

The Glomerular Mesangium

I. KINETIC STUDIES OF MACROMOLECULAR UPTAKE IN NORMAL AND NEPHROTIC RATS

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ABSTRACT This study was designed to define quantitatively the function of the rat glomerular mesangium in the uptake and processing of intravenously administered protein macromolecules (radiolabeled aggregated human IgG, AHIgG-¹²⁵I), to relate this function to that of the general reticuloendothelial system, and to examine the effects of increased glomerular permeability to protein on the mesangial cell system.

Mesangial localization of human IgG as demonstrated by immunofluorescent microscopy showed good correlation with concentrations of AHIgG-¹²⁵I in preparations of isolated glomeruli. In normal rats the concentrations of AHIgG-¹²⁵I in glomeruli were similar to those of lung, liver, and spleen and demonstrated a rapid decrease with increasing time intervals after aggregate administration.

In rats given aminonucleoside of puromycin a marked increase in mesangial uptake of aggregates was found while studies of nephrotic lungs, liver, spleen, and blood showed no such differences. Glomerular levels of AHIgG-¹²⁵I in aminonucleoside animals could not be correlated with the quantity of proteinuria.

Nephrotic and control animals given unaggregated human IgG showed little glomerular localization by immunofluorescent microscopy; no difference in the concentration of this protein in nephrotic as compared to control glomerular isolates was found.

Thus, the mesangium in normal animals functions in a manner analogous to that of the general reticuloendothelial system. In nephrotic rats the mesangial uptake

of macromolecules is markedly increased, a finding not observed in other tissues.

INTRODUCTION

The mesangium is a poorly understood part of the glomerulus occupying, in man, approximately 8% of the glomerular surface area and containing 25% of the total number of glomerular cells (1). The mesangial cells occupy an intercapillary position separated from the capillary lumen, internally, by the endothelial cell cytoplasm and bounded externally by the glomerular basement membrane (2). Light, immunopathologic, and electron microscopic observations of the human kidney in a variety of diseases including anaphylactoid purpura nephritis (3), chronic membranoproliferative nephritis with hypocomplementemia (4), diabetic nephropathy (5), acute poststreptococcal nephritis (6), and lupus erythematosus (7) have demonstrated significant mesangial alterations. However, at present only very limited knowledge of the function of the mesangial system and its relationship to glomerular pathology is available.

Studies of the glomerular localization and transport of macromolecular tracer materials including thorotrast (8), ferritin (2), colloidal carbon (9), and globin (10) have demonstrated mesangial uptake of these substances after their injection into the circulation of normal animals. Thus, Farquhar and Palade have suggested that one function of the mesangium is the removal of filtration residues from the subendothelial space (2). More recently, Michael, Fish, and Good (11) showed that aggregated human gamma globulin, a polydisperse population of macromolecules having many of the properties of antigen-antibody complexes (12), localizes in the mouse glomerulus after intravenous injection. Immunofluorescent studies demonstrated that, within a few hours, there was transport of these aggregates to the

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mesangial region from which they gradually disappeared over the next 36–72 hr.

The purpose of this paper is to describe a method of quantitating mesangial macromolecular uptake and to describe the effects on this mechanism of increased glomerular permeability to protein as induced by aminonucleoside of puromycin (PA).¹ We found that mesangial function in normal rats is similar to that of the general reticuloendothelial system, whereas nephrotic animals demonstrate increased mesangial macromolecular uptake.

METHODS

Preparation of radiolabeled aggregated human gamma globulin and nonaggregated human gamma globulin

Human immune serum globulin² (Cohn fraction II), kindly provided by the American National Red Cross, was labeled with ¹²⁵I³ by the chloramine-T⁴ method of McConahey and Dixon (13) with one modification as suggested by these authors,⁵ i.e., 0.15 M phosphate buffer pH 7.0 was employed instead of 0.05 M phosphate buffer. 1 mCi of ¹²⁵I was used to label each 25 mg of human IgG; 1.0 g of unlabeled human IgG was added at the end of the labeling procedure before removal of free ¹²⁵I on a 30 ml Dowex 1-X8 (100–200 mesh) column.⁶ The efficiency of labeling was approximately 55% and the quantity of free ¹²⁵I in the final product was less than 3%. From this labeled protein heat-aggregated human IgG was prepared according to the methods of Christian (14, 15) and Ishizaka, Salmon, and Fudenberg (12) with certain modifications. To each 100 ml of a 2% human IgG solution, in 0.15 M saline buffer containing 0.01 M sodium phosphate (phosphate-saline buffer), approximately 0.2 mCi of labeled protein was added. This mixture was then heated to 63°C for 30 min and cooled to room temperature. 40 ml of 2.18 M sodium sulphate was added and the mixture was stirred for 30 min. From this point on all procedures were carried out at 4°C. The resulting precipitate obtained by centrifugation at 3000 g for 30 min was resuspended in small quantities of cold phosphate-saline buffer and dialyzed against this buffer for 24 hr with three bath changes. The dialyzed protein solution was centrifuged at 104,000 g for 90 min and the pellet was resuspended in cold buffer and centrifuged at 5000 g to remove insoluble protein aggregates. The concentration of the aggregated human IgG-¹²⁵I (AHIgG-¹²⁵I) was determined by the biuret reaction and adjusted to contain 25 mg of protein per milliliter and merthiolate was added to a final concentration of 1:10,000. The efficiency of the aggregation process was approximately 25%. The free ¹²⁵I of the final AHIgG-¹²⁵I preparation was less than 1% and the

specific activity of the material was about 0.1 μ Ci per milligram protein. The AHIgG-¹²⁵I was stored at 4°C and used within 1 wk of preparation. Bacterial cultures of this material were consistently negative.

