Striking glomerular changes are seen electron microscopically in rat kidneys after acute uranyl nitrate poisoning. More interesting than the several glomerular epithelial cell reactions to this injury, perhaps, is the development of the nodular centrolobular lesion containing collagen fibers.

The glomerular epithelial cell changes include loss of foot processes, the appearance of dense deposits in foot processes or in the larger portions of cytoplasm which replace lost foot processes, hyaline droplets, other cytoplasmic bodies with varying internal membranes and granules, myelin figures, and cytoplasmic vacuoles. The functional significances of these changes and of the observed proteinuria, polyuria, and oliguria are discussed.

The nodular centrolobular lesion was identified with a trichrome stain after embedding in methacrylate. As seen with the electron microscope it consists of several components lying between the central dense layer of the basement membrane and endothelial cells. Separately distinguished components include inter-capillary cells and their processes lying in intimate contact with extracellular (a) small dense granules 30 m1i in diameter, (b) large bodies 400 ma in diameter, vaguely forming rough cords, and (c) collagen fibers, randomly oriented and appearing within four days after injection of uranium. The intercapillary cells, often containing clear vacuoles with very dense and thick osmiophilic walls, may later prove to be endothelial cells but their intimate association with collagen fibers raises other alternatives.

As part of a systematic investigation of reactions in the ultrastructure of the kidney I. under physiologic and pathologic conditions, glomerular changes in rat kidneys during acute uranium poisoning have been studied by electron microscopy. Some other aspects of this investigation are also being reported (2, 3, 27).

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Nineteen Sprague-Dawley rats were used in preliminary experiments to determine which of several methods of fixation would yield the best results for electron microscopy. The method selected consisted of dripping buffered osmium tetroxide on the cortical surface of the decapsulated kidney (16). Twenty-six rats received subcutaneously 14.4 mg/kg of uranyl nitrate hexahydrate, UO₂(NO₃)₂·6H₂O, dissolved in saline solution. These arid 26 control rats were sacrificed after 18, 24, and 36 hours, and 2, 3, 4, 5, and 6 days. The urine and protein outputs were measured daily in 17 rats. Polyuria and proteinuria were found to reach a peak at the 3rd and 4th days respectively after uranium injections. Blocks for light microscopy were fixed in Zenker-formol and paraffin sections 2 thick were stained as described elsewhere (3). For electron microscopy rectangular blocks about 1 x 1 x 3 mm were cut so that the surface of the kidney formed one long side of the block. The block was oriented for sectioning so that the surface of the kidney that had been fixed by dripping formed one edge of the section. Dripping enabled comparison of superficial well-fixed layers with deeper ones. With only rare exceptions in the present study, sections showing the entire depth of the blocks appeared well fixed. Sections were cut with a Porter Blum microtome using a glass knife. An RCA EMU-3B electron microscope was used at 50 kV.

A rapid toluidine blue staining method was useful in correlating hyaline droplets noted by light microscopy (3) with bodies seen in the electron micrographs (2). For correlation of a centrolobular glomerular lesion staining blue in paraffin sections with a trichrome stain (3), the adaptation of a trichrome stain to osmium-fixed tissues was necessary. After this lesion was located in a block by the rapid toluidine blue stain, the trichrome stain was performed on methacrylate sections 1 thick as follows:

1. Acetone rinse, 5—10 sec.
2. Hydration with tap water.
3. Weigert iron hematoxylin, 2 min.
4. Tap water rinse.
5. Saturated aqueous Orange G in 5% phosphotungstic acid, 2—3 min.
6. Tap water rinse.
7. Phosphotungstic acid 5%, 15—30 min.
8. Tap water rinse.

![Fig. 1](image-url)
(5 g Wood Stain Scarlet (Dupont), 1 g Fast Green, and 2 g Acid Fuchsin in 100 cc of 7° acetic acid.)

