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# Absence of focal glomerulosclerosis in aging analbuminemic rats

CLARICE KAZUE FUJIHARA, DIRCE MARIA ZANETTA PAPATERRA LIMONGI, HELENA COUTINHO FRANCO DE OLIVEIRA, AND ROBERTO ZATZ (With the Technical Assistance of Regina Maria Padilha and Marinete Miristeni dos Santos)
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**Fujihara, Clarice Kazue, Dirce Maria Zanetta Papaterra Limongi, Helena Coutinho Franco de Oliveira, and Roberto Zatz.** Absence of focal glomerulosclerosis in aging analbuminemic rats. *Am. J. Physiol.* 262 (*Regulatory Integrative Comp. Physiol.* 31): R947–R954, 1992.—The Nagase analbuminemic rat (NAR), a mutant of the Sprague-Dawley (SD) strain, exhibits high levels of plasma cholesterol (Chol), thrombocytosis, and enhanced platelet aggregability, which might promote glomerulosclerosis (GS). To determine whether NAR are more susceptible than SD rats to aging GS, young (3-mo-old) and aging (18-mo-old) SD rats and NAR were studied. In young NAR, glomerular pressure and glomerular volume were lower, whereas total and high-density lipoprotein plasma Chol levels were higher than in young SD rats. Aging SD rats developed glomerular hypertension and hypertrophy. Less glomerular enlargement and subnormal glomerular pressures were seen in aging NAR. Enhanced platelet aggregation developed in aging SD rats, approaching the values seen in NAR. Similarly elevated levels of low-density lipoprotein Chol were seen in additional SD rats and NAR studied at 12 mo of age. Plasma triglyceride (TG) levels were lower in NAR at this age. Only SD rats developed proteinuria and exhibited GS and glomerular lipid deposits at 18 mo of age. Reduced glomerular wall stress due to lower glomerular pressure and volume as well as lower TG levels may explain the absence of GS in aging NAR despite plasma lipid and platelet abnormalities.

glomerular pressure; glomerular volume; plasma cholesterol; Nagase analbuminemic rat; Sprague-Dawley rat

IN HUMANS and several rat strains (5,7), progressive glomerulosclerosis (GS) develops with advancing age. The mechanisms underlying this disorder are unclear. Conceivably, any of the mechanisms currently believed to mediate glomerular injury may be involved in the pathogenesis of aging GS. These include glomerular hypertension (2, 3, 13), glomerular hypertrophy (10), intraglomerular coagulation (15, 21), and lipid accumulation (13, 14, 17, 18), among others. However, the possible pathogenetic association of these factors with the process of aging GS has not been systematically examined.

In the Nagase analbuminemic rat (NAR), a mutant of the Sprague-Dawley (SD) strain (20), at least two of these pathogenetic mechanisms may conspire to promote glomerular injury. First, these animals are characterized by marked elevations in plasma cholesterol, an abnormality that has been incriminated in the pathogenesis of GS in the obese Zucker rat (17) and in rats with renal ablation (13, 18). In addition, NAR exhibit pronounced thrombocytosis and enhanced platelet aggregation (13). These abnormalities may also facilitate the development of GS, since platelet aggregation and fibrin deposits have been described in association with progressive glomerular injury (21). No conspicuous tendency toward the development of spontaneous GS has been observed in 3- (13) or 12-mo-old NAR (16). Studies of glomerular morphology of NAR beyond 12 mo of age are lacking. However, GS of unusual severity associated with renal insufficiency was observed in NAR 60 days after five-sixths renal ablation (13), suggesting that hypercholesterolemia and platelet abnormalities may aggravate glomerular injury in these animals.

In this study, we sought to determine whether the possible pathogenetic effect of these lipid and platelet abnormalities would render NAR more susceptible than SD controls to the development of age-related GS. We also examined the role of arterial pressure variations as well as the possible detrimental effects of intraglomerular mechanisms such as intracapillary hypertension, tuft enlargement, and lipid accumulation.

## METHODS

*Experimental groups.* Forty-seven SD rats and 46 NAR were utilized in this study. All rats were adult males and had free access to standard (24% protein) chow and tap water. NAR were originally purchased from Clea Japan (Tokyo, Japan). Four groups of rats were examined in this study: young (3-mo-old) SD rats (body wt range 261–349 g) (*SDY*), young NAR (279–330 g) (*NARY*), aging (18-mo-old) SD rats (437–556 g) (*SDAg*), and aging NAR (464–644 g) (*NARAg*). All groups were subjected to micropuncture or platelet functional studies as described below. A separate cohort of 29 SD rats and NAR was utilized for lipid studies (see below).

