pore diameter of 0.5 $\mu$m and stored in appropriate aliquots for intravenous injection into dogs. Different batches of these anticanine kidney antibodies (IgG) were tested for specificity against kidney antigens of dogs, humans, rats, and goats. Heavy precipitation at optimal concentrations occurred with antigens derived from dog kidneys. Some crossreaction with human kidney antigens was found.

*Histopathologic studies.* At the time of death or killing, appropriate blocks of kidney were fixed in 10% buffered formalin, embedded in paraffin and stained by the periodic acid–Schiff and hematoxylin and eosin method. Others were snap-frozen for immunofluorescent studies. Frozen sections were air-dried, fixed in acetone for 5 min and washed twice with phosphate buffered saline (pH 7.1). Commercially obtained fluorescein conjugated goat anti-rabbit IgG globulin (Behring Diagnostics, Sommerville, NJ) was applied to sections for 45 min. Slides were washed with phosphate buffered saline three times before being mounted with glycerol in saline and examined with a Zeiss micro-

scope equipped with an HBO 200 W mercury lamp as an ultraviolet source.

*Results. Pathological changes.* All dogs showed pathologic changes in the kidney consistent with time that elapsed following the last injection of the anti-dog kidney antibody, which was demonstrable by immunofluorescence to have a linear distribution along the basement membrane of glomeruli in all dogs (Fig. 1B).

The changes were most marked in dog B that died 26 days following the first series of injections. This animal had a severe glomerulonephritis superimposed on which there was terminally a severe acute tubular necrosis (Fig. 1C and D), associated with anasarca and cardiac failure.

The pathologic changes in the kidneys of dogs M, S, and H were minimal, but definite changes occurred in the glomeruli including irregular thickening of the basement membranes, mesangial cell proliferation and, occasionally, focal fibrosis of the glomerular tuft. These represent healed lesions and were associated with no active degenerative or inflammatory pathologic changes of the tubules, glomeruli, or blood vessels or interstitium. Characteristic lesions are shown in Figs. 1E and F.

*Hemodynamic changes.* Dog M (Fig. 2) showed the most marked hypertensive response to the treatment. After the first course of injections, blood pressure rose from 142/103 to 177/106. After the second treatment,

