Spontaneous Glomerular Sclerosis in Aging Sprague-Dawley Rats

I. Lesions Associated With Mesangial IgM Deposits

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The present studies examined the pathogenesis of focal glomerular sclerosis in aging rats. A marked difference in development of the lesion was noted between males and females, and strain variability was an important factor. Increased glomerular basement membrane permeability with loss of selectivity unrelated to changes in glomerular sialoprotein occurred with aging and was accompanied by increasing proteinuria. Non-complement-fixing mesangial deposits of rat IgM were present after 1 month of age and were also found in lesser amounts in germfree rats. Fluoresceinated eluates of rat kidneys did not have antibody activity against rat serum or tissue antigens. There was no evidence for a pathogenetic role of IgM deposits. Rat IgG, IgA, IgE, C3, and fibrin were occasionally found in sclerotic areas. Analysis of multiple histologic sections revealed a close correlation between aging and glomerular pathology, with a poor correlation between tubular damage and aging. Glomerular damage appeared to be the initial event leading to tubular damage. Indirect evidence suggests that a relative thymic deficiency may play an important role in the pathogenesis of the lesion. (Am J Pathol 85:277–302, 1976)

NUMEROUS REPORTS HAVE DESCRIBED spontaneous renal lesions in aging rats.[1-8] The incidence of associated proteinuria among strains has differed,[7,9,10] and males have been most severely affected.[3,7,11,12] Many environmental and genetic factors appear to be capable of altering the onset and course of the disease.[1-4,7,9-20] While proteinuria in most experimental animal models is induced by humorally mediated renal damage, there is little evidence linking the aging nephropathy of rats to immune mechanisms.[21-23] In addition, no toxic substances or metabolic abnormalities have been discovered to account for this spontaneous disease process. Recent studies have likened the glomerulopathy of aging in rats to focal glomerular sclerosis in man [6,7] and have implicated mesangial deposits of IgM as a possible factor in the pathogenesis of this lesion.[6] These studies further suggest that the sclerotic lesions result from mesangial overload and dysfunction from a persistent increase in glomerular permeability and consequent proteinuria. In the present paper, we report

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Supported in part by a grant from the Virginia Heart Association to Dr. Bolton.

Accepted for publication July 1, 1976.

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the results of our study of the spontaneous lesion of focal glomerular sclerosis of aging in the male Sprague-Dawley rat and provide data suggesting that the lesion does not occur as a consequence of IgM deposition but more likely as a natural consequence of aging, which in turn is related to genetic and environmental factors. While superficially similar to focal sclerosis in man, the lesion does not appear to represent an entity comparable to human focal glomerular sclerosis.

Materials and Methods

Animals

Random bred albino male and female Sprague-Dawley rats were used for most of these studies. The majority of the animals were male, and ranged in age from immediately newborn, 1 through 6 weeks, and 3, 4, 6, 9, 12, 18, 22, and 24 months. Germfree male Sprague-Dawley rats were 12 months of age, as were Fischer (F-344) rats. Adult Lewis rats, 12 months of age, were also used. Animals were obtained mainly from Flow Laboratories and maintained in the University of Virginia Vivarium under standard conditions with a regular diet.

Urine Protein and Blood Chemistries

Animals were periodically placed in metabolic cages to collect 24-hour urine samples. The total urine protein (TUP) content was determined using 3% sulfosalicylic acid with bovine serum albumin as a standard.[24] The upper limit of normal for young rats in our laboratory is 10 mg 24 hours (6.4 ± 3.6 mg 24 hrs, mean ± 3 SD). Total urine protein determinations were made for 184 male Sprague-Dawley rats, with the smallest number, 16, at age 24 months. Total urine protein determinations were made for 60 female Sprague-Dawley rats, for 12 male and 12 female Fischer rats at 1 year, and for 10 male and 6 female Lewis rats. Blood urea nitrogen, serum creatinine, total serum protein, and serum albumin levels were determined by routine laboratory methods. Individual concentrated rat urines and sera were periodically analyzed by electrophoresis in 1% agarose gel, and pooled urine and sera of animals adjusted to the same concentration were analyzed by cellulose acetate protein electrophoresis.

Kidney Tissue

Tissue obtained by open renal biopsy or sacrifice was divided into three portions.[25] One specimen was fixed in neutral formalin, the second snap-frozen on a cork in isopentane cooled with dry ice, and the third portion fixed in 2% osmium tetroxide for 45 minutes, dehydrated with ethanol, and embedded in Araldite. Paraffin-embedded specimens were serially sectioned at 3 μ, and sequential strips of sections were stained with hematoxylin and eosin (H&E), periodic acid-Schiff reagent (PAS), and colloidal iron for sialoprotein. Kidneys from 87 male Sprague-Dawley rats of various ages were examined, as was a small number from each of the other groups noted above.

Histologic Grading

All of the serial sections were examined to determine the degree and extent of histologic changes. The specimens were examined without knowledge of the animal's age or the quantity of protein excreted. Four specific areas were analyzed: a) Glomeruli were graded 0 to 4+, with 1+ representing increased mesangial matrix; 2+ for segmental sclerosis, 3+

for sclerosis with collapse, and 4+ for obsolescence. Marked thickening of Bowman's capsule increased the grade by 0.5+. b) Tubular histology was graded from 0 (normal) to 1+ for an isolated area of tubular atrophy and increased tubular basement membrane thickening, up to 4+ for multiple areas of atrophy, interstitial cellular infiltration, and fibrosis. c) Colloid casts were graded 0 for none, 1+ for one cast /20 glomeruli, 2+ for up to four casts, 3+ for five to eight casts, and 4+ for more than 8 casts /20 glomeruli. d) Colloidal iron specimens were graded 4+ for normal staining as found in 3-month-old and younger animals, 3+ for a moderate diffuse decrease in staining, progressively to 0 for no staining within most glomeruli.

Immunofluorescence Studies

Rat IgG was isolated by DE-52 cellulose chromatography of a 33⅓% ammonium sulfate precipitation of whole rat serum using .01 M sodium phosphate buffer, pH 7.5, for elution.[26]

Rabbit Antirat F(ab')²

Rat IgG was dissolved in .07 M acetate buffer, pH 4.5, and .05 M NaCl. A 1 50 of the globulin weight of pepsin was dissolved in .1 M acetate and added to the stirred globulin solution.[27] After stirring for 50 hours at 37 C, the soluble portion was brought to neutrality and dialyzed against isotonic phosphate-buffered saline, pH 7.2 (PBS). After precipitation with sodium sulfate, the precipitate was dialyzed against water and lyophilized. Two hundred micrograms were emulsified with complete Freund's adjuvant and utilized to immunize rabbits. Sera were harvested at 30 days and recognized five lines in the globulin region by immunoelectrophoresis (IEP) against fivefold concentrated rat immunoglobulins.

Rabbit Antirat IgM Antiserum

The fraction of sulfate-precipitated rat immunoglobulins eluted from a DE-52 column with 1 M sodium chloride was concentrated by pressure dialysis and separated on a Sephadex G-200 column using retrograde flow with PBS as the eluant.[26] The ascending portion of the first peak was refractionated and examined by IEP. Two lines in the globulin region were seen using anti-whole rat antiserum, but only a single line using anti-rat F(ab')2. Thirty lines produced by IEP anti-F(ab')2 were cut out, washed repeatedly in PBS, emulsified with complete Freund's adjuvant, and injected into the footpads of rabbits. The resultant antiserum recognized one line in the IgG region and another line (rat IgM) near the well. After absorption with gluteraldehyde cross-linked rat IgG,[28] a single line was recognized by IEP.

Rabbit Antirat IgA

Seventy-two hours after delivery, suckling rats were separated from their mothers for 12 to 15 hours and then permitted to nurse for 2 hours.[26] The newborn rats were then sacrificed, and the stomach contents were collected and mixed with PBS. After centrifugation and dialysis to produce clarified colostrum, the vacuum-concentrated colostrum was separated using a 2.5 × 90 cm column with Sephadex G-200 equilibrated with PBS. The first peak contained rat IgA and occasionally IgM. Specimens shown not to contain IgM were mixed with an equal volume of complete Freund's adjuvant and used to immunize rabbits. The resulting antiserum was appropriately absorbed to yield a single line by Ouchterlony immunodiffusion.

Rabbit Antirat IgE Antiserum

Young Sprague-Dawley rats were injected subcutaneously with .2 ml of larvae of *Nippostrongylus brasiliensis* (2000 to 2500 larvae). Larvae were reinjected every 3 weeks

and animals were exsanguinated 6 to 8 days after the last reinfection. The techniques of Ogilvie[29,30] were used for the harvesting and maintenance of *N. brasiliensis*. Worm antigen was prepared by collecting adult worms from the intestines of infected rats 9 to 10 days after the infection. Passive cutaneous anaphylaxis (PCA)[31] was performed utilizing the worm antigen and hyperimmune sera from rats repeatedly infected with *N. brasiliensis*. Two tenths milliliter of worm antigen (1000 worm equivalents ml) mixed with .5 ml of a .5% solution of Evans blue dye was used as a challenge 72 hours after the injection of serially diluted hyperimmune serum intracutaneously into the backs of normal test animals. The skin tests were read 30 minutes after sacrifice of the animals by reflecting the skin and measuring the area of bluing. Serum collected and pooled from rats with high PCA titers was precipitated with 33⅓% ammonium sulfate and chromatographed on a DE-52 column 2.5 × 45 cm using step-wise elutions.[32] The peak eluted in the .05 M phosphate buffer, pH 5.0, was concentrated by pressure dialysis and dialyzed against PBS. This fraction, which contained high titers of IgE by PCA analysis, was placed on a Sephadex G-200 column 2.5 × 85 cm and eluted with PBS. The fractions collected at 4 ml tube were each analyzed for the presence of IgE by PCA testing and by IEP after concentrating. Those portions of the eluted immunoglobulin containing high PCA activity were pooled. Purified rat IgG was ultracentrifuged at 105,000*g*, and 5 mg of the aggregate-free supernatant was injected intravenously into rabbits.[33] One milligram protein of the IgE-rich fraction was mixed with an equal quantity of complete Freund's adjuvant and injected into the footpads of the same rabbits. Three weeks later, a subcutaneous injection of IgE-rich antigen and 10 mg of ultracentrifuged IgG were given again and the serum was harvested 10 days later. IgG and a small line next to the well were identified by the antiserum against hyperimmune serum from rats infected with *N. brasiliensis*. After repeated absorptions, no lines were demonstrable, but the antiserum was capable of neutralizing PCA reactions in a titer of greater than 1 : 320 and was not blocked by antibodies against other rat immunoglobulins. Antiserum to rat IgG, C3, fibrinogen, and albumin were prepared as previously described.[34] All of the antisera were fluorescein (FITC) labeled by the method of Clark and Shepard,[35] and then the appropriate absorptions as described above were performed. F : P ratios ranged from 0.8 to 1.5.

