STRUCTURAL AND HISTOCHEMICAL OBSERVATIONS OF LIVER AND KIDNEY IN ALEUTIAN DISEASE OF MINK

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In 1941 a mutation occurred in the mink population on an Oregon ranch which led to the development of a new series of colors in mink furs. This mutation, which expressed itself as a homozygous recessive, was designated as "Aleutian" (symbol *aa*). The Aleutian mink, along with several substrains which have since been developed, have been collectively designated as blue mink. Along with lack of vigor manifested in several ways, the *aa* mink exhibited a special syndrome first described as "hepatonephritis" by Hartsough and Gorham [1] and which has come to be known as Aleutian disease (AD). Helmboldt and Jungherr [2] reported gross and microscopic lesions in mink which died during a spontaneous outbreak on a ranch in Connecticut. They observed plasma cell infiltrations in the liver, kidneys and other organs along with widespread periarteritis. Obel [3] studied the histologic characteristics of a similar mink disease. Because of the presence of large numbers of plasma cells in parenchymal organs, she considered the condition to be a plasma cell myeloma. Henson, Leader and Gorham [4] reported striking abnormalities in the serum proteins, including an extreme rise in gamma globulin in mink with AD.

Filtered suspensions of spleen and other tissue from affected animals have been used in successful transmission.[5–8] Titrations of spleen by mink inoculation in this laboratory have given levels of $10^{5.5}$ ID^{50} per ml. of tissue suspension when titrated in Aleutian (*aa*) genotypes. Also, preliminary trials in this laboratory indicated that the disease could be transmitted to susceptible animals by a fraction sedimentable in the ultracentrifuge with a force of $95{,}000 \times g$ for 1 hour.[9]

The occurrence of plasma cell proliferation, periarteritis, fibrinoid degeneration of arteries, glomerulonephritis and hypergammaglobulinemia suggested that this disease might have mechanisms similar to

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those responsible for some of the connective tissue diseases of man. Since the pathogenesis of these diseases is not understood, it became apparent that this "experiment in nature" might be employed to help clarify some of these problems.

The authors are presently conducting comprehensive studies of several hundred animals affected by natural and experimental Aleutian disease in all stages from the incipient to the terminal in order to elucidate the natural history, gross and microscopic lesions, and measurable deviations of various metabolic functions. This material is not yet available in complete form and will be the subject of a later report. The present paper deals with abnormalities in the livers and kidneys in advanced cases. The major objective is to analyze the lesions in these organs so that the syndrome can be more clearly related to human collagen diseases producing prominent lesions in these organs.

Material and Methods

Necropsy was performed when animals were killed for removal of pelts. Death was caused by inhalation of cyanide gas. Tissues were placed immediately in Carnoy's solution and in cold neutral 10 per cent formalin. All animals selected for this study had gross lesions of AD, which included the following: kidneys somewhat enlarged with gray flecks in the cortex in milder forms of the disease, but becoming shrunken, dimpled, and showing cortical cysts in more advanced stages. Livers were yellow-orange with irregular surfaces, sometimes showing small cysts. Lesions in other organs were splenomegaly, enlarged lymph nodes, and sometimes ulcers of gastric mucosa and of the gingival borders. Abnormal serum protein distribution with a drop in albumin and marked increase in gamma globulin were constant findings.[4] All affected animals had proteinuria. There were 16 animals in this study, including 3 normal Aleutian mink to serve as controls.

Hematoxylin and eosin and van Gieson stains were used for observation of general tissue changes. Histochemical procedures applied were the periodic acid-Schiff (PAS) reaction,[10] colloidal iron-periodic acid-Schiff, with Mowry's modification of the Hale reaction,[11] Feulgen reaction, methyl-green-pyronine (before and after application of ribonuclease), methyl violet, ninhydrin-Schiff, toluidine blue and alcian blue with chromotope 2R, according to Wagner and Shapiro,[12] before and after testicular hyaluronidase digestion.

Observations

Structural Alterations

The liver lesions affected principally the duct system, with only minor changes in the parenchyma. Vascular lesions will be described separately. The most constant and obvious deviations from normal were proliferation and dilatation of small bile ducts accompanied by a halo of inflammatory cells. The nuclei in the bile duct epithelium were slightly vesicular and stained less intensely with hematoxylin and the Feulgen reaction than did the nuclei of nonproliferating duct cells. There was a marked increase in the number of these small bile ducts, which appeared

to develop by budding of existing ductules. In the mildest lesions, a few small duct cells were surrounded by plasma cells. In the advanced stages, the ducts became dilated to a diameter as much as 2 mm. (Figs. 1 and 2). They were grossly visible when present in large numbers. The material within the bile ducts did not have the pigmented appearance of bile, but stained pink with eosin and contained variable quantities of polysaccharide as shown by the PAS reaction. In some instances there appeared to be impingement upon the hepatic parenchyma by the growing bile ducts. Pericholangial fibrosis was seen but was not sufficiently intense to be considered cirrhosis.

In the tissues surrounding the dilated bile ducts, there was an accumulation of leukocytes predominantly of mononuclear type, mostly plasma cells. The plasma cells appeared mature and varied from those with the classical cartwheel nucleus and slightly basophilic cytoplasm to large cells with very pink cytoplasm. The nucleus was pushed to one side and showed various stages of degeneration. These cells frequently contained Russell bodies (Figs. 3 and 4). Their development, histochemical characteristics and variations are described later in this paper. In the areas of cellular infiltration there were occasional accumulations of bile pigment and hemosiderin within mononuclear cells.

In some instances small areas of necrosis without surrounding inflammatory response were seen (Fig. 5). The necrotic lesions varied from very small size to 2 or 3 mm. and appeared to be uniformly circular in shape. An intact fibrous framework remained within them. The sinusoids appeared to be functional and were dilated and filled with blood; in effect they replaced the space previously occupied by hepatic parenchymal cells. Intact Kupffer cells were not observed within the necrotic tissue. The parenchymal cells at the margins of the degenerated areas exhibited progressive pyknosis and karyorrhexis. Occasional lesions revealed neutrophil infiltration, associated with a concurrent bile duct inflammation, but this was not characteristic. The lesions were not anatomically associated with any specific part of the lobule, with the portal vein branches, or the inflammatory cells surrounding the proliferating bile ducts. Bacteria were not observed within the lesions.

