ISOLATION AND CHARACTERIZATION OF CELLS OF A PRIMARY CULTURE OF RAT RENAL GLOMERULI

O. V. Gabrilevskaya, A. A. Ivanov,
S. M. Terekhov, and E. M. Shilov

UDC 612.465.1.085.23

KEY WORDS: renal glomeruli; cell culture; mesangial and epithelial cells; immunofluorescence

A new technqiue has recently been introduced to study the biology and pathology of the kidneys, and involves the use of cultures of separate cell types of the renal glomeruli. By the use of this method our knowledge of the physiology, biochemistry, and endocrinology of the renal glomeruli has been greatly widened and much basically new information has been obtained on the immunology and immunopathology of the kidneys and the mechanisms of immune inflammation in the glomeruli and progression of renal disease in man [1, 5].

Three types of renal glomerular cells have now been isolated and maintained as pure lines: epithelial, mesangial, and endothelial cells,. There is no generally accepted method of obtaining pure lines of these cell types. The suggested methods differ in the procedure of isolating glomeruli from the renal cortex, obtaining primary cultures of renal glomerular cells, and of isolating the separate types of cells [2-5].

The aim of this investigation was to determine optimal conditions for obtaining a primary culture of renal glomerular cells and of establishing the nature of these cell types.

EXPERIMENTAL METHOD

Random-bred albino rats, male and female, or male Wistar rats weighing 150-200 g were used. Different variants of isolation of the glomeruli from the renal cortex, treating the glomeruli with enzymes, and the substrates for their culture were compared (Table 1).

The extirpated kidneys were placed in Hanks' balanced salt solution (HBSS) with antibiotics (pencillin 1000 U/ml, streptomycin 1000 μg/ml). The glomeruli were isolated from the cortex by two methods. In the first [3, 4], fragments of cortex were gently pressed with a rubber spatula through a stainless steel sieve with pores measuring 110 μ, and then

TABLE 1. Conditions of Isolation and Culture of Renal Glomeruli

Conditions compared	Variant of isolation and culture of glomeruli					
	I	II	III	IV	V	VI
Isolation of glomeruli						
rubbing through a sieve	+	–	+	+	+	+
trypsinization and sieving	–	+	–	–	–	–
Treatment of glomeruli with enzymes:						
without treatment	+	+	–	–	–	–
with trypsin or chymotrypsin	–	–	+	+	–	–
with collagenase	–	–	–	–	+	+
Substrate for culture						
plastic	+	+	+	–	+	–
plastic + gelatin	–	–	–	+	–	+

I. M. Sechenov First Moscow Medical Institute. Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bochkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 1, pp. 57-60, January, 1990. Original article submitted December 14, 1988.

