PROGRESSIVE INTERCAPILLARY GLOMERULOSCLEROSIS IN THE MOUSE, RAT, AND CHINESE HAMSTER, ASSOCIATED WITH AGING AND X-RAY EXPOSURE

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When the kidneys of CAF_1 and BALB/c mice were examined histologically to determine the late effects of whole-body irradiation and of aging, a progressive lesion was found which involved principally the intercapillary supporting cells or the so-called mesangium of the glomeruli. Similar changes were also seen in the glomeruli of A/Vi mice, Chinese hamsters, and AC(I) rats. Contrary to the observations of Lamson and associates [1,2] in Wistar rats, Furth and colleagues [3] in LAF_1 mice, and Cole, Nowell and Ellis [4] in LAF_1 mice, sclerosis of small glomerular vessels was rarely seen in our normal and irradiated CAF_1 and BALB/c mice. Since, to our knowledge, the glomerular lesions found in our study have not hitherto been described, a histologic investigation was made in an effort to trace their histogenesis and to determine what effect late irradiation may have on their progress.

MATERIAL AND METHODS

Animals

Mice were obtained from the stock of the Cancer Research Genetics Laboratory, University of California, Berkeley. Three kinds were used: inbred strains BALB/c and A/Vi, and the CAF_1 hybrid from crossing BALB/c females with A/Vi males. The rats were of the A × C/9935 (Irish agouti) inbred strain, bred in our laboratory. The Chinese hamsters were obtained through the courtesy of Dr. George Yerganian, Children's Cancer Research Foundation, Boston. The two younger animals were from BUY-11 mothers; the two older were from BUY-7 and BUY-8 mothers.

All of the animals were housed in drawer-type cages with wire mesh floors and were fed Purina Laboratory Chow Checkers and tap water *ad libitum*.

The BALB/c mice were sacrificed during the course of an experiment in which mortality rate and tumor incidence were under study.[5] The CAF_1 mice 385 days of age and older were from a similar experiment not yet reported.

Irradiation

A single exposure to the entire body was given to some of the animals. The radiation factors were: 250-kvcp x-rays, HVL 1.5 mm. Cu, exposure dose rate in soft

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tissue ~ 45 r per minute. The radiation dose in Table I is the exposure dose in soft tissue. The absorbed dose in rads would be about 5 per cent less.

Tissue Technique

The tissues were fixed immediately after sacrifice in a modified Tellyesniczky's fluid (737 cc. of 95 per cent alcohol, 100 cc. of neutral formalin, 50 cc. of glacial acetic acid, 200 cc. of water). After 24 hours the tissue was transferred to 80 per cent alcohol, dehydrated in amyl acetate, and embedded in paraffin. Sections were cut at 4 μ and were stained as follows: trichrome stain of Gomori; Weigert's elastic tissue stain; hematoxylin and eosin; the Kramer and Windrum[6] method for metachromasia, using concentrated sulfuric acid; Mowry's colloidal iron-periodic acid-Schiff stain[7]; Alcian blue; crystal violet; Lison's toluidine blue; periodic acid-Schiff (PAS) stain (Lillie's modification); Wilder's reticulum stain; modified * Rinehart and Abul-Haj stain for mucopolysaccharides[8]; Van Gieson stain; azocarmine of Heidenhain; Peers's modification of Mallory's phosphotungstic acid-hematoxylin (PTAH); and Weigert's stain for fibrin. In addition, preparations were examined with phase contrast microscopy.

Observations and Results

Structure of Normal Mouse Glomerulus

In order to evaluate the changes in the glomeruli of aging mice, an understanding of the normal structure of the mouse glomerulus was essential. A series of normal young mice was sacrificed at ages 36 to 254 days, and the kidneys were prepared as described above.