*Platelet aggregation studies.* To examine platelet function, eight rats from *group SDY*, eight from *group NARY*, eight from *group SDAg*, and eight from *group NARAg* were anesthetized with pentobarbital sodium (50 mg/kg ip), and a total of 4 ml blood was collected from the abdominal aorta with 0.8-mm needles and plastic syringes. A 0.5-ml blood aliquot was anticoagulated with sodium EDTA, and 20 $\mu$l of blood were diluted to 1:100 in 1% ammonium oxalate. Platelets were then counted utilizing a phase-contrast microscope (8). To analyze platelet aggregation properties, the remaining blood was anticoagulated with 3.8% sodium citrate (vol/vol 9:1) and centrifuged at ~150 *g* for 10 min to obtain platelet-rich plasma (PRP). The remaining blood was then centrifuged at ~1,300 *g* for an additional 20 min to obtain platelet-poor plasma (PPP). Platelet counts were performed in PRP as described. PRP was then diluted with PPP until a standard platelet count of 300,000/mm$^3$ was obtained, and platelet aggregation was evaluated utilizing an aggregometer (Minigator, Payton Associates, Toronto, Canada). Platelet aggregation was quantitatively expressed as the maximal percentage change in optical density after addition of ADP (3 $\mu$M/ml) (6).

*Micropuncture studies.* Eight rats from *group SDY*, eight from *group NARY*, eight from *group SDAg*, and eight from *group NARAg* were anesthetized with Inactin (100 mg/kg body wt ip) and placed on a temperature-controlled micropuncture

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table. Rectal temperature was maintained at 37 ± 0.5°C. The femoral artery was cannulated with PE-50 polyethylene tubing for periodic blood sampling as well as for determination of mean arterial pressure (MAP) and baseline hematocrit. MAP was monitored utilizing a P23 Db Statham transducer connected to a chart recorder (model 611, Beckman Instruments, Schiller Park, IL). After tracheostomy, the jugular veins were catheterized for plasma and inulin infusion. Saline solution containing inulin (7.5 g/dl) was infused at the rate of 1.5 ml/h throughout the experiment. In both SD rats and NAR, respective homologous plasma was infused intravenously throughout the study to replace surgical losses, as described previously (13). The left kidney was exposed by a subcostal incision, freed from the adrenal gland and perirenal fat, and immobilized by means of a Lucite holder. The renal surface was continuously bathed with isotonic saline solution. The left ureter was catheterized with PE-10 polyethylene tubing. Hydraulic pressures in cortical structures were determined employing a servo-nulling system (model V, Instrumentation for Physiology and Medicine, San Diego, CA). Glomerular hydraulic pressure was measured by a stop-flow technique (1). Urine collections of 20- to 30-min duration were obtained for the determination of whole kidney glomerular filtration rate (GFR). Blood aliquots (100 μl) were obtained at the middle of the experiment for determination of plasma total protein and cholesterol concentrations. At the end of the experiment, the left kidney was weighed and fixed in 10% formaldehyde solution in phosphate buffer for specific histochemical analysis of glomerular lipid deposition. The right kidney was perfusion fixed in situ with 3% glutaraldehyde in phosphate buffer for histological examination and estimation of glomerular tuft volume.

*Plasma lipoprotein studies.* In a separate set of 3-mo-old SD rats ($n = 6$) and NAR ($n = 6$) anesthetized with pentobarbital sodium (50 mg/kg ip), 10 ml of blood were obtained from the abdominal aorta to determine total cholesterol and triglyceride (TG) concentrations as well as cholesterol distribution among very low-density (VLDL), low-density (LDL), and high-density (HDL) lipoproteins. Blood collections were performed after a 12-h fast to minimize the influence of plasma chylomicra, thus restricting our observations to the endogenous lipoprotein pathway. To examine the variation with age of plasma lipoprotein profiles, this procedure was repeated in 12-mo-old SD rats ($n = 9$) and NAR ($n = 8$). Plasma lipoproteins were separated by preparative discontinuous density gradient ultracentrifugation utilizing the following density ranges: below 1.006 g/ml (VLDL), 1.006–1.063 g/ml (LDL), and 1.063–1.210 g/ml (HDL). Chylomicra still remaining in plasma were removed by a similar ultracentrifugation procedure, carried out previously for 30 min. Fractions were stored in a deepfreeze for subsequent determination of cholesterol concentration. Details of the ultracentrifugation procedure are given elsewhere (24).

*Follow-up studies.* At 3, 6, 9, 12, and 18 mo of age, mean systemic arterial pressure was evaluated in aging SD rats and aging NAR using a tail-cuff method (30). Rats were then placed in metabolic cages for determination of 24-h urinary protein excretion rate by the sulfosalicylic method.

*Histological techniques.* In both short- and long-term studies, renal tissue was processed as follows. *1)* Renal tissue was perfusion fixed with glutaraldehyde. After fixation, two midcoronal slices of renal tissue, 2–3 mm thick, were embedded in paraffin and 3-μm-thick sections were stained by the periodic acid-Schiff (PAS) reaction. The renal tissue was inspected under light microscopy at a final magnification of ×400. At least 300 glomeruli were randomly examined per kidney. The extent of glomerular injury was evaluated by counting glomeruli exhibiting sclerotic lesions and expressing this amount as a percentage of the total number of glomeruli examined. The method utilized to estimate average glomerular tuft volume in glutaraldehyde-perfused renal tissue is described elsewhere (13). *2)* Renal tissue was fixed with formaldehyde solution. Histochemical analysis of glomerular lipid deposition was performed in 8-μm-thick frozen sections stained with oil red O and counterstained with hematoxylin.

