

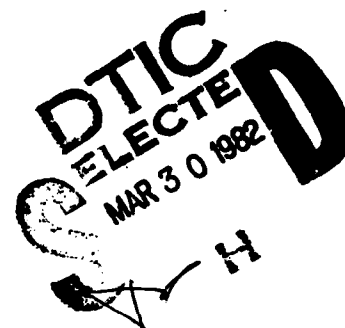
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Sequential Postmortem Changes of Glomeruli

Their Detection by Scanning Electron Microscopy

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• Postmortem changes were studied by scanning electron microscopy (SEM) in renal tissue from five species of animals. Specimens were collected at intervals up to 24 hours after death. Six distinct morphological alterations were identified in the renal glomerulus, including capillary loop constriction, changes in microvillus morphologic features, podocyte surface blebs, swelling or fusion of pedicels, podocyte erosion, and retraction or loss of pedicels. Glomerular size remained nearly constant in all five species. The results of these studies demonstrate that pathologic evaluation by SEM of tissue specimens from the kidney is acceptable up to 60 minutes post mortem.

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The morphologic characteristics of cellular autolysis have been well documented by transmission electron microscopy (TEM).¹⁻⁶ There is little information, however, on autolytic

architectural changes in specimens viewed by scanning electron microscopy (SEM). In one study, our institute examined glomeruli by SEM in human postmortem renal specimens for changes associated with acute renal failure.⁷ To distinguish lesions of the glomerulus from autolytic postmortem changes, a concurrent study was conducted using animal tissue collected under conditions that simulated those under which postmortem human specimens are collected. This study was conducted to determine the sequential autolytic changes during a 24-hour period, and to establish the time limit during which postmortem specimens would be acceptable for examination by SEM.

MATERIALS AND METHODS

Five species of animals were used in this study: five common laboratory rabbits (*Oryctolagus cuniculus*), three mixed-breed goats, five Sprague-Dawley rats, five Swiss ICR mice, and three mongrel dogs.

The animals were killed using minimum lethal doses of pentobarbital sodium, and kidney specimens were taken 0, 15, 30, 60, and 90 minutes and 2, 3, 4, and 24 hours after death. The corpses were kept at room temperature for the first four hours after death, then were refrigerated at 4 to 6 °C until the 24-hour specimen was taken.

All specimens were fixed for SEM in 2.5% glutaraldehyde in 0.1M cacodylate sodium buffer solution at a pH of 7.3 and at 4 °C for 24 hours. Fixed specimens were washed overnight in buffer, dehydrated in graded ethyl alcohol-water solutions to absolute ethyl alcohol, then through graded ethyl alcohol-trichlorotrifluoroethane (Freon 113) solutions to absolute trichlorotrifluoroethane. The specimens were dried by the critical-point method, using monochlorotrifluoromethane (Freon 13). Dried specimens were coated with gold-palladium and examined at either 10 or 20 kV.

Each specimen was examined for morphological changes by SEM and compared with the zero-minute specimens. All glomeruli in each specimen were examined to determine both the type and extent of morphological alterations. The number of glomeruli ranged from as few as seven to as many as 36. These alterations were judged by the following criteria: (1) severity of change, and (2) distribution (ie, focal or diffuse). The observed changes were considered focal if they were present only in segments of a glomerulus, and if not all glomeruli in the specimen were affected. The alterations were considered diffuse if they were found over the entire glomerulus, and if nearly all glomeruli in the specimen showed the same change.

RESULTS

The morphological observations are given in Table 1. Comparing the postmortem specimens with the zero-min-

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Table 1.—Observed Glomerular Changes*						
Time After Death	Constriction of Capillary Loops	Microvillus Morphology	Podocyte Surface Blebs	Swelling or Fusion of Pedicels	Podocyte Erosion	Retraction or Loss of Pedicels
15 min	Dog, goat, mouse, rabbit, rat: 1+ to 2+, D	Dog, goat, mouse, rabbit, rat: 1+, D	Dog, goat, mouse, rabbit, rat: 1+ to 2+, F	Mouse: 1+, F	...	...
30 min	Same change	Same change	Same change	Goat, mouse: 1+, F	Rabbit: 1+, F	...
60 min	Same change	Same change	Same change	Goat: 2+, F; mouse: 1+, F	Rabbit: 1+, F	...
90 min	Same change	Same change	Same change	Dog: 1+, F; goat: 2+, F; mouse: 1+, F; rabbit: 2+, F; rat: 2+, F	Dog: 1+, F; goat: 1+, F; mouse: 2+, F; rabbit: 1+, F; rat: 1+, F	Rat: 1+, F
2 hr	Same change	Same change	Same change	Dog: 1+, F; goat: 2+, F; mouse: 1+, F; rabbit: 2+, D; rat: 2+, F	Dog: 1+, F; goat: 1+, F; mouse: 2+, F; rabbit: 1+, F; rat: 1+, F	Goat: 1+, F; rabbit: 1+, F; rat: 1+, F
3 hr	Same change	Same change	Same change	Dog: 2+, D; goat: 2+, D; mouse: 1+, F; rabbit: 2+, D; rat: 2+, F	Dog: 1+, F; goat: 1+, F; mouse: 3+, F; rabbit: 2+, F; rat: 2+, F	Goat: 1+, F; mouse: 2+, F; rabbit: 1+, F; rat: 1+, F
4 hr	Same change	Same change	Same change	Dog: 2+, D; goat: 3+, D; mouse: 1+, F; rabbit: 2+, D; rat: 2+, F	Dog: 1+, F; goat: 1+, F; mouse: 3+, F; rabbit: 2+, F; rat: 2+, F	Goat: 1+, F; mouse: 3+, F; rabbit: 1+, F; rat: 2+, F
24 hr	Same change	Same change	Same change	Dog: 3+, D; goat: 3+, D; mouse: 2+, F; rabbit: 2+, D; rat: 2+, D	Dog: 2+, D; goat: 1+, D; mouse: 3+, F; rabbit: 2+, D; rat: 2+, F	Goat: 2+, D; mouse: 4+, D; rabbit: 2+, F; rat: 4+, D

*1+ indicates minimal; 2+, mild; 3+, moderate; 4+, severe; D, diffuse; and F, focal.