Three cellular elements were clearly defined in the glomerulus of a normal mouse: a visceral epithelial layer which covered the surface of the glomerular tuft, an inner lining of endothelial cells, and a third type of cell, the intercapillary cell (IC cell), or mesangial cell, which formed a highly developed supporting structure in the glomerulus (Fig. 1). The epithelial cells had pale, finely granular chromatin structure and delicate nuclear membranes; the cytoplasm appeared finely granular and was faintly basophilic. The endothelial cells were small, with oval to flat nuclei of coarse chromatin structure, and there was scant cytoplasm. With the Rinehart-Abul-Haj stain, a refractive, pale lemon-yellow membrane formed a thin layer on the inner surface of the basement membrane; this inner membrane is interpreted as being the attenuated extension of the endothelial cytoplasm. The IC cells contained abundant cytoplasm which stained orange-yellow with the cochineal of the Rinehart-Abul-Haj stain and brownish-yellow with PTAH. The cytoplasm was attenuated, branched, and appeared to fuse with adjacent IC cells. The nuclei were larger than those of the endothelial cells, and showed moderate pleomorphism and irregularity. They exhibited a coarse chromatin pattern and a prominent nucleolus. These cells were

* The colloidal iron used in the Rinehart-Abul-Haj stain was prepared according to the Müller modification, as suggested by Mowry.[7] This proved to be more stable than the preparation originally used, and the results were easily reproducible.

embedded in a ground substance, staining light blue with colloidal iron and pink with the PAS procedure.

In sections obtained from the hilus of the glomerulus, which included the afferent arterioles, the IC cells appeared continuous with the perithelial elements surrounding the arteriole and extended into the glomerulus about the main branches of the afferent vessels. The IC cells formed a thick mantle about the dilated intraglomerular portion of the afferent artery. From the hilus, the cells fanned out in fingerlike processes, 3 to 5 per section, and extended almost to the peripheral margin of the glomerular tuft, forming delicate columns, or the so-called axes, supporting the glomerular capillary loops (Fig. 2). In sections taken at right angles to the axes, the IC cells were distributed fairly evenly. These were clearly identifiable by cytoplasmic staining, nuclear structure, and their relation to the basement membrane and ground substance.

The orientation of the IC cells with respect to the capillary wall and the basement membrane was investigated by means of PAS stains, colloidal iron, the sulfonation technique of Kramer and Windrum,[6] and phase contrast microscopy. Kramer and Windrum demonstrated that when tissues were treated briefly with concentrated sulfuric acid, basement membranes and ground substance were stained metachromatically as a result of sulfonation of the acid polysaccharides. With this technique, the afferent artery showed a well defined basement membrane beneath the endothelium. A distinct latticework of similar structure extended outward from the basement membrane to enclose the perithelial cells. This structure could be followed into the glomerulus where the continuity of the basement membrane of the afferent vessel with that of the main capillary branches was clearly demonstrable. The latticework of basement membrane substance in the outer wall of the afferent vessel could be traced into the glomerulus where it formed a complex network enclosing the IC cells at the hilus. As one approached the central and peripheral portions of the tufts, this orientation was difficult to follow. However, it was possible to demonstrate strands from the capillary basement membrane that ramified among the IC cells (Fig. 3). These findings with light microscopy are in agreement with those of Yamada,[9] who, with electron microscopy, found that the basement membrane of the capillary wall continued over the IC cells and, in addition, sent out small processes which formed a coarse, spongelike network enmeshing the IC cells. As stated by Mueller,[10] fingerlike projections extending from the basement membrane around and through the cells of the stalk are present in electron micrographs of most observers; he is of the opinion, however, that these may be due to tangential sections of wrinkled membranes.

In thin sections at the peripheral capillary tufts, cytoplasmic processes of the IC cells appeared to penetrate the basement membrane of the capillary wall, breaking its continuity and establishing intimate contact with the endothelium. This relationship was also observed by Yamada.[9] Inasmuch as the IC cells are enveloped by basement membrane substance arising from the capillary basement membrane, one cannot conclude that these cells are within the capillary lumen because they are within the confines of the basement membrane.

In respect to the existence of the third type of cell described—the IC cells—the authors' views are consistent with the observations of Policard, Collet and Giltaire-Ralyte,[11] Zimmerman,[12] MacCallum,[13] Kimmelstiel,[14] McManus, Lupton and Graham,[15] Yamada,[9] Churg and Grishman,[16] and Jones.[17] They are opposed to the views of Hall,[18] Dalton,[19] Rinehart, Farquhar, Jung and Abul-Haj,[20] Mueller, Mason and Stout,[21] Farquhar and associates,[22,23] Kurtz,[24] Pease,[25] and Vimtrup,[26] who recognize only two types of cells forming the capillary structure of the glomerulus, namely, endothelium and epithelium. Since the controversy on the structure of the normal glomerulus has been well covered in the excellent review of the older literature by McGregor[27] and in the recent literature by Mueller,[10] the reader is referred to these articles for further information.