Lesions in the kidney involved glomeruli, tubules, interstitial tissues and blood vessels. Most seriously damaged were the proximal convoluted tubules, which suffered both from primary damage and disruption because of focal infiltration and proliferation of mononuclear cells. The range of severity of the changes was as follows:

1. In the mildest stage there was an accumulation of mononuclear cells in the interstitial tissues of the cortex. These infiltrations were patchy in distribution and appeared to surround, distort and displace the

proximal convoluted tubules. The cell populations varied but consisted mostly of plasma cells. In some instances there were considerable numbers of monocytes with pale indented nuclei. Some lymphocytes were also present. In more advanced lesions the arrangement suggested granuloma formation.

2. There was a concurrent progressive degeneration of the proximal tubules characterized by thickening, wrinkling and splitting of the basement membrane (Figs. 6 and 7). The normal membranes were thin and straight and were immediately adjacent to each other with very little intervening stroma.

3. As the changes advanced there was increasing severity of tubular distortion along with evidence of blockage as indicated by the presence of tubular casts and distention in the portions of the nephron upstream from the lesions. In areas of most severe damage the only surviving tubular structures were distal tubules and collecting ducts.

4. The ultimate consequences were complete destruction of the affected nephrons with marked dilatation of Bowman's space and atrophy of the glomerular tufts. Repair attempts evidenced by tubular hyperplasia were occasionally seen.

In addition to the obstructive features, there were progressive changes in the glomeruli. These consisted of irregular thickening and splitting of the basement membranes of glomerular tufts. These changes chiefly affected the mesangium in the tuft, with slight proliferation of endothelial cells in some instances. Bowman's capsule was affected to a lesser degree. There was no evidence of epithelial crest formation in the lining of Bowman's capsule. The glomerular changes paralleled in some degree the tubular lesions, although not all animals which showed advanced tubular alterations had severe glomerular lesions.

Eight of the 13 animals in the affected group had blood vessel abnormalities (Figs. 8 and 9). These lesions, which affected principally the medium-sized muscular arteries, consisted of mononuclear periarteritis, disruption of elastic membranes and smooth muscle structures, necrotizing reaction in the wall and deposition of fibrinoid substances. Arteries of all parts of the body were affected; veins were not involved. For descriptive purposes the changes have been divided into 3 degrees. These are intended to connote severity rather than progression in time.

1. The mildest lesion was a focal disruption of the internal elastic membrane and degeneration of smooth muscle cells. A halo of mononuclear cells, mostly plasma cells, surrounded the vessel.

2. In the more severe lesion, there was a pink amorphous fibrinoid deposit which obliterated the structures of the tunica media completely. Scattered through the degenerating area were bluish fragments of debris

derived from disintegrating nuclei. The lesion occasionally involved the entire circumference of the vessel, and the lumen was completely obscured (Fig. 8). In many of the larger affected vessels, however, the lesions involved only some segments of the wall while tangential portions remained normal (Fig. 9). Here the perivascular accumulation of mononuclear inflammatory cells was more abundant in the area of necrosis. Longitudinal sections of arteries demonstrated a nodular or segmental distribution of the lesions.

3. In the most severe stage of the vascular lesions, amorphous debris containing nuclear fragments was no longer manifest. The lesions consisted of fibrillar deposits arranged in a circular lamellated pattern. Smooth muscle had disappeared. There remained a mantle of perivascular mononuclear cells. The vascular lumen was reduced or obliterated. Cortical infarction was not evident even though there was extensive vascular involvement in many kidneys.

In all areas of plasma cell infiltration there were abnormal plasma cells, consistent in appearance with Mott cells,[13] Russell bodies[14] or grape cells.[15] Successive stages of development of these cells were readily observed (Figs. 3 and 4). The cytoplasm of plasma cells first became swollen and stained light pink with PAS. Later the cytoplasm stained brighter with PAS and the nucleus was displaced to an eccentric position. Some evidence of vacuolation or globule formation in the cytoplasm appeared mostly at the periphery. What seemed to be the end stage consisted of cells with swollen vacuolated cytoplasm containing many large foamy granules or bodies. The nucleus was pyknotic (Fig. 4).

Histochemical Observations

Hepatic parenchymal cells exhibited only limited deviations from normal with the techniques used. The secretions in the lumens of proliferating bile ducts, however, exhibited interesting properties. In some animals there was a strong pink staining with colloidal iron-PAS; in others there was a variegated appearance of this substance, with some regions pink and others showing a strong blue reaction with colloidal iron. Wide variations often occurred within the same tissue section. There was also a strong PAS-positive staining in surviving stroma in areas where hepatic parenchyma had undergone necrosis (Fig. 5). A varying intensity of PAS reaction was evident in the collagen surrounding proliferating bile ducts (Fig. 2). A summary of histochemical changes in the arteries is shown in Table I.

In portions of arteries undergoing active necrosis there was intense coupling with the Schiff reagent. When the colloidal iron-PAS technique was applied to the same vessel, there was a strong PAS-positive reaction

in the center of the lesion; its edge was stained by colloidal iron (Fig. 9B) and stained blue with alcian blue. In lesions affecting the entire circumference of vessels there tended to be a laminated mixture of blue and pink staining, although some advanced lesions were entirely pink and some, especially less severely affected regions, were mostly blue. The

• TABLE I

HISTOCHEMICAL REACTIVITY OF VASCULAR FIBRINOID

	Color reaction	Indication
Polysaccharide		
Schiff reaction	No color	Free aldehydes not present
Periodic acid-Schiff (PAS)	Pink	Oxidized 1,2 glycols
Periodic acid-phenylhydrazine-Schiff	No color	Block of aldehydes (oxidized 1,2 glycols)
Colloidal iron-PAS (Fig. 9B)	Blue-pink	Mixture of AMP and NMP
Alcian blue 8GS (Fig. 9C)	Blue	AMP present
Toluidine blue, pH 4.5, 0.1%, 20° C.	Colorless	Highly acidic AMP not present
Toluidine blue, pH 2, 3%, 70° C.	Pink	Hydrolysis of masking protein
Methyl violet	Unstained	No metachromasia
Crystal violet	Unstained	No metachromasia
Thioflavine T	No fluorescence	Amyloid not present
* Testicular hyaluronidase digestion	Staining with PAS, colloidal iron-PAS and alcian blue not changed	Polysaccharides not digested
Proteins		
Chromotrope 2R (Fig. 9C)	Focal red areas	Basic proteins present
Feulgen	Focal pink areas	DNA fragments
Methyl green-pyronine	Faint pink	Suggestive of RNA
Ninhydrin-Schiff (Fig. 9D)	Pink	Reactive amino groups
Sulfhydryl	Dark brown	SH groups present
Hematoxylin and eosin	Red	Proteins present
* Trypsin digestion (1 mg./ml.), pH 8.2, 37° C., 1 to 3 hr.	Partial digestion	Proteolysis
* Ribonuclease followed by methyl green-pyronine	No pink areas	Removal of RNA
Trypsin followed by hyaluronidase	Almost total digestion	Proteolysis and AMP digestion