Immunofluorescence Microscopy

A Zeiss epifluorescence microscope was utilized to examine specimens. It is equipped with a 50-W mercury lamp and dichroic mirrors exciting at 490 mμ and transmitting at 520 mμ. Fluorescence was graded 0 to 4+ with 1+ representing mesangial deposits affecting some lobules, 4+ with heavy mesangial deposits throughout all mesangial regions of all glomeruli, and 2 to 3+ intermediate in terms of distribution and intensity. Deposits with other distributions were similarly graded relative to intensity of fluorescence.

Elution Studies

One hundred fifty 6- to 18-month-old male Sprague-Dawley rats were exsanguinated from the aorta, the kidneys stripped of the capsules after weighing, and the cortices separated and minced in iced PBS. After homogenization for 3 minutes in a Waring microblender at 4 C, the tissue was repeatedly washed in PBS with centrifugation until the supernatant was clear. The sediment was eluted with ten volumes of .02 M citrate-citric acid saline buffer pH 3.2 for 3 hours at 37 C.[36] After centrifugation, the supernatant was brought to pH 7.3 with sodium hydroxide and dialyzed against PBS for 48 hours with frequent changes of buffer. The eluate was concentrated by negative pressure dialysis to 1 cu cm and then split into two aliquots. The first aliquot was fluorescein labeled using the method of Clark and Shepard.[35] The eluate was examined against frozen sections of rat thymus, lung, brain, liver, adrenal gland, heart, spleen ilium, jejunum, skin, kidney, testis, muscle, and consolidated lung of rats with chronic pneumonia. It was further character-

ized by Ouchterlony immunodiffusion with normal rat serum and the various antirat antisera noted above.

Autohemolysins

Ten 18-month-old rats were bled into heparinized tubes, and after repeated washing in cold PBS, the red cells were individually examined for surface antibodies using serial dilutions of fluoresceinated antirat IgM. Aliquots of red cells from each of these animals were reacted with Bromelase (American Hospital Supply Corp., Miami, Fla.) to remove any surface-bound protein and then were examined by indirect immunofluorescence using serial dilution of autologous rat serum. The eluate was similarly examined in both of these systems.

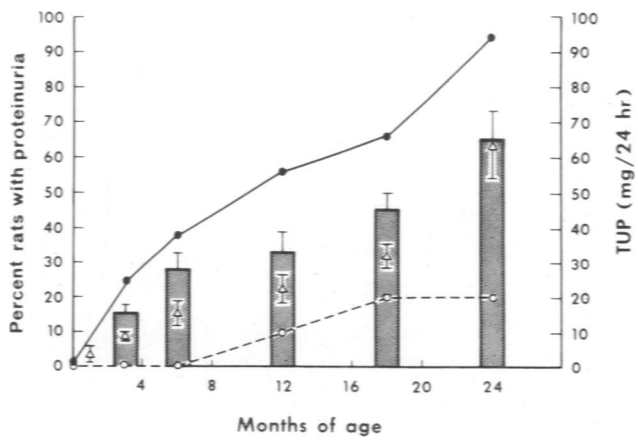
Complement Fixation

In vivo complement fixation was monitored using the FITC-labeled antirat C3. *In vitro* complement fixation was determined by cutting 4-μ-thick cryostat sections and incubating unfixed tissue with various dilutions of fresh and heat-inactivated human, rabbit, and guinea pig complement as described by Burkholder.[37] Complement fixation was also examined after partial elution of cryostat sections with 2.5 M KSCN. Positive controls of antinuclear factor containing serum and kidneys from rats with autologous immune complex nephropathy were used.[34]

Results

Urine Protein Excretion

Protein in neonatal rats and those up to 2 weeks of age was not detectable in bladder urine by dipstick testing. By 3 months, 25% of the male rats had proteinuria in excess of 10 mg/day, and the number of male animals with proteinuria progressively increased to 38% at 6 months (Text-figure 1). This number had increased to 56% at 12 months, 66% at

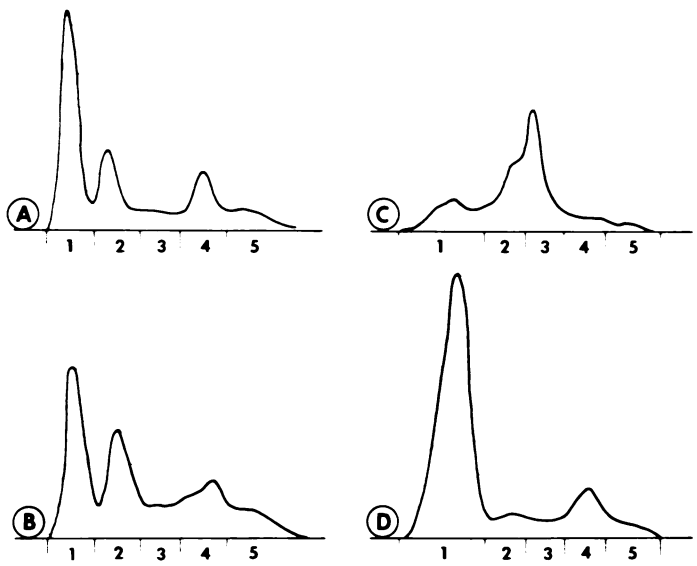


TEXT-FIGURE 1—Relationship between age and sex, and total urine protein (TUP) excretion in Sprague-Dawley rats. The values for protein excretion are presented as the mean ± SE. (Males, *solid circles*; females, *open circles*; TUP in proteinuric males, *shaded columns*; proteinuria, all males, *open triangles*)

18 months, and 94% at 24 months of age. In striking contrast are the results of urine protein determinations in smaller groups of female rats, in which abnormal proteinuria appeared in 20% at 18 months of age. Not only did the number of proteinuric male animals increase, but the quantity of protein excreted by these rats similarly increased, with a mean of 15 mg at 3 months increasing to a mean of 65 mg at 24 months. Examination of the electrophoretic pattern of young nonproteinuric rat urine revealed the essential absence of albumin, with the bulk of the protein being an α-globulin, probably of tubular origin (Text-figure 2). In contrast, as the rats aged, albumin began to appear in the urine and at a still later time, the full spectrum of serum proteins could be demonstrated in urine.

Serum Chemistries

The normal initial protein electrophoresis of young pooled rat serum shown in Text-figure 2 changed into a pattern typical of the nephrotic syndrome with decreased serum albumin and elevated α-globulin levels. Despite the decrease in serum albumin, the total serum protein remained normal (Table 1). Similarly, no deterioration of blood urea nitrogen (BUN) or serum creatinine was observed in any of the animals at any age.



TEXT-FIGURE 2—Protein electrophoresis of pooled male Sprague-Dawley rat serum and urine at equivalent protein concentrations. **A**—Serum from 3-month-old nonproteinuric rats. **B**—Serum from 18-month-old proteinuric rats. **C**—Urine from 3-month-old nonproteinuric animals. **D**—Urine from 18-month-old proteinuric rats. Albumin, *1*; α-1-macroglobulin, *2*; α-2-macroglobulin, *3*; β-globulins, *4*; γ-globulins, *5*)