In summary, the histologic basis for recognition of a third type of cell in the glomerulus is as follows: (a) continuity of the IC cell with perithelial elements of afferent arterioles; (b) selective staining of the cytoplasm in IC cells; (c) envelopment of IC cells in ground substance; (d) relation of the IC cell to the basement membrane of the capillary and to the intercapillary basement membrane; (e) arrangement of IC cells to form supporting structures to glomerular capillary loops.

Description of Glomerular Changes in the Aging and Irradiated Mouse

The principal alterations were found in the intercapillary cells and spaces. The Rinehart-Abul-Haj stain and the PTAH stain proved most valuable in studying these cells and the ground substance. The cytoplasm of the IC cells stained selectively with these stains, exhibiting a brilliant orange-yellow color with the cochineal component of the former and brownish-yellow with the latter. The ground substance stained light blue with colloidal iron, and the contours of the basement membranes were clearly defined. There was a gradual thickening of the mesangial axis, owing to an increase in the number and size of the IC cells. In the early lesions, the IC cells maintained their normal orientation (Figs. 4 and 5); later they became irregularly grouped in clusters and exhibited irregularity in size and shape of their nuclei. An appreciable increase in

cytoplasm was noted (Fig. 6). Only rarely, a concentric arrangement of these cells was noted. PAS-positive substance in the form of irregular fibrillar material was seen in increasing quantities engulfing the IC cells. Rarely, small clumps of dense hyaline material were seen, staining positively with PAS and deep blue with azocarmine. With the increase of PAS-positive material in the intercapillary spaces, there was a gradual loss of colloidal iron-positive-staining ground substance. Reticulum stain showed the presence of fine and coarse threads of silver impregnated fibers in the thickened intercapillary spaces in the hilus, but rarely in the peripheral portions of the glomeruli. Collagen fibers were extremely rare; when present, these were found in the hilus and not in the peripheral areas.

Although the basement membrane appeared thickened with PAS stain and with azocarmine, with phase contrast microscopy and the Kramer-Windrum technique this was not verified. Phase contrast microscopy of PAS-stained slides clearly showed a basement membrane of approximately normal diameter embedded in PAS-positive material. This observation casts some doubt that the PAS reagent and colloidal iron actually stain the basement membrane. It is more likely that the contours of the membranes are visualized as a result of staining of the mucopolysaccharides deposited on their surfaces. Sections of advanced lesions stained with periodic acid-silver methenamine showed the presence of knob-like excrescences on their outer surfaces, imparting a coarsely serrated appearance to the basement membranes. Whether this represents actual thickening of the membranes or deposits of mucopolysaccharides in the interstices of the foot processes is not clear.

In far advanced lesions, the main body of the glomerulus was occupied by thickened mesangium with compression and obliteration of the capillaries. The capillary loops remained patent only along the peripheral margins of the glomerulus (Fig. 7). In PAS-stained sections and with phase contrast microscopy, these late stages showed folding and moderate wrinkling of the basement membrane. Collapse of the capillaries produced areas composed of closely matted strands of basement membrane (Fig. 8).

The endothelial cells did not show any evidence of proliferation. There was no indication of an intracapillary deposition of fibrin or hyalin. Inflammatory exudate was seen neither within nor outside the capillaries. The epithelium exhibited no evidence of proliferation.

The basement membrane of Bowman's capsule appeared thickened in aging animals; this thickening, however, did not always parallel the glomerular alterations. There was no evidence of proliferation of the parietal layer. As a rule, the changes in the IC cells preceded by a long

interval the thickening of the basement membrane of Bowman's capsule. With increasing severity of the lesions, secondary changes were seen in the other parts of the kidney: cystic tubular atrophy was common, large numbers of casts appeared in the collecting tubules, and moderate interstitial fibrosis was noted. Only very rarely was there sclerosis of the afferent arterioles or interlobular arteries. Subintimal hyaline deposits were not observed in the afferent arterioles. Examination of the medulla showed no indication of inflammation or of the amyloid papillonephritis described by Dunn.[28] The lesions of spontaneous nephritis in mice described by Kirschbaum, Bell and Gordon[29,30] were not encountered in any of our animals.

