

Mesangial Function in Ureteral Obstruction in the Rat: ***BLOCKADE OF THE EFFERENT LIMB***

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At all time intervals, concentrations of AHlgG^{125I} in isolated glomeruli from control animals were similar to values obtained from nonobstructed kidneys of UUL(U) and UUL(R) rats: a linear decrease in concentration over a period of 24 h was observed when the logarithm of glomerular AHlgG^{125I} concentration was plotted against time. Aberrations in the kinetics were apparent in obstructed kidneys but not in liver, spleen, or blood concentrations of AHlgG^{125I}: (a) At 2 h in all obstructed kidneys, glomerular concentration of AHlgG^{125I} was markedly [...]

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Mesangial Function in Ureteral Obstruction in the Rat

BLOCKADE OF THE EFFERENT LIMB

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ABSTRACT The kinetics for mesangial uptake and transport of radiolabeled aggregated human immunoglobulin (IgG) (AHIgG¹²⁵I) deviated markedly from normal in male Sprague-Dawley rats with ureteral obstruction. Four experimental groups, each containing 25 rats, were used: (a) bilateral ureteral ligation (BUL) with release of one ureter 24 h later; (b) unilateral ureteral ligation with release 24 h later [UUL(R)]; (c) unilateral ureteral ligation without release (unreleased) [UUL(U)]; (d) uremia-control, which consisted of rats with ligated left ureter and a severed right ureter. A similar number of sham-operated rats served as control for each group. AHIgG¹²⁵I (45 mg/100 g body wt) was given intravenously 1 h after release of the ureteral obstruction (25 h after ureteral obstruction or sham surgery). Groups of five control and five experimental animals were sacrificed at 2, 4, 8, 16, and 24 h after injection.

At all time intervals, concentrations of AHIgG¹²⁵I in isolated glomeruli from control animals were similar to values obtained from nonobstructed kidneys of UUL(U) and UUL(R) rats: a linear decrease in concentration over a period of 24 h was observed when the logarithm of glomerular AHIgG¹²⁵I concentration was plotted against time. Aberrations in the kinetics were apparent in obstructed kidneys but not in liver, spleen, or blood concentrations of AHIgG¹²⁵I: (a) At 2 h in all obstructed kidneys, glomerular concentration of AHIgG¹²⁵I was markedly reduced. (b) In BUL (released or unreleased), glomerular concentrations of AHIgG¹²⁵I

from 4 to 16 h were \cong 10-fold those in UUL(U) or UUL(R) kidneys. (c) The significant decline in glomerular concentration between 4 and 16 h in control and non-obstructed kidneys was not observed in UUL(R), UUL(U), or BUL (released or unreleased) kidneys; in all obstructed kidneys, a plateau in glomerular concentrations of AHIgG¹²⁵I was observed between 4 and 16 h. (d) After 16 h at a time when the blood level of AHIgG¹²⁵I had decreased to 3% of initial values, there was progressive fall in glomerular AHIgG¹²⁵I. Similar results were obtained in the uremia-control group in rats, which indicated that uremia per se had no measurable effect on mesangial kinetics. These studies demonstrate that ureteral occlusion induces alterations in mesangial uptake (afferent limb) and egress (efferent limb) of macromolecules. Particularly evident is the "blockade" of the efferent limb which is demonstrable at high blood levels of AHIgG¹²⁵I. These alterations in the transit of macromolecules through the mesangium may be mediated in part by the hemodynamic changes that accompany ureteral obstruction.

INTRODUCTION

The glomerular mesangium occupies an intercapillary position and consists of mesangial cells and an amorphous fibrillar material, the mesangial matrix (1). Anatomically, it is separated from the capillary lumen by the endothelium and limited by the mesangial reflexion of the glomerular basement membrane (2). The contiguity with the lacis cells of the juxtaglomerular (JG)¹ zone and the presence of actomyosin suggest that it may play a role in the regulation of the glomerular

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¹*Abbreviations and nomenclature used in this paper:* AHIgG¹²⁵I, radiolabeled aggregated human IgG; BUL, bilateral ureteral ligation; BUN, blood urea nitrogen; GFR, glomerular filtration rate; JG, juxtaglomerular; P_{GC}, glomerular capillary pressure; RBF, renal blood flow; SNGFR, single nephron GFR; UUL(R), unilateral ureteral ligation with left ureter released; UUL(U), unilateral ureteral ligation with left ureter unreleased.

blood flow (3, 4). In addition, morphological studies with a variety of probes have demonstrated movement of macromolecules into the mesangium by way of the endothelial fenestra and the mesangial endothelial interface (5-8). This mesangial uptake (afferent limb) is dependent in part upon type, size, and blood level of the macromolecule (8). The mechanisms controlling the clearing of macromolecules (efferent limb) are incompletely understood, but depend upon phagocytosis, passage by way of the glomerular stalk to the JG region, and possibly regurgitation into the glomerular circulation (8).