Table 1—Serum Biochemistry Characteristics in Male Sprague-Dawley Rats

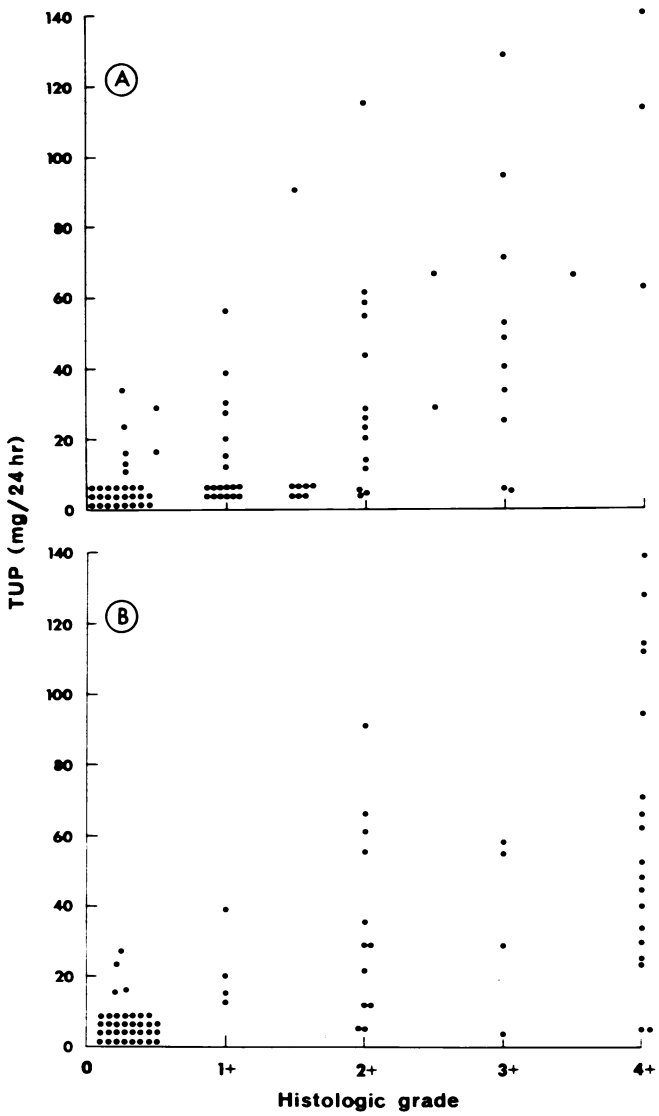
Months of age	Creatinine (mg/dl)	BUN (mg/dl)	Total protein (g/dl)	Serum albumin (g/dl)
6	0.6 ± .03	22.7 ± 1.1	7.8 ± .2	3.5 ± .2
18	1.0 ± 0.1	22.5 ± 2.2	7.8 ± .5	2.2 ± .2
24	0.8 ± 0.2	26.0 ± 5.6	6.7 ± .2	2.8 ± .7

Values are expressed as the mean ± SE.

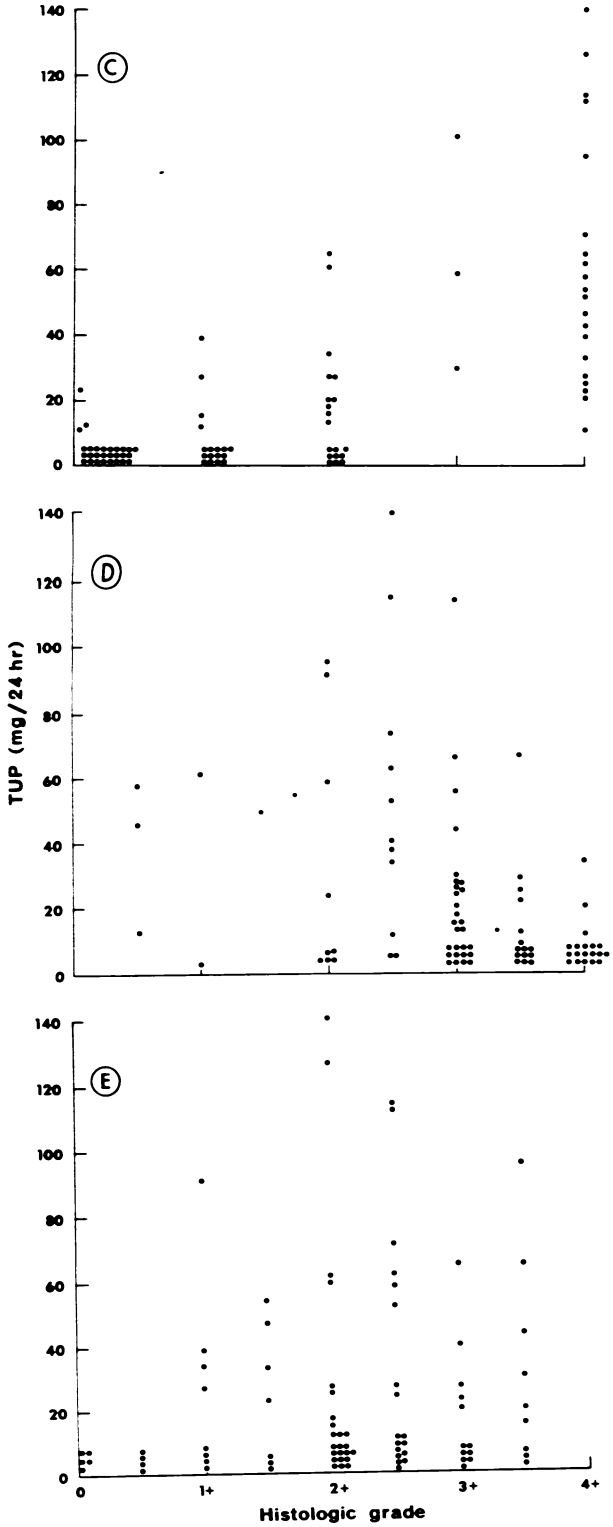
Histologic Findings

Newborn animals had small, compact glomeruli which appeared slightly hypercellular when compared to specimens from older animals. No thickening of basement membranes of the tubules, glomeruli, or Bowman's capsule was ever found, and there was no evidence of tubular atrophy or colloid casts. As the animals aged, there was progressive simplification of the glomeruli with enlargement. Occasional colloid casts, focal tubular atrophy, and increased mesangial matrix were noted at 3 to 6 months (Figure 1A). With continued aging, increasing numbers of casts, tubular atrophy, and glomerular changes were seen (Figure 1B). Interstitial mononuclear cell infiltration became more common after 1 year of age (Figure 2), as did progressive thickening of Bowman's capsule, at times to extreme degrees. Similarly, progressive mesangial changes were obvious; there were areas of segmental sclerosis and eventual collapse (Figure 3A). Obsolescence of glomeruli was found in severely affected animals. Adhesions between tufts and capsules became more common, as did the presence of infrequent crescents (Figure 3B). Vascular changes were minimal. Staining with colloidal iron was present in a 4+ pattern of intensity in animals from birth. While there was a slight decrease of staining with aging, little correlation could be demonstrated between the age of the rat and colloidal iron staining. Indeed, in old rats, staining with colloidal iron was very strong, 3+ in most instances. Of more interest was the change in the pattern of colloidal iron staining. Despite similar degrees of staining intensity, rats of older age groups had markedly widened areas of mesangium which did not stain. Thus, even though near-normal staining was evident in the filtering side of the glomerular basement membrane, obvious increases in the mesangium were apparent in aging animals.

When the association between histologic changes and protein excretion was examined, several conclusions could be drawn. As noted above, proteinuria increased with age in both incidence and quantity. When the total urinary protein was plotted against glomerular changes, a rough correlation was apparent (Text-figure 3A), with rats demonstrating mild



TEXT-FIGURE 3—Relationship between the histologic grade and urinary protein excreation. The TUP (mg day) is represented on the Y-axis and the histologic grade on the X-axis. **A**—Glomerular grade. **B**—Tubular grade. **C**—Colloid casts. **D**—Colloidal iron. **E**—Rat IgM.


C
D
E
TUP (mg/24 hr)
0
20
40
60
80
100
120
140
0
1+
2+
3+
4+
Histologic grade

degrees of glomerular damage having lesser degrees of proteinuria. Again, as glomerular damage increased, the quantity of proteinuria increased, but the scatter was quite wide with numerous animals having 2 to 3+ changes with moderate proteinuria. A similar pattern was seen comparing total urinary protein and tubular damage (Text-figure 3B), but few animals with 2+ or greater tubular damage had normal protein excretion. Examination of the number of colloid casts and proteinuria showed not unexpectedly the same correlation as tubular pathology (Text-figure 3C). That is, clinical proteinuria was associated with large numbers of colloid casts. Thus, both tubular damage and colloid cast formation correlated well with proteinuria, although the scatter of points was quite wide. When an attempt was made to relate loss of sialoprotein to the quantity of proteinuria, no correlation was obvious (Text-figure 3D). Most animals had bright blue staining of 3 to 4+ intensity, regardless of proteinuria, while some animals had minimal proteinuria but a marked decrease in colloidal iron staining. When the relationship between proteinuria and IgM deposits was plotted (Text-figure 3E), no correlation was evident and indeed, the heaviest proteinuria was noted in animals with 2 to 3+ deposits, while those at the extremes with heavier deposits or few deposits had lesser degrees of proteinuria. Thus, attempts to correlate proteinuria with histologic changes or IgM deposits revealed a general trend toward more severe abnormalities with greater degrees of proteinuria, but the scatter of points was extremely wide in all instances.