The glomerular changes in untreated and in irradiated mice were similar in structure and development. To estimate the degree of involvement, the glomeruli were graded as follows: 0, normal; I, doubtful (Fig. 4); II, mild (Fig. 5); III, moderate (Fig. 6); IV, marked (Fig. 7). The grade for a kidney was the sum of numerical grades in 25 glomeruli chosen at random. No differences were found between the results for males and females, and the data for both sexes in each group were therefore pooled.

The data relating intercapillary sclerosis to age are summarized in Table I for the 3 kinds of mice studied. Each entry in the table consists of the average grade for a group, the standard deviation of the grade, and, in parentheses, the number of animals in the group. In the case of the nonirradiated mice, it was clear that sclerosis increased progressively with age during the first 2 years of life. The maximum degree may have occurred somewhat earlier in the BALB/c strain than in the CAF_1 hybrid. With quantitative grading, differences could be established during the first year of life.

Irradiation effected an increase in the degree of sclerosis, but only after a latent period during which no differences were noted. The duration of the latent period in the CAF_1 hybrid depended on the age of the animals at the time of irradiation. Animals irradiated at age 385 days did not differ in degree of sclerosis from the controls one year later (age 650 to 799 days), but did differ from them 490 days later (age 800 to 949 days); animals irradiated at age 550 days did not differ from the controls 175 days later, but did differ 325 days later (age 800 to 949 days); animals irradiated at age 730 days did not differ 140 days later.

In the case of the BALB/c strain irradiated at age 435 days, a significant increase in sclerosis was noted 290 days later (age 650 to 799 days).

For some of the animals graded in Table I, the weights of the kidneys and hearts were available. The data, analyzed separately for males and

TABLE I

GRADES OF INTERCAPILLARY GLOMERULAR SCLEROSIS AS A FUNCTION OF AGE AND X-RAY EXPOSURE

Mouse	Dose (r)	Age at exposure (days)	Age at sacrifice (days) 36 to 99	100 to 299	300 to 499	500 to 649	650 to 799	800 to 949
CAF_1	0		7 ± 3 (16)	17 ± 6 (8)		38 ± 16 (14)	55 ± 15 (10)	56 ± 16 (13)
	550	385				40 ± 9 (6)	57 ± 10 (8)	89 ± 8 (4)*
		550				41 ± 13 (15)	55 ± 8 (6)	71 ± 7 (12)*
		730					47 ± 17 (10)	48 ± 10 (13)
BALB/c	0		10 ± 3 (9)	13 ± 4 (7)	44 ± 8 (8)		47 ± 17 (12)	
	550	435					59 ± 11 (16)†	
A/Vl	0		8 ± 2 (8)	19 (2)	25 ± 12 (8)			

Each entry states the mean ± the standard deviation of the grade, followed in parentheses by the number of animals graded. Two thirds of the observations fall within the range of grades bounded by the mean ± standard deviation.

*$P \leqq 0.01$. †$P \leqq 0.05$

females, showed that the radiation-induced increase in sclerosis was not consistently associated with either an increase or a decrease in cardiac or renal weight (Table II).

TABLE II

INTERCAPILLARY GLOMERULOSCLEROSIS AND ORGAN WEIGHT

Mouse	Sex	X-ray dose (r)	Age at sacrifice (days)	No. of mice	Kidney		Heart (mg.)
					Grade	Weight (mg.)	
CAF_1	M	0	800 to 949	5	59	484	165
		550		4	71	552	158
	F	0	800 to 949	7	53	423	140
		550		7	76	409	150
BALB/c	M	0	683 to 786	6	47	583	177
		550		9	59	541	157
	F	0	683 to 786	6	47	428	130
		550		9	59	421	129

Glomerular Changes in the Aging Rat and Chinese Hamster

Examination of a small series of young and old rats and Chinese hamsters led to the conclusion that a significant increase in intercapillary glomerular sclerosis occurred in these animals with age. The appearance of the progressive lesion was strikingly similar to that observed in mice. The IC cells were well developed in both species, and the mesangium could be readily identified. It appeared, however, that the mesangium was more highly developed in the mouse than in the rat, the hamster being intermediate. The animals were examined as follows: 4 rats aged 80 days; 4 rats aged 835 days; 2 hamsters aged 66 days; 2 hamsters aged 745 days. The differences between the young and old animals were striking; the degree of intercapillary sclerosis was not as marked, however, as in the mouse.