* Enzymes supplied by Worthington Biochemical Corporation, New Jersey.
AMP = acid mucopolysaccharide. NMP = neutral mucopolysaccharide.
DNA = deoxyribonucleic acid. RNA = ribonucleic acid.

region staining with alcian blue coincided with the distribution of blue reaction with colloidal iron (Figs. 9B and 9C), although it was somewhat more extensive. Metachromasia with toluidine blue was seen only after heat-acid hydrolysis.[16] The ninhydrin-Schiff (Fig. 9D) reaction was positive in wide areas coinciding with those reacting with PAS, colloidal iron and alcian blue. Chromotrope 2R (Fig. 9C), on the other hand, was deposited only in the most active portion near the center of the lesions, the area of intense PAS positivity.

The fine structure of the muscular portion of arterial walls was readily seen in PAS stained sections because of the thin pink trabeculations of the cement substance between each smooth muscle cell. Disruptions in these trabeculae could be noted before necrosis or polysaccharide deposition were apparent and very probably constituted the first morphologic evidence of damage to the blood vessel walls. Lesions of this type were seen in the afferent arterioles of renal glomeruli more frequently than the severe types of lesions with fibrinoid degeneration.

The cytoplasm of normal mature plasma cells was slightly basophilic and stained very lightly in hematoxylin and eosin stained preparations. Some plasma cells exhibited slight metachromasia with toluidine blue, a light pink color with the PAS reaction, bright red with chromotrope 2R and bright green with light green. In some of the lesions these cells appeared to be enlarged and exhibited increasing intensity of cytoplasmic reaction with PAS, colloidal iron, chromotrope 2R and light green (Table II). With toluidine blue, however, vacuoles appeared in the cytoplasm, until in the largest form the cells appeared reticulated, with only the interfaces of the globules maintaining metachromasia (Figs. 3 and 4).

• TABLE II

HISTOCHEMISTRY OF CYTOPLASM OF PLASMA CELLS AND RUSSELL BODIES

Polysaccharide	Pearse [14]	Zlotnick [15]	This report	Constitution
Periodic acid-Schiff	+	+	+	1,2 glycols
PAS after diastase		+		NMP present
Colloidal iron			+ (around globules)	AMP present
Alcian blue			+ (weak around globules)	AMP present
Toluidine blue		− (in globules)	+ (around globules)	AMP present
Biocol reaction		−		Negative for free acids (in globules)
OsO_4 (nonpolar solvent)		−		
OsO_4 (aqueous)		Brown		Glucosamine
Chromotrope 2R			+	Basic protein
Ninhydrin-Schiff			+	Free amino groups
Hematoxylin and eosin			+	Protein
Light green			+	
Methyl green-pyronine		− (in globules)		
Before RNAse	+ (around globules)		+ (around globules)	RNA around globules
After RNAse	−		−	
Feulgen			−	No DNA

Histochemical Reactions in Glomeruli. There were abnormal mucopolysaccharide deposits in the glomeruli of all animals affected by AD. The severity varied from slight to complete obliteration of the entire glomerular structure. In PAS stained material it was easy to follow the basement membrane of the glomerulus as it entered the tuft and became a part of the mesangium.[17] In normal glomeruli this membrane was thin, sharply delineated and slightly wavy. In AD, it exhibited increased thickness and irregularity. When it was followed into the tuft, the membranous nature was lost, and instead there was pink stippled or amorphous material representing abnormal deposits of polysaccharide (Fig. 10). The uniform thickening usually associated with membranous glomerulonephritis was not seen. Staining with the ninhydrin-Schiff reagent indicated a very extensive deposition of pink amorphous substance (Fig. 11). The Feulgen reaction disclosed DNA fragments indicating nuclear destruction (Fig. 12). Chromotrope 2R was not deposited in any constant manner in the glomeruli. The basement membrane of Bowman's capsule was affected in a fashion similar to the portion that entered the tuft. It became increasingly PAS-positive, wrinkled and occasionally fragmented into several layers.

Histochemical Reactions in the Renal Cortex Exclusive of Glomeruli. As pointed out in the description of structural abnormalities, there were displacement and disruption of the tubules, which appeared to be related to the proliferation or infiltration of mononuclear inflammatory cells. These changes could be very clearly followed with the PAS stain (Figs. 6 and 7) where the tubular basement membrane appeared bright red, and could be observed in normal kidneys to be thin, clear and regular. In AD it underwent progressive wrinkling and thickening which was apparent both in areas of cell infiltration and in regions which did not show inflammation. In regions of thickest plasma cell accumulation, one could see that many tubular basement membranes were completely interrupted, with resultant breakdown and dissolution of the tubules themselves. Proximal convoluted tubules were identified with the PAS stain by the prominent brush border on the lumen surface of the cells. The portion of the nephron chiefly damaged in this process consisted of the proximal convoluted tubule since nearly all surviving tubular elements in badly damaged areas consisted of distal convoluted tubules. Within the tubule lumens, especially upstream from the areas of damage, there were heavy casts which reacted as a mixture of pink and blue staining substance when colloidal iron-PAS was used, indicating a mixture of neutral and acid mucopolysaccharides. There was intense staining of this substance with ninhydrin-Schiff and eosin, but not with chromotrope 2R.

Discussion

In a review of the possible role of the connective tissue in hypersensitivity, Wagner [18] agreed with the original hypothesis of Klemperer that "collagen disease" was a conceptual term and did not imply pathogenetic definition. The type and distribution of pathologic changes were emphasized by the use of this term, serving to stimulate thought about the causes. The conditions most commonly included in this group are rheumatic fever, systemic lupus erythematosus, generalized scleroderma, rheumatoid arthritis, dermatomyositis, periarteritis nodosa and serum sickness. There is much overlapping of clinical and laboratory manifestations, which complicates clear definition of the entities. While Aleutian disease of mink has characteristics resembling several of these, the lesions are not completely compatible with any one of them.