Whether the hemodynamic determinants of glomerular filtration modulate the mesangial traffic of macromolecules has not been defined. Unilateral and bilateral ureteral ligation are known to induce alterations in these determinants, particularly renal blood flow (RBF), glomerular capillary pressure (P_{GC}), and tubular pressure (9-14). Ureteral obstruction is also accompanied by changes in the renal interstitium and renal lymph flow (15, 16). We have studied mesangial uptake and disappearance of radiolabeled aggregated human immunoglobulin (Ig)G (AHIgG¹²⁵I), a polydispersed population of macromolecules with characteristics similar to those of antigen-antibody complexes, in normal rats and in rats with unilateral and bilateral ureteral ligation of 24-h duration. In the experiments reported here, we have shown a marked alteration in mesangial uptake and egress of macromolecules after ureteral obstruction of 24-h duration. Specifically, ureteral occlusion induces a "blockade" in the transport of AHIgG¹²⁵I out of the mesangium (efferent limb) which is demonstrable at high blood levels of AHIgG¹²⁵I.

METHODS

Preparation of AHIgG¹²⁵I

Human immune serum globulin (E. R. Squibb and Sons, New Brunswick, N. J.) was labeled with ¹²⁵I (New England Nuclear, Boston, Mass.) by the chloramine-T method of McConahey and Dixon (17). The efficiency of labeling was $\approx 55\%$, and the quantity of free ¹²⁵I in the final product was $< 3\%$. From this labeled protein, AHIgG¹²⁵I was prepared by heat aggregation according to the methods of Christian (18) and Ishizaka and Ishizaka (19) as modified by Mauer et al. (6, 7).

The final concentration of AHIgG¹²⁵I was adjusted to contain 60 mg of protein/ml; merthiolate was added to a final concentration of 1:10,000. Free ¹²⁵I in this preparation was $< 1\%$, and the specific activity of the solution was 0.1 mCi/mg of protein. The AHIgG¹²⁵I was stored at room temperature and used within a week of its preparation. Immediately before the injection of AHIgG¹²⁵I to animals, the solution was centrifuged at 5,000 g to remove insoluble aggregates.

Experimental design

Male Sprague-Dawley rats (Bio-Lab Corp., St. Paul, Minn.) weighing 190-210 g were used in all experiments. The animals were housed in cages and allowed free access to water and standard Purina rat chow (Ralston Purina Co., St. Louis, Mo.) up to the time of the experiment.

Experimental and control groups (Fig. 1). Four experimental groups, each consisting of 25 animals, were used: Group 1, bilateral ureteral ligation (BUL) in which both ureters were obstructed and 24 h later the left ureter was released. Group 2, unilateral ureteral ligation in which the left ureter was obstructed and 24 h later was released [UUL(R)]. Group 3, unilateral ligation in which the left ureter was obstructed for 24 h but not released [UUL(U)]. Group 4, "uremia-control," effect of uremia on the mesangial kinetics of AHIgG¹²⁵I in obstructed and unobstructed kidneys; after overnight dehydration, the right ureter was severed and allowed to drain into the peritoneal cavity while the left ureter was ligated and released 24 h later. For each of the four experimental

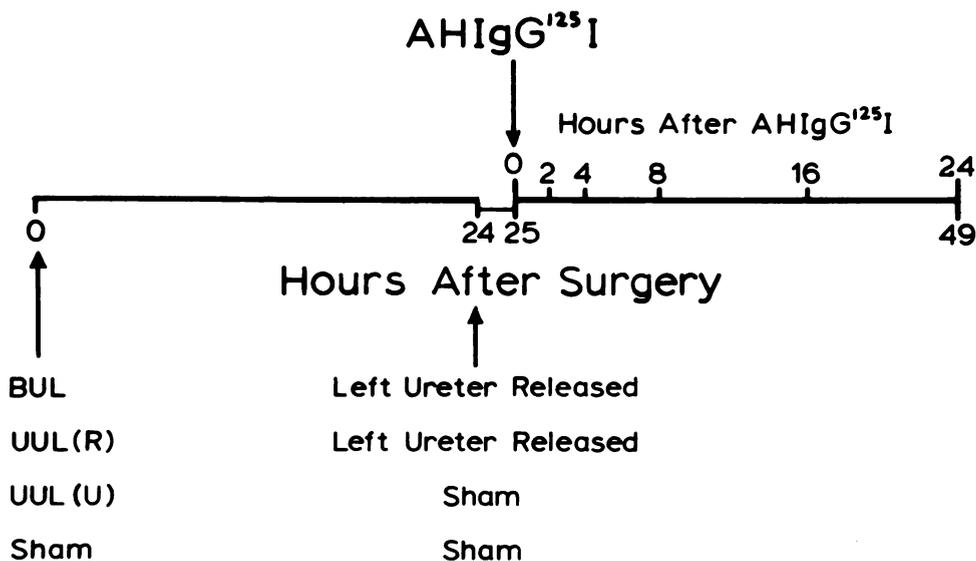


FIGURE 1 Schematic representation of the experimental design.

groups, 25 sham-operated rats served as controls. A total of two separate experiments were performed. Physiological studies were carried out in six control animals and in groups of six animals with BUL, UUL(R), and UUL(U), 24 h after ureteral occlusion.