Nonaggregated human IgG-¹²⁵I (NAHIgG-¹²⁵I) of similar specific activity to the AHIgG-¹²⁵I was also adjusted to 25 mg of protein per milliliter.

Experimental design

Kinetic studies of mesangial function in normal and nephrotic rats. Nine groups of 10 rats each (male, Sprague-Dawley, 100 g) were given daily subcutaneous injections of 1.5 mg of PA⁷ in 1.0 ml of 0.9 M NaCl for 10 days and for each of these groups an additional 10 rats served as controls. After the last injection, all aminonucleoside animals had marked proteinuria and were clinically nephrotic. 24 hr after the last PA dose, the nephrotic animals and their concurrent controls were each given AHIgG-¹²⁵I in a dose of 0.25 mg per gram body weight by tail vein injection. Phenenergan was administered intraperitoneally to the animals 5 min before the AHIgG-¹²⁵I injection in a dose of 0.005 mg per gram body weight, since this was shown to decrease the incidence of death after aggregate administration. Nephrotic and control animals were sacrificed in groups of 10 at 1, 2, 4, 16, 36, and 72 hr and 1 wk (168 hr) after aggregate administration. Two groups of 10 nephrotic and 10 control animals each were studied 8 hr after AHIgG-¹²⁵I injection. All the animals survived the aggregate injection except for four aminonucleoside animals in the 36 hr group.

The relationship of mesangial function to proteinuria in rats with aminonucleoside nephrosis. Three groups of 10 rats each were given daily subcutaneous injections of 1.5 mg of PA. The first group received PA for 4 days, the second for 6 days, and the third for 10 days. 30 animals in three groups of 10 rats each served as concurrent controls. Urine was collected from PA and control animals by placing them in metabolic cages without food but with free access to water for 24 hr. The cages were rinsed with water and values for 24 hr urinary excretion of protein from this total collection were determined by the biuret reaction on trichloroacetic acid (TCA)-precipitable protein (16). The animals were then given AHIgG-¹²⁵I in a dose of 0.25 mg per gram body weight intravenously and sacrificed 8 hr later by cervical dislocation.

Nonaggregated protein injection in normal and nephrotic rats. 20 animals were given PA for 10 days and were then injected intravenously with NAHIgG-¹²⁵I in a dose of 0.25 mg per gram body weight. 20 normal rats served as controls and received only the NAHIgG-¹²⁵I injections. All animals in this experiment were sacrificed at 8 hr after NAHIgG-¹²⁵I administration.

Immunofluorescent studies

Immediately after sacrifice the kidneys were removed and a cortical slice was placed in formalin for paraffin embedding and subsequent staining with hematoxylin and eosin and periodic acid-Schiff (PAS) reaction. Part of the kidney was immediately frozen in isopentane precooled in liquid nitrogen, sectioned at 4 μ in a Lipshaw cryostat, and processed for immunofluorescence with appropriate controls according to methods previously described (6) using fluorescein-isothiocyanate-labeled rabbit antisera against human

¹ Abbreviations used in this paper: AHIgG-¹²⁵I, radiolabeled aggregated human IgG; GBM, glomerular basement membrane; NAHIgG-¹²⁵I, radiolabeled nonaggregated human IgG; PA, aminonucleoside of puromycin; PAS, periodic acid-Schiff; RES, reticuloendothelial system; TCA, trichloroacetic acid.

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IgG. The amount of immunofluorescence was arbitrarily graded as negative, trace, 1+, 2+, 3+, and 4+; photographs were taken as previously described (6).

Glomerular isolation

Each glomerular isolate was prepared from a pool of the kidneys of five PA or control animals as previously described (17). A mash of decapsulated kidneys was pushed through a 150 mesh monel screen,⁸ the resulting suspension was poured through a 100 mesh screen to exclude large tissue fragments and tubules, and the glomeruli were collected on a 200 mesh screen, washed vigorously with a jet of 0.15 M sodium chloride, and rinsed into a 50 ml centrifuge tube. An aliquot was examined by phase contrast microscopy to ensure that at least 85% purity of the preparation was obtained. The glomeruli were then washed four times with distilled water, lyophilized, weighed, and counted in a crystal scintillation counter for ¹²⁵I. The data were expressed as micrograms AHIgG-¹²⁵I per milligram dried glomeruli plus or minus standard error. In four homogenized glomerular preparations more than 98% of the counts were precipitable by TCA.