Of considerable interest was bile duct proliferation. Popper and Schaffner [19] described several causes for this, including (a) contact with proliferating connective tissue as in postnecrotic cirrhosis, (b) humoral stimulation after removal of part of the liver, (c) stimulation by carcinogens such as butter yellow, and (d) chronic ethionine intoxication. Stowens [20] described a similar response in partial occlusion of bile ducts as in congenital bile duct atresia. It is well recognized in cattle that vitamin A deficiency can result in proliferation. However, these conditions do not exhibit the marked degree of bile duct proliferation and dilatation manifested in Aleutian disease. In AD, which does not usually produce icterus, the budding biliary radicles were filled with a secretion which stained pink with eosin, contained acid and neutral mucopolysaccharides, as shown by colloidal iron and PAS stains, and protein, as indicated by a positive ninhydrin-Schiff reaction. This material did not appear to be normal bile, nor was there evidence of bile stasis in canaliculi. Thus, we assumed that excretion of bile continued during the period of bile duct proliferation. From these observations we believe that the proliferating bile ducts in AD developed as lateral buds with very little pressure exerted upon existing ductules.

It is often impossible to indicate pathogenetic mechanisms in studies of human and animal necrotizing angiitis by conventional histologic observations. However, loss of internal structure, with fibrinoid deposits in artery walls surrounded and infiltrated by plasma cells and other mononuclear cells, suggested an immunologic type of damage. The common denominator of fibrinoid alteration in vessel walls and connective tissue has served to develop the concept of collagen disease, especially when studied by histochemical methods.

The histochemical studies (Table I) showed that the fibrinoid in

Aleutian disease contained no free aldehyde groups, but there were polysaccharide constituents with available 1,2 glycol groups. Positive reactions with alcian blue and colloidal iron further confirmed the presence of acid mucopolysaccharides. The mixture of blue and pink staining in the fibrinoid following the iron-PAS reaction indicated a mixture of acid and neutral mucopolysaccharides. Lack of metachromasia with weak, acidic toluidine blue, crystal violet and methyl violet suggested that the acid mucopolysaccharides present were not highly acidic and with a significant negative charge. The demonstration of metachromasia by strongly acidic, concentrated toluidine blue at 70° C. indicated the presence of blocking proteins. Hydrolysis of these proteins uncovered enough negative groups to allow for metachromasia. Digestion with testicular hyaluronidase proved unsuccessful in changing mucopolysaccharide reactions. This strongly indicated a stable complex of mucopolysaccharides with other structures, namely proteins.

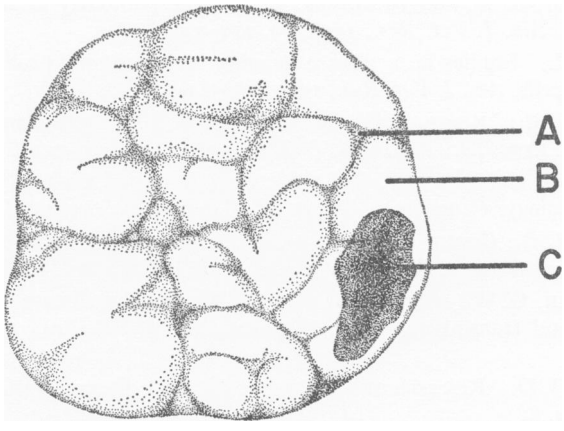
The protein reactions indicated the presence of basic proteins with reactive NH_2 and SH groups. Scattered fragments of DNA and RNA were noted with a random dispersion throughout the fibrinoid. Trypsin digestion alone partially removed the fibrinoid. However, the sequential application of trypsin and hyaluronidase resulted in almost complete removal of the fibrinoid. It would appear that the fibrinoid was largely composed of a rather stable protein-acid mucopolysaccharide complex. The general pattern of reactivity was similar to the fibrinoid in human polyarteritis and rheumatic fever but quite unlike that in systemic lupus erythematosus and generalized scleroderma.

The cytologic features of the plasma cell in this disease assumed considerable significance in view of the tremendous amounts of gamma globulin produced and the possible relationship of the globulin to the pathogenesis of the disorder. The cytochemistry of Russell bodies has been discussed by Pearse [14] and Zlotnick, Gerichter and Nir.[15] The data contained in Table II make comparisons and add to the previous information by indicating the results of additional histochemical reactions.

Electron microscopy of Russell bodies by Bessis [13] and Welsh [21] appear to give validity to the hypothesis that the dilated endoplasmic reticulum constitutes areas of very active protein synthesis, probably of gamma globulin.

The presence in Aleutian disease plasma cells of globules containing a substance histochemically compatible with glyco or mucoproteins surrounded by layers of ribonucleic acid indicates antibody synthesis, perhaps abnormal antibodies (Text-fig. 1). Since Aleutian disease has now been reproduced by the injection of a cell-free sedimentable filtrate,[9] several questions concerning the antigen-plasma cell-antibody

relationship need to be answered. What manner of aberration occurs in protein synthesis resulting in hypergammaglobulinemia and excessive accumulation of globulin within certain cells? If these are indeed antibody molecules, against what antigen are they directed? If the antigen is a virus, why does progression of the disease continue in spite of tre-



TEXT-FIG. 1. A plasma cell contains fully developed "Russell bodies." A. Interface of cytoplasmic globules: pyroninophilic, metachromatic with toluidine blue, stains with colloidal iron and alcian blue. B. Center of cytoplasmic globule: positive with PAS, ninhydrin-Schiff, chromotrope 2R, and light green; not metachromatic with toluidine blue. C. Pyknotic nucleus.

mendous rises of antibody (serum globulin) level? What is the relationship of the antibody to the degenerative lesions in the connective tissue and arteries? What is the site of virus proliferation within the tissues?

Whatever the ultimate answers may be to these questions, Aleutian disease constitutes a convenient reproducible model which can provide information of great value in elucidation of the pathogenesis and perhaps etiology of related conditions in man.

SUMMARY

Results of detailed studies of selected significant tissue alterations in advanced Aleutian disease of mink have been presented. A series of staining procedures and sequential histochemical techniques were directed toward an analysis of bile duct proliferation, renal tubular and glomerular degeneration and an arteritis accompanied by fibrinoid alterations. Associated with these changes was an intense plasmocytosis with the formation of Russell bodies. The combination of hypergammaglobulinemia, plasmocytosis and arteritis with fibrinoid necrosis suggested a possible hypersensitivity mechanism. Since this spontaneous disease of mink is most probably of viral etiology, the fact that it closely simulates

some of the "collagen" diseases of man presents an intriguing system for further investigations.