Surgical procedure. Ureteral ligation, bilateral or unilateral, was performed under light ether anesthesia. The ureters were carefully dissected, and a small piece of polyethylene tubing was placed along side of the ureter. The ureter and tubing were tied together with 3-0 silk suture. In this way, minimal damage was induced to the ureter by the ligature. After recovery from surgery, the animals were returned to their cages and allowed free access to water but no food. In sham-operated control animals for each of the groups, the ureters were dissected free of surrounding tissues but not ligated. Release of ureteral ligation was accomplished under light ether anesthesia. Care was taken to ensure that the released ureter was dissected clear of any adhesion.

Administration of AHlgG¹²⁵I. All animals received AHlgG¹²⁵I (45 mg/100 g body wt) intravenously 24 h after ureteral ligation or sham operation. However, the AHlgG¹²⁵I was given 1 h after ureteral release to BUL, UUL(R), and uremia-control rats. Groups of five experimental and five sham-operated control rats were sacrificed at 2, 4, 8, 16, and 24 h after the administration of AHlgG¹²⁵I.

Tissue and blood analysis. The control and experimental animals to be killed 24 h after the administration of AHlgG¹²⁵I were placed in metabolic cages, and all urines were collected. Protein-bound ¹²⁵I in urine was investigated by the addition of 2 ml of 10% TCA to 2 ml of urine. From each of the experimental and control animals sacrificed at the various time intervals, the kidneys were removed; the left and right kidneys were pooled separately for determination of AHlgG¹²⁵I in preparations of isolated glomeruli (see below). In addition, small wedge sections were taken from left and right kidneys of all control and experimental animals for histologic studies. Liver and spleen from experimental and control animals were also removed for determination of AHlgG¹²⁵I. The blood obtained at the time of sacrifice was used for determination of blood urea nitrogen (BUN), protein bound ¹²⁵I, and for sucrose density gradient centrifugation.

Renal histology

Wedge-shaped renal tissue obtained at the time of sacrifice was prepared for light and immunofluorescence microscopic studies by techniques previously described (20). The intensity of immunofluorescence was arbitrarily graded from negative to 3+. Within each specimen the intensity and pattern of fluorescence in superficial and juxtamedullary glomeruli were compared.

Quantitation of AHlgG¹²⁵I in preparations of isolated glomeruli

Glomerular isolates were prepared separately from pools of five left and five right kidneys from experimental and control animals sacrificed at the different time intervals after administration of AHlgG¹²⁵I. The kidneys were decapsulated and medulla was separated from cortex. The mash of decapsulated cortex was pushed through a 150-mesh screen (Michigan Dynamics, Div. of Ambac Industries, Inc., Garden City, Mich.). The resulting suspension was poured through a 100-mesh screen to remove large tissue fragments; the glomeruli were then collected on 200- and 325-mesh screens successively, and washed vigorously with 0.15 M sodium chloride into a 50-ml centrifuge tube. Glomerular preparations were examined by phase microscopy to insure at least an 85% purity.

The glomeruli were then washed, lyophilized, and weighed and counted in a Biogamma II counter (Beckman Instruments, Inc., Fullerton, Calif.) for ¹²⁵I. The data were expressed as micrograms of AHlgG¹²⁵I per milligram of dried glomeruli. Protein-bound ¹²⁵I in combined supernates from glomerular washes was <0.16% of the measured radioactivity in a given pool of glomeruli, which indicates that mesangial-trapped AHlgG¹²⁵I was not released during the washing procedure.

The contamination of isolated glomeruli by blood was demonstrated to be minimal (0.002 ml serum/mg glomeruli) in separate experiments after administration of nonaggregated 7S IgG¹²⁵I; correction of glomerular concentration of AHlgG¹²⁵I for entrapped serum resulted in minimal differences from uncorrected values (<7.0%).²

Quantitation of AHlgG¹²⁵I in preparations of liver and spleen

Liver and spleen were removed from each animal at the time of sacrifice. The tissues were thoroughly washed in isotonic saline and dried in a ventilated oven at 85°C for 24 h. The dried organs were weighed, placed in 1.0 N sodium hydroxide that contained 0.2 N sodium deoxycholate, and then dissolved by heating at 95°C for 1 h. The dissolved tissue was then counted for ¹²⁵I; the data were expressed as micrograms of AHlgG¹²⁵I per milligram of dried tissue. No attempt was made to correct for residual blood contamination in liver and spleen because the AHlgG¹²⁵I content in these organs was used to exclude major differences in reticuloendothelial function among experimental groups. We have not demonstrated differences in these data after perfusion of the liver with saline until free of blood.²

Quantitation of AHlgG¹²⁵I in blood

Whole blood (0.1 ml) was added to 2.0 ml of 0.1% sodium carbonate to produce hemolysis. After total counts for ¹²⁵I were obtained, 2.0 ml of 10% TCA was added to produce protein precipitation. The samples were centrifuged at 5,000 g, and the supernate was separated and counted to determine free ¹²⁵I; protein bound counts were calculated by subtraction.

