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ABSOLUTE VOLUMES OF GLOMERULAR CELLS AND GLOMERULAR COMPARTMENTS IN THE NORMAL RAT KIDNEY

Mary C. Soosaipillai, John F. Bertram, Graeme B. Ryan

Department of Anatomy and Cell Biology, University of Melbourne, Parkville, Victoria 3052, Australia

ABSTRACT

Alterations in the size of renal glomeruli, in the number and size of glomerular cells, and in the size of the major glomerular compartments (matrix, capillary lumina, urinary space) occur in various renal disorders. We have previously used unbiased stereological methods to estimate glomerular size and the total number of glomeruli and individual glomerular cell types in the kidneys of seven normal female Sprague-Dawley rats. In the present study we combined the earlier data with volume density estimates obtained using traditional point counting methods at the electron microscopic level to estimate the average volumes of individual glomerular cell types and of the major glomerular compartments. Surface density estimates of the plasma membrane of glomerular epithelial cells (GECs) were used to estimate average GEC surface area. To estimate volume and surface densities, one thin section from each of six randomly selected renal corpuscles was analyzed from each rat. Each section provided approximately 20 electron micrographs (final magnification x23,500). Estimates were: renal corpuscle volume $8.588 \pm 1.084\ \mu m^3 \times 10^5$ (mean ± SD), GEC volume $584 \pm 105\ \mu m^3$, endothelial cell volume $151 \pm 39\ \mu m^3$, mesangial cell volume $149 \pm 31\ \mu m^3$, parietal epithelial cell volume $182 \pm 49\ \mu m^3$, matrix volume $3.841 \pm 0.604\ \mu m^3 \times 10^4$, capillary lumina volume $2.664 \pm 0.378\ \mu m^3 \times 10^5$, urinary space volume $3.467 \pm 0.509\ \mu m^3 \times 10^5$, and GEC surface area $5.211 \pm 0.967\ \mu m^2 \times 10^3$. This study has provided baseline data for the volumes of individual glomerular cells and the major glomerular compartments. This data will be useful in identifying changes in glomerular dimensions in disease.

Key words: disector, glomerulus, kidney, morphometry.

INTRODUCTION

The morphology of renal glomeruli is known to change in a variety of renal disorders. These changes often involve alterations in the numbers and sizes of individual glomerular cell types (GECs, endothelial cells, mesangial cells, parietal epithelial cells), and the volumes of the glomerular matrix, capillary lumina, and urinary space. Knowledge of the normal quantitative morphology of glomeruli should prove useful in detecting alterations in glomerular structure. Such information is also essential for a full understanding of the molecular mechanisms used by glomerular cells to maintain and regulate their architecture.

Recently, we used unbiased stereological methods to estimate mean glomerular volume and the number of individual glomerular cell types in the kidneys of seven normal rats (Bertram *et al.*, 1992). In the present study we used traditional point counting methods to estimate the volume densities of the four cell types, matrix, capillary lumina and urinary space in the renal corpuscles of these same seven rats. We also estimated the surface density of GEC plasma membrane. Through combining these density estimates with estimates of renal corpuscle volume and cell number, we have obtained unbiased estimates of the absolute volumes of the four cell types, as well as matrix, capillary lumina, and urinary space, and have also produced an unbiased estimate of GEC surface area.

MATERIALS AND METHODS

Animals and perfusion procedure

Seven female Sprague-Dawley rats weighing 215 ± 16 g were obtained from the Department of Pathology Animal House, University of Melbourne. These were the same animals used in our previous study (Bertram *et al.*, 1992). Urine protein excretion rates were within normal limits.

Rats were anaesthetized with sodium pentobarbitone (5 mg/100 g body weight; Nembutal, Abbott Laboratories, Australia) given intraperitoneally. Kidneys were perfused for 30 secs at 180 mmHg pressure with Hanks' Balanced Salt Solution, followed by a mixture of 4% paraformaldehyde and 1% glutaraldehyde in 0.1M phosphate buffer (pH=7.4) for 5 mins. Animals were then sacrificed via exsanguination.

Tissue sampling

Following perfusion, the left kidneys were weighed and cut into 1 mm-thick transverse slices using a razor blade fractionator (Baddeley *et al.*, 1986). Every second slice was taken for light microscopy (LM). The cortex on the remaining slices was diced and about 20 blocks per animal were randomly selected for transmission electron microscopy (TEM). The entire cortex was sampled. No attempt was made to restrict the study to a subpopulation of glomeruli, such as juxtamedullary glomeruli, for example.