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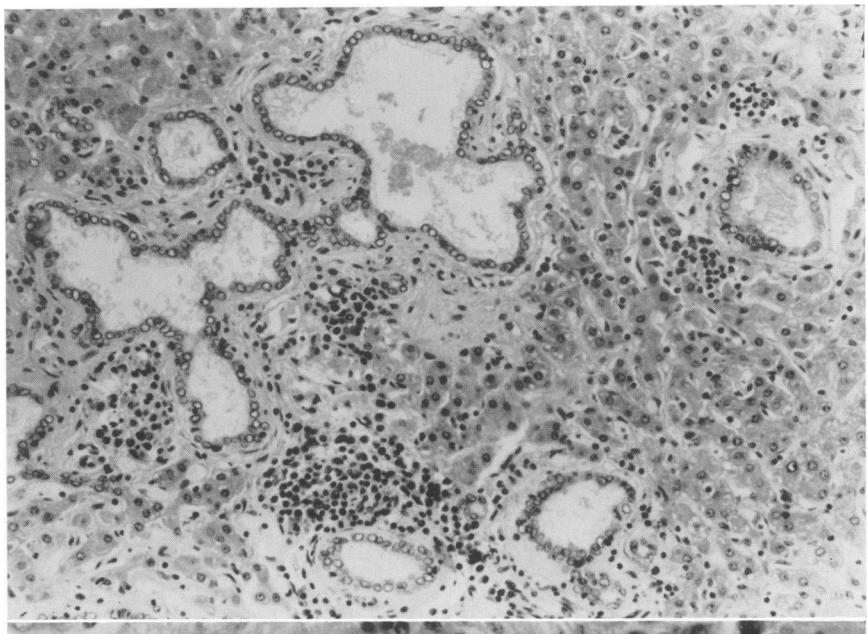
[*Illustrations follow*]

Legends for Figures

FIG. 1. Bile duct proliferation and dilatation. Plasma cells have accumulated around the ducts. Hematoxylin and eosin stain. × 180.

FIG. 2. Colloidal iron-periodic acid-Schiff (PAS) stained proliferating bile duct. An intense pink reaction (arrows) suggests an older connective tissue. Growth of the ductule may be outward at other points. The lumen contains mucopolysaccharide. × 450.



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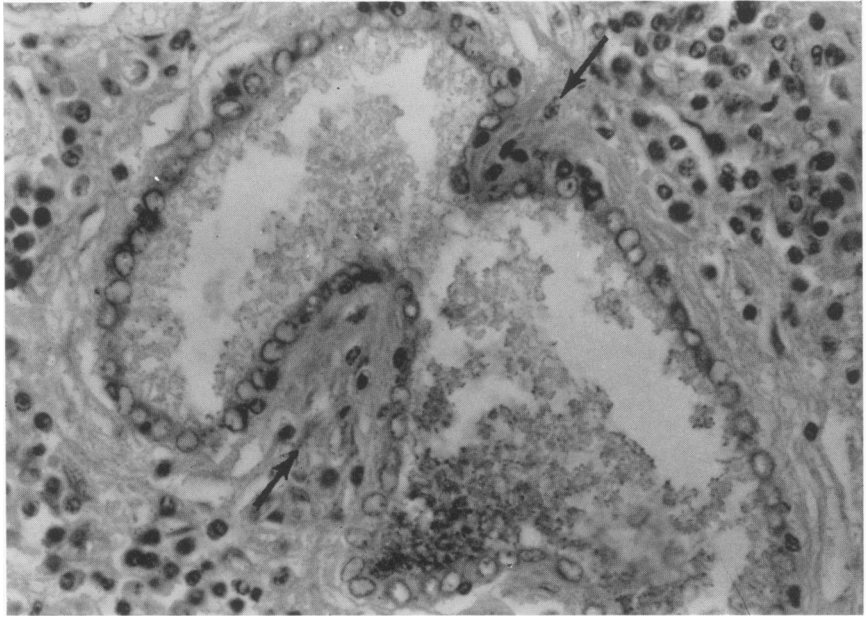

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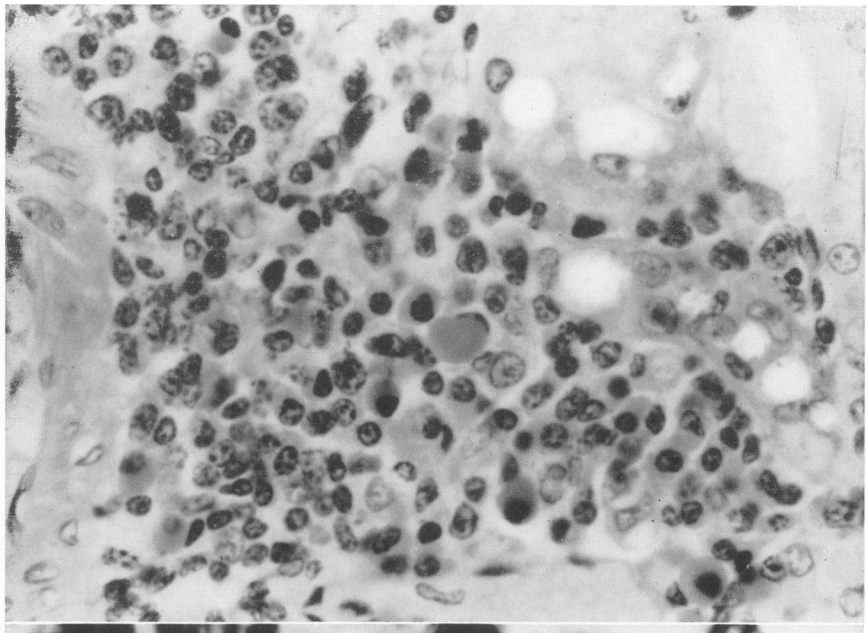