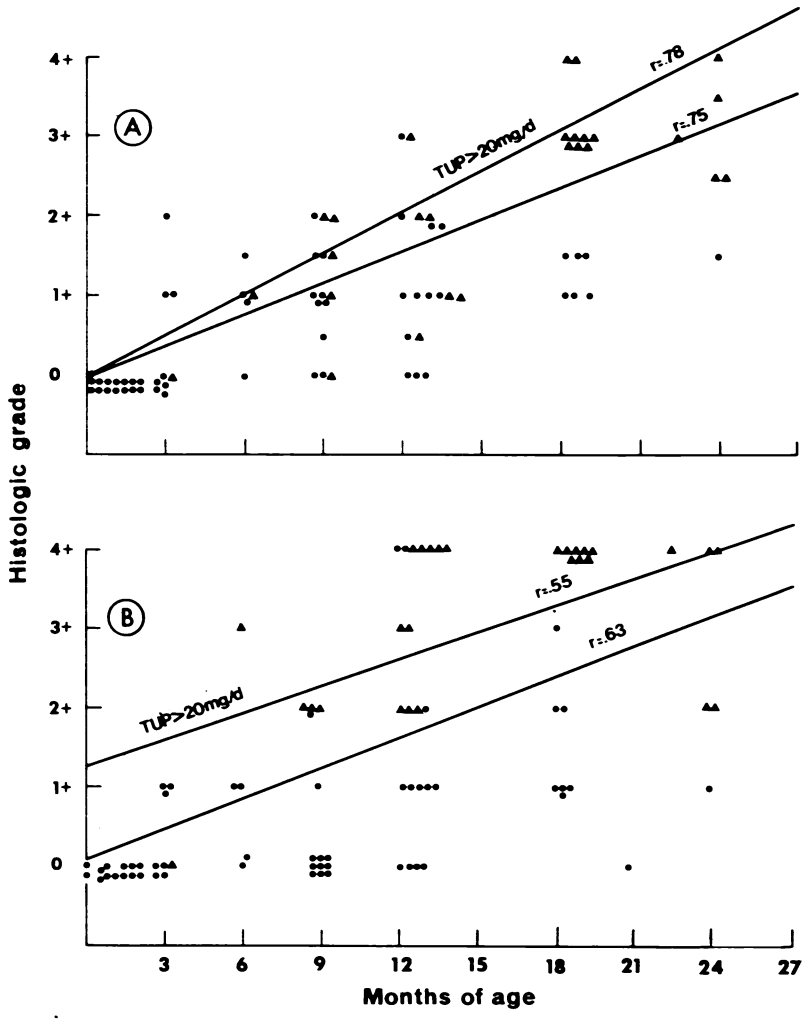
The relationship between histologic changes and the age of the rats is illustrated by the scattergrams in Text-figure 4A–E. It is important to remember that rats were selected for study to provide a spectrum of ages, and more importantly, a spectrum of protein excretion relative to each age group. As can be seen in Text-figure 4A–E, as rats aged there were progressive increases of tubular atrophy, colloid casts, and glomerular sclerotic changes. These changes were unusual and generally mild prior to 9 months of age, and more severe changes were only seen beginning at approximately 1 year. These changes progressed in severity with advancing age. Grades 3 to 4+ were essentially confined to animals over 12 months of age, and usually over 18 months of age. Grade 2 corresponded to age 9 to 12 months. Less severe damage showed little correlation with age. In any individual rat, severity of tubular and glomerular changes were not necessarily directly related, although in general both increased with advancing age and were of approximately equal severity.

The linear regression lines in all animals (Text-figure 4A) ($r = .75$, $y = -.0339 + .1278\,x$) were only slightly different from the line determined in considering only those animals excreting more than 20 mg/day of protein

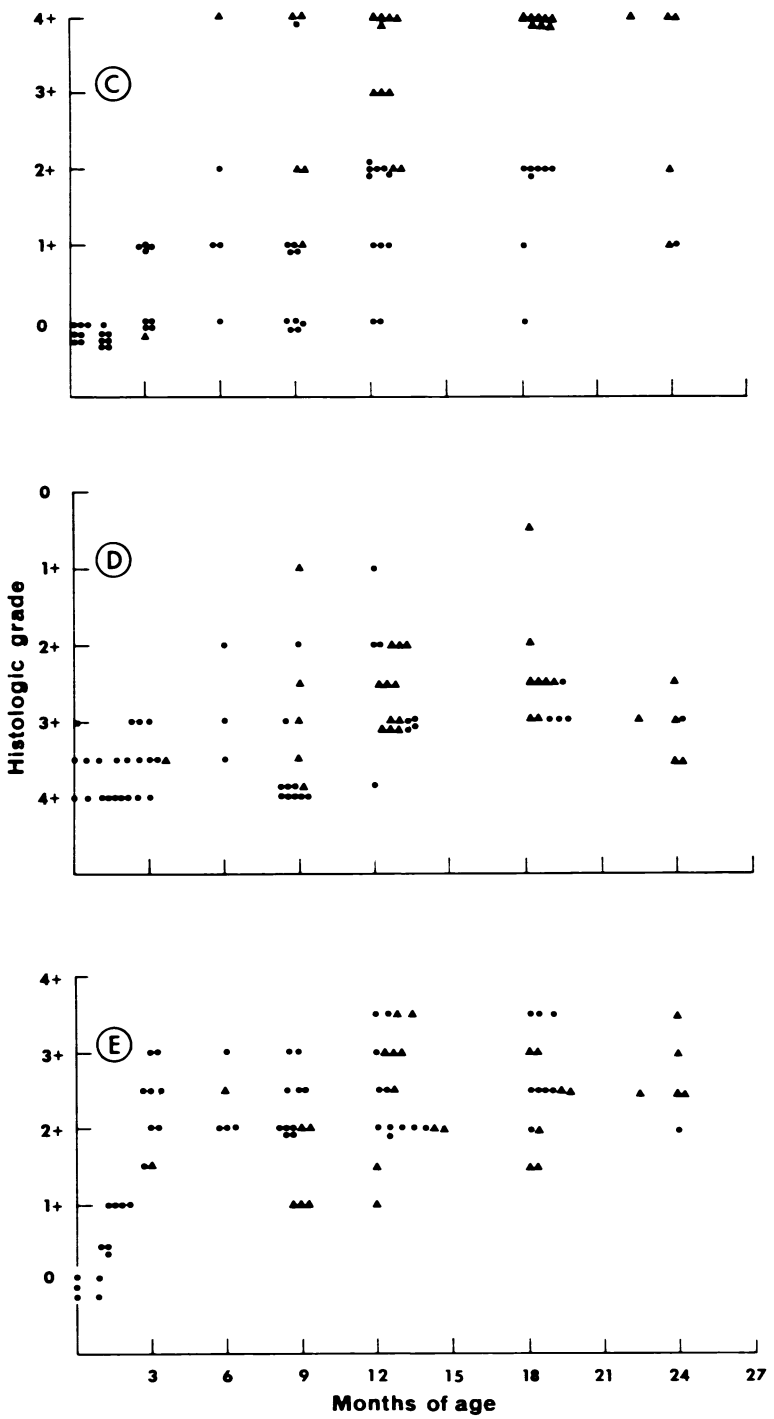
(r = .78, y = .0497 + .1504 x). Analysis of tubular changes relative to aging demonstrated a similar trend (Text-figure 4B), although the correlation coefficient was low (r = .63, y = −.0698 + .1438 x). Consideration of tubular changes in aging rats with total urinary protein >20 mg/day showed a similar pattern, but the line was essentially parallel to the line drawn for all rats. Further, the scatter of points for both proteinuric and nonproteinuric animals with tubular changes were quite wide, unlike the glomerular changes. A very wide scatter of points irrespective of proteinuria or age was seen with colloid casts (Text-figure 4C) and colloidal iron stains (Text-figure 4D). The data comparing the deposits of rat IgM and aging are given in Text-figure 4E. Neonatal animals and rats up to 3 weeks of age had no demonstrable deposits. In the fourth week, very trace amounts of IgM were first detectable in the mesangium. By 6 weeks, this had increased to 1+ intensity, and by the third month after birth deposits of 2 to 3+ intensity were found in all animals. While the amount of IgM tended to increase with aging, the distribution was extremely variable. The deposits of IgM were mesangial in location and on occasion assumed a pseudolinear pattern in a subendothelial location (Figure 4). The distribution of deposits varied within the same specimen in younger animals up to 9 months of age but thereafter tended to be uniformly deposited with equal intensity in the mesangium of all glomeruli. Rat complement was seldom seen except in segmental lesions, usually associated with fibrin (Figure 5) and less often IgA. Fibrin was also found in occasional crescents (Figure 5). A few animals had trace deposits of IgE, but albumin was not demonstrated other than in casts and protein reabsorption droplets. Complement was also present in a linear pattern along tubular basement membranes in most rats as a normal finding.

Elution and Complement Fixation Studies

Microelution of cyrostat sections with either pH 3.2 .02 M citrate buffer, or 2.5 M KSCN resulted in loss of most of the IgM. Elution with PBS or normal saline did not alter staining for IgM. When cyrostat sections were evaluated for their ability to fix complement *in vitro* with several different sources of complement, no fixation could be demonstrated. Both the FITC-antirat IgM and FITC-eluate from 300 rat kidneys failed to stain kidney or any other tissues as described in Materials and Methods. Some peripheral lymphocytes did demonstrate typical membrane staining for IgM but were negative with the eluate. No red cell–bound antibodies could be demonstrated with anti-IgM or eluate at 4 C or 37 C using a panel of 10 different 18-month-old rats. Some mononuclear cells in rat blood took up FITC-labeled products on incubation.



TEXT-FIGURE 4—Relationship between the histologic grade and age in male Sprague-Dawley rats. The histologic grade is given on the Y-axis and months of age on the X-axis. (TUP $\leq$ 20 mg/day, *solid circles*; TUP > 20 mg/day, *solid triangles*). **A**—Glomerular grade with regression line for all animals (r = .75, y = −.0329 + .1278x, 95% confidence level, $.104<\beta<.152$) and for those animals excreting >20 mg protein/day (r = .78, y = .0497 + .1504x, 95% confidence level, $.105<\beta<.195$). **B**—Tubular grade with regression line for all animals (r = .63, y = −.0698 + .1438x, 95% confidence level, $.106<\beta<.182$) and for those animals with TUP >20 mg/day (r = .55, y = 1.1759 + .1222x, 95% confidence level, $.052<\beta<.192$). **C**—Colloid casts. **D**—Colloidal iron. **E**—Rat IgM.


C
D
E
Histologic grade
0
1+
2+
3+
4+
3
6
9
12
15
18
21
24
27
Months of age

These were probably aggregates, since reexamination using an ultracentrifuged preparation abolished this phenomenon. The eluate had no demonstrable antibody activity against pooled normal rat serum.