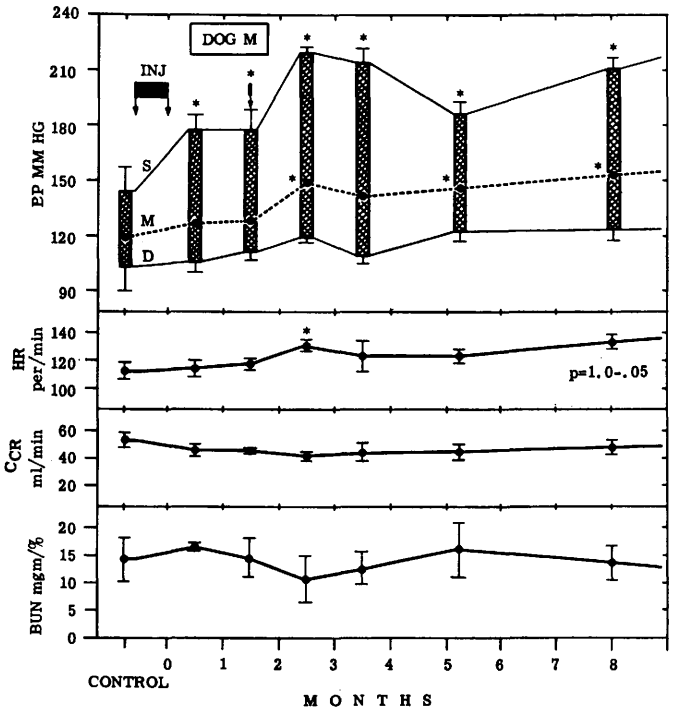


FIG. 2. Hemodynamic alterations resulting from injection of rabbit anti-canine kidney antibodies (IgG). Injection series 1: 1 ml (13 mg antibody globulin/ml) on first day, 1 ml 2 days later, and 1 ml 17 days later. Injection 2: 0.5 ml of 14.2 mg/ml antiserum 64 days later. *S*, systolic arterial blood pressure (mm Hg); *M*, mean arterial pressure; *D*, diastolic blood pressure. *HR*, heart rate (beats per min); $C_{Cr}$, endogenous creatinine clearance taken to measure GFR. Abscissa: time (mo) after completion of first injection series. Values are average of groups of observations made during monthly intervals, $\pm$ 1 SEM. Number of observations per interval ranged from 4 to 8 (av 6). Asterisks, denote $p$ values $< 0.05$. ($t$ and $p$ values) calculated for samples with different numbers in each group: Snedecor, G. W., "Statistical Methods," p. 90. Iowa State Univ. Press, Ames (1962).

TABLE I. Renal Function Changes During Hypertension of Chronic Immune Nephritis (Dog M).[a]

| | Control (av ± 1 SEM) | During hypertension (av ± 1 SEM) | $p$ | % Change |
|---|---|---|---|---|
| $C_{Cr}$ (ml/min) | 52.7 ± 5.7 | 44.1 ± 1.80 | NS | −16 |
| $C_{Na}$ (ml/min) | 0.36 ± 0.11 | 0.20 ± 0.025 | .05 | −44 |
| $\frac{C_{Na}}{C_{Cr}} \times 100$ | 0.70 ± 0.15 | 0.48 ± 0.07 | NS | −31 |
| $\frac{\dot{V}}{C_{Cr}} \times 100$ | 0.92 ± 0.23 | 0.64 ± 0.055 | NS | −31 |
| $C_{H_2O}$ (ml/min) | − 1.13 ± 0.29 | − 1.00 ± 0.034 | NS | −12 |
| $U/P_{Osm}$ | 3.49 ± 0.46 | 5.10 ± 0.17 | .01 | +46 |
| $\frac{CK}{C_{Cr}} \times 100$ | 16.74 ± 5.2 | 17.70 ± 0.84 | NS | + 5 |

[a] Control: $N = 4$; during hypertension: $N = 42$.

about 1 mo later, it rose to 220/118 and remained persistently elevated until the animal was sacrificed approximately 9 mo after the initial treatment. There was minimal reduction in creatinine clearance (Table I), and the BUN never exceeded 20 mg%, a finding consistent in dogs H and S. Animal H did not show changes in blood pressure until the fourth series of injections (Fig. 3). Then it remained moderately elevated for approximately the last 2 mo of observation.

During treatment, all dogs tended to show a decrease in creatinine clearance ($C_{Cr}$), taken to measure glomerular filtration rate. PAH clearances measured at intervals of approximately 3, 4, 6, 7.5 mo (H and S), and 9 mo (M), after the start of IgG injections were in the high range of normal and averaged as follows:

M: 16.5 ml/min/kg (range, 14.8–17.2);
H: 17.8 ml/min/kg body wt (14.9–23.7);
S: 16.8 ml/min/kg (15.0–17.4).

The respective $C_{Cr}$ averaged: 2.7 (2.0–3.9 ml/min/kg), 2.34 (1.5–3, 35 ml/min/kg), and 2.7 (2.2–3.1 ml/min/kg). These values are below the average expected in normal female dogs, *viz,* 4.3 (2.6–5.6 ml/min/kg) (17). Hence, the filtration fraction (FF) was noticeably reduced to an overall average of 0.153, well below the expected normal average value of 0.317 in unanesthetized female dogs (17).

Dog B responded more acutely and severely to the injection of IgG (Fig. 4). Indications of transient renal failure were observed during the treatment: BUN increased to a high value of 38.5 mg%, and $U/P_{Osm}$ decreased to about half of normal. A marked increase in protein excretion was observed during this phase, and total plasma proteins decreased significantly. However, remission of the acute changes was indicated 12 to 20 days after start of injection, when the blood pressure began to rise. The above parameters had returned to control several days before the animal's demise on Day 26. No renal clearance studies were conducted in this animal.

*Water and electrolyte handling.* Dog M (Table I) showed a significant decrease (44%) in $C_{Na}$, which was related to the decrement in GFR. Expressed as the fractional clearance, the average decrement was 31%. Fractional urine volume also averaged 31% less than control during the period of hypertension. $Tc_{H_2O}$ was not noticeably altered, and $U/P_{Osm}$ averaged 5.10.