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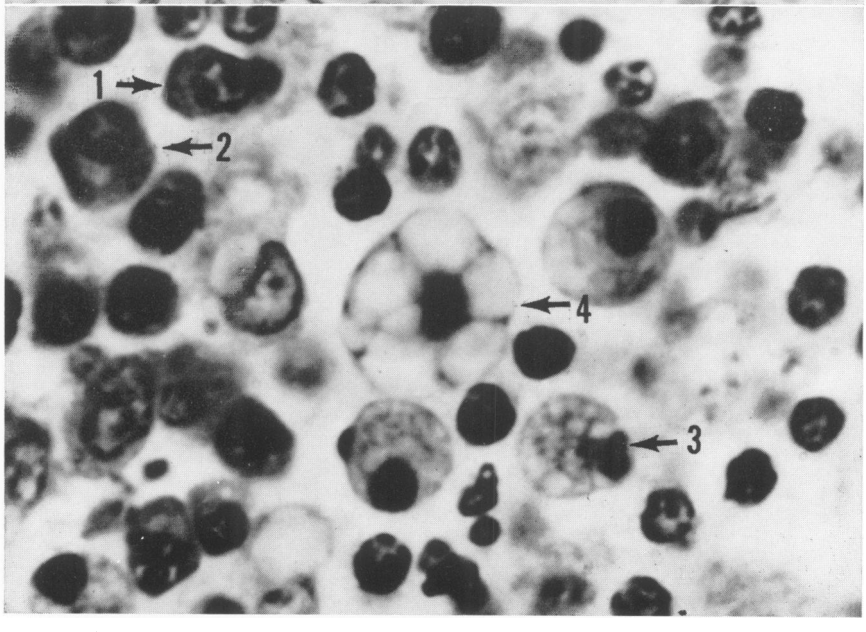

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FIG. 3. A Feulgen stain with light green counterstain shows plasma cell accumulation in the liver. Larger cells with intensely staining cytoplasm develop into "grape cells" containing Russell bodies. × 720.

FIG. 4. Toluidine blue stain of fully developed "grape cells." Plasma cells 1 and 2 have slightly metachromatic cytoplasm. In 3 and 4 there is vacuolation with loss of staining in globules but with metachromasia retained at the interfaces. × 1,800.

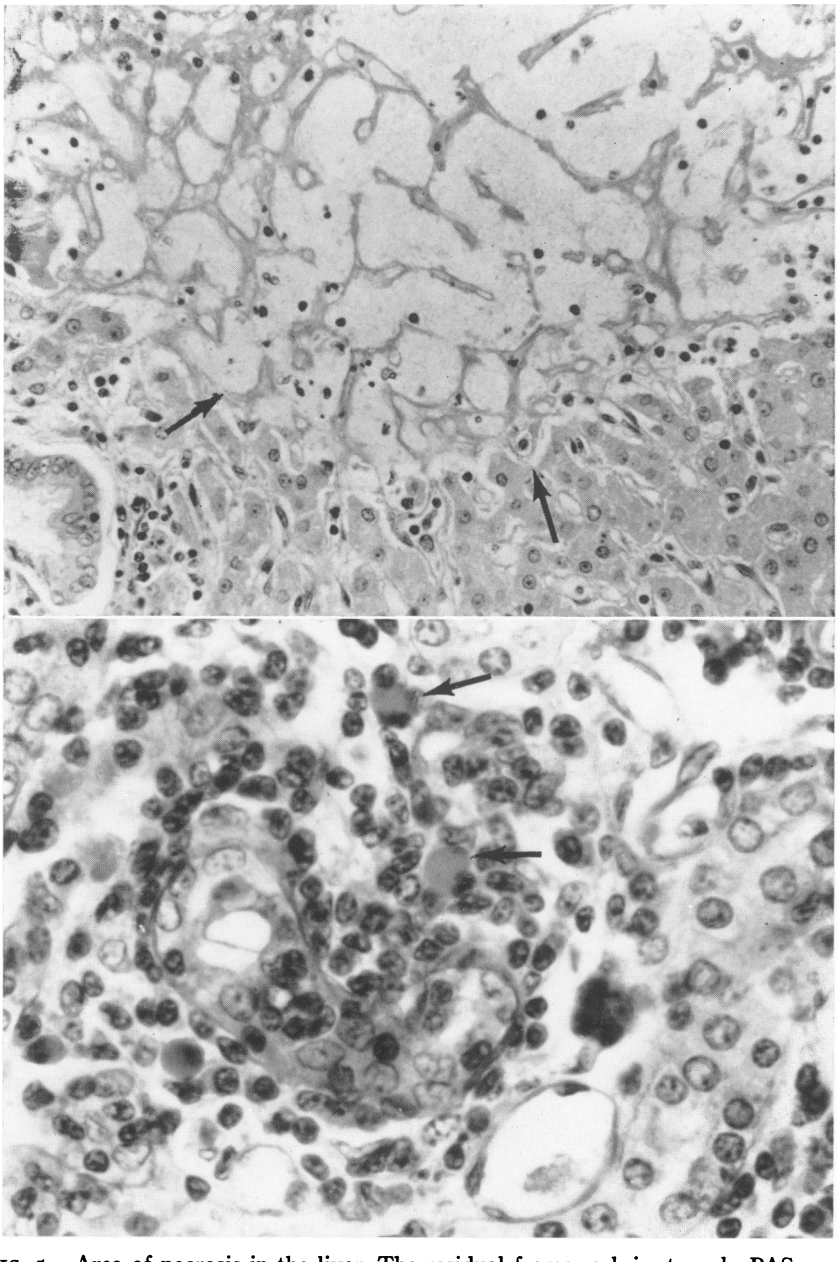

FIG. 5. Area of necrosis in the liver. The residual framework is strongly PAS positive. Tissue reactivity is lacking at the margin. PAS stain. × 250.

FIG. 6. Colloidal iron-PAS stain of convoluted tubule. There is accumulation of plasma cells. Arrows indicate "Russell cells" with PAS-positive cytoplasm. × 720.

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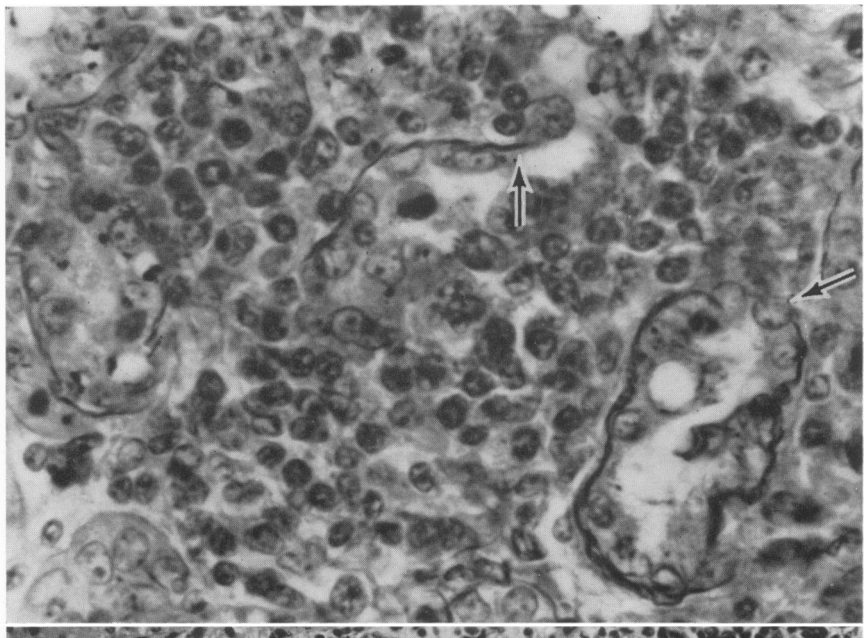