Serum (0.2 ml) was layered on a 12-ml linear sucrose gradient (10-50% sucrose). Gradients were centrifuged at 36,000 rpm for 16 h at 4°C with a SW 40 rotor in a Beckman ultracentrifuge (model L5-65, Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). Fractions (0.5 ml) were collected through a hole in the bottom of the tube and counted for ¹²⁵I. The gradient curves were integrated, and blood levels of AHlgG¹²⁵I >7S were calculated (21).

Determination of RBF and arterial pressure

RBF was determined in six animals in each of the following groups: (a) control animals: RBF was determined in the left kidney 24 h after the sham operation; (b) BUL animals: 1 h after release of the left ureter, RBF was measured in both kidneys; (c) UUL(R) animals: RBF was separately determined in both kidneys 1 h after the release of the left ureter; and (d) UUL(U) animals: RBF was measured in both kidneys.

Animals were anesthetized with sodium pentobarbital (50 mg/kg of body wt) intraperitoneally, and the femoral artery was cannulated with PE 50 polyethylene tubing. Arterial pressure was monitored with a Statham P23 D6 pressure transducer (Statham Instruments Inc., Oxnard, Calif.) connected to a Hewlett-Packard recorder (model 7702b, Hewlett-Packard Co., Palo Alto, Calif.). RBF was measured by a small-

² Keane, W. F., and L. Raji. Unpublished observations.

diameter flow transducer (model EP 401.5 or EP 401.8) connected to a square-wave electromagnetic flow meter (model 501, Carolina Medical Electronics, Inc., King, N. C.) and a Hewlett-Packard recorder. RBF was expressed as milliliters per minute and also as milliliters per minute per 100 g body weight.

Micropuncture studies

24 h after either unilateral or bilateral ureteral obstruction, animals were prepared for micropuncture studies as previously described (22). Isotonic saline that contained 20 mg/ml of inulin was infused via a jugular vein catheter (PE 50) at a rate of 2 ml/h. Blood pressure was monitored by a second catheter placed in the left carotid artery. The ureter was released and then cannulated with PE 50 tubing. Clearance periods and micropuncture measurements were started 45 min after ureteral cannulation and were completed within 30–50 min. Two 15- to 20-min clearance periods were obtained. Blood was withdrawn at the midpoint of the urine collection for inulin determination.

In each rat, tubular collections and stop-flow measurements were obtained from four to eight tubules. From randomized tubular puncture sites, single nephron filtration rate was calculated from the tubular flow rate and the tubular:plasma ratio of inulin. With a servo-nulling device, the hydrostatic pressure was measured with micropipettes (OD:3–5 microns) in the proximal tubules, in the first surface convolution distal to Bowman's space. Pressures were measured under free-flow conditions and after blockage of the tubular lumen with Sudan black-stained paraffin oil. At the end of the period, 1 ml of arterial blood was withdrawn for measurement of the systemic protein concentration. P_{GC} was estimated with the stop-flow technique of Gertz as modified by Allison et al. (23). Estimation of P_{GC} by stop-flow technique in normal rats has been shown to be equivalent to that measured by direct capillary puncture (24, 25). Tubular fluid volume was measured with a previously calibrated micropipette. Inulin concentrations in blood, tubular fluid, and urine were determined in duplicate by the microfluorometric method (22). In UUL(U) rats, no micropuncture studies were performed. Inulin clearance was determined in the right unobstructed kidney 24 h after the ligation of the left kidney.

Statistical analysis

Where applicable, data are expressed as mean \pm standard error of the mean. Physiologic data were compared in control and experimental animals with the Student's *t* test. Linear regression in the experimental and control animals was performed, and slopes were compared by standard techniques.

RESULTS

Blood levels of >7S AHlgG¹²⁵I were determined in control and experimental animals (Fig. 2). No significant differences were observed among the groups at any time interval. By 24 h, circulating levels of >7S AHlgG¹²⁵I were <1% of the 2-h value. Protein-bound ¹²⁵I was not present in urine of either control or experimental animals.

Kinetic studies of mesangial function

Quantitative studies of AHlgG¹²⁵I in glomerular isolates. In control animals sacrificed at different time

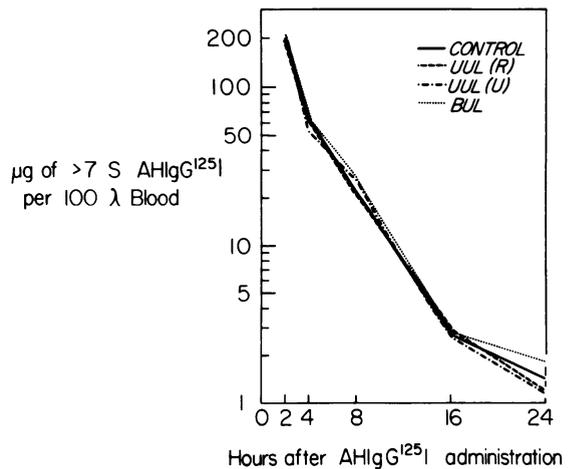


FIGURE 2 The concentrations of >7S AHlgG¹²⁵I in blood of control and experimental animals determined at various time intervals. Each plot represents the mean value of data obtained from two separate experiments.