When the desired intensity was obtained, sections were rinsed and placed for 2 to 3 minutes in a solution of 2% aniline blue in 1% acetic acid. Then sections were dehydrated with absolute alcohol, cleared with toluene, and mounted. Nuclei and other basophilic structures appeared pale brown, acidophilic structures red, and collagen blue.

OBSERVATIONS

Glomeruli of the control animals (Fig. 1) have structures similar to those already described (6, 15—17, 30). The nature of certain cells in the center of glomerular lobules is still unclear. Cells and processes lying beneath endothelial cells in the center of the glomerular lobules may represent the mesangium or may be endothelial cells not in contact with the lumen of the capillary in the plane of the section. Because the true nature of these cells is not settled, they will be referred to as “intercapillary cells” in this paper. In the normal glomerular epithelial cells there are certain cytoplasmic structures such as small vacuoles (30) and various other cytoplasmic bodies, including a few hyaline droplets.

The changes found in the uranium-treated animals are described below. Some of these changes appear to be largely exaggerations in either number or size of otherwise normally-occurring structures.

Glomerular epithelium

Loss of foot processes or pedicles of epithelial cells may occur over some glomerular capillaries in the latter part of the experimental period (Figs. 2, 3, and 10). This may occur alone but appears more often associated with dense cytoplasmic deposits (Figs. 3 and 10), or with the centrolobular lesion described below.

Hyaline droplets are frequently found in glomerular epithelium in paraffin sections about the third day after uranium injection (3). The same droplet has been identified

FIG. 2. This and subsequent pictures all represent glomeruli of rats treated with uranium. Extensive loss of foot processes (FP) is associated with focal deposits of an irregular dense material. The cytoplasm of intercapillary cells (I) appears considerably less dense than adjacent endothelial cell cytoplasm (End) lining the capillary. Five days after uranium injection. 20,000.

FIG. 3. An irregular dense deposit lies in an epithelial cell which has lost some foot processes (FP). A hyaline droplet (H) is seen in another epithelial cell. Five days after uranium injection. 14,000. Fig. 4. A group of hyaline droplets of different sizes and densities (H) lies in the left upper corner. An alteration of epithelial cytoplasm (Ep) is possibly related to the formation of hyaline droplets. Foot processes are lost in a small area (FP). Five days after uranium injection. x 12,000.
by both light and electron microscopy (2). In electron microscopy the droplets are moderately osmiophilic and homogeneous but occasionally some denser material is seen in them (Figs. 3, 4, and 5). In some, only portions of a single outer membrane are visible.

Cytoplasmic bodies appearing as small and medium-sized round structures, sometimes with incomplete boundaries, are frequently present in the cytoplasm along with hyaline droplets and other structures to be described. Particularly when small, some rounded masses seem to be formed by whorls of membranes. In other instances they appear to contain finely divided particles. The relationship of these structures to hyaline droplets is uncertain.

Myelin figures are found in the glomerular epithelium, frequently formed around one or more dense round bodies resembling hyaline droplets (Fig. 5). Surrounding the droplets is a zone containing a few vesicles and membranes. These in turn are surrounded by a thick outer layer of concentric membranes. Each membrane is about 40 Å thick. The outer membranes tend to be grouped in pairs (Fig. 6).

Cytoplasmic vacuoles appeared with increasing frequency from 2 to 5 days after injection (Fig. 7). They become so large and numerous in some instances that they obliterate the capsular space. Some of these vacuoles are over 10 μ in diameter. They are bounded by an osmiophilic membrane and sometimes are irregular in shape. The density of the intraluminal material is low. It is not always possible to distinguish vacuoles from Bowman’s space because both may be irregular and contain light material. These vacuoles appear at about the same time and sometimes in the same cells as the hyaline droplets. They may be found also in the same glomeruli that show centrolobular lesions, as seen in Fig. 7 and described below, but without relation to this lesion.