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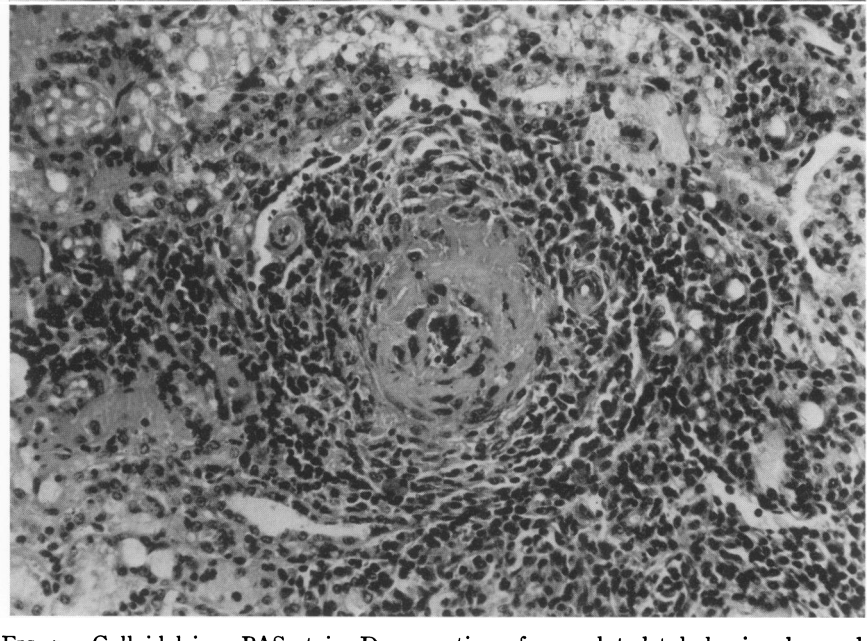

FIG. 7. Colloidal iron-PAS stain. Degeneration of convoluted tubules is advanced. Arrows point to discontinuity in the basement membrane. The remaining membrane is thickened and irregular. × 600.

FIG. 8. A periarteritis is characterized by severe fibrinoid necrosis and obstruction of the lumen. Hematoxylin and eosin stain. × 225.

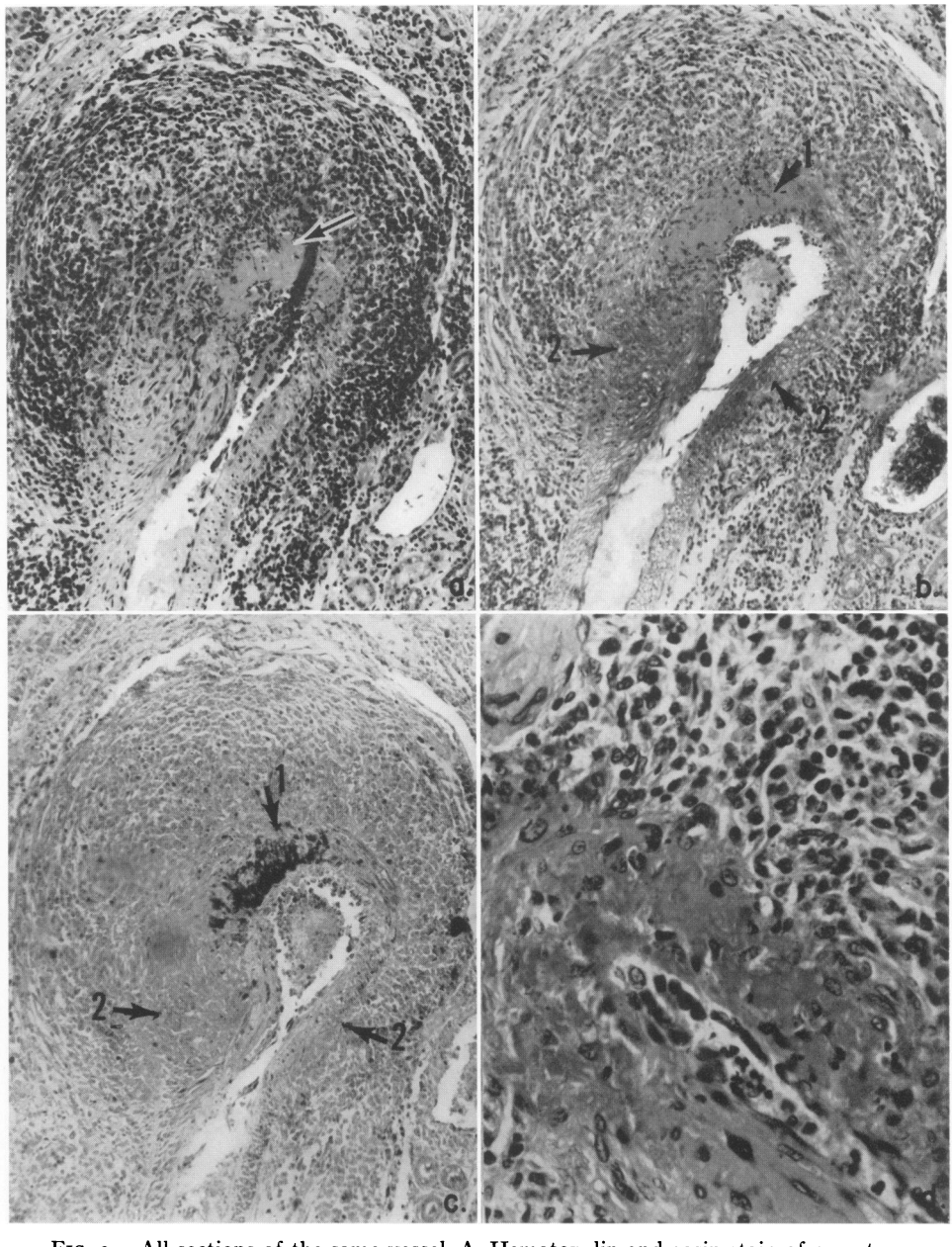

FIG. 9. All sections of the same vessel. A. Hematoxylin and eosin stain of an artery in the kidney shows periarteritis and fibrinoid necrosis (arrow). The lesions have a tangential distribution with some segments remaining normal. × 110. B. Colloidal iron-PAS stain. The arrow 1 indicates a PAS-positive area; arrows 2 are stained by the colloidal iron. × 110. C. The region indicated by arrow 1 is stained pink by chromotrope 2R; the area of arrows 2 are stained by alcian blue. × 110. D. A diffuse reaction occurs with the ninhydrin-Schiff stain. × 375.