Preparation of lung, liver, spleen, and blood for counting for ¹²⁵I

Samples of lung, liver, and spleen were obtained from each animal at the time of sacrifice. These tissues were washed twice in isotonic saline, dried in a ventilated oven overnight at 85°C, weighed, and dissolved in 1.0 N sodium hydroxide containing 0.2 N sodium deoxycholate with heating at 95°C for 1 hr. The dissolved tissue was then cooled to room temperature and counted for ¹²⁵I and the data expressed as micrograms AHIgG-¹²⁵I per milligram dried tissue.

Blood was obtained by cardiac puncture in ether-anesthetized animals just before sacrifice. 50- or 100-μl whole blood samples were added to 2.0 ml of 0.1% sodium carbonate to produce hemolysis. After total counts for ¹²⁵I were obtained, 2.0 cc of 10% TCA was added to produce protein precipitation. The samples were centrifuged at 5000 g and the supernatant separated and counted to determine free ¹²⁵I; protein-bound counts were calculated by subtraction.

RESULTS

Kinetic studies of mesangial function in normal and nephrotic rats

Immunofluorescent microscopy. All aminonucleoside rats in this experiment received PA for 10 days. Nephrotic and control rats were sacrificed at intervals of from 1 to 168 hr after AHIgG-¹²⁵I administration. At 1 hr all animals studied had human IgG widely distributed within the glomerular and peritubular capillaries. The glomerular staining appeared to be localized to the capillary lumen, probably in a subendothelial location (Fig. 1a). At 2 hr little peritubular and glomerular capillary luminal staining was evident and IgG was present along the endothelium and within the mesangium. By 4 hr human IgG was found almost entirely within the

glomeruli and in a mesangial distribution with staining in the center of the glomerular lobules in an arborizing pattern which radiated toward the glomerular hilum in the region of the afferent and efferent arteriole. Rarely, arteriolar staining was seen (Fig. 1b); no fluorescence was seen in peritubular capillaries, tubular lumina, Bowman's space, in the area of the capillary loop, or within or along the glomerular basement membrane (GBM). Thus from 4 to 168 hr the results in Table I may be considered as a comparison, between PA and control kidneys, of the intensity of mesangial deposits of IgG. These results reveal increased mesangial localization of human IgG at each of the time periods from 4 to 168 hr (Fig. 1c and d). As the intensity of mesangial fluorescence diminished with time the axial and central portion of the mesangium lost the staining more rapidly, leaving only the peripheral areas positive for IgG (Fig. 1c and f).

Glomerular isolations. Glomerular levels of AHIgG-¹²⁵I were similar in control and nephrotic animals sacrificed 1 hr after aggregate administration (Fig. 2). Thereafter levels in nephrotic glomeruli were consistently higher than control values. The AHIgG-¹²⁵I content of nephrotic glomeruli rose steadily during the early hours after injection to peak at 8 hr at a mean (four preparations) of 5.04 ± 0.57 μg AHIgG-¹²⁵I per mg glomeruli; control values generally fell over this time period to a mean (four preparations) of 0.535 ± 0.025 μg AHIgG-¹²⁵I per mg glomeruli.

After 8 hr there were no differences between the falloff slope derived from the data of the PA glomerular isolates (-1.232 ± 0.137) and that of control preparations (-0.856 ± 0.206) ($0.1 < P < 0.2$).

Lung, liver, spleen, and blood. During the first 8 hr after the administration of AHIgG-¹²⁵I, at a time when there were striking differences in glomerular uptake between control and PA animals, there were no significant differences in lung, liver, or spleen uptake (Fig. 2). At subsequent time periods slight differences ($P < 0.01$) were seen only at 168 hr in lung (control values higher than PA) and at 36 hr in liver (PA values higher than control).

Blood levels of aggregates decreased rapidly from the values at 1 hr so that by 8 hr only 4.9% of the 1-hr level was still circulating in PA animals and 5.2% in control. Significant differences did not occur at the 1-, 2-, 4-, or 8-hr time periods (Fig. 3). However at 16, 36, 72, and 168 hr blood levels in PA animals were consistently and significantly lower than those in control rats ($P < 0.001$).

Comparison of falloff rates from lung, liver, spleen, and blood. Simple linear regression analyses of falloff slopes derived from data for lung, liver, spleen, and blood from the 8- to 168-hr time periods showed no

⁸ Michigan-Dynamics, Inc., Detroit, Mich.