DISCUSSION

The glomerular alterations described in this paper are similar in many respects to those described by Kimmelstiel [14] in man. Kimmelstiel described broadening of the supporting connective tissue of the glomeruli which at first appeared fibrillar and later hyaline but with very little change in the basement membrane. He was not convinced that there was an actual increase in the number of supporting connective tissue cells. These lesions were not associated with arterial disease. It must be emphasized that the renal changes described later by Kimmelstiel and Wilson [31] as a form of intercapillary glomerulosclerosis associated with diabetes differ strikingly from those observed in mice in the present

study. As pointed out by Allen,[32] the lesions of diabetic intercapillary glomerulosclerosis result from laminated hyaline thickening of the capillary wall. Farquhar, Hopper and Moon [22] also indicated that the primary process in the disease was a thickening of the basement membrane and deposition of interendothelial hyalin. Bell [33] believed that the nodular hyaline lesions were intracapillary formations and that the intercapillary hyaline masses were the product of the inner capillary basement membrane. Horn and Smetana [34] found that the lesions of intercapillary glomerulosclerosis might occur in arteriosclerotic kidneys independently of diabetes. The lesions observed in mice do not show dependency on or association with arteriolar or arterial sclerosis. They differ from the diabetic intercapillary sclerosis in the absence of intracapillary hyaline masses and the lack of significant thickening or splitting of the basement membranes.

Differentiation from the acute and subacute forms of glomerulonephritis associated with intercapillary changes deserves mention. Churg and Grishman [35] described edema and mononuclear cell infiltration of the intercapillary spaces and, later, the appearance of fibrils in this area. These were also associated with endothelial proliferation and intracapillary exudation, neither of which was observed in our material. In the subacute forms, changes were described in the intercapillary space which were similar to those seen in intercapillary glomerulosclerosis in mice, namely, deposition of PAS-positive fibers among the IC cells, enlargement of the intercapillary spaces, and compression of the capillaries. Other alterations accompanying these lesions, however, were lacking in progressive intercapillary glomerulosclerosis in mice. There was no evidence of intracapillary hyaline deposits, splitting of the basement membrane, thickening of the basement membrane as a result of hyaline deposits, or of inflammatory changes in Bowman's capsule. Bell [33] stated that in nearly all cases of chronic glomerulonephritis hyaline tissue was found between the capillaries which he, however, traced to the inner basement membrane of the capillaries. Moreover, there were no features in the glomeruli of our mice indicative of previous acute or subacute inflammation.

The pathogenesis of the glomerular lesion under consideration is obscure. Examination of other viscera failed to give any clue that this was a part of a general systemic disease. There was no evidence of diabetes mellitus on the basis of histologic changes in the pancreas. There was no indication of glomerular inflammation at any period in the development of the lesion. Amyloidosis was found in only 2 animals in the series. Systemic and renal arteriosclerosis was not a contributing factor since CAF_1 and BALB/c mice given sublethal doses of x-rays did not

develop significant changes in the renal vessels. Also, these animals do not develop a significant degree of arteriosclerosis spontaneously with age, unlike the LAF_1 mouse and Wistar rat. In the case of the latter two animals, serial sacrifices have not yet established whether or not intercapillary glomerulosclerosis develops progressively or precedes the development of arteriosclerotic lesions and hypertension.

In the absence of demonstrable cause, the possibility that intercapillary glomerulosclerosis is an aging process deserves consideration. In support of this theory are the following observations: (1) All animals beyond the age of 3 months showed evidence of intercapillary sclerosis. (2) The degree of involvement increased progressively with the age of the animal. (3) Lesions of similar structure were demonstrated in 2 strains of mice, 1 strain of rats, and the Chinese hamster. Should this contention prove to be correct, intercapillary glomerulosclerosis may be of considerable value in the experimental study of aging, since the lesions can be readily quantitated.

The effect of x-ray exposure was to increase the degree of intercapillary glomerulosclerosis after a considerable latent period whose duration depended on the age of the animal at the time of exposure. It appeared that irradiation at 1 year of age was followed by a longer latent period than irradiation at 1.5 years of age. Since the life span of the unirradiated CAF_1 mouse is about 2.4 years, the present experiments have dealt with the effects of irradiation during the latter half of life. It would be of interest to determine if irradiation during the early part of life leads to an effect at an earlier age or after a shorter latent period.