Findings in Other Rats

Renal biopsies from female Sprague-Dawley rats at various ages from birth to 24 months of age demonstrated a similar pattern of intensity of staining for IgM, despite the absence of proteinuria (Text-figure 1) and mild histologic changes. Similarly, examination of male and female Lewis rats up to 1 year of age demonstrated deposits of IgM of a slightly lesser intensity than in the Sprague-Dawley rats, but still of significant intensity. None of these animals had abnormal proteinuria. Twelve male and 12 female Fischer (F-344) rats 1 year of age were also studied. Deposits of IgM were present with the typical granular mesangial pattern of distribution. None of the female Fischer rats demonstrated abnormal proteinuria, while 67% of the males had abnormal protein excretions of more than 10 mg 24 hours associated with the characteristic histopathology seen in Sprague-Dawley rats. Six germfree 1-year-old Sprague-Dawley male rats were examined and typical deposits of mesangial IgM were present, but in diminished quantities compared to normal rats of the same age. The deposits were generally of 1+ intensity and occasionally of as much as 2+ intensity.

Discussion

Spontaneous nephropathy in the aging albino rat has been described by many investigators. In 1928, Newburgh and Curtis [1] produced severe renal lesions in albino rats using a diet enriched with beef liver. Blatherwick and Medlar [3] extended these observations in 1937 and noted the occurrence of mild histologic abnormalities in some of their old rats fed a stock diet. Saxton and Kimball [4] were the first authors to focus attention directly on the association between aging and nephrosis in the rat. They also confirmed the effect of a high-protein diet in accelerating the disease and shortening the life-span, and demonstrated the beneficial effect on both with moderate food restriction. They also corroborated Blatherwick and Medlar's illustration of a difference in disease incidence and severity related to sex.

Since these early reports describing the clinical and histopathologic aspects of this disease, numerous other studies have been published detailing factors which influence the disease process. The etiology and, indeed, the pathogenesis of this spontaneous lesion remain obscure. As noted above, dietary factors appear to be quite important in the develop-

ment of the lesion. Animals allowed a stock diet *ad libitum* have a higher incidence than restricted rats and conversely, rats fed high-protein diets (especially high in casein) develop the nephropathy at an earlier age and with a greater severity.[1-4,7,13-16,18-20] Male animals are much more likely to develop the disease than females and, while diet can alter the course in females, this factor has much less influence than in males.[3,7,11,12] Male rats castrated before puberty have been reported to have protein excretion rates similar to females. However, if castrated as adults, the protein excretion falls, but still remains markedly abnormal.[12] Androgen therapy increases protein excretion in both castrated males and normal females. Other hormonal influences may be important, as shown by the studies of Addis.[38] Adrenalectomy decreases spontaneous proteinuria in males but not in females, and it blocks the proteinuriogenic effect of infused renin seen in normal animals. Further, the addition of thyroid supplements may hasten the development of the disease.[38] Genetic influences also appear to be important.[7,9,10] Most authors have studied the disease in random bred albino rats, including Sprague-Dawley, Yale strain, Slonaker-Addis, Wistar, and Holtzman. As noted in our present study, the inbred Lewis strain of rats developed little proteinuria with aging. This is in agreement with experiments in which we have followed this strain of rats as controls. On the other hand, the Fischer (F-344) inbred strain of rats appears to develop proteinuria and histologic damage at an accelerated rate compared to random bred Sprague-Dawley animals. Growth factors other than diet alone can influence the disease process. Unilateral nephrectomy accelerates the appearance of the lesion in females as well as males, but the acceleration is markedly greater in young rats with developing kidneys.[14,18,20,39] Another manipulation that will enhance the development of sclerosis is irradiation.[18,40] Again, this is more marked in unilaterally nephrectomized animals and can be ameliorated by dietary restriction. The mechanism by which these various factors influence the development of the lesion is not known.

Male Sprague-Dawley rats excrete more protein than females at about 2 months of age and the quantity increases with senescence. Not only does the amount increase, but the number of males developing "abnormal" proteinuria continues to rise throughout the life of the colony. In some reports, females have developed proteinuria similar to males at 1 year of age.[10] However, in our present series none of the females excreted abnormal amounts until 18 months of age. At this time, only 20% of females had abnormal proteinuria, compared to 66% of males. Young proteinuric animals excreted mainly albumin, while old proteinuric animals excreted protein in a profile like normal rat serum, demonstrating progressive loss

of glomerular basement membrane selectivity. Concomitantly, the serum albumin level fell, but the total level of serum protein remained normal because of increased production of α-globulins. This confirms the evolution of selective to nonselective proteinuria described by Couser *et al.*[6] and Perry *et al.*[10] The small amount of protein excreted in young nonproteinuric rats is α-globulin, stains for mucoprotein, and presumably originates from tubular cells. Studies by Perry *et al.*[10] and Sellers *et al.*[11] document the renal origin of the protein and demonstrate that prostatic and testicular secretions are not responsible for the proteinuria. These changes in pattern of protein excretion thus appear to occur on an age-related continuum. The relationship between these excretion pattern changes and glomerular or tubular damage remains to be clarified.

The histologic changes of the present study are similar to those previously reported.[5,6,9,19,20,41,42] We have attempted to draw conclusions regarding the interrelationships between proteinuria, aging, and the histologic lesions by studying a large number of animals in order to decrease variability. While the predominant pathologic changes of aging in man are vascular and ischemic, there is little evidence in the rat for pathology in blood vessels.[41,42] Instead, there is progressive development of tubular atrophy, interstitial infiltration with mononuclear cells, interstitial fibrosis, thickening of Bowman's capsule, mesangial accentuation followed by segmental sclerosis of isolated capillary loops and then by segments of tufts, and finally obsolescence of glomeruli. Adhesions between tufts and Bowman's capsule may be found, and crescents are not uncommon. The tubular atrophy is associated with colloid cast formation. These observations raise an important etiologic question: Which is damaged first, the glomerulus or the tubule? In the early report by Blatherwick and Medlar,[3] the authors postulated that toxic substances are filtered and reach the distal tubule, causing them to disintegrate "as a result of overwork or as a toxic phenomenon." This perceptive hypothesis can be interpreted both as initial glomerular basement membrane damage and leak with distal damage, or normal glomerular basement membrane function with primary tubular dysfunction. Some investigators suggest that the tubular system is the initial site of damage and the glomerular damage is secondary.[7,43] This is based upon finding increased tubular mitotic figures with aging while glomeruli are normal [43] and there is protection afforded by sodium restriction.[7] Other studies suggest that primary glomerular dysfunction occurs, perhaps secondary to increased glomerular basement membrane permeability with increased filtration of macromolecules.[6,20,42] Mesangial processing capacity could be overloaded, leading to glomerular damage with consequent tubular pathology.[6] We have selected rats of

different ages with a spectrum of degrees of proteinuria to ascertain if we could distinguish a relationship between the histologic changes, proteinuria, and aging. More specifically, we were interested in attempting to delineate further the relationship between glomerular damage, tubular pathology, and proteinuria in aging. The various scattergrams in Textfigures 3 and 4 depict the results of our categorization. The closest correlations existed between glomerular pathology and aging. When this association was examined in terms of only those animals excreting protein greater than 20 mg/day, there was little difference from the group as a whole while, as described in Results, the lines for these two groups relative to tubular pathology were parallel. These findings imply to us that glomerular pathology and proteinuria are increasing in the same population of rats and that this increase occurs as a function of age. The parallel lines of tubular changes suggest that the tubular pathology and proteinuria occur independently with aging and, in view of the association noted above for glomerular pathology, might well support the concept that primary glomerular damage results in secondary tubular pathology.

Dense glomerular basement membrane deposits similar to those seen in autologous immune complex nephropathy in rats are not found in this spontaneous lesion.[22] Glomerular basement membrane alterations with nonspecific mesangial trapping of certain serum proteins as seen in aminonucleoside nephrosis is similarly not present in this disease.[44] Analysis of our own data and that of other authors leads us to conclude that the alterations of glomerular basement membrane permeability occur as a function of age, are accelerated by agents producing a state of premature senescence, and retarded by methods of increasing longevity. Alterations of the sialoprotein "barrier" to filtered protein appears to have no role in this process.[6,45,46] The similarity of regression lines for both proteinuric and nonproteinuric rats increasing with age—while the tubular histologic grade paralleled proteinuria increasing with age—strongly implies that glomerular damage precedes proteinuria and tubular atrophy. Indirect information about the relationship between increased glomerular basement membrane permeability and focal sclerosis in rats is provided by the studies of Mauer *et al.*[47] These authors reported glomerular histologic abnormalities in alloxan diabetic rats which were considered very similar to premature aging. Yet, despite heavy immunoglobulin and complement deposits in diabetic rats, no abnormal proteinuria developed relative to the normal increase in proteinuria with aging in control rats. Again, this incriminates primary senescent mesangial damage independent of altered glomerular basement membrane permeability.

Immunoglobulin E has been shown to be an important intermediary in

the pathogenesis of acute serum sickness in rabbits.[48] Basophils sensitized with IgE release a platelet-activating factor upon contact with the sensitizing antigen. Platelets containing vasoactive amines are consequently released after this interaction. Treatment of rabbits with either acute or chronic serum sickness with histamine and serotonin antagonists provides protection from the deposition of circulating immune complexes.[49] A similar, though less significant, effect of vasoactive amines in autologous immune complex nephropathy in rats has been demonstrated.[34] While this sequence of events may well not result acutely in any soluble IgE complexes, the possibility exists that a life-long continuous activation of this system, even at low levels, might eventually lead to soluble complexes with localization in the kidney. Whether IgE complexes might induce renal damage is a moot point. Nonetheless, examination of tissue from more than 100 animals revealed only occasional regions of staining for IgE, probably in areas of sclerosis. These findings only indicate that we were unable to detect glomerular bound IgE and still do not eliminate the possibility that IgE-mediated release of vasoactive amines might play some role in the mesangial deposition of IgM.