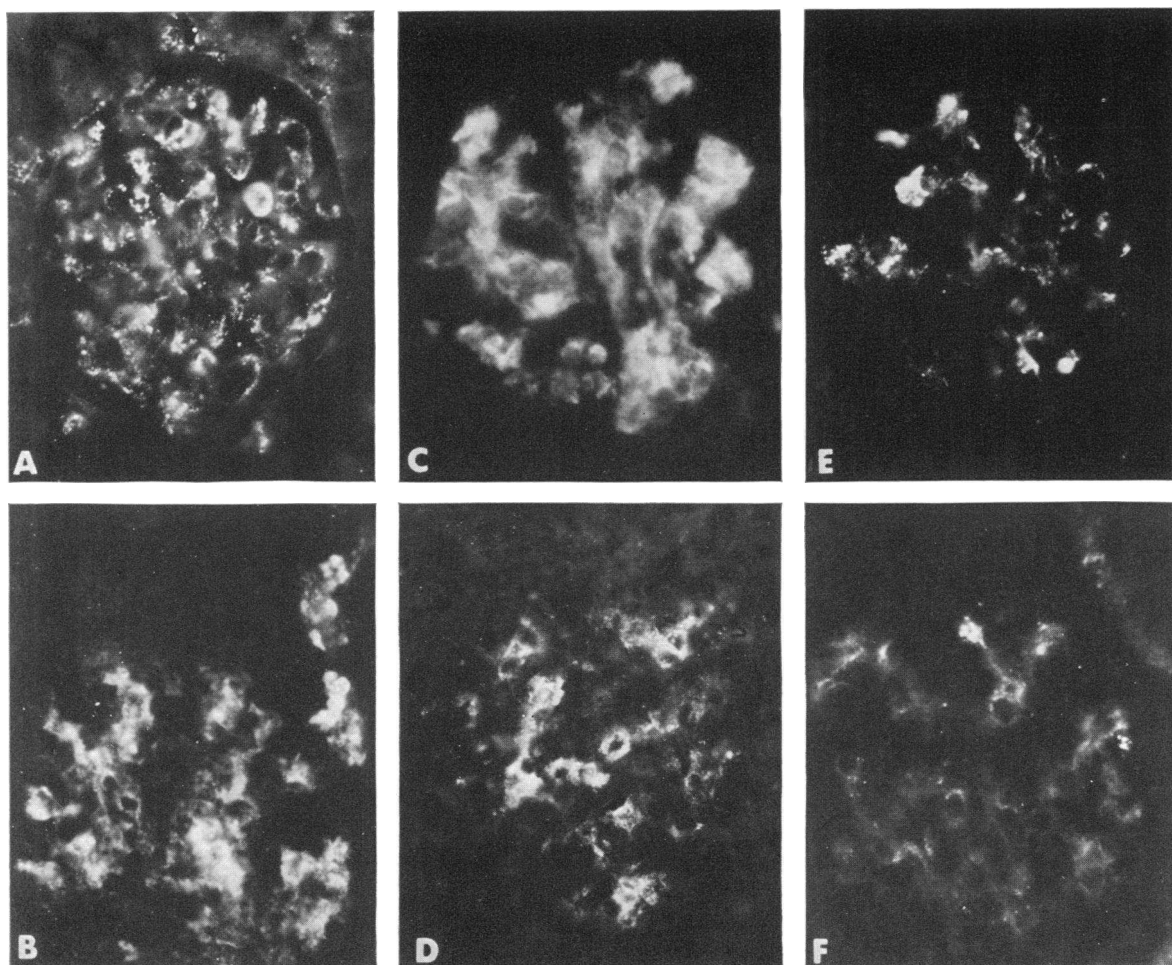


FIGURE 1 Immunofluorescent photomicrographs of glomeruli from: (a) a control rat 1 hr after AHIgG-¹²⁵I administration showing localization of human IgG along the capillary endothelium and in peritubular capillaries; (b) a nephrotic rat 4 hr after AHIgG-¹²⁵I administration showing localization of human IgG in the glomerular mesangium and afferent arteriole; (c) a nephrotic rat 8 hr after AHIgG-¹²⁵I administration showing intense staining for human IgG only in the glomerular mesangium; (d) a control rat 8 hr after AHIgG-¹²⁵I administration showing considerably less glomerular mesangial localization of human IgG as compared with Fig. 1c; (e) a nephrotic rat 36 hr after AHIgG-¹²⁵I administration showing a reduction of the human IgG localized in the glomerular mesangium as compared with Fig. 1b and Fig. 1c; (f) a control rat 36 hr after AHIgG-¹²⁵I administration showing less glomerular mesangial localization of human IgG as compared with the 36 hr nephrotic glomerulus (Fig. 1e) and the 8 hr control glomerulus (Fig. 1d). The tubular staining seen above the glomerulus represents autofluorescence.

significant differences between tissues of PA and control groups.

Relationship of mesangial function to proteinuria in rats with aminonucleoside nephrosis

Urinary protein excretion. Values for 24 hr urinary excretion of protein in animals treated with PA for 4 days did not differ significantly from controls (Table II). 6-day PA rats had minimal proteinuria but differed

significantly from controls ($P < 0.001$). After 10 days of PA administration all rats were clinically nephrotic and the mean urinary protein excretion was more than 60 times that of controls (Table II).

Immunofluorescent microscopy. 8 hr after AHIgG-¹²⁵I administration the pattern of human IgG localization in the kidneys of PA and control animals was qualitatively similar and was present only in the glomerular mesangium.