intervals, the concentration of glomerular AHlgG¹²⁵I was highest at 2 h, which was the first time period evaluated (Figs. 3 and 4). Thereafter, a progressive decrease in concentration was observed that was linear when expressed as the logarithm of glomerular AHlgG¹²⁵I concentration vs. time after injection. By 4 h, glomerular AHlgG¹²⁵I had decreased to 68% of the 2-h value and by 24 h to 14%. Similar plots of glomerular AHlgG¹²⁵I vs. time were found on analysis of glomeruli from kidneys of sham-operated control animals and nonob-

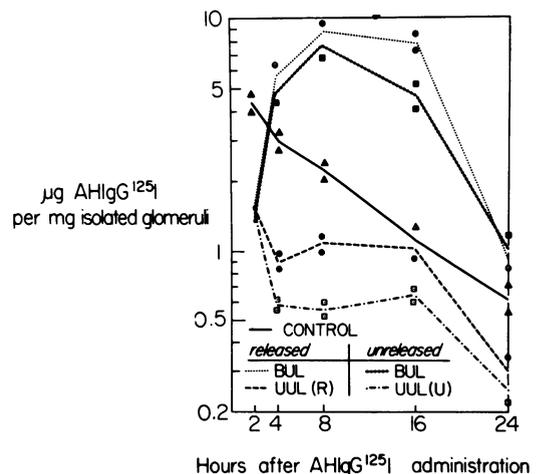


FIGURE 3 The concentration of AHlgG¹²⁵I in glomeruli isolated from control kidneys and from kidneys with ureteral obstruction. Each plot represents the mean value of data obtained from two separate experiments. Each point represents the concentration of glomeruli from five kidneys in each group at the time intervals noted. Data points at 2 h are not represented to avoid crowding; variations between experiments were similar to that observed at other time intervals.

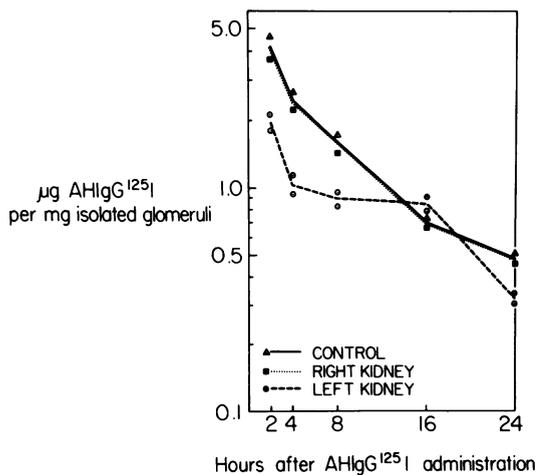


FIGURE 4 Mesangial concentrations of AHlgG¹²⁵I in glomeruli isolated from control animals and from animals with one ureter ligated for 24 h and then released (left kidney) and the other ureter severed and allowed to drain into the peritoneal cavity (right kidney). The concentration of AHlgG¹²⁵I in blood, spleen, and liver (not shown) were not different from values obtained in BUL, UUL(R), and UUL(U) animals.

structed kidneys of UUL(R) and UUL(U) animals. At 2 h, glomerular AHlgG¹²⁵I in UUL(R), UUL(U), and both BUL kidneys, was $\approx 30\%$ of the levels observed in control animals (Fig. 3). Between 2 and 4 h, the concentration increased in both BUL kidneys but decreased further in UUL(U) and in UUL(R) kidneys.

In all obstructed kidneys, glomerular concentrations of AHlgG¹²⁵I remained relatively constant between 4 and 16 h. However, in BUL (released or unreleased), glomerular concentration of aggregates from 4 to 16 h was ≈ 10 -fold those in UUL(U) and UUL(R) kidneys. In all experimental groups, after 16 h, at a time when the blood levels of AHlgG¹²⁵I had decreased to $< 3\%$ of initial values, there was a marked decrease in mesangial AHlgG¹²⁵I.

The BUN was elevated in animals with BUL and in the uremia-control rats 184.67 ± 10.61 and 155.0 ± 11.3 mg/dl, respectively. Mesangial kinetics in the latter group were similar to that observed in UUL(R) rats (Fig. 4), which demonstrated that uremia per se did not influence mesangial kinetics of AHlgG¹²⁵I.