*Analytic techniques.* Protein concentration in systemic plasma was determined by the biuret reaction (19). Inulin concentrations in plasma and urine samples were assessed by the anthrone technique (29). Plasma cholesterol and TG concentrations were determined by an enzymatic method utilizing commercially available kits (Sera-Pak, Ames Division, Miles do Brasil, and TG-Enz-Color, Biodiagnostica, São Paulo, Brazil).

*Statistical analysis.* To evaluate the overall effects of aging as well as differences between the two strains of rats examined in this study, data were analyzed according to a two-way (2 × 2) factorial design (28) associated with simultaneous pairwise comparisons according to the Bonferroni method (28). Differences in tail-cuff pressure and urinary protein excretion rate were assessed by a multigroup repeated measurements design also associated with pairwise comparisons (28). Because protein excretion rate and the prevalence of GS lesions were not normally distributed, log (for proteinuria) and arcsin (for % sclerosis) transformations were performed before statistical analysis. All results are expressed as means ± SE. Differences were taken as significant at $P \leq 0.05$.

## RESULTS

Systemic and renal hemodynamic parameters obtained for young and aging SD rats and NAR are summarized in Table 1. Body growth of similar magnitude was observed with age in SD rats and NAR. MAP tended to be lower in NAR than in age-matched SD controls, although this difference was statistically significant only in aging rats. Age-related renal enlargement was observed in both SD rats and NAR, although renal size was always smaller in the latter. Systemic plasma protein concentration was slightly lower in NAR than in SD rats, particularly in 18-mo-old rats. As previously reported for this strain

Table 1. *Systemic and renal hemodynamic parameters in young and aging rats*

| Group | Body Wt, g | Left Kidney Wt, g | MAP, mmHg | $P_{Prot}$, g/dl | GFR, ml/min | Pressures, mmHg | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | $P_{sf}$ | $\pi_A$ | $P_{GC}$ |
| *SDY* | 298±12 | 1.25±0.06 | 118±4 | 5.7±0.1 | 1.47±0.10 | 32±1 | 21±0.4 | 53±1 |
| *NARY* | 311±7 | 0.94±0.04* | 109±5 | 5.5±0.1 | 1.26±0.03 | 31±1 | 16±0.4* | 47±1* |
| *SDAg* | 508±13† | 1.80±0.10† | 124±3 | 5.8±0.2 | 1.66±0.13 | 38±2† | 21±0.7 | 59±2† |
| *NARAg* | 522±19† | 1.33±0.07*† | 102±3* | 5.2±0.1* | 1.36±0.09 | 30±1* | 15±0.4* | 45±1* |

Values are means ± SE; $n = 8$/group. Groups: *SDY*, young (3-mo-old) Sprague-Dawley rats; *NARY*, young Nagase analbuminemic rats; *SDAg*, aging (18-mo-old) SD rats; *NARAg*, aging NAR. MAP, mean arterial pressure; $P_{Prot}$, systemic plasma protein concentration; GFR, glomerular filtration rate; $P_{sf}$, stop-flow pressure; $\pi_A$, systemic plasma oncotic pressure; $P_{GC}$, glomerular capillary hydraulic pressure. * $P < 0.05$ NAR vs. SD. † $P < 0.05$ aging vs. young.

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(26), plasma oncotic pressure was 5–6 mmHg lower in NAR compared with SD controls.

Although GFR increased numerically with age in both SD rats and NAR, no significant difference among groups could be demonstrated. As shown previously (13, 26), glomerular hydraulic pressure was lower in young NAR than in SD controls. This difference was exacerbated with age, since a significant elevation in glomerular hydraulic pressure was observed in aging SD rats, whereas it remained essentially unaltered with age in NAR.

Nonhemodynamic risk factors potentially involved in the pathogenesis of GS are listed in Table 2. As expected, total plasma cholesterol concentration was nearly twice as high in young NAR than in SD controls. This difference was largely attenuated in aging rats, since plasma cholesterol levels were markedly elevated in aging SD rats compared with young SD rats, whereas a slight decrease in cholesterol levels was observed in aging NAR. Glomerular volume was lower in young NAR than in young SD rats ($0.91 \pm 0.01 \times 10^6$ vs. $1.14 \pm 0.06 \times 10^6$ $\mu m^3$, $P < 0.05$). Age-related glomerular enlargement was observed in both SD rats and NAR, but to a larger extent in SD rats. As a result, glomerular volume was much lower in aging NAR than in aging SD rats ($1.39 \pm 0.08 \times 10^6$ vs. $1.94 \pm 0.05 \times 10^6$ $\mu m^3$, $P < 0.05$). As shown previously (13), young NAR exhibited increased platelet count and enhanced platelet aggregation compared with SD controls. Thrombocytosis persisted and was even exacerbated in NAR. In aging SD rats, platelet aggregation was enhanced and rendered indistinguishable from that observed in NAR.