Immunoglobulin A has been shown to be a common type of glomerular deposit in man, especially in focal glomerulonephritis with hematuria (Berger's disease) and in Henoch-Schoenlein purpura.[51] The pathogenetic significance of the IgA is unknown. In Berger's nephritis, no glomerular changes may be found or minimal proliferation of mesangial cells may be present, while in Henoch-Schoenlein purpura variable degrees of proliferative nephritis are seen. Obviously, other factors than the IgA are involved. In the present studies of rats, IgA was seldom found except in areas of sclerosis, despite the presence of heavy deposits of mesangial IgM in a distribution similar to that seen for IgA in the human diseases described above. The findings of fibrin were similar to those of IgE and IgA, except that the frequency was somewhat greater and occasional crescents and areas of periglomerular fibrosis were demonstrated. These deposits of immunoglobulins and fibrin would appear to be only in some of the sclerotic areas and not to bear any relationship to the IgM deposits or to the pathogenesis of the lesion.

The role of IgM in the pathogenesis of this lesion is unclear. It is associated with occasional deposits of fibrin, IgG, C3, and rarely IgE or IgA. Generally, however, only IgM is present. It cannot be eluted with PBS or normal saline but can be removed with acid elution or potassium thiocyanate, suggesting that it may represent an immune complex. The deposits do not fix complement *in vivo* or *in vitro*. Further, the failure to find other serum proteins in the same distribution as IgM augers against

nonspecific protein trapping. The IgM is found in both proteinuric and nonproteinuric animals after 1 month of age, in both males and females, and in several different strains of rats, some of which develop proteinuria and sclerosis while others do not. It is present in germfree animals, albeit in lesser quantities. These findings are similar to those reported by Markham *et al.*[52] in mice and by Poskitt *et al.*[53] in monkeys. Attempts to elute the antibody and identify an endogenous antigen capable of producing an autoimmune phenomenon as described by Linder *et al.*[54] have thus far been unsuccessful. The failure to find IgM deposits at birth or during the first few weeks of life indicates that either the IgM is not being trapped or that an immune response with consequent IgM complexes has not yet been mounted. Since IgM is not present at birth,[55] the latter phenomenon appears more likely. The antigen which elicits the IgM is as yet unknown, although the finding of IgM (even in lesser quantities) in germfree rats suggests that an endogenous antigen may be involved. Another possibility is that the IgM complexes are a heterogeneous group containing IgM combined with a variety of exogenous and endogenous antigens. The present findings by immunofluorescence are dissimilar to those reported by Elema and Arends.[7] These authors described a spontaneous glomerular hyalinosis and sclerosis in Wistar rats which resembled focal glomerular sclerosis in man. Deposits of immunoglobulins and complement were found only in a pattern suggestive of insudated material and not in the mesangial locations described in our rats. The pattern of IgM distribution in our Sprague-Dawley rats is very similar to that reported by Couser *et al.*[6] However, unlike those authors, we were unable to correlate the quantity of deposited IgM with the proteinuria of the animals. This may be related to the small number of animals in each age group examined by fluorescence in Couser's study. At the present time, the evidence is suggestive that the IgM represents an elutable immune complex being processed by the mesangium, but there is no evidence to incriminate the IgM or complexes in the pathogenesis of glomerular sclerosis in rats.

The aging process in animals and man has been associated with changes in immune function [56-58] and an autoimmune phenomenon has been postulated to be important in the pathogenesis of this renal lesion in rodents.[8,59,60] Indirect support for this is the fact that rodents develop numerous autoantibodies with aging associated with decreased cellular immune competence and a higher incidence of various types of specific autoimmune diseases in senescence.[61,62] Further, Linder *et al.*[54] and Porter *et al.*[63] described autoerythrocyte antibodies complexed with red cell antigens in mesangial deposits in mice, while Porter *et al.*[63] documented

the occurrence of viral antigen in mesangial deposits. Couser *et al.*[6] and our present work document the presence of IgM in mesangial deposits in rats, and the current evidence supports the concept that the IgM is present as some type of complex. The IgM deposits in these rats have not been shown to have antibody activity against rat serum or a variety of tissue, nuclear, and red cell antigens, nor have any IgM anti-red cell antibodies been demonstrated, either in the serum of these rats or on circulating red cells. No activity against lung tissue of rats with active consolidation with chronic pneumonia was demonstrable, although this common illness induced by a variety of pleuropneumonia-like organisms is very common in all rats.[64] The mesangial IgM appears thus to be an incidental finding, and the humoral immune system does not seem culpable in the pathogenesis of the lesion. However, the role of the cellular immune system is less clear. The ability of neonatal thymectomy and irradiation to accelerate the lesion is evidence of participation of the cellular immune system in this process. Starvation results in amelioration of the sclerosis and significantly protects the animals.[4,15,16,18] Starvation also produces involution of lymphoid organs and lymphopenia, and a reduction in antibody production of IgM and IgG, especially IgG.[58,65] These two situations (i.e., protection with depressed immune response in starvation and acceleration with depressed immune capacity with aging) seem incompatible, but work by Folch and Waksman [66] has demonstrated a "splenic suppressor cell" of thymic origin which is present in large numbers at birth, but declines with age. Thymectomy eliminates this population of cells; thus, if the aging changes were immunologically related, the evidence would implicate alteration of the suppressor cell or other cell populations closely linked to this cell.[66,67] Additionally, there appears to be decreasing amounts of thymic hormone with increasing age,[68] thus implicating a decrease in cell-mediated immunity from lack of this substance. Inhibition of the development of autoimmunity by giving young syngeneic thymocytes further incriminates the role of cell-mediated immunity in this process.[69-71] Thus, indirect evidence would implicate the cell-mediated immune system as an etiologic factor in glomerular sclerosis of senescence in rats if the immune system is involved.

Despite the development of proteinuria and progressive histopathology, aging rats maintain essentially normal levels of serum creatinine and die of nonrenal diseases. This clinical course is markedly different from the focal glomerular sclerosis seen in man, where progressive renal failure within a short period of time is the rule, regardless of therapy.[72-75] The deposits of IgM in rats are mesangial and usually diffuse, with minimal amounts of other immunoglobulins, complement, and fibrin. Similar dif-

fuse mesangial IgM deposits have been noted in a few patients.[75] More commonly, the deposits of serum proteins in focal glomerulosclerosis in man are extremely focal and segmental, with complete sparing of many glomeruli and involvement of perhaps isolated tufts in other glomeruli.[72-74] Thus, focal sclerotic lesions in man and rats generally bear little resemblance to each other by immunofluorescence or clinical findings. Further, while the proteinuria and histologic changes are both age and sex-related in rats, little such association is present in focal glomerulosclerosis in man. Spontaneous glomerular sclerosis in Sprague-Dawley rats is for the most part dissimilar to focal glomerulosclerosis in man; nonetheless, the further study of the pathogenesis of this process may provide greater insight into the factors which are involved in the induction of glomerular sclerosis in general. The information obtained may eventually have applicability in man to those glomerulopathies with focal sclerosis which are associated with a significant risk of progressive renal dysfunction terminating in renal failure.

References

1. Newburgh, LH, Curtis AC: Production of renal injury in the white rat by the protein of the diet. Arch Intern Med 42:801–821, 1928
2. Medlar EM, Blatherwick NR: The pathogenesis of dietary nephritis in the rat. Am J Pathol 13:881–896, 1937
3. Blatherwick NR, Medlar EM: Chronic nephritis in rats fed high protein diets. Arch Intern Med 59:572–596, 1937
4. Saxton JA, Kimball GC: Relation of nephrosis and other diseases of albino rats to age and to modification of diet. Arch Pathol 32:951–965, 1941
5. Berg BN: Spontaneous nephrosis with proteinuria, hyperglobulinemia and hypercholesterolemia in the rat. Proc Soc Exp Biol Med 119:417–420, 1965
6. Couser WG, Stilmant MM: Mesangial lesions and focal glomerular sclerosis in the aging rat. Lab Invest 33:491–501, 1975
7. Elema JD, Arends A: Focal and segmental glomerular hyalinosis and sclerosis in the rat. Lab Invest 33:554–561, 1975
8. Couser WG, Stilmant MM: The immunopathology of the aging rat kidney. J Gerontol 31:13–22, 1976
9. Kraus B, Cain H: Über eine spontane Nephropathie bei Wistar-ratten: Die licht und elektronenmikroskopischen Glomerulum veränderungen. Virchow Arch [Pathol Anat Histol] 363:343–358, 1974
10. Perry SW: Proteinuria in the Wistar rat. J Pathol Bacteriol 89:729–733, 1965
11. Sellers AL, Goodman HC, Marmorston J, Smith M: Sex difference in proteinuria in the rat. Am J Physiol 163:662–667, 1950
12. Linkswiler H, Reynolds MS, Baumann CA: Factors affecting proteinuria in the rat. Am J Physiol 168:504–508, 1952
13. Rumsfeld HW Jr: Role of dietary protein in normal rat proteinuria. Am J Physiol 184:473–478, 1956
14. Kennedy GC: Effects of old age and over-nutrition on the kidney. Br Med Bull 13:67–70, 1957
15. Simms HS, Berg BN: Longevity and the onset of lesions in male rats. J Gerontol 12:244–252, 1957