Dog H (Fig. 3) showed a tendency for fractional urine volume and Na clearance to decrease during the last 2 mo of the study, coinciding with the upward trend in blood pressure. Dog S (normotensive) showed no significant alterations in the above parameters, and none of the animals showed significant changes in fractional K clearance.

*Body water compartment measurements.*

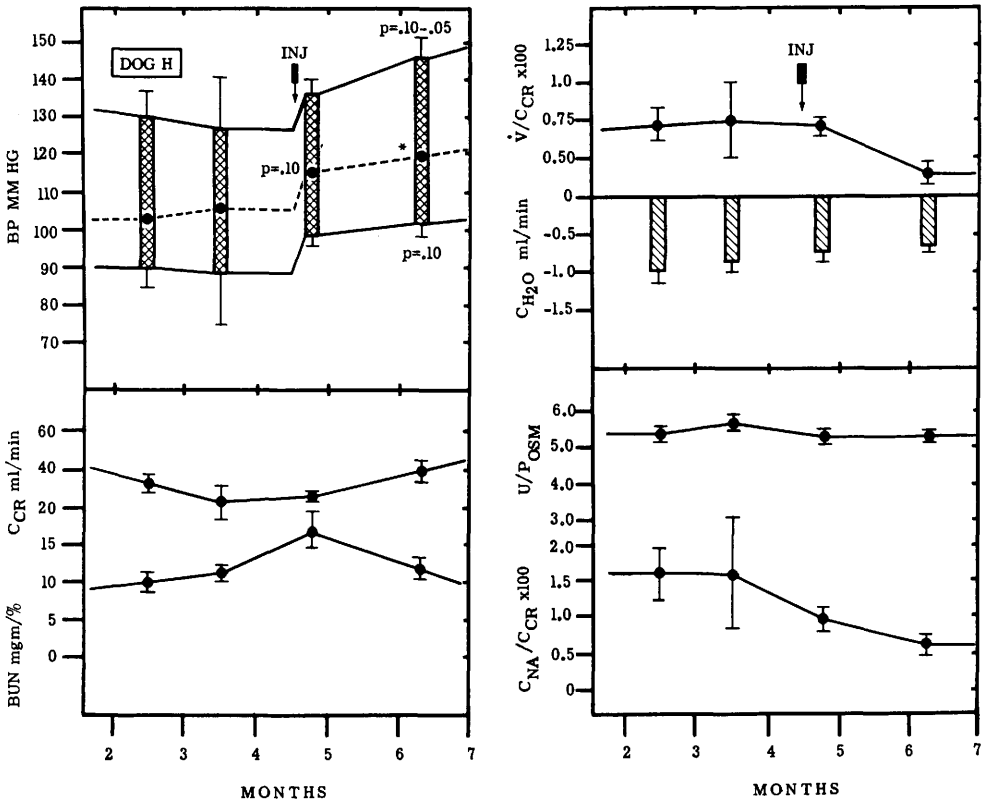


FIG. 3. Hemodynamic and renal functional data in an animal which developed hypertension after 4 series of injections of IgG. Injection series 1: 0.5 ml on first day (14.2 mg/ml), 0.5 ml on fifth day, and 0.5 ml on seventh day. Injection 2: 0.5 ml on Day 31. Injection series 3: total of 3 ml of antiserum between Days 61 and 63. Injection 4: 3 ml at 5 mo after initial injection series. Number of observations per interval, 4 to 9 (av 6). *BUN*, blood urea nitrogen; $V/C_{Cr} \times 100$, fractional urine flow; $C_{H_2O}$, free water clearance; $U/P_{Osm}$: ratio of urinary to plasma osmolality; $C_{Na}/C_{Cr} \times 100$, fractional sodium clearance.

The mean and range of blood volume in normal dogs is 90.0 (56–135) ml/kg, and for inulin space, 198 (145–251) ml/kg body wt (18). Dogs H and S, measured 77 and 283 days after initial treatment, averaged 123 and 119 ml/kg, respectively, for blood volume, and 236 and 259 ml/kg, for inulin space. Dog M was measured at 123 and 284 days, showing averages of 95 ml/kg for blood volume, and 230 ml/kg for inulin space. Hence, values averaged above the expected control mean, but were within the normal range.