Plasma TG and total cholesterol concentrations as well as cholesterol fractions are given in Table 3. TG concentration was numerically lower in young NAR compared with SD rats. This difference was more evident and became statistically significant at 12 mo of age, since plasma TG levels were numerically increased in SD rats and decreased in NAR. Values obtained in this set of rats for plasma total cholesterol concentration were comparable to those shown in Table 2. No significant difference in VLDL-C was shown between SD rats and NAR at 3 mo of age. At 12 mo of age, VLDL-C remained stable in SD rats, whereas a marked decrease was seen in NAR. LDL-C levels were higher in young NAR than in age-matched SD rats, and increased with age in both SD rats and NAR. However, because this elevation was proportionally larger in SD rats, no difference was observed between SD rats and NAR at 12 mo of age. HDL-cholesterol concentration was markedly elevated in young NAR compared with SD controls. With age, HDL-cholesterol levels decreased in NAR and increased in SD rats. No difference in HDL-cholesterol was observed between SD rats and NAR at 12 mo of age.

Table 2. *Nonhemodynamic risk factors for glomerular injury in young and aging rats*

| Group | Chol, mg/dl | $V_G$, $\mu^3 \times 10^6$ | PC, $\times 10^3$/mm$^3$ | PA, %ΔOD |
|---|---|---|---|---|
| *SDY* | 63±3 | 1.14±0.06 | 590±60 | 48±3 |
| *NARY* | 127±7* | 0.91±0.01* | 798±60* | 66±2* |
| *SDAg* | 92±8† | 1.94±0.05† | 654±18 | 65±2† |
| *NARAg* | 114±3 | 1.39±0.08*† | 1,007±28*† | 69±3 |

Values are means ± SE; $n$ = 8/group. Groups: same as in Table 1. Chol, serum cholesterol concentration; $V_G$, mean glomerular tuft volume; PC, platelet count; PA, platelet aggregation to ADP (3 μM/ml); %ΔOD, percentage change in optical density after ADP addition. † $P <$ 0.05 aging vs. young. * $P < 0.05$ NAR vs. SD.

Table 3 *Plasma levels of triglyceride, cholesterol, and cholesterol fractions in 3- and 12-mo-old rats*

| | $n$ | TG | Chol | VLDL-C | LDL-C | HDL-C |
|---|---|---|---|---|---|---|
| | | | *3-mo-old rats* | | | |
| *SD* | 6 | 169±5 | 73±2 | 12±1 | 16±1 | 45±3 |
| *NAR* | 6 | 137±9 | 140±6* | 18±4 | 27±2 | 94±5* |
| | | | *12-mo-old rats* | | | |
| *SD* | 9 | 198±25 | 117±11† | 14±2 | 45±5† | 59±5 |
| *NAR* | 8 | 102±8* | 111±6† | 5±1*† | 46±4*† | 60±4*† |

Values are means ± SE in mg/dl. SD, Sprague-Dawley rats; NAR, Nagase analbunemic rats. Chol, serum cholesterol concentration; TG, triglyceride; VLDL-C, LDL-C, and HDL-C, cholesterol fractions associated with the very low-density, low-density, and high-density plasma lipoprotein fractions, respectively. *$P < 0.05$ NAR vs. SD. †$P < 0.05$ aging vs. young.

The evolution of awake systemic arterial pressure and urinary protein excretion rate in aging rats is illustrated in Fig. 1. Tail-cuff pressure (Fig. 1*A*) was slightly but persistently lower in NAR compared with SD controls, especially toward the end of the study, in agreement with observations made in aging anesthetized animals (Table 1). Progressive proteinuria (Fig. 1*B*) developed in aging SD rats, exceeding 100 mg/24 h at 18 mo of age. By contrast, protein excretion levels in NAR remained close to baseline throughout the study.

The frequency of glomerular lesions was negligible in both young SD rats and young NAR, <1% of glomeruli examined showing segmental sclerotic lesions in either group. GS was equally infrequent in aging NAR (0.59 ± 0.20%, $P > 0.4$ vs. *NARY*). By contrast, GS was observed in 9.9 ± 2% of glomeruli examined in aging SD rats ($P <$ 0.05 vs. *SDY* or *NARAg*). The morphological aspect of glomerular lesions found in aging SD rats is illustrated in Fig. 2*A*, showing segmental collapse of capillary loops, accumulation of periodic acid-Schiff-positive material, and adhesions to Bowman's capsule. By contrast, virtually all glomeruli examined in aging NAR were morphologically inconspicuous as shown in Fig. 2*B*. Glomerular lipid deposition, consisting of coarse granules of focal and

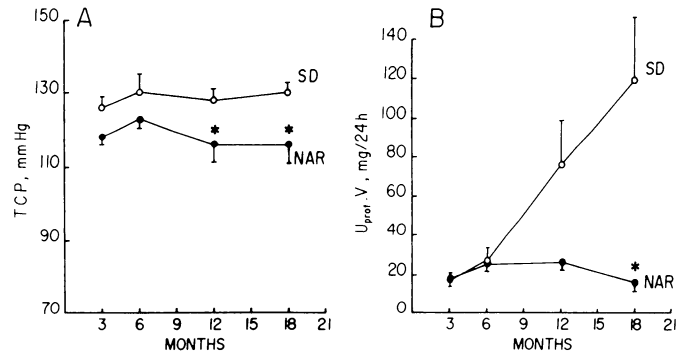


Fig. 1. Serial determinations of tail-cuff pressure (TCP, *A*) and urinary protein excretion rate ($U_{Prot}V$, *B*) in Sprague-Dawley rats (SD; $n$ = 8) and Nagase analbuminemic rats (NAR; $n$ = 8).