Fig 1.—Mouse glomerulus taken zero minutes post mortem. It is typical of all control samples in this study. Note normal capillary loops, with well-defined interdigitating pedicels ($\times 3,000$).



Fig 2.—Capillary constriction characterized by narrowing of loops producing ridge-like appearance in specimen from dog 15 minutes post mortem ($\times 3,000$).





Fig 3.—Rat glomerulus 30 minutes post mortem shows rounding and shortening of podocyte surface microvilli (arrows) ($\times 3,000$).



Fig 4.—Podocyte surface blebs from rabbit 30 minutes post mortem. Blebs vary in size and shape ($\times 3,000$).

Fig 5.—Left, Specimen taken 30 minutes post mortem from goat shows early podocyte swelling ($\times 3,000$). Right, Specimen 24-hours post mortem from dog shows more severe swelling and fusion of pedicels ($\times 3,000$).





Fig 6.—Rabbit glomerulus three hours post mortem shows areas of podocyte erosion (arrows) exposing intracellular contents ($\times 3,000$).

Table 2.—Comparison of Glomerular Size, μm					
Time, hr	Goat	Dog	Rabbit	Rat	Mouse
0	62.0×51.7	62.4×48.4	45.3×37.8	50.7×42.3	33.7×25.3
24	61.5×48.1	65.5×49.2	47.7×37.2	49.4×42.8	34.8×27.8

Fig 7.—Left, Mouse glomerulus from 90-minute postmortem specimen. Arrows show areas of early loss of pedicels exposing basement membrane ($\times 3,000$). Right, Rat glomerulus 24 hours post mortem shows severe loss of pedicels exposing large areas of basement membrane. Only few remaining pedicels are seen in upper edge of micrograph ($\times 3,000$).



The first three changes appeared as early as 15 minutes post mortem in all five species in a slight to mild degree, and remained nearly constant in distribution and severity throughout the 24-hour period. The last three changes were variable, appearing at different times in different species and with random distribution and severity. Only one change, retraction or loss of pedicels, was present in four species but was not seen in dogs. Of the three changes that became progressively severe with increased time post mortem, swelling or fusion of the pedicels was the most prominent in all five species. As is evident in the results given in Table 1, no significant differ-

All glomeruli were measured in each specimen collected at zero-minute and at 24 hours to determine if any shrinkage or swelling occurred. Table 2 gives the results of each species. The number of glomeruli measured ranged from nine to 28, with an average of 17. None of the animal groups showed significant change in glomerular size.

The present study was undertaken to establish a norm for the evaluation by SEM of pathological lesions in human renal postmortem specimens. A number of architectural changes were seen in our human specimens,⁷ but no studies are available to establish criteria for classifying these changes as pathological or autolytic artifacts. The use of five species of animals enabled us to determine that the observed autolytic changes were probably universal in the mammalian kidney. Simulating conditions used in obtaining human postmortem tissue specimens provided observations that I and my colleagues believe accurately mimicked the clinical situation. The results derived from these two facets of the study provided a reasonable guideline for evaluating the architectural changes seen in our human patients.

Postmortem autolytic changes do not seem to be as dramatic in the architecture of the renal glomerulus when viewed by SEM as do the ultrastructural changes observed in TEM specimens. Indeed, some glomeruli in the 24-hour specimens appeared normal. Table 2 indicates no significant change in glomerular size up to 24 hours. Based on the morphological observations shown in Table 1, it seems that postmortem renal specimens obtained within one hour after death are suitable for pathological evaluation by SEM.

1. Ito S: Light and electron microscopic study of membranous cytoplasmic organelles, in Harris RJC (ed): *The Interpretation of Ultrastructure*. New York, Academic Press Inc, 1962, pp 129-147.
2. Ito S: Post-mortem changes of the plasma membrane, in *Proceedings of the Fifth International Congress on Electron Microscopy*. New York, Academic Press Inc, 1962, p L-5.
3. Ashworth CT, Stembridge VA: Utility of formalin-fixed surgical and autopsy specimens

- for EM. *Am J Clin Pathol* 1964;42:466-480.
4. Glauret AM, Glauret RH: Araldite as an embedding medium for electron microscopy. *J Biophys Biochem Cytol* 1968;4:191-194.
5. Phase DC: *Histological Techniques for Electron Microscopy*. New York, Academic Press Inc, 1960, pp 15-29.
6. Trump BF, Goldblatt PJ, Stowell RE: An electron microscopic study of early cytoplasmic alterations in hepatic parenchymal cells of mouse liver during necrosis. *Lab Invest* 1962;11:986

7. Langlinais PC, Myers WD, Merrill RH: Scanning electron microscopic observations on glomeruli. *Arch Pathol Lab Med* 1980;104:308-312.

8. Churg J, Grisham E: Electron microscopy of glomerulonephritis, in Grunomann E (ed): *Current Topics in Pathology: Glomerulonephritis*. Berlin, Springer-Verlag, 1976, pp 109-116.

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