TABLE I
Intensity of Glomerular Staining for Human IgG at Various
Time Intervals after Aggregated Human
IgG (AHlgG-¹²⁵I) Administration

Hours after AHlgG- ¹²⁵ I admin- istration		Intensity of glomerular staining for human IgG				
		Trace	1+	2+	3+	4+
1	PA*				5	
	Control			2	3	
2	PA			2	3	
	Control		2	3		
4	PA					5
	Control			1	4	
8	PA				3	2
	Control			5		
16	PA			1	4	
	Control		5			
36	PA		2	3		
	Control	2	3			
72	PA		3	2		
	Control	3	2			
168	PA	1	4			
	Control	3	2			

* Five animals were studied from each of the PA and control groups. The numbers represent the number of animals per group with a given intensity of fluorescence on a scale of trace to 4+. PA animals received aminonucleoside for 10 days.

The intensity of mesangial staining for IgG was similar within the three control groups (Table II). Of the 30 control kidneys studied 25 had intensity ratings of

1+ or 2+, five were 3+, and none were 4+. No clear distinction could be made between the kidneys of 4-day, 6-day, and 10-day aminonucleoside animals. However, in these specimens the intensity of mesangial staining was clearly greater than controls. 27 kidneys were rated at 3+ or 4+, only three were 2+, and none were 1+.

Glomerular levels of AHlgG-¹²⁵I. Values for AHlgG-¹²⁵I in glomeruli isolated from 4-, 6-, and 10-day aminonucleoside rats were considerably higher than those obtained from control animals (Table II). However, one of the control values from the 4 day experiment (3.19 μ g AHlgG-¹²⁵I per mg glomeruli) was much higher than that obtained in more than 20 control glomerular isolations. For this reason the 4-day PA studies were repeated and values from experimental animals of 4.23 and 5.88 μ g AHlgG-¹²⁵I per mg glomeruli were clearly higher than concurrent controls which were 1.62 and 1.74.

There was excellent correlation between the intensity of mesangial fluorescence and glomerular levels of AHlgG-¹²⁵I obtained by counting glomerular isolates (Table II).

AHlgG-¹²⁵I in lung, liver, spleen, and blood. There were no significant differences in the levels of AHlgG-¹²⁵I in liver, spleen, or blood on comparing 4-day aminonucleoside animals with controls (Table III). The level in the lungs of control animals in the 4 day PA experiment were higher than control (0.001 < P < 0.005).

In the 6 day PA experiment lung, spleen, and blood studies showed no differences; however the AHlgG-¹²⁵I level in the liver of nephrotic animals was higher than controls (0.001 < P < 0.005) (Table III).

Studies of lung, liver, spleen, and blood levels in 10-

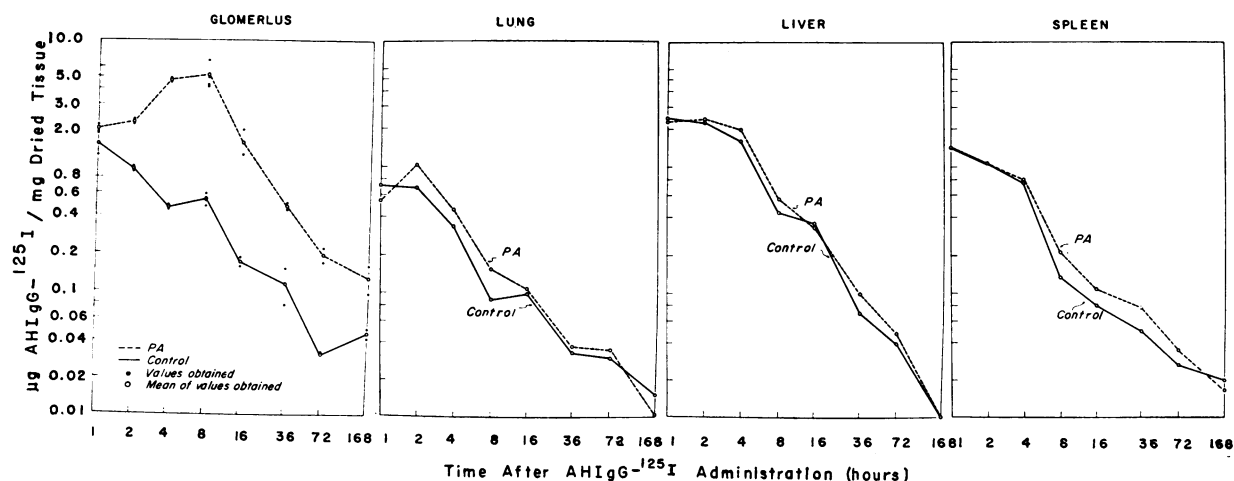


FIGURE 2 AHlgG-¹²⁵I in preparations of isolated glomeruli and in the lungs, livers, and spleens in normal and nephrotic rats at varying time intervals after aggregate administration.