Centrolobular lesions containing collagen

Within four days after uranium injection a large number of glomeruli show focal alterations in the centrolobular areas. These were characterized elsewhere by the

FIG. 5. Myelin figures (My) in an epithelial cell. Some figures are very large and surround one or more hyaline droplets (H). Some hyaline droplets are also present outside myelin figures. In the left upper corner is a portion of the centrolobular lesion (CL) which is described in the text. This lesion is situated on the luminal side of the dense layer of the basement membrane (BMD). Collagen fibers (CF) are present among other components of the lesion. Six days after injection of uranium. x 24,000.

FIG. 6. This is a higher magnification of part of the upper myelin figure in Fig. 5. It shows the organization of the myelin figures, which are composed of membranes about 40 Å thick, frequently running in pairs. X 110,000.
formation of blue-staining masses in paraffin sections (3). Similar blue masses are present in methacrylate sections stained by the trichrome procedure described above. In light microscopy of these sections, the blue-staining areas appear to be composed of irregular masses frequently grouped together in the form of rough cords (Fig. 8). By the use of a thick and thin serial sectioning technique (2), the blue masses are found to correspond in the electron micrographs to a complex structure containing several elements including collagen fibers (Figs. 5, 7, and 9—12).

In electron micrographs these lesions are found centrally placed in the glomerular — lobule on the luminal side of the central dense layer of the basement membrane and beneath the endothelial cytoplasm (Fig. II). Occasionally they extend peripherally along the basement membrane toward the capillary lumen. The area involved may be larger than a cross-section of a single capillary. The morphologic composition of a centrolobular lesion is highly variable from area to area. Five closely related components are here distinguished: (1) intercapillary cells, some of which may have a lighter cytoplasm than adjacent endothelial cells lining capillaries (Fig. 2), and which may show groups of clear vacuoles with very dense and thick osmiophilic walls, probably lipid in nature (Figs. 9 and 10); (2) small cytoplasmic processes from inter-capillary cells lying in intimate contact with the extracellular portions of the lesion (Fig. 11); (3) extracellular groups of small dense granules without a sharp boundary, about 30 mu in diameter (Fig. 7); (4) large, poorly-demarcated, irregular, extracellular bodies up to 400 mu in diameter, lying separately or in groups and sometimes giving the impression of forming rough cords (Fig. II); (5) collagen fibers, probably the most striking component of the lesion (Figs. 5, II, and 12).

These fibers with the morphologic characteristics of collagen have a major periodicity which varies in different instances from about 390 to 690 A. These periods are usually shorter than the recognized average of 640 A but within the range of collagen fibers observed by others (24). A clear subperiodicity of 5 or 6 bands of varying density arranged in a definite polarized order serves as confirmation of the collagenous nature of these fibers (Fig. 12). They vary considerably in length, the ends of some are tapered, and they seem to lie pointing at random in different directions. Some lie close to intercapillary cells or cell processes.

FIG. 7. Marked vacuolization of epithelial cytoplasm (V) is apparent in the upper part of this picture. Extensive loss of foot processes (FP) is also seen. The lower portion of the figure shows the general distribution of the centrolobular lesions (CL). These appear as irregular light areas with several dark rough cords and large granules. Small granules about 30 m in diameter are best seen in the upper centrolobular lesions. In intimate association with these lesions are intercapillary cells (I). Five days after uranium injection. x5000.
DISCUSSION

Because epithelial vacuoles, hyaline droplets, loss of foot processes, and the appearance of the centrolobular lesion become prominent in glomeruli during and after the development of polyuria and proteinuria, it seems possible that these changes may be related to the latter.

Loss of foot processes has been found in pure nephrosis (28, 29), mixed glomerulonephritis and nephrosis (7), serum sickness nephritis (8), and nephrotoxic nephritis (20). Loss of foot processes has been related with massive proteinuria (29). The dense cytoplasmic deposits occurring in epithelial cells are of unknown significance. They have been illustrated in association with loss of foot processes in some renal diseases (7, 29) and in the present experiments.