 0007-4888/90/0001-0076$12.50

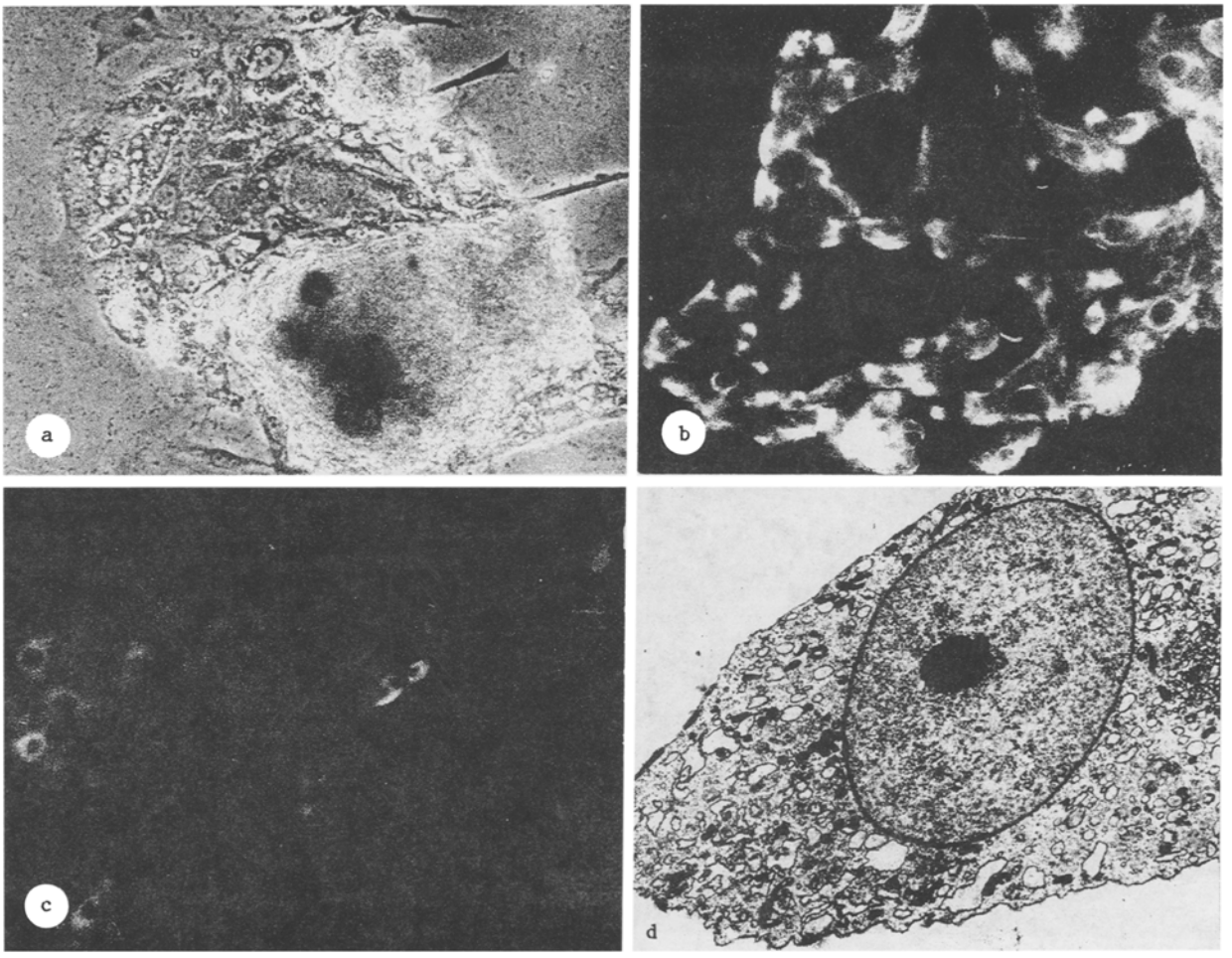

Fig. 1. Epithelial cells in culture of renal glomerulus. a) Phase-contrast microscopy, b) specific fluorescence with antibodies to vimentin, c) no reaction with antiserum to myosin, d) electron microscopy, 3200 ×.

rubbed through a sieve with pores measuring 110 and 40 μ. The material retained on the last sieve was washed in HBSS at 4°C and 50 g. In the second method fragments of cortex were subjected to enzymic digestion in 0.125-0.25% trypsin solution (Moscow Poliomyelitis Research Institute) or 0.025-0.05% solution of chymotrypsin (Leningrad Meat Combine) at 37°C and with constant mixing for 30-60 min. The supernatant was passed successively through 110-μ and 40-μ sieves. The glomeruli were collected from the 40-μ sieve.

When the glomeruli were isolated by the method of rubbing through a sieve, they subsequently either were not treated with enzymes or they were treated with a 0.125-0.25% solution of trypsin (0.025-0.05% solution of chymotrypsin) at 37°C for 30 min, or in a solution of collagenase, 750 U/ml (type IV, from Sigma, USA) at 37°C for 30-60 min. After treatment with enzymes, the glomeruli were washed in HBSS at 4°C and at 50 g.

Plastic tissue culture plates with 12 or 24 wells (Flow Laboratories, England) were used. Either the plates were used untreated or the wells were treated beforehand with a 1-2% solution of gelatin in HBSS for 2 h at room temperature.

The complete culture medium in all cases consisted of Eagle's medium (Moscow Poliomyelitis Research Institute), supplemented with a 20% (v/v) mixture of human umbilical serum and bovine serum (1:1), and containing glutamine (2 mM), insulin (0.66 U/ml), penicillin (100 U/ml), streptomycin (100 μg/ml), and amphotericin (2.5 μg/ml). The glomeruli were planted out in the wells with a density of 300/cm^2 surface and cultured at 37°C in a humid atmosphere of air with 5% CO_2. The primary cultures were subcultured by brief treatment of the cells with 0.05% trypsin (1:250, from Serva, West Germany) and 0.02% EDTA.