Immunofluorescence and light microscopy

At 2 h, human AHlgG, detected with fluorescein isothiocyanate-labeled goat anti-human IgG, was present primarily within the mesangium but also in a sub-endothelial distribution. By 4 h, human IgG was found almost entirely within the mesangium. Minimal fluorescence was seen in peritubular capillaries, but none was observed in tubules or Bowman's space. This pattern of fluorescence was similar in control and experimental kidneys. However, the intensity of fluorescent

staining was different in the control and experimental kidneys at the various time intervals but showed an excellent correlation with the quantitative determination of AHlgG¹²⁵I in isolated glomeruli. In control and nonobstructed kidneys of UUL(U) and UUL(R), mesangial immunofluorescence was intense (2–3+) at 2, 4, and 8 h after administration of AHlgG¹²⁵I, with a decrease to trace amounts by 24 h. In obstructed kidneys from UUL(R) and UUL(U), the intensity of immunofluorescence (1+) was the same at all time periods and became negative by 24 h. Evaluation of BUL kidneys, whether released or unreleased, revealed striking fluorescence (3+) at 4, 8, and 16 h, with a lesser intensity (1+) at 2 and 24 h. In addition, tissue obtained by wedge biopsy from control and experimental animals revealed that at all time intervals studied there were no differences in the intensity of mesangial staining between cortical and deep glomeruli. Light microscopy revealed only tubular dilatation, mild epithelial cell flattening, and interstitial edema in obstructed kidneys.

AHlgG¹²⁵I in liver and spleen

Despite striking differences in mesangial AHlgG¹²⁵I between control and experimental animals, there were no major differences in AHlgG¹²⁵I concentrations in the liver and spleens among control and UUL(U), UUL(R), and BUL rats (Fig. 5). In both organs there was a progressive decrease in AHlgG¹²⁵I concentration, and, by 24 h, $< 1\%$ of the initial concentration was present. Linear regression analysis of the slopes derived from data for liver and spleen showed no significant differences between experimental and control animals.

Physiological studies

RBF was significantly decreased in all models of ureteral obstruction (Table I). The RBF of unreleased kidneys in BUL and UUL(U) animals was reduced to

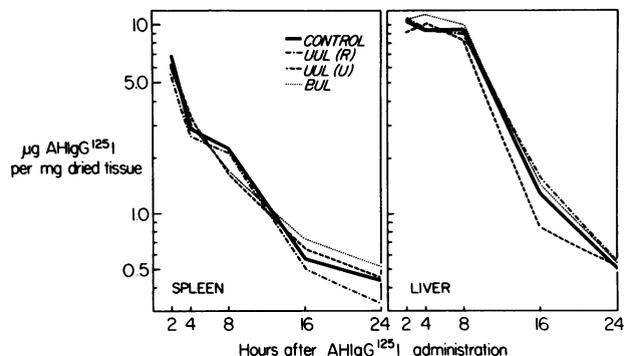


FIGURE 5 The concentration of AHlgG¹²⁵I in livers and spleens of control and experimental animals sacrificed at various time intervals. Each plot represents the mean value of data obtained from two separate experiments.

TABLE I
*Renal Blood Flow in Control and Experimental Animals**

	Control	BUL		UUL(R)		UUL(U)	
	LK	LK(R)	RK(U)	LK(R)	RK(N)	LK(U)	RK(N)
<i>ml/min</i>	5.90±0.18	5.07±0.19‡§	4.16±0.20¶¶	4.60±0.23‡	6.22±0.13**	3.54±0.30¶	5.85±0.23**
<i>ml/min/100 g body wt</i>	2.84±0.03	2.44±0.11‡§	1.73±0.11¶¶	2.03±0.08‡	3.08±0.11**	1.47±0.06¶	3.02±0.04**

LK, left kidney; RK, right kidney; (R), released; (U), unreleased; (N), normal.

* *n* = 6.

‡ *P* < 0.05 compared with control.

§ *P* < 0.05 compared with LK of UUL(R).

¶ *P* < 0.001 compared with control.

¶¶ Not significantly different from LK of UUL(U).

** Not significantly different from control.

50% of control kidneys. In released kidneys of BUL and UUL(R) animals RBF was 86% and 71% of control kidneys, respectively. Compared with control as well as UUL(R) and UUL(U), BUL animals had a higher arterial pressure and BUN (Table II).

Comparison of glomerular dynamic factors between released kidneys of BUL and UUL(R) animals is presented in Table II. After ureteral release, the hydrostatic pressures in the proximal tubules were lower than normal in both models. Compared with control rats, single nephron glomerular filtration rate (SNGFR) and the calculated P_{GC} were reduced in the released kidneys of BUL and UUL animals. It is apparent that in both of these models the reduced P_{GC} can be explained by vasoconstriction of the afferent arteriole. Calculations of resistance changes across the afferent arteriole from the changes in blood flow and pressures indicated that the increase in resistance was similar in both UUL(R) and BUL kidneys. However, P_{GC} in released

kidneys of BUL animals was higher than in the released kidneys of UUL(R) animals. This increase can be explained by the higher mean arterial pressure observed in BUL animals (Table II). However, SNGFR was not significantly different between BUL and UUL(R) kidneys. This suggests that the glomerular capillary ultrafiltration coefficient, may be lower in BUL than UUL(R) kidneys. Whole kidney glomerular filtration rate (GFR) in both models was proportionately more decreased than SNGFR, which suggests nephron GFR heterogeneity, although back leak of glomerular filtrate (13) or sample errors may be contributory factors. Although these variables conceivably could affect the accuracy of the data, the methods and results are similar to that reported by other investigators (9, 10, 12).