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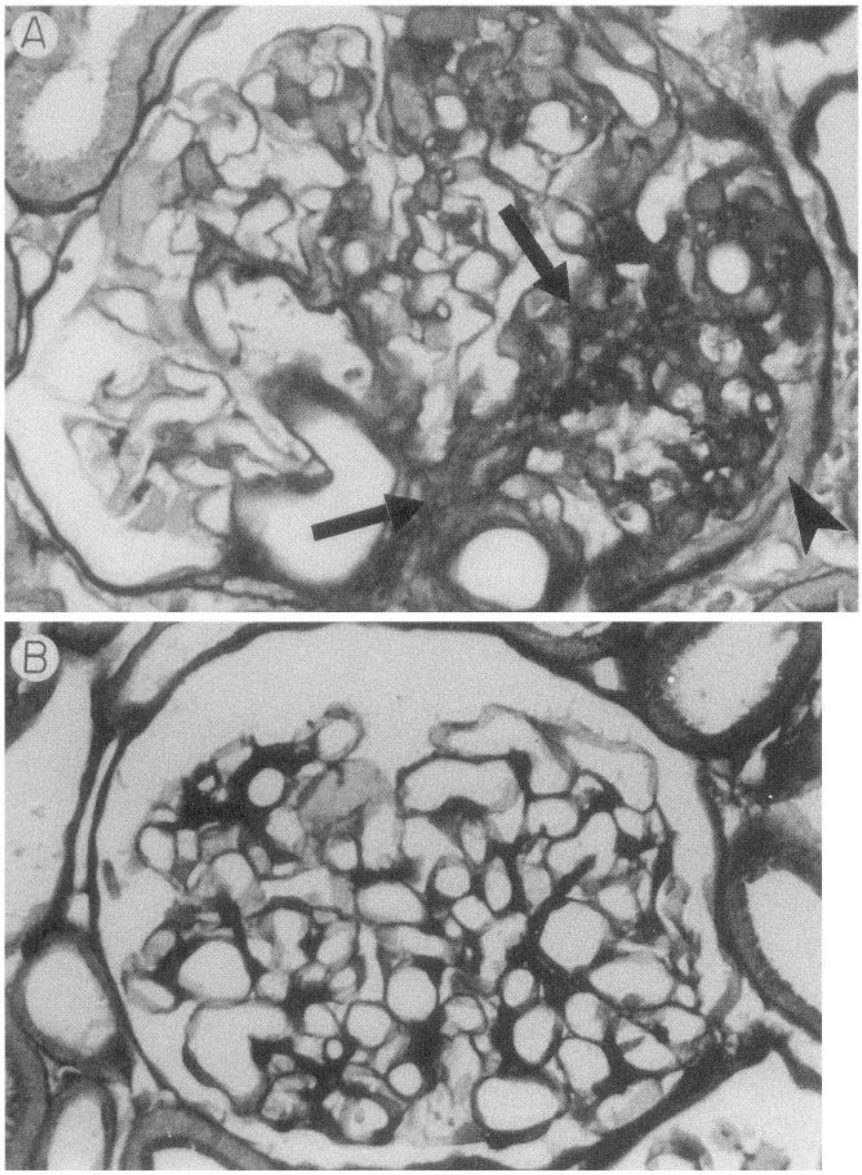


Fig. 2. *A*: typical segmental sclerotic lesion (arrows) observed by light microscopy in 18-mo-old SD rat (*SDAg*) showing collapse of capillary loops, deposition of periodic acid-Schiff (PAS)-positive material, and adhesion to Bowman's capsule (arrowhead). *B*: glomerulus from 18-mo-old NAR (*NARAg*) showing no signs of sclerotic injury. PAS in 3-$\mu$m-thick section; ×500. * $P < 0.05$, *SDAg* vs. *NARAg*.

segmental distribution, was readily observed in aging SD rats as shown in Fig. 3*A*. By contrast, no lipid deposition was detected in glomeruli of aging NAR (Fig. 3*B*). Focal areas of tubular atrophy were occasionally observed in both SD rats and NAR.

Statistical analysis revealed that the age-related increases observed in SD rats with respect to glomerular hydraulic pressure, glomerular tuft volume, plasma total cholesterol concentration, and percentage GS were associated with a significant interaction between group (strain) and aging (treatment) effects. A similar interaction was shown in relation to the age-related decreases in TG, VLDL, and HDL-cholesterol observed in NAR.