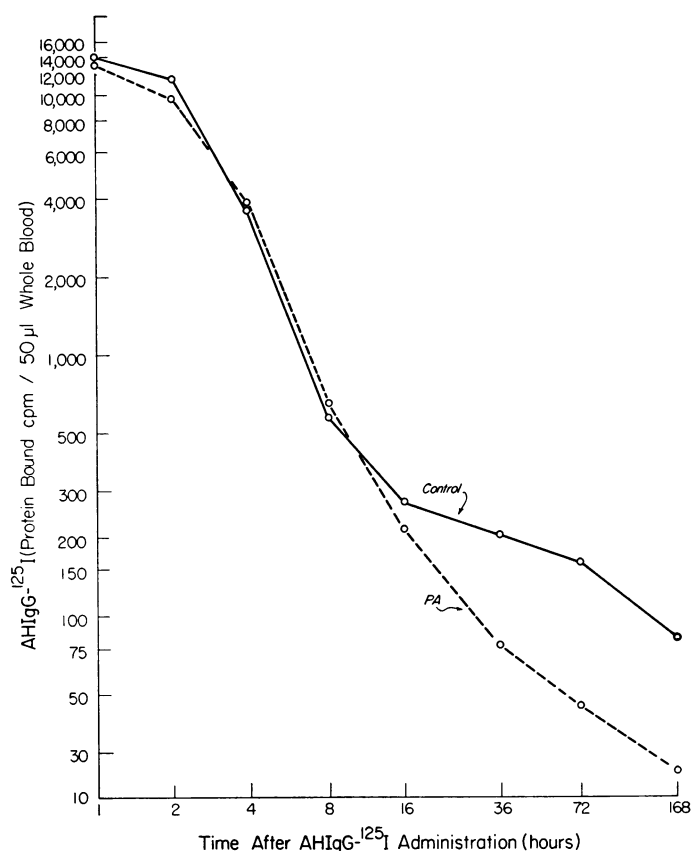


FIGURE 3 AHlgG- 125 I in the blood of normal and nephrotic rats at varying time intervals after aggregate administration.

day nephrotic animals and controls were presented above (Fig. 2).

Nonaggregated protein injection in normal and nephrotic rats

In this experiment, 20 10-day aminonucleoside animals and 20 controls received NAHlgG- 125 I and were sacrificed 8 hr later. Immunofluorescent microscopy revealed little renal localization. Slight fluorescence was seen in the peritubular capillaries of most animals and minimal staining was seen in glomerular capillary lumina. Mesangial localization in trace quantities was observed in some animals. There was no difference in the immunofluorescent findings of nephrotic as compared to control kidneys.

Glomerular levels of NAHlgG- 125 I were similar in both groups with mean nephrotic and control values of 1.00 and 0.93 μ g NAHlgG- 125 I per mg glomeruli respectively (Table IV). No differences were found in liver, spleen, or blood values but there was a slightly higher concentration of NAHlgG- 125 I in the lungs of nephrotic rats ($P < 0.01$).

DISCUSSION

The mesangial or "third cell" system of the glomerulus has been well defined anatomically as occupying a strategic intercapillary position in the glomerular stalk with cytoplasmic processes extending for some distance and in several directions between the endothelial cell and the GBM (2). Individual mesangial cells are separated from one another by amorphous fibrillar PAS-positive material of unknown composition, the mesangial matrix. Despite the important involvement of this cell system in several pathological states little is known of its functional significance. Substances entering the glomerulus via the afferent arteriole may be filtered, excluded and passed into the efferent arteriole, or may be "trapped" in the glomerulus in or along the GBM, or may pass into the mesangium. The study of the mesangial mechanisms involved in the glomerular handling of macromolecular substances has previously been approached by anatomical techniques (2, 8, 9, 10, 11). In this report an attempt was made to apply quantitative methodology to the examination of the kinetics of mesangial uptake (afferent

TABLE II
Relationship of Mesangial Uptake of Aggregated Human IgG (AHI-¹²⁵I) to Proteinuria in Rats after Aminonucleoside of Puromycin (PA) Administration

			Intensity of mesangial staining for human IgG by immunofluorescent microscopy‡				Glomerular AHIgG- ¹²⁵ I	
		Urinary protein excretion	1 +	2 +	3 +	4 +		
		mg/24 hr*					µg/mg glomeruli§	
Day 4 of PA admin.	PA	3.3	0	1	6	3	3.33	4.23
	N = 10	(1.9–6.1)					4.36	5.88
		N = 10						
	Control	2.1	2	6	2	0	3.19	1.62
	N = 10	(1.6–3.0)					1.49	1.74
		N = 7						
Day 6 of PA admin.	PA	16.0	0	1	4	5	9.24	
	N = 10	(3.8–75)					5.92	
		N = 10						
	Control	2.4	4	5	1	0	1.12	
	N = 10	(1.2–7.1)					2.09	
		N = 7						
Day 10 of PA admin.	PA	109	0	1	4	5	4.59	
	N = 10	(22.7–204)					5.91	
		N = 10						
	Control	1.7	4	4	2	0	1.08	
	N = 10	(0.2–4.9)					1.38	
		N = 8						

* Protein excretion expressed as mean and range. N = number of animals tested.