Microscopy

Total glomerular number was estimated using a LM physical disector/fractionator combination, and the total number of cells per average glomerulus was estimated using a LM optical disector/Cavalieri combination (see Bertram and Nurcombe 1992; Cruz-Orive and Weibel 1990; Gundersen *et al.*, 1988). Physical disectors of unknown thickness were used at the TEM level to estimate the total number of individual cell types in an average renal corpuscle. These methods are described in detail in Bertram *et al.* (1992).

To estimate volume and surface densities, one technically perfect thin section was collected from each of six randomly selected renal corpuscles from each rat. Each section provided 20 electron micrographs, taken systematically throughout the entire renal corpuscle. Micrographs were measured at a final magnification of approximately x23,500. A micrograph of a carbon replica grating (21,600 lines/cm) was obtained during each microscope session and used to calibrate micrographs.

Estimating average renal corpuscle volume

Average renal corpuscle volume (V_{CORP}) was estimated using:

$$V_{CORP} = \frac{V_{V\,(CORP,KID)}}{N_{V\,(GLOM,KID)}} \quad (1),$$

where $V_{V(CORP,KID)}$ was the volume density of renal corpuscles in the kidney and was estimated using traditional stereological point counting methods at the light microscopic level, and $N_{V(GLOM,KID)}$ was the numerical density of glomeruli in the rat kidney. See Bertram *et al.* (1992) for further details on estimating $N_{V(GLOM,KID)}$.

Estimating volume densities

The volume densities of seven compartments of the renal corpuscle were determined using traditional point counting methods (Weibel 1979). An orthogonal test grid drawn on

transparent plastic and comprising 84 points each separated by 2 cm was used. PCS System II software (Pentcheff and Bolender 1985; Pentcheff 1987) running on an IBM AT-compatible microcomputer was used to collect and store data.

Points overlying the following compartments of renal corpuscles were counted: glomerular epithelial cells (GEC); endothelial cells (ENDO); mesangial cells (MES); parietal epithelial cells (PEC); glomerular matrix (MATRIX), including points falling on the glomerular basement membrane; capillary lumina (CAP); and urinary space (US).

Volume densities were estimated as exemplified in equation 2 for GECs:

$$V_{V\,(GEC,CORP)} = \frac{P_{GEC}}{P_{CORP}} \qquad (2),$$

where P_{GEC} was the number of points on GECs and P_{CORP} was the total number of points overlying the renal corpuscle.

Estimating absolute volumes

To estimate the absolute volumes of renal corpuscle compartments, the volume density of the compartment was multiplied by V_{CORP}. For example, to estimate the absolute volume of matrix in an average renal corpuscle we used:

$$V_{MATRIX} = V_{V\,(MATRIX,CORP)} \times V_{CORP} \qquad (3).$$

To estimate the absolute volumes of individual cell types, cell volume density was multiplied by V_{CORP}, and this product was divided by the total number of that cell type per average glomerulus (from Bertram *et al.*, 1992). For GECs the equation was:

$$V_{GEC} = \frac{V_{V\,(GEC,CORP)} \times V_{CORP}}{N_{(GEC,CORP)}} \qquad (4).$$

Estimating surface density

To estimate the surface density of GEC plasma membrane in renal corpuscles, a standard intersection counting method was used. The micrographs and orthogonal grid used to estimate volume densities were employed.

The surface density of GEC plasma membrane was determined using:

$$S_{V\,(GEC,CORP)} = \frac{2I}{P_{CORP} \times 2 \times d} \qquad (5),$$

where I was the number of intersections between the test grid and the plasma membrane, P_{CORP} was the total number of points overlying the renal corpuscle, and d was the length of each test line corrected for magnification.

Estimating the absolute surface area of GECs

The absolute surface area of GECs was estimated using:

$$S_{GEC} = \frac{S_{V\,(GEC,CORP)} \times V_{CORP}}{N_{(GEC,CORP)}} \qquad (6).$$

Statistics

Values are means ± standard deviations. The inter-individual coefficient of variation (CV) is the standard deviation expressed as a percentage of the mean.

RESULTS

Mean renal corpuscle volume was 8.588 ± 1.084 $\mu m^3 \times 10^5$. Estimates of renal corpuscle volume for the seven rats are presented in Table 1. Estimates of volume densities and absolute volumes of glomerular matrix, capillary lumina and urinary space are given in Table 2. Volume densities and absolute volumes of the four cell types are given in Table 3. The surface density and absolute surface area of GEC plasma membrane are presented in Table 4.

Table 1. Estimates of average renal corpuscle volume (V_{CORP}) for the seven normal rats.

Animal	1	2	3	4	5	6	7	Mean	SD	CV (%)
V_{CORP} $\mu m^3 \times 10^5$	8.448	8.672	9.053	6.928	10.534	8.137	8.343	8.588	1.084	12.6

Table 2. Estimates of volume densities and absolute volumes of glomerular matrix, capillary lumina (CAP), and urinary space (US).