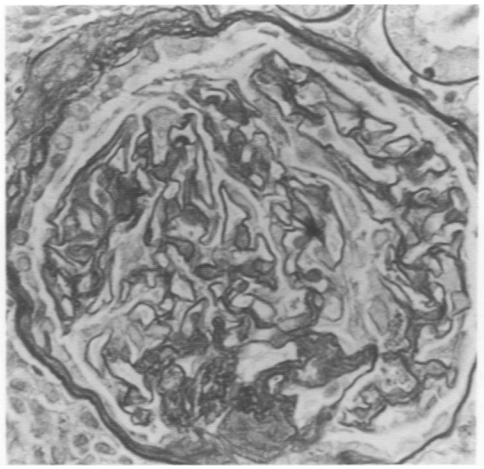
16. Berg BN, Simms HS: Nutrition and longevity in the rat. II. Longevity and onset of disease with different levels of food intake. J Nutr 71:255–263, 1960
17. Foley WA, Jones DCL, Osborn GK, Kimeldorf DJ: A renal lesion associated with diuresis in the aging Sprague-Dawley rat. Lab Invest 13:439–450, 1964
18. Wachtel LW, Cole LJ, Rosen VJ: X-ray induced glomerulosclerosis in rats: Modification of lesion by food restriction, uninephrectomy and age. J Gerontol 21:442–448, 1966
19. Lalich JJ, Faith GC, Harding GE: Protein overload nephropathy. Arch Pathol 89:548–559, 1970
20. Lalich JJ, Allen JR: Protein overload nephropathy in rats with unilateral nephrectomy. II. Ultrastructural study. Arch Pathol 91:372–382, 1971
21. Masugi M: Über die experimentelle Glomerulonephritis durch das specifische Antinierenserum: Ein beitrag zur pathogenese der diffusen Glomerulonephritis. Beitr Pathol Anat Allgem Pathol 92:429–437, 1934
22. Heymann W, Hackel DB, Harwoods S, Wilson SG, Hunter JL: Production of nephrotic syndrome in rats by Freund's adjuvants and rat kidney suspension. Proc Soc Exp Biol Med 100:660–669, 1959
23. Dixon FJ, Feldman JD, Vazquez JJ: Experimental glomerulonephritis: The pathogenesis of a laboratory model resembling the spectrum of human glomerulonephritis. J Exp Med 113:899–919, 1961
24. Davidson J, Henry JB: Clinical Diagnosis by Laboratory Methods, Fourteenth edition. Philadelphia, W. B. Saunders Co., 1969, p 48
25. Bolton WK, Sande MA, Normansell DE, Sturgill BC, Westervelt FB Jr: Ventriculojugular shunt nephritis with *Corynebacterium bovis*. Am J Med 59:417–423, 1975
26. Stechschulte DJ, Austen KF: Immunoglobulins of rat colostrum. J Immunol 104:1052–1062, 1970
27. Nisonoff A: Enzymatic digestion of rabbit gamma globulin and antibody and chromatography of digestion products. Meth Med Res 10:134–141, 1964
28. Avrameas S, Ternynck T: The cross-linking of proteins with gluteraldehyde and its use for the preparation of immunoadsorbents. Immunochemistry 6:53–66, 1969
29. Ogilvie BM: Role of adult worms in immunity of rats to *Nippostrongylus brasiliensis*. Parasitology 55:325–335, 1965
30. Ogilvie BM: Reagin-like antibodies in rats infected with the nematode parasite *Nippostrongylus brasiliensis*. Immunology 12:113–131, 1967
31. Ovary Z: Cutaneous anaphylaxis in the albino rat. Br J Pharmacol 15:293–301, 1960
32. Stechschulte DJ, Orange RP, Austen KR: Immunochemical and biologic properties of rat IgE. I. Immunochemical identification of rat IgE. J Immunol 5:1082–1086, 1970
33. Spiegelberg HL, Weigle WO: The production of antisera to human gamma G subclasses in rabbits using immunological unresponsiveness. J Immunol 101:377–380, 1968
34. Bolton WK, Spargo BH, Lewis EJ: Chronic autologous immune complex glomerulopathy: Effect of cyproheptadine. J Lab Clin Med 83:695–704, 1974
35. Clark HF, Shepard CC: A dialysis technique for preparing fluorescent antibodies. Virology 20:642–644, 1963
36. McPhaul JJ, Dixon FJ: Characterization of human anti–glomerular basement membrane antibodies eluted from glomerulonephritic kidneys. J Clin Invest 49:308–317, 1970
37. Burkholder PM: Complement fixation in diseased tissues. I. Fixation of guinea pig complement in sections of kidney from humans with membranous glomerulonephritis and rats injected with anti-rat kidney serum. J Exp Med 114:606–616, 1961
38. Addis T, Marmorston J, Goodman HC, Sellers AL, Smith M: Effect of adrena-

lectomy on spontaneous and induced proteinuria in the rat. Proc Soc Exp Biol Med 74:43–46, 1950
39. Striker GE, Nagle RB, Kohnen PW, Smuckler EA: Response to unilateral nephrectomy in old rats. Arch Pathol 87:439–442, 1969
40. Guttman PH, Kohn HI: Progressive intercapillary glomerulosclerosis in the mouse, rat and Chinese hamster associated with aging and x-ray exposure. Am J Pathol 37:293–304, 1960
41. Andrew W, Pruett D: Senile changes in the kidneys of Wistar Institute rats. Am J Anat 100:51–69, 1957
42. Gray JE, Weaver RN, Purmalis A: Ultrastructural observations of chronic progressive nephrosis in the Sprague-Dawley rat. Vet Pathol 11:153–164, 1974
43. Sworn MJ, Fox M: Renal age changes in the rat compared with human renal senescence: An autoradiographic study. Invest Urol 12:140–145, 1974
44. Unanue E, Dixon FJ: Experimental glomerulonephritis. IV. Participation of complement in nephrotoxic nephritis. J Exp Med 119:965–982, 1964
45. Rosenquist TH, Bernick S: Histochemistry of renal basal lamenae: Adolescent compared with senescent rats. J Gerontol 26:176–185, 1971
46. Couser WG, Stilmant MM, Darby C: Autologous immune complex nephropathy. I. Sequential study of immune complex deposition ultrastructural changes, proteinuria, and alterations in glomerular sialoprotein. Lab Invest 34:23–30, 1976
47. Mauer SM, Michael AF, Fish AJ, Brown DM: Spontaneous immunoglobulin and complement deposition in diabetic rats. Lab Invest 27:488–494, 1972
48. Benveniste J, Henson PM, Cochrane CG: Anaphylactic reactions and deposition of circulating immune complexes. Inflammation: Mechanisms and Control. Edited by IH Lepow, PA Ward. New York, Academic Press, Inc., 1972, p 179
49. Kniker WT: Modulation of the inflammatory response *in vivo*: Prevention or amelioration of immune complex disease.[48] pp 335–336
50. Berger J, Hinglais N: Les depots intercapillaires d'IgA-IgG. J Urol Nephrol (Paris) 74:694–695, 1968
51. Urizar RE, Michael A, Sisson S, Vernier RL: Anaphylactoid purpura. II. Immunofluorescent and electron microscopic studies of the glomerular lesions. Lab Invest 19:437–450, 1968
52. Markham RV, Sutherland JC, Mardiney MR Jr: The ubiquitous occurrence of immune complex localization in the renal glomeruli of normal mice. Lab Invest 29:111–120, 1973
53. Poskitt TR, Fortwengler HP Jr, Bobrow JC, Roth JC: Naturally occurring immune complex glomerulonephritis in monkeys (*Macaca irus*). I. Light, immunofluorescence and electron microscopic studies. Am J Pathol 76:145–159, 1974
54. Linder E, Pasternack A, Edgington TS: Pathology and immunology of age associated disease of mice and evidence for an autologous immune complex pathogenesis of the associated renal disease. Clin Immunol Immunopathol 1:104–121, 1972
55. van Breda Vreisman PJC, Feldman JD: Rat γM immunoglobulins: Isolation and some biological characteristics. Immunochemistry 9:525–534, 1972
56. Mackay I: Ageing and immunological function in man. Gerontology 18:285–304, 1972
57. Walford RL: Immunologic theory of aging: Current status. Fed Proc 33:2020–2027, 1974
58. Yunis EJ, Fernandes G, Stutman O: Susceptibility to involution of the thymus-dependent lymphoid system and autoimmunity. Am J Clin Pathol 56:280–292, 1971
59. Guttman PH, Wuepper KD, Fudenberg HH: On the presence of γG and γ1C globulins in renal glomeruli of aging and neonatally x-irradiated mice. Vox Sang 12:329–339, 1967