† The PA and control groups contain 10 rats each. The numbers represent the number of animals per group with a given intensity of fluorescence on a scale of 1+ to 4+.

§ Each value represents the concentration of AHIgG-¹²⁵I in one glomerular isolation from a pool of kidneys of five animals. All animals were sacrificed 8 hr after AHIg-¹²⁵I administration.

|| Values derived from repeat of the 4 day PA experiment.

limb) and loss (efferent limb) of a macromolecular material in the normal and injured glomerulus.

The glomerular levels of intravenously administered radiolabeled immunoglobulin aggregates (AHIgG-¹²⁵I) were measured in isolated glomeruli from normal and nephrotic rats and results of these studies were correlated with qualitative immunofluorescent observations. Using the latter technique, the findings, in mice, of Michael et al. (11), which demonstrated the importance of the mesangium in the glomerular handling of protein macromolecules, were confirmed. In the early time periods after aggregate administration considerable quantities of IgG were seen in the region of the peripheral capillary loop. The immunofluorescent technique does not allow precise localization of this material which may have been in the capillary lumen, the GMB, or the sub-endothelial space. Previous electron microscopic studies, however, support the latter possibility (18). 4 hr after infusion, the IgG was localized entirely within the glomerulus and was confined almost totally to the mesangial area. In normal rats peak glomerular concentrations of AHIgG-¹²⁵I are comparable to those of lung, liver, and spleen and the falloff rates from these tissues are similar. These data suggest that, at least with large quantities of circulating macromolecules, the mesangial cell system is an analogue of the general reticuloendothelial system (RES). Benacerraf, McCluskey, and Patras (9) demonstrated glomerular localization of colloidal carbon only after prior "overloading" of the RES with repeated large doses of this substance. We have not studied the relationship of the RES to mesangial function with smaller doses of AHIgG-¹²⁵I.

The mechanism of macromolecular uptake and transport by the mesangium in normal animals is unclear.

TABLE III
Aggregated Human IgG (AHIgG-¹²⁵I) in Lung, Liver, Spleen, and Blood in Aminonucleoside (PA) and Normal Rats

	Days of PA administration	PA	Control	P
Lung, µg AHIgG- ¹²⁵ I/mg (dry weight)	4	0.12±0.01*	0.16±0.005	0.001 < P < 0.005
	6	0.11±0.01	0.10±0.01	>0.1
Liver, µg AHIgG- ¹²⁵ I/mg (dry weight)	4	0.58±0.04	0.57±0.04	>0.1
	6	0.60±0.03	0.39±0.05	0.001 < P < 0.005
Spleen, µg AHIgG- ¹²⁵ I/mg (dry weight)	4	0.20±0.04	0.30±0.04	0.05 < P < 0.1
	6	0.13±0.02	0.16±0.01	>0.1
Blood protein bound, cpm/50 µl whole blood	4	1713±131	1843±135	>0.1
	6	1910±134	1867±115	>0.1

All animals were sacrificed at 8 hr after AHIgG-¹²⁵I administration.

* N = 10 for all lung, liver, spleen, and blood studies. Number presented is mean of 10 values plus or minus standard error.

Farquhar, Wissig, and Palade found ferritin in the mesangial matrix and in vacuoles within the cytoplasm of mesangial cells within 15 min after injection into the circulation of normal rats (18). Vernier and Birch-Anderson (19) demonstrated the rapid transport of ferritin and colloidal carbon into the mesangial cells of the human fetus minutes after intravascular administration. Similar results have been obtained with other substances including thorotrast, human globin, and colloidal iron. Michael et al. (11) observed by electron microscopy that, in mice, ferritin-labeled protein aggregates appeared within channels between glomerular mesangial cells as opposed to direct cellular phagocytosis. The precise relationships of particle type, size, and charge to the mesangial transport and phagocytic mechanisms remain to be determined.

The mechanism of loss of aggregates from the mesangium with time is unknown but may be due to local digestion and/or transport out of glomerulus. The recent demonstration by Barajas (20) that the mesangium extends into and fills the space between the afferent and efferent arterioles at the glomerular hilum and has extensive contact with the distal tubule and macula densa provides an anatomical pathway out of the glomerulus which could be related to the renal lymphatic system.

Rats made nephrotic by 10 days of PA administration have a marked increase in the mesangial localization of IgG by immunofluorescent microscopy and by direct counting of AHIgG-¹²⁵I in preparations of isolated glomeruli. This confirms the electron microscopic observations of Farquhar and Palade (21) demonstrating greater mesangial deposition of intravenously administered ferritin in rats made nephrotic with PA. Peak glomerular uptake in nephrotic rats occurred at 8 hr after aggregate administration, whereas glomerular levels in control rats fell over this time period so that at 8 hr AHIgG-¹²⁵I in nephrotic glomeruli was 9.4 times that found in control preparations. Levels in nephrotic glomeruli were increasing while blood levels of AHIgG-¹²⁵I were rapidly falling suggesting that, in aminonucleoside rats, mesangial uptake can occur with relatively small quantities of circulating aggregates. Our simultaneous studies of AHIgG-¹²⁵I in lung, liver, spleen, and blood in nephrotic and control animals showed no depression of RES function in nephrotic animals. Indeed blood levels of AHIgG-¹²⁵I after the 8 hr time period were consistently lower in nephrotic animals. This could reflect urinary loss of protein fragments, increased protein catabolism, increased glomerular uptake, or slight RES stimulation, which could not be detected by our techniques. After 8 hr, the falloff of AHIgG-¹²⁵I from nephrotic glomeruli with time was parallel to that of controls indicating that there was greater mesangial uptake of aggregates rather than diminished rate of loss.

