Growth and Maintenance of Glomerular Cells *In Vitro*[1] (35177)

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The glomerulus contains 3 cell types, epithelial, mesangial, and endothelial. Their exact function in the normal kidney and their importance in the pathogenesis of renal disease is unclear. Various functions have been assigned to each cell type from studies using *in vivo* manipulations and observation in disease states. Because it is difficult, if not impossible, to evaluate the interaction of various extraglomerular, plasma, and cell factors on the gomerulus or to assess the exact function and interrelationship of the three resident cell types in health and disease, it would be desirable to isolate the cells and study their individual behavior.

Kidney cell lines derived from renal tubular epithelium long have been established and currently are used on a routine basis in many tissue culture laboratories (1), but glomerular cells have not been specifically separated and propagated. Isolation of viable glomeruli and their maintenance *in vitro* was recently reported by Bernik (2). All three cell types survived in culture for prolonged periods, but cellular proliferation was minimal and the cells were not subcultured.

In the present study, glomeruli were isolated from the kidneys of several species and placed in tissue culture medium. Three morphologically distinguishable cell types were seen in the outgrowth. The largest number resembled those on the external aspect of the GBM and thus appeared to be glomerular epithelial cells. A variable number of intraglomerular cells were also identified. A third type of cell which occasionally appeared in the outgrowth resembled smooth muscle cells.

Materials and Methods. Isolation. Kidney tissue was obtained from rats, dogs, adult humans, and human fetuses and processed under sterile conditions by a modification of the Krakower–Greenspon method (3). Fragments of cortex were minced and buttered through a 100-mesh stainless steel screen which was then rinsed with Eagle's minimal essential medium (MEM) containing penicillin and streptomycin. The suspension was centrifuged at slow speed in a table top centrifuge for 2 min. The supernate was aspirated and discarded. The sediment was resuspended in MEM and the centrifugation was repeated twice. The sediment was then resuspended and poured onto a 150-mesh stainless steel screen and washed with MEM. A 200-mesh screen was used for fetal and rat kidneys. The retained glomeruli were washed off the screen with MEM and resuspended. The suspension was centrifuged once again for 2 min and the supernate discarded. The sediment was composed of large numbers of glomeruli which were generally free of capsular and tubular debris.

Culture conditions. The glomerular suspensions were placed in Weymouth's medium supplemented with nonessential amino acids, sodium pyruvate, D-glutamine, penicillin, streptomycin, and 30% heat-inactivated newborn calf serum. Enough media to just cover the culture plate was added to facilitate contact with the flask surface and hasten their attachment. 30-mm plastic tissue culture flasks and glass bottles were used. When cells covered the flask surface, they were subcultured or frozen.

Morphologic techniques. Light micrographs of viable cultures were taken with a microscope equipped with phase optics. For electron

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microscopy the cultures in plastic flasks were washed with saline and covered with 2% OsO_4 containing 0.1 *M* s-collodine buffer (pH 7.2). The flasks were washed with a graded series of alcohol and a thin layer of epoxy resin was placed over the culture. After partial polymerization, capsules filled with resin were inverted over glomeruli and cell outgrowths. After complete polymerization, flask were removed from the oven and the capsules were broken off of the flasks surface with a twisting motion. This operation had to be done before the resin cooled completely or a cleavage plane between the cell layer and the flask was not achieved. The blocks were thin-sectioned, being careful to retrieve the first few sections. Alternatively, the face of the block was sawed off and fragments were reimbedded perpendicular to the original plane.

Results and Discussion. The ease of preparation of glomeruli with intact cells depended on the kidney tissue obtained. Normal tissue yielded large quantities of satisfactory glomeruli. Tissue obtained from pateints with chronic renal disease was more difficult to process since the cortico-medullary junction was obscure and the tissue was fibrous and difficult to push through the screen. Despite these problems, glomeruli with viable cells were isolated from all but the most shrunken and fibrotic kidneys. The glomeruli obtained were free of capsules and tubular debris. The debris that was present after preparation generally stained with trypan blue.

Attachment and cell proliferation was most rapid with human fetal glomeruli. They attached to the culture flask in 3–5 days and an outgrowth of cells appeared in 5–7 days. Adult human, goat, mouse, and dog glomeruli attached in approximately 7 days, showing definite outgrowth in approximately 10 days. Proliferating cells covered the culture flask by about 25 days, the exact time depending on the number of glomeruli explanted. Rat glomeruli grew more slowly, requiring 3–5 weeks to attach to the flasks.

Light microscopy. Glomeruli remained identifiable for several passages. The cell outgrowth around the glomeruli consisted of large, irregularly shaped cells (Fig. 1). Near the glomerulus they often appeared to overlap or be several layers deep. The cytoplasm was not vacuolated except in one instance where the source of the glomeruli was a diseased kidney. The general cell morphology has remained stable for nearly 20 passages.

Electron microscopy. The appearance of cells resident on the exterior of the glomerular tufts was quite similar to that of normal glomerular epithelial cells. Complex interdigitations with adjacent cells were frequent, and they maintained a close relationship to the glomerular basement membrane. Free ribosomes were prominent cyctoplasmic features and many profiles of the Golgi apparatus were present. Mitochondria, endoplasmic reticulum, and lysosomes were not conspicuous.

The cells residing within the loops of the glomerular basement membrane contained mitochondria, myelin figures, and many single membrane-bounded structures with a dense matrix resembling lysosomes. Smooth and rough-surfaced endoplasmic reticulum were common features. Since glomeruli remained recognizable for few passages, it was not possible to determine if these cells proliferated. Cells with similar cytoplasmic organelles were not seen in the outgrowth.