DISCUSSION

After the administration of AHIgG¹²⁵I to the normal rat, kinetic studies have shown a rapid increase in

TABLE II
Micropuncture Data, and BUN in Control and Experimental Animals

	UV	C _{in}	A	SNGFR	P _t	SFP	P _{gc}	MAP	BUN
	<i>μl/min</i>	<i>ml/min</i>	<i>mmHg</i>	<i>nl/min</i>	<i>mmHg</i>	<i>mmHg</i>	<i>mmHg</i>	<i>mmHg</i>	<i>mg/dl</i>
Control (<i>n</i> = 6)									
Mean	3.96	0.90	14.10	26.05	13.10	39.75	53.82	126.5	25.52
SEM	±1.55	±0.19	±1.10	±2.05	±0.41	±0.25	±0.15	±1.5	±1.04
BUL (<i>n</i> = 6)									
Mean	29.13	0.18	13.41	10.43	10.62	25.91	39.33	147.4	184.67
SEM	±5.06	±0.03	±0.79	±1.25	±0.81	±1.57	±1.82	±1.8	±10.61
UUL(R) (<i>n</i> = 6)									
Mean	1.36	0.14	13.82	9.84	8.85	16.68	30.50	119.4	23.69
SEM	±0.81	±0.02	±0.22	±0.96	±1.55	±0.84	±1.50	±1.2	±1.21
<i>P</i> value									
Control vs. BUL	<0.01	<0.001	NS	<0.001	NS	<0.01	<0.001	<0.001	<0.001
Control vs. UUL(R)	<0.05	<0.001	NS	<0.001	<0.05	<0.05	<0.001	NS	NS
BUL vs. UUL(R)	<0.005	NS	NS	NS	NS	<0.05	<0.01	<0.001	<0.001

UV, urine volume; C_{in}, inulin clearance; A, plasma oncotic pressure; P_t, tubular pressure; SFP, stop-flow pressure; MAP, mean arterial pressure.

mesangial uptake within a period of 2–4 h after administration, followed by a decline over the subsequent 24 h. The initial uptake, which occurs during a period of relatively high blood levels, and the subsequent falloff can be described in terms of afferent and efferent limbs, respectively (8). These phases are not separate events because efferent mechanisms likely come into play during the initial uptake period, and, therefore, no time line can clearly separate one phase from the other.

Prior studies have demonstrated that uptake of AHlgG, immune complexes, and other macromolecules may be increased (*a*) after the elevation of the blood level, either by raising the dose or by impairing systemic mononuclear phagocytic uptake (5, 7, 26, 27), and (*b*) after the administration of anti-GBM antibody or aminonucleoside of puromycin (6, 7). In both situations the efferent limb appears to be intact. The localization of AHlgG in the mesangium is dependent upon delivery rate to the capillary lumen (the concentration in the blood times glomerular blood flow) as well as on transfer from the lumen to the mesangium. In all models of ureteral obstruction reported here, we observed a marked decrease in 2-h mesangial AHlgG¹²⁵I. Because at the time, blood levels of AHlgG¹²⁵I in experimental and control animals were high, this reduced uptake suggests a decrease in either delivery of AHlgG¹²⁵I or in transfer from the capillary to the mesangium (lumen-mesangial transfer) or in both. Indeed, a decrease in delivery rate of aggregates produced by a 50% reduction in RBF secondary to aortic constriction causes a proportionate decrease of the same magnitude in the mesangial AHlgG¹²⁵I.² The reduction in RBF to 50% of normal in UUL(U) and in unreleased BUL kidneys could be the reason for diminished 2-h uptake of AHlgG¹²⁵I in these models. However, this does not explain completely the similar decrease in 2-h mesangial AHlgG¹²⁵I in released BUL and UUL(R) kidneys, which have a higher RBF, 86% and 71% of normal, respectively. Hence, a similar decrease in 2-h mesangial AHlgG¹²⁵I in all experimental models despite different RBF would suggest that impaired lumen-mesangial transfer of aggregates may also be a contributing factor. If this assumption is correct, lumen-mesangial transfer of aggregates in BUL kidneys is less impaired than in UUL kidneys.

Mesangial AHlgG¹²⁵I in BUL kidneys increased in the period from 2–4 h, whereas in UUL(R) and UUL(U), mesangial AHlgG¹²⁵I decreased. The reason for this difference is unknown, although several explanations may be entertained. First, this may be a consequence of differences in capillary pressure and/or permeability. The capillary pressure was lower than normal in all models of ureteral obstruction of 24-h duration but was higher in BUL and UUL(R) kidneys. In addition, although not specifically determined in these experiments,

the glomerular capillary ultrafiltration coefficient appeared lower in BUL than UUL(R). Second, the higher concentration of AHlgG¹²⁵I at 4 h in BUL compared with UUL may result from the differences in uptake described above superimposed on efferent mesangial blockade (see below). Third, these findings may be related to differences in the time-course of uptake, i.e., rapid in UUL so that maximal uptake had already occurred by 2 h, and retarded in BUL with maximum uptake at 4–8 h. However, this latter hypothesis does not explain the plateau at 4–16 h in UUL animals.