Growth of the culture was monitored by phase-contrast microscopy. The culture, fixed with acetone, was counterstained with azure II—methylene blue—fuchsine. The culture of renal

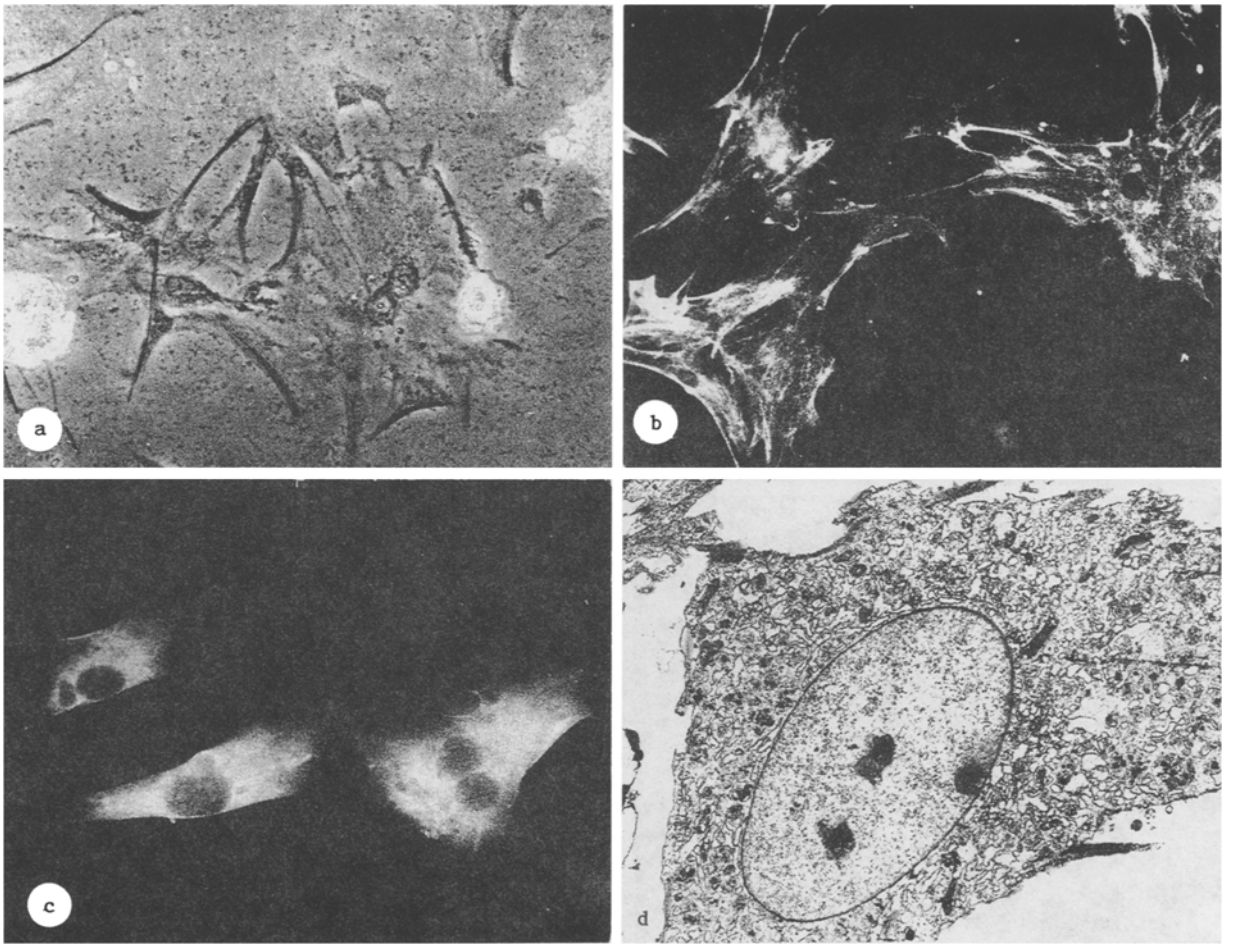

Fig. 2. Mesangial cells in culture of renal glomerulus. a) Phase-contrast microscopy, b) specific fluorescence with antibodies to vimentin, c) specific reaction with antiserum to myosin, d) electron microscopy, 2500 ×.

glomerular cells on coverslips was fixed by Karnovsky's method, using plane-parallel embedding. Ultrathin sections, stained with uranyl acetate and lead citrate, were examined in the EM 410 electron microscope (Philips, The Netherlands).