The cells comprising the monolayer shared many features with those on the external aspect of the glomeruli (Fig. 2). The cells interdigitated with those on the glomeruli and formed junctional complexes at many points (Fig. 3). They would thus appear to be of the same cell type. A regular external lamina was not seen except between layers of cells. Even in this location a closely associated external lamina was not apparent.

A third cell type was occasionally seen in the outgrowth. These cells were elongate, contained a substantial amount of endoplasmic reticulum, formed multiple layers and often had a regular external lamina (Fig. 4). Peripheral dense bodies and microfilaments were present but not prominent. These features suggest that the cells are related to smooth muscle cells. Whether they are derived from the mesangium or from the vessels at the hilum of the glomerulus remains to be determined.

Isolation of these several glomerular cell types from diverse species, including humans, for the first time allows a study of their individual features. It may be possible to determine the role each plays in the synthesis of the glomerular basement membrane and other extracellular material. Finally, these cells may provide the basis for an *in vitro*

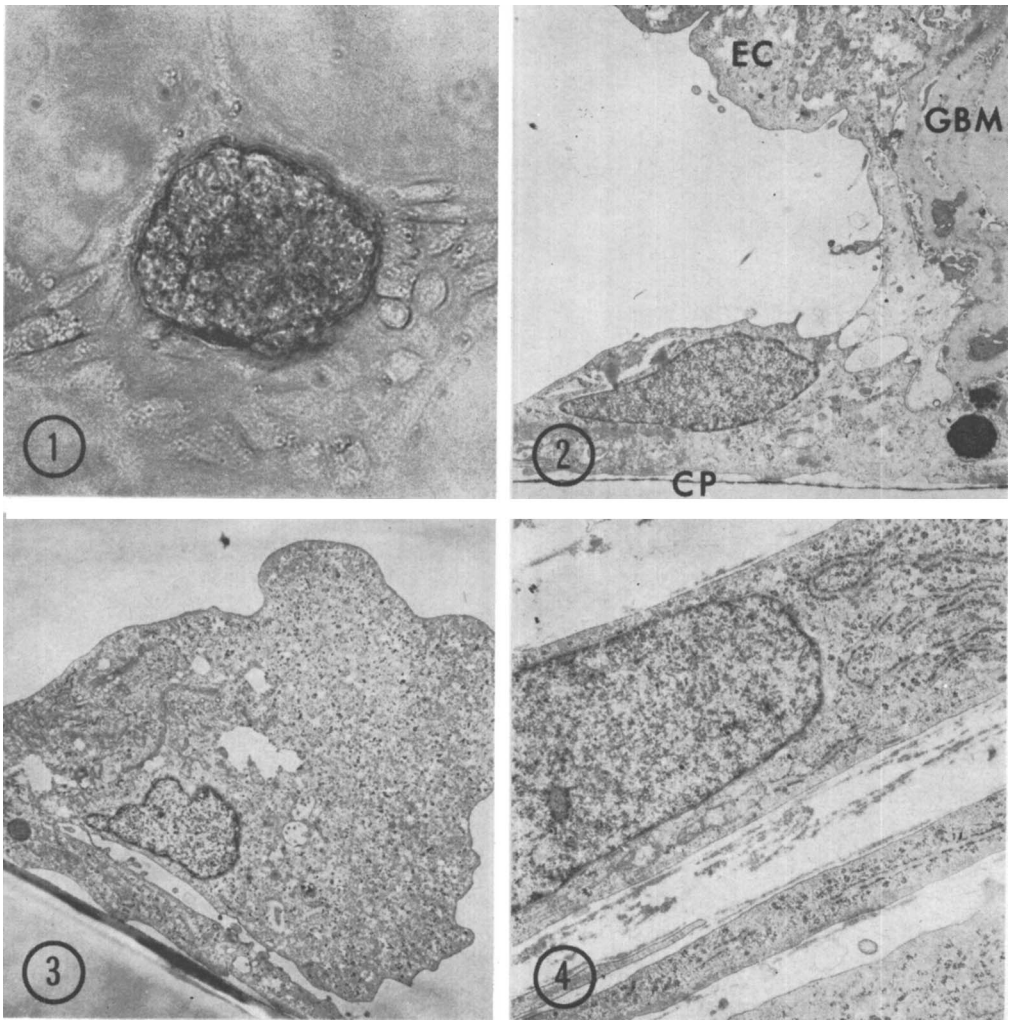

FIG. 1. Rat glomerulus six weeks after explantation. Large, irregularly-shaped cells surround the glomerulus. Phase contrast micrograph, ×200.

FIG. 2. Electron micrograph of the junction between cells (EC) covering a human glomerulus and its cellular outgrowth onto the culture plate surface (CP). The cells are morphologically similar and cell–cell contacts are frequent. (The collapsed layers of glomerular basement membrane (GBM) are on the right). ×1500.

FIG. 3. A single cell at a distance from a glomerulus (human). Like the cells resident on the glomerulus, the cytoplasm contains many free ribosomes, few lyososmes or mitochondria, and a prominent Golgi zone. ×5000.

FIG. 4. At the margin of a glomerulus three cells are seen. They are characterized by an elongate shape, prominent rough-surfaced endoplasmic reticulum, peripheral dense bodies, a modest number of microfibrils, and an external lamina. They are very similar to smooth muscle cells. ×10,300.

assay of factors important in the pathogenesis of glomerulonephritis, transplantation reactions, and other renal responses to injury.

Summary. Glomeruli with viable cells were isolated from rats, dogs, and humans in large numbers by a modification of the Karkower–Greenspon method. Cellular outgrowths having the characteristics of epithelial cells were observed. A second cell type was identified within glomerular loops and remained intact as long as glomeruli were recognizable. A third cell type which resembled smooth muscle cells was seen occasionally in the outgrowth.

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