Summary

A form of diffuse intercapillary glomerulosclerosis is described in CAF_1, BALB/c, and A/Vi mice. The lesion is progressive, unrelated to inflammatory or systemic disease, and develops independently of arteriosclerosis. A similar progressive lesion has been observed in the Chinese hamster and the AC(I) rat. Following whole-body x-ray exposure of mice, intercapillary glomerulosclerosis is significantly increased. The effect of the radiation, however, is not immediate but is greatly delayed. It is suggested that these progressive renal changes are a morphologic manifestation of aging rather than of a specific disease entity.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

Figures 1, 2, 4, 5, 6, and 7 are color photographs of sections prepared by the Rinehart-Abul-Haj stain. Figures 3 and 8 are of sections stained by the periodic acid-Schiff (PAS) method and combined with phase contrast visualization.

FIG. 1. BALB/c female mouse, age 498 days. High magnification of a portion of a glomerulus showing relation of a third type of cell, IC cell, to the capillary wall. The IC cells have abundant orange cytoplasm. The nuclei of the endothelial cells are seen lining the capillary wall. Note different tinctorial features in the cytoplasm of the epithelial cells located at the outer margins and of the IC cells. × 1,350.

FIG. 2. CAF_1 female mouse, age 181 days. Branching, fingerlike processes of the axis extend outward from the hilus. Note the concentration of IC cells about the central vessel at the hilus and the continuity of these cells with the mesangial cells forming the axis. × 675.

FIG. 3. CAF_1 female mouse, age 683 days. Note the presence of intercapillary basement membrane substance embedded in a PAS-positive fibrillar network enveloping IC cells. The basement membrane of the capillary wall is clearly defined and is of uniform thickness. × 675.

FIG. 4. A/Vi female mouse, age 359 days. Section through a glomerulus at right angles to the hilus. The distribution of IC cells is fairly normal. The presence of a few cell clusters and of cells with swollen cytoplasm establishes this as grade I, a doubtful lesion. × 675.

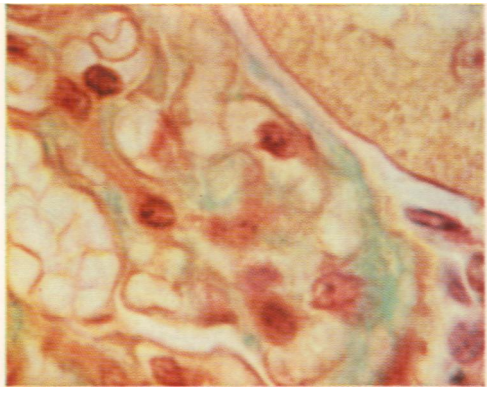
FIG. 5. BALB/c female mouse, age 683 days. Grade II lesion showing clusters of IC cells, moderate increase in the number of these cells, and an increase in cytoplasm. Capillary lumens are only slightly compressed. × 675.

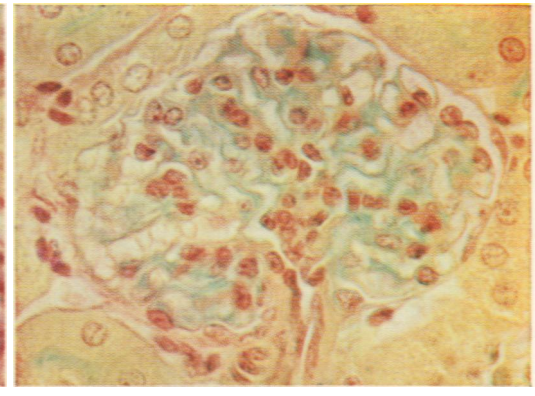
FIG. 6. CAF_1 female mouse, age 885 days. Grade III lesion showing marked thickening of the mesangium. Note the nuclear pleomorphism. Although capillary walls are compressed, many loops are still patent. × 675.

FIG. 7. CAF_1 female mouse, age 949 days. Grade IV lesion showing marked proliferation of mesangial cells with compression of the capillaries in the central portion. Only capillary loops at outer margins are patent. Note the loss of colloidal iron-staining ground substance. × 675.

FIG. 8. CAF_1 female mouse, age 885 days. Advanced lesion showing wrinkling of the basement membrane, compression of the basement membranes of capillary walls, and an increase in intercapillary PAS-positive material. × 675.