*Plasma renin and aldosterone levels.* Control renin plasma concentrations averaged 5.23 ± 2.6 (SEM) ng/ml/3.0 hr. Values measured at average intervals of 90, 160, and 250 days after the initial antiserum treatment averaged as follows: M, 2.63 ± 0.65; H, 1.81 ± 0.56; S, 3.41 ± 0.65.

Control plasma aldosterone averaged 8.42 ± 2.3 (SEM) ng/%. Values measured at the above time intervals averaged: M, 6.3 ± 1.6; H, 3.80 ± 1.1; S, 6.80 ± 1.6.

*Plasma proteins and urinary protein excretion.* Figure 5 shows that urinary protein excretion was increased in all three chronic animals, but excretion rates were much less than in dog B, who died acutely (Fig. 4). The middle panel shows that the urinary loss was not great enough so that the animals could not compensate to maintain normal plasma protein concentrations.

*Catecholamines; cholesterol.* Circulating catecholamine concentrations assayed from

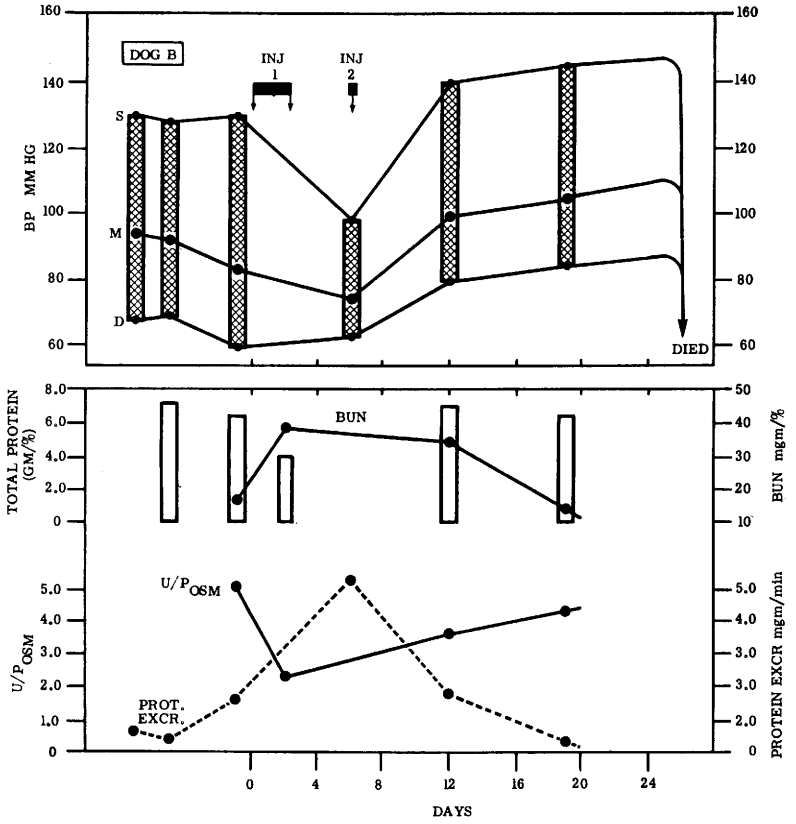


FIG. 4. Hemodynamic alterations and accompanying changes in plasma and urinary proteins, BUN, and $U/P_{Osm}$ resulting from immune globulin treatment in a male dog. Injection series 1: 1 ml/day for 3 days (14.0 mg/ml of Igb); Series 2: 1 ml.

systemic venous blood samples taken 66 days (H, S), and 112 days (M) after the initial IgG injection series were: H: 55.3 ng/100 ml; S: 36.2; and M: 37.6. A second series of analyses done 172 days later yielded the following average values: H: 46.0; S: 51.0; M: 32.6 ng/100 ml. The range of normal values found here in dogs is: 29–49 ng/100 ml.

Plasma cholesterol measured 6 to 8 mo after treatment had been started showed the following values: H: 237 mg%; S: 127 mg%; M: 188 mg%. These values indicate no hypercholesterolemia at this time.