Animal	$V_{V\,(MATRIX,CORP)}$	$V_{V\,(CAP,CORP)}$	$V_{V\,(US,CORP)}$	V_{MATRIX} $\mu m^3 \times 10^4$	V_{CAP} $\mu m^3 \times 10^5$	V_{US} $\mu m^3 \times 10^5$
1	0.043	0.332	0.378	3.616	2.803	3.190
2	0.050	0.275	0.403	4.371	2.387	3.500
3	0.048	0.288	0.434	4.372	2.610	3.926
4	0.045	0.313	0.397	3.090	2.165	2.753
5	0.038	0.320	0.403	3.950	3.370	4.245
6	0.055	0.317	0.380	4.443	2.580	3.091
7	0.037	0.327	0.427	3.045	2.731	3.562
Mean	0.045	0.310	0.403	3.841	2.664	3.467
SD	0.007	0.021	0.021	0.604	0.378	0.509
CV (%)	14.9	6.7	5.3	15.7	14.2	14.7

Table 3. Volume densities and absolute volumes of glomerular epithelial cells, endothelial cells, mesangial cells and parietal epithelial cells in seven normal rat kidneys.

Animal	$V_{V\,(GEC,CORP)}$	V_{GEC} μm^3	$V_{V\,(ENDO,CORP)}$	V_{ENDO} μm^3	$V_{V\,(MES,CORP)}$	V_{MES} μm^3	$V_{V\,(PEC,CORP)}$	V_{PEC} μm^3
1	0.126	733.5	0.056	217.4	0.041	153.6	0.025	273.5
2	0.149	687.8	0.049	157.5	0.043	189.2	0.031	224.1
3	0.105	535.5	0.049	116.9	0.048	140.3	0.028	161.3
4	0.127	523.3	0.042	119.9	0.055	159.8	0.022	162.9
5	0.129	428.7	0.046	140.4	0.045	144.1	0.020	140.7
6	0.136	625.2	0.039	186.8	0.052	167.6	0.022	167.3
7	0.124	555.3	0.038	118.7	0.029	88.5	0.018	144.4
Mean	0.128	584.2	0.045	151.1	0.045	149.0	0.024	182.0
SD	0.013	104.7	0.007	38.8	0.008	31.3	0.004	48.8
CV (%)	10.5	17.9	14.3	25.7	18.6	20.9	18.6	26.8

Table 4. Surface density and absolute surface area of GEC plasma membrane in seven normal rat kidneys.

Animal	1	2	3	4	5	6	7	Mean	SD	CV (%)
$S_{V\,(GEC,CORP)}$ μm^{-1}	0.971	1.212	0.928	0.980	0.971	1.160	0.946	1.024	0.113	11.0
S_{GEC} $\mu m^2 \times 10^3$	5.657	5.591	4.746	4.041	6.865	5.333	4.243	5.211	0.967	18.6

DISCUSSION

This study provides estimates of the volume densities and absolute volumes of the four cell types normally found in renal corpuscles (GECs, endothelial cells, mesangial cells, parietal epithelial cells), and the three other major compartments of renal corpuscles (matrix, capillary lumina, urinary space). Volume density estimates were combined with unbiased estimates of renal corpuscle volume to obtain unbiased volume estimates.

It is important to note that no attempt was made in this study to correct the volume density and absolute volume estimates for the well-known effects of tissue processing for microscopy on tissue dimensions (Bahr *et al.*, 1957; Bertram *et al.*, 1986). However, specimens were processed using a rigorously standardized protocol. The shrinkage of soft tissues embedded in Araldite/Epon is generally of the order of 10-15%.

It is difficult to compare the present unbiased estimates of glomerular cell volume and glomerular compartment volume with previous estimates. In most of the earlier studies, assumptions of glomerular shape, size and size distribution were required to estimate glomerular volume with traditional stereological methods. Moreover, in those studies that estimated glomerular cell volume, biased methods were used to count cells (nuclei), and subsequently estimate the volumes of cells. Some studies were restricted to subpopulations of glomeruli, such as juxtamedullary glomeruli. In the present study, no assumptions were required of the shape, size or size distribution of glomeruli or nuclei, and the total population of glomeruli was analysed.

The present study also provides estimates of the absolute surface area of the GEC plasma membrane. Again, a traditional stereological method (intersection counting) was combined with unbiased estimates of glomerular number and GEC number to obtain the estimate. Alterations in GEC morphology are observed in a variety of glomerular disorders, and this parameter may serve as a useful index of GEC architecture.

The present data provide a baseline for studies on glomerular development, cell biology and pathology. We are currently using these methods to assess glomerular morphology in acute and progressive models of glomerular disease.

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