The cell composition was assessed under a "Fluovert" microscope (West Germany) with monoclonal antibodies to intermediate filaments: cytokeratin, desmin, and vimetin, and to the common leukocytic antigen CL (Dakopatts, Denmark), with rabbit antibodies to factor VII (Bergin, West GermanY), to myosin (the serum was obtained from V. S. Rukosuev, All-Union Cardiologic Scientific Center, Moscow), and to hematopoietic cells from rat bone marrow (AKKM-2; serum obtained from T. V. Vasil'eva, Msocow University). Fab-fragments against mouse and rabbit immunoglobulins, labeled with FITC (Dakopatts, Denmark), were used as secondary antibodies.

EXPERIMENTAL RESULTS

The six variants of isolation and culture of the renal glomeruli (Table 1) were compared with respect to following parameters: The degree of separation of glomeruli from the renal cortex, the viability of the cells in isolated glomeruli, and the presence of growth in the primary cell culture. The best results were obtained with variant VI. The remaining variants did not give satisfactory results. The use of trypsin to isolate the glomeruli or for their enzyme treatment always gave a low yield of glomeruli and cells of low viability. Transplanting glomeruli untreated with collagenase or on to plastic not covered with gelatin led to a low percentage of adhesion of the glomeruli and to poor growth.

Under optimal cultural conditions (variant VI) adhesion of the glomeruli began on the 2nd-3rd day and the first cell growth was observed on the 3rd-4th day after transplantation. Under the light microscope, cultures of renal glomeruli contained two types of cells. The

Table 2. Distribution of Antigenic Markers in Cells of Renal Glomerular Culture

Marker	Cells	
	epithelial	mesangial
Vimentin	+	+
Cytokeratin	—	—
Desmin	—	+
Myosin	—	+
Factor VIII	—	—
CL antigen	—	—
AKKM-2	—	—*

Legend. *)Single fluorescent cells unconnected with monolayer of mesangial cells.

first type consisted of polygonal cells, forming a monolayer around the adherent glomerulus (Fig. 1a). On electron-microscopic investigation, a few microvilli could be seen on the surface of the cells, the euchromatic nucleus was often located eccentrically, and the nucleolus was well defined. In the cytoplasm there were small, dilated cisterns of the rough endoplasmic reticulum, a well developed Golgi complex, small mitochondria, lysosomes, and lipid inclusions (Fig. 1d). Myelin bodies were present in the cytoplasm of some cells. Immunofluorescence microscopy revealed only vimentin (Fig. 1b, c). The reaction with other antibodies was negative (Table 2). These cells were identified as epithelial. Cells of the second type were spindle-shaped and covered with processes (Fig. 2a). On electron microscopy they contained an oval euchromatic nucleus with two or three nucleoli, a well developed rough endoplasmic reticulum two or three Golgi complexes, large mitochondria, and small osmiophilic granules. Bundles of microfibrils could be seen at the periphery, under the plasma membrane. Immunofluorescence microscopy revealed vimentin, desmin, and myosin (Fig. 2b, c). On the basis of these results these cells could be identified as mesangial, of smooth-muscle type. Besides the two types of cells described above, solitary cells unconnected with the main sheet of cells, and reaction with AKKM-2, were found in the primary culture, evidence of their bone-marrow origin.

As a result of this investigation optimal conditions were defined for obtaining a primary culture of rat renal glomeruli, and two types of cells were isolated and characterized: epithelial and mesangial of smooth-muscle nature. By subsequent subculture, a pure line of mesangial cells of smooth-muscle type was obtained.

LITERATURE CITED

1. E. M. Shilov, N. B. Gordovskaya, A. A. Ivanov, et al., Ter. Arkh., No. 11, 150 (1987).
2. C. H. Dubois, J. B. Foidart, M. B. Hautier, et al., Eur. J. Clin. Invest., 11, 91 (1981).
3. D. H. Lovett, R. B. Sterzel, M. Kashgarian, et al., Kidney Int., 23, 342 (1983).
4. C. Melcion, L. Lachman, P. D. Killen, et al., Transplant. Proc., 141, 559 (1982).
5. G. E. Striker and L. J. Striker, Lab. Invest., 53, 122 (1985).