Ureteral release did not substantially alter mesangial kinetics in either BUL or UUL kidneys, which suggests that the factors inducing abnormal kinetics persisted after release of ureteral obstruction. Other studies have shown that alterations in glomerular function persist for at least 24 h after release of ureteral obstruction (28).

The mechanisms that control the disappearance of macromolecules from the mesangium or efferent limb are unclear. In previous studies, intracellular degradation by mesangial cells was postulated as a primary mechanism (1, 29). However, the predominant localization of certain macromolecules such as antigen-antibody complexes (30) and aggregated proteins (5) within the mesangial channels and not within mesangial cells suggests that this may not be the main mechanism. Passage by way of the glomerular stalk to the JG zone has been clearly demonstrated with morphologic techniques, although the pathway thereafter is obscure (31, 32). It is possible that drainage into the lymphatics, interstitium, or tubules may occur, although none of these routes has been proven. Regurgitation into the glomerular circulation is theoretically possible but has not been shown by morphologic techniques. For any given blood level of macromolecules, mesangial concentration is determined by a dynamic process that depends upon the balance between the afferent and efferent limb. As previously noted, after intravenous injection into normal animals, blood and glomerular levels of AHlgG¹²⁵I decreased linearly over 24 h. The disappearance of macromolecules in obstructed kidneys from UUL(R), UUL(U), and BUL animals was markedly different from normal: a common feature in all three groups was the lack of a significant decrease in mesangial AHlgG¹²⁵I between 4 and 16 h despite the progressive decrease in blood levels of AHlgG¹²⁵I. These findings suggest a blockade of the mesangial efferent limb. The level of mesangial AHlgG¹²⁵I at which the 4 to 16-h plateau occurred was different among the experimental models, and, in each model, a function of the mesangial concentration of AHlgG¹²⁵I present at 4 h. In that respect, in both the released and unreleased kidneys of BUL animals, this plateau was 10-fold greater than that

observed in UUL(U) or UUL(R). The observed alterations in mesangial kinetics were a result of intrarenal factors because there were no differences in the blood levels of AHIgG¹²⁵I among any of the groups. During the 16- to 24-h period of time, a progressive decrease in mesangial AHIgG¹²⁵I occurred in obstructed as well as control kidneys.

The reason for the blockade in the transport of AHIgG¹²⁵I out of the mesangium of kidneys with ureteral obstruction is unclear. During the 4- to 16-h time period, the concentration of AHIgG¹²⁵I in the blood was relatively high, which suggests that the lack of decrease in mesangial concentration was dependent upon high levels of circulating AHIgG¹²⁵I. However, during the 16- to 24-h time period, a significant decrease in mesangial concentration occurred in all models associated with blood levels that were <3% of initial values. These observations suggest at least two mechanisms for disposal of macromolecules from the mesangium: one which is abrogated by ureteral obstruction and is detected at high levels of circulating AHIgG¹²⁵I and the other which is not affected by ureteral occlusion and is evident at low blood concentrations. One possibility to explain these observations is that ureteral obstruction impairs traffic by way of the glomerular stalk to the JG region. At high blood levels, glomerular concentrations change little because of a dynamic equilibrium between the circulating and mesangial pools of AHIgG¹²⁵I. As the blood concentration decreases to low values at the 16- to 24-h time period, this equilibrium becomes imbalanced, and, despite continued ureteral obstruction, egress from the mesangium occurs. This loss may be a consequence of regurgitation of AHIgG¹²⁵I into the circulation by way of the glomerular capillary and/or degradation after pinocytosis and phagocytosis.

Whereas previous physiologic and morphologic studies in animals with ureteral obstruction suggest the nephron function may be heterogenous (10), we were unable to demonstrate differences between cortical and juxtamedullary glomerular uptake of AHIgG¹²⁵I in any of the models using semiquantitative immunofluorescence microscopy. However, with this technique, subtle differences in mesangial AHIgG¹²⁵I may not be appreciated. From the studies performed in uremia-control rats, it is apparent that the differences in the mesangial kinetics between BUL, UUL, and control animals cannot be explained by uremia per se. In addition, in other studies we have shown that rats made acutely uremic with mercury chloride have normal mesangial uptake and release of AHIgG¹²⁵I (33).

The analysis of physiologic parameters measured in these experiments suggests that no single factor can explain the observed differences in mesangial kinetics. These studies do, however, demonstrate that the altera-

tions induced by ureteral obstruction markedly influence the afferent and efferent limb of the mesangium. The possibility that some of these changes in mesangial kinetics may be related to retention or increased production of vasoactive materials cannot be excluded. Experimental data have shown that hydronephrotic kidneys produce increased amounts of the vasodilator prostaglandin E₂ and the vasoconstrictor thromboxane A₂ (34-36). It is interesting to speculate that these mediators or similar vasoactive substances, including angiotensin II (37), could modulate the mesangial afferent and/or efferent limbs by acting upon the contractile elements of the mesangium.

Clinically, the experiments reported here may have a bearing upon the recent report of Mathew et al. (38), who demonstrated that transplanted kidneys with persistent ureteral reflux develop mesangial proliferative changes, findings not present in the original kidneys.

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