## DISCUSSION

Progressive proteinuria was observed in aging SD rats, reaching values in excess of 100 mg/24 h in 18-mo-old rats. Accordingly, focal and segmental GS as well as glomerular lipid accumulation were readily seen in aging SD rats at the end of the study. These findings corroborate previous observations that in SD rats as well as in other rat strains, obsolescence of a large number of glomeruli occurs in association with the aging process (5, 7). By contrast, results obtained in aging NAR failed to support our initial hypothesis that these animals might be more prone to the development of GS than SD controls. Renal function was well preserved in these rats, whereas urinary protein excretion rates remained stable throughout the study and only negligible glomerular injury was demonstrated at 18 mo of age, suggesting that this strain is resistant to the process of aging GS. Our results diverge from those recently reported by Joles et al. (16) in 12-mo-old SD rats and NAR. In contrast to the trivial amounts of nonglomerular injury observed in our study, these investigators described the occurrence of more severe tubulointerstitial injury in NAR than in SD rats, which might relate to a slight but significant decrease in creatinine clearance in these animals. Also in disagreement with our results was the description in that study of only slight GS in both SD rats and NAR. However, it must be

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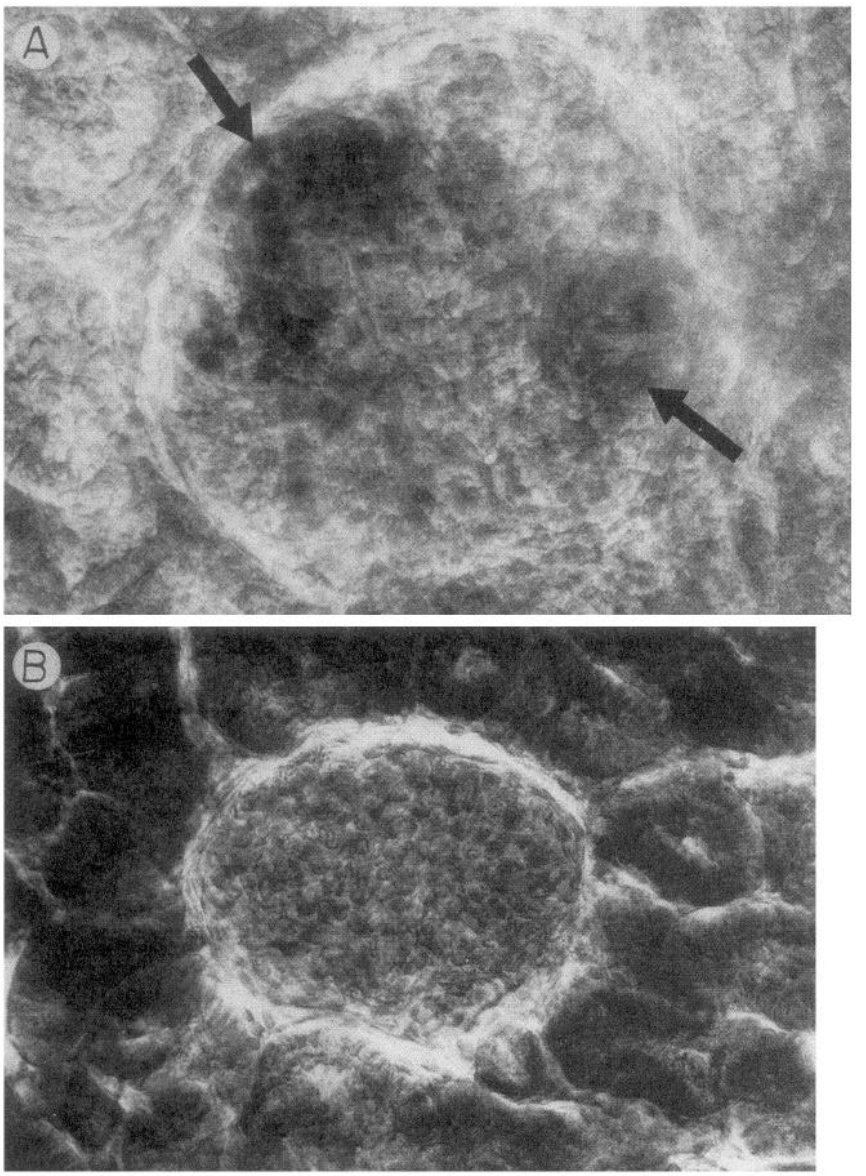


Fig. 3. Specific histochemical analysis of glomerular lipid deposition in 18-mo-old rats. *A*: segmental deposition of lipid granules in *SDAg* (arrows). *B*: no lipid deposition in *NARAg*. Oil red O in 8-$\mu$m sections; ×500.

noted that the renal histology was examined in that study at 12 mo of age, whereas the present morphological observations, performed at 18 mo of age, may reflect a more advanced stage of the process of aging GS, clearly manifest in SD rats but not in NAR.