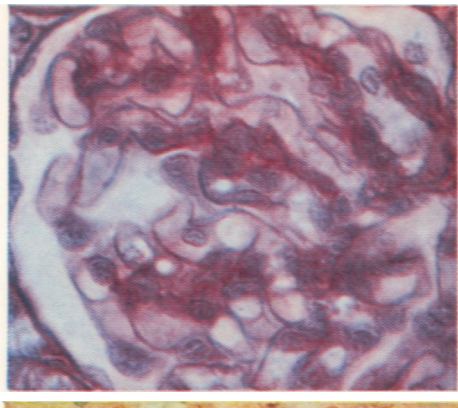
1

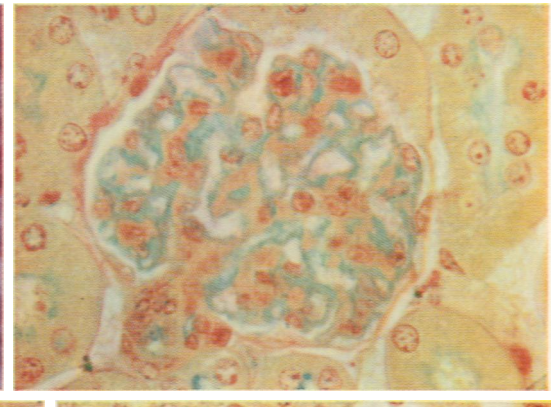




2

3





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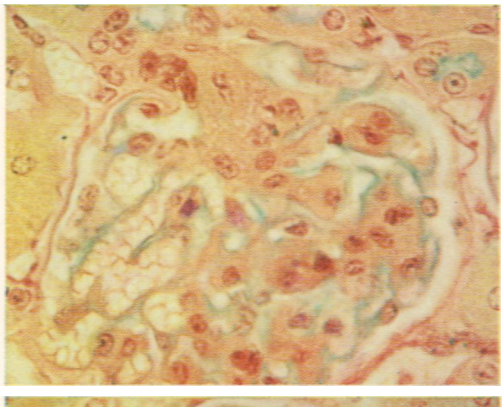
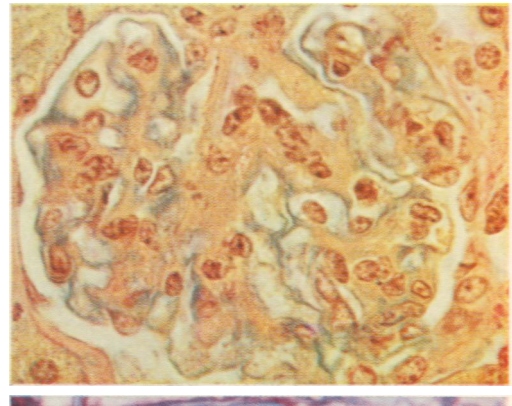
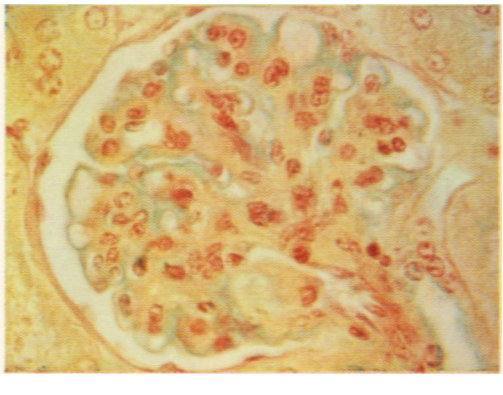
5

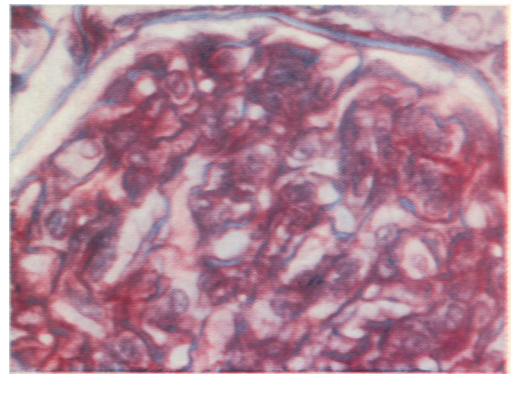




6

7





8