*Discussion.* The major finding of the present investigation was that hypertension can result following repeated mild immunologic injuries in the absence of acute renal failure. All animals observed showed proteinuria and hematuria following injections, even the animal that did not develop hypertension over prolonged periods of observation and with repeated sequences of injection of IgG. None of the animals examined showed increase in plasma renin, nor was plasma aldosterone altered significantly.

Fukuda, Green and Vander (1) reported no significant change in total blood volume, and cardiac output remained essentially unchanged as elevation of arterial blood pressure occurred, indicating increase in TPR. In our series, blood volume and extracellular fluid volume increased but remained in the normal range.

In searching for possible mechanisms to explain the observed hypertension, a significant manifestation was the decrease in Na clearance in association with a trend for GFR to decline. Fractional urine volume declined in association with this. Urinary concentrat-

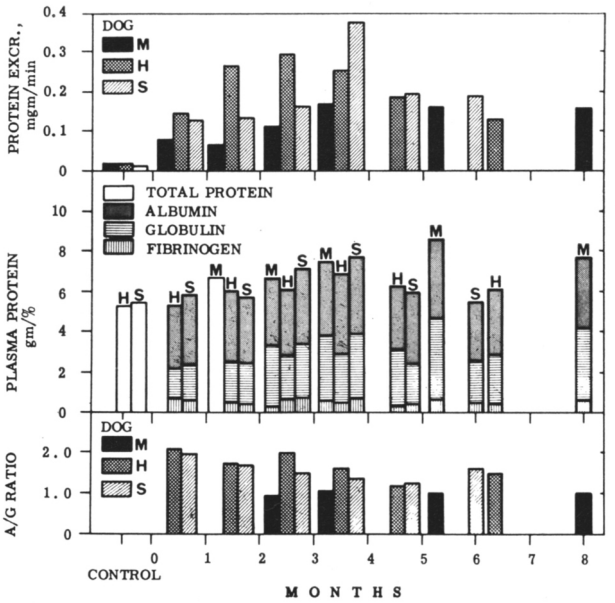


FIG. 5. Changes in urinary protein excretion, plasma protein fraction, and A/G ratio during treatment with IgG. Values represent averages taken during monthly intervals (av of 6 determinations/mo), after the control period (av of 4 determinations).

ing ability remained good, however, as evidenced by the high $U/P_{Osm}$ ratios in the chronic dogs.

Since increased aldosterone release is probably not basic to the mechanism of sodium retention in our series, the alternatives which remain are increased efficiency of reabsorption with the observed tendency for GFR and sodium load to be reduced (glomerulotubular imbalance). Another possibility is that there might be diminution of action of a sodium-losing hormonal or humoral substance. These views find support in the writings of Kassirer (19) and Shapiro (20). Vasoactive lipids have been extracted from renal medullary tissue which have depressor properties and probably belong to the prostaglandin family. It is well known that the prostaglandins exert a sodium-losing action on the cells of the nephron (21–24). One may, therefore, speculate that with the renal vascular changes associated with glomerulonephritis, production, and/or release of the medullary prostaglandins may in some manner be impaired.

In conclusion, the mechanism of the observed hypertension is not understood at present. Fukuda, Green and Vander (1) found an increase in total peripheral vascular resistance (TPR), with normal cardiac output and blood volume. The kidneys participated in the increase in TPR (25). It does not seem likely that increased angiotensin II production was involved in the development of hypertension, and we observed no striking increases in catecholamines. In the absence of evidence for increased production of vasopressor substances, the remaining alternative is that pressor mechanisms somehow became more sensitized to existing levels of vasopressor substances during the hypertension of glomerulonephritis. This could be the consequence of reduced circulating antipressor (depressor) substances, such as the prostaglandins (26–30).

*Summary.* Development of hypertension in unanesthetized dogs in response to rabbit anti-canine kidney glomerular antiserum (IgG) was examined over periods of time totalling 7.5–9 mo. The dosage of IgG was such as to cause minimal renal functional alterations, and BUN remained normal. Two of three dogs studied developed hypertension. Plasma renin and aldosterone value were not increased. Tentative mechanisms for the ob-

served hypertension are discussed in the light of possible failure of the kidney medulla to produce and release depressor material.

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