60. Peter CP: Possible immune origin of age-related pathological changes in long-lived mice. J Gerontol 28:265–275, 1973
61. Kishimoto S, Shigemoto S, Yamamura Y: Immune response in aged mice. Changes of cell-mediated immunity with aging. Transplantation 15:455–459, 1973
62. Konen TG, Smith GS, Walford RL: Decline in mixed lymphocyte reactivity of spleen cells from aged mice of a long-lived strain. J Immunol 110:1216–1221, 1973
63. Porter DD, Porter HG, Cox NA: Immune complex glomerulonephritis in one year old C57 BL 6 mice induced by endogenous murine leukemia virus and erythrocyte antigens. J Immunol 111:1626–1633, 1973
64. Gay FW, Maguire ME, Baskerville A: Etiology of chronic pneumonia in rats and a study of the experimental disease in mice. Infect Immun 6:83–91, 1972
65. Chandra RR: Antibody formation in first and second generation offspring of nutritionally deprived rats. Science 190:289–290, 1975
66. Folch H, Waksman BH: The splenic suppressor cell. I. Activity of thymus-dependent adherant cells: changes with age and stress. J Immunol 113:127–139, 1974
67. Steinberg AD, Law LD, Talal N: The role of NZB NZW F_1 thymus in experimental tolerance and autoimmunity. Arthritis Rheum 13:369–377, 1970
68. Bach JF, Dardenne M, Bach M: Demonstration of a circulating thymic hormone in mouse and man. Transplant Proc. 5:99–104, 1973
69. Teague PO, Friou GJ: Antinuclear antibodies in mice. II. Transmission with spleen cells: inhibition or prevention with thymus or spleen cells. Immunology 17:665–675, 1969
70. Denman AM, Barnes RD: Hypothesis: Cooperating and controlling functions of thymus-derived lymphocytes in relation to autoimmunity. Lancet 2:135–140, 1971
71. Gershwin ME, Steinberg AD: Suppression of autoimmune hemolytic anemia in New Zealand (NZB) mice by syngeneic young lymphocytes. Clin Immunol Immunopathol 4:38–45, 1975
72. Habib R: Focal glomerular sclerosis (editorial). Kidney Int 4:355–361, 1973
73. Hyman LR, Burkholder PM: Focal sclerosing glomerulopathy with segmental hyalinosis: A clinicopathologic analysis. Lab Invest 28:533–544, 1973
74. Matalon R, Katz L, Gallo G, Waldo E, Cabaluna C, Eisinger RP: Glomerular sclerosis in adults with nephrotic syndrome. Ann Intern Med 80:488–495, 1974
75. Newman WJ, Tisher CC, McCoy RC, Gunnells JC, Krueger RP, Clapp JR, Robinson RR: Focal glomerular sclerosis: Contrasting patterns in children and adults. Medicine 55:67–87, 1976

Acknowledgments

The authors would like to acknowledge the excellent technical aid of D. Shifflett and the secretarial assistance of B. Parks.



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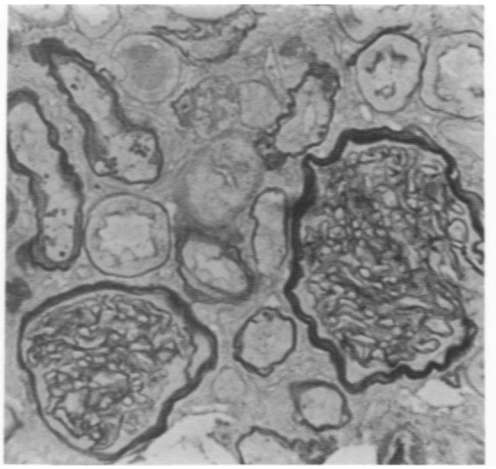

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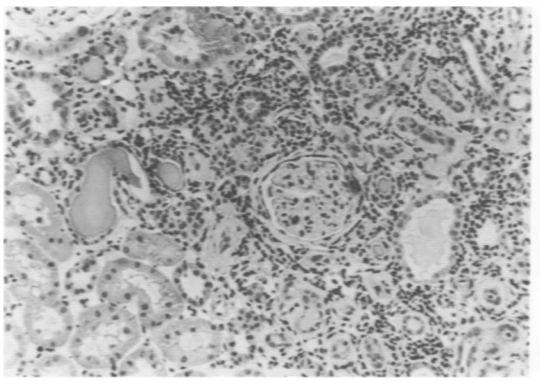

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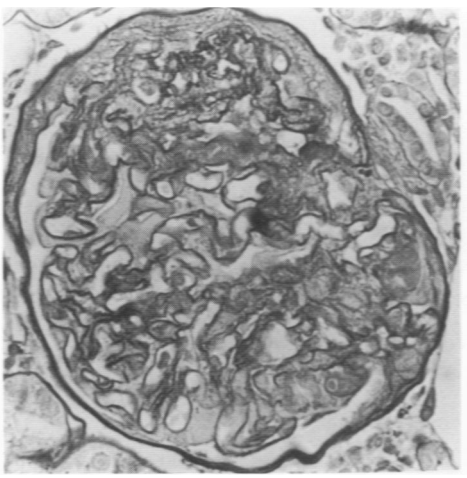

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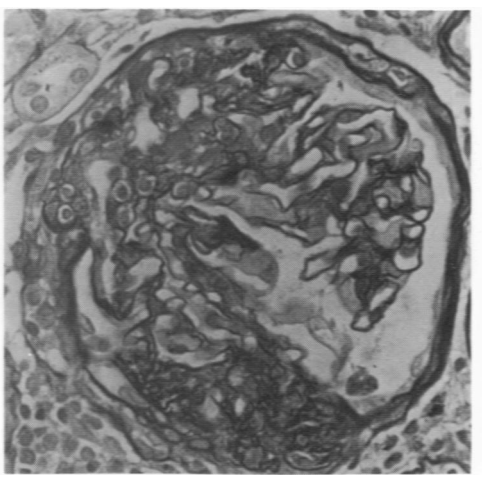
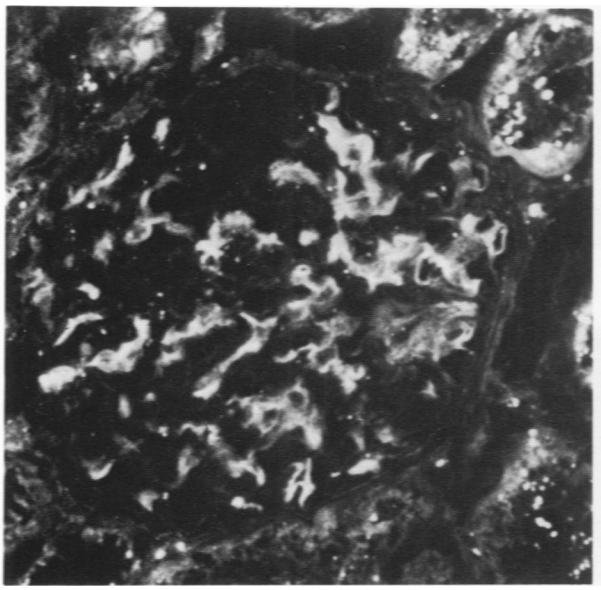
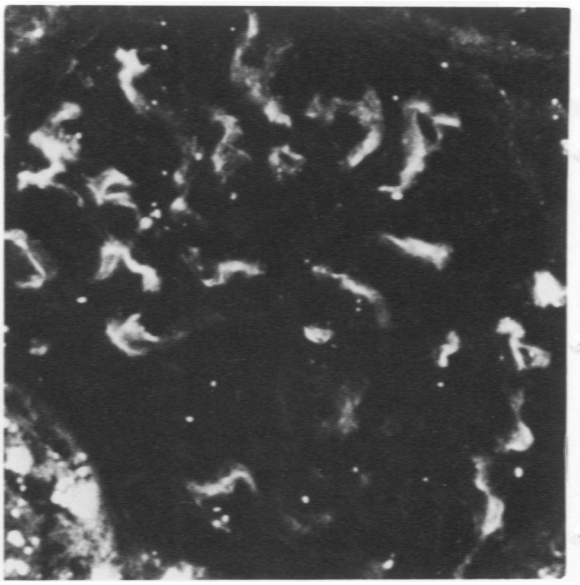


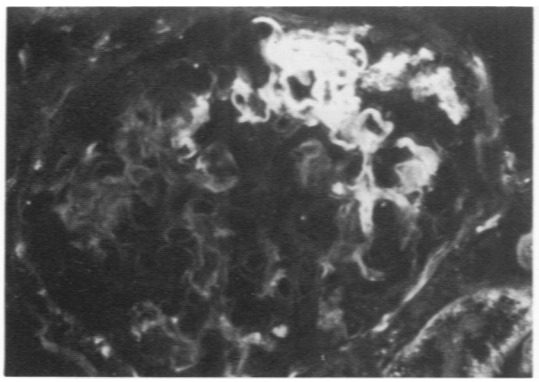
3B

Figure 1A—Glomerulus from a 6-month-old Sprague-Dawley male rat showing increased mesangial matrix (PAS, × 350). **B**—Section from a 12-month-old rat illustrating tubular atrophy and prominant thickening of Bowman's capsule (PAS, × 490). **Figure 2**—Section of an 18-month-old rat showing interstitial cellular infiltrate, colloid casts, and a glomerulus with periglomerular fibrosis (H&E, × 110). **Figure 3A**—Glomerulus from an 18-month-old rat illustrating a crescent with mesangial sclerosis and adhesions between tufts and the capsule (PAS, × 380). **B**—Glomerulus from an 18-month-old rat demonstrating adhesions, with mesangial sclerosis and segmental collapse (PAS, × 370).

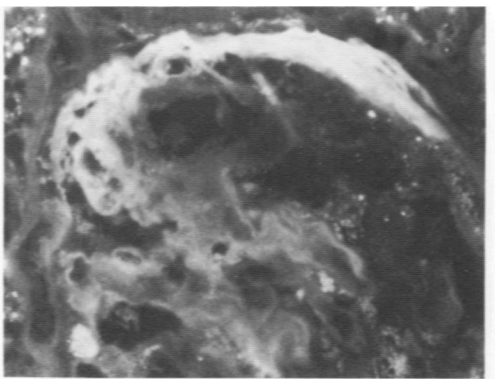


4A





5A



5B

Figure 4A—Glomerulus from an 18-month-old rat stained for IgM. Mesangial deposits of 3+ intensity are illustrated. Tubular autofluorescence is a normal finding. (× 380) **B**—Glomerulus from the same rat demonstrating 1+ mesangial IgM deposits (× 640). **Figure 5A**—Glomerulus from an 18-month-old rat demonstrating 3+ segmental fibrin deposition in an area of sclerosis (× 340). **B**—Glomerulus from a 12-month-old rat showing fibrin in a crescent (× 530).