Obstruction of glomerular capillary lumina by microthrombi has been suggested to constitute an important step in the process of experimental GS. Support for this hypothesis was provided by the finding of intraluminal platelet aggregates in glomeruli of rats with five-sixths renal ablation (13, 21) as well as by the protective effect of heparin administration to these animals (15). In the present study, the enhanced platelet aggregation observed in aging SD rats may have facilitated the development of GS in these animals. However, because thrombocytosis and enhanced platelet activity were also demonstrated in young as well as in aging NAR, the striking differences between SD rats and NAR with respect to glomerular morphology cannot be explained on the basis of differences in platelet activity alone. Nevertheless, a role for intraglomerular coagulation in the pathogenesis of GS in aging SD rats cannot be excluded by these data.

Age-related elevation of serum cholesterol concentration, in particular of the fraction transported in circulating LDL particles, has been observed in humans as well as in other species (25). In the present study, aging SD rats exhibited marked elevation of total plasma cholesterol and of its HDL and particularly LDL fractions. These abnormalities might result from the marked proteinuria encountered in these animals as frequently observed in nephrotic subjects. However, the levels of proteinuria measured in 18-mo-old SD rats were relatively modest compared with those usually observed in nephrotic rats (11). Accordingly, plasma protein concentration in aging SD rats was no different from values observed in young animals, suggesting that age-related urinary protein losses exerted little systemic impact and cannot explain the lipid abnormalities observed in these rats. Additional support for this contention is provided by the finding that hypercholesterolemia of similar magnitude was already

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encountered in SD rats at 12 mo of age, when proteinuria was considerably lower than at 18 mo of age.

Elevated levels of circulating cholesterol may strongly influence the development of progressive glomerular injury. In the obese Zucker rat, persistent hypercholesterolemia is associated with early development of severe GS, which can be prevented by prolonged therapy with lipid-lowering agents (17). Hypercholesterolemia has also been observed in rats subjected to five-sixths renal ablation (18). Therapy with lipid-lowering drugs was associated with marked attenuation of glomerular injury in these animals (18). The pathogenetic influence of plasma cholesterol may be related to its distribution among lipoprotein fractions rather than to its total concentration. Gröne and co-workers (14) have recently shown that administration of a 5% cholesterol diet to SD rats promotes an elevation of VLDL-cholesterol concentration without hypercholesterolemia. Lipid abnormalities were associated with the development of GS lesions in these rats. Elevations of VLDL- and LDL-cholesterol have been associated with the initiation and progression of atherosclerosis (27), a process in many respects analogous to GS (12), whereas elevations in HDL-cholesterol may exert a protective effect (27). In this study, the marked elevation in LDL-cholesterol encountered in 12-mo-old SD rats may have participated in the subsequent development of progressive proteinuria and GS observed in these animals. A pathogenetic role of glomerular lipid accumulation in SD rats is further suggested by the finding of lipid droplets in the glomeruli of these animals. Hypercholesterolemia of similar magnitude was observed in aging as well as in young NAR. However, although in young NAR LDL-cholesterol corresponded to less than one-third of the HDL-cholesterol fraction, this ratio rose to nearly 80% in 12-mo-old NAR, in accordance with values reported previously (16). These values are also similar to those encountered in this study in 12-mo-old SD rats. Despite these similarities between 12-mo-old SD rats and NAR with respect to plasma cholesterol distribution, only negligible GS developed in NAR at 18 mo of age. Thus neither plasma levels of cholesterol nor its distribution among lipoprotein fractions can adequately explain the marked divergence between aging SD rats and NAR with respect to glomerular morphology. The development of glomerular injury related to abnormalities in plasma cholesterol may require the concurrence of other pathogenetic mechanisms, in particular hemodynamic stress. Gröne and co-workers (14) showed that uninephrectomy, a maneuver that did not influence plasma cholesterol levels, markedly aggravated GS in rats fed a cholesterol-rich diet. In addition, induction of two-kidney, one-clip hypertension in these rats was associated with exacerbation of diet-induced GS in the unclipped kidney while glomerular structure was preserved on the clipped side. We have recently demonstrated (13) that removal of five-sixths renal parenchyma in NAR is associated with glomerular hypertension, intraglomerular thrombosis and severe glomerular scarring. Therapy with the angiotensin I-converting enzyme inhibitor enalapril promoted attenuation of glomerular hypertension without alteration of circulating cholesterol levels. Nevertheless, glomerular injury was largely prevented in treated animals.

In the present study, plasma TG concentration tended to rise with age in SD rats and to decline in NAR, TG levels in 12-mo-old NAR reaching only half those observed in SD rats of the same age. These results are at variance with those previously reported by Joles and co-workers in 12-mo-old NAR (16). However, it must be noted that because plasma lipid analysis in that study was performed in the fed state, a considerable fraction of circulating TG was probably carried by chylomicra. In the present study, a 12-h fast was imposed before plasma lipids were examined. Our data may therefore reflect more accurately the presence of TG in the endogenous pathway of lipoprotein metabolism. Because low levels of circulating TG in 12-mo-old NAR were associated with the subsequent development of only trivial GS, the possibility arises that TG levels may be related to the development of aging GS, although the pathogenetic mechanisms that might mediate such effect are unknown. Evidence as to the role of hypertriglyceridemia in the pathogenesis of atherosclerosis and GS is presently inconclusive. Although in at least one study high TG levels have been shown to predict the development of coronary disease (9), other investigators have failed to characterize hypertriglyceridemia as an independent risk factor for the development of atherosclerosis (22). In the obese Zucker rat, in which GS is associated with hypercholesterolemia and severe hypertriglyceridemia, pharmacological treatment of hyperlipidemia promoted marked amelioration of glomerular injury (17). However, preservation of glomerular structure correlated with lowering of plasma cholesterol rather than TG levels.