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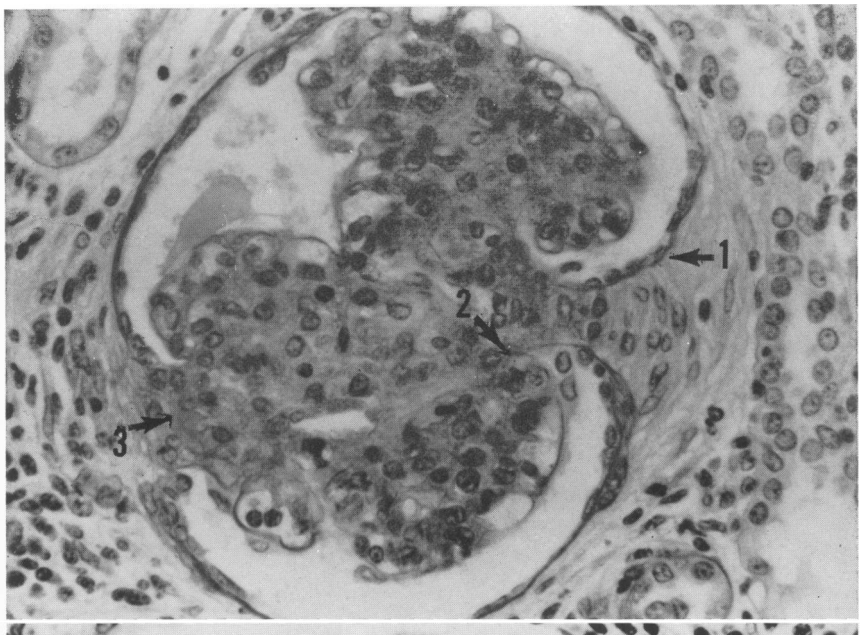

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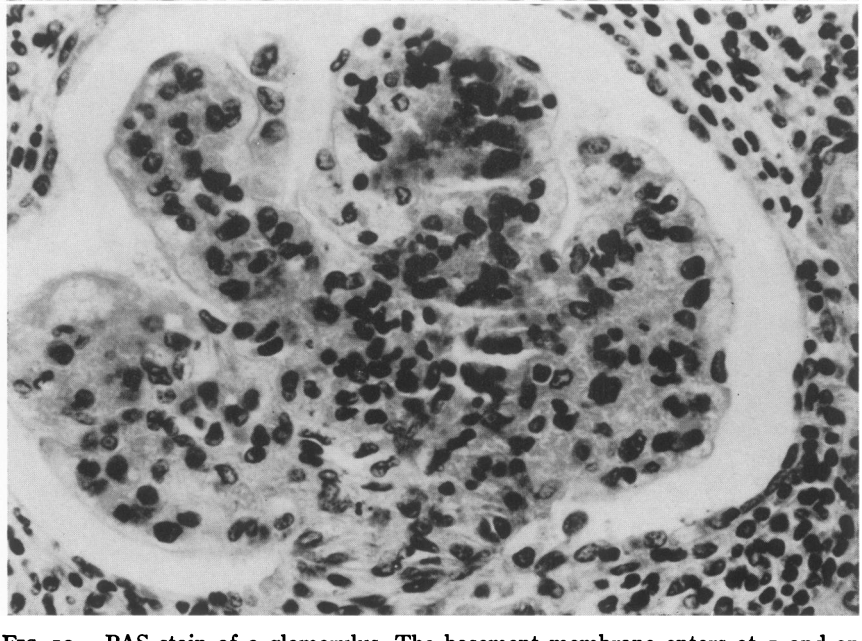

FIG. 10. PAS stain of a glomerulus. The basement membrane enters at 1 and appears to split at 2. There is diffuse deposition of mucopolysaccharide. Synechia, 3. × 440.

FIG. 11. Ninhydrin-Schiff stain. An abnormal amount of protein appears in the glomerulus. × 440.

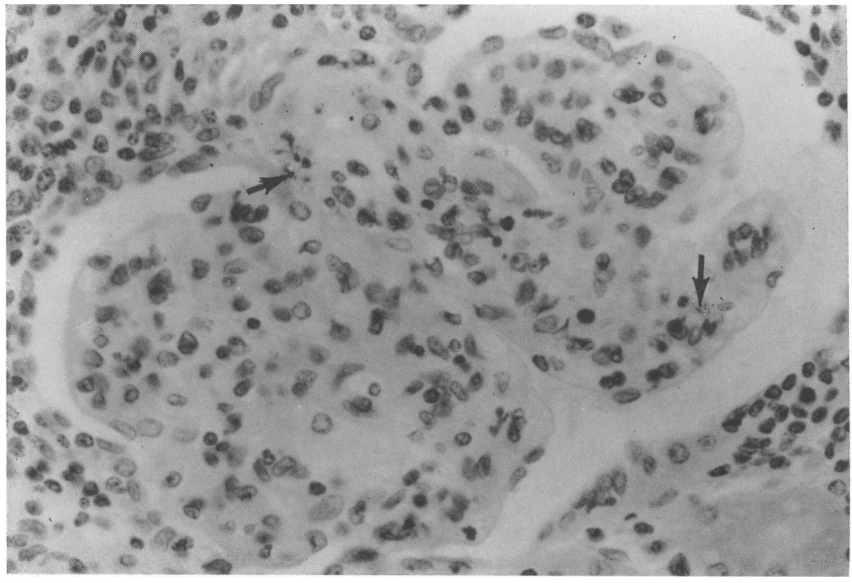

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FIG. 12. Feulgen reaction. Extensive pyknosis (arrows) and karyorrhexis are manifest. × 440.