TABLE IV
Nonaggregated Human IgG (NAHIgG-¹²⁵I) in Glomeruli, Lung, Liver, Spleen, and Blood in Aminonucleoside (PA) and Normal Rats

	PA	Control	P
Glomeruli, μg NAHIgG- ¹²⁵ I/mg (dry weight)	1.22* 0.94 0.84	1.00 0.99 0.80	Not done
Lung, μg NAHIgG- ¹²⁵ I/mg (dry weight)	3.79 ± 0.11‡	3.05 ± 0.22	<0.01
Liver, μg NAHIgG- ¹²⁵ I/mg (dry weight)	1.39 ± 0.07	1.75 ± 0.22	0.05 < P < 0.1
Spleen, μg NAHIgG- ¹²⁵ I/mg (dry weight)	1.64 ± 0.06	1.52 ± 0.11	>0.1
Blood protein bound, cpm/50 μl whole blood	4135 ± 186	4136 ± 190	>0.1

PA animals received aminonucleoside for 10 days and all animals were sacrificed 8 hr after NAHIgG-¹²⁵I administration.

* Each value represents one glomerular isolation from a pool of kidneys of 5 or 10 animals.

‡ N = 10 for all lung, liver, spleen, and blood studies. Number presented is mean of 10 values plus or minus standard error.

One possible explanation of the greater uptake in aminonucleoside animals is glomerular endothelial cell damage with increased permeability to macromolecules and consequent exposure of the mesangium to larger quantities of circulating aggregates. However, electron microscopic observations of this cell in PA rats have not revealed pathologic alterations (22, 23). Further, with the dose of AHIgG-¹²⁵I used in these studies there were no marked differences between nephrotic and control animals in the capillary localization seen in the early time periods after aggregate injection. However, we have found massive capillary type localization in nephrotic animals given larger doses of aggregates (0.5 mg per gram of body weight), while controls showed only mesangial localization (unpublished data). Alternately, the mesangial function may be stimulated directly by PA itself or by increased glomerular capillary permeability to protein. Our studies of the relationship of the quantity of proteinuria to mesangial activity demonstrated increased mesangial uptake after 4 days of PA administration when there was no significant proteinuria. It is possible, however, that increased glomerular permeability to protein was present at this time, but because of tubular reabsorption of protein, this was not reflected in the urine of these animals. There was no difference in the uptake with mild proteinuria (6 days of PA administration) as compared to marked proteinuria (10 days of PA).

Immunofluorescent studies of kidneys after administration of nonaggregated IgG (NAHIgG-¹²⁵I) demonstrated

little glomerular localization with most of the IgG present in vascular lumina throughout the tissue. The levels of NAHIgG-¹²⁵I in preparations of isolated glomeruli 8 hr after infusion probably reflect the large quantity of circulating labeled protein present at this time. The absence of significant mesangial deposits of IgG in either normal or nephrotic animals and the lack of differences between these groups by direct glomerular counts suggest that the phenomena observed with AHIgG-¹²⁵I are specific for macromolecular material.

Germuth, Valdes, Senterfit, and Pollack (24) demonstrated that, in rabbits developing acute serum sickness nephritis, cortisone inhibited the development of glomerulitis and immune complexes localized preferentially in the mesangium as opposed to the subepithelial portion of the capillary loop. These authors theorized that the effect of cortisone was to diminish glomerular basement membrane permeability to circulating immune complexes thus preventing transmigration of these complexes across the filtration barrier for plasma proteins. Our studies clearly demonstrate that increased permeability to protein in aminonucleoside nephrosis is associated with increased mesangial uptake of circulating macromolecules.

It is known that the administration of aminonucleoside results, in most rats, in chronic renal disease with progressive mesangial hyperplasia and lobular sclerosis (25), which is associated with mesangial deposition of host (rat) immunoglobulins and to a lesser extent, complement components (26, 27). It is possible that in these animals endogenous circulating immune complexes gain access to the mesangium and eventually produce chronic changes. Focal sclerosis, mesangial proliferative changes, stalk hyalinization, and, eventually, glomerular fibrosis can occur in human glomerulonephritis and nephrotic syndrome. Although there is no direct way of measuring mesangial function in man, the further application of quantitative techniques to the study of mesangial function in animals may provide insights into the relationships between the mesangial cell system and glomerular disease.

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