Elevation of systemic (4) and intraglomerular (2, 3, 13) hydraulic pressures has been suggested to adversely affect glomerular structure in several experimental models and may have influenced the ultimate development of glomerular injury in the present study. The slight arterial hypotension observed in NAR may have contributed to the preservation of glomerular structure observed in these animals at 18 mo of age. Nevertheless, studies performed in Milan hypertensive rats (7) have demonstrated that GS can occur independent of systemic arterial hypertension. In addition, recent observations have shown that persistent pharmacologically induced arterial hypotension may be insufficient to prevent GS in diabetic rats unless normalization of glomerular hydraulic pressure is concomitantly achieved (3). Previously reported evidence linking glomerular hypertension to the development of aging GS has been inconclusive so far. In a preliminary study, Anderson and co-workers (3) showed elevations in glomerular hydraulic pressure and marked albuminuria in 24-mo-old Munich-Wistar rats compared with young controls. Prolonged treatment of these animals with a converting enzyme inhibitor was associated with reversal of glomerular hypertension and limitation of urinary albumin excretion. In another preliminary report, Reckelhoff and co-workers (23) obtained normal glomerular pressures in 21-mo-old SD rats and suggested that the pathogenesis of aging GS may be unrelated to

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glomerular hemodynamic abnormalities. However, protein excretion rates or glomerular morphological changes were not reported in their study. In the present study, glomerular hydraulic pressure and glomerular morphology were examined simultaneously in both young and aging SD rats and NAR. Glomerular hydraulic pressure in young SD rats was comparable to that obtained in other studies (18). With age, significant glomerular hypertension developed in SD rats. This abnormality, perhaps in association with alterations in plasma lipid composition, may explain the ultimate development of lipid accumulation and glomerular injury in these animals. Glomerular hydraulic pressure was low in young analbuminemic rats compared with values observed in SD rats and remained stable in aging NAR. Because no GS was seen in these rats, these findings support the hypothesis that glomerular hemodynamic alterations may have been central to the pathogenesis of GS observed in this study.

In recent years, glomerular hypertrophy has also been implicated as one of the factors potentially involved in the process of progressive GS. Enlargement of the glomerular tuft has been described in association with several experimental models of GS (3, 10). In addition, prevention of glomerular hypertrophy has been shown to limit the development of progressive glomerular injury in rats with five-sixths renal ablation (10). One of the mechanisms whereby glomerular enlargement might promote glomerular injury is by increasing wall tension, in accordance with the Laplace relationship and in association with glomerular hypertension (10). In this study, glomerular size was slightly smaller in young NAR than in age-matched SD controls. Because glomerular hydraulic pressure was also lower in young NAR, glomerular wall tension would be expected to be substantially attenuated in these animals compared to SD controls. This difference is likely to have been exacerbated with aging, since marked glomerular hypertension and glomerular hypertrophy developed in SD rats, whereas glomerular hypertrophy of lesser magnitude and glomerular hypotension were seen in aging NAR. Thus persistently reduced mechanical stress on the glomerular wall may account for the lack of glomerular injury observed in these animals.

Lifelong mesangial accumulation of albumin, the most abundant circulating protein, may conceivably exert a noxious effect on the mesangium and contribute to the development of aging GS. Therefore the virtual absence of plasma albumin in NAR may help explaining the lack of aging GS in these rats. This particular aspect was not addressed in the present study. However, it must be noted that severe glomerular scarring was previously shown to develop in NAR after five-sixths renal ablation (13). This finding suggests that mesangial albumin deposition may not constitute a prerequisite for the development of GS in these animals.

In summary, NAR appear to be resistant to the development of aging GS, despite the presence in these animals of enhanced platelet activity and age-related elevation of circulating LDL-cholesterol levels. This finding may be explained by an association between lowered glomerular hydraulic pressure and only modest enlargement of the glomerular tuft, resulting in low mechanical stress on the glomerular walls. Additional protection may result from the low TG levels observed in these animals.

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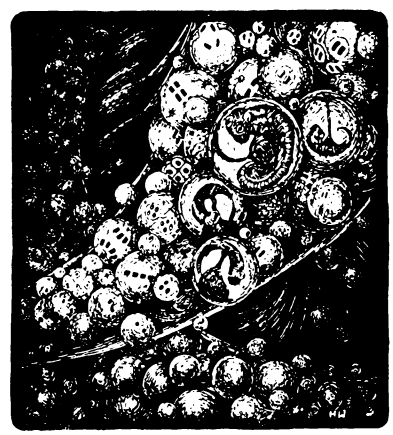

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