Hyaline droplets have been found in epithelial cells of glomeruli of uranium-treated animals (2, 3, 10), in subacute glomerulonephritis (5), and in apparently healthy human beings (6). Although there has been considerable work regarding the significance of hyaline droplets in the proximal convoluted tubules (9, 11, 14, 21), their significance in the glomerular epithelium is not known. In the present work these droplets appear identical in the electron micrographs to hyaline droplets seen in the proximal tubules of uranium-treated rats (27). In glomeruli they become more abundant when proteinuria is maximal. This suggests that the droplets in the glomeruli may represent cellular uptake of protein as seems to be the case with proximal tubule cells (11, 14). Glomerular epithelial localization has been found after the injection of labeled foreign protein (11). Hyaline droplets have been considered to include also mitochondrial material or structures (9, 21, 22).

Cytoplasmic bodies with varying kinds of internal structures have been found in epithelial cells of the glomerulus as well as in those of the proximal tubules (27).
These bodies are similar to those found in alveolar macrophages in various conditions (25).

Myelin figures have been found with the electron microscope in other cells reading to various stimuli (19, 25), and in the tubular epithelium following uranium injection (27).

The cytoplasmic vacuoles occasionally found in control animals were sufficiently increased in size and number after uranium injection to be related to it. Similar but less marked vacuolization has been found in glomeruli from normal human beings (6), and in nephrotic and nephritic glomeruli (5, 7, 28). These vacuoles may be concerned with fluid transport, as has been suggested for the apical and central vacuoles appearing in the proximal tubule cells (27). Some functional similarities between glomerular epithelium and proximal convoluted tubule cells might be expected on the basis of embryologic and morphologic similarities.

The centrolobular lesions have not been described by electron microscopy in experimental animals (13, 23, 29) or in human beings (4, 5, 7, 28). Of the known lesions in human renal disease the one which this experimental lesion most nearly resembles by light microscopy is chronic glomerulonephritis of the lobular type (l).

A dense material interpreted as fibrinoid has been described between the basement membrane and the endothelium. Sometimes this material replaces the basement membrane in disseminated lupus erythematosus and subacute glomerulonephritis (7, 28). A more homogeneous deposit in the centrolobular area, believed to be in the cytoplasm of endothelial cells, was found in diabetic glomerulosclerosis (4).

The most remarkable feature of the centrolobular lesion in uranium-treated rats is the appearance of collagen fibers. Such fibers are absent in the controls and have not been described by others in normal glomeruli. Collagen has been described in amyloidosis and glomerulonephritis. In these instances, however, the glomeruli were so distorted that they were difficult to recognize (26). Moreover, no illustrations or description of the position of these fibers were given.

In the present experiments the position of collagen fibers on the capillary side of the basement membrane is unusual when compared to their position in the rest of the body. The close relation of collagen to intercapillary cells suggests that these cells.

Fig. II. Cytoplasmic processes of intercapillary cells (I) are in intimate contact with other components of a relatively small centrolobular lesion. The boundaries of these processes are not clear in some places because of the plane of cutting. The space around the cytoplasmic processes contains irregular dense large granules (OG), and collagen fibers (CF). The lesion extends from endothelium (End) to the dense layer of the basement membrane (BMD). Six days after injection of uranium. 24,000.

Fig. 12. Collagen fibers in a centrolobular lesion. Five and sometimes six subperiods can be identified. Six days after injection of uranium. 120,000.
may be different from endothelial cells and may have the potentiality of producing collagen in response to proper stimuli. Although other interpretations are possible, the appearance of collagen fibers in association with the processes of intercapillary cells is an observation to be added to other evidence favoring intercapillary cells being different from endothelial cells (5, 30). Intercapillary or mesangial cells have been thought to be related to smooth muscle cells (30). There is electron microscopic evidence that smooth muscle cells